Encyclopedia of Cardiovascular Research and Medicine

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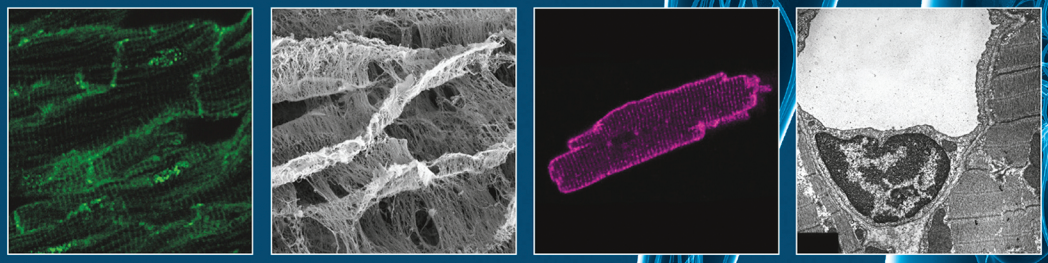

1 VOLUME

ENCYCLOPEDIA OF
CARDIOVASCULAR RESEARCH AND MEDICINE

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CARDIOVASCULAR RESEARCH AND MEDICINE

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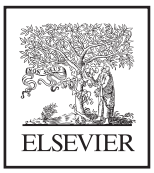

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PREFACE

The burden of cardiovascular disease is growing across the globe as the leading noncommunicable cause of morbidity and mortality. This is despite outstanding research that has improved our understanding of cardiovascular biology, the determinants of disease, and development of new therapies. We are optimistic that the next generation of scientists and clinicians will further these efforts and ultimately bring about changes that will reduce risk and improve outcomes for patients worldwide. We have assembled this encyclopedia with the explicit intent to provide this generation with easy access to past and current wisdom, with the hope that this helps to accelerate innovation in cardiovascular medicine.

This encyclopedia represents both a dream and a labor of love. The dream is to create a scientific compendium that recognizes that the methods and tools used to access and study the scientific literature have changed, with a burgeoning impact of digital technology—the so-called pocket book encyclopedia that is accessible on a smart phone fitting into medical overalls, and that can also be read as a textbook at a desk! These changes in the access of publications have been accompanied by the evolving need for studying different aspects of biology and disease as stand-alone chapters not necessarily read sequentially. Furthermore, as editors we recognize the fundamental importance of conveying knowledge through brilliantly illustrated texts that are profusely embedded with links to key references. The critical importance to use multimedia tools and to provide downloadable slides for educational purposes is reflected in this encyclopedia. Additionally, we want to ensure the feasibility of updating text, figures, and other reading material iteratively even after a publication date, recognizing the rapid advent of information and huge strides made in cardiovascular medicine every day. Thus, this work represents the end of the beginning of a live text that will metamorphose over time, being guided by the rapid strides and advances in both cardiovascular medicine and publication science.

Overall, we conceptualized this work as providing broad coverage of science and also serving the expansive needs of a readership that is quite diverse, that is, encompassing medical students, residents, fellows, and postdoctoral scientists interested in cardiovascular medicine. We hope the labor of love of an outstanding team of editors and a stellar group of authors is readily evident to the broad readership we have targeted.

Assembling this compendium had many challenges. Early on we made the choice to cover the broad range of cardiovascular biology, pathophysiology, epidemiology, and treatment of disease. We sought to assure equal coverage to foundational cardiovascular cellular and molecular biology as well as state-of-the-art treatments. Covering the breadth inevitably led to some overlap between some chapters, and yet we certainly have some residual gaps. We hope to have the opportunity to fill these gaps in future editions. There is also inevitable variation in the style and scope of articles in this multiauthor publication. We and the section editors felt it was more important to allow our expert authors freedom in how they chose to present their topic.

We invite feedback on how this encyclopedia can be improved as a resource for trainees worldwide. Undoubtedly, as noted above, there are gaps in content areas that need to be bridged in future iterations of this encyclopedia. We look forward to hearing from and listening to our readership about how the text can be better organized or better compiled to serve their scholarship better in future years.

This project was made possible through the hard work of many people from across the globe. We were pleased to have section editors and authors from around the world. Drs. Kessler and Schunkert, renowned cardiologists with an outstanding record of accomplishment in genetic research, have compiled a series of chapters on genetics and genomics. Dr. Baliga, a distinguished cardiologist and educator, has conceptualized a tour de force of chapters that capture all aspects of the heart failure syndrome, whereas Dr. DiSalvo, a master clinician and educator, has synthesized a fine tapestry of chapters focusing on diagnostic testing with key sections on cardiac imaging, interventions, and device therapy. Dr. Raj, an outstanding clinical investigator and

electrophysiologist, brought together an exemplary collection of chapters that offer coverage of a broad spectrum of topics in cardiac electrophysiology. Drs. Major and Liaw, both highly accomplished basic scientists and educators, were instrumental in identifying topics of fundamental importance to the field of cardiovascular biology and medicine. Dr. Lenihan, a pioneering clinical scientist and outstanding cardiologist, brought together a special set of articles that represent interactions between cardiovascular and other fields of medicine. Dr. Gale, a celebrated cardiologist with expertise in comparative effectiveness research and clinical cardiology, offers a series of stellar chapters underscoring various aspects of the pathogenesis and management of atherosclerotic cardiovascular disease. Dr. Kokubo, a leading preventive cardiologist with specialization in cardiovascular epidemiology, has orchestrated state-of-the-art chapters on cardiovascular epidemiology.

We would like to thank our colleagues at Elsevier who provided steadfast support of an exemplary nature throughout this long journey. Last but not the least, the editors would like to thank their families, their mentors, and their respective institutions for the constant encouragement, advice, and support over the years.

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A

Adult Congenital Heart Disease

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Glossary

ACHD *Adult congenital heart disease*: Field of medicine dealing with adult survivors of cardiovascular birth defects relating to their prior pathology as well as consequences of prior interventions.
ASD *Atrial septal defect*: A communication between the atria resulting from incomplete formation of the interatrial septum.
CHD *Congenital heart disease*: Field of medicine dealing with cardiology resulting from embryologic errors and structural problems present since birth.
CoA *Coarctation of the aorta*: A narrowing of the aorta, which may be defined or focal, commonly near to the juxtaductal region.
ES *Eisenmenger syndrome*: Disease state in which a previously left-to-right shunting lesions reverses and becomes right-to-left.
FALD *Fontan-associated liver disease*: Unique state of liver dysfunction found in the majority of patients who undergo single-ventricle palliation.
PAH *Pulmonary arterial hypertension*: Elevated blood pressure in the pulmonary vasculature.
PDA *Patent ductus arteriosus*: A fetal blood vessel connecting the pulmonary artery to the descending aorta.
VSD *Ventricular septal defect*: A communication between the atria resulting from incomplete formation of the interventricular septum.

Introduction

Congenital heart disease (CHD) is a term used to describe structural malformations of the heart and/or great vessels present since birth. CHD is the most common major birth defect present in approximately 8/1000 live births (Bernier et al., 2010; Marelli et al., 2014; Shuler et al., 2013). The term CHD has been used for decades and is generally accepted in reference to children, as prior to the past few decades, few palliative/surgical options were available that permitted survival into adulthood. In the past 50 years significant advances in medical, surgical, and interventional treatments have changed the face of CHD. There are now more adults living with CHD than children, making the field of adult congenital heart disease (ACHD) a relatively new subspecialty that focuses on the unique care needs of the adult patient who has survived with CHD. Adults with CHD vary in their presentation to the healthcare system, with many asymptomatic to those with significant cardiovascular disease including late sequelae from the original CHD anatomy and/or repair. Commonly, these patients may experience heart failure, arrhythmia including malignant variants, and extracardiac involvement. Each ACHD patient has unique needs reflective of underlying anatomy, prior surgical

procedures, catheter interventions, and other comorbid disease. Heterogeneity in any of these factors contributes to the complex care that this special population requires.

Etiology

There is no readily identifiable precise cause of CHD. However, there are some important associations with CHD. For instance, maternal infections such as rubella are associated with increased risk of CHD in the fetus. Pregnant women with diabetes mellitus, whether gestational or pregestational, are known to have fetuses with higher rates of CHD (Simeone et al., 2015). Some medications are associated with increased risk of CHD: isotretinoin, lithium, anticonvulsant medications, folic acid antagonists, and thalidomide among others (Ruedy, 1984). Genetics, and more specifically cardiogenetics, is an evolving field. While there are no universal known genetic associations with CHD, research has shown that CHD can result from: single-nucleotide polymorphisms, microdeletions, duplications, single-gene mutations, and aneuploidy (Fahed et al., 2015; Emer et al., 2015; Su et al., 2016). Trisomy syndromes are relatively common; for instance, Trisomy 21 (Down's syndrome) is associated with midline defects involving the endocardial cushions such as partial and complete atrioventricular septal defects. Meanwhile, specific genetic mutations in TBX5, NKX2-5, TLL1, and others have been associated with other CHD lesions. Regardless of the cause, once the alteration in normal embryologic development is sustained, CHD can result.

Embryology

To understand CHD, one must have a brief understanding of the embryologic basis of cardiac development. Errors at any of the steps in normal cardiovascular development may lead to CHD or even fetal demise. Cardiogenesis is dependent on multiple transcription factors and proteins interacting at specific times and locations throughout gestation and still is not completely understood. Cells destined to become cardiac structures are identified as early as 15 days and form a cardiac tube at approximately 21 days gestation. It develops into a sinus venosus structure, which ultimately forms atria, which receive venous structures as well as multiple primitive structures (Fig. 1). Over the following week, the bulboventricular region separates from the primitive atrium and ventricle, and the endocardial cushion forms and fuses dividing the atrioventricular canal into two segments. Around the same time, a septum forms in the primitive atrium both from the endocardial cushions and also from the ventral wall of the ostium primum. Also, the primitive ventricle undergoes septation via a distinct series of events, which lead to left and right ventricles forming side-by-side. The interventricular septum has contributions from neural crest cells of the endocardial cushion, the bulboventricular flange, and the inferior edge of the spiral septum of the conotruncus, which is the outflow chamber for the primitive ventricles. The conotruncus undergoes spiral septation allowing the aortic valve to move posteriorly to the left ventricle and the pulmonary valve to align with the anterior right ventricle. This series of developmental folds and morphologic relationships form the structurally recognizable normal anatomic heart and great vessels.

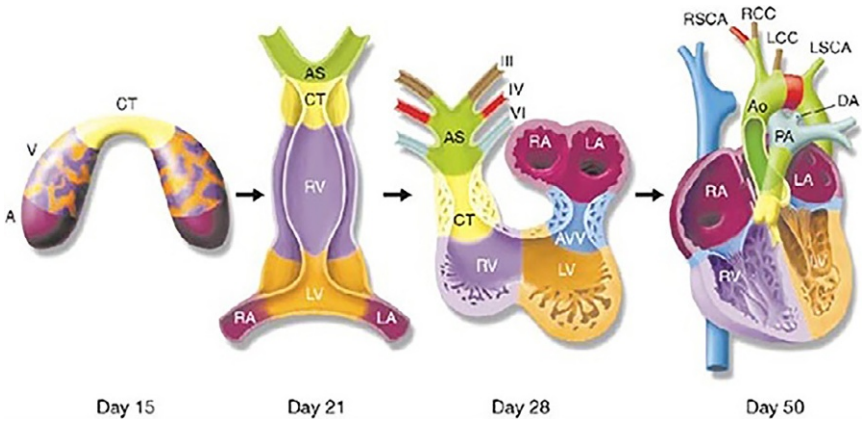

Fig. 1 Schematic of cardiac morphogenesis. Illustrations depict cardiac development with color coding of morphologically related regions, seen from a ventral view. Cardiogenic precursors form a crescent (left-most panel) that is specified to form specific segments of the linear heart tube, which is patterned along the anterior–posterior axis to form the various regions and chambers of the looped and mature heart. Each cardiac chamber balloons out from the outer curvature of the looped heart tube in a segmental fashion. Neural crest cells populate the bilaterally symmetrical aortic arch arteries (III, IV, and VI) and aortic sac (AS) that together contribute to specific segments of the mature aortic arch, also color coded. Mesenchymal cells form the cardiac valves from the conotruncal (CT) and atrioventricular valve (AW) segments. Corresponding days of human embryonic development are indicated. *A*, atrium; *Ao*, aorta; *DA*, ductus arteriosus; *LA*, left atrium; *LCC*, left common carotid; *LSCA*, left subclavian artery; *LV*, left ventricle; *PA*, pulmonary artery; *RA*, right atrium; *RCC*, right common carotid; *RSCA*, right subclavian artery; *RV*, right ventricle; *V*, ventricle.

Nomenclature

There are multiple systems of nomenclature to describe CHD, and none of them is universally accepted. Herein we will use the presence of cyanosis at birth (saturation ≤85%) as the initial delineation (cyanotic vs acyanotic lesions). An *acyanotic* lesion is typically the result of an obstructive lesion or a left-to-right shunt. Among *cyanotic* lesions, a right-to-left shunt must be present. For the interested reader, complex nomenclature practices can be reviewed (Anderson et al., 1984; Van Praagh, 1972).

Acyanotic Lesions

Obstructive lesions

Obstructive lesions include those affecting the right ventricular outflow tract such as pulmonary stenosis, and those affecting the left ventricular outflow tract, such as aortic stenosis (subvalvular, valvular, and supravalvular) and coarctation of the aorta (CoA). By definition, obstructive lesions restrict forward flow of blood. Symptoms are largely dependent on the location and severity of obstruction. Pulmonary stenosis is among one of the most common types of CHD, and it is variable in its pathology, occurring below, at, or above the valve level. Pathology varies and can include valvular dysplasia and/or doming of the pulmonary valve leaflet due to abnormal coaptation and valve function. Depending on the extent of obstruction, atrial level shunting may occur if an interatrial communication persists. If this is the case, oxygen saturation may be affected until the obstruction is palliated. Commonly, even after palliation, poststenotic dilation of the main pulmonary artery is seen.

In left ventricular outflow tract obstruction pathology is similar and can occur below, at, or above the valve level. Valvular pathology can include unicupsid, bicuspid, tricuspid, and quadricuspid aortic cusp variants with or without valvar dysplasia. Like pulmonary stenosis, poststenotic dilation commonly is seen (here of the aorta). Discrete and long-segment CoA subtypes may influence the type of intervention timing as well as age of presentation (including whether or not there is a well-developed spinal arterial system and dependent upon the presence of collateral blood vessels) (Fig. 2). Blood flow inferior to the diaphragm is

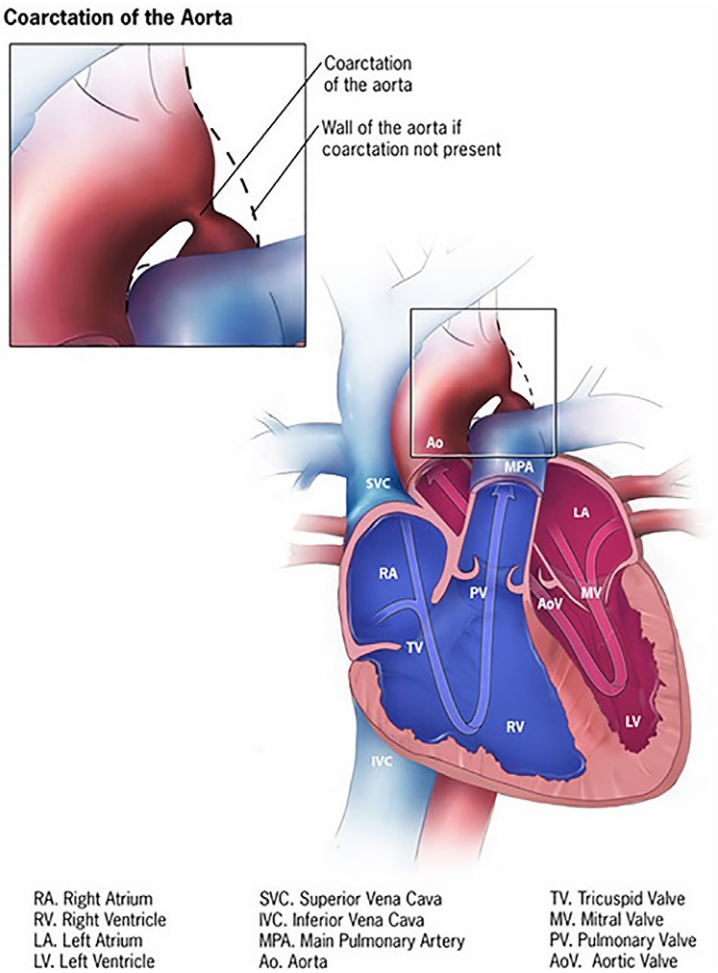

Fig. 2 Coarctation of the aorta is demonstrated here and represents an obstructive lesion of the aorta, which affects distal blood flow.

compromised by this lesion when the patent ductus arteriosus (PDA) closes, and collateralization occurs to supply blood distal to the level of obstruction. The proximal segment is sometimes dilated and is exposed to increased blood pressure while the distal segment is often diminutive because of decreased flow and with subsystemic pressure. It is not uncommon for adults to present with CoA undiagnosed. They typically have few if any symptoms, and the only finding may be hypertension.

Left-to-right shunts

Atrial septal defects (ASDs) constitute the most common ACHD lesion and occur when there is a defect in the septum between the atria. There are four major types, and all of them lead to varying degrees of the same shunting physiology (Fig. 3). ASDs typically lead to volume overload of the right atrium and ventricle. Over decades this volume-loading lesion may lead to elevated right-sided pressure and resistance, and in a minority of patients pulmonary arterial hypertension (PAH) may occur.

Ventricular septal defects (VSDs) are another common type of intracardiac shunt. Given the embryologic origin of the ventricular septum, a VSD can form in several locations. Commonly VSDs are seen in the membranous septum, which lies beneath the aortic valve. Other defect locations include: conal septum, adjacent to the tricuspid valve and within the muscular septum, and muscular septum (Fig. 4). Defects vary in size, number, and shape, making their physiology and anatomy strikingly disparate. Lesions can coalesce and atrioventricular canal defects occur when the endocardial cushions fail to fuse properly. This leads to failure of the mitral valve to form correctly, and a cleft in the left-sided atrioventricular valve is always present.

PDA is the persistence of a fetal vessel called the ductus arteriosus, which normally involutes in the first few days after birth. It is an artery connecting the aorta to the pulmonary artery (Fig. 5). Its persistence is more commonly seen in premature infants but may be seen in full term infants as well. The muscular artery's persistence leads to variable outcomes. If tortuous and narrow, then it acts as a resistor and protects the pulmonary vascular bed; however, it may be broad and straight, which may lead to pulmonary overcirculation and/or PAH early in life.

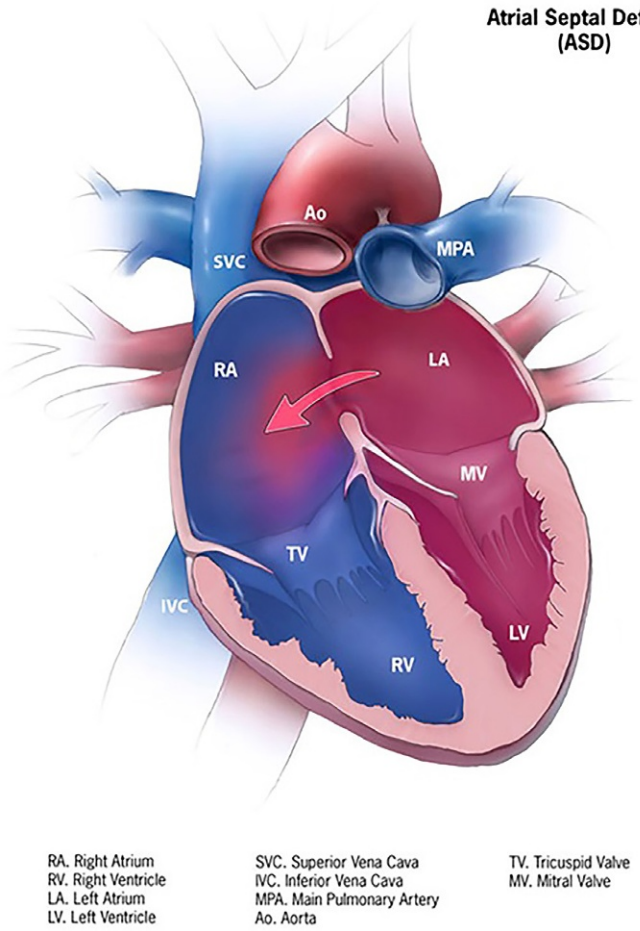

Fig. 3 Atrial septal defect is demonstrated here and represents a deficiency in the atrial septum that permits shunting of blood at this level.

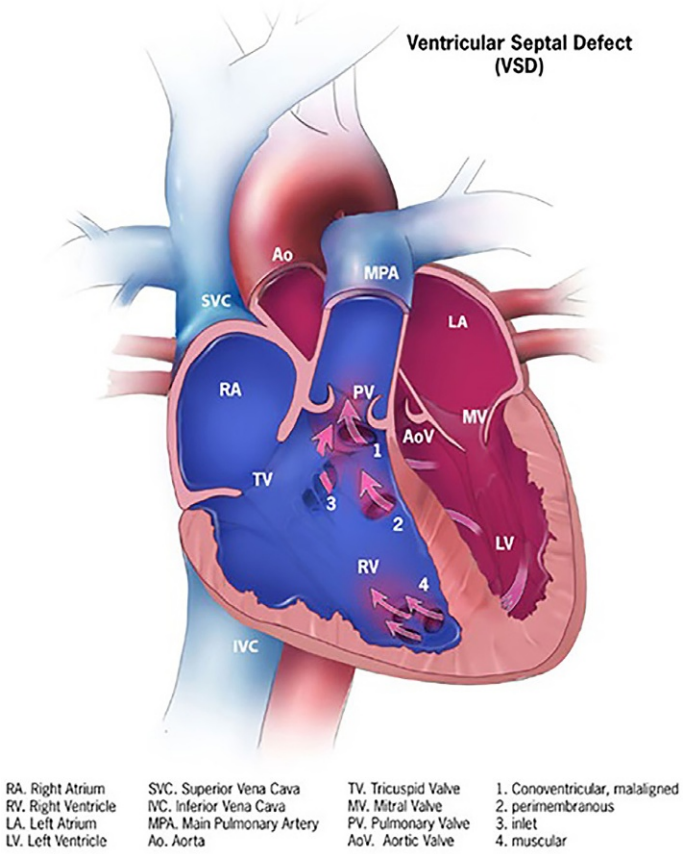

Fig. 4 Ventricular septal defect (VSD) is demonstrated here and represents a deficiency in any area of the ventricular septum that permits shunting of blood at this level. Levels of shunting include subarterial VSD (1), membranous VSD (2), inlet VSD (3), and muscular VSD (4).

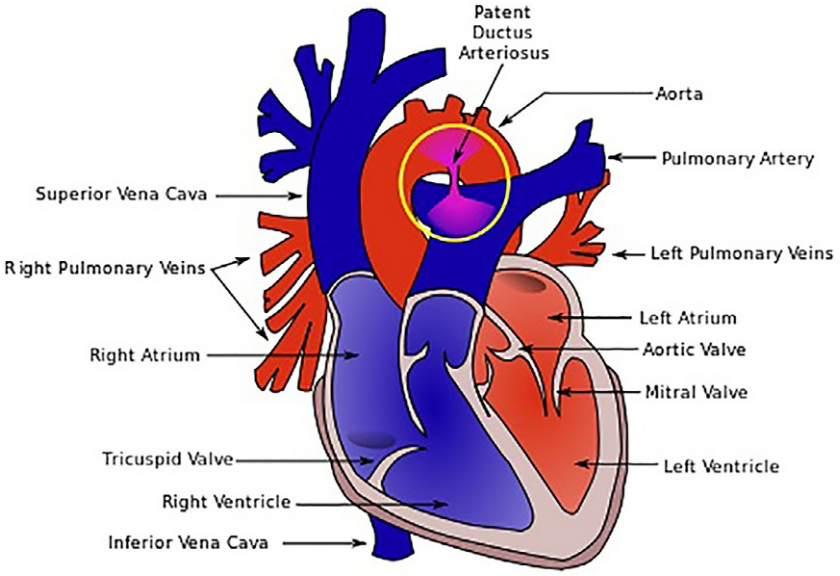

Fig. 5 Patent ductus arteriosus is demonstrated here and represents the continued existence of a fetal structure, which connects the aorta to the pulmonary artery.

Cyanotic Lesions

Cyanotic CHD requires the presence of right-to-left or deoxygenated-oxygenated blood shunting. This scenario may exist in the form of parallel circulations when two usually interdependent circulations do not distribute and receive blood from one another appropriately. Instead, they work in isolation and recirculate their own blood that each pumps out redundantly. Cyanotic CHD occurs in the presence of such parallel circulations, obstruction leading to decreased pulmonary blood flow, or mixture of arterial or venous blood.

In the unrepaired state (typically infants), Tetralogy of Fallot is the most common scenario where right-to-left shunting of blood occurs. This pathology occurs because of anterior–superior deviation of the conal septum leading to the presence of a VSD, pulmonary stenosis, right ventricular hypertrophy, and overriding aorta (Fig. 6). Coronary artery anomalies are sometimes seen, which may complicate surgical repair. ASDs are often present, which may lead to further shunting depending on the degree of right ventricular diastolic dysfunction. Lesions associated with pulmonary valve atresia also lead to right-to-left shunting whether at the atrial or ventricular level. Another cause of cyanotic CHD is complete transposition of the great arteries, where the aorta and pulmonary artery insert into the right and left ventricles, respectively. This ventriculoarterial discordance leads to a complicated physiologic state in which oxygenated blood is recirculated to the lungs and deoxygenated blood recirculates within the body. ASDs and VSDs as well as the PDA serve as shunts to allow for oxygenation of deoxygenated blood and circulation of oxygenated blood. This serious condition is incompatible with prolonged survival without surgical intervention. Any adult seen with this condition in the unrepaired state has survived strictly from mixing of oxygenated and deoxygenated blood at any the level of remaining shunt. Finally, mixture of arterial and venous blood may lead to net deoxygenated blood. Some examples of this are: atrioventricular valve atresia concurrent with an unrepaired shunt, total anomalous pulmonary venous return, in some cases partial anomalous pulmonary venous return, arteriovenous malformations, and truncus arteriosus. In truncus arteriosus for instance, cardiac output from the left and right ventricles join in a common outflow artery, termed the truncus. The lung gets blood via pulmonary arteries that emanate from the truncus arteriosus and sometimes also by PDA. The lungs are exposed to high pressure and volume overload, making early surgical repair important for normal cardiopulmonary development and competence.

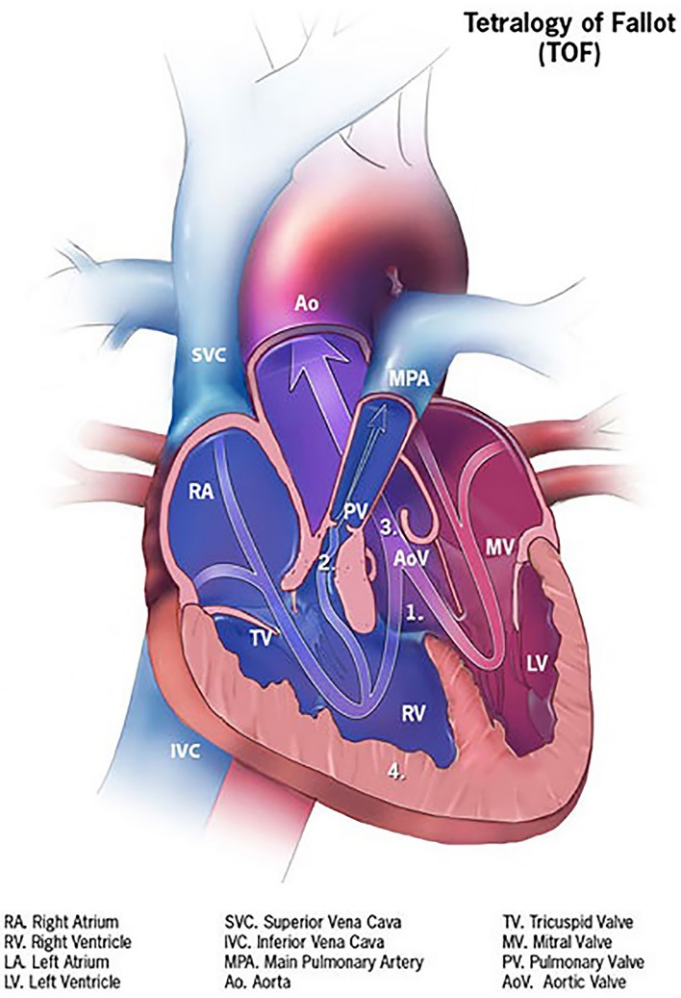

Fig. 6 Tetralogy of Fallot (TOF) is demonstrated here and is characterized by a series of findings, which occurs secondary to anterior deviation of the conal septum. It is exemplified by ventricular septal defect (1), pulmonary stenosis (2), aortic override (3) and right ventricular hypertrophy (4).

Single-ventricle patients represent a unique subpopulation of those with ACHD. These patients can have a diverse array of underlying CHD but ultimately have a similar palliative plan. Whether the systemic or pulmonary output is compromised, the first stage of surgical palliation ensures adequate cardiac output for both systems, adequate venous blood admixture at the atrial level and the integrity of the aortic arch (Fig. 7A). The *second stage*, commonly called a Bidirectional Glenn procedure or Bidirectional cavopulmonary anastomosis, redirects some of the systemic venous blood directly to the pulmonary arteries while permitting deoxygenated infradiaphragmatic blood flow to return to the heart (Fig. 7B). Finally, in the *third stage* which is commonly referred to as the Fontan completion, the deoxygenated venous blood that had been returning to the heart is redirected to the pulmonary artery leading to a circuit with only one pump and passive pulmonary blood flow as is required without a subpulmonary ventricle (Fig. 7C). Late findings in the Fontan-palliated patient are similar despite underlying native anatomy. The current standard modification performed is the extracardiac Fontan procedure (Fig. 8). We are just beginning to learn of both the late-cardiac and

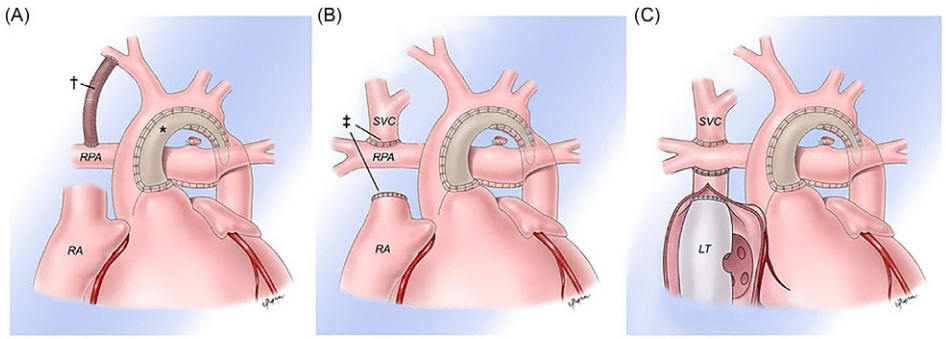

Fig. 7 The traditional three step palliation is demonstrated here. In A, the modified Blalock-Taussig-Thomas shunt is shown connecting the right subclavian artery to the right pulmonary artery along with an aortic arch reconstruction. In B, the superior vena cava is redirected to the pulmonary arteries and the Blalock-Taussig-Thomas shunt is taken down. In C, the sub-diaphragmatic blood is redirected to passively drain into the pulmonary artery.

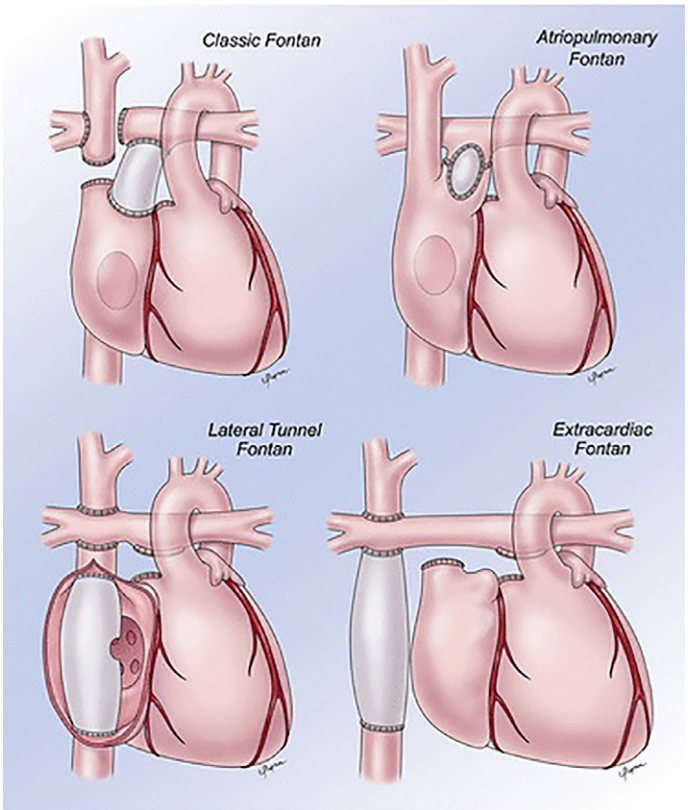

Fig. 8 Iterations of the third palliative surgery step are demonstrated here. The Classic Fontan was initially utilized with sub-diaphragmatic blood flowing only to the left lung and using the right atrium as a conduit. In the Atriopulmonary Fontan, the right atrial appendage is anastomosed directly to the main pulmonary artery. In the Lateral Tunnel Fontan, sub-diaphragmatic blood utilizes native right atrial tissue within the baffle as it ascends to the pulmonary artery. In the Extracardiac Fontan, a conduit is placed outside of the heart, which connects sub-diaphragmatic blood to the pulmonary artery.

extracardiac manifestations that result from this surgical procedure, as the first patients surviving this operation typically had the procedure in the early 1970s (Van Praagh, 1972).

Treatment

Prior to the 1950s, surgical interventions were not possible, and CHD was a harbinger of early death. However, with the advent of cardiopulmonary bypass, surgical interventions have become increasingly more complex and common. Since the 1960s, however, catheter interventions have been utilized and changed diagnosis and treatment of CHD (Rashkind and Miller, 1966). Since the 1970s, such procedures have eliminated the need for some open surgical procedures (King and Mills, 1974).

Transcatheter interventions are multiple and the details of them are beyond the scope of this article. They may be used to relieve valvar obstruction, dilate blood vessels, place stents, insert devices to occlude septal defects, coil anomalous vessels, and insert new valves, among other procedures. The benefits of transcatheter interventions are several: avoidance of thoracotomy/sternotomy including effects on respiratory mechanics, no requirement for cardiopulmonary bypass and therefore offer neurologic protection and potentially avert late neurocognitive abnormalities and shorter hospitalization, among others. The ability to place valve homografts in the pulmonary and aortic positions has revolutionized the treatment of CHD specifically as it relates to transcatheter pulmonary valve utilization particularly in Tetralogy of Fallot. It would be expected that these types of interventions will continue to develop and improve, offering more patients procedural success for treatment of CHD.

Surgical interventions for CHD are widespread and specific to the underlying type of CHD. Similar to transcatheter interventions, valvular interventions and septal defects may be intervened upon but in this case with direct visualization. Although percutaneous options are increasing, there are times where surgical intervention is preferred for precise repair, for example, when defect repair is in a location that intimately interfaces with the conduction system. Creation of shunts and placement of conduits largely remain procedures that require surgical intervention; however, newer percutaneous options are in study and may be on the horizon in the near future. Interventions on arteries and veins to ensure patency or redirect flow are also routinely preferentially performed surgically. Staged repairs over weeks to years are commonly utilized alone or in concert with transcatheter interventions, particularly in complex CHD such as the single-ventricle population. In the setting of dire circumstances, heart transplantation may be performed by congenital heart surgeons when other options are deemed too risky.

Cardiac Manifestations in ACHD

ACHD patients face several cardiovascular problems as they age. This may be the result of residual congenital lesions, or a result of the surgical palliation performed in childhood. Congestive heart failure is not uncommon, and although tolerable in youth, over time the same pressure–volume relationships may not be as well tolerated (Engelings et al., 2016). There remains a paucity of data about use of traditional heart failure medications in the CHD population, and it remains an important area of research (Book and Shaddy, 2014; Gurvitz et al., 2016). Medications may slow down this progression but reversal may not be possible (Stout et al., 2016). CHD patients with valvular heart disease typically progress independent of CHD and can compound existing hemodynamic derangements. Arrhythmia is another late common cardiac problem for many CHD patients (Moore, 2014). These issues can arise from scar tissue secondary to the original repair, for instance, the ventriculotomy scar in Tetralogy of Fallot, or from the abnormal hemodynamic milieu. PAH is a dreaded complication of CHD and is known to affect overall survival (Manes et al., 2014; Dimopoulos et al., 2014). This occurs when the mean pulmonary artery pressure is $\geq$25 mmHg, pulmonary capillary wedge pressure is $\leq$15 mmHg, and pulmonary vascular resistance is $>$3 Wood units. One common way that PAH develops is from an unrepaired nonrestrictive shunt. In this condition, such as is the case with an unrepaired VSD, a shunt that once went left-to-right reverses to right-to-left secondary to the increased pulmonary vascular resistance. This condition is known as Eisenmenger's syndrome (ES) and is associated with increased mortality and several extracardiac manifestations of CHD (Dimopoulos et al., 2014).

Extracardiac Manifestations of ACHD

ACHD patients have pathology extending beyond the cardiopulmonary system. For those who have single-ventricle physiology, the ingenious palliative Fontan operation has permitted survival into adulthood for those who otherwise might have died young; however, late problems in adulthood are commonplace (Hsu, 2015; Cohen et al., 2013). These patients typically remain at least mildly cyanotic and secondary erythrocytosis is common, as is coagulopathy. Peripheral skin and soft tissue changes, particularly in the legs, are frequent due to passive and often congestive systemic venous return via the Fontan circuit. Liver involvement is common, and in fact has its own unique term: Fontan-associated liver disease (FALD), because it is unlike other liver disease due to common mechanisms such as hepatitis or alcohol abuse. Nearly all adults with a prior Fontan will have histopathology consistent with liver fibrosis, if not cirrhosis (Pundi et al., 2016). Hyperenhancing liver nodules are not uncommon in the Fontan population, and there is an increased incidence of hepatocellular carcinoma compared to other types of CHD (Wells et al., 2016). Protein losing enteropathy may also occur in adulthood and is characterized by fluid retention, low albumin, and is generally poorly tolerated with no good treatment strategies available (Hsu, 2015).

In patients who have ES or cyanosis from unrepaired CHD, there is hematologic dysfunction characterized by coagulopathy and increased bleeding risk (Tay et al., 2011). Secondary erythrocytosis is responsible for microthorombi formation and the increased cell turnover of this state can also lead to gout and cholelithiasis (Shiina et al., 2011). Despite having increased hemoglobin and hematocrit, iron deficiency is common and contributes to bleeding diatheses and reduced oxygen delivery (Tay et al., 2011). Renal dysfunction manifests as reduced glomerular filtration rate and proteinuria. Severe infections are also more common with ES, in particular, cerebral abscesses and infective endocarditis. ES is truly a multisystem disease, even though the initial insult is cardiac in nature.

Other systemic problems that are frequently seen in ACHD patients include: orthopedic problems such as scoliosis, respiratory insufficiency/dysfunction typically in the form of restrictive lung disease commonly related to prior surgery, and renal dysfunction (Cohen et al., 2013; Fredriksen et al., 2001; Buelow et al., 2013). Many of these comorbid disease states are only beginning to be discovered, and we continue to evaluate their significance in this special population.

Conclusions

In summary, CHD is a heterogeneous disease state, where variable presentation is affected not only by the initial lesion, but also by surgical repair, and the normal aging process. Adults with CHD are a unique group of patients that require specialized cardiac and extracardiac medical care. It is crucial that these patients are cared for by physicians with expertise in their type of cardiovascular disease.

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Adverse Impact of Delayed Electrical Activation of the Heart and Benefits of Cardiac Resynchronization

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Introduction

Heart failure (HF) is a cardiovascular condition with a high mortality, ≈50% of people diagnosed with HF will die within 5 years (Writing Group Members et al., 2016). Mortality declines have been attributed to evidence-based approaches with implementation of multiple medical therapies targeting various pathophysiologic mechanisms and use of implantable cardiac defibrillators (ICD) for management of malignant ventricular arrhythmias (Merlo et al., 2014). One prognostic feature for HF patients is the duration of the QRS that is recorded on the surface 12-lead electrocardiogram. A QRS duration of ≥120 ms occurs in 30% of HF patients, and a prolonged QRS (Kashani and Barold, 2005) reflects slowed/delayed intraventricular conduction, which promotes abnormal activation sequence of the ventricular walls. This dyssynchronous activation leads to immediate and long-term adverse hemodynamic effects and adverse cellular and structural remodeling (Shaver et al., 1988). Attempts to reverse this pathophysiology by minimizing cardiac dyssynchrony is the goal of pacing both ventricles, termed biventricular (BiV) pacing, and thus providing cardiac resynchronization therapy (CRT). The purpose of this review article is to discuss the experimental and clinical findings associated with CRT, as well as advances in CRT technology (McAlister et al., 2007; Epstein et al., 2013).

Adverse Hemodynamics of Broad QRS

Left Bundle Branch Block Conduction Pattern

Native left bundle branch block conduction

With activation of the ventricle in the setting of a left bundle branch block (LBBB) pattern, the left ventricle (LV) is no longer activated via the left bundle branch and Purkinje fibers. Instead, electrical activation spreads from the right bundle branch.

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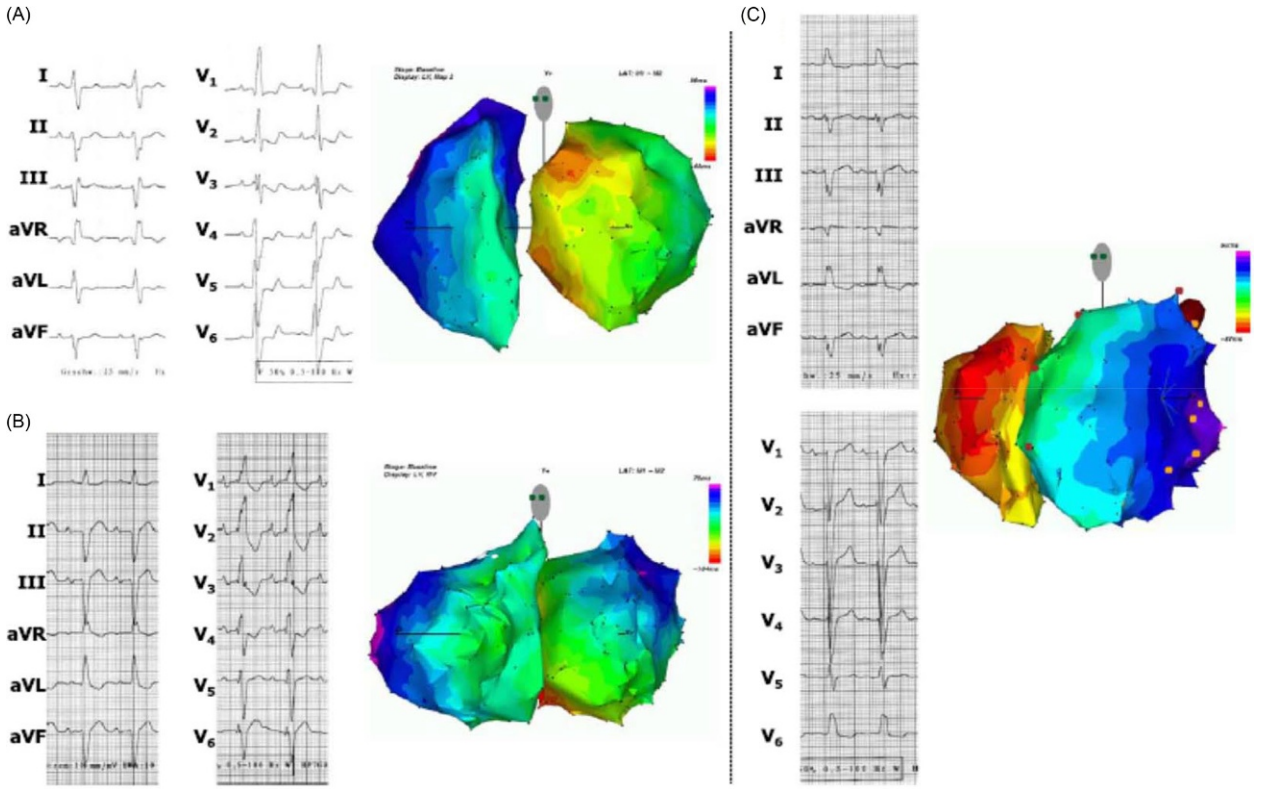

Fig. 1 Electroanatomic mapping and surface 12-lead ECG in a patient with right bundle branch block (RBBB; A), in a patient with RBBB masking left bundle branch block (LBBB; B), and in a patient with LBBB (C). The activation sequence is color coded by 10-ms isochrones (*red* indicates the earliest activation region, and *purple* indicates the latest activation region). With permission from Wolters Kluwer health, Inc. Auricchio A, et al. Does Cardiac Resynchronization Therapy Benefit Patients With Right Bundle Branch Block. Circulation: Arrhythmia and Electrophysiology 2014;7:532–542.

After conduction through the right bundle, which activates the right ventricle (RV) first, the LV is subsequently activated via slow muscle-to-muscle communication from the septum over to the lateral LV wall (**Fig. 1**). Since this activation is not through the rapid His–Purkinje system, the conduction time is slow (reflected by a prolonged QRS), and activation of the septum and the lateral LV walls does not occur concomitantly but rather sequentially, or dyssynchronously, leading to wasted cardiomechanics. This activation pattern has been confirmed in an elegant canine study (Russell et al., 2013). **Fig. 2** demonstrates how the dyssynchronous electrical activation of the LV causes the early activated septum to contract against a reduced load, which leads to prestretch of the LV lateral wall. This prestretch increases contractile force of the LV free wall, which in turn paradoxically stretches the septum later in systole. Both types of systolic stretching can be considered wasted work, which is the ratio of negative and positive work. This wasted mechanics leads to decreased maximum rates of rise and fall of the LV pressure (dP/dt_{max} and dP/dt_{min}), which in turn leads to increased end-diastolic volume (EDV) and end-systolic volume (ESV) and decrease of ejection fraction (EF) (Leyva et al., 2014).

Right ventricular pacing-induced LBBB

The DAVID (Dual Chamber and VVI Implantable Defibrillator) trial (Wilkoff et al., 2002) demonstrated that dual-chamber pacing (DDD mode at a rate of 70 ppm) was inferior to bradycardia-initiated RV pacing (VVI mode at 40 ppm). This surprising result reflects that pacing a high percent of time from the RV activates the RV and LV in a pattern identical to native conduction with a LBBB. Thus active intervention with DDD pacing, resulting in RV pacing, created a dyssynchronous and deleterious impact on RV and LV hemodynamics that did not occur with VVI RV pacing at a much lower frequency of RV stimulation (Akerstrom et al., 2014). The long-term effects of RV pacing have been described in many studies (Karpawich et al., 1999; Adomian and Beazell, 1986). These reports confirm that RV pacing causes adverse remodeling which leads to increased EDV and reduction in EF; correction of single-site RV pacing by addition of a LV lead for BiV pacing reverses these adverse effects (Leon et al., 2002).

Nonleft Bundle Branch Block Conduction Delay

Right bundle branch block

In HF patients with right bundle branch block (RBBB) (Fantoni et al., 2005), the earliest ventricular activation is at the septum (**Fig. 1A**) and, after a considerable delay (50–70 ms), the activation arrives at the RV septum. The activation front then proceeds toward the RV anterior wall and then to the right lateral wall and the outflow tract, which are the latest activated regions. In RBBB conduction, the LV walls are synchronously activated via the His-left bundle branch and Purkinje system.

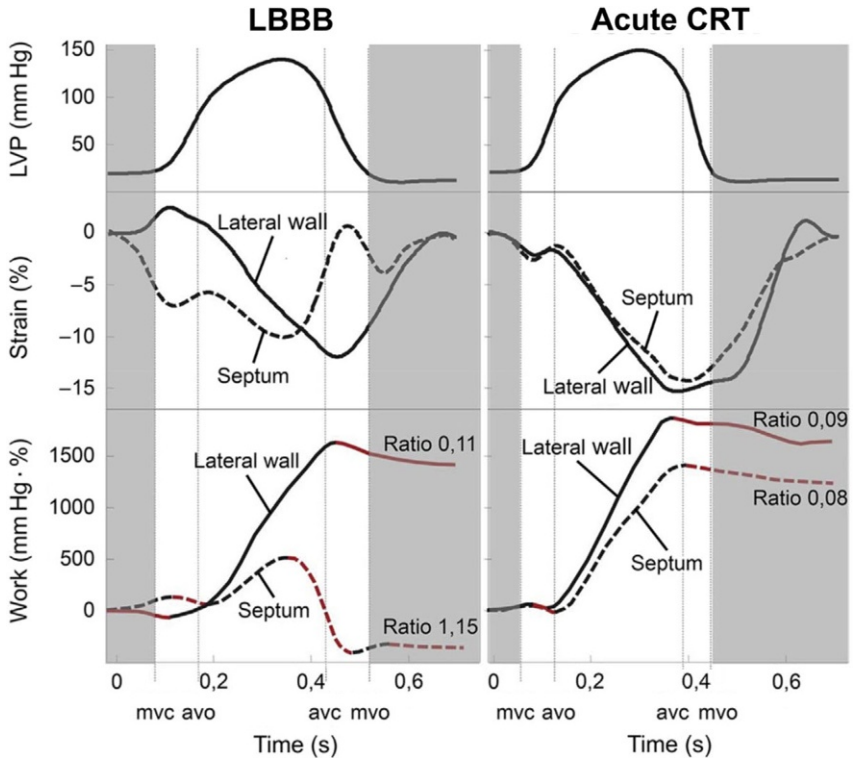

Fig. 2 Estimated LV pressure (top), strain (middle), and calculation of wasted work for the septum and lateral wall (bottom) in a representative patient with left bundle branch block (LBBB; left) and after treatment with cardiac resynchronization therapy (CRT; right). *Vertical lines* indicate valvular events measured by echocardiography. Negative and positive work during systole is marked as *red* and *black*, respectively. Wasted work ratios (WWRs) were calculated using cumulated work from MVC to MVO. *LVP*, left ventricular pressure; *MVC*, mitral valve closure; *MVO*, mitral valve opening. With permission from Russell K, et al. Am J Physiol Heart Circ Physiol 2013;305:H996–H1003.

In some HF patients with RBBB, there is a specific electrocardiographic pattern defined by Rosenbaum et al. (1973) as RBBB masking LBBB (Fig. 1B). In patients with RBBB masking LBBB, electroanatomic mapping data have demonstrated that not only RV activation is abnormally delayed, but also LV activation is delayed to a same degree as in patients presenting with LBBB (Fantoni et al., 2005).

The hemodynamic effects of RBBB are not well understood in humans. In the 1980s, Yasui et al. (Yasui et al., 1985) induced experimental RBBB in dogs with normal hearts and in dogs with moderate and severe RV hypertrophy. RBBB increased RV end-diastolic pressure, RV dP/dt_{max}, and stroke volume. This deterioration was related to the duration of the QRS complex. In multiple studies (Byrne et al., 2007; Takamatsu et al., 2008), a RBBB conduction does not significantly alter LV EF; however, there is dyssynchronous activation and deterioration in RVEF.

Non-specific interventricular conduction delay

There is no hemodynamic data in patients with non-specific interventricular conduction delay, which is a term applied to conduction patterns that are not compatible with a typical morphology of a bundle branch block or fascicular block (Eschalier et al., 2015). In an epidemiologic study (Aro et al., 2011), non-specific interventricular conduction delay pattern was associated with increased all-cause mortality, increased cardiac mortality, and elevated risk of arrhythmic death ($P < 0.001$).

Benefit of CRT

Alteration in Hemodynamics

The goal of CRT is to help correct/minimize the adverse impact of electrical dyssynchrony. By simultaneously delivering a pacing impulse to the RV and LV, the intended goal is to resynchronize activation of the ventricular walls. Since activation by the pacing stimuli is still via a muscle-to-muscle communication, rather than through the His–Purkinje system, the conduction pattern with BiV pacing is certainly not better than when compared to native conduction with a narrow QRS, but the intent of BiV pacing is to enhance synchrony when compared to native conduction with a wide QRS. A visual depiction of cardiac resynchronization is shown in Fig. 3 (Ghosh et al., 2011).

In an animal model of BiV pacing, total activation time and QRS duration is shortened compared with LBBB conduction and BiV stimulation results in an immediate improvement in hemodynamics (Strik et al., 2012b). Acutely, BiV pacing increases dP/dt_{max}

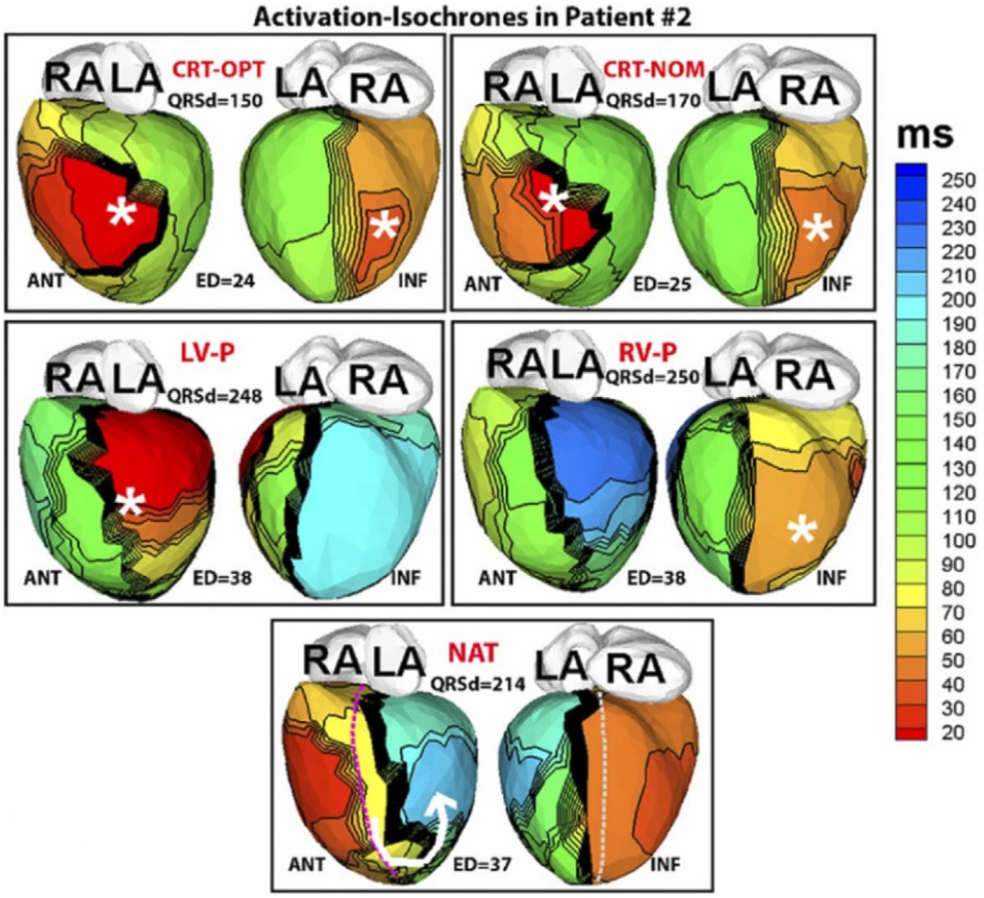

Fig. 3 Activation-isochrone maps in a patient who had a wide QRS (QRS duration QRSd = 215 ms) preimplant and responded well to CRT. Isochronal lines are depicted in *black*. *Thick black lines* indicate conduction block, while crowded *isochronal lines* indicate slow conduction. Pacing sites are indicated by asterisk. Each panel shows anterior (left, ANT) and inferior (right, INF) four-chamber views. Epicardial activation sequences are imaged during (1) optimal CRT settings (CRT-OPT), (2) nominal CRT settings (CRT-NOM), (3) LV pacing (LV-P), (4) RV pacing (RV-P), and (5) native sinus rhythm (NAT). QRS duration (QRSd) and electrical dysynchrony index (ED) computed from the epicardial activation maps (in milliseconds) are shown for each rhythm. *RA*, right atrium; *LA*, left atrium. The septal aspect of the epicardium is shown by *dotted lines* (*purple*, ANT view and *gray*, INF view) in the NAT panel. With permission from Ghosh S, et al. Heart Rhythm 2011;8(5):692–629.

often to pre-LBBB levels in animal model (Strik et al., 2012a) (**Fig. 2**) and increases about 10%–30% in human studies (Leclercq et al., 1998; Kass et al., 1999). An important additional finding is that this increase in dP/dt is without affecting LV end-systolic or end-diastolic pressures. This increase in dP/dt is thus without compromise in Starling mechanics. With increased dP/dt, there is also an increase in pulse pressure, cardiac index, and stroke work (Auricchio et al., 1999; Leclercq et al., 1998; Gold et al., 2005; de Roest et al., 2013). However, it should be noted that BiV pacing does not necessarily reduce QRS duration (see **Fig. 3**) (Leclercq et al., 1998; Kass et al., 1999; Lambiase et al., 2004). Also, hemodynamic improvement does not correlate to a specific change in QRS duration or morphology (Verbeek et al., 2002; Gold et al., 2012).

Chronically, BiV pacing results in extensive reverse-remodeling, reflected by improvements in pressure–volume loops (improvement of ESV and EDV and LV EF) recorded after 6–12 months of CRT (**Fig. 4**) (Steendijk et al., 2006,; de Roest et al., 2013). Although dP/dt_{max} improves in comparison to prepacing measurements, the value does not recover to normal (Vernooy et al., 2007).

Alterations in Cellular Physiology

The molecular aspects of reverse remodeling are explained in **Fig. 5** (Kirk and Kass, 2015) and **Table 1**. CRT helps reverse adverse changes in calcium handling (Aiba et al., 2009; Sachse et al., 2012; Nishijima et al., 2007; Li et al., 2015), increase expression of β receptors (Chakir et al., 2009; Vanderheyden et al., 2008; Chakir et al., 2011; Feldman et al., 1988), and change ion channel expression (Valdivia et al., 2005; Wagner et al., 2006; Aiba et al., 2013; Chakir et al., 2008; Nuss et al., 1999; Markowitz et al., 2009). CRT also helps increase sarcomere force by increasing sensitivity to calcium stimulation (Kirk et al., 2014; Shah et al., 2014; Orrego et al., 2011), increasing and organizing α-actinin (an integral myofilament Z-disk protein) (Lichter et al., 2014), and reducing cleavage of tethering protein desmin (Agnetti et al., 2014). CRT has been demonstrated to improve glucose metabolism (Lionetti et al., 2007; Lionetti et al., 2009; Nowak et al., 2003) and mitochondrial oxygen consumption (Agnetti et al., 2010). Lastly, various

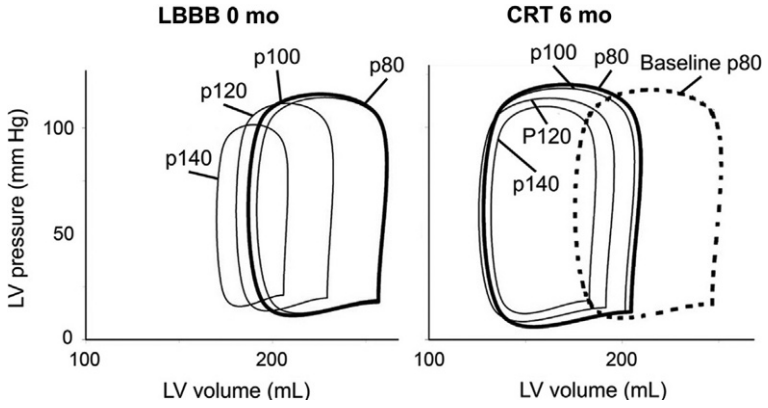

Fig. 4 Mean pressure–volume loops at baseline and after 6 months of CRT. Mean pressure–volume loops are based on mean end-systolic and end-diastolic pressures and volumes and are shown at heart rates 80, 100, 120, and 140 bpm. With permission from Steendijk P, et al. Circulation 2006;113(10):1295–1304.

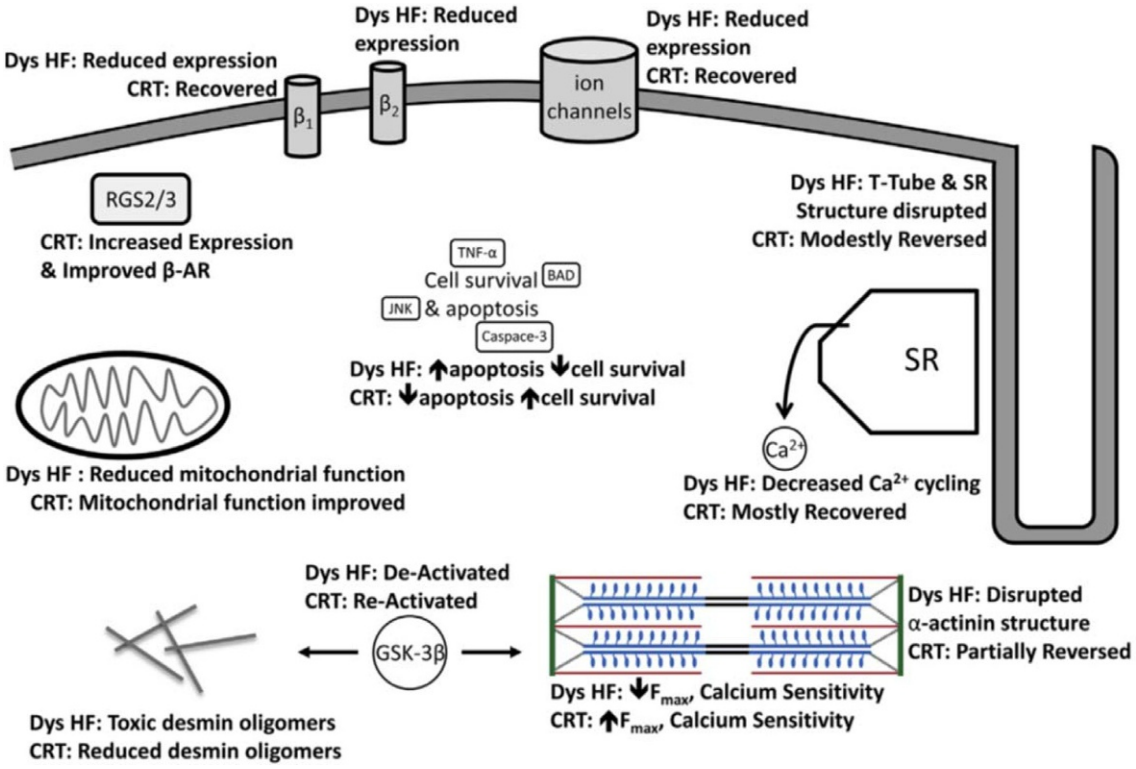

Fig. 5 Summary of molecular alterations in dyssynchronous HF (Dys HF) and CRT. *β-AR*, β-adrenergic receptor; *SR*, sarcoplasmic reticulum. With permission from Kirk JA and Kass DA. Card Electrophysiol Clin 2015;7(4):585–597.

antiapoptotic mechanisms are initiated with CRT: reduction in tumor necrosis factor α (Rordorf et al., 2014; Limongelli et al., 2014), increase in micro RNA-30d (Melman et al., 2015), and reduction in proapoptotic protein annexin-A5 (Ravassa et al., 2010).

Implantation of Biventicular Pacing System

Left Ventricular Lead Placement

When implanting a BiV pacing system, it is recommended to place the RV lead first because it is less likely to dislodge during manipulation of other leads, provides information about the position of the tricuspid valve and right atrial size, and can be used for backup pacing if, in the setting of LBBB, the right bundle suffers mechanical trauma with subsequent complete heart block.

Table 1 Chronic molecular changes seen with CRT

Molecular substrate	*Changes with HF*	*Changes with CRT*
Calcium handling	Restructuring of T-tubules	Improved structure
	Downregulation of sarcoplasmic reticulum	Improved structure
	Downregulation of ryanodine receptor	Improved structure
	Decreased calcium transients	Enhanced transients
β1 receptor	Downregulation	Upregulation
β2 receptor	Increased inhibitory G protein which inhibits β2 expression	Upregulation of RGS2/RGS3 which reduces inhibitory G protein hence upregulating the β2 receptor expression
Multiple ion channels	Increase in late activated Na currents	Reversal of current
Sarcomere force (F)	Reduced Ca sensitivity due to reduction in phos by GSK-3b	Increased calcium sensitivity by improved phos by GSK-3b
	Decreased α-actinin	Increased α-actinin partially
	Desmin reduction and increase in toxic cleavage products	Reversal of desmin migration and degeneration
Glucose metabolism	Uptake, hibernation, metabolic activity and energy turnover reduced	Normalization and harmonization of metabolomics
	Decreased anerobiosis	Increased mitochondrial oxygen consumption
Apoptosis	HF is pro-apoptotic via increase in TNF-α, cardiotrophin-1, Annexin-A5 and reduction in microRNA-30d	Reversal of these pro-apototic changes

CRT, cardiac resynchronization therapy; *GSK-3b*, glycogen synthase kinase 3b; *HF*, heart failure; *Na*, sodium; *phos*, phosphorylation; *RGS*, regulator protein of G protein; *TNF*, tumor necrosis factor.

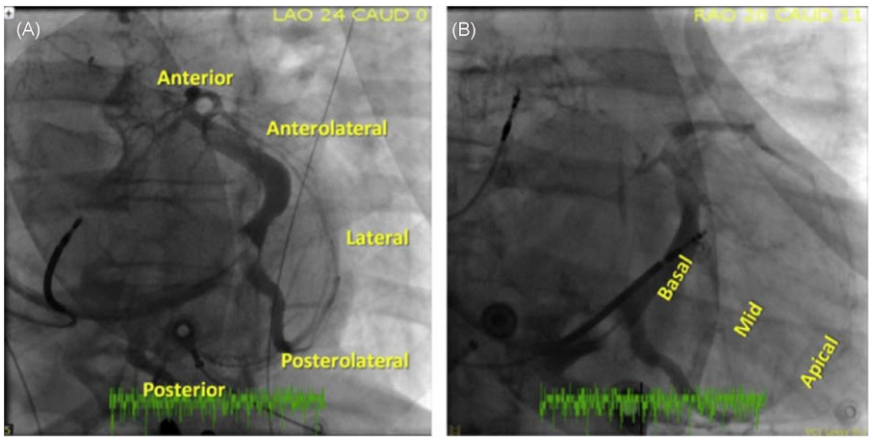

Fig. 6 Angiographic classification of CS anatomy. (A) The left anterior oblique (LAO) projection enables segmentation of the LV along the short axis, defining anterior, anterolateral, lateral, posterior, and posterior segments of the CS and its veins. (B) The right anterior oblique (RAO) projection shows the LV along the long axis, defining basal, mid, and apical segments. With permission from Roka A, et al. Card Electrophysiol Clin 2015;7(4):635–647.

LV pacing is achieved by delivering a pacing electrode to the LV epicardium via branches of the coronary sinus (CS). To cannulate the CS, venous access is achieved via central vein (typically axillary or subclavian vein), and guiding sheaths are manipulated into the right atrium under fluoroscopic guidance. Often through the use of a steerable electrophysiological catheter that is positioned in the CS body, guiding sheaths are advanced over the catheter into the CS. Once in the CS, a CS venogram is completed by balloon occlusion and hand-injection of contrast via a distal port (**Fig. 6**). The branches of the CS are named by their anatomic location, and there is considerable variation in anatomy. By defining the CS anatomy, the guiding sheaths and LV pacing electrode can be positioned into the most desired location. Most LV leads are implanted using over the wire technique, using a flexible coronary guidewire. For veins with acute takeoff, preshaped telescopic sheaths can be used (Jackson et al., 2013). Certain techniques like venoplasty (Luedorff et al., 2009) (angioplasty of vein using balloon catheter over wire), dilatation (Abben et al., 2010) of the collateral veins, and various other techniques using coronary balloons and guidewires can increase implantation success rate from the expected success rate of 92%–95%, to as high as 99%.

The goal of CS pacing is to position the lead in a lateral, basal, or mid-location, with a reasonable capture threshold (typically $<2/2.5$ V at 0.5 ms) and without phrenic nerve stimulation. Since the lateral region of the LV is activated last, the goal is to stimulate this region by the pacing electrode. In some patients, access to the CS is challenging due to presence of venous valves, such as the Thebesian valve located at the superior position of the CS os and the valve of Vieussens that is more distal in the CS (Noheria et al., 2013; Da Costa et al., 2012; Habib et al., 2009).

Table 2 Complication percentages in major clinical trials

Trial	*Year*	*Total complication (%)*	*Unsuccessful implant (%)*	*LV lead related (%)*
MIRACLE	2002		8	6
MIRACLE ICD	2003	28	7.80	5.60
CONTAK CD	2003	2		
PATH CHF II	2003		7	
MIRACLE ICD II	2004	22	10.90	10
COMPANION	2004	8–10	10.90	1.6–1.8
PAVE	2005		14.30	11
CARE-HF	2005			12.50
HOBIPACE	2006			6.70
RethinQ	2007			3.50
REVERSE	2008	16.30		10
Albertsen et al	2008		0	20.80
MADIT-CRT	2009		7.50	4
PACE	2009		8.30	
RAFT	2010		5.30	8.10
COMBAT	2010		5.90	3.30
PREVENT- HF	2011			6
NARROW CRT	2013		3.30	
EchoCRT	2013	17.30		3.50
LESSER EARTH	2013			21.40
BLOCK HF	2013	14	6.30	6.40
BIOPACE	2015			14.80

BIOPACE, Biventricular Pacing for Atrioventricular Block to Prevent Cardiac Desynchronization; *BLOCK HF*, Biventricular versus Right Ventricular Pacing in Heart Failure Patients with Atrioventricular Block; *CARE-HF*, Cardiac Resynchronization-Heart Failure; *COMBAT*, Conventional versus Multisite Pacing for BradyArrhythmia Therapy; *COMPANION*, Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure; *HOBIPACE*, Homburg Biventricular Pacing Evaluation; *EchoCRT*, Echocardiography Guided Cardiac Resynchronization Therapy; *LV*, left ventricular; *MADIT-CRT*, Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy; *MIRACLE*, Multicenter InSync Randomized Clinical Evaluation; *MIRACLE ICD*, Multicenter InSync ICD Randomized Clinical Evaluation; *MIRACLE ICD II*, Multicenter InSync ICD Randomized Clinical Evaluation II; *MUSTIC*, Multisite Stimulation in Cardiomyopathy; *NARROW CRT*, Cardiac Resynchronization Therapy Defibrillator Versus Dual-Chamber Implantable Cardioverter-Defibrillator in Ischemic Cardiomyopathy With Narrow QRS Study; *PACE*, Pacing to Avoid Cardiac Enlargement; *PATH-CHF*, Pacing Therapies for Congestive Heart Failure; *PREVENT-HF*, Preventing ventricular dysfunction in pacemaker patients without advanced heart failure; *RAFT*, Resynchronization–Defibrillation for Ambulatory Heart Failure Trial; *RethinQ*, Resynchronization Therapy in Normal QRS; *REVERSE*, Resynchronization Reverses Remodeling in Systolic Left Ventricular Dysfunction.

Complications of CRT

There are multiple complications with the procedure and the device during and after implantation. Complications are listed in **Table 2**; yet two large metaanalyses provide greater insight (McAlister et al., 2007; van Rees et al., 2011). In about 6123 patients (McAlister et al., 2007), CRT implant success rate was ~93% with periimplant mechanical complications occurring in 4.3% of procedures, and periimplant mortality of 0.3% of patients. Pneumothorax is reported in 0.5%–0.9% cases (van Rees et al., 2011; Peterson et al., 2009). The LV lead is a cause of many of the periimplant mechanical complications and mortality. The complications from LV lead have ranged from 1.9% to 4.6% based on multiple studies (Leon et al., 2005b; Alonso et al., 2001; Hansky et al., 2002; Nof et al., 2008), and this is contributed equally by coronary vein dissection (1.3%) and coronary vein perforation (1.3%) (van Rees et al., 2011). Lastly, lead dislodgement of the LV lead has been recorded from as low as 2.9% to as high as 10.6% in various clinical trials (van Rees et al., 2011). During the follow-up period of various trials (**Table 3**), 5% of devices malfunctioned, 1%–2% of patients had infections in the implant site requiring hospitalization, 6%–7% patients had lead-related problems, and pocket hematoma occurred in 2.4% of CRT recipients (van Rees et al., 2011). Phrenic nerve stimulation, which is another common complication of the LV lead, occurs in as many as 13% of patients and is more common at midlateral/posterior, and LV apical sites (Biffi et al., 2013).

Clinical Trials of CRT (Table 3)

Trials Enrolling NYHA Class III and Ambulatory Class IV With LV EF <35%

The first report utilizing CRT was by Cazeau et al. in 1993 (Cazeau et al., 1994). Multisite Stimulation in Cardiomyopathy (MUSTIC) was the first randomized study on CRT (Linde et al., 2002) and was followed by the first blinded randomized controlled trial, the Multicenter InSync Randomized Clinical Evaluation (MIRACLE) study (Abraham et al., 2002). The MIRACLE trial demonstrated that CRT improves HF symptoms and quality of life, and these results were confirmed by the MIRACLE-ICD (Young et al., 2003) and the Pacing Therapies for Congestive Heart Failure study (PATH-CHF) (Auricchio et al., 2002).

Table 3 Randomized Trials of CRT arranged in chronological order with positive results presented in the last column

Trial	*Year*	*Pts*	*Study Design*	*Follow up (mo)*	*Enrollment QRS duration (ms)*	*Enrollment NYHA Class*	*EF (%)*	*% LBBB*	*SR/AF included?*	*Results (only significant values)*
MUSTIC-SR	2002	58	Single-blinded, crossover CRT pacing on or off	12	≥150	III Ischemic and nonischemic	≤35	87	SR	6-min walked distance increased by 20% ($P=0.0001$); QOL improved by 36% ($P=0.0001$); NYHA class improved by 25% ($P=0.0001$)
MUSTIC AF	2002	43	Single-blinded crossover CRT pacing on or VVI pacing	12	≥200	III Ischemic and nonischemic	≤35	NR	AF	6-min walked distance increased by 17% ($P=0.004$); QOL improved by 32% ($P=0.002$); NYHA class improved by 27% ($P=0.0001$)
MIRACLE	2002	453	Double-blinded, parallel control CRT pacing on or off	6	≥130	III, IV Ischemic and nonischemic	≤35	NR	SR	6-min walked distance increased (+39 vs. +10 m, $P=0.005$); NYHA class improved ($P<0.001$); QOL improved (−18.0 vs. −9.0 points, $P=0.001$); exercise time improved (+81 vs. +19 s, $P=0.001$); HF hospitalization reduced (8% vs. 15%, $P<0.05$); intravenous medications use reduced (7% vs. 15%, $P<0.05$); EF improved (+4.6% vs. −0.2%, $P<0.001$)
PATH CHF	2002	42	Single-blinded, crossover CRT pacing on or off	12	≥120	III, IV Ischemic and nonischemic	NR	97	SR	VO_2 increased ($P<0.05$); 6-min walked distance increased ($P<0.05$)
MIRACLE ICD	2003	369	Double-blinded, parallel control CRT-D with CRT pacing on or off	6	≥130	III, IV Ischemic and nonischemic	≤35	94	SR	QOL improved (−17.5 [−21 to −14] vs. −11.0 [−16 to −7], $P=.02$); NYHA class improved (−1 [−1 to −1] vs. 0 [−1 to 0], $P=.007$); VO_2 increased (1.1 vs. 0.1 mL/kg/min, $P=.04$); exercise time improved (+56 vs. −11, $P<0.001$)
Contak CD	2003	490	Single-blinded Phase I: crossover CRT-D with CRT pacing on or off Phase II: parallel control	6	≥120	II, III, IV Ischemic and nonischemic	≤35	46	SR	VO_2 increased (0.8 vs. 0.0 mL/kg/min, $P=0.030$); 6-min walked distance increased (+35 vs. +15 m, $P=0.043$); LV systolic volume improved (−4.0 mm vs. −0.7 mm, $P<0.001$); LV diastolic volume improved (−3.4 mm vs. −0.3 mm, $P<0.001$); LV ejection fraction improved (5.1% vs. 2.8%, $P=0.020$)
PATH CHF II	2003	89	Single-blinded, randomized, crossover controlled CRT in 120–150 vs >150 ms QRS duration with 3 mo active and then 3 mo inactive pacing	6	≥120	II Ischemic and nonischemic	≤35	88	SR	VO_2 increased 2.46 mL/min/kg ($P<0.001$); anaerobic threshold increased 1.55 mL/min/kg ($P<0.001$); QOL improved 8.1 points ($P=0.004$); 6-min walked distance increased 47 m ($P=0.024$)
MIRACLE ICD II	2004	186	Double-blinded, parallel control CRT-D with CRT pacing on/off	6	≥130	II Ischemic and nonischemic	≤35	83	SR	EF improved ($P=0.02$); LV systolic volume improved ($P=0.01$); LV diastolic volume improved ($P=0.04$); NYHA class improved ($P=0.05$)
COMPANION	2004	1520	Randomized CRT-D vs. OPT or CRT-D vs. OPT	~24	≥120	III, IV Ischemic and nonischemic	≤35	86	SR	Reduced all-cause mortality and hospitalization (CRTP 0.81, CRTD 0.8, $P<0.001$);QOL improved ($P<0.001$); NYHA class improved ($P<.001$); VO_2 increased ($P<0.001$)

(*Continued*)

Table 3 (Continued)

Trial	*Year*	*Pts*	*Study Design*	*Follow up (mo)*	*Enrollment QRS duration (ms)*	*Enrollment NYHA Class*	*EF (%)*	*% LBBB*	*SR/AF included?*	*Results (only significant values)*
PAVE	2005	184	Randomized single blinded CRT-P vs. dual chamber for chronic AF with AVN ablation for rate control	24	NR	I, II, III	NR	NR	AF	6-min walked distance increased (31% vs. 24%, $P=0.04$) (better in low EF and NYHA II, III); EF better (46% vs. 41%, $P=0.03$)
CARE-HF	2005	813	Randomized CRT-P vs. OPT	~30	≥120	III, IV Ischemic and nonischemic	≤35	95	SR	Reduced all-cause mortality and hospitalization (CRT 0.54, $P<0.001$); QOL improved (−10, $P<0.001$); NYHA class improved (0.6, $P<0.001$)
HOBIPACE	2006	30	Randomized blinded, crossover CRT CRT (on vs. off) with indication of pacing and low EF	6	NR	Ischemic and nonischemic	≤40	NR	30% AF	LV systolic volume improved (−16%, $P<0.001$); LV diastolic volume improved (−9%, $P=0.022$); EF improved (22.1%, $P<0.0002$); 6-min walked distance increased (31% vs. 24%, $P=0.04$); QOL improved (−18.9%, $P=0.01$); VO_2 increased (+12.5%, $P<0.0002$); pro-BNP improved (−31%, $P<0.002$)
RethinQ	2007	172	Double blind, parallel control CRT-D vs. ICD	6	<130	III Ischemic and nonischemic	≤35	NR	NR	VO_2 increased in >120 ms QRS group not in <120 ms group ($P=0.02$ vs. $P=0.45$)
REVERSE	2008	610	Single-blinded, parallel control CRT-D with CRT pacing on or off	12	≥120	I, II Ischemic and nonischemic	≤40	61	SR	LV end-systolic volume index improved ($P<0.0001$); time-to-first HF hospitalization delayed ($P=0.03$)
Albertsen et al.	2008	50	Randomized blinded CRT-P on vs. off chamber system with one indication of pacing due to heart block	12	NR	NR	NR	8	SR	Dyssynchrony improved ($P=0.02$); pro-BNP improved ($P=0.02$)
MADIT-CRT	2009	1820	Randomized CRT-D vs. ICD	29	≥130	I, II Ischemic and nonischemic	≤30	71	SR	Reduced all-cause mortality and HF hospitalization (CRT 0.66, $P<0.001$); women, QRS >150, lower NYHA class, non-AF better outcomes; LV systolic volume improved (57 mL vs. 18mL, $P<0.001$); LV diastolic volume improved (52 mL vs. 15 mL, $P<0.001$); EF improved (11% vs. 3%, $P=0.020$)
PACE	2009	177	Randomized blinded CRT-P on vs. off chamber system with one indication of pacing due to heart block	12	NR	NR	>45	NR	SR	EF improved (62% vs. 54%, $P<0.001$); LV systolic volume better (35.7 mL vs. 25.6 mL, $P<0.001$) outcomes similar at 2 years
RAFT	2010	1798	Double-blinded, randomized CRT-D vs. ICD	40	≥120	II, III Ischemic and nonischemic	≤30	72	SR/AF/ paced	Reduced all-cause mortality and HF hospitalization (CRT 0.75, $P<0.001$) mortality analysis not significant for NYHA III, QRS >150, lower NYHA class, LBBB better outcomes

COMBAT	2010	60	Randomized blinded, crossover CRT CRT (on vs. off) with indication of pacing and low EF	17.5	NR	II, III, IV Ischemic and nonischemic	≤40	NR	SR	NYHA class improved ($P<0.001$); LV systolic volume improved ($P<0.01$); LV ejection fraction improved ($P<0.001$); quality-of-life improved ($P<0.01$)
PREVENT-HF	2011	108	Randomized blinded CRT-P vs. dual chamber system with one indication of pacing	12	NR	I, II	NR	NR	10% AF	No difference in outcomes
NARROW CRT	2013	120	Randomized CRT-D vs. ICD	16	<120	II, III Ischemic only	≤35	NR	SR	Reduced composite HF mortality, VF and HF hospitalization (CRT 0.75, $P<0.028$); LV systolic volume improved (30 mL, $P=0.003$); LV ejection fraction improved (12%, $P<0.001$)
EchoCRT	2013	809	Parallel control CRT-D with CRT pacing on or off	24	<130	III, IV Ischemic and nonischemic	≤35	NR	SR	More deaths in CRT group ($P=0.02$); no benefit seen in any subgroup
LESSER EARTH	2013	85	Parallel control CRT-D with CRT pacing on or off	12	<120	Symptoms of HF and a 6-min walk <400 m Ischemic and nonischemic	≤35	NR	SR	Negative
BLOCK HF	2013	691	Randomized blinded CRT-P vs. dual chamber system with one indication of pacing due to heart block	37	NR	I, II, III	≤50	33%	52.8% AF	Reduced all-cause mortality and HF hospitalization (CRT 0.79, $P<0.05$)
BIOPACE	2015	1810	Randomized blinded CRT-P vs. dual chamber system with one indication of pacing (class 1)	67	NR	NR	NR	17%	25% AF	No change in mortality and HF hospitalization

AF, atrial fibrillation; *AVB*, Atrioventricular Block; *BIOPACE*, Biventricular Pacing for Atrio-ventricular Block to Prevent Cardiac Desynchronization; *BLOCK HF*, Biventricular versus Right Ventricular Pacing in Heart Failure Patients with Atrioventricular Block; *CARE-HF*, Cardiac Resynchronization-Heart Failure; *COMBAT*, Conventional versus Multisite Pacing for BradyArrhythmia Therapy; *COMPANION*, Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure; *CRT*, cardiac resynchronization therapy; *CRT-D*, cardiac resynchronization therapy combined with a defibrillator; *CRT-P*, cardiac resynchronization therapy combined with a pacemaker; *HF*, heart failure; *HOBIPACE*, Homburg Biventricular Pacing Evaluation; *ICD*, implantable cardioverter-defibrillator; *IVCD*, intraventricular conduction delay; *LBBB*, left bundle branch block; *EchoCRT*, Echocardiography Guided Cardiac Resynchronization Therapy; *EF*, left ventricular ejection fraction; *MADIT-CRT*, Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy; *MIRACLE*, Multicenter InSync Randomized Clinical Evaluation; *MIRACLE ICD*, Multicenter InSync ICD Randomized Clinical Evaluation; *MIRACLE ICD II*, Multicenter InSync ICD Randomized Clinical Evaluation II; *MUSTIC*, Multisite Stimulation in Cardiomyopathy; *mo*, months; *NARROW CRT*, Cardiac Resynchronization Therapy Defibrillator Versus Dual-Chamber Implantable Cardioverter-Defibrillator in Ischemic Cardiomyopathy With Narrow QRS Study; *NR*, not recorded; *NYHA*, New York Heart Association; *OPT*, optimal pharmacological therapy; *PACE*, Pacing to Avoid Cardiac Enlargement; *PATH-CHF*, Pacing Therapies for Congestive Heart Failure; *PREVENT-HF*, Preventing ventricular dysfunction in pacemaker patients without advanced heart failure; *Pts*, patients; *QOL*, quality of life; *RAFT*, Resynchronization-Defibrillation for Ambulatory Heart Failure Trial; RethinQ, Resynchronization Therapy in Normal QRS; *REVERSE*, Resynchronization Reverses Remodeling in Systolic Left Ventricular Dysfunction; *SR*, sinus rhythm; *VF*, ventricular fibrillation; VO_2, maximal oxygen consumption; *VVI*, Ventricular sensed, Ventricular paced, Inhibitory.

A paradigm shift in compelling evidence in favor of CRT in patients with moderate to severe HF was the Cardiac Resynchronization—Heart Failure (CARE HF) (Cleland et al., 2005) and the Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure (COMPANION) (Bristow et al., 2004) trials that randomized patients to optimal medical therapy versus CRT (with a pacemaker in CARE HF; and, with or without a defibrillator in COMPANION). The endpoints of these two trials were "hard endpoints": all-cause mortality and hospitalization. It is noteworthy that only a few clinical trials demonstrated a reduction in total mortality with CRT and that the benefit of CRT was primarily based on the composite endpoint of all-cause mortality and hospitalization. Nonetheless, as HF hospitalizations are a major socioeconomic burden, reduction in hospitalization is a significant finding; also, metaanalyses (McAlister et al., 2007) support that CRT does result in a reduction in total mortality (**Table 3** and **Fig. 7**).

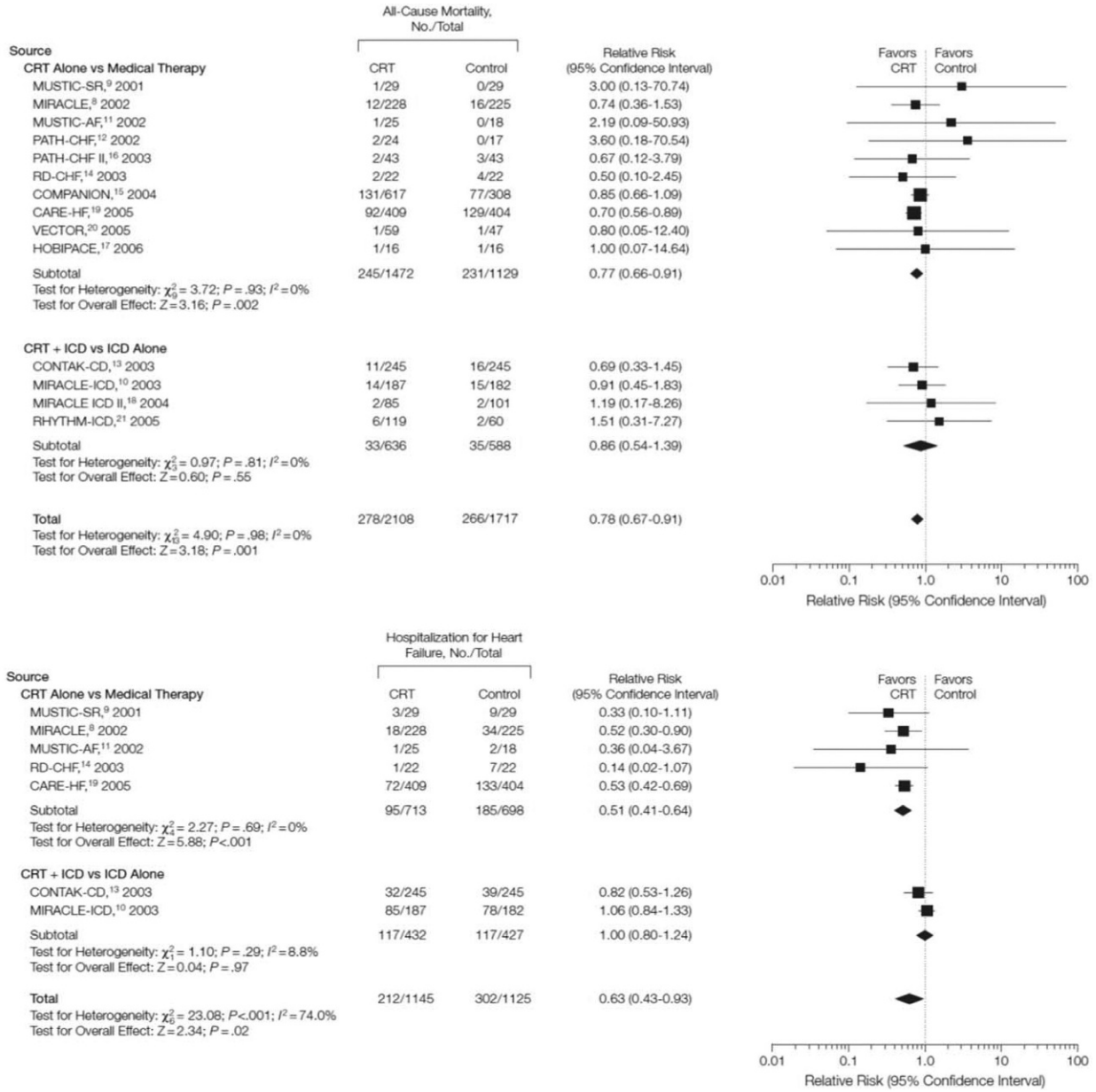

Fig. 7 Effect of CRT on all-cause mortality and proportion of patients hospitalized for heart failure (randomized trial data). *CRT*, cardiac resynchronization therapy; *ICD*, implantable cardioverter-defibrillator. Size of data markers indicates the weight of the study. With permission from McAlister FA, et al. JAMA 2007;297(22): 2502–2514.

Trials Enrolling NYHA Class I and II With LV EF <35%

The next group of patients evaluated was those with mild HF (NYHA Class I and II). The clinical trials enrolling this population of patients were MIRACLE-ICD II (Abraham et al., 2004), PATH-CHF II (Auricchio et al., 2003), Contak CD trial (Higgins et al., 2003), Resynchronization Reverses Remodeling in Systolic Left Ventricular Dysfunction (REVERSE) trial (Linde et al., 2008), Multicenter Automatic Defibrillator Implantation Trial with CRT (MADIT-CRT) (Moss et al., 2009), and Resynchronization/Defibrillation for Ambulatory Heart Failure (RAFT) study (Tang et al., 2010). These trials consistently validated the benefits of CRT in patients with NYHA Class I and II symptoms. These trials have led to guidelines giving weaker recommendations for CRT in patients with ischemic cardiomyopathy with left ventricular ejection fraction (LVEF) ≤30%, QRS ≥150 ms and LBBB with NYHA Class I and Class II HF. Also patients with nonischemic cardiomyopathy with LBBB and QRS ≥120 ms with NYHA class II HF have a weak indication for CRT (Fig. 8).

High Degree of RV Pacing

In the DAVID trial (Wilkoff et al., 2002), RV pacing created a LBBB activation pattern and resulted in adverse cardiac performance. In a similar manner, clinical trials have demonstrated the value of CRT when used in patients with a high percent (>40%) of single-site RV pacing. The Biventricular Pacing for Atrioventricular Block and Systolic Dysfunction trial (BLOCK-HF) (Curtis et al., 2013) studied patients with NYHA class I–III HF and LV EF ≤50% with AV block, requiring frequent RV pacing (>40%). Patients with RV-only pacing, in comparison to patients with CRT, had more HF events, worsened LV function, and higher total mortality. Other smaller studies with a similar inclusion criteria of >40% RV pacing reported only minor clinical benefits, and no survival benefit, for

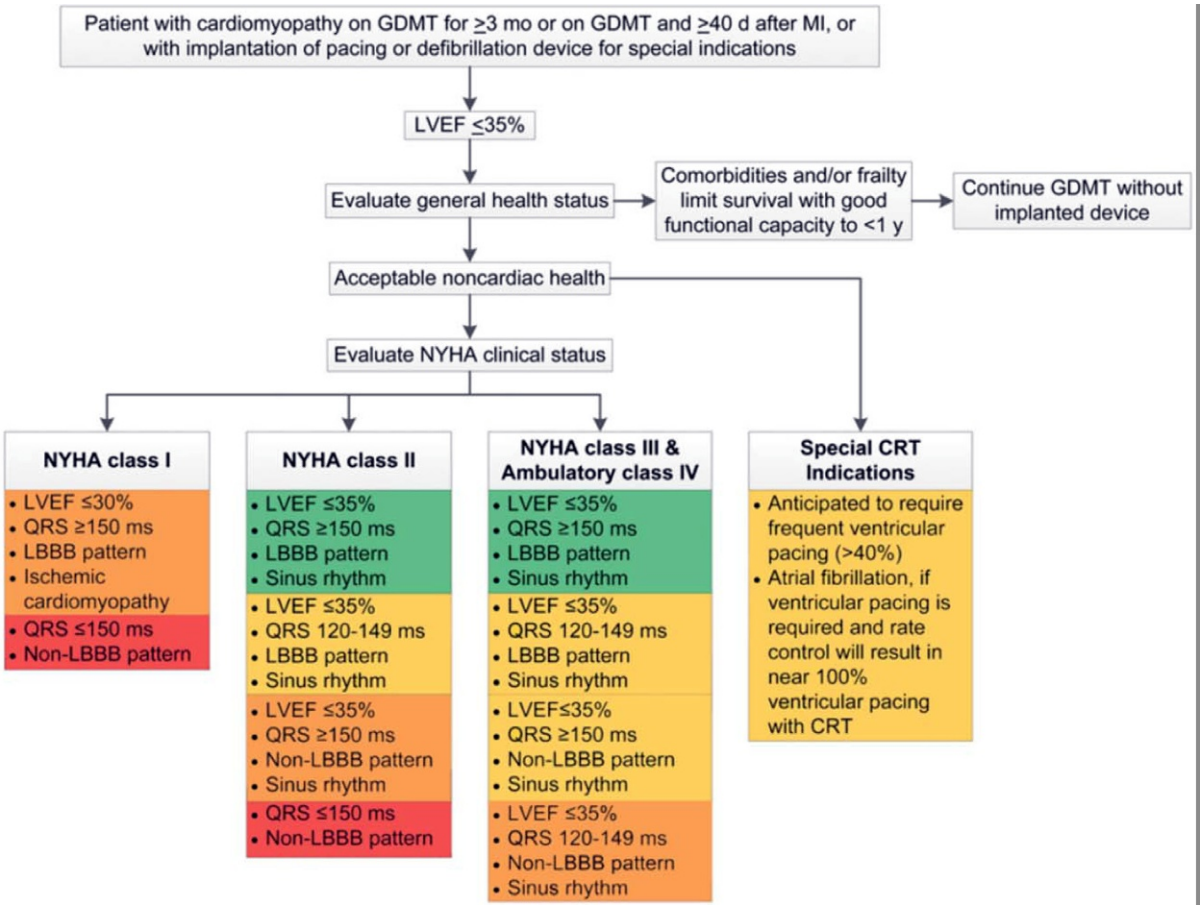

Fig. 8 2012 Indications for CRT therapy—algorithm. *CRT*, cardiac resynchronization therapy; *CRT-D*, cardiac resynchronization therapy defibrillator; *GDMT*, guideline-directed medical therapy; *ICD*, implantable cardioverter-defibrillator; *IV*, intravenous; *LV*, left ventricular; *LVEF*, left ventricular ejection fraction; *LBBB*, left bundle-branch block; *MI*, myocardial infarction; *NYHA*, New York Heart Association; *Green color*, Class 1; *Yellow color*, Class 2a; *Orange*, Class 2b; *Red*, Class 3 (or contraindicated). Every colored box is one patient with all those features and not individual characteristics except *red* where each individual characteristic makes for a contraindication. With permission from Epstein AE, et al. J Am Coll Cardiol 2013;61(3):e6–e75.

CRT compared to dual chamber pacing (Albertsen et al., 2008; Yu et al., 2009b; Stockburger et al., 2011; Kindermann et al., 2006; Martinelli Filho et al., 2010). However, BIOPACE (Biventricular pacing for atrIo-ventricular BlOck to Prevent cArdiaC dEsynchronization) trial (Funck et al., 2014) which was recently concluded suggests that there was no significant benefit for CRT over dual chamber pacing.

Patient Criteria for CRT

HF Patients

Consistent selection criteria for CRT have been LV EF of $\leq$35%, HF symptoms despite optimal medical therapy, and a broad QRS of $>$120 ms (Epstein et al., 2013) (**Table 4**; Vernooy et al., 2014). However, although these were the typical inclusion criteria, subsequent indications were modified to suggest the use of CRT in patients with a broader QRS duration, such as $\geq$150 ms. In general, CRT can be utilized for patients with advanced HF symptoms and a QRS of 120–150 ms, yet for mild HF symptoms, a broader QRS is recommended, $\geq$150 ms. The current guidelines for selection (summarized in **Fig. 8**) are hence based on the QRS duration, morphology, cause of HF, HF symptoms, and the need for high percent RV pacing.

Dyssynchrony Detected by Echocardiography

Numerous trials have evaluated if echocardiographic parameters are useful to identify patients who would respond to CRT. Even though echocardiographic dyssynchrony remains a prognostic marker for CRT response (Delgado et al., 2008; Hara et al., 2012; Haugaa et al., 2014; Gorcsan et al., 2004; Gorcsan et al., 2007; Gorcsan et al., 2010; Kutyifa et al., 2013; Risum et al., 2013; Sogaard et al., 2002; Suffoletto et al., 2006; Tanaka et al., 2010; Yu et al., 2009a), using echocardiographic data to guide therapy has not been shown to be of benefit (Chung et al., 2008); guidelines (European Heart Rhythm et al., 2012; Epstein et al., 2013) currently do not support echocardiographic mechanical dyssynchrony to influence patient selection.

Use of CRT in HF Patients With a Narrow QRS

Another area of investigation was the use of CRT in patients with HF but with only minimal QRS prolongation, but with evidence of echocardiographic dyssynchrony. Several studies and subgroup analysis (MADIT-CRT (Goldenberg et al., 2014), EchoCRT (Ruschitzka et al., 2013), Narrow CRT (Muto et al., 2013) trial, LESSER EARTH (Thibault et al., 2013), and RethinQ trial (Beshai et al., 2007) (Resynchronization Therapy in Normal QRS)) reported that CRT implantation using echocardiography-detected mechanical dyssynchrony as criteria in patients with QRS duration $<$120–130 ms does not improve HF outcomes. In particular, the EchoCRT trial (Ruschitzka et al., 2013) was stopped early due to increased mortality in the arm randomized to CRT, reinforcing the improved outcomes associated with narrow native QRS conduction rather than BiV pacing.

Table 4 Criteria for selection of patients and application of CRT

Criteria for patient selection	*RCT evidence*	*Single-center evidence*
QRS morphology	LBBB is the primary electrical substrate for CRT	Hearts without LBBB might be amenable to CRT if LV activation is delayed
Mechanical dyssynchrony	Adds no predictive value to CRT response in patients with definite (or definite lack of) electrical substrate	Might be predictive of CRT response in patients with intermediate QRS duration and non-LBBB morphology
Ischemic etiology and scarring	In patients with LBBB, ischemic etiology does not reduce clinical CRT response, but echocardiographic response might be reduced	LV pacing in a scarred region substantially impairs CRT response
LV lead position	No data from multi-center RCTs available	LV lead in a late-activated region improves CRT response, especially in patients with intermediate QRS duration and non-LBBB morphology of the QRS complex; the latest-activated region might differ between RV pacing and intrinsic LBBB rhythm
Percentage cumulative pacing	Almost any heart beat lost to biventricular pacing reduces the benefit of CRT, especially when the cumulative percentage is $<$95%	Atrioventricular-nodal ablation can substantially increase CRT benefit in patients with atrial fibrillation
Atrioventricular and interventricular optimization	No evidence of clinical benefit from atrioventricular or interventricular optimization, or from device-based algorithms Only the AdaptiveCRT algorithm has been associated with long-term benefit, and only in patients in whom LV fusion pacing was performed	Optimization of atrioventricular and interventricular delay improves acute hemodynamic response

CRT, cardiac resynchronization therapy; *LBBB*, left bundle branch block; *LV*, left ventricular; *RCT*, randomized controlled trial; *RV*, right ventricular.
With permission from Vernooy K, et al. Nat Rev Cardiol. 2014;11(8), 481–493.

High Percent RV Pacing

Whether CRT should be used for all patients requiring high percent of RV pacing is unclear. Evidence supports the use of CRT among patients with a depressed LV EF; however, in patients without HF or a depressed EF, the role of CRT among this population is not resolved. In favor of CRT is the concern for single-site RV pacing to promote a cardiomyopathy; the con opinion is that the benefit of CRT in patients without HF and without depressed LV EF has not been demonstrated. Other clinical features may impact cardiovascular performance in patients with preserved EF and chronic RV pacing such as gender, coronary artery disease, and other comorbidities (Herweg et al., 2015; Khurshid et al., 2014; Zhang et al., 2008; Dreger et al., 2012; Shimano et al., 2007; Ahmed et al., 2014; Hori et al., 2011; Daoud, 2015; Sweeney et al., 2003).

Nonresponse to CRT: Causes and Solutions

Although CRT helps improve symptoms and survival in most patients with HF, a large percent (30%–40%) of CRT recipients do not obtain clinical benefit and are considered clinical nonresponders (European Heart Rhythm et al., 2012; Yu and Hayes, 2013). The response rate hinges on five key elements (discussed later): appropriate patient selection, CRT implant techniques, appropriate CRT programming, patient follow-up, and multidisciplinary approach.

Nonresponse: Appropriate Patient Selection

Appropriate patient selection has been established by clinical trials (**Table 3**) and is summarized in the Appropriate Use Guidelines (Russo et al., 2013). The goal is to provide CRT to patients on optimal medical therapy and demonstrate dyssynchrony (determined solely by the QRS duration and morphology), presence of HF symptoms, and persistently depressed EF. Even with these guidelines, other features can contribute to nonresponse.

A common and important cause of nonresponse is reduction in the percent of BiV pacing. Since BiV pacing is the therapy, a low percent of BiV pacing (<96%) has been associated with increased HF, mortality, and need for transplant (Koplan et al., 2009; Hayes et al., 2011). For example, atrial fibrillation is common (~30%) (Dickstein et al., 2009) in HF population, and when AF occurs in patients with a CRT device, the irregular and often rapid native conduction through the His–Purkinje system appropriately results in inhibition of BiV pacing. In this setting, HF is exacerbated not only by the arrhythmia-induced absence of atrial contractility, irregular ventricular contraction, and rapid ventricular rate but also by the absence of CRT (Daubert, 2004; Wilton et al., 2011). Based on studies (Doshi et al., 2005), many investigators (Gasparini et al., 2008) recommend atrioventricular junction ablation in these patients to ensure 100% BiV pacing, while others support a conservative initial strategy, optimizing the medical treatment and programming the device to obtain a high percentage of ventricular pacing, and recommend reserving atrioventricular junction ablation only for patients with poor heart rate control (Tolosana et al., 2008; Delnoy et al., 2007; Khadjooi et al., 2008; Tolosana et al., 2012).

In a manner similar to AF, frequent premature ventricular contractions (PVCs) can inhibit BiV pacing (Cheng et al., 2012) and leads to worse outcomes (Ruwald et al., 2014). In a small study Lakireddy and colleagues (Lakkireddy et al., 2012) showed that radiofrequency ablation of PVC foci improves LV EF and NYHA class and promotes reverse remodeling hence enhancing CRT efficacy in nonresponders.

Other features that have been associated with reduced response to CRT include patients with dilated LV and severe mitral regurgitation indicate a lower probability of response (Diaz-Infante et al., 2005; Sweeney and Prinzen, 2008). The EAARN score which is based on preimplant risk factors (LV EF <22%; age ≥70 years, AF, renal dysfunction, and basal NYHA functional class IV) demonstrated an excellent prognosis and low mortality in patients with less risk factors, whereas those patients with three or more risk factors had a high mortality despite the benefits of the therapy (Khatib et al., 2014).

Patients with ischemic cardiomyopathy seem to have a poorer response to CRT and worse LV reverse remodeling (Diaz-Infante et al., 2005; Shanks et al., 2011). Poor conduction across extensive myocardial scar tissue (Sweeney and Prinzen, 2008) and ineffective LV remodeling of scar tissue (Ypenburg et al., 2007) may be the key determinants of the decreased response to CRT.

Female sex has been associated with higher response rates. The difference is thought to be due to difference in comorbidities, risk factors, response to medical treatment, greater nonischemic cardiomyopathy, higher LBBB morphology, and less myocardial scar (Loring et al., 2013b; Arshad et al., 2011; Leyva et al., 2011; Loring et al., 2013a; Bleeker et al., 2005).

Nonresponse: Appropriate CRT Implant Techniques

Even after selection criteria are met, lack of response to CRT is sometimes related to an inappropriate LV lead location (almost 21% of patients in one study) (Mullens et al., 2009). Apical lead position has been associated with increased mortality (Singh et al., 2011), whereas lateral, basal, or mid-ventricular position have shown good outcomes (Thebault et al., 2012; Singh et al., 2011).

Type of LV lead implanted in the CS has a great variability in outcomes. Typically, the LV lead is a bipolar lead, but recently quadripolar pacing electrodes have been developed. These four independent electrodes allow programming additional vectors and multiple pacing configurations, including multipoint pacing, which is pacing simultaneously from greater than two electrodes on the LV lead. Multiple studies of quadripolar pacing and multipoint pacing have described a lower rate of hospitalizations for HF

(Forleo et al., 2015), lower rate of surgical revision (Forleo et al., 2015), improved hemodynamics (**Fig. 9**) (Rinaldi et al., 2014; Pappone et al., 2015; Pappone et al., 2014; Zanon et al., 2016; Boriani et al., 2016; Forleo et al., 2016; Ploux et al., 2014; Shetty et al., 2014), overall good acute response to CRT (Rinaldi et al., 2014; Pappone et al., 2015; Zanon et al., 2016), improved mortality outcomes (Behar et al., 2015; Turakhia et al., 2016), and less complications (Forleo et al., 2011; Mehta et al., 2012; Boriani et al., 2016) (**Fig. 10**). There are clinical trials like MultiPoint Pacing IDE Study (Tomassoni, 2016) (MPP-IDE) and MOre REsponse on Cardiac Resynchronization Therapy with MultiPoint Pacing Study (Leclercq, 2016) (MORE-CRT MPP) which are underway which will clarify the role of quadripolar pacing in CRT. Preliminary data from the MPP-IDE study (Tomassoni et al., 2016) suggest that it is safe and shows high clinical response (87%) if the spacing between the electrodes is $\geq$30 mm with a 5 ms LV delay.

A goal of LV lead placement is to activate the latest activated region of the LV during native conduction. There are two techniques reported to help guide proper positioning of the LV lead during implantation. The first technique is the use of echocardiography. Echocardiographic guidance consists of radial strain timing toward the latest activation and away from radial strain segments with less than 10% thickening, considered to be likely scar (Khan et al., 2012; Saba et al., 2013; Marek et al., 2014; Sade et al., 2014). A second technique to guide LV lead placement is the measurement of the Q-LV lead timing. Q-LV timing refers to the time from onset of the surface QRS to the intracardiac electrogram recorded from the LV lead when placed in the final position of the CS branch. The goal is a Q-LV time of at least 50% of the QRS duration. Higher Q-LV represents higher likelihood of response and reduced timing (<50%) is associated with worse clinical outcome (Singh et al., 2006).

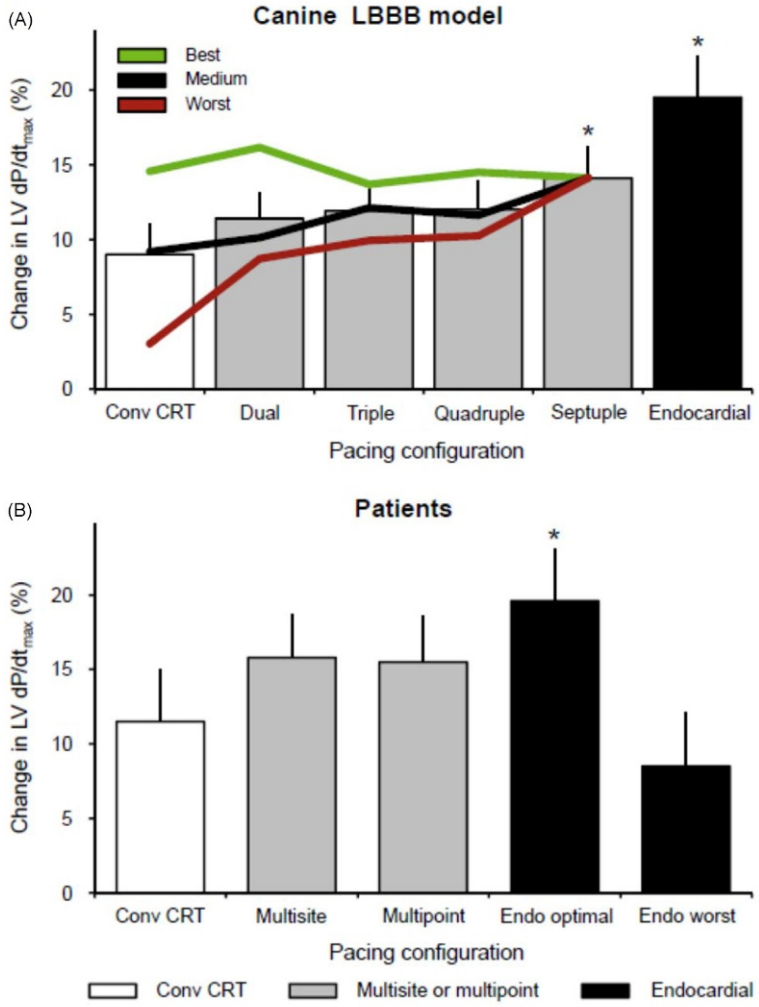

Fig. 9 Percentage change in LV dP/dt_{max} versus baseline during conventional (Conv) CRT (*white bars*), multisite or multipoint (*gray bars*), and endocardial pacing (*black bars*) measured in a canine LBBB model (A) and in patients (B). The values in the bars are presented as mean + SEM. The three lines in A are the pooled data for the two "worst," the three "intermediate," and two "best" single LV pacing sites (*red*, *black*, and *green*, respectively). $P \leq 0.05$ versus conventional CRT. With permission from Ploux S, et al. Heart Rhythm 2014;11(1):119–125; Shetty AK, et al. Europace 2014;16(6):873–879.

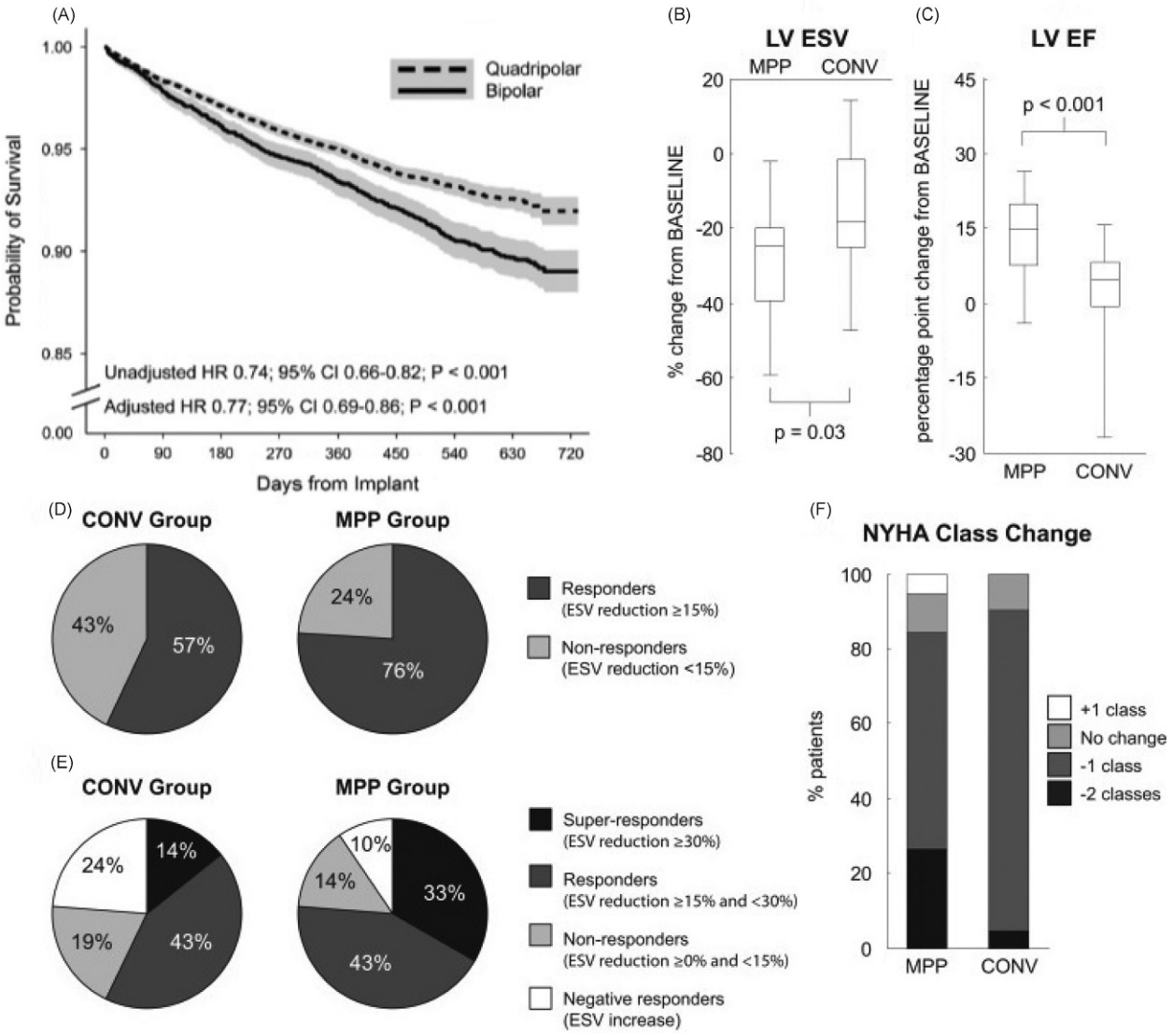

Fig. 10 (A) Kaplan Meier survival (lines) from all-cause mortality is shown with 95% CI (*shaded area*). Use of the quadripolar system was associated with a lower risk of death compared to the bipolar system. The quadripolar group was more heavily censored because these patients were implanted later in the observation period. (B, C, F) Change in echocardiographic and clinical parameters from BASELINE to 12-month follow-up. Boxplots show the median (central mark), 25th and 75th percentiles (box edges), and minimum and maximum values (whiskers). After 12 months, patients in the MPP group had significantly improved LV end systolic volume (LVESV) and LVEF compared to patients in the CONV group. (D and E) Response in MPP and CONV groups. CRT response at 12 months was defined by end-systolic volume LVESV decrease $\geq$15% relative to BASELINE and alive status. An additional analysis divided patients into super-responders with reduction in LVESV of $\geq$30% relative to BASELINE and negative responders with increase in LVESV relative to BASELINE or death during the observation period. *CI*, confidence interval; *CONV*, conventional cardiac resynchronization therapy; *CRT*, cardiac resynchronization therapy; *HR*, hazard ratio; *LVEF*, left ventricular ejection fraction; *LVESV*, left ventricular end-systolic volume; *MPP*, multipoint pacing; *NYHA*, New York Heart Association. With permission from Turakhia MP, et al. JACC: Clinical Electrophysiology 2016; Pappone C, et al. Heart Rhythm 2015;12(6):1250–1258.

Nonresponse: Appropriate CRT Programming

Finally, response to CRT has been suggested to be improved through techniques to optimize atrioventricular synchrony as well as synchrony of LV and RV events (termed VV timing). The two main techniques are echocardiography and the use of intracardiac electrogram events.

Echocardiography is used to time mechanical activation of events to guide CRT programming. Unfortunately, this technique has not been shown to be of benefit when applied universally but may be successful for an individual patient in whom other techniques to improve response to CRT have failed **Table 5** (Auger et al., 2013; Leon et al., 2005a; Cleland et al., 2005; Ellenbogen et al., 2010; Martin et al., 2012b; Sawhney et al., 2004; Chung et al., 2008; Abraham et al., 2012).

Table 5 Summary of the various methods of AV and VV interval optimization

	AV optimization	*Clinical trial using the algorithm*	*Results*	*VV optimization*	*Clinical trial using the algorithm*	*Results*
Echocardiography	Mitral inflow (Ritter method, iterative method, "fast and simple")	InSync III trial	Increased exercise capacity but no change in overall symptomatology	LV M-Mode (septal-posterior wall motion delay)	Abraham WT, et al. Am Heart J 2012;164 (5):735–41	NYHA class and HF composite score improved in VV optimized mode
		CARE-HF				
		SMART-AV	No difference in 6-mo change in LVESV, NYHA class, QOL, or 6-min walk			
		Adaptive CRT trial	Adaptive CRT was noninferior to echo optimization in clinical composite score (73.6% vs. 72.5% improved)			
	Aortic VTI	Sawhney NS, et al. Heart Rhythm 2004;1 (5):562–7	Mild improved in NYHA class, quality of life, ejection fraction	Tissue Doppler imaging	PROSPECT	No difference in optimized versus standard therapy
		Adaptive CRT trial	Adaptive CRT was noninferior to echo optimization in clinical composite score (73.6% vs. 72.5% improved)	Aortic VTI	InSync III trial	Increased exercise capacity but no change in overall symptomatology
					AdaptiveCRT trial	Adaptive CRT was noninferior to echo optimization in clinical composite score (73.6% vs. 72.5% improved)
Intracardiac electrogram-based	SmartDelay™	SMART-AV	No difference in 6-mo change in LVESV, NYHA class, QOL, or 6-min walk	Expert Ease™	DECREASE-HF	Non inferior compared to standard algorithm
		PEGASUS-CRT	No change in outcomes compared to standard delay			
	QuickOpt™	FREEDOM	No difference in 12-mo clinical composite score	QuickOpt™	FREEDOM	No difference in 12-mo clinical composite score
	AdaptivCRT™	Adaptive CRT trial	Noninferior to echo optimization in clinical composite score (73.6% vs. 72.5% improved)	AdaptivCRT™	Adaptive CRT trial	Noninferior to echo optimization in clinical composite score (73.6% vs. 72.5% improved)
QRS based	Fusion optimized intervals (FOI)	Arbelo E, et al. J Cardiovasc Electrophysiol 2014; 25(3):283–92	Improved acute hemodynamics	Fusion optimized intervals (FOI)	Arbelo E, et al. J Cardiovasc Electrophysiol 2014; 25(3):283–92	Improved acute hemodynamics

CARE-HF, Cardiac Resynchronization-Heart Failure; *DECREASE-HF*, Device Evaluation of CONTAK RENEWAL 2 and EASYTRAK 2: Assessment of Safety and Effectiveness in Heart Failure; *FREEDOM*, Frequent Optimization Study Using the QuickOpt Method; *HF*, heart failure; *NYHA*, New York Heart Association; *PROSPECT*, Predictors of Response to CRT study; *SMART-AV*, SmartDelay Determined AV Optimization; *VTI*, velocity time integral.

With permission from Cobb DB and Gold MR. Card Electrophysiol Clin 7 (2015) 765–779; Brabham WW, et al. J Arrhythm 2013; 29:159.

Another method to enhance AV/VV synchrony is based on intracardiac electrograms. These studies—SmartDelay™ (Ellenbogen et al., 2010), Quickopt™ (Abraham et al., 2010), and Adaptive CRT (Martin et al., 2012b)—did not demonstrate significant improvement when optimization was guided by altering the timing of activation of intracardiac electrograms (2016; Ellenbogen et al., 2010; Martin et al., 2012a; Rao et al., 2007; Abraham et al., 2012), but the AdaptivCRT™ (Daoud et al., 2016) have shown small modest benefits in reducing readmission (Singh et al., 2013) and noninferiority to echocardiography-guided synchronization techniques (Birnie et al., 2013) but no difference in mortality or morbidity (Martin et al., 2012b). QRS shortening using various algorithms have been associated with good clinical response and echocardiographic improvement (Lecoq et al., 2005; Hsing et al., 2011). Finally, fusion-optimized intervals (Arbelo et al., 2014), which uses fusion with intrinsic conduction to achieve the shortest possible QRS by BiV pacing with the intrinsic rhythm, have been found to improve acute hemodynamic response, but it is unclear if this will translate into long-term benefit (Arbelo et al., 2014) (**Table 4**).

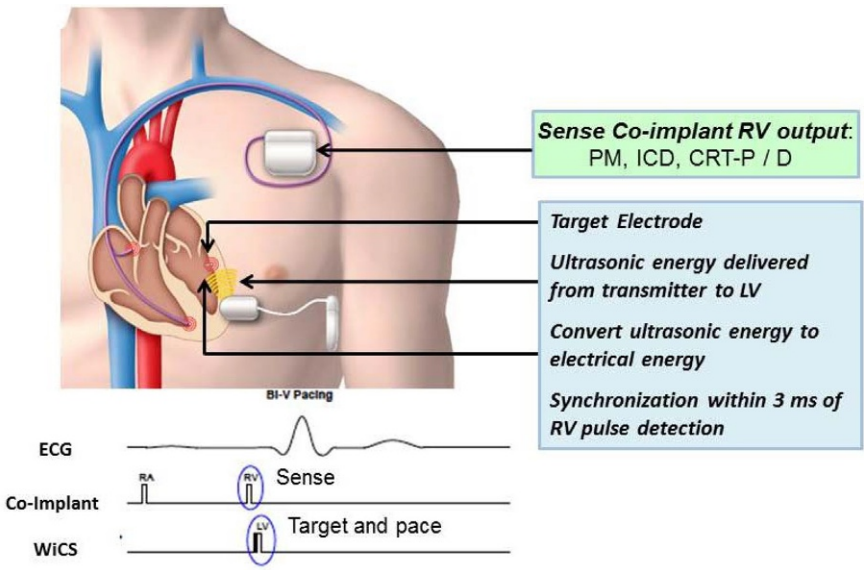

Fig. 11 Ultrasound-based wireless cardiac stimulation system (WiCS, EBR Systems), which includes a 9-mm leadless pacing electrode implanted at the endocardial mid-lateral LV free wall positioned using a retrograde aortic approach with a steerable 12 F sheath. The electrode is activated by a submuscular ultrasonic transmitter synchronized to a right ventricular pacing pulse of a standard pacemaker or implantable cardioverter defibrillator. *CRT-P/D*, cardiac resynchronization therapy with pacemaker/defibrillator; *ECG*, electrocardiogram; *ICD*, implantable cardiovertor-defibrillator; *LV*, left ventricle; *PM*, pacemaker. With permission from EBR systems website (http://www.ebrsystemsinc.com).

Nonresponse: Patient Follow-Up and Multidisciplinary Approach

Once the device is placed in a well-selected patient with proper programming with good remote monitoring, continued care and optimization of the patient care and device are required, which are best managed by a multidisciplinary approach (Hindricks et al., 2014; Mullens et al., 2009; Altman et al., 2012) (imaging, device programming, HF, and social support).

Future Technology

Future technology of CRT is to focus upon better stimulation models of the LV. MPP-IDE study (Tomassoni, 2016) and MORE-CRT MPP (Leclercq, 2016) are large multicenter clinical trials that are underway which compare standard quadripolar pacing to MPP. Another area of investigation for LV stimulation is direct LV pacing via an endocardial pacing electrode. The recently completed AlSync study (Morgan et al., 2016), assessed LV endocardial pacing achieved by LV lead placement via a transseptal puncture then across the mitral valve. This technique was successful in deploying the lead, but, even while on anticoagulation, patients had a high rate of stroke. Yet this study is launching a new area of investigation into direct LV endocardial stimulation and potentially offers access to all regions of the LV instead of only regions accessible by the CS branches, faster impulse propagation in the endocardium than compared to the epicardium, faster LV depolarization, and more physiologic LV stimulation (Bordachar et al., 2010). Additionally, direct LV pacing may be achieved by leadless stimulation powered by a remote source of ultrasound (**Fig. 11**) (Reddy et al., 2015). The role of CRT in non-LBBB patients has not been the sole study population for any CRT trial. The currently enrolling ENHANCE-CRT trial (Singh, 2013) randomizes patients with a non-LBBB native conduction to CRT implantation directed by the implanter's preference versus implant guided by Q-LV timing. Finally, the future of device optimization is also being evaluated with the advent of SonR hemodynamic sensor, which is embedded in the right atrial lead. This sonar sensor listens to the heart sounds as a surrogate for contractility. Based on this information, the AV and VV timing intervals are programmed on a weekly basis and has been shown to reduce HF hospitalization and mortality and improve the quality of life and NYHA class in ~1000 patients who were studied in the RESPOND-CRT study (Brugada et al., 2016).

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AL Cardiac Amyloidosis: Classification, Diagnosis, and Treatment

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Introduction

The systemic amyloidoses are a group of disorders characterized by organ deposition of misfolded protein fragments. Natural history, clinical manifestations, and treatment options for these disorders vary widely depending on the type of the abnormal protein. Cardiac amyloidosis is a disease of extracellular deposition of amyloid protein, often resulting in a restrictive cardiomyopathy. Cardiac involvement with amyloidosis most often occurs from misfolded immunoglobulin light chains (AL), produced by bone marrow plasma cells, or transthyretin (ATTR), formerly called prealbumin, manufactured by the liver. The heart may also rarely be involved with secondary (AA) amyloidosis, resulting from chronic inflammation, but this is not a common contributor to cardiac amyloidosis. Cardiac involvement may occur as part of a syndrome of systemic multiorgan involvement, as is frequently the case in AL amyloidosis, but the heart may also be the sole organ involved as typically seen in ATTR wild-type (formerly, senile) cardiac amyloidosis. It is imperative to accurately recognize cardiac involvement in systemic amyloidosis, as therapeutic options are constrained by reductions in cardiac function, and the degree of impairment serves as the principal determinant of prognosis and clinical course.

After a brief discussion about the historical background of the systemic amyloidoses and an introduction to other non-AL types of cardiac amyloidosis, this chapter will explore in depth the pathophysiology, diagnostic approach, and treatment options for AL cardiac amyloidosis.

Background

In 1854, the German pathologist Rudolph Virchow coined the term "amyloid" to describe macroscopically abnormal brain tissue which stained pale blue with iodine and violet after sulfuric acid was added. He postulated that this staining pattern suggested the presence of cellulose and derived the term "amyloid" from the Latin "amylum" and the Greek "amylon" (Cohen, 1986). Subsequent studies demonstrated the lack of carbohydrate in amyloid and it was eventually characterized as a protein. In 1927, light microscopic studies revealed the characteristic apple-green birefringence of amyloid protein when stained with Congo Red, and when viewed under polarized light (Divry and Florkin, 1927). Subsequent electron microscopy studies in the 1950s described the following common fibrillar ultrastructure underlying amyloid protein of diverse origins: all amyloid deposits comprise of nonbranching, rigid fibrils about 80–100 A in width and of varying lengths (Cohen and Calkins, 1959). These fibrils, in turn, are composed of filamentous subunits and are organized in a beta-pleated sheet configuration (Bonar et al., 1969; Glenner et al., 1974; Sunde and Blake, 1998). The nonfibrillar components of amyloid include serum amyloid P component, proteoglycans, and apolipoprotein E which is found in several types of amyloid. Serum amyloid P (SAP) is a pentagonal alpha globulin which is nearly identical to its counterpart found in amyloid tissue (Cathcart et al., 1965). As will be discussed later, SAP can serve as a target for both imaging and potentially dissolution of amyloid deposits.

Despite the common fibrillar ultrastructure, there is significant biochemical heterogeneity in amyloid deposits and more than 36 distinct amyloidogenic proteins have been identified in the animal kingdom (Sipe et al., 2016). However, three of these proteins play major causative roles in human amyloidosis. In the 1970s, amino acid sequencing of amyloid fibrils identified primary amyloidosis as being derived from immunoglobulin light chain fragments. This was initially termed amyloid B and is now referred

to as AL amyloid (Benditt et al., 1971). The same lab performed amino acid sequencing of amyloid tissue from patients with acute and chronic inflammation and identified a protein that they called amyloid A (Benditt et al., 1971). Finally, Costa et al. (1978) and Skinner and Cohen (1981) reported that transthyretin (formerly known as prealbumin) played a prominent role in the amyloid fibrils found in familial amyloid polyneuropathy. Since then, greater than 100 transthyretin (TTR) single-nucleotide polymorphisms have been identified (Connors et al., 2003), although a much smaller proportion have been implicated in cardiac amyloidosis. The systemic amyloidosis shares some homology with other protein folding diseases such as Alzheimer's dementia (precursor protein amyloid beta or Aβ) in that the final ultrastructure of the deposited protein is amyloid. This homology affords potential targets for both imaging and therapeutic intervention. There is no known association between the systemic amyloidosis and Alzheimer's dementia.

Transthyretin (TTR) Cardiac Amyloidosis

Formerly known as prealbumin, *TTR* is protein synthesized and secreted into the circulation by the liver, and plays a minor role in the transport of vitamin A (retinol) and thyroxine. Normally circulating as a stable tetramer, aging (wild-type ATTR) or genetic mutations (mutant ATTR) can cause the protein to dissociate into monomers which misfold and aggregate first as monomers and then oligomers with subsequent organ deposition. Wild-type (formerly referred to as senile systemic or senile cardiac) ATTR amyloidosis predominantly affects the heart with a striking male predominance (Ruberg and Berk, 2012). Prevalence increases with age, as demonstrated by numerous autopsy series that estimate up to 25% of patients over the age of 80 have demonstrable amyloid deposition by histopathology (Cornwell et al., 1983; Tanskanen et al., 2008). Recent evidence suggests that ATTR wild-type amyloidosis is an unrecognized cause of heart failure in the elderly (Mohammed et al., 2014), and may affect up to 10% of elderly heart failure patients (Gonzalez-Lopez et al., 2015).

In contrast, the prevalence and disease expression of mutant ATTR amyloidosis varies by mutation. Disease manifestations generally involve some combination of sensory and motor small fiber polyneuropathy and/or cardiomyopathy. Mutations, which represent single-nucleotide polymorphisms that follow an autosomal dominance inheritance pattern with variable penetrance, tend to have significant geographic variations with most pathological mutations resulting in significant polyneuropathy. Worldwide, three mutations are most commonly associated with cardiac amyloidosis. V30M (valine substitution for methionine at position 30; Met 30) is most common in Portugal, Sweden, and Japan, and its expression varies by geography, age, parental transmission, and gender (Rapezzi et al., 2010). The T60A (threonine to alanine substitution) mutation is also an important cause of cardiac amyloidosis and polyneuropathy and is particularly prevalent in Northwest Ireland and the United Kingdom (Reilly et al., 1995). In contrast, the V122I mutation (valine to isoleucine substitution) mutation is the most common mutation affecting the heart primarily, with an allele carrier rate of 3%–4% in the African American population (Jacobson et al., 1990; Jacobson et al., 1997). Penetrance of ATTR V122I amyloidosis is age dependent, and many patients do not live to reach the age of disease manifestation, typically the late 6th or 7th decade of life.

The diagnosis of amyloidosis relies on the identification of amyloid tissue by Congo red staining from an affected organ or from a surveillance biopsy typically taken from subcutaneous abdominal fat or the gastrointestinal tract, most commonly. Recent evidence suggests that ATTR amyloidosis can be diagnosed accurately without a tissue biopsy with use of the diagnostic bone-avid nuclear tracers Tc^{99m} pyrophosphate or Tc^{99m} DPD (3,3-diphosphono-1,2-propanodicarboxylic acid) and the absence of a plasma cell dyscrasia (Gillmore et al., 2016). Since treatment options for TTR and AL amyloidosis are completely different, identification of the precursor protein is imperative once amyloidosis has been identified. Immunohistochemistry can aid in identifying immunoglobulin κ and λ light chain fragments, TTR and AA protein (Ruberg and Berk, 2012); however diagnostic accuracy of immunohistochemistry is dependent upon the skill and experience of the pathology service at a given hospital. **Fig. 1** is an example of immunohistochemical stains identifying the origin of amyloid proteins in patients with cardiac amyloidosis. Precursor proteins are perhaps most accurately identified using liquid chromatography-tandem mass spectrometry at specialized amyloid centers (Vrana et al., 2009). Once TTR has been identified as the precursor protein, wild-type ATTR may be differentiated from mutant ATTR by sequencing of the *TTR* gene.

Treatment of ATTR cardiac amyloidosis hinges on management of heart failure symptoms. Diuretics are the mainstay of treatment as patients with amyloid cardiomyopathy do not typically tolerate treatment with ace inhibitors, angiotensin receptor blockers, beta blockers, or digoxin. Liver transplant may halt the progression of variant TTR amyloid cardiomyopathy, but organ progression is well recognized after transplantation of the liver alone (Ruberg and Berk, 2012). Small molecule inhibitors of TTR dissociation, including diflunisal (FDA approved, off-label indication) and tafamidis (European Medicines Authority approved), or agents that suppress TTR expression may be useful alternatives to liver transplantation in mutant ATTR amyloidosis and provide effective treatment options for ATTR cardiac amyloidosis (Maurer et al., 2015).

AL Cardiac Amyloidosis

Systemic AL amyloidosis is caused by a plasma cell dyscrasia resulting in overproduction and accumulation of immunoglobulin lambda (more commonly) or kappa light chains. These light chains are the precursor proteins resulting in amyloid fibril formation and organ deposition. Unlike multiple myeloma, a related plasma cell disorder, patients with AL amyloidosis usually have <20%

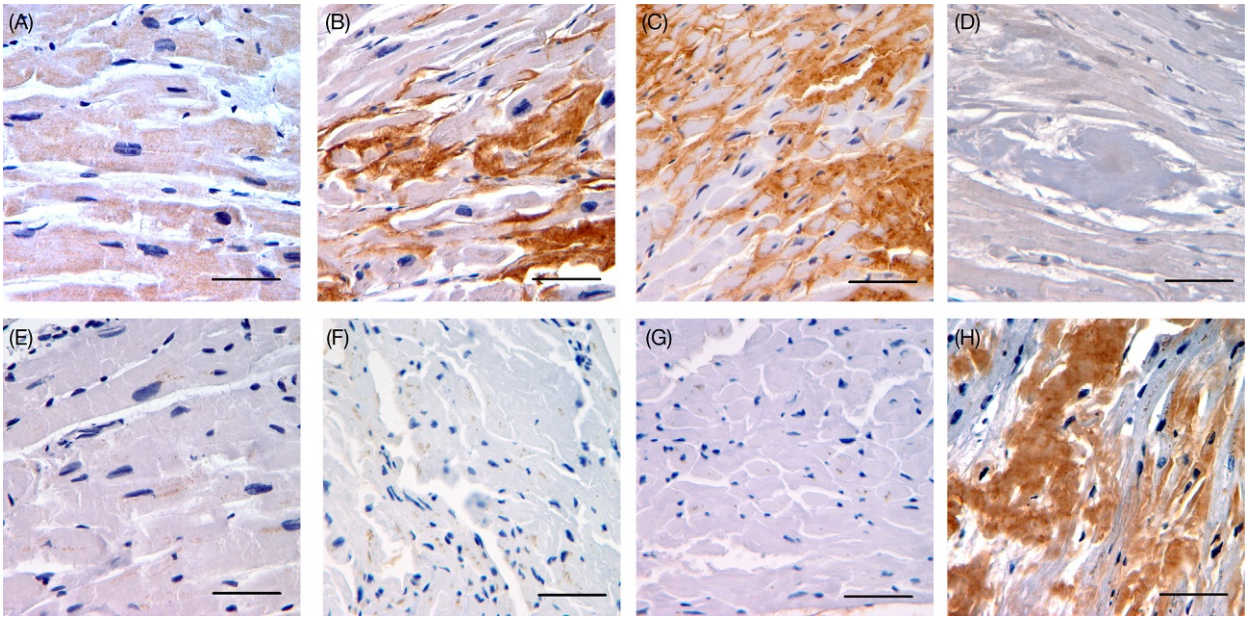

Fig. 1 Characteristic immunohistochemical staining for transthyretin (TTR, top row) and light chain (LC, bottom row) amyloid protein under light microscopy using control (A and E), wild-type (senile, SSA) transthyretin amyloidosis (B and F), variant transthyretin (ATTR) (C and G), and AL amyloidosis (D and H) cardiac biopsy samples. Control samples do not stain with either TTR or LC stains (A and E). TTR stains reveal significant peri-cellular staining in both wild-type (SSA) and variant ATTR samples (B and C), but not in the AL amyloidosis sample (D). In contrast, AL amyloid cardiac samples are markedly positive for LC stains (H), but transthyretin samples (F and G) do not reveal LC staining. Reproduced by permission from Greene, M. J., Sam, F., Soo Hoo, P. T., Patel, R. S., Seldin, D. C. and Connors, L. H. (2011). Evidence for a functional role of the molecular chaperone clusterin in amyloidotic cardiomyopathy. *American Journal of Pathology*, **178**, 61–68.

involvement of their bone marrow with a plasma cell clone. However, about 10% of patients with AL amyloidosis may have multiple myeloma (Hasserjian et al., 2007). AL amyloidosis may involve multiple organs, but renal and cardiac AL amyloidosis is the most common, with cardiac involvement seen in approximately 50% of cases (Merlini and Bellotti, 2003). The presence of cardiac involvement is the most important determinant of survival for patients with AL amyloidosis as historically, patients with untreated AL cardiac amyloidosis and congestive heart failure experience an overall median survival of only 6 months (Kyle et al., 1992). In sharp contrast, contemporary treatment with modern chemotherapeutic regimens, including high-dose melphalan-based chemotherapy and stem cell transplantation (discussed below) in eligible patients can extend median survival to >5 years, and for some, beyond a decade or longer (Madan et al., 2012).

Cardiac amyloidosis is an infiltrative disorder, characterized by expansion of the extracellular space due to amyloid fibril deposition. The resulting increase in biventricular wall thickness leads to ventricular stiffness, manifesting as an infiltrative, restrictive cardiomyopathy. Atrial infiltration is common, predisposing patients to atrial fibrillation, particularly in conjunction with elevated LA pressures from restrictive LV filling. Atrial infiltration also contributes to atrial electromechanical dissociation, despite the presence of normal sinus rhythm on ECG, with an associated risk of atrial thrombus formation and thromboembolism (Stables and Ormerod, 1996; Feng et al., 2007, 2009). Diffuse valvular thickening is often noted as well, resulting in regurgitant lesions but not stenosis. Additionally, perivascular deposition may be seen with arterial and arteriolar involvement. Coronary microvascular dysfunction is an uncommon but well-recognized feature in cardiac amyloidosis that can precipitate ischemia and infarction (Tsai et al., 2011). Sensitive measurements of cardiac perfusion by PET imaging have demonstrated diminished coronary flow reserve in patients with suspected small vessel amyloid infiltration (Dorbala et al., 2014a). The histologic distribution of amyloid infiltration and the resultant restrictive cardiomyopathy is generally similar in TTR and AL cardiac amyloidosis; however, the pattern of amyloid deposition can differ with diffuse pericellular and reticular deposits in AL amyloidosis and nodular deposits in TTR amyloidosis (Kristen et al., 2016).

In addition to interstitial infiltration, there is clear evidence for direct light chain toxicity to the cardiomyocyte which may contribute to organ dysfunction and the rapidly progressive nature of this disease (Brenner et al., 2004; Shi et al., 2010; Diomede et al., 2014). Further investigation has suggested that this light chain proteotoxicity derives from lysosomal dysfunction and impairment of autophagy which leads to the generation of reactive oxygen species and impaired intracellular calcium homeostasis (Guan et al., 2014). This sequence of events eventually leads to cell death. Amyloidogenic light chain-induced cell necrosis and apoptosis also lead to abnormalities in arteriolar reactivity that can contribute to microvascular dysfunction (Migrino et al., 2011), and may precipitate an imbalance in the generation and degradation of extracellular matrix protein undermining the organized structure of the cardiac contractile apparatus (Biolo et al., 2008). Thus, there is ample evidence that direct light chain toxicity contributes to the pathophysiology of AL cardiac amyloidosis, in addition to the restrictive physiology caused by direct extracellular protein infiltration.

Clinical Features of AL Cardiac Amyloidosis

Symptoms of heart failure, particularly dyspnea on exertion and profound peripheral edema, are the most common presenting symptoms of cardiac amyloidosis (Falk et al., 2016). These are manifestations of high filling pressures in the setting of a restrictive cardiomyopathy. Exertional syncope is an advanced sign of an inability to augment cardiac output with exertion and is associated with a particularly poor outcome (Chamarthi et al., 1997). Atrial infiltration and soft tissue infiltration may precede symptoms of heart failure by several years and present as atrial fibrillation and bilateral carpal tunnel syndrome respectively, although they are commonly unrecognized as signs of systemic amyloidosis. The prevalence of atrial fibrillation is about 10% in AL cardiac amyloidosis and up to 40% in patients with wild-type TTR cardiac amyloidosis (Longhi et al., 2015). Peripheral edema, in addition to being a sign of diastolic heart failure, is also commonly seen due to nephrotic syndrome associated with renal amyloidosis. Therefore, heart failure with signs of nephrotic syndrome is a strong indicator of systemic AL amyloidosis and must be evaluated accordingly.

Systemic thromboembolic events, particularly ischemic strokes, may also be the initial signs of cardiac amyloidosis (Zubkov et al., 2007). Both venous and arterial thromboembolic events may be seen with AL amyloidosis (Freeman et al., 2012). Intracardiac thrombi are common and were present in one-third of patients with cardiac amyloidosis in one autopsy study (Feng et al., 2007). Advanced diastolic dysfunction and atrial fibrillation were most strongly associated with intracardiac thrombi; however anticoagulation can mitigate the likelihood of systemic embolization (Feng et al., 2009).

In addition to arterial thromboembolic disease, microvascular involvement of coronary and peripheral arterioles may result in angina and claudication, including jaw claudication. Peripheral and autonomic neuropathies are common due to nervous system involvement. Low cardiac output coupled with an autonomic neuropathy often leads to significant hypotension and orthostasis, greatly complicating diuresis (Sugiyama et al., 2014; Matsuda et al., 2011).

The physical exam in patients with AL amyloidosis may reveal orthostatic hypotension. There may be significant tongue enlargement. Macroglossia is seen in up to 10% of patients with AL amyloidosis, and is considered pathognomonic for the AL type. Periorbital ecchymosis may be subtle, but can be seen in AL amyloidosis (Falk et al., 2016). The liver may be enlarged due to hepatic infiltration with amyloid protein or due to right heart failure. Peripheral neuropathy may be noted, is predominantly sensory, and may be subtle in patients with AL amyloidosis. This is in contrast to patients with mutant ATTR amyloidosis who often have a progressive sensorimotor peripheral neuropathy, depending on the particular transthyretin mutation.

The cardiac exam reveals signs of elevated filling pressures. Ascites and peripheral edema may be a part of anasarca that is seen in patients with nephrotic syndrome due to renal involvement. As will be discussed below, valvular thickening and valvular regurgitation are relatively common and these murmurs may be auscultated as well. S3 and S4 gallops, which are commonly associated with diastolic heart failure, are not usually appreciated in cardiac amyloidosis until advanced heart failure with systolic dysfunction ensues.

Electrocardiogram

Low limb voltages on ECG, especially in the setting of increased left ventricular wall thickness on echocardiogram, is a classic feature of cardiac amyloidosis. Unlike the myocyte hypertrophy, which leads to increased QRS voltages in hypertrophic cardiomyopathy, the extracellular volume expansion due to amyloid fibril deposition is responsible for the low QRS voltages in the setting of increased wall thickness in cardiac amyloidosis. Depending on the method used to define low QRS voltage, the prevalence of low voltage in cardiac amyloidosis ranges from 60% using the Sokolow index (S wave in V1 + R wave in V5 or V6 $<$1.5 mV) to 34% using QRS voltage $<$0.5 mV in each limb lead and 13% when using QRS voltage $<$1.0 mV in each precordial lead (Cyrille et al., 2014). Pseudo infarct patterns are common as well and can be seen in approximately 50% of patients with AL cardiac amyloidosis (Murtagh et al., 2005). Pseudo infarct patterns may also be seen with an unusual QRS axis in cardiac amyloidosis. In addition, atrioventricular block may be seen in up to 22% of patients with cardiac amyloidosis. Intraventricular conduction delays and bundle branch blocks are also common ECG features of cardiac amyloidosis (Huang et al., 2015). Finally, ECG-defined left ventricular hypertrophy can also be observed in approximately 10%–15% of patients with AL amyloidosis, but this likely reflects preexisting hypertensive heart disease, with superimposed amyloidosis (Murtagh et al., 2005). **Fig. 2** is an example of characteristic ECG abnormalities seen in cardiac amyloidosis.

Echocardiography

Two-dimensional echocardiography is the cornerstone of diagnosis of cardiac amyloidosis and is the imaging modality which most often raises suspicion for cardiac involvement (**Fig. 3**). Indeed, international consensus criteria for the identification of cardiac involvement in AL amyloidosis have relied upon a septal thickness more than 12 mm though this is changing with contemporary imaging techniques and the advent of biomarker data (Gertz et al., 2005). Symmetrically increased LV wall thickness, often $\geq$15 mm, in the setting of a nondilated ventricle, can be an indicator of cardiac amyloidosis, but is not specific for the type of amyloidosis (Rapezzi et al., 2009). Wall thickness is generally more prominent in patients with transthyretin cardiac amyloidosis at

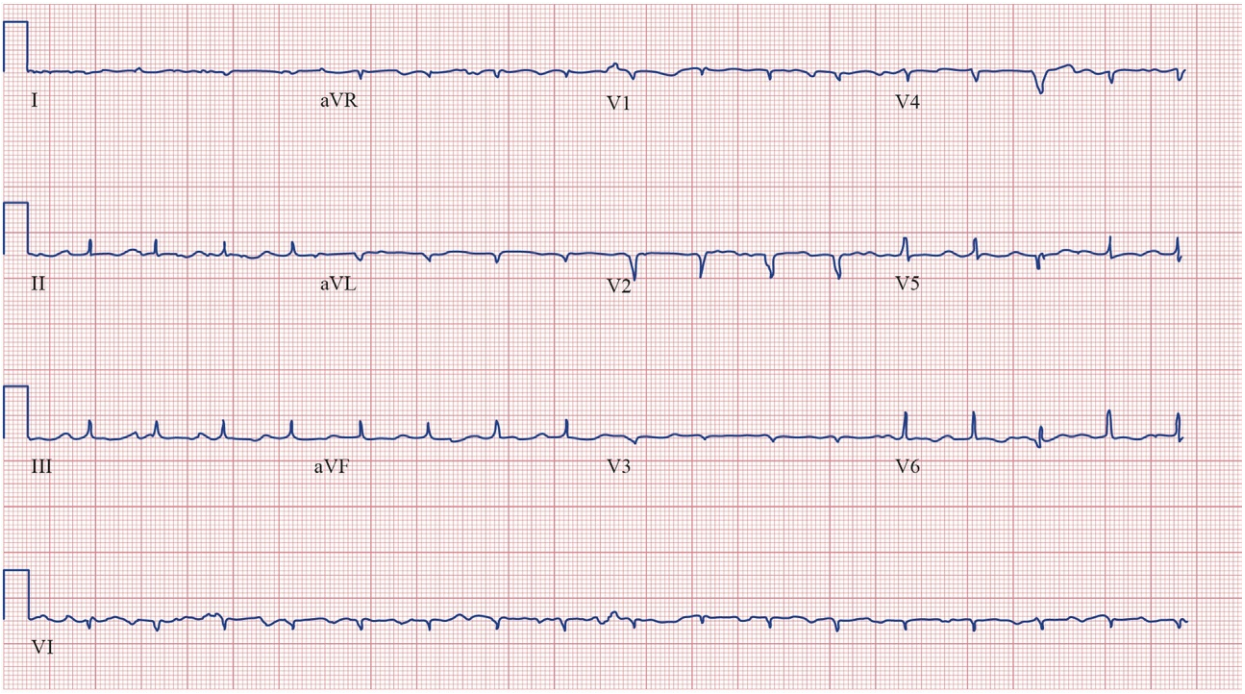

Fig. 2 Electrocardiography in cardiac amyloidosis. ECG from a patient with cardiac amyloidosis reveals low voltages, 1st degree AV delay, and a septal infarct pattern.

diagnosis, as patients with AL amyloidosis tend to become symptomatic earlier on in the disease course. RV wall thickness is also commonly increased due to amyloid infiltration (Cacciapuoti, 2015). Indices combining low QRS voltage on ECG with increased LV wall thickness have high sensitivity and specificity for diagnosis of cardiac amyloidosis (Cheng et al., 2011). Historically, "granular sparkling" or "speckling" of the myocardium was felt to be a marker of amyloid infiltration, and hence, felt to be diagnostic of cardiac amyloidosis. With advances in digital image analysis techniques, myocardial speckling has a low sensitivity and specificity for diagnosis of cardiac amyloidosis (Picano et al., 1991; Rahman et al., 2004).

Global LV systolic function, as assessed by the ejection fraction, is usually preserved until advanced stages of disease, but impairments in systolic deformation as measured by global longitudinal strain are often evident early in the course of the disease (Piper et al., 2010). Global longitudinal strain (GLS) is an accurate marker of systolic dysfunction in cardiac amyloidosis and can help discriminate between early and more advanced stages of the disease (Bellavia et al., 2007). In addition to a decline in global longitudinal strain, there are regional differences in systolic deformation with cardiac amyloidosis such that longitudinal strain is relatively preserved at the apex and is more significantly diminished at the basal and mid LV cavity (Senapati et al., 2016). An apical to basal ratio >2.1 conferred high discriminative capacity for cardiac amyloidosis identification (Liu et al., 2013). Furthermore, baseline two-dimensional global longitudinal strain is an independent predictor of survival in patients with AL cardiac amyloidosis with a global longitudinal strain value of approximately -11% accurately differentiating survivors from nonsurvivors at a follow-up of over 1200 days (Buss et al., 2012). Longitudinal strain measures can also identify an early cardiac improvement following treatment for AL amyloidosis as patients achieving complete response demonstrated significant improvement in the apical to basal longitudinal strain ratio, compared to nonresponders (Salinaro et al., 2016).

Amyloid infiltration results in biventricular stiffening and diastolic dysfunction, often presenting as heart failure with preserved EF (HFpEF). The trans-mitral flow pattern is abnormal due to diastolic dysfunction with elevated filling pressures (pseudonormalized or a restrictive pattern) and atrial mechanical dysfunction, even in the setting of sinus rhythm (Mohty et al., 2013). Low or even absent trans-mitral A velocities may commonly be encountered in patients with an advanced restrictive cardiomyopathy (Dubrey et al., 1995) despite sinus rhythm. A trans-mitral A velocity of less than 30 cm/s was associated with intracardiac thrombi in AL cardiac amyloidosis patients without atrial fibrillation (Feng et al., 2009). A consequence of advanced diastolic dysfunction and high filling pressures is atrial enlargement, which, unlike ventricular dilation, is commonly seen in cardiac amyloidosis and may be an independent predictor of decreased survival (Mohty et al., 2011).

In addition to atrial infiltration, there is often infiltration of the interatrial septum. Increased RV wall thickness and RV systolic dysfunction, as denoted by a tricuspid plane systolic excursion (TAPSE) <1.4 cm, may be seen and each are independent predictors of adverse outcomes (Bodez et al., 2016). Valvular thickening is often noted due to amyloid deposition and this, along with elevated filling pressures, may lead to significant valvular regurgitation (Mohty et al., 2013). Small pericardial effusions are common, but cardiac tamponade in cardiac amyloidosis is very rare (Navarro et al., 1992; Bodez et al., 2016).

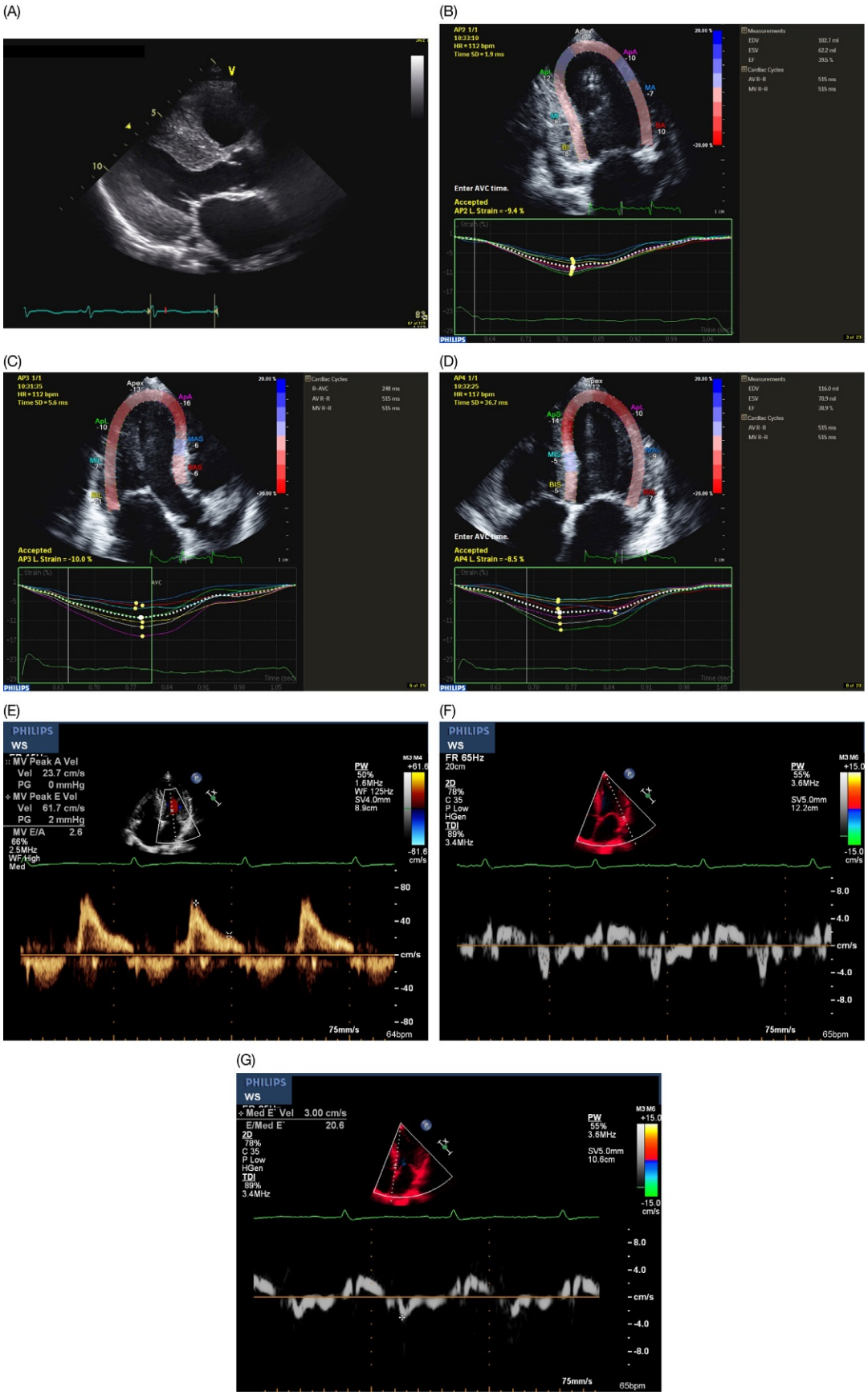

Fig. 3 Echocardiography in cardiac amyloidosis. Parasternal long-axis view depicting severely increased wall thickness, with granular sparkling, which may be seen in cardiac amyloidosis (A). Longitudinal strain in apical 4-, 2- and 3-chamber views showing severely reduced global longitudinal strain (GLS) of -9.3% (B–D). Diastology in amyloidosis, revealing a restrictive trans-mitral filling pattern and low mitral annulus tissue Doppler velocities (E–G). Low trans-mitral A velocity is suggestive of atrial electromechanical dissociation and may increase the risk for atrial clot formation (see text).

Cardiac Magnetic Resonance (CMR) Imaging

While echocardiography is appropriately the first-line imaging test used to detect cardiac amyloidosis, cardiac MRI (CMR) is useful in equivocal cases and can help risk stratify patients beyond the usual clinical and laboratory risk markers.

As discussed above, cardiac amyloidosis involves the interstitial deposition of protein fibrils leading to an expansion of the extracellular volume (ECV) and eventually, myocardial fibrosis (Ishikawa et al., 1996). Late gadolinium enhancement (LGE) imaging using CMR has proven effective in identifying ECV expansion and endomyocardial fibrosis (Wu et al., 2001; Moon et al., 2003; McCrohon et al., 2003). Cardiac amyloidosis has a characteristic pattern of diffuse subendocardial LGE with CMR that is associated with clinical heart failure (Ruberg et al., 2009) and survival (Austin et al., 2009). At autopsy, this subendocardial pattern of LGE appears to correlate more with amyloid deposits rather than fibrosis (Maceira et al., 2005). However, many different patterns from subendocardial to transmural have been seen in cardiac amyloidosis (Syed et al., 2010), and therefore the presence of LGE in a noninfarct pattern in a patient with systemic amyloidosis should be considered highly suggestive of cardiac involvement (**Fig. 4**). In fact, in patients with multiple myeloma, CMR has sensitivity and negative predictive values of 100% and specificity and positive predictive values of 80% and 81%, respectively, for diagnosis of AL cardiac amyloidosis (Bhatti et al., 2016). Additionally, LGE by CMR is an independent predictor of mortality in patients with AL cardiac amyloidosis and has prognostic value beyond the usual clinical and laboratory data (Boynton et al., 2016).

In addition to LGE, myocardial and blood pool handling of gadolinium products are also abnormal in cardiac amyloidosis (Maceira et al., 2005). This results in a higher blood T1 and a lower myocardial T1 in cardiac amyloid patients with a smaller difference in T1 between blood and myocardium. For this reason, contemporary LGE imaging in cardiac amyloidosis is best performed using the phase-sensitive inversion recovery (PSIR) technique that has superior accuracy compared to conventional LGE imaging (Fontana et al., 2015).

LGE is very useful for differentiating abnormal from normal myocardium, but this presupposes regions of normal are present. Amyloidosis is a diffuse disease, and in many instances, there is no "normal" myocardium to contrast. For this reason, quantitative imaging techniques including T1 mapping and extracellular volume fraction (ECV) determination have been recently explored in cardiomyopathic disease such as amyloidosis. Extracellular volume (ECV) measurement from pre- and postcontrast T1 mapping has shown promise for detection of cardiac amyloidosis (Mongeon et al., 2012; Banypersad et al., 2013). Similarly, native T1 quantification, without the need for contrast, can differentiate AL cardiac amyloidosis from AL amyloidosis without cardiac involvement, albeit with considerable overlap (Karamitsos et al., 2013). Of the two quantitative techniques, ECV appears the most reproducible (not subject to field strength or technique differences) and confers the highest resolution to determine the severity of amyloid deposition (Fontana et al., 2015).

Diminished left atrial function as determined by CMR, expressed by a LA EF of $<16\%$, is associated with a worse functional status, biomarkers, and increased two-year mortality in patients with cardiac amyloidosis (Mohty et al., 2016). Impairment of coronary microvascular function can also be demonstrated by first-pass perfusion differences between AL cardiac amyloid patients with normal LVEF, AL cardiac amyloid patients with LV systolic dysfunction, and healthy controls (Li et al., 2016). Finally, while a CMR scoring system was useful in differentiating AL cardiac amyloidosis from TTR cardiac amyloidosis, the reproducibility of this measure was only fair, limiting its applicability (Dungu et al., 2014).

Nuclear Scintigraphy

In 1977, the bone-avid tracer technetium diphosphonate was found to bind to amyloid deposits, thus providing a potent imaging tool for visualizing the distribution of amyloid protein (Kula et al., 1977). This led to the development of several other

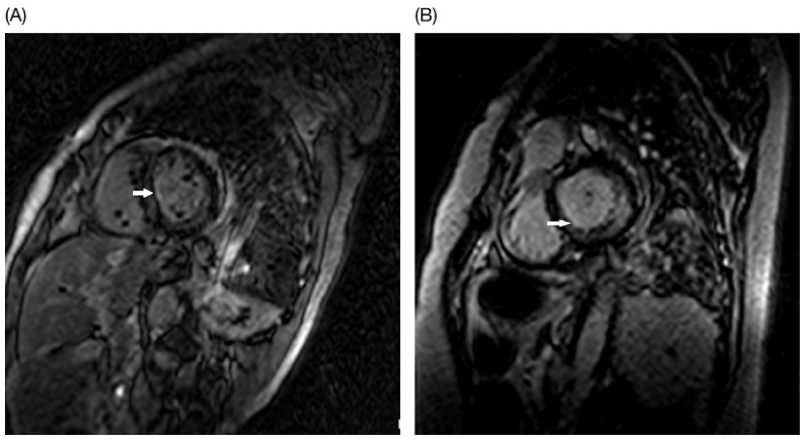

Fig. 4 Short-axis cardiac MR images revealing a patchy subendocardial late gadolinium enhancement (LGE) pattern (*white arrows*) consistent with cardiac amyloidosis in two separate subjects.

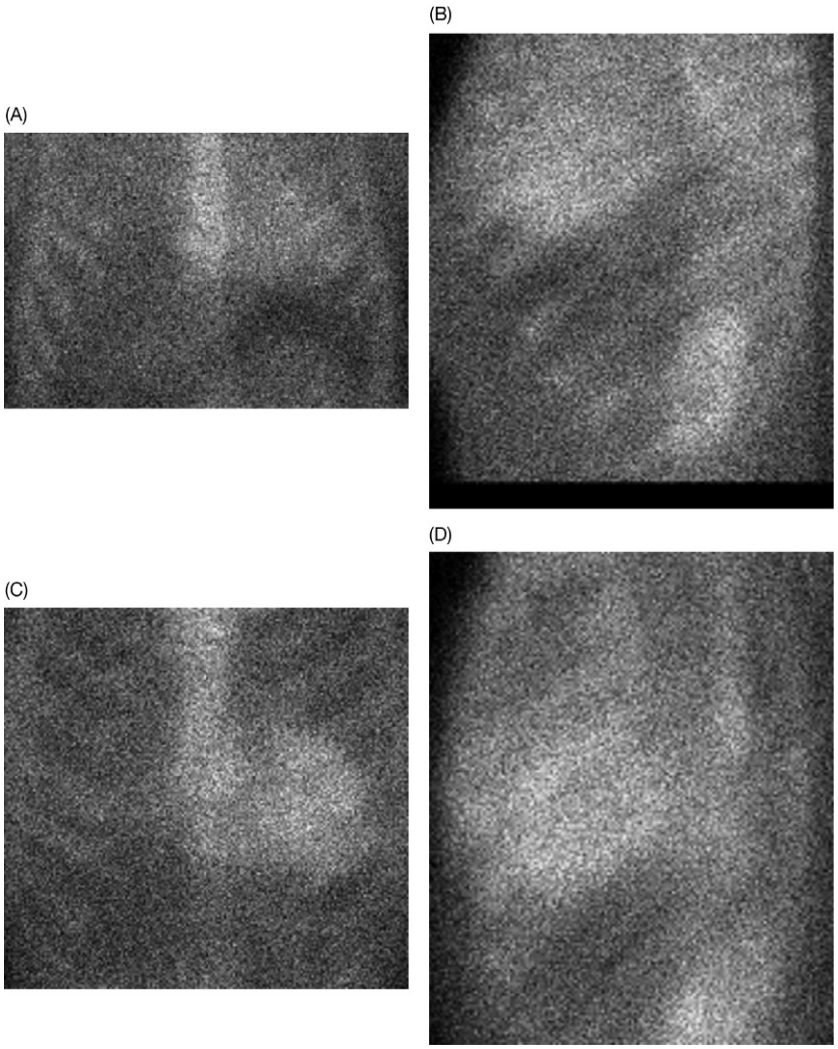

Fig. 5 Tc99m pyrophosphate scans in AP and lateral projections revealing a qualitative Grade 1 (equivocal) uptake (A and B) in a patient with AL amyloidosis, and Grade 3 (strongly positive) uptake (C and D) by the heart when compared to the surrounding ribs in a patient with ATTR wild-type amyloidosis.

phosphate-based isotopes, including ^{99m}Tc-PYP (pyrophosphate) and ^{99m}Tc-DPD (3,3-diphosphono-1,2-propanodiacarboxylic acid), which have a specific avidity for ATTR amyloid deposits. An international consensus document, supported by data from over 1000 patients, has confirmed the utility of the bone-avid compounds for the highly accurate identification of ATTR amyloidosis (Gillmore et al., 2016), and differentiation from AL amyloidosis. ^{99m}Tc-PYP is now starting to be used clinically in the United States and is highly useful for the detection of ATTR cardiac amyloidosis, but it is not useful for identification of AL (**Fig. 5**). A PYP scan that does not show uptake, or shows mild grade 1 uptake, is completely consistent with AL amyloidosis.

In addition, there is increasing evidence that amyloid-specific PET tracers can identify cardiac amyloidosis, specifically the AL type. Dorbala and colleagues reported the utility of 18-F florbetapir to identify cardiac involvement in AL disease (Dorbala et al., 2014b), and similarly, the 11-C Pittsburgh B compound (PiB) has been shown effective in AL identification (Lee et al., 2015).

Pepys and colleagues in the United Kingdom developed a radiolabeled serum amyloid P (SAP) tracer which binds amyloid deposits (Hawkins et al., 1990). This I^{123}-labeled SAP scan has the unique advantage of being able to map the distribution of amyloid within the body, thus providing an accurate estimate of amyloid burden that can be followed with treatment. However, this scan is not useful for identification of cardiac amyloidosis.

Table 1 summarizes the main features of the various imaging modalities used to detect cardiac amyloidosis.

Cardiac Biopsy/Histology

While advances in imaging have greatly enhanced our ability to detect cardiac amyloidosis and to differentiate AL cardiac amyloidosis from ATTR cardiac amyloidosis, histology from a tissue biopsy remains the gold standard for diagnosis of amyloidosis. In fact, it is essential that amyloid be identified by Congo red (or similar) amyloidosis specific stain prior to any chemotherapy

Table 1 Summary of imaging findings used to diagnose cardiac amyloidosis

Modality	*Imaging features*
Echocardiogram	1. Increased ventricular wall thickness 2. Advanced diastolic dysfunction 3. Granular sparkling of the myocardium may be seen 4. Apical to basal strain ratio >2 5. Global longitudinal strain <−11% associated with increased mortality 6. Pericardial effusion may be present
Cardiac MRI	1. Diffuse or subendocardial late gadolinium enhancement (LGE) 2. LGE is in a noncoronary pattern 3. Extracellular volume (ECV) expansion by T1 mapping
Tc99m pyrophosphate scan	1. Cardiac uptake greater than uptake by surrounding ribs (grade 3 uptake) is diagnostic of transthyretin cardiac amyloidosis in the setting of an absence of a plasmal cell dyscrasia 2. AL cardiac amyloidosis may have no uptake or mild uptake, not equal to surrounding ribs (grade 0 or 1)

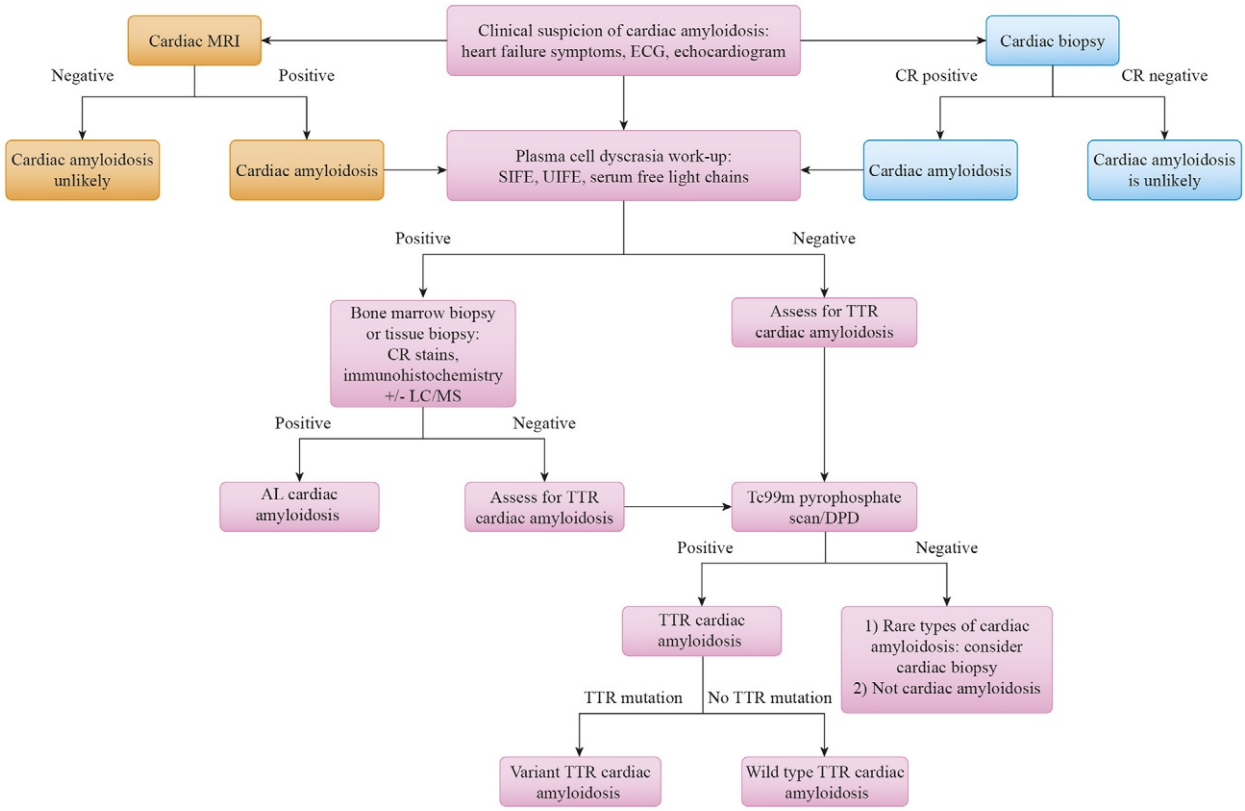

Fig. 6 Decision algorithm for diagnosis of cardiac amyloidosis.

administration. It is also critical to define the precursor protein by blood testing or tissue specimen analysis prior to treatment. Monoclonal gammopathies (MGUS) are relatively common in ATTR amyloidosis, occurring in up to 10% of patients (Connors et al., 2016). As such, AL and ATTR can be confused if the proper diagnostic algorithm is not followed. Figure 6 describes a diagnostic approach to identification of cardiac amyloidosis and differentiation between AL and ATTR cardiac amyloidosis.

Abdominal fat aspirate can be safely performed in the outpatient setting and, in experienced hands, Congo red staining has a sensitivity of 70%–90% for diagnosis of systemic AL amyloidosis (Gertz et al., 1988; Libbey et al., 1983). Thus, cardiac amyloidosis can be inferred in the setting of consistent noninvasive cardiac testing results and a positive extra-cardiac biopsy.

The histologic techniques employed to type the precursor protein include immunohistochemistry and immunofluorescence, immunogold electron microscopy, and laser dissection, liquid chromatography tandem mass spectrometry (LC/MS/MS) which is considered the gold standard for diagnosis. LC/MS/MS is only performed at a few specialized centers worldwide. Immunohistochemisty and immunofluorescence are commonly performed, but accuracy varies with experience, while immunogold electron

microscopy is very uncommonly performed. In most circumstances, an abdominal fat aspirate yields too little tissue for typing, while solid organ biopsies such as renal or gastrointestinal are more commonly pursued.

In patients with a plasma cell dyscrasia with equivocal cardiac imaging findings, or when AL cardiac amyloidosis needs to be differentiated from ATTR cardiac amyloidosis in the setting of a MGUS, an endomyocardial biopsy should be performed. All endomyocardial biopsies undergo Congo Red or Alcian Blue staining to assess for amyloidosis. Immunohistochemistry and immunogold electron microscopy is performed for confirmation and to identify the precursor protein. As above, mass spectrometry may be performed at specialized centers, if the above investigations are equivocal or unavailable. At experienced centers, cardiac biopsies can be performed with a very low risk of complications. However, with the advances in imaging summarized above, endomyocardial biopsies are no longer routinely required for the diagnosis of cardiac amyloidosis. Quantification of amyloid deposition degree in the heart is challenging and difficult to perform reproducibly, but has been reported (Kristen et al., 2016).

Prognosis

The prognosis of patients with AL amyloidosis has dramatically improved over time, with a four-year overall survival of around 90% for successfully treated patients with stem cell transplantation in the contemporary era (Muchtar et al., 2017). For patients with cardiac involvement successfully treated with stem cell transplantation, median overall survival is >10 years (Madan et al., 2012). The Mayo Biomarker Stage is a risk score using pretreatment serum cardiac troponin T (*c*TnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) to stratify patients. Using threshold values of *c*TnT <0.035 microg/L and NT-proBNP <332 ng/L, patients were classified in stages I, II, or III, if both biomarkers were negative, if one was positive, or if both were positive, respectively. In the original derivation cohort, around a third of the patients were classified in each category. Median survivals were 26.4, 10.5, and 3.5 months (Dispenzieri et al., 2004). This score was subsequently enhanced with the addition of a fourth metric, the absolute difference in serum free light chains. Patients were assigned one point each for free light chain difference $\geq$18 mg/dL, *c*TnT $\geq$0.025 ng/mL, and NT-proBNP $\geq$1800 pg/mL, resulting in stages I through IV with a total of 0–3 points respectively. This modified staging system resulted in improved risk stratification with mean overall survival ranging from 94.1 months for stage 1–5.8 months for stage IV (Kumar et al., 2012). Stage 1 patients likely have no clinically important cardiac amyloidosis. These staging systems help to highlight patients at the highest risks for death for whom intensification of therapy must be considered.

Therapy

The goal of medical therapy in AL cardiac amyloidosis is twofold: relieving symptoms of the infiltrative cardiomyopathy through diuresis and optimization of hemodynamics, and treatment of the underlying plasma cell dyscrasia which is the source of the amyloidogenic light chains. While there is excellent evidence-based data for chemotherapeutic regimen selection, there is only anecdotal experience for symptom-based therapy.

Standard cardiomyopathy therapy with beta blockers and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers is not well tolerated in AL cardiac amyloidosis, owing to hypotension. In fact, a common presentation scenario is a patient in whom a beta blocker was administered for cardiomyopathy who subsequently developed profound hypotension and fatigue. AL amyloidosis patients depend greatly on heart rate and contractility to maintain cardiac output, and beta blockade interferes with this adaptation. Autonomic neuropathy is commonly seen in patients with AL cardiac amyloidosis, increasing the incidence of hypotension and postural symptoms with the use of afterload reducing agents. Low-dose beta blockers may be used with caution for rate control in cardiac amyloid patients with atrial fibrillation or atrial flutter. If tolerated, doses typically should not exceed 50 mg daily of metoprolol. Tolerance of high dose beta blockade suggests that cardiac amyloidosis is not present or not clinically important.

For atrial dysrhythmias, particularly fibrillation or flutter, amiodarone may be used for rhythm control in these patients, and is the most widely selected agent. Other antiarrhythmic agents are generally avoided, but some can be used with caution including propafenone and dofetilide. Patients with amyloid cardiomyopathy are very sensitive to digoxin, which often leads to bradycardia. Digoxin has significant affinity for amyloid fibrils, to which it binds strongly in cardiac tissue from patients with cardiac amyloidosis (Rubinow et al., 1981). This interaction likely increases the local concentration of digoxin, leading theoretically to its adverse effects in these patients, and for this reason has been largely avoided clinically. Similarly, calcium channel blockers bind to amyloid fibrils, resulting in a potentiation of their negative inotropic effects, and worsening systolic heart failure in patients with cardiac amyloidosis (Pollak and Falk, 1993; Gertz et al., 1985).

Loop diuretics are often the mainstay of heart failure therapy. Increasing doses of diuretic are often required as the disease progresses. Significant right-sided heart failure often leads to ascites and gut edema. Thus, diuretics such as torsemide and bumetanide may have better oral absorption in these patients. Thiazide diuretics such as metolazone may be used to potentiate the effect of loop diuretics. Aldosterone antagonists including spironolactone and eplerenone are well tolerated and may be used adjunctively for diuresis and for treating hypokalemia which may result from aggressive diuresis. Patients with AL amyloidosis commonly have renal involvement which manifests as nephrotic syndrome. These patients may have profound lower extremity edema without other signs of heart failure. Diuretics are also used in these patients for treatment of their edema. A particularly challenging case is of the patient with AL cardiac amyloidosis with autonomic dysfunction leading to significant symptomatic

orthostatic hypotension. Such patients often require therapy with midodrine to support their blood pressure so they can be diuresed. Another challenging phenotype is the AL amyloidosis patient with LV outflow obstruction owing to chamber size and wall thickness, mimicking hypertrophic cardiomyopathy (Dinwoodey et al., 2008). There is no intentional experience with septal reduction surgery in AL amyloidosis; a small case series has been reported of patients with presumed HCM who were found to have cardiac amyloidosis by cardiac histology at surgery (Helder et al., 2014).

Atrial and ventricular arrhythmias are common in patients with cardiac amyloidosis. Atrial arrhythmias may be amenable to catheter ablation, but the risk of recurrence remains high, especially in the setting of atrial fibrillation (Tan et al., 2016), and the continued high risk of thromboembolic events warrants continued treatment with therapeutic anticoagulation. No clear guidelines exist for placement of ICDs and the rates of appropriate ICD therapies are high in patients with AL cardiac amyloidosis (Hamon et al., 2016). However, the overall mortality remains high, with pulseless electrical activity (PEA) being the dominant cause of death. Studies to date have not shown a survival benefit with ICD implantation for primary or secondary prevention (Kristen et al., 2008; Lin et al., 2013). Therefore, ICDs are likely not associated with a significant mortality benefit and their routine use in AL cardiac amyloidosis is not recommended. Our general practice is to place devices for true secondary prevention episodes including aborted sudden death and/or documented sustained VT/VF.

Definitive therapy is aimed at treating the underlying plasma cell dyscrasia. Since light chain toxicity contributes to the pathogenesis of cardiac amyloidosis, reducing the burden of circulating light chains leads to an improvement in cardiac function, even if the underlying amyloid deposits persist (Palladini et al., 2006). The goal of antiplasma cell therapy is the attainment of the complete hematologic response (CR), defined as the normalization of the affected light chain concentration, normalization of the bone marrow, and absence of monoclonal bands in serum and urine. Attainment of CR has been shown as a marker of long-term survival and organ-specific improvement. In the heart, a 30% reduction in NT-pro-BNP defines the organ-specific response, and this has been associated with a slight reduction in wall thickness and improvement in LVEF (Madan et al., 2012). The alkylating agent melphalan and the proteasome inhibitor bortezomib are most commonly used as first-line chemotherapy, the latter is often used in combination with cyclophosphamide and dexamethasone as part of the CyBorD regimen. First-line induction chemotherapy with CyBorD results in a hematologic response in around 60% of patients, with a cardiac response seen in about 20% of patients (Mikhael et al., 2012; Venner et al., 2012). Patients with cardiac involvement have a lower rate of achieving complete remission, with a response rate of about 40%, but with a two-year survival of 67% in responders (Palladini et al., 2015). Another class of immunomodulatory drugs, derived from thalidomide, and known as IMiDs have shown some promise in the treatment of patients with multiple myeloma and AL amyloidosis (Knight, 2005). Lenalidomide and pomalidomide are two drugs in this class that have been used as second-line agents in the treatment of AL amyloidosis. A recent phase 2 trial of pomalidomide and dexamethasone in patients who had previously been treated with melphalan, bortezomib, or other agents revealed a hematologic response rate of 68% and a survival benefit (Palladini et al., 2017). High-dose melphalan chemotherapy followed by autologous stem cell transplantation (HDM/SCT) represents a major therapeutic advance in the field, especially for patients without significant cardiac involvement as assessed by serum cardiac biomarkers (Comenzo et al., 1996). The hematologic response rate (CR or partial response) to HDM/SCT exceeds 70%, with a median survival of 7.6 years and more than half of all responders enjoying a prolonged survival of greater than 10 years (D'Souza et al., 2015; Sanchorawala et al., 2015). It is important to note that HDM/SCT is selected as the treatment of choice in patients deemed to be at acceptable risk for the procedure; thus the highest risk and sickest patients are not treated with this regimen. Transplant-related mortality remains high in patients with significant cardiac involvement (Mayo biomarker stage 3) and these patients are generally excluded from consideration of ASCT, except at specialized referral centers. In fact, the only randomized clinical trial of HDM/SCT vs. melphalan and dexamethasone did not identify a survival benefit, largely related to excessive peri-transplant mortality in the SCT arm (Jaccard et al., 2007).

Patients with advanced cardiac involvement also do not tolerate full doses of chemotherapy, and often need to undergo reductions in CyBorD and melphalan doses. While the majority of patients with AL cardiac amyloidosis do not achieve complete hematologic response with chemotherapy, those who do can enjoy prolonged survival (Palladini et al., 2015).

New antiplasma cell therapies are currently in clinical trials. These include the second-generation oral proteasome inhibitor ixazomib, and multiple myeloma drugs such as the proteasome inhibitor carfilzomib, the antiplasma cell antibody daratumumab, and the antibody elotuzumab, which stimulates the immune system by activating a lymphocyte signaling pathway (Palladini and Merlini, 2016). Another novel approach currently in development involves RNA interference targeting lambda light chain production and hence reducing the burden of amyloidogenic protein (Zhou et al., 2014). In addition to targeting clonal plasma cell populations and reducing the production of light chains, there is also interest in targeting amyloid deposits and reducing organ damage. The antibiotic doxycycline can inhibit amyloidogenesis and has been shown to improve survival when added to standard chemotherapy in a small trial (Wechalekar et al., 2015). Finally, immune therapy consisting of manufactured antibodies that are specific for misfolded light chain have shown promise. The monoclonal antibody NEOD001 (Prothena) targets amyloid fibrils and a phase I/II trial in AL amyloidosis patients resulted in a cardiac response rate of 50% (Gertz et al., 2016). A phase 3 trial of NEOD001 is currently underway. Other amyloid-specific antibodies are presently in development.

Orthotopic heart transplantations (OHT) have historically been controversial in patients with cardiac amyloidosis due to the risk of amyloidosis recurrence in the transplanted organ and the risk of progression of the noncardiac underlying disease. Without treatment of the underlying plasma cell dyscrasia, patient's who undergo OHT in cardiac amyloidosis have a one-year survival of around 60% and a five-year survival of 30% (Dubrey et al., 2001). This initial experience with OHT occurred in the context of aggressive treatment of the plasma cell dyscrasia followed by a heart transplant. Unfortunately, this strategy is not applicable to most patients with advanced heart failure who would be eligible for OHT because they would be too high risk for HDM/SCT.

Nevertheless, in carefully selected patients without other extra cardiac manifestations of amyloidosis, there was a transplant-related mortality of 18% and median survival was 76 months after heart transplant and 57 months after stem cell transplant (Lacy et al., 2008).

A more contemporary strategy is to first perform OHT followed by HDM/SCT. As reported by Semigran and colleagues, this approach yields a 5-year survival rate of 60% that is similar to nonamyloid cardiomyopathy treated with OHT (Dey et al., 2010; Gray Gilstrap et al., 2014). Another study from the National Amyloidosis Centre in the United Kingdom reported a median survival of 9.7 years when OHT was followed by HDM/SCT (Sattianayagam et al., 2010). These efforts and others have resulted in the inclusion of cardiac amyloidosis as an acceptable indication of OHT in the 2016 Listing Criteria of the International Society for Heart and Lung Transplantation (Mehra et al., 2016).

Conclusion

Amyloidosis is a disease of protein misfolding that leads to end organ deposition and dysfunction. In AL amyloidosis, abnormal plasma cell production of amyloidogenic light chains commonly deposits in organs such as the heart and kidneys. Heart involvement leads to significant morbidity and, without effective treatment, leads to reduced survival. Thus, early determination of cardiac involvement is critical. Echocardiography forms the cornerstone of noninvasive diagnosis of cardiac amyloidosis. Cardiac MRI is more sensitive, particularly for diagnosis of early or subclinical cardiac amyloidosis and is often used when echocardiography is equivocal. Endomyocardial biopsy remains the gold standard for diagnosis of cardiac amyloidosis, and can also help identify the origin of the amyloid protein by immunohistochemistry or laser microdissection and mass spectrometry. While early experience with this disease suggested a median survival of only 1 year in the context of heart failure symptoms, advances in plasma cell therapy have dramatically extended survival to beyond 10 years.

Aggressive therapy in advanced disease patients with tandem heart and autologous stem cell transplantations can significantly prolong survival, especially in those patients who achieve hematologic remission. Newer treatment approaches are targeting amyloid fibrils with the goal of disrupting amyloid deposits, and prompting reabsorption, in end organs. In contrast to just 20 years ago, AL amyloidosis is now a treatable disease, and in particular if recognized early in its course, long-term survival can be achieved.

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Further Reading

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Alcohol

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Introduction

Over the last two decades, multiple observational and experimental studies have suggested an association between light-to-moderate alcohol consumption and a reduced risk of cardiovascular disease (CVD), mainly driven by a decrease in the risk of coronary heart disease (CHD) and ischemic stroke. While it is difficult to design randomized controlled trials (RCTs) to evaluate the causal relationship between alcohol consumption and the risk of CVD, accumulating evidence strongly suggests a causal relationship between light-to-moderate alcohol consumption and a reduced risk of CVD. However, alcohol intake is also known to have complex physiological, psychological, and social effects, and health care providers should remain cautious about recommending alcohol consumption to their patients for reducing the risk of CVD.

In this article, we shall discuss recent evidence on the association between alcohol consumption and the risks of multiple types of CVD and intermediate CVD end points, along with the underlying mechanisms. We shall also consider the associations between alcohol consumption and the risk of CVD in subpopulations classified according to the sex, ethnicity, comorbidities, etc. and whether the type of alcoholic beverage may have a bearing on the association between alcohol consumption and the risk of CVD. Finally, we shall provide some guidance for patient counseling and discuss the public health implications and potential adverse effects of recommending alcohol consumption for CVD prevention.

Definition of Alcohol Consumption

Alcoholic beverages contain different amounts of alcohol, and the amount of alcohol in the same types of alcoholic beverages may also vary among countries. Thus, "light-to-moderate alcohol consumption" may be defined differently in different research projects. Based on the American Heart Association (AHA) guideline and the European Society of Cardiology (ESC) guideline, moderate alcohol consumption is defined as intake of 20–30 g of pure alcohol/day for men and intake of 10–20 g of pure alcohol/day for women, and "heavy drinking" is defined as consumption of 15 drinks (each drink containing roughly 14 g of pure alcohol) or more per week for men and consumption of eight drinks or more per week for women. Furthermore, "binge drinking" is defined as consumption of five or more drinks on a single occasion for men and consumption of four or more drinks on a single occasion for women, according to the statement of the National Institute on Alcohol Abuse and Alcoholism (**Table 1**).

The pathology and etiology differ among various types of CVD; therefore, the association between alcohol consumption and the risk of CVD also varies according to the type of CVD under discussion. We shall now discuss the association between alcohol consumption and the risk of CVD for various types of CVD.

Table 1 Typical standard serving sizes of various alcoholic beverages

Beverage	*Ounces (oz)*	*Milliliters (mL)*	*Approximate grams of pure alcohol per serving (g)*
Wine	4	118.3	10.8
Beer	12	354.9	13.2
Liquor/spirits	1.5	44.4	15.1

Alcohol and CVD

Coronary Heart Disease

The favorable association between light-to-moderate alcohol consumption and CVD risk is mainly driven by the reduced risk of atherosclerotic CVDs, namely, CHD and ischemic stroke, associated with such consumption.

Multiple observational studies from diverse regions and countries have reported a J- or U-shaped association between alcohol consumption and the risk of CHD and CHD mortality. Despite the great heterogeneities in the population characteristics, size, study duration, and adjustments for confounding factors, a large number of observational studies have consistently reported an association between moderate alcohol consumption and a roughly 20–45% reduction in the risk of CHD as compared with that in nondrinkers (Kono et al., 1991; Mancia et al., 2013; Marques-Vidal et al., 1996; Mukamal et al., 2005b; Steyn et al., 2005). A meta-analysis of 29 observational studies involving 549,504 subjects reported a pooled adjusted relative risk (RR) (95% confidence interval (CI)) of CHD in alcohol drinkers versus nondrinkers of 0.71 (0.66–0.77); it also revealed that the greatest degree of risk reduction for CHD was associated with consumption of 15–29.9 g/day (Ronksley et al., 2011). There is always the argument about potential confounding by healthy lifestyle factors in light-to-moderate drinkers and the potential bias arising from inclusion of sick quitters to the nondrinking referent group. However, the findings of studies in which lifelong nondrinkers and ex-drinkers were evaluated separately argue against this "healthy-drinker hypothesis" (Ronksley et al., 2011) and suggest the robustness of the favorable association between light-to-moderate alcohol consumption and the risk of CHD.

The potential underlying mechanism for the J-shaped association between alcohol consumption and the risk of CHD still remains to be clearly elucidated. It is speculated that elevation of the serum levels of high-density lipoprotein cholesterol (HDL-C) and improvement of the glucose metabolism by moderate alcohol consumption, as discussed later, are the main factors responsible for the J-shaped association between alcohol consumption and the risk of CHD. In addition, the effects of alcohol consumption on the coagulation/fibrinolysis systems (Brien et al., 2011; Salem and Laposata, 2005; Collins et al., 2009; Wannamethee et al., 2003; Zhang et al., 2000; Ridker et al., 1994), oxidative stress status (Addolorato et al., 2008; Blackhurst and Marais, 2006; Estruch et al., 2011), inflammation (Albert et al., 2003; Avellone et al., 2006; Wang et al., 2008), and endothelial function (Suzuki et al., 2009; Teragawa et al., 2002) may also play important roles in the potential favorable effect of moderate alcohol consumption on the risk of CHD. However, previous studies, including experimental studies and clinical studies, have not yielded consistent results, and the potential effects of moderate alcohol consumption on these parameters still remain under debate.

Stroke

Stroke is classified as ischemic stroke (embolic stroke, thrombotic stroke, and lacunar stroke) or hemorrhagic stroke (intracerebral hemorrhage and subarachnoid hemorrhage). The pathophysiology differs among different types of strokes; therefore, the relationships between alcohol consumption and the risk of each type of stroke may also be different.

Ischemic stroke shares important risk factors with arteriosclerotic CHD; therefore, the favorable effect of alcohol consumption on the risk of ischemic stroke is similar to that on the risk of CHD. A meta-analysis of 35 observational studies that evaluated the relationships between alcohol intake and the risks of ischemic stroke and hemorrhagic stroke separately demonstrated a J-shaped association between alcohol consumption and the risk of ischemic stroke. The moderate drinkers (alcohol intake, 12–24 g/day) showed the greatest degree of risk reduction for ischemic stroke as compared with the risk in nondrinkers, with an RR (95% CI) of 0.72 (0.57–0.91) (Reynolds et al., 2003). In contrast, heavy drinkers (alcohol intake, >60 g/day) showed an increased risk of ischemic stroke, with an RR (95% CI) of 1.69 (1.34–2.15) as compared with nondrinkers (Reynolds et al., 2003). However, the associations between alcohol consumption and each subtype of ischemic stroke (embolic stroke, thrombotic stroke, and lacunar stroke) still remain unclear. Some observational studies that have attempted to evaluate the relationships between alcohol consumption and each subtype of ischemic stroke have failed to reveal any differential associations between alcohol consumption and the risks of each subtype of ischemic stroke (Mukamal et al., 2005a; Ohira et al., 2006). One of the reasons for this may be the lack of sufficient statistical power to detect the associations because of the relatively small numbers of patients with each subtype of ischemic stroke. In this context, it must be noted that the different pathophysiology of each subtype of ischemic stroke may also differentially affect the risk reduction associated with alcohol consumption. For example, among the major risk factors for embolic ischemic stroke is atrial fibrillation (AF) and for lacunar ischemic stroke is hypertension (HTN). Both AF and HTN are associated with heavy alcohol drinking, as discussed later. Therefore, we need further study to establish strong evidence for the associations between alcohol consumption and the risk for each of the subtypes of ischemic stroke.

In contrast to ischemic stroke, no J-shaped association has been detected between alcohol consumption and the risk of hemorrhagic stroke; in fact, heavy alcohol consumption has been shown to be consistently associated with an increase in the risk of hemorrhagic stroke, regardless of the subtype (cerebral hemorrhage or subarachnoid hemorrhage). A previous meta-analysis conducted by Reynolds found an increased risk of hemorrhagic stroke, regardless of the subtype, associated with heavy drinking (>60 g/day), with an RR (95% CI) of 2.18 (1.48–3.20), and also revealed a positive linear association between alcohol consumption and the risk of hemorrhage stroke (Reynolds et al., 2003). In regard to the subtypes of hemorrhagic stroke, another meta-analysis of 25 observational studies reported an increased risk of intracranial hemorrhage associated with heavy alcohol consumption (mean cutoff 56 g/day), with an odds ratio (OR) (95% CI) of 3.36 (2.24–5.12) (Ariesen et al., 2003). A meta-analysis by Feigin reported an increased risk of subarachnoid hemorrhage associated with heavy drinking (>150 g/week), with an OR (95% CI) of 2.1 (1.5–2.8) (Feigin et al., 2005). The fact that HTN, the risk of which is also increased in heavy drinkers, is among the strongest risk factors for hemorrhagic stroke may be one of the underlying reasons for the positive association between alcohol consumption and the risk of hemorrhagic stroke. As for other possible mechanisms, increased platelet activity and prothrombotic activity induced by alcohol consumption may also affect this association (Deykin et al., 1982; Mukamal et al., 2001, 2005b; Hendriks et al., 1994; Rimm et al., 1999).

Heart Failure

Heart failure (HF) has a complex pathophysiology and varied etiologies, including CHD, HTN, valvular disease, arrhythmias, and cardiomyopathy (including alcoholic cardiomyopathy (ACM)). Nonetheless, a number of studies have reported a U-shaped association between alcohol consumption and the risk of HF. The Framingham Heart Study reported a lower risk of HF associated with moderate alcohol consumption (8–14 drinks/week for men and 3–7 drinks/week for women), with a hazard ratio (95% CI) of 0.41 (0.21–0.81) for men and 0.49 (0.25–0.96) for women (Walsh et al., 2002). Because one of the strongest predisposing factors for HF is CHD, this association could be driven by the association between moderate alcohol consumption and a lower risk of CHD. The Physicians' Health Study reported the absence of an association between moderate alcohol consumption and the risk of HF in patients without antecedent CHD (Djousse and Gaziano, 2007). On the other hand, the Cardiovascular Health Study reported a lower risk of HF associated with moderate alcohol consumption even after accounting for myocardial infarction (Bryson et al., 2006). Therefore, it still remains to be clarified whether the lower risk of HF associated with moderate alcohol consumption is due to suppression of the risk of CHD by the alcohol intake. However, a reduced risk of HF associated with moderate alcohol consumption has been reported quite consistently, and it is possible that some other factors, including systemic arterial dilation (Greenberg et al., 1982) and reduction of the pulmonary arterial wedge pressure (Vlachopoulos et al., 2003), are also responsible for the favorable association between moderate alcohol consumption and the risk of HF.

Alcoholic Cardiomyopathy

Chronic and excessive alcohol consumption leads to ACM. ACM is classified among the acquired forms of dilated cardiomyopathy (DCM) and is diagnosed through the exclusion of other causes in patients with a long history of heavy alcohol consumption. The reported prevalence of ACM among DCM patients is about 23–47% (Guzzo-Merello et al., 2014). Even though the precise amount of alcohol intake and duration of exposure to alcohol that can cause ACM have not yet been established, intake of over 80 g/day of alcohol for at least 5 years has been used as a prerequisite for establishing the diagnosis of ACM in many studies. However, it should be noted that this amount does not take into account the sex or body mass index, even though the heart in women may be affected by consumption of smaller amounts of alcohol due to the lower BMI of women as compared with men. The mechanism underlying the development of ACM has not yet been clearly elucidated; however, changes in the level of oxidative stress, alterations of the mitochondria, protein synthesis, gene expression and calcium transport, apoptosis, and changes in the myofilament structure and function associated with alcohol and acetaldehyde accumulation are some of the speculated putative mechanisms (Guzzo-Merello et al., 2014).

Atrial Fibrillation

A phenomenon called "holiday heart" has been reported to be associated with binge drinking (Greenspon and Schaal, 1983; Rich et al., 1985). Also, chronic heavy alcohol consumption has been consistently reported to be associated with an increased risk of development of AF (Conen et al., 2008; Mukamal et al., 2005c). On the other hand, inconsistent results have been reported in regard to the association between moderate alcohol consumption and the risk of AF (Samokhvalov et al., 2010). Nonetheless, several meta-analyses have reported a positive linear association between alcohol consumption and the risk of AF (Kodama et al., 2011; Larsson et al., 2014; Samokhvalov et al., 2010). A recent meta-analysis of prospective studies involving 79,019 subjects reported a linear association between alcohol consumption and the risk of AF, with an RR (95% CI) of 1.12 (1.02–1.23) associated with consumption of 7–14 drinks/week, of 1.18 (1.03–1.35) associated with consumption of 15–21 drinks/week, and of 1.43 (1.25–1.65) associated with consumption of >21 drinks/week (p for trend <0.0001) as compared with nondrinkers; in addition, an increment of 1 drink/day was associated with an 8% increase in the risk of AF (Larsson et al., 2014). Therefore, it seems that not only heavy drinking but also moderate drinking is associated with an increased risk of AF. The mechanism of alcohol-related AF is complex, and several factors appear to influence this association: decreased vagal tone,

Table 2 Association of alcohol intake and risk of developing cardiovascular disease (CVD)[a]

Coronary heart disease (CHD)	• J-shaped association • Moderate drinking is associated with 20–45% reduction of CHD
Ischemic stroke	• J-shaped association • Moderate drinking is associated with 30% reduction of ischemic stroke • Increased risk by heavy drinking
Hemorrhagic stroke	• Increased risk with heavy drinking
Subarachnoid hemorrhage (SAH)	• Increased risk with heavy drinking
Congestive heart failure (CHF)	• J-shaped association • Light-to-moderate alcohol intake is associated with 30–50% reduction of CHF
Alcoholic cardiomyopathy (ACM)	• Increased risk with over 80 g/day drinking during at least 5 years
Atrial fibrillation (AF)	• Increased risk with heavy drinking • Increased risk with binge drinking

[a]Compared with nondrinkers.

increased adrenergic drive, cardiac conduction interference, and refractory period shortening are some of the speculated mechanisms (Tonelo et al., 2013; **Table 2**).

Alcohol and Intermediate CVD End Points

HTN and Blood Pressure

Even though several clinical studies have reported a decrease of the blood pressure (BP) by acute alcohol consumption (Abe et al., 1994; de Lorimier, 2000), chronic heavy alcohol consumption is causally associated with elevation of the BP and HTN. A number of observational studies, including meta-analyses, have consistently reported a higher risk of HTN in heavy alcohol drinkers (Taylor et al., 2009). A meta-analysis of 12 observational studies conducted by Taylor reported a linear dose–response relationship between alcohol consumption and the risk of HTN in men, with an RR of 1.09 for consumption of 10 g/day, of 1.57 for consumption of 50 g/day, and of 2.49 for consumption of 100 g/day. Also, several RCTs have suggested an association between alcohol consumption and elevation of the BP (Taylor et al., 2009). For example, Zilkens reported that alcohol consumption for 4 weeks raised the ambulatory systolic BP, regardless of the type of alcoholic beverage consumed. They reported an increase of the systolic BP by 1.7 and 2.2 mmHg in association with heavy consumption of beer (41 g of alcohol) and red wine (39 g of alcohol), respectively (Zilkens et al., 2005). Moreover, a meta-analysis of 15 RCTs reported a dose-dependent decrease of the BP associated with a reduction of alcohol consumption in heavy drinkers, with a significant reduction of the mean (95% CI) systolic and diastolic BP by −3.31 (−2.52 to −4.10) and −2.04 mmHg (−1.49 to −2.58 mmHg), respectively (Xin et al., 2001). It is estimated that in about 5–24% of cases, HTN is associated with alcohol consumption (Friedman et al., 1983; Milon et al., 1982). However, in regard to the association between light-to-moderate alcohol consumption and the risk of BP elevation/HTN, a previously reported meta-analysis by Taylor reported a lower risk of HTN among light alcohol drinkers (<5 g/day), after which a linear dose–response relationship was found, with RRs of 1.81 and 2.81 for alcohol consumption of 50 and 100 g/day, respectively, among women (Taylor et al., 2009). Furthermore, an observational study conducted by Okubo reported a J-shaped association between alcohol consumption and BP changes, with a threshold effect at 18 mL (=14.2 g)/day (Okubo et al., 2001). Therefore, it seems at least moderate alcohol consumption may not have any effect of inducing HTN or increasing the BP.

In regard to the mechanism of elevation of the BP by heavy alcohol consumption, no solid evidence has been established. However, increase in the sympathetic nervous activity, stimulation of the renin–angiotensin–aldosterone system, and increase of the intracellular Ca^{2+} concentration in the vascular smooth muscle, increase in oxidative stress, and endothelial dysfunction are some of the speculated mechanisms underlying the elevation of the BP by heavy alcohol consumption (Marchi et al., 2014).

Dyslipidemia and Lipids

Alcohol consumption is known to increase the plasma level of HDL-C, which is established as a major protective factor against CHD. Many observational studies and RCTs, including meta-analyses, have reported a dose–response association between alcohol consumption, including heavy alcohol consumption, and the plasma HDL-C level. A meta-analysis of 47 RCTs showed a mean difference from the baseline serum HDL-C of 0.072 mmol/L (95% CI; 0.024–0.119) for alcohol consumption of 12.5–29.9 g/day and of 0.103 (0.042–0.540) and 0.141 mmol/L (0.042–0.240) for alcohol consumption of 30–60 and >60 g/day, respectively (p for trend = 0.013) (Brien et al., 2011). It is regarded that alcohol may increase the plasma HDL-C level by modulating the HDL composition, HDL function, and/or HDL-related metabolic factors (Brinton, 2012); however, the mechanism underlying the increase of the serum HDL-C by alcohol has not yet been clearly elucidated. Also, the reason for the lack of any beneficial effect of the increase of the plasma HDL-C level by heavy alcohol consumption on the CVD risk remains unclear. However, it is speculated that the adverse effects by heavy alcohol consumption, including elevation of the BP, increase of the oxidative stress, endothelial dysfunction, and inflammation, may offset any beneficial effect of elevated HDL-C on the risk of CVD.

On the other hand, low-density lipoprotein cholesterol (LDL-C), a risk factor for CHD, may not be remarkably affected by alcohol consumption. A meta-analysis of short-term RCTs has reported the absence of any significant association between alcohol consumption and the plasma LDL-C levels (Brien et al., 2011). However, this meta-analysis showed high heterogeneity, and given the inconsistent results from some observational studies, the association of alcohol consumption with the serum LDL-C appears to vary according to the population characteristics. Observational studies carried out in a Japanese population and Danish population reported an inverse association between alcohol consumption and the plasma LDL-C (non-HDL-C) level (Tolstrup et al., 2009; Wakabayashi and Groschner, 2009). In contrast, an observational study carried out in an elderly Italian population reported a positive association between alcohol consumption and the serum LDL-C. Further investigations are needed to clarify the association between alcohol consumption and the serum LDL-C (Perissinotto et al., 2010).

Multiple observational studies have reported an association between heavy alcohol drinking and increased plasma triglycerides (TG) (Park and Kim, 2012; Ruidavets et al., 2010; Tolstrup et al., 2009). However, in addition to the reported linear relationship between alcohol consumption and TG, some studies have shown a J-shaped association between alcohol consumption and the serum TG (Ruidavets et al., 2002; Whitfield et al., 2013). RCTs of carbohydrate-free alcohol reported a reduction of the serum TG by moderate alcohol consumption in women (Davies et al., 2002). However, the inconsistent results warrant further exploration of the relationship between alcohol consumption and the serum TG, along with mechanistic explanations.

Type 2 Diabetes Mellitus and Glucose Metabolism

Moderate alcohol consumption appears to have a favorable effect on glucose metabolism. Multiple studies have reported beneficial effects of moderate alcohol consumption on the insulin sensitivity, fasting blood glucose, and hemoglobin $A1_C$ levels (Davies et al., 2002; Harding et al., 2002; Maraldi et al., 2006; Sierksma et al., 2004). A number of observational studies, including two meta-analyses, have shown a U-shaped association between alcohol consumption and the risk of type 2 diabetes mellitus (T2D) (Baliunas et al., 2009; Rimm et al., 1999). A recent meta-analysis of 20 cohort studies also reported a U-shaped relationship between alcohol consumption and the risk of T2D. As compared with the case in lifetime abstainers, moderate alcohol consumption (22 g/day in men and 24 g/day in women) was associated with a reduced risk of T2D among both men and women (RR (95% CI) in men, 0.87 (0.76–1.00); RR in women, 0.60 (0.52–0.69)). Some trials have also reported improvement of the insulin sensitivity associated with consumption of red wine in a healthy population (Beulens et al., 2006; Sierksma et al., 2004). The report from the Nurses' Health Study of attenuation of the positive association between glycemic load and the incidence of T2D by higher alcohol consumption ($\geq$15 g/day) (Mekary et al., 2011) lends support to the evidence that moderate alcohol consumption has a favorable effect on the risk of T2D. The biological mechanism underlying the U-shaped relationship between alcohol consumption and T2D is still a matter of debate. Improvement of the insulin sensitivity by elevation of the adiponectin levels induced by moderate alcohol consumption is speculated as one of the potential mechanisms for this association (Beulens et al., 2006, 2008; Imhof et al., 2009).

Alcohol and the Risk of CVD/Intermediate CVD End Points in Subpopulations Classified According to Variables Such as the Sex, Ethnicity, and Genetic Factors

Available data from diverse populations (different sex and ethnicities) have consistently shown a lower risk of CHD in light-to-moderate alcohol drinkers. Yet, it should be noted that the magnitude and threshold of the protective effect of moderate alcohol consumption against CHD may differ according to sex and ethnicity. For example, the definition of moderate alcohol consumption differs between men and women (20–30 g/day for men and 10–20 g/day for women), due to the generally smaller body size, lower levels of alcohol-metabolizing enzymes, and higher proportion of body fat in women as compared with men. In fact, some studies have reported this difference; they have demonstrated that a higher amount of alcohol consumption was associated with a greater degree of risk reduction for CHD or intermediate CHD end points in men as compared with women (Di Castelnuovo et al., 2002; Taylor et al., 2009). In regard to the differences in the effect of alcohol consumption on the risk of CVD related to the ethnicity, the majority of observational studies that have reported a favorable association between moderate alcohol consumption and CHD risk are reported from Western countries. As one of the exceptions, the study on a Kaiser Permanente cohort reported an inverse association between alcohol consumption and the risk of HF in Caucasians, Asians, and African Americans (Klatsky et al., 2005). Furthermore, the interaction between genetic factors and the effect of alcohol on the risk of CVD should be also considered. Alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) are key enzymes metabolizing alcohol, and there are significant genetic differences in the rate of alcohol metabolism between Asian and Western populations. Some studies have evaluated the interaction between alcohol consumption and the ADH/ALDH expression status, as genetic factors, on the risk of CVD. For example, Hines reported that in subjects who were homozygous for the slow-oxidizing ADH3 allele, moderate alcohol consumption was associated with an increase in the serum levels of HDL-C (Hines et al., 2005). Yet, the results of studies evaluating the interactions between genetic factors and alcohol consumption on the risk of CVD are not consistent (Heidrich et al., 2007; Jensen et al., 2009; Whitfield et al., 2003). There might be many genes affecting the association between alcohol consumption and the risk of CHD, and each of these genes may play a small role, but a potentially larger role in combination with multiple other genes. Further evidence is needed to shed further light on the impact of genetic factors on the association between alcohol consumption and the risk of CVD.

Alcohol and CVD Risk in Subjects at a High Risk for CVD

The potential cardioprotective effect of moderate alcohol consumption is observed not only in the healthy population but also in subjects at high risk for CHD, such as patients with T2D, HTN, etc. Patients with a history of CHD also appear to benefit from moderate alcohol consumption for secondary prevention of CHD and protection against CHD mortality. However, the potential beneficial effect of alcohol consumption on the risk of CHD is limited to moderate alcohol consumption, and excessive drinking has adverse effects, for example, in patients with T2D. Perneger reported that consumption of more than 2 drinks/day was associated with a fourfold higher risk of end-stage renal disease among diabetic patients (Perneger et al., 1999), and Kohner reported a higher incidence of diabetic retinopathy among alcohol drinkers as compared with those who abstained from alcohol (Kohner et al., 1998).

Also, the potential interaction of alcohol with medications should be considered. Many patients with a history of CVD and also those at a high risk for CVD take multiple medications. For example, the effect of the anticoagulant warfarin is enhanced by alcohol consumption, which may increase the risk of major bleeding (Fiumara and Goldhaber, 2009). Also, alcohol consumption can enhance the effects of vasodilators such as hydralazine and nitroglycerin to cause hypotension.

Difference in Potential Cardioprotective Effect Among Different Types of Alcoholic Beverages

It still remains controversial as to whether there are differences in the cardioprotective effect among different types of alcoholic beverages. Since the majority of observational studies have demonstrated a consistently positive association between moderate alcohol consumption and a reduced risk of CVD regardless of the type of alcoholic beverage, it may be reasonable to assume a cardioprotective effect of pure alcohol per se. Observational studies evaluating the effects of moderate consumption of red wine in France (Renaud et al., 1998), beer in Germany and Hawaii (Keil et al., 1997; Kagan et al., 1981), and distilled spirits in Japan (Kitamura et al., 1998) have all shown an ~45% reduction in the risk of CHD as compared with that in nondrinkers. A large-scale observational study, the Copenhagen City Heart Study, showed a J-shaped relationship between alcohol consumption and all-cause mortality among both beer and sprit drinkers (Gronbaek et al., 2000). A meta-analysis of 11 observational studies analyzing the effects of drinking wine, beer, and spirits reported a similar J-shaped relationship between wine and beer consumption and the incidence of CVD (Costanzo et al., 2011).

On the other hand, each type of alcoholic beverage contains different nonalcohol components that could exert additive cardioprotective effect over that of alcohol itself. For example, red wine is enriched in polyphenols such as anthocyanins, flavan-3-ols, and resveratrol, which may exert beneficial effects on the lipid profile and platelet aggregation. Beer also contains a variety of nonalcohol components, such as amino acids, minerals, and some kinds of polyphenols such as phenolic acid, prenylated chalcones, flavonoids, and catechins, different from those in red wine. In contrast, spirits do not contain any significant amounts of polyphenols as compared with red wine and beer.

The aforementioned nonalcohol components, especially polyphenols, can cause discrepancies in the effects of different types of alcoholic beverages on the risk of CVD. For example, a randomized crossover trial reported that moderate consumption of red wine had greater antioxidant effect as compared with that of gin (Estruch et al., 2011). Also, one RCT showed improved endothelial function associated with consumption of red wine, but not with that of beer or vodka (Huang et al., 2010). Therefore, alcoholic beverages enriched in polyphenols, especially red wine, may have enhanced cardioprotective effects as compared with alcoholic beverages not containing or containing lower amounts of polyphenols.

Guidance for Patient Counseling and the Public Health Implications of Alcohol Consumption

While there are hardly any RCTs demonstrating the causal relationship between moderate alcohol consumption and a reduced risk of CVD, accumulating evidence from many observational and experimental studies strongly suggests such a causal relationship.

However, from a public policy standpoint, encouragement of patients for moderate alcohol consumption should be weighed carefully by physicians, as alcohol consumption also has numerous physiological and psychological adverse effects. Not only can indiscriminate alcohol consumption increase the risk of some CVDs such as cerebral hemorrhage, ACM, AF, and HTN, it can also increase the risk of noncardiac diseases such as liver cirrhosis and of some types of cancer (breast, colorectal, and hepatocellular cancer) (Giovannucci et al., 1995; WCRFAIFC Research, 2010). In fact, in the United States, nearly 88,000 people die annually from alcohol-related causes, making alcohol the fourth leading preventable cause of death (Stahre et al., 2014). In a survey conducted in 2014, 16.3 million adults aged 18 years or older (6.8% of the population in this age group) were detected to have an alcohol use disorder (SAMHSA, 2014b). Therefore, recommendation to start moderate alcohol consumption for reducing the risk of CHD may not be judicious. However, given the fact that 87.6% of people aged 18 years or older have had alcohol at some point in their lifetime and that 56.9% of the entire population reported having consumed alcohol during the previous month in the United States (SAMHSA, 2014a), health care providers may advise people who are seeking to improve their cardiovascular health that light-to-moderate alcohol consumption may reduce the risk of CHD. As a matter of fact, major organizations such as the AHA and the ESC have refrained from promoting blanket recommendation of alcohol consumption for the prevention of CHD but recommend limitation of alcohol consumption to 1 or 2 drinks/day (moderate drinking) to individuals who choose to consume alcohol (Krauss

et al., 2000; Goldberg et al., 2001; Mosca et al., 2011; Perk et al., 2012). Under this circumstance, it must be borne in mind that there are also certain populations that should not/are not allowed to drink any alcohol, for example, children and adolescents, pregnant women, patients with a history of alcohol abuse, patients with severe liver disease and/or certain other health conditions, and persons with specific religious beliefs.

Needless to say, health care providers should give advice on alcohol consumption after carefully taking into account the characteristics of individual subjects (sex, age, lifestyle, risk of CVD and intermediate CVD end points, potential for abuse/addiction, the presence/absence of liver disease, etc.); in other words, blanket recommendation to all cannot be provided, and the recommendation should be tailored to the needs of individual subjects.

Conclusion

Accumulating evidence strongly suggests that light-to-moderate alcohol consumption is associated with a reduced risk of CVD, especially CHD. However, given the potential adverse physiological and psychological effects of alcohol consumption, health care providers should remain cautious while recommending light-to-moderate alcohol consumption to their patients for reducing the risk of CVD, by carefully taking into account the characteristics of individual patients.

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SAMHSA (2014b) *2014 National Survey on Drug Use and Health (NSDUH).* Table 5.8A—Substance dependence or abuse in the past year among persons aged 18 or older, by demographic characteristics: Numbers in thousands, 2013 and 2014.
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Alcoholic Cardiomyopathy

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Introduction

Alcohol or ethanol is a toxin responsible for a significant proportion of the total disease burden in the modern world, especially in economically advanced societies. Chronic consumption of large quantities of alcohol leads to a dose-related increase in mortality, morbidity and a reduction in quality of life. Due to its' biochemical nature and the small molecular size, ethanol is easily absorbed in the proximal digestive tract and freely traverses into the intracellular environment where it affects organelles and various metabolic pathways. Evidence of the detrimental effect of alcohol on the cardiovascular system and especially the myocardium has been available for a long time, but new insight into what constitutes risk for developing alcoholic cardiac dysfunction has recently been achieved (Laurent and Edwards, 2014). Starting from clinical and epidemiological observations, the concept of alcoholic heart muscle disease or "alcoholic cardiomyopathy" (ACM) emerged and this condition is currently considered one of the most common causes of nonhereditary dilated cardiomyopathy (Japp et al., 2016).

Pathological Effects of Alcohol on the Myocardial Cells

Ingested ethanol (CH_3-CH_2-OH) is rapidly absorbed in the stomach and upper part of the small bowel and, once present in the systemic circulation, it diffuses freely into cells throughout the organism. The main site of metabolism is the liver where alcohol dehydrogenase and aldehyde dehydrogenase act sequentially to transform alcohol into acetaldehyde and then nontoxic acetate in a stepwise, rate-limited fashion (Edenberg, 2007). Both alcohol and acetaldehyde induce toxic effects on skeletal and myocardial muscle cells through a variety of mechanisms that lead to myopathy and cellular necrosis (Piano and Phillips, 2014). A variety of pathogenic pathways have recently been hypothesized but contradictory findings have rendered the identification of a key central mechanism responsible for ACM difficult (Rodriguez et al., 2015; Seiva et al., 2009; Matyas et al., 2016). Increased oxidative stress, impaired mitochondrial bioenergetics, accelerated protein catabolism, and altered calcium handling leading to apoptosis and myocardial cell death are the main putative mechanisms involved.

Oxidative stress is currently the most studied hypothesis of alcohol-induced cardiac dysfunction (Seiva et al., 2009). Experimental studies have shown that repeated exposure to ethanol results in excess free radical production, decreased glutathione levels, increased catalase activity, and signs of lipid peroxidation and protein damage (Matyas et al., 2016). Antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase are responsible for the protection of the cellular environment from oxidative stress by stimulating the breakdown of deleterious reactive oxygen species (ROS) (Espinosa-Diez et al., 2015). Chronic ethanol exposure requires a rise in enzymatic activity in order to counteract ROS and a deficiency in antioxidant function has been documented in both experimental models (Fahimi et al., 1979; Kino, 1981) and patients with ACM and sudden cardiac death (Vikhert et al., 1986). Proteins are major targets of oxidative stress (Davies, 2016) and myofilament dysfunction has been traced back to oxidative stress (Ribiere et al., 1992; Edes et al., 1986; Avner et al., 2010; Santos et al., 2011 Apr 1).

Recently, the importance of an alcohol-induced increase in the expression of cytochrome P450 2E1 (CYP2E1) has been underlined (Jing et al., 2012). CYP2E1 offers an alternative pathway for alcohol metabolism and elegant studies have demonstrated a reduction in oxidative stress by pharmacological inhibition of the cytochrome (Zhang et al., 2013). Use of agents with antioxidant properties or inhibition of reactive-oxygen generation resulted in alleviation or prevention of functional or ultrastructural myocardial damage in several studies (Khanna et al., 2007; Rao et al., 2015).

Chronic ethanol feeding was found to result in increased expression of angiotensin II and its' AT1 receptor leading to the assumption that angiotensin II plays a critical role in alcohol-induced cardiac nitrate damage, cell death, remodeling, and cardiomyopathy (Tan et al., 2012; Cheng et al., 2006). Genetic susceptibility to the development of left ventricular failure in alcohol consumers due to a polymorphism of the angiotensin-converting enzyme has also been proposed (Fernández-Solà et al., 2002).

Mitochondrial damage observed in cardiomyocytes after chronic alcohol exposure is a common finding in histopathological studies. In addition to generating reactive oxygen species mitochondria are also targets for free radicals and suffer from a reduced capacity for DNA repairing (Marzetti et al., 2013). Analysis of tissue fragments collected through cardiac biopsies has shown myocardial mitochondrial enlargement and disorganization as well as increased numbers and altered intracellular distribution (Ferrans et al., 1965; Hibbs et al., 1965). Fatty acid ethyl esthers, which are products of alcohol interaction with fatty acids, are found in increased concentrations in the circulation and cardiomyocytes of alcohol abusers. These molecules can attach to mitochondria and disrupt their function (Kaphalia et al., 2004). Perturbation of lipid metabolism and intracellular accumulation of triglycerides (Zhong et al., 2012) may also contribute to decreased myocardial contractility (Yoerger et al., 2006).

There are several other important molecular mechanisms linked to cardiac injury in chronic alcohol abuse such as increase in intracellular calcium (Samak et al., 2016 Dec 13), decrease of myofilament sensitivity to calcium, excitation-contraction coupling, decrease of contractile proteins, and disproportion between myofibrils and neurohormonal signaling (Guzzo-Merello et al., 2014). An investigation of differentially expressed microRNAs and their value in predicting ACM is also ongoing and has yielded promising results so far (Jing et al., 2015).

Many of these pathogenic pathways converge in apoptosis and, indeed, myocyte loss leading to ventricular dysfunction and dilatation seems to be the driving force of ACM (Fernandez-Sola et al., 2006). Overexpression of pro-apoptotic proteins has been demonstrated in a myocyte cell culture system exposed to alcohol (Chen et al., 2000) but this finding has not yet been confirmed by other studies (Jänkälä et al., 2001). Alcohol also affects cardiac repair mechanisms by inhibiting myocyte proliferation through the decrease in myocardial expression of insulin-like growth factor-1 (Borrisser-Pairó et al., 2013). However, the inherent difficulty of reproducing the effects of long-term alcohol abuse in animal models with only weeks of toxic exposure underlines the caution required in adapting these findings to clinical research and practice.

Apart from the direct toxic effect on the cardiomyocytes which leads to ACM, alcohol may also induce heart damage indirectly, by acting on different components of the cardiovascular system (**Fig. 1**).

Histopathological Changes

A direct relationship between unhealthy patterns of alcohol use and cardiac dysfunction was first inferred from clinical experience and necroptic studies performed in the 19th century (Münzinger, 1877). Since then several studies have confirmed the typical macroscopic and microscopic aspects of ACM by autopsy or endomyocardial biopsy. These histopathological findings have been

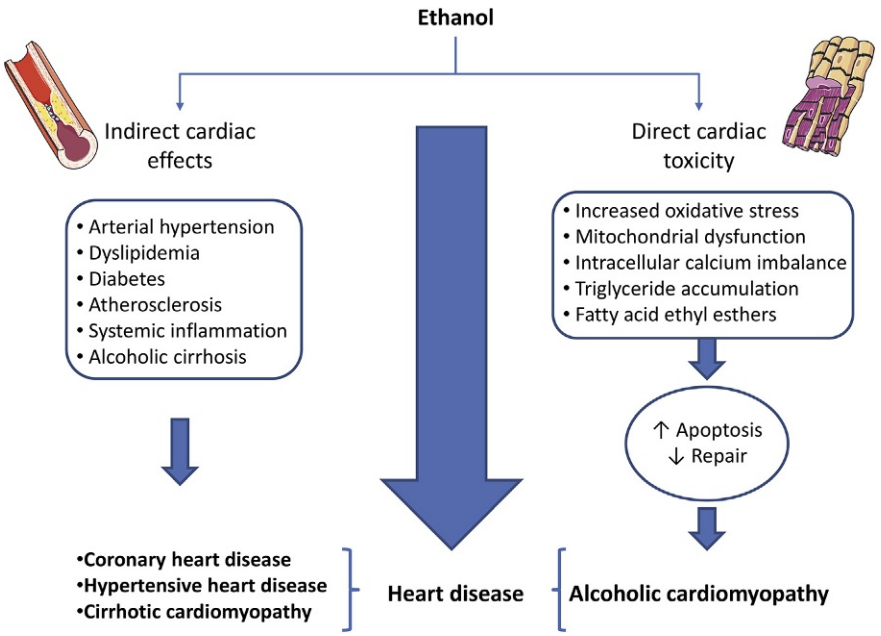

Fig. 1 The effects of chronic ethanol consumption on the pathogenesis of heart disease. Ethanol directly affects the structure and function of the cardiac muscle leading to alcoholic cardiomyopathy but also acts as trigger or risk factor for other conditions associated with heart disease.

thoroughly described and are concordant between reports but they are not pathognomonic (Maisch, 2016). In patients with ACM the heart chambers are generally dilated with increased overall mass and thinned ventricular walls. Interstitial patchy fibrosis and fatty infiltration are common (Tobin et al., 1967). Cardiomyocytes are atrophic or hypertrophic, with distorted myofibrils, enlarged nuclei, pronounced dilation of sarcoplasmic reticulum, lipid accumulation, and mitochondrial enlargement with crest alterations (Teragaki et al., 1993; Fernandez-Sola et al., 1994). This appearance is not specific to alcoholic cardiomyopathy and cannot differentiate it from idiopathic or chronic inflammatory cardiomyopathy.

Definition of Alcoholic Cardiomyopathy

Cardiomyopathy is currently defined as a disease of the heart muscle that is "sufficient to cause structural and functional myocardial abnormality in the absence of coronary artery disease, hypertension, valvular disease, and congenital heart disease" (Elliott et al., 2008; Arbustini et al., 2014). The direct implication of this definition is that correct diagnosis requires exclusion of other frequent conditions that lead to cardiac dysfunction. Chronic alcohol consumption is probably responsible for a significant proportion of cases of nonfamilial cardiomyopathy, but the lack of a specific diagnostic test hinders proper epidemiologic conclusions. This problem is compounded by three key issues: the fact that the cut-off for alcohol use predisposing to cardiomyopathy is still debated, the concurrent alcohol-induced conditions that affect heart structure and function, and the low reliability of self-reported alcohol use.

Excessive Alcohol Use

Assessment of alcohol consumption is based on the standard "drink" containing a fixed amount of pure alcohol. According to the U.S. Department of Health "moderate drinking is up to 1 drink per day for women and up to 2 drinks per day for men" while heavy drinking is defined by 5 or more drinks on the same occasion on each of 5 or more days in the past 30 days (health.gov).

The relationship between dose and risk of the developing cardiovascular disease in alcohol abusers is the focus of intense study. The current opinion is that alcohol is related to cardiovascular risk according to a J or U-shaped curve (Costanzo et al., 2010; Petrone et al., 2014). While alcohol abuse is clearly a risk factor for cardiovascular events, an increasing amount of evidence points to a reduced cardiovascular mortality following low-to-moderate alcohol intake. This has led to the assumption that complete abstinence may not be compulsory even in chronic alcohol abusers with organ damage (Guzzo-Merello et al., 2015b; Nicolás et al., 2002). However, a high degree of bias is frequent even in large studies reporting the benefits of moderate consumption (Fillmore et al., 2007) and recent data suggests that these benefits may be overestimated or absent altogether (Gonçalves et al., 2015; Knott et al., 2015; Fernández-Solà, 2015).

It is generally acknowledged that consumption of at least 80 g alcohol/day for a minimum of 5 years is required in order to consider alcohol as the cause of dilated cardiomyopathy (Urbano-Marquez et al., 1989; Lee and Regan, 2002). While laboratory measurements of mean corpuscular volume, liver enzymes or carbohydrate deficient transferrin (Arndt, 2001) can be helpful, the diagnosis of chronic alcohol abuse in clinical practice and large studies hinges on patient history, in-depth interviews and questionnaires. The notoriously unreliable patient self-reporting of alcohol use probably results in systematic underestimation of consumption which could affect our conception of the relevant cut-off for dangerous use. Variation in individual rates of alcohol metabolism, patterns of drinking and choice of drink, gender and body mass index also play significant roles in individual susceptibility to alcohol-induced organ damage. Furthermore, the existence or development of comorbidities can contribute to cardiac dysfunction in this vulnerable population. Chronic excessive alcohol use disturbs the metabolism of lipids and carbohydrates, alters vascular motor function and has a proinflammatory effect (Athyros et al., 2008). This translates into higher risk of atherosclerosis and hypertension with ensuing coronary artery disease and ischemic heart disease, further complicating the diagnostic work-up.

Diagnosis and Main Features

ACM is a form of dilated cardiomyopathy characterized by the presence of left ventricular dilatation and dysfunction, sometimes accompanied by similar right ventricular alterations. The exact prevalence of alcoholic cardiomyopathy is difficult to ascertain due to its' long initial asymptomatic period, the need to exclude other causes of heart disease, and the reliance on self-reported alcohol consumption. An alcoholic etiology is considered likely in 20%–40% of cases of idiopathic cardiomyopathy (Laonigro et al., 2009).

While it can remain asymptomatic for years (Cerqueira et al., 1991), chronic severe alcohol-induced injury to the myocardium can manifest through a classical clinical picture of low-output heart failure. New onset fatigue, exertional dyspnea and orthopnea, peripheral edema, nocturia, and increased or irregular heart rate are frequently encountered on first presentation in these decompensated patients (McKenna et al., 1998).

The earliest echocardiographic abnormalities in asymptomatic heavy drinkers are an increase in end-systolic and diastolic left ventricular (LV) volumes and diastolic dysfunction. Only a minor fraction of the patients develop thickening of the posterior wall and systolic dysfunction (Zambrano et al., 1974; Lazarević et al., 2000). A symptomatic diastolic dysfunction is a relatively frequent

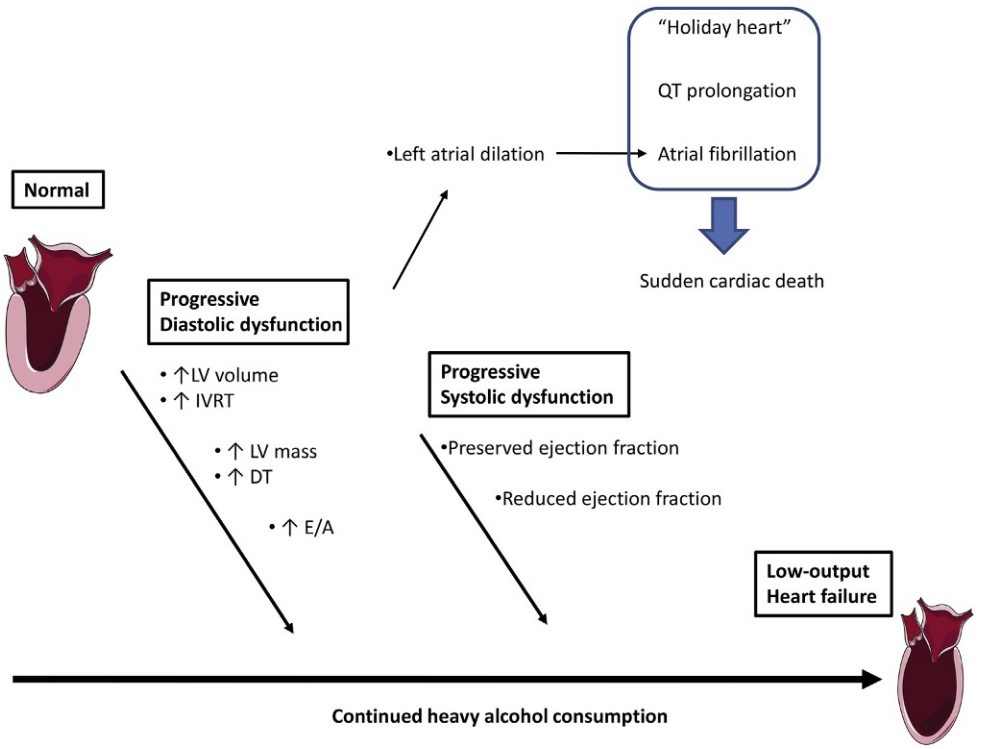

Fig. 2 The progression of cardiac dysfunction in chronic heavy alcohol consumption. Initial echocardiographic abnormalities such as altered diastolic function of the left ventricle can slowly progress toward overt heart failure. An increased risk of life-threatening arrhythmia is documented in patients with alcoholic cardiomyopathy and includes acute and chronic rhythm disturbances.

finding in chronic alcohol abusers (Kupari et al., 1990; Fernández-Solà et al., 2000) and there is some evidence that the duration of consumption and cumulative dose of alcohol are directly associated with the severity of the diastolic dysfunction (Urbano-Marquez et al., 1989). Lazarević et al. reported that echocardiographic changes in alcoholics were progressive (**Fig. 2**): an initial increase in LV volume and prolongation of the isovolumetric relaxation time are followed by an increase in LV mass and prolongation of the deceleration time, with subsequent decrease in the E\A ratio (Lazarević et al., 2000).

A mild decrease in left ventricular ejection fraction is initially asymptomatic and has been demonstrated by comparing heavy drinkers to moderate drinkers or abstainers (Yousaf et al., 2014; Li et al., 2016a). A further reduction in the systolic function marks the progression from heart failure with preserved ejection fraction (>50%) toward heart failure with reduced ejection fraction (<50%), with ominous prognostic implications (Ponikowski et al., 2016).

Left atrial dilatation and functional impairment are more frequently observed in heavy drinkers (Kocabay et al., 2015) and their role as an intermediary phenotype linking left ventricular dysfunction to the development of atrial fibrillation is a novel and intriguing concept (McManus et al., 2016). While binge drinking has been known to induce arrhythmia ("holiday heart syndrome" (Ettinger et al., 1978)), atrial fibrillation is much more common with chronic heavy drinkers with underlying cardiomyopathy being more susceptible (Samokhvalov et al., 2010). Studies have also revealed that alcohol abusers have prolonged QT intervals (Li et al., 2016b) and these patients are more predisposed to the development of ventricular tachycardia and sudden cardiac death (Wannamethee and Shaper, 1992; Guzzo-Merello et al., 2015a). This predilection is probably due to the combination of the direct toxic effect of alcohol on the cardiomyocytes and the structural and functional alterations of the myocardium and conduction system in ACM.

The prevalence of left ventricular dysfunction and dilated cardiomyopathy in chronic consumers is not gender-biased but women seem to be more susceptible to the toxic effects of alcohol even when accounting for differences in body mass index. Consistently lower total lifetime doses of ethanol in women than in men with ACM are reported in epidemiologic studies showing that LV ejection fraction might be affected more and earlier in women exposed to the same doses of alcohol (Urbano-Márquez et al., 1995; Fernández-Solà et al., 1997).

Recent progress in characterizing ACM has been made possible through technological developments of functional cardiac imaging such as cardiovascular magnetic resonance with late gadolinium enhancement (LGE CMR) and echocardiographic speckle-tracking. These techniques have been validated in patients with nonischemic dilated cardiomyopathy (Mewton et al., 2011; Iles et al., 2015) and the regional differences in signal intensity on LGE CMR allow the assessment of myocardial fibrosis and are of prognostic importance (Yamada et al., 2014; Chimura et al., 2016).

Prognosis and Therapy

Excessive alcohol consumption is responsible for 5.1% of the global burden of disease and injury (World Health Organization, 2014) and accounts for 1 in 10 deaths among working-age adults in the United States (Stahre et al., 2014). Cardiovascular events make up an important fraction of alcohol-related morbidity and ACM has been shown to influence outcome. Initial long-term follow-up data showed higher survival rates in patients with alcoholic etiology who discontinued drinking compared to patients with idiopathic cardiomyopathy (Guzzo-Merello et al., 2014; Brinkley et al., 2014). In contrast, studies performed after the introduction of beta-blockers, angiotensin-converting enzyme inhibitors, and implantable devices report worse transplant-free survival in chronic alcohol consumers (Prazak et al., 1996; Guzzo-Merello et al., 2015b). However, all studies are in concord on the value of maintaining abstinence in reducing morbidity and mortality in alcoholic cardiomyopathy (Gavazzi et al., 2000) and reversibility of mild to moderate ventricular dysfunction is to be expected in abstainers.

Alcohol reduction or total abstinence are the mainstay in the treatment of ACM as they can improve systolic and diastolic function and prevent further cardiovascular complications. In other regards, the management of ACM is the management of the resulting congestive heart failure. Conventional pharmacotherapy is based on beta-blockers, angiotensin-converting enzyme inhibitors, aldosterone antagonists, and loop diuretics. Implantable devices and surgical ventricular remodeling are sometimes indicated, with heart transplant being the curative alternative. Stem-cell therapy with intracoronary injection of $CD34^{+}$ cells resulted in improvement of systolic function, increased exercise tolerance, and a significant survival benefit in a long-term follow-up study (Vrtovec et al., 2013). A recent randomized controlled trial with a one year follow-up confirmed a mild improvement in LV ejection function and clinical symptoms in patients treated with autologous bone-marrow derived cells and granulocyte colony-stimulating factor (Hamshere et al., 2015). Novel and unconventional therapies are currently being explored in order to alter and possibly reverse the natural history of the disease (Sisakian, 2014; Fernández-Solà and Planavila Porta, 2016).

Alcoholic Cardiomyopathy and Cirrhosis

Assessment of the specific influence of alcohol on the cardiovascular function is more difficult in alcohol abusers with chronic liver disease. Proof of this is the fact that the hyperdynamic circulation typical of cirrhosis first described 60 years ago was initially attributed to chronic alcohol use, the overwhelming cause of liver disease at that time (Kowalski and Abelmann, 1953). Later studies revealed that etiology was irrelevant in the pathogenesis of the cardiohemodynamic disturbances encountered in cirrhotic patients (Møller and Bernardi, 2013).

The partial overlap between alcoholic cardiomyopathy and the more recently described "cirrhotic cardiomyopathy" (Wiese et al., 2014), an entity characterized by a latent cardiac dysfunction and inadequate response to stress, poses a distinct problem to the clinician and researcher. Since ethanol abuse is frequent in cirrhotic patients, distinguishing between the role of alcohol and liver disease in cardiovascular dysfunction can be impossible in the clinical setting. As the phenotype may change with disease progression the differential diagnosis becomes even more problematic. Echocardiography, electrocardiography, and serum biomarkers of cardiac dysfunction are common tools and essential to the detection of cardiac impairment. Left ventricular dysfunction is the most studied abnormality encountered in both alcoholic and cirrhotic cardiomyopathies. Prolongation of the QTc interval, electromechanical uncoupling and elevation of pro-brain natriuretic peptide or troponins do not help differentiate between the two.

However, despite their similarities there are some differences worth noting. ACM is a form of dilated cardiomyopathy with typical albeit nonspecific histopathological changes and leads to low-output heart failure and an increased risk of arrhythmia and sudden cardiac death. By contrast, cirrhotic cardiomyopathy is, at least initially, characterized by high-output heart failure due to a hyperdynamic circulatory state with minimal histopathological changes and no reported increase in arrhythmic events. A slow but gradual progression to overt heart failure is more frequent in ACM while cirrhotic patients generally manifest reversible cardiac dysfunction when confronted with acute intravascular volume change or further vasodilation (e.g., insertion of transjugular intrahepatic portosystemic shunt, sepsis, paracentesis, liver transplantation).

A noteworthy point is the observation that cirrhosis is not a prerequisite for ACM as structural and functional alterations compatible with ACM have been described in chronic consumers of alcohol without advanced liver disease. This would suggest the existence of various yet uncharacterized phenotypes that determine different patterns of end-organ damage in the case of sustained injury due to alcohol. Further investigation into the cell-specific mechanisms that predispose the hepatocyte and cardiomyocyte to alcohol-induced damage seems worthwhile.

Lacking stringent diagnostic criteria the integration of all the data from clinical and paraclinical investigations is mandatory for distinguishing between these two entities, with the understanding that a complete differentiation may not be feasible in all cases.

Summary and Conclusions

Chronic alcohol abuse increases cardiovascular morbidity and mortality. Ethanol and its metabolites negatively influence cardiac function both directly, through their toxic cellular effect, and indirectly, by disrupting the complex metabolic and hemodynamic

homeostasis. Alcoholic cardiomyopathy is a form of dilated cardiomyopathy that occurs after years of chronic high-dose alcohol consumption. Diagnosing this condition is hindered by the lack of pathognomonic signs and a specific diagnostic test. Further difficulties in identifying and managing alcoholic cardiomyopathy stem from the long period of clinical latency, subtle initial manifestations and frequent cardiovascular comorbidities that cloud the clinical picture. Echocardiography and electrocardiography are useful in detecting ACM, but differentiating it from cirrhotic cardiomyopathy in patients with concomitant advanced liver disease is difficult. A slow progression toward overt low-output heart failure and unfavorable outcome is frequent but reversible in abstainers. Cessation of alcohol consumption is paramount for improving prognosis and standard pharmacological and interventional therapy is indicated according to the severity of heart failure. Further research into the pathogenesis of ACM and exploring novel therapies are vital to improving the management and care of these patients.

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Amyloid Cardiomyopathy

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Introduction

Amyloidosis is a unique, yet remarkably heterogeneous group of diseases characterized by the deposition of misfolded protein precursors in the extracellular space in various tissues. Amyloid fibrils of diverse protein origins are resistant to proteolytic cleavage under physiological condition, and the characteristic uniformity of beta-pleated sheet configuration among all forms of amyloidosis forms the basis of characteristic staining of amyloid fibrils by Congo red, a feature essential for establishing the diagnosis on histopathologic examination (Fig. 1; Picken, 2010). Widely divergent precursor proteins (more than 30 currently known) can cause amyloidosis and lead to diseases belonging to infectious (prion disease), neoplastic (immunoglobulin light-chain systemic amyloidosis), neurodegenerative (Alzheimer's disease), and hereditary (familial amyloidosis) groups of disorders (Sipe et al., 2012). The heart is a frequently involved organ in systemic amyloidosis, and amyloid cardiomyopathy is the most common cause of death in these patients.

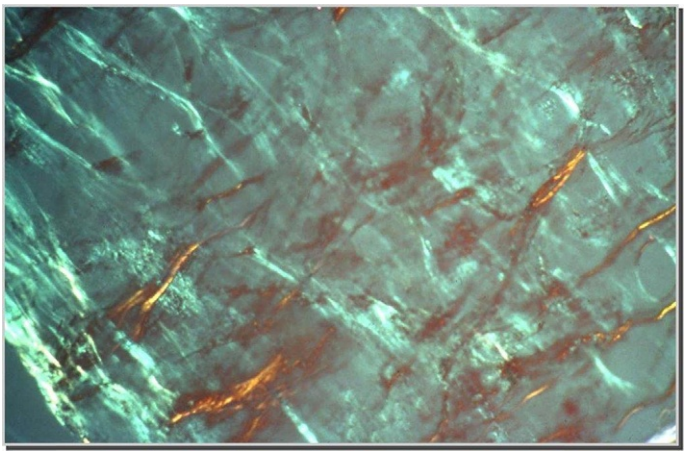

Fig. 1 Apple green birefringence of Congo red stained endomyocardial biopsy under polarized microscopy.

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Amyloid Heart Disease

Heart is one of the most common organs involved in amyloidosis. Amyloidogenic proteins can involve any part of the heart, including the myocardium (atrial, ventricular, and interventricular septum), the valvular tissue, the perivascular space around and within small blood vessels, and the conduction system (Falk and Dubrey, 2010). Gross pathological examination of the heart infiltrated with amyloidosis reveals characteristic thickening of all four cardiac chambers and interventricular septum (Fig. 2). Biatrial enlargement with small- to normal-sized left ventricular cavity is a unique feature of amyloid cardiomyopathy. Microscopically, amyloid deposition is seen in the extracellular space, which results in separation and distortion of myocardial cells and electromechanical dissociation. It is important to recognize that different amyloid proteins can result in similar pathological findings and there is no characteristic deposition pattern in the myocardium unique to any subtype. Furthermore, cardiac involvement can occur at any stage of the disease and may be the initial presentation, incidentally discovered during evaluation of other symptoms or discovered on postmortem examination (Falk, 2005).

Types of Amyloid Cardiomyopathies

Various forms of cardiac amyloidosis and the clues to differentiate them are tabulated in Table 1, and the common types are elaborated later.

Immunoglobulin Light-Chain (AL) Amyloid Cardiomyopathy

Immunoglobulin light-chain amyloidosis (primary systemic amyloidosis or AL amyloidosis), the most common systemic amyloidosis in the United States and Western Europe, is a neoplastic disease of plasma cells, where transformed clonal plasma cells in the bone marrow produce amyloidogenic light chains. With an approximate incidence of 2500 new cases each year, AL is a very rare disease (Kyle et al., 1992). Mostly, AL occurs de novo, but in a minority of patients, it can evolve from preexisting multiple myeloma (the most common plasma cell malignancy) and non-Hodgkin lymphomas such as Waldenström's macroglobulinemia. Cardiac involvement is often very advanced and is seen in 50% of the cases (Gertz et al., 1999). AL cardiomyopathy is the most aggressive form of amyloid heart disease and is the sole reason for the poor prognosis associated with amyloidosis. Patients with AL frequently present with congestive heart failure (CHF) from restrictive physiology. Peripheral edema from right heart dysfunction is a very common feature often associated with ascites and hepatomegaly. Atrial dysrhythmias are common and can be complicated by mural atrial thrombus. Embolic stroke may be the first presentation of the disease (Seldin et al., 2011). Ventricular arrhythmias also

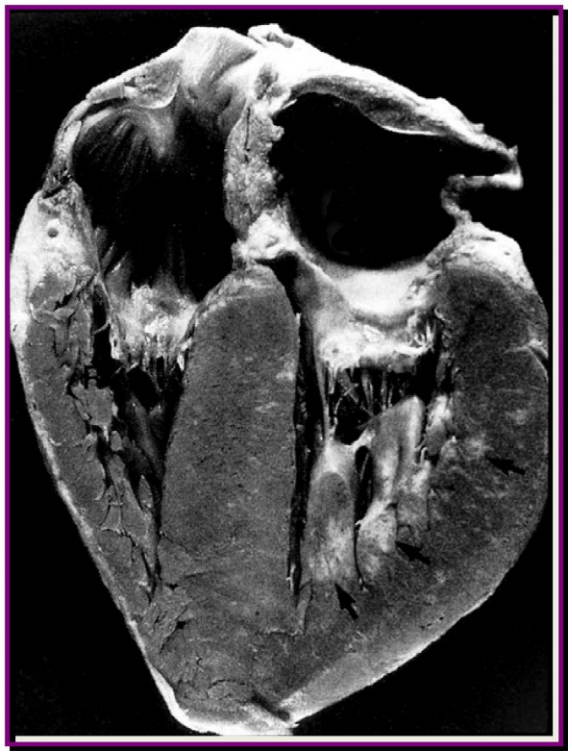

Fig. 2 Heart from an AL patient. Note thickened interventricular septum and left ventricular posterior wall.

Table 1 Common types of cardiac amyloidosis

Characteristics	*AL*	*ATTR Mutant*	*ATTR Wild*	*AA*	*AANF*
Common name amyloidosis	Primary (immunoglobulin light-chain) systemic amyloidosis	Familial transthyretin amyloidosis	Senile systemic amyloidosis	Secondary amyloidosis	Isolated atrial
Incidence/ prevalence	2000–3000 cases per year	Variable in different races Val122Ile variant in 4% of African–Americans Val30Met variant in Caucasians	Males older than 70 years Incidence increases with age	Uncommon	Common
Precursor protein	Immunoglobulin light chain	Mutant TTR	Wild-type TTR	SAA protein	Atrial natriuretic peptide
Clinical organ involvement	Heart, kidney, liver, peripheral nerves, autonomic nerves, GI tract, soft tissue	Heart, nerves, GI tract, autonomic nerves	Heart, rarely nerves	Kidney, liver, GI tract. Very rarely heart	Cardiac atria
Salient features	Low QRS voltage Pseudoinfarct pattern on ECG IVS thickness seldom >1.9 cm Rapid progression	IVS thickness frequently severe	IVS thickness frequently severe Relatively indolent disease		
Treatment	Chemotherapy (standard or stem-cell transplant)	Liver transplant	Supportive care	Treat inflammation	No specific treatment
Prognosis/ survival	Poor, 3–5 months if present with CHF	8–10 years from presentation	5–8 years from diagnosis	Generally good	Good

IVS, interventricular septum; *TTR*, transthyretin.

Table 2 Syndromes in AL amyloidosis (Mayo Clinic experience)

Syndrome	*Patients, %*
Nephrotic or nephrotic and renal failure	30
Hepatomegaly	24
Congestive heart failure	22
Carpal tunnel syndrome	21
Neuropathy	17
Orthostatic hypotension	12

occur, but are not a common presenting feature, possibly because patients succumb to sudden cardiac arrest before the diagnosis is established. Patients can also present with typical angina pectoris from myocardial ischemia due to amyloid infiltration of small vessel walls, which can, at times, be very frustrating as cardiac catheterization reveals normal epicardial coronaries (Falk and Dubrey, 2010; Ogawa et al., 2001). Multiorgan involvement is very common in AL patients. The kidney, peripheral nerves, autonomic nerves, liver, and GI tract are frequent extracardiac sites of disease (Table 2; Gertz et al., 1999). Soft-tissue infiltration is rare but gives rise to classic clinical exam findings such as shoulder pad sign, muscle infiltration (Figs. 3 and 4), macroglossia (Fig. 5), and periorbital purpura (Kyle and Greipp, 1983). Small- and medium-sized blood vessel ischemia from involvement by amyloid can cause symptoms of jaw claudication, and patients can be misdiagnosed as having giant cell arteritis. Mucocutaneous bleeding from amyloid angiopathy and acquired deficiency of circulating coagulation factors, in particular factor X, can be a prominent presenting feature (Choufani et al., 2001).

Wild-Type Transthyretin Cardiomyopathy

Also called senile systemic amyloidosis (SSA), wild-type transthyretin is caused by systemic deposition of amyloid fibrils derived from a native (wild-type) circulating plasma protein transthyretin (TTR) (Westermark et al., 1990). For reasons that remain unclear, SSA is predominantly seen in males. Autopsy series have commonly identified wild-type TTR amyloid deposits in the hearts of older males with some series reporting an incidence as high as 25% in subjects 80 years of age or older. Many patients with SSA may not develop clinically significant end-organ damage, despite histological or echocardiographic evidence of amyloidosis. SSA typically presents with progressive decline in energy and symptoms of CHF, often with normal systolic function (Ng et al., 2005). The diagnosis is often delayed in these patients until they have multiple hospitalizations for CHF. Increased myocardial thickness is often blamed on systemic hypertension or hypertrophic cardiomyopathy. Almost half of SSA patients may have symptomatic

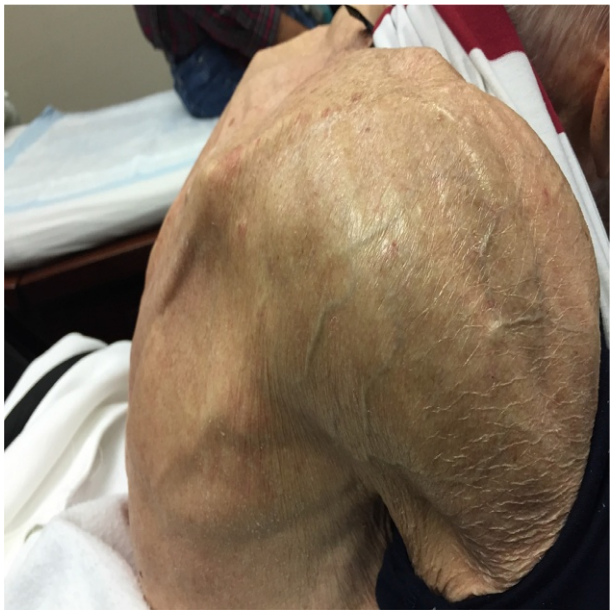

Fig. 3 Shoulder fat-pad sign in a patient with advanced AL.

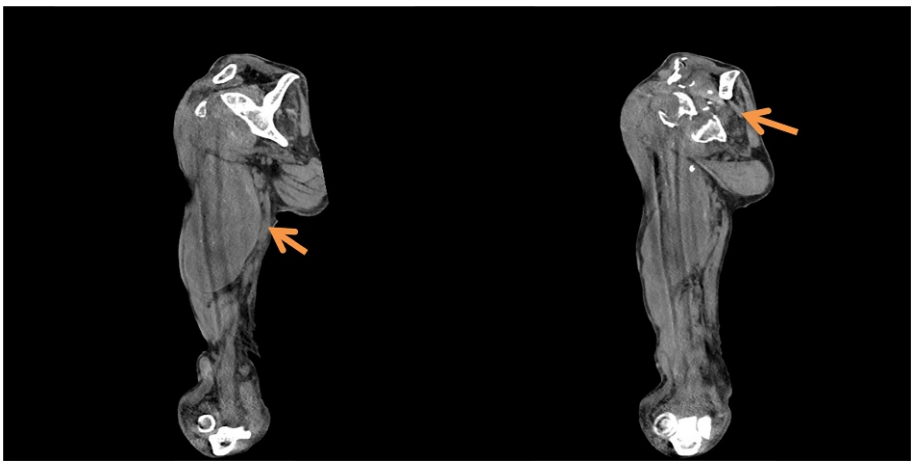

Fig. 4 Amyloid arthropathy.



Fig. 5 Macroglossia from amyloid infiltration of tongue. This can be a formidable problem in patients with AL.

median neuropathy presenting as carpal tunnel syndrome. This is in contrast to familial TTR amyloidosis, where manifestations of peripheral neuropathy and gastrointestinal involvement are frequently seen in addition to cardiomyopathy. With the increasing availability and frequent use of echocardiography, the recognition of SSA is expected to increase significantly in near future.

Familial Amyloid Cardiomyopathy

In heredofamilial amyloidosis, the amyloid deposits are derived from misfolded forms of several proteins such as TTR, apolipoprotein, fibrinogen, and leukocyte chemotactic factor 2 (Gertz et al., 1992). Familial ATTR is the most common type of familial amyloid cardiomyopathy (FAC). More than 100 pathogenic mutations have been described in the *TTR* gene on chromosome 18 (Connors et al., 2000). In familial ATTR, the specific TTR mutant determines the clinical presentation, and the peripheral nerves and the heart are the most common sites of involvement. Autonomic dysfunction and GI tract involvement can be severe and can cause significant malabsorption and protein-losing enteropathy (Plante-Bordeneuve and Said, 2000; Benson, 2005). One particular TTR variant allele, Val122Ile, is found in 4% of African–Americans and is a frequently overlooked cause of cardiomyopathy in African–American males (Jacobson et al., 1997). Despite the high prevalence of this variant, the overall prevalence and incidence of FAC in African–Americans remains low, possibly owing to failure to recognize the etiology of the CHF. In Caucasians, the Val30Met mutant is the most commonly reported TTR variant with high penetrance presenting as early-onset (30–40 years of age) peripheral neuropathy and relatively late-onset (50–60 years of age) cardiomyopathy (Tojo et al., 2008). The most common TTR mutant in the Caucasian population of the United States is T60A.

Variants of fibrinogen A-alpha, apolipoprotein A-I, and A-II account for other rare types of familial amyloidosis and can involve the heart; however, renal involvement predominates in these non-TTR familial amyloidoses (Benson et al., 2001; Hamidi Asl et al., 1998).

Secondary Systemic Amyloid (AA) Cardiomyopathy

Secondary systemic (AA) amyloidosis results from excessive production and misfolding of the acute-phase reactant serum amyloid A protein. AA is common in developing countries and in the Mediterranean basin due to a high prevalence of chronic infections and auto-inflammatory disorders, such as familial periodic fever syndromes (van der Hilst et al., 2005). In the United States and Western Europe, it is rare and usually results from unrecognized or treatment refractory chronic autoimmune disorders such as rheumatoid arthritis and inflammatory bowel disease (Hazenberg and van Rijswijk, 2000). Nephrotic syndrome is the most common presentation, but involvement of gastrointestinal and hepatobiliary systems leading to malabsorption syndrome and/or hepatosplenomegaly can be seen. Involvement of endocrine organs, such as thyroid and adrenal gland, can be seen and may result in adrenal insufficiency (Hazenberg and van Rijswijk, 2000). Cardiac involvement in AA is rare and even if severe by echocardiogram, seldom results in clinically significant cardiomyopathy.

Isolated Atrial Amyloidosis

Isolated atrial amyloidosis (IAA) results from deposition of atrial natriuretic peptide derived amyloid in cardiac atria (Millucci et al., 2012). Unlike other cardiac amyloidoses, this form is almost always localized to the atria and can only be diagnosed on histological examination of atrial tissue. The incidence of IAA increases with age and appears to be more common in patients with atrial diseases such as rheumatic heart disease. Recent studies have reported a very high incidence of IAA on the histopathologic examination of the explanted hearts from patients with dilated and hypertrophic cardiomyopathies and raise important questions about the possible role of IAA in the pathogenesis of these conditions (Millucci et al., 2011).

Pathogenesis of Amyloid Cardiomyopathy

Organ tropism of specific amyloid proteins is poorly understood and is illustrated by familial ATTR as certain TTR mutations result in exclusive familial amyloid polyneuropathy (FAP) while others in exclusive FAC, yet still, others cause FAP/FAC overlap syndromes. This organ tropism is also recognized in AL where restricted usage of certain variable regions of light-chain gene segments during immunoglobulin gene rearrangements can result in generation of amyloidogenic light chains with particular affinity for the kidney (λ_{6a}*family*) or heart (λ_{III}*family*) (Perfetti et al., 2002, 2012). It is likely that in addition to the characteristics of the amyloidogenic protein, organ distribution is also determined by local factors operating in the involved organ.

Historically, mechanical infiltration of the myocardium and resultant physical myocardial distortion was identified as the main mechanism underlying cardiac dysfunction. Recent advances in molecular biology and genetic engineering have provided several pieces of evidence that suggest that additional mechanisms are operating and may be more important in myocardial damage than simply the infiltration. Myocardial thickening and mass is more pronounced in SSA than in AL cardiomyopathy, yet patients with SSA cardiomyopathy survive for many years with just supportive care, while survival of AL patients with comparable or even "milder" cardiac involvement by imaging studies is considerably shorter. This is further corroborated by animal studies whereby the infusion of amyloidogenic light chains purified from AL patients resulted in significant myocardial dysfunction in the absence of

amyloid fibril deposits in mice (Liao et al., 2001; Mishra et al., 2013). The exact cellular mechanism underlying this toxicity is not known; however, increased generation of oxygen free radicals and induction of cardiomyocyte apoptosis have been suggested as possible causes (Brenner et al., 2004).

Diagnosis of Amyloid Cardiomyopathy

Amyloidosis is a great masquerader and like many rare diseases is very often diagnosed at late stages. This is especially true in AL where the rapid disease progression coupled with a lack of specificity in patient's symptoms results in delayed diagnosis and poor outcomes. Amyloidosis and in particular AL should be suspected in patients presenting with proteinuria, unexplained CHF (especially diastolic heart failure), new onset peripheral neuropathy especially with bilateral carpel tunnel syndrome, new onset autonomic dysfunction, otherwise unexplained weight loss, and hepatomegaly in the absence of known liver disease (Gertz et al., 1999; Table 2).

Patients should undergo a thorough physical examination with particular attention to signs of volume overload, orthostatic hypotension, macroglossia, amyloid purpura, hepatosplenomegaly, peripheral edema, and sensory neuropathy. Initial laboratory studies should include complete blood counts, renal and liver function profiles, serum electrolytes, coagulation profile, serum protein electrophoresis with immunofixation, 24 h urine protein electrophoresis, immunofixation, and nephelometric measurement of serum immunoglobulin-free light chains and troponin T and N-terminus of the prohormone brain natriuretic peptide (NT-ProBNP) (Gertz, 2010). The common abnormalities noted in these initial studies in amyloidosis, particularly in AL patients, are summarized in Table 3. The diagnosis of amyloidosis must be confirmed by histological examination of an optimal biopsy. Sites to consider for the biopsy include abdominal wall subcutaneous fatty tissue (fat-pad aspirate and fat-pad biopsy), vascular submucosa of the GI tract (minor salivary glands in mouth, labial soft tissue, and rectal biopsy), or bone marrow biopsy. In our experience, at the Mayo Clinic, a combination of bone marrow biopsy and abdominal subcutaneous fat-pad aspirate yields the diagnosis of AL amyloidosis in 85% of patients (Gertz et al., 1999).

Endomyocardial biopsy is the gold standard for the diagnosis of cardiac amyloidosis. Heart biopsy is not needed when less invasive biopsy has established a diagnosis of amyloidosis (Pellikka et al., 1988). The biopsy tissue should be stained with amyloid-binding stains such as Congo red or sulfated Alcian blue. Optimal staining by an experienced pathologist is important as we have seen false positive and false negative cases when biopsies done elsewhere are reviewed at the Mayo Clinic. Once a histological diagnosis is established, the next step is to determine the type of amyloid protein. Immunohistochemistry has been most commonly used for amyloid typing; however, its sensitivity is relatively low especially for AL. At the Mayo Clinic, we routinely use laser capture microdissection and mass spectrometry, the gold standard proteomic typing technique, to determine the subtype in all patients with amyloidosis (Klein et al., 2011; Brambilla et al., 2012; Vrana et al., 2009). The accurate determination of amyloid type is especially important to exclude familial ATTR as it can clinically mimic light-chain amyloidosis, and up to 10% of patients with non-AL cardiomyopathy can have coexisting monoclonal gammopathy of undetermined significance, which can lead to the misdiagnosis of AL in these patients (Comenzo et al., 2006). If TTR is identified as the precursor protein, DNA testing should be performed on peripheral blood to identify a possible gene mutation to differentiate between SSA and familial ATTR (Table 4).

After establishing the diagnosis, the next step in the management of patients is to determine the extent of organ involvement. The heart is the most important organ involved in amyloidosis; therefore, determination of the presence and degree of cardiac amyloidosis is imperative. Various diagnostic procedures providing critical clues to the diagnosis and severity of cardiac amyloidosis are reviewed later.

Electrocardiography

Electrocardiography (ECG) abnormalities are frequently overlooked in amyloidosis patients. Low QRS voltage can be seen in up to two-thirds of patients with AL (Hongo et al., 2000). It is less common in non-AL amyloid heart disease. Low ECG voltage in the presence of the echocardiographic findings of increased myocardial thickness is highly suggestive of advanced amyloid heart

Table 3 Recommended diagnostic testing for a histological diagnosis of amyloidosis

- CBC, creatinine level, alkaline phosphatase level
- Serum and 24 h urine total protein, electrophoresis, and immunofixation
- Serum immunoglobulin-free light-chain assay
- Marrow biopsy
- Quantitative immunoglobulins
- N-terminal pro-B natriuretic peptide level, serum troponin levels, uric acid level
- Echocardiography with Doppler
- Confirmation that amyloid deposits are of Ig origin (i.e., laser capture microdissection followed by mass spectroscopy)

CBC, complete blood cell count.

Table 4 Common abnormalities in AL cardiomyopathy and their prognostic impact

Prognostic factor	*Comment*
Clinical findings:	
● Congestive heart failure and exertional syncope	● Patients presenting with exertional syncope have a median survival of 2 months
● Jaundice	● Jaundice and hyperbilirubinemia is usually a preterminal finding
Laboratory findings:	
● Thrombocytosis	● Indicator of splenic involvement
● Howell–Jolly bodies	● Indicator of advanced disease
● Elevated bilirubin	● Often a preterminal finding
● Elevated creatinine	
● Elevated free light-chain levels	● Important factors that have been incorporated into four-stage prognostic model
● Elevated troponin T	
● Elevated BNP (NTpro-BNP)	
Echocardiogram findings:	
● Interventricular septum thickness	● Median survival 1 versus 4 years if thickness $>$ or $<$1.5 cm
● Short mitral deceleration time	● Poor outcome for patients with deceleration $<$150 ms
● Decreased fractional shortening	● Poor outcomes for patients with fractional shortening $<$20%

disease. Bundle branch abnormalities can be seen in up to 40% of patients with ATTR. Additional ECG abnormalities include loss of the R-wave in leads V1–V3, resulting in a "pseudoinfarction" pattern. Patients with a pseudoinfarction pattern on ECG frequently undergo an unrevealing coronary angiogram. Rarely, patients can present with true myocardial ischemia due to amyloid infiltration of small intramyocardial arteries (Hongo et al., 2000).

Cardiac Biomarkers

Myocardial damage from amyloidosis results in leakage of intracellular proteins and enzymes into the circulation. Cardiac troponin T (cTnT) and NT-ProBNP are two such markers that have made a significant impact in the diagnosis and monitoring of patients with AL cardiomyopathy (Merlini et al., 2016; Palladini et al., 2010). The Mayo Clinic staging system is a prognostic scoring system that has been derived based on the levels of serum-free light chains, cTNT, and NT-ProBNP in 801 previously untreated AL patients and can separate patients into four prognostic groups with median survival of patients with stage I, II, III, and IV disease of 94, 40.3, 14, and 5.8 months, respectively (Fig. 6; Kumar et al., 2012a).

Cardiac Imaging

Cardiac imaging remains the cornerstone of evaluation of patients suspected of having amyloid heart disease.

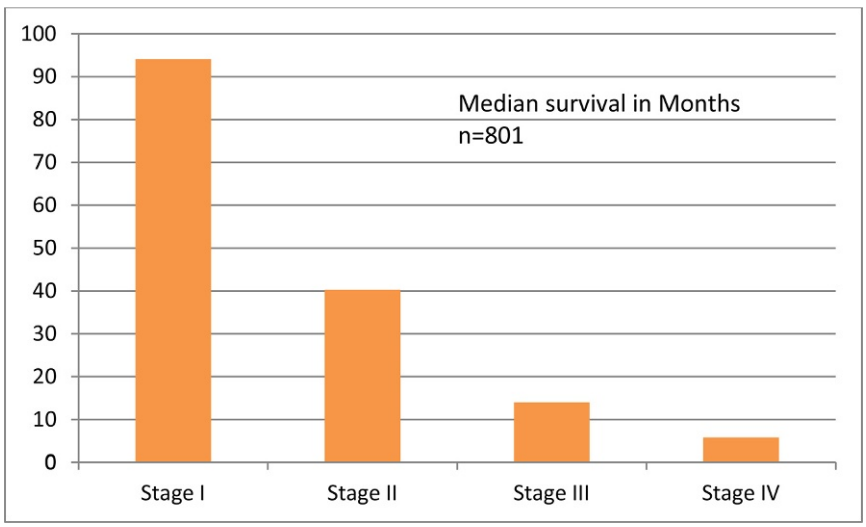

Fig. 6 Prognostic staging system in AL—patients are assigned a score of 1 for each of dFLC $\geq$ 18 mg/dL, cTnT $\geq$ 0.025 ng/mL, and NT-ProBNP $\geq$ 1800 pg/mL leading to scores of 0–3 points creating stages I through IV, respectively. *dFLC*, difference of involved and uninvolved light chains; *cTnT*, cardiac troponin T; *NT-ProBNP*, N-terminus of the prohormone BNP.

Echocardiography

A 2-D echocardiogram can be an invaluable tool for evaluating patients with amyloid cardiomyopathy (Falk and Dubrey, 2010; Tsang and Lang, 2010). Normal ejection fraction and absence of LV dilatation in patients presenting with heart failure should lead cardiologists to suspect cardiac amyloidosis. Thickening of the right ventricular free wall and biatrial enlargement, along with diffuse valvular thickening, are all features suggestive of amyloid cardiomyopathy (Tsang and Lang, 2010). In advanced stages, atrial standstill and intramural thrombus can be seen (Fig. 7).

Measurement of myocardial flow velocities with Doppler almost always reveals longitudinal systolic dysfunction, despite "preserved" LV ejection fraction calculated from myocardial contraction along its short axis (Elliott et al., 2000). The impairment of long-axis LV contraction is especially visible in strain and strain rate imaging (Koyama and Falk, 2010).

Echocardiography with standard Doppler also provides important prognostic information. In a Mayo Clinic series of 132 AL patients, an interventricular septal (IVS) thickness of >1.5 cm was associated with a median survival of <1 year as compared with that of 4 years in patients with an IVS thickness of <1.5 cm (Klein et al., 1991). Transmitral Doppler studies serve as a good index of restrictive physiology seen in amyloid cardiomyopathy, and a short mitral deceleration time (<150 ms) has been associated with shorter survival in AL patients. Basal systolic strain measured on regional strain and strain rate assessments is superior to Doppler flow measurements in predicting clinical outcomes (Koyama and Falk, 2010). A large observational study identified longitudinal strain and 2-D global longitudinal strain as important prognostic factors in patients with amyloid cardiomyopathy (Buss et al., 2012). Importantly, the 2-D global longitudinal strain remained an independent predictor of prognosis even in patients with preserved LV ejection fraction (Koyama and Falk, 2010). Novel echocardiographic techniques, such as feature- and speckle-tracking imaging, allow dynamic frame-by-frame myocardial motion analysis during the cardiac cycle and can help to distinguish between amyloid cardiomyopathy, hypertensive heart disease, and hypertrophic cardiomyopathy (Tsang and Lang, 2010). Evolving echocardiographic techniques are expected to help differentiate between various forms of diastolic dysfunction and represent an opportunity for early diagnosis of amyloid heart disease that can have a profound impact on outcome in amyloid cardiomyopathy.

Magnetic resonance imaging

Contrast-enhanced cardiac magnetic resonance imaging (MRI) is a promising diagnostic modality and is being increasingly utilized in the assessment of myocardial diseases. Besides accurate measurements of myocardial thickness, the kinetics of gadolinium enhancement can provide very specific clues about cardiac amyloidosis. The finding of ventricular wall and IVS thickening associated with diffuse, delayed gadolinium enhancement of the subendocardium is very suggestive of cardiac amyloidosis (Fig. 8; Vanden Driesen et al., 2006). Phase-contrast MRI, like Doppler echocardiography, can provide useful information about the flow dynamic parameters such as mitral deceleration times, early to late ratio of LV diastolic filling and mitral peak inflow velocity (Maceira et al., 2005). Novel techniques, such as magnetic resonance (MR) relaxometry, can characterize the physical

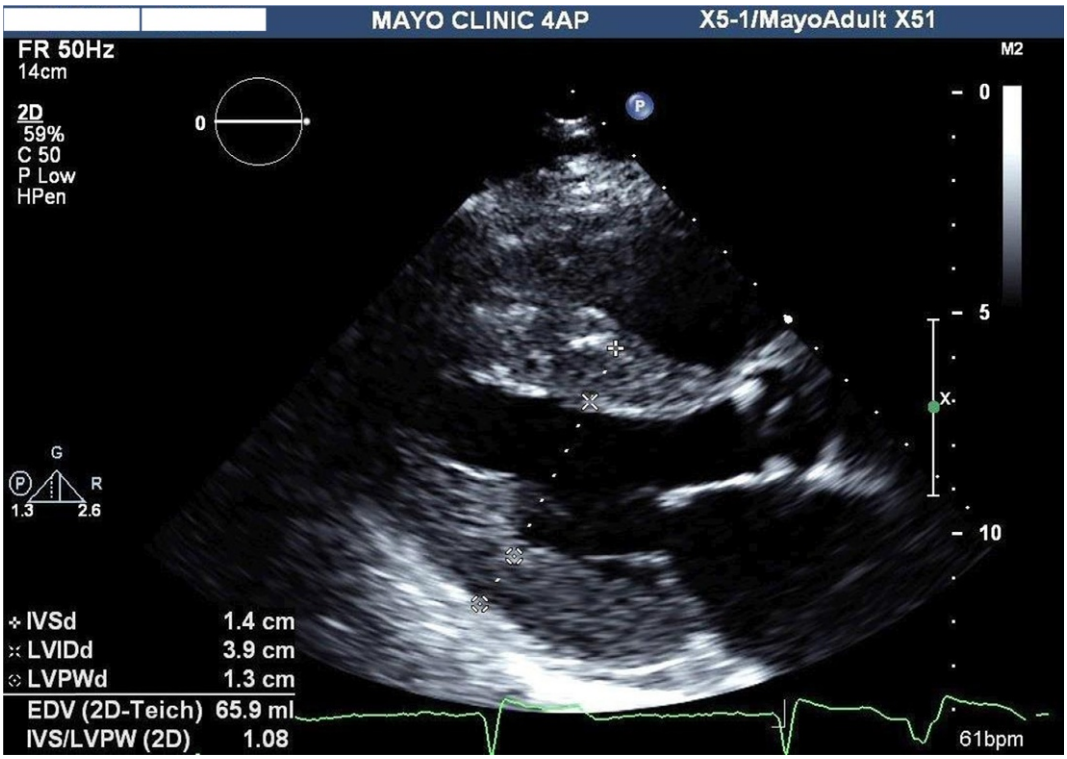

Fig. 7 Echocardiogram in a patient with AL cardiomyopathy.

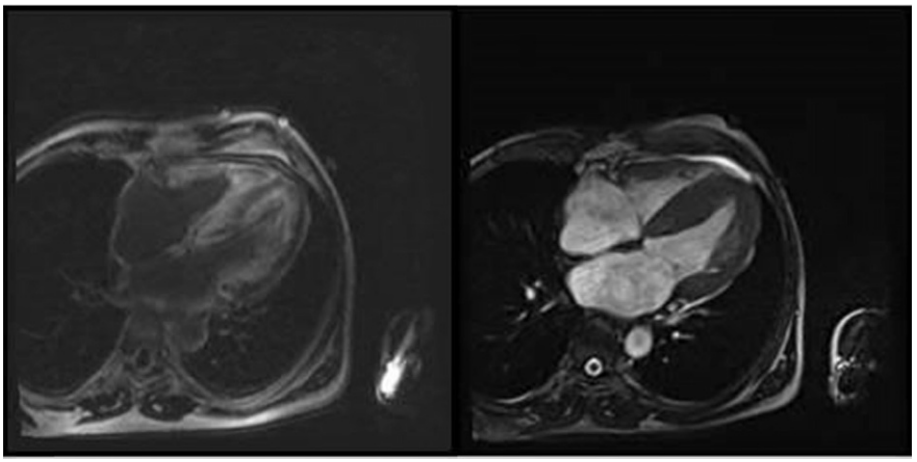

Fig. 8 A and B, MRI in amyloid cardiomyopathy.

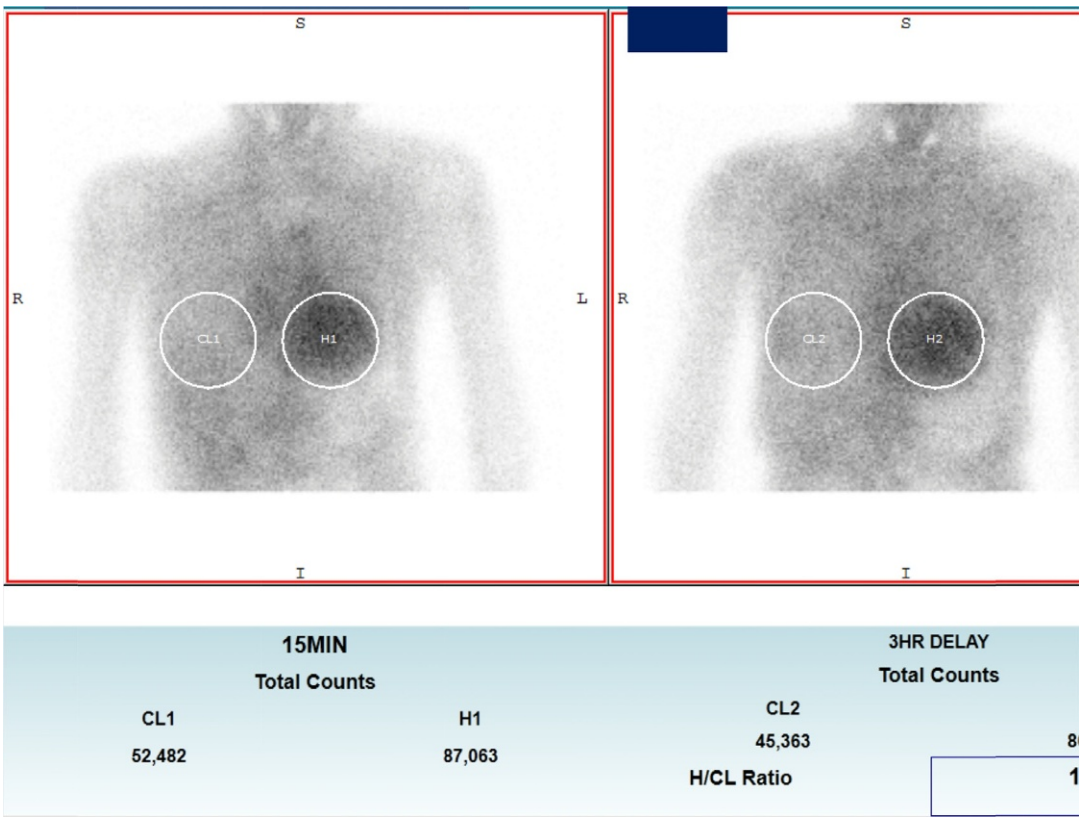

Fig. 9 Technetium 99 PYP scan in ATTR cardiomyopathy.

composition of the tissues and assess their dynamic properties (Rubinshtein et al., 2009). By providing functional assessment of the heart, these newer MR techniques can not only help establish early diagnosis but also serve as a useful tool for assessment of organ response to treatment. Despite these important advancements, the use of cardiac MR in amyloidosis may be limited due to the presence of pacemaker/defibrillators. Renal impairment increases the risk of gadolinium-induced nephrogenic systemic sclerosis.

Nuclear-medicine-based imaging

^{123}I–labeled SAP component scanning (SAP scan) is the prototype of nuclear medicine tests used for imaging tissue amyloid burden (Hawkins et al., 1998). This test does not accurately assess myocardial involvement. Technetium (^{99m}Tc)-based isotopes can identify cardiac involvement with TTR amyloidosis but have variable sensitivity and specificity for other types; for example, technetium pyrophosphate is a sensitive marker of cardiac amyloidosis with preferential myocardial uptake in ATTR compared with AL (Fig. 9).

Intense Tc99m PYP uptake in a patient with echocardiographic evidence of amyloid cardiomyopathy and no evidence of plasma cell dyscrasia is a strong evidence of TTR cardiac amyloidosis. Recently, in a small study, *N*-[methyl-^{11}C]2(4′methylamino-phenyl)-6-hydroxybenzothiazole (^{11}CPIB)-based PET imaging appeared promising as it identified cardiac amyloid deposits in all patients with AL or ATTR amyloid heart disease, while it was negative in healthy controls (Glaudemans et al., 2009; Antoni et al., 2013). The role of nuclear-based techniques in assessing systemic and, more importantly, cardiac amyloid burden is evolving. Additional studies are needed to help identify the appropriate isotope-scanning technique that can not only assess the disease burden but also reliably differentiate between various types of amyloidosis.

Management of Amyloid Cardiomyopathy

Management of amyloid heart disease depends upon the type of amyloid.

AL Cardiomyopathy

Treatment of AL involves the use of systemic chemotherapy to treat the underlying plasma cell dyscrasia to eliminate the production of amyloidogenic light chains. Systemic therapy improves organ function, quality of life, and survival of patients. Serum-free light-chain assay and cardiac biomarkers (*c*TnT and NT-proBNP) are invaluable in monitoring response to treatment (Gertz et al., 2005). While the ultimate goal of therapy in AL is improvement in organ function, the main aim of systemic therapy is to achieve hematologic response as indicated by reduction in the circulating burden of amyloidogenic light chains. Hematologic response precedes the organ response and has been shown to be a strong predictor of not only organ improvement but also overall survival (Gertz et al., 2007). Patients who achieve a complete hematologic remission (CR; i.e., normal serum-free light-chain levels and a normal K/L ratio and no evidence of clonal plasma cells) have a 66%–86% chance of organ improvement and 5-year survival approaching 70% (Dispenzieri et al., 2006). Improvement in organ function and survival is also noted in patients who achieve partial hematologic remission (i.e., at least 50% reduction from baseline in involved free light-chain levels).

Oral melphalan and dexamethasone (MelDex) has been the most common regimen used for the treatment of AL and has demonstrated hematologic response rates of over 60%, with 30%–40% of patients achieving CR. Organ improvement is noted in a third of patients with median survival as long as 5 years (Palladini et al., 2004; Sanchorawala et al., 2010). Treatment with newer antiplasma cell agents, such as proteasome inhibitors and immunomodulatory drugs, has demonstrated significant activity in patients with relapsed AL. Bortezomib has demonstrated hematologic response rates of 80% or more in combination with steroids and alkylating agents, and these combinations are moving to the frontline treatment of AL (Kastritis et al., 2010). Lenalidomide and pomalidomide are thalidomide analogs and have demonstrated response rates of over 50% in patients with relapsed disease (Palladini et al., 2012). While these newer agents are highly effective in AL, their use can be challenging because of potential cardiotoxicity, and their safety and superiority over MelDex have not been prospectively demonstrated. Myeloablative chemotherapy followed by autologous peripheral blood stem-cell rescue (stem-cell transplant (SCT)) has been very effective in eliminating the amyloidogenic plasma cell clone. In a large series of patients, SCT has demonstrated overall hematologic response rates of 75% and CR rates of 38% (Gertz et al., 2010). Median overall survival of patients who achieved CR was not reached, while it was 107 months for those with partial hematologic remission. Patients who achieved CR after SCT have been reported to have a 10-year survival approaching 60% (Sanchorawala et al., 2007). While SCT is very effective, it is associated with a high risk of complications including a high treatment-related mortality. Early experience reported a mortality in excess of 20% in the United States and >40% in a small European series. High transplant-related mortality has been attributed to inclusion of patients with advanced cardiac disease. The availability of cardiac biomarkers and the use of stringent selection criteria have significantly improved the transplant-related mortality to <5% in experienced centers (Gertz et al., 2011; Comenzo and Gertz, 2002). At the Mayo Clinic, active New York Heart Association class III or IV heart failure, an LV ejection fraction <45%, advanced cardiac stage by biomarkers, a systolic BP <90 mmHg, and more than two organ involvement are considered contraindications for SCT. In patients with less severe involvement, a risk-adapted approach is used to select an appropriate dose of melphalan as conditioning for SCT. Using these stringent criteria, only 25% of amyloidosis patients qualify for SCT at diagnosis (Sher et al., 2016a).

Generally, if an AL patient is a suitable candidate and bone marrow plasma cell percentage is <10%, we recommend proceeding with SCT as soon as the diagnosis is made. In all other patients, combination therapy with MelDex or with newer agents is recommended.

Immunotherapy is being actively explored for the treatment of AL. Recently, in a phase I/II study NEOD 001, a monoclonal antibody against epitopes expressed by amyloidogenic light chains has been found to be well tolerated by AL patients who achieved control of their plasma cell dyscrasia with systemic therapy. Improvement in biomarkers of organ dysfunction was seen in several patients, and this is undergoing active investigation in combination with systemic therapy in newly diagnosed patients in a phase III study (Gertz et al., 2016). Daratumumab, a monoclonal antibody against CD 38, a protein routinely expressed on plasma cells in multiple myeloma and AL, has shown to be remarkably effective in AL including in patients with who progressed after SCT and multiple other treatments (Sher et al., 2016b).

Supportive care remains the cornerstone of management of AL cardiomyopathy and includes managing the cardiac manifestations of the disease and complications of systemic chemotherapy. Management of heart failure can be very challenging in AL, as these patients cannot tolerate several medications used for routine therapy of heart failure from other causes.

Angiotensin-converting-enzyme inhibitors (ACEIs) and angiotensin receptor blockers are particularly difficult to use due to the risk of hypotension. The negative inotropic effect of beta-blockers can precipitate acute CHF in a patient with stable heart failure. Digitalis derivatives and atrioventricular nodal-blocking calcium channel blockers can cause significant dysrhythmias as their intracardiac concentration may increase due to increased binding to amyloid fibrils. Patients with atrial hypokinesis and standstill are at risk of developing mural thrombus and subsequent embolization and stroke (Gertz et al., 2015). Such patients should be treated with anticoagulation with close attention to the risk of bleeding as it is significantly increased in AL patients. Orthostatic hypotension can be a problem and should be managed with the use of compression stockings, fludrocortisone, droxidopa, and alpha-adrenergic agonist midodrine. Volume overload can be a recurrent and difficult to treat problem; salt restriction and use of loop diuretics and metolazone along with strict monitoring of blood pressure and orthostatic responses can be helpful in difficult cases. We prefer torsemide as the diuretic of choice. Patients with dysrhythmias pose a particular challenge. The role of cardiac pacemakers in AL cardiomyopathy is controversial, as no clear survival advantage has been demonstrated with their use as primary prevention or as treatment of common cardiac dysrhythmia. Pacemaker placement should be considered on an individual basis especially in patients who present with high-grade atrioventricular block. Unlike non-AL cardiomyopathies, even automatic implantable cardioverter–defibrillator may not help the failing heart in AL as electromechanical dissociation is common (Kristen et al., 2008; Lin et al., 2013).

Doxycycline has been shown in in vitro studies to inhibit the formation of amyloid fibrils and to disaggregate formed fibrils by disrupting the beta-pleated structure of the amyloid fibrils. Mayo Clinic examined the survival in patients following stem-cell transplantation based on biological randomization of the anti-infective prophylaxis in the first year following ASCT Of 455 evaluated patients; 77% received oral penicillin that is the standard of care for antibacterial prophylaxis following ASCT at Mayo Clinic, while 23% received oral doxycycline as the prophylaxis agent due to history of penicillin allergy. Among patients who achieved hematologic response to ASCT, oral doxycycline was associated with a longer overall survival (median not reached compared with 161 months, respectively, $P = 0.04$) (Kumar et al., 2012b).

Heart transplantation is an option in AL; however, the vast majority of patients with AL cardiomyopathy will not qualify for a heart transplant due to significant extracardiac involvement, rapid disease progression, and the lack of control of the plasma cell dyscrasia (Kpodonu et al., 2005; Hosenpud et al., 1991). Moreover, the outcomes of heart transplantation in AL cardiomyopathy have been inferior compared with that for other cardiomyopathies. These poor outcomes are primarily due to progression of AL, as a heart transplant does not address the production of amyloidogenic light chains. Use of SCT after heart transplant has been reported in a small series with 1- and 5-year heart transplant survival rates of 82% and 65%, respectively (Lacy et al., 2008). These numbers do not compare favorably to the outcomes achieved by heart transplant for other conditions. A heart transplant is feasible in highly selected patients with AL cardiomyopathy; the ideal candidate will be a younger patient with isolated or predominant heart involvement who is also a candidate for SCT within 6–12 months after cardiac transplant.

Familial/Hereditary Amyloid Cardiomyopathy

The management of FAC consists of supportive care and addressing the source of amyloidogenic protein. The liver is the most dominant source of production of amyloidogenic protein precursors, and as such, orthotopic liver transplantation (OLT) is the only proved treatment for ATTR, fibrinogen-related and apolipoprotein-related amyloidosis (Suhr et al., 2005). Mutant ATTR is the most common indication for liver transplantation for familial amyloidosis worldwide. It is very important to recognize that efficacy of OLT is dependent upon the particular TTR mutation. The 5-year survival after OLT is 82% in patients with Val30Met TTR variant compared with 59% in non-Val30Met patients. Interestingly, progression of amyloid heart disease may occur in patients with ATTR after liver transplantation (Herlenius et al., 2004). This is caused by deposition of wild-type TTR produced from the transplanted liver on already established nidus of amyloid deposits in the heart. This phenomenon is particularly noticeable in the Ala60 mutation where progression of amyloid cardiomyopathy is common after liver transplantation. Combined liver and heart transplantation should be considered in this situation if no other significant systemic involvement is noted. Unlike ATTR, fibrinogen- and apolipoprotein A-related amyloid cardiomyopathy does not appear to progress significantly after liver transplantation.

Recent advancements in our understanding of the pathogenesis of amyloidosis have resulted in the emergence of novel therapies in ATTR. Stabilizing TTR agents such as tafamidis meglumine, a small molecule that stabilizes the native TTR tetramer, and diflunisal, a NSAID, has shown to inhibit dissociation to an amyloid fibril monomer in laboratory studies (Lozeron et al., 2013). Tafamidis has been approved in Europe and Japan for the treatment of FAP (caused by ATTR) by demonstrating improved symptom control in these patients and is under active clinical testing in FAC patients. Recently, messenger RNA-based silencing of the TTR mRNA with short interfering RNA (siRNA) has been shown to significantly decrease the circulating levels of circulating TTR (Suhr et al., 2015). Early clinical trials have confirmed the feasibility of this therapy, and an ongoing large phase III study will help assess the effectiveness of this modality compared with the current standard of supportive care. If proved successful, these therapies may obviate the need for liver transplantation.

Heart failure in ATTR is generally milder, and medications such as ACEIs and beta-blockers are better tolerated in ATTR compared with AL. Outcomes of cardiac transplant in non-AL amyloid cardiomyopathies are significantly better compared with those in AL cardiomyopathy; therefore, a diagnosis of cardiac amyloidosis early in the disease course will result in better organ transplantation outcomes. All patients with FAC must be offered genetic counseling and testing for early diagnosis in first-degree relatives.

Wild-Type TTR Cardiomyopathy

Treatment of SSA is predominantly supportive. The usual principles of management of CHF apply in SSA. As a majority of these patients are older, cardiac transplantation is not routinely used in SSA, except in the occasional younger patient with good organ system function and the absence of advanced systemic amyloidosis. Trials of tafamidis and siRNA are being planned in SSA.

Prognosis of Amyloid Heart Disease

The prognosis of cardiac amyloidosis is highly dependent upon the underlying amyloid protein. AL patients with symptomatic CHF and advanced cardiac stage, as determined by biomarkers, have a median survival of 3–5 months. Despite significant advancements made in antiplasma therapy in the last two decades, the early mortality (~40% in 1 year) has not significantly changed in AL, with death usually resulting from progressive cardiac failure and/or fatal arrhythmias. In patients with less severe cardiac involvement, systemic chemotherapy improves survival. The staging system based on cardiac biomarkers and serum-free light chains provides critical information about prognosis as patients with stage I cardiac disease have a median survival of 94 months compared with 5.8 months for stage IV disease (Fig. 6; Kumar et al., 2012a). The prognosis is significantly better in familial and SSA cardiac amyloidosis despite advanced cardiac infiltration as assessed by imaging studies compared with AL. In familial ATTR, the type of mutation in the TTR gene influences prognosis; for example, in a large series of patients with the Ala60 variant, median survival from the time of diagnosis was 3.4 years. Generally, median survival in SSA and familial ATTR ranges between 5 and 8 years.

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Anemia in Heart Failure

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Introduction

While anemia is a common comorbidity seen in patients with heart failure (HF), its role in prognosis and therapy is evolving. The prevalence of anemia in HF with reduced ejection fraction (HFrEF) has been reported to be 10%–50% in inpatient cohorts and 19%–23% in outpatient cohorts (Triposkiadis et al., 2016). Many studies have shown anemia is an independent predictor of mortality in HF patients (Berry et al., 2016; Sanchez-Torrijos et al., 2006; Szachniewicz et al., 2003; Groenveld et al., 2008). Anemia has been shown to correlate with reduced exercise capacity (Ebner et al., 2016; Kalra et al., 2003) and NYHA (Silverberg et al., 2000) class in heart failure patients. In this article, we will focus on iron deficiency (ID) anemia, which is the most common form of anemia. We will discuss the importance of separating ID from ID anemia, particularly in the population of HF patients. We also summarize the two major randomized controlled trials in the area of ID in congestive heart failure. Finally, we will discuss the impact and management of anemia of chronic disease and the impact of erythropoietin supplementation in HF patients.

Etiologies of Anemia

The World Health Organization defines anemia as a condition in which the number of red blood cells is insufficient to meet the body's physiologic needs (WHO, 2011). The circulating volume of red blood cells is represented by surrogate lab values, most commonly hemoglobin and hematocrit. The World Health Organization defines anemia as a hemoglobin <13.0 g/dL in a male and as a hemoglobin <12.0 g/dL in a nonpregnant female (WHO, 2011). Anemia has multiple causes which can be broken down into two general categories: excessive red blood cell loss or inadequate RBC production. Chronic or acute blood loss, autoimmune destruction of blood cells, and mechanical hemolysis are a few of the more common causes of excessive red blood cell loss. Causes of inadequate production include ID, hemoglobinopathies, nutritional deficiencies, bone marrow malignancies, chronic kidney disease, chronic infection, and chronic inflammation. However, of all these varied etiologies, ID anemia is the most common form of anemia among all patient groups and is extremely common in patients with heart failure (WHO, 2011; Tim Goodnough et al., 2017; Cleland et al., 2016) (**Fig. 1**).

Hemodynamic Effects of Anemia

The hemodynamic contribution of anemia to the pathophysiology of patients with HF is multifactorial. Most intuitively, a change in hemoglobin directly changes the oxygen carrying capacity of blood, impacting the end-organ delivery of oxygen. Oxygen delivery also depends on cardiac output and vascular distribution, which can be altered by anemia-induced decreases in systemic vascular resistance. In essence, anemia causes increased oxygen extraction by tissues, and requires a higher cardiac output and a more rapid circulatory time to keep up with demand (Duke and Abelman, 1969). In a study by Anand et al., four anemic patients with congestive symptoms and high cardiac output underwent hemodynamic and neurohormonal evaluations. Their results were compared with normal controls as well as HF controls without anemia (Anand et al., 1993). Anemic patients had a 50% reduction in renal blood flow, with a hormonal response that consisted of plasma increases in norepinephrine, renin–angiotensin–aldosterone hormones, and atrial natriuretic peptides (Anand et al., 1993). Similar hormonal derangements are well described in the clinical syndrome of HFrEF and contribute to disease progression. In patients with heart failure, concomitant neurohormonal and

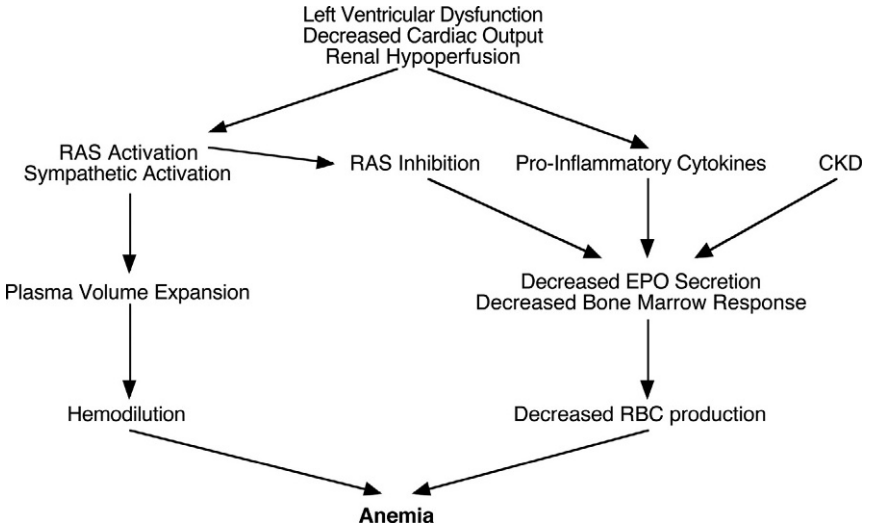

Fig. 1 Potential causes of anemia in chronic heart failure. (RAS: Renin-Angiotensin-Aldosterone System, CKD: Chronic Kidney Disease, EPO: Erythropoeitin, RBC: Red Blood Cell). With permission from Wolters Kluwer Inc., Tang and Katz (2006), Anemia in Chronic Heart Failure. *Circulation 113*, 2454–2461.

proinflammatory cytokine activation favors the development of anemia of chronic disease. This manifests as altered iron utilization, inappropriate erythropoietin production, and a depression in hematopoiesis.

Iron Defiency Without Anemia

Another important concept in congestive heart failure is not only that of anemia, but also of ID, with and without clinical anemia. Two-third of the body's iron is stored in the hemoglobin of red blood cells: 25% is stored in mobilizable stores as ferritin, and 15% is bound to myoglobin in muscle tissue and a variety of enzymes involved in oxidative metabolism (Abbaspour et al., 2014). The importance of iron in oxidative metabolism and other processes like fatty acid oxidation may provide an explanation as to why ID even without anemia can have consequences in the patient with heart failure (Jankowska et al., 2011; Haas and Brownlie, 2001) at the cellular level. Low cellular iron reserves can result in a decrease in activation of the mitochondrial respiratory chain. This leads to reduced ATP production, causing a reduction in exercise capacity and increasing fatigue (Bayeva et al., 2013; Guzman Mentesana et al., 2014).

Mechanisms of ID and Anemia

The mechanisms of ID and anemia in HF are multifactorial and an area of active investigation. Iron absorption, storage, and distribution through the body is a tightly regulated system that is controlled mainly by two pathways: the conservation of iron from the destruction of red blood cells through the mononuclear phagocytic system, and the absorption of ingested iron in the gut (Tim Goodnough et al., 2017; Ebner and von Haehling, 2013). Hepcidin, a peptide hormone synthesized by the liver, is felt to be the "master regulator" of iron metabolism in the body (Singh et al., 2011). Hepcidin induces a block in duodenal iron absorption and recycling through the mononuclear phagocytic system (Singh et al., 2011). In HF, there is an increase in TNF-alpha and IL-6 which can contribute to ID and anemia in several ways: reduced erythropoietin production in the kidney, hepcidin-induced failure of iron absorption from the gut, and hepcidin-induced trapping of iron in iron stores of macrophages (Tim Goodnough et al., 2017; Silverberg et al., 2011). In the initial phases of HF, hepcidin is upregulated, which results in the development of ID (Jankowska et al., 2013). Over time, as the disease process progresses, the opposite occurs with hepcidin downregulation, potentially in an attempt to reverse prior ID (Jankowska et al., 2013). There may also be a contribution from heart failure-induced cardiorenal syndrome which could worsen or potentiate ID in mechanisms similar to that of CKD (Silverberg et al., 2011).

There have been two pivotal clinical trials conducted to determine the impact of ID and anemia on patients with heart failure.

Ferric Carboxymaltose in Patients With Heart Failure and ID (FAIR-HF)Anker et al., 2009

Anker et al. were the first to study ID with or without anemia in a multicenter, double-blind, randomized, placebo-controlled parallel group study conducted across 75 sites (Anker et al., 2009). Major inclusion criteria in the study included stable ambulatory HF patients with ID, with NYHA class II symptoms and left ventricular ejection fraction (LVEF) <40% or NYHA class III symptoms

and LVEF <45%. Patients were included if they had ID, defined as a ferritin <100 ng/mL or ferritin 100–300 ng/mL with an iron saturation of <20%, and a hemoglobin between 9.5 and 13.5 g/dL. Patients with uncontrolled hypertension, other significant heart disease, inflammation, or significantly impaired liver or renal function were excluded. Patients were randomized in a 2:1 fashion to receive ferric carboxymaltose versus placebo. Those randomized to the treatment iron received ferric carboxymaltose dose equivalent of 200 mg of iron weekly until iron repletion was achieved and then every 4 weeks during the maintenance phase. A total of 459 patients were enrolled and 304 patients were in the treatment arm, with primary endpoints being improvement in patient global assessment (PGA) at week 24 and improvement in NYHA class. At 24 weeks, 50% of the treatment group were improved by PGA, while only 28% of the placebo group were improved by PGA ($P<0.001$). NYHA class had also improved to I or II in 47% of the treatment group as compared with 30% in the placebo arm at 24 weeks ($P<0.001$). Secondary outcomes of 6-minute walk time distance (6MWT), Kansas City Cardiomyopathy Questionnaire (KCCQ) score, and EuroQol five dimensions questionnaire (EQ-5D) were all improved at 24 weeks in the treatment group as well. There was no difference in side effects leading to premature discontinuation in either group. FAIR-HF was the first large-scale randomized controlled trial that showed iron repletion would be a viable target for HF therapy.

Beneficial Effects of Long-Term Intravenous Iron Therapy With Ferric Carboxymaltose in Patients With Symptomatic Heart Failure and ID (CONFIRM-HF) Ponikowski et al., 2015

Based on the improvement in 6MWT (a secondary endpoint) in the FAIR-HF trial, the investigators of CONFIRM-HF set out to evaluate iron repletion in ambulatory iron deficient heart failure patients using 6MWT as their primary endpoint (Ponikowski et al., 2015). CONFIRM-HF was a multicenter, double-blind, randomized, placebo-controlled, parallel group study conducted over 41 sites. Major inclusion criteria included ambulatory HF patients with ambulatory NYHA class II or III symptoms, EF <45%, BNP >100, or NT-proBNP >400, who were iron deficient as defined by ferritin <100 ng/mL or 100–300 ng/mL with TSAT <20%, and a hemoglobin <15 g/dL. Patients with uncontrolled hypertension, infection, clinical evidence of current malignancy, or significantly impaired liver or renal function were excluded. There was no lower limit to hemoglobin but patients having an immediate need for transfusion were excluded. Patients were randomized in a 1:1 fashion to receive ferric carboxymaltose or placebo. Patients in the treatment arm received between 500 and 2000 mg of iron within the first 6 weeks of the study, and thereafter patients received 500 mg of iron each at weeks 12, 24, and 36 if ID was still present. A total of 304 patients were randomized with 152 in the treatment arm and 152 in the placebo arm. The primary endpoint of the study was 6MWT at 24 weeks. At baseline, 6MWT was similar between treatment and placebo groups [288 +/−98 m vs. 302 +/−97 m, respectively]. At 24 weeks, there was a significant improvement of 6MWT by 33 +/−11 m when compared with the placebo arm ($P=0.002$). Functional and quality of life secondary endpoints included change in PGA, 6MWT distance, fatigue score, KCCQ, and EQ-5D at 6,12,24,36, and 52 weeks. PGA was improved at week 12 onward, NYHA from week 24 onward, 6MWT distance at weeks 36 onward, fatigue score week 12 onward, KCCQ at 12, 24, and 52 weeks, and EQ-5D at 36 weeks. Outcome-related secondary endpoints showed no mortality benefit seen in the 1-year follow-up period, however there was a statistically significant reduction in the combined secondary endpoint of hospitalizations due to worsening HF or death. There was no difference in the side-effect or safety endpoints leading to premature discontinuation in either group. The CONFIRM-HF study showed using the functional endpoint of 6MWT those patients who were iron replete independent of anemia status improved their functional capacity. At 24 weeks, there was a difference in 6MWT of 33 m +/−11 m. This was statistically significant at each follow-up interval in the secondary analysis (out to 52 weeks), which confirmed the findings in the secondary analysis of FAIR-HF.

Anemia of Chronic Disease

Defined by a reduced serum iron and transferrin concentration and a low total iron binding capacity along with a normal to high ferritin level, anemia of chronic disease is a prevalent entity in the heart failure population, with incidence varying in the literature. Erythropoiesis is dependent on a number of mechanisms, many of which can be altered in patients with heart failure. Erythropoietin is produced in the kidney. The primary stimulus for erythropoietin production is low serum oxygen content (Anand, 2008), which occurs due to a reduction in renal blood flow and glomerular filtration rate in patients with HF. However, the amount by which erythropoietin production rises is blunted in comparison with the severity of anemia (Opasich et al., 2005).

In addition, heart failure results in an overall active proinflammatory state, including elevated levels of tumor necrosis factor-α, interleukin (IL)-6, CRP, and other proinflammatory cytokines, which inhibit erythropoiesis in the kidney via a series of complex mechanisms (Jelkmann, 2009).

In patients with heart failure who develop anemia without ID, alternate methods of therapy are often considered. Trials using darbepoetin alfa (Swedberg et al., 2013; Pfeffer et al., 2009) to correct hemoglobin to a target level of 13.0 g/dL showed no reduction in death, cardiovascular events, or HF hospitalizations, but did result in a concomitant increase in fatal and nonfatal strokes. The medical community has therefore steered away from the use of EPO-analogs to treat mild anemia in heart failure.

Recommendations

Anemia and, more specifically, ID have been shown to be associated with worse outcomes and symptoms in heart failure patients. The treatment of ID in particular can help improve quality of life and improve functional capacity. Currently, the only guidelines for screening for ID are provided by a consensus document of the Spanish Society of Internal Medicine and Spanish Society of Cardiology. They recommend laboratory screening for ID by monitoring ferritin and iron saturation is performed at diagnosis of HF, during episodes of decompensation, and annually for stable outpatients with HF (Manito et al., 2016). We feel these are reasonable recommendations; the current body of evidence currently only supports iron repletion in stable outpatients with heart failure. There is no evidence for or against treatment or monitoring of ID in the inpatient setting, but given the potential for improved outcomes and the relatively low risk of harm, we generally replete patients with intravenous iron in the inpatient setting, when appropriate. In those diagnosed with ID, we would also recommend screening at 3 months after repletion to ensure iron stores continue to be robust. This also allows for a determination of whether more aggressive iron repletion is necessary or whether the patient can go onto a maintenance regimen of iron repletion. In addition to these recommendations, patients with ID should undergo age-appropriate screening for other common causes of ID, that is, gastrointestinal losses due to bleeding sources, including malignancy. There is limited data for the route of iron repletion and all positive evidence for iron repletion has been studied via the parenteral route. The IRON-HF study compared iron repletion of eight patients with IV iron and seven patients with oral iron. It showed that both preparations corrected the hemoglobin levels, but parenteral iron only was associated with an increase in peak VO2 over a 3-month follow-up period (Beck-da-Silva et al., 2013). Based on the current body of evidence we would recommend when possible in a real world setting to use parenteral iron and if not possible then default to oral iron repletion.

Future

Recent evidence has suggested that iron can be a novel target at least for symptoms of congestive heart failure. The mechanisms of ID are multifactorial and per our discussion earlier, there is a suggestion that parenteral treatment of ID in stable outpatients with congestive heart failure leads to functional and symptomatic benefits. The signal toward improvement of hospitalizations in CONFIRM-HF is intriguing and lays the groundwork for future larger scale studies to investigate this question. Currently, there are several interesting studies in the area of parenteral iron repletion which will answer important questions. Preliminary data from the effect of ferric carboxymaltose on exercise capacity in patients with iron deficiency and chronic heart failure (EFFECT-HF—NCT01394562) study (completed in 2016) show an improvement in peak oxygen consumption (VO2), an important prognostic marker in heart failure, with IV Iron repletion versus placebo. The study to compare ferric carboxymaltose with placebo in patients with acute heart failure and iron deficiency (AFFIRM-AHF—NCT02937454) will aim to evaluate the effect of iron infusion on the population of patients hospitalized with acute heart failure. Primary endpoints will include HF hospitalizations after randomization and composite HF hospitalizations with an endpoint of cardiovascular death. Future research will likely continue to evaluate the beneficial effects of iron repletion not only on symptoms, but also on morbidity- and mortality-related outcomes.

Another important question for future research is the difference between parenteral iron and oral iron for treatment in HF patients. This remains an important logistical question, as ease of implementation and cost remain major differences between the two routes of repletion. The oral iron repletion effect on oxygen uptake in heart failure (IRONOUT-HF) study seeks to improve understanding of the question by comparing oral iron repletion to placebo in 225 patients with NYHA II–IV HF and LVEF $\leq$ 40%. Preliminary results have suggested no change in peak VO2 with oral iron repletion compared with placebo. Continued research into iron repletion for ID should seek to answer the above questions, along with seeking to definitively answer the question of whether there is a mortality or morbidity benefit to iron repletion. The question of how to manage patients with anemia who are not iron deficient is a difficult one, and ongoing close monitoring of these patients will have to occur, along with ensuring they are appropriately replete in Vitamin B12 and folic acid stores.

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Angiogenesis

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Glossary

Angiogenesis The sprouting of new blood capillaries from preexisting vasculature
Hypoxia Insufficient tissue oxygen abundance
Mural cell Cells that surround EC, including pericytes and vascular smooth muscle cells
Notch A cell surface signaling molecule involved in many important developmental processes including vascular branching and maturation
Pericyte Specialized mural cells which surround microvascular EC
Phalanx cell Quiescent, closely interlocked EC, present in stable blood vessels
Shear stress The physical force exerted by blood flow in vessels
Stalk cell Proliferating EC forming the base of a new vessel sprout
Tip cell The EC selected to guide the advancement of new vessel sprouts
Vasculogenesis De novo formation of vascular cells during embryogenesis

Introduction

The vascular system has permitted the evolution of complex multicellular organisms by overcoming the limitations of diffusion as a means of delivering oxygen and metabolic substrates. It has been estimated that the adult human vasculature has a surface area similar to a soccer pitch, emphasizing its pervasiveness and capacity to influence health (Aird, 2005). However, the vasculature is much more than a passive transport system; for example, it actively regulates tissue perfusion, leukocyte trafficking, and hemostasis (Aird, 2005). More recently, it has even been shown to regulate processes such as bone marrow stem cell quiescence (Itkin et al., 2016) and systemic metabolism (Kubota et al., 2011). Hence, we have also evolved highly regulated processes to ensure that the vascular system remodels appropriately in response to changing physiological demands.

Angiogenesis, the sprouting of new capillaries from the existing microvasculature, was first described in the 18th century, but its potential as a therapeutic target was not proposed until the 1970s when Judah Folkman hypothesized its requirement for cancer growth (Folkman, 1971). We now recognize abnormal angiogenesis to contribute to many diseases (Carmeliet, 2003), which are often characterized by insufficient or excessive neovascularization. However, this distinction may be overly simplistic since it

neglects the importance of new vessel quality, and overlooks the cooccurrence of "excessive" and "insufficient" angiogenesis in some disease processes (e.g., diabetes). This may explain why therapeutic manipulation of angiogenesis has not been uniformly successful (Potente et al., 2011), and supports the need for more nuanced therapeutic approaches. In this article, we discuss contemporary understanding of the process of angiogenesis, outline how this is dysregulated in common diseases, examine current therapeutics, and speculate how recent advances may improve therapeutic approaches.

The Origins of the Vascular System

In vertebrates, the vascular system develops early during embryogenesis in a process called vasculogenesis, where angioblasts (vascular progenitor cells) form de novo from the mesoderm and aggregate as a primordial capillary plexus. Vessel sprouting enlarges this primitive vasculature, which then remodels and matures into a hierarchical network of arteries, veins, and capillaries, which is required for effective circulation of blood (Potente et al., 2011). However, arteriovenous specification is determined before the onset of blood circulation, suggesting regulation by genetic factors, rather than shear stress generated by flow. In zebrafish, the cardinal vein develops by segregation and selective migration of venous angioblasts from the common vessel primordium, which later forms the dorsal artery, and this process is highly regulated (Herbert et al., 2009).

Differential sensitivity to vascular endothelial growth factor (VEGF), with subsequent alterations in Notch signaling, appears important in initiating endothelial cell (EC) arteriovenous fate (Swift and Weinstein, 2009). Moreover, EphrinB2 and its corresponding receptor EphB4 are also differentially expressed in arteries and veins before the onset of circulation (Wang et al., 1998; Gerety et al., 1999), and their interaction regulates angioblast migration and arteriovenous segregation (Herbert et al., 2009). Notch signaling suppresses venous fate by enhancing EphrinB2 expression, and simultaneously inhibiting EphB4 expression. Notch signaling in arteries is stimulated by VEGF, while venous phenotype appears to be actively promoted by chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII), which suppresses Notch, so promoting EphB4 expression (Kuijper et al., 2007).

Adjacent to the blood-containing vasculature, the body develops a lymphatic system during embryogenesis to maintain fluid homeostasis. COUP-TFII-expressing venous EC give rise to lymphatic EC, which migrate and build primitive lymph sacs to develop the lymphatic vasculature further (Yang and Oliver, 2014).

Defining Angiogenesis

The formation of new capillaries from preexisting blood vessels is termed angiogenesis, and is distinct from vasculogenesis, which only occurs in utero. Angiogenesis is traditionally defined as the process of capillary sprouting from preexisting vasculature, and is most strongly induced by low tissue oxygen tension (hypoxia), although can be promoted by other biological processes, as discussed later. Besides classical "sprouting angiogenesis," new vessels can also emerge from preexisting ones by "splitting angiogenesis," often termed intussusception; in this case, transluminal pillars develop, which expand to separate two new vessels. Intussusception occurs as a result of altered blood flow during development or physical exercise, and under pathological conditions (e.g., tumorigenesis) (Makanya et al., 2009; Gianni-Barrera et al., 2013). Remodeling of the vasculature by either form of angiogenesis is essential during postnatal development, and in response to many physiological phenomena, to ensure the vascular system is matched to the changing local demands.

The Process of Angiogenesis

Sprouting angiogenesis occurs in response to proangiogenic molecular signals (in particular, VEGF), which activate EC, resulting in the extension of protrusions called filopodia that sense the origins of angiogenic signals (Phng and Gerhardt, 2009). EphrinB2 has been shown to be essential for VEGF/VEGF receptor (VEGFR) internalization, and accordingly VEGF signal transduction (Sawamiphak et al., 2010; Wang et al., 2010). Moreover, Neuropilin1 acts as a coreceptor for VEGF and is pivotal in effecting normal VEGFR activation (Lanahan et al., 2013; Bjo et al., 2014), further emphasizing the intricate regulation of angiogenic growth factor signaling.

Upon growth factor signaling, EC must break down their enveloping basement membrane, which they share with mural cells (pericytes and vascular smooth muscle cells), in order to allow formation of a "tip cell" that guides the new vessel sprout. To achieve this, tip cell filopodia secrete matrix metalloproteases (MMPs), such as MMP1 (Potente et al., 2011), to enzymatically digest basement membrane proteins. This liberates further proangiogenic factors to support sprouting, although antiangiogenic factors are also released to prevent excessive sprouting and ensure adequate stability of neovessels (Nyberg et al., 2005). Mural cell detachment is facilitated by release of Angiopoietin 2 (Ang2) from EC (Maisonpierre et al., 1997), which is thought to be stored in Weibel–Palade bodies (Augustin et al., 2009). Ang2 competitively binds the EC receptor Tie2, which disrupts the Ang1/Tie2 signaling required to maintain vascular quiescence (discussed later) (Maisonpierre et al., 1997).

Once an endothelial tip cell has emerged, it is followed by proliferating endothelial "stalk cells," which extend the new sprout toward proangiogenic growth factor gradients detected by the tip cell. The decision of EC to adopt tip or stalk status is critical to

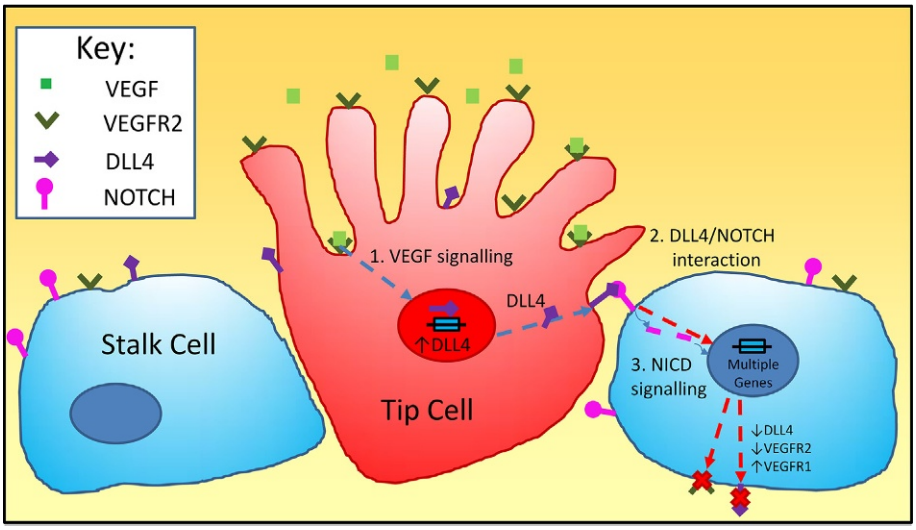

Fig. 1 Lateral inhibition during tip/stalk selection. During angiogenic sprouting, endothelial cells (EC) with higher levels of VEGF signaling express greater concentrations of cell surface Dll4, which interacts with Notch on neighboring EC. This molecular interaction results in cleavage of the Notch intracellular domain (NICD), which translocates to the nucleus and modulates gene expression, resulting in suppression of VEGFR2 and Dll4, along with increased expression of the antiangiogenic VEGF receptor VEGFR1. This communication is dynamic and bidirectional, with the cells expressing most Dll4 at any instant becoming tip cells, which actively inhibit tip cell fate in their neighbors.

effective angiogenesis, since an excess of either phenotype would cause excessive or inadequate sprout formation, respectively. Therefore, an intercellular communication system has evolved whereby EC compete to suppress tip cell status in their neighbors, in a process called "lateral inhibition," which has parallels across biology (e.g., branching during lung or glandular development) (Fig. 1). Critical to this intercellular competition are the cell surface molecules Notch and Delta-like ligand 4 (DLL4), which are differentially expressed in tip and stalk cells, with higher DLL4 and lower Notch in "successful" tip cells. Proangiogenic VEGF signaling via VEGFR2 enhances EC surface expression of DLL4, which binds to Notch1 receptors on neighboring EC. This binding causes enzymatic cleavage of Notch, freeing its intracellular domain to induce gene expression changes, including feedback inhibition of DLL4 and VEGFR2 (Phng and Gerhardt, 2009). Accordingly, only those EC with the greatest DLL4 expression can become a leading tip cell, whereas remaining cells are forced to adopt stalk status (Jakobsson et al., 2010). Notch signaling also influences intercellular adhesion mediated by VE-cadherin, further promoting tip/stalk cell rearrangement during angiogenesis (Bentley et al., 2014), and importantly, tip/stalk fate is dynamic and frequently changes during the sprouting process (Jakobsson et al., 2010). The ratio of tip cells to stalk cells is of great importance in establishing adequate vasculature, with deletion of either DLL4 or Notch 1 being embryonically lethal (Krebs et al., 2004). Interestingly, the excessive sprouting associated with DLL4 inhibition results in decreased tumor perfusion and size (Thurston et al., 2007).

The developing sprout needs to establish a lumen and integrate within appropriately perfused circuits if it is to make a persistent contribution to the vasculature; inadequately perfused sprouts will rapidly regress. Lumen formation by stalk cells is established by processes called cell and cord hollowing, in which cellular vesicle transport and repulsive membrane interactions, respectively, create a space for blood flow between or within EC (Strilić et al., 2009). New vessel circuits are then established by sprout anastomosis, a phenomenon where neighboring tip cells fuse by forming VE-cadherin junctions, which can be orchestrated by tissue macrophages (Fantin et al., 2010).

New vessel maturation and stabilization is achieved by basement membrane matrix deposition and mural cell recruitment (Fig. 2). Platelet-derived growth factor (PDGF) is secreted by tip and stalk cells and binds to heparan sulfate proteoglycans at the cell surface (Abramsson et al., 2007). PDGF, together with the epidermal growth factor HB-EGF, stimulates mural cell migration and proliferation (Stratman et al., 2010), resulting in pericyte recruitment occurring simultaneous to sprout elongation (Armulik et al., 2011). PDGF/PDGF receptor-β (PDGFRβ) signaling is crucial to stabilize EC-pericyte interactions in the neovasculature, and disruption of this signaling pathway is lethal during embryogenesis (Lindahl et al., 1997). After initial pericyte recruitment, Ang1/Tie2 signaling is crucial to the maturation process, and also maintains pericyte coverage in quiescent vessels; developmental defects in either Ang1 or Tie2 have been demonstrated to be lethal (Suri et al., 1996). Interestingly, recent research has also demonstrated that reciprocal regulation of Tie1/2 receptors is required during sprouting angiogenesis (Savant et al., 2015), with the orphan receptor Tie1 mediating complex regulation of Tie2 activity, and hence Ang signaling. In quiescent EC, Tie1 expression is reduced, allowing Tie2 signaling in response to pericyte-derived Ang1. Tie1 expression increases in remodeling in stalk cells, where it unexpectedly sustains Tie2 activation, yet greater Tie1 expression in tip cells disrupts Tie2 activity, which is required for normal sprouting. Hence complex Tie1/Tie2 interactions ensure breakdown of the basement membrane and mural cell detachment so that sprout formation can be initiated, but also promote effective maturation and quiescence of new sprouts. EphrinB2 signaling is also involved in mural cell recruitment, with global- (Adams et al., 1999) and mural cell-specific (Foo et al., 2006) deletion causing

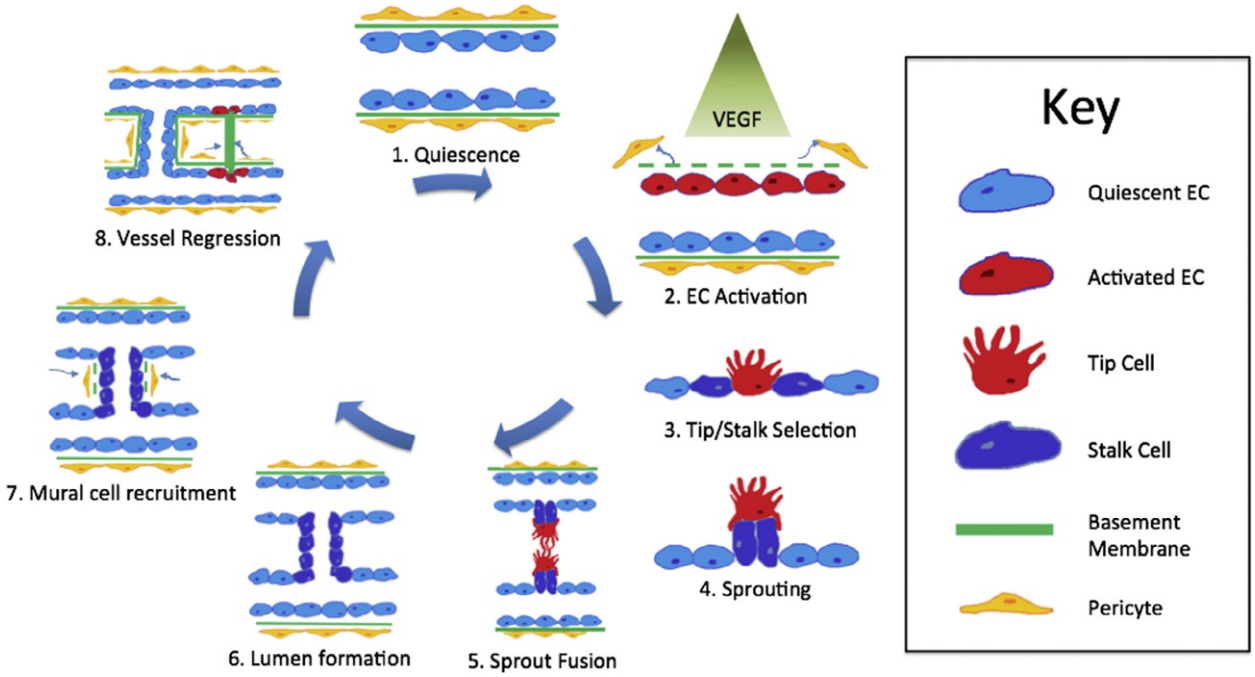

Fig. 2 Progression from vascular sprout to mature vessel. When a quiescent vessel (1) experiences increased concentrations of proangiogenic growth factors (2), endothelial cells (EC) become activated, releasing cytokines and enzymes that promote pericyte shedding and basement membrane degradation. EC with the highest levels of VEGF signaling become selected as tip cells, which actively induce stalk cell phenotype in their neighbors (3—see also **Fig. 1**). Tip cells migrate toward the greatest concentrations of proangiogenic growth factors, with following stalk cells proliferating to form a vascular sprout (4). Sprouts then anastomose (5) with the assistance of macrophages to form vascular loops, which then form perfused lumens (6) that mature as migrating pericytes envelop the vessel and contribute to basement membrane deposition (7). Finally, neovessels experiencing low flow selectively regress (8), with this cycle continuing iteratively until a mature vascular plexus is formed, which matches local blood supply to demand.

vascular anomalies including defective mural cell attachment, along with embryonic or postnatal lethality. Pericyte recruitment progresses alongside vascular basement membrane deposition, with both EC and pericytes secreting collagen type IV, laminin and fibronectin, thereby further enhancing neovascular stabilization (Stratman et al., 2009).

EC within new vessels further mature by developing a quiescent "phalanx" phenotype, characterized by closely interlocked nonmigratory nonproliferative cells, which is promoted by Notch signaling (Ehling et al., 2013). Blood flow then promotes remodeling of the vasculature, with regression or "pruning" of poorly perfused vessels. The expression of Krüppel-like factor 2 (KLF2) mediates blood flow-induced changes in EC gene transcription, such that when a vessel is not perfused, shear stress and hence KLF2 signaling is low, and the vessel regresses (Potente et al., 2011). Depending on the vascular bed, cell adhesion molecules such as VE-cadherin and N-cadherin, and tight junctions allow cell–cell contact and barrier function. VE-cadherin also diminishes VEGFR2 signaling to suppress proangiogenic VEGF activity (Potente et al., 2011), and in order to survive in this quiescent milieu, EC and pericytes express prosurvival signals, such as VEGF and Ang1, respectively. Thereby, VEGF operates in an autocrine manner, activating the PI3K/Akt pathway in EC, to promote survival (Lee et al., 2007; Warren and Iruela-Arispe, 2010).

Modulation of Angiogenesis

Hypoxia is probably the most potent stimulus for angiogenesis, and the expression of hypoxia-inducible factors (HIFs) and prolyl hydroxylase domain (PHD)-containing enzymes allow exquisite cellular oxygen sensitivity. Adequate tissue perfusion permits oxygen-dependent hydroxylation of HIFs by PHDs, which targets them for proteasomal degradation. When oxygen is limited, PHDs remain inactive, so HIFs are not degraded and can alter gene transcription (e.g., increased VEGF) to increase angiogenesis, and so oxygen supply (Aragones et al., 2009; Ivan et al., 2001). HIF1α is required for hypoxia-induced autocrine VEGF/VEGFR2 expression, and accordingly EC proliferation and migration (Tang et al., 2004). Hence, oxygen levels modulate VEGF expression, and adequate oxygenation of phalanx EC reduces VEGF expression to a prosurvival level. Further, HIF1α can modulate EC fate; activation of DLL4/Notch signaling by HIF1α suppresses COUP-TFII, and supports arterial cell fate (Diez et al., 2007), which may be important in augmenting tissue perfusion. The role of EC HIF2α is less well understood, but appears only to partially overlap HIF1α function, and is crucial in promoting maturation of new arteries in a Notch-dependent manner (Skuli et al., 2012).

Low oxygen levels are often accompanied by decreased nutrient supply, which can also modulate angiogenesis (**Fig. 3**). For example, AMP-dependent kinase (AMPK) is activated by energy depletion, associated with hypoxia and glucose deprivation,

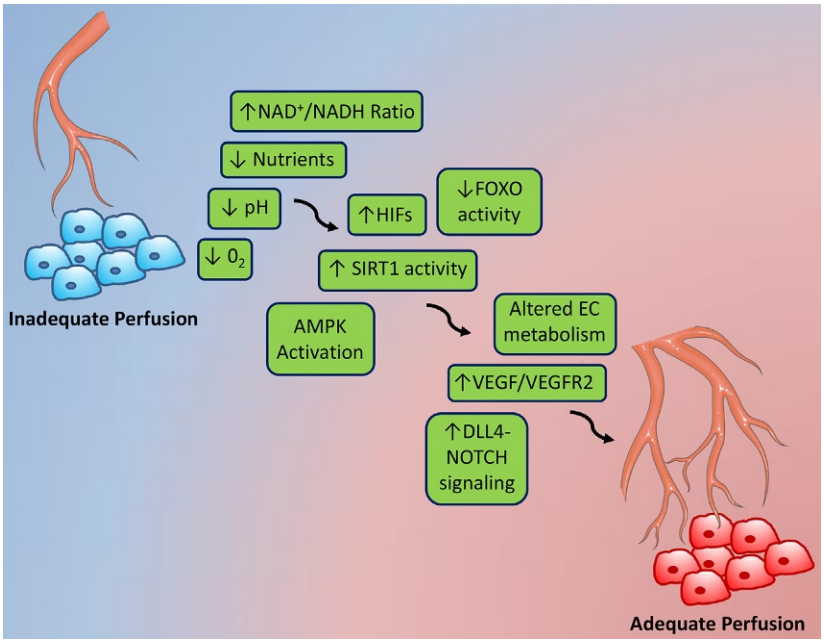

Fig. 3 Processes that induce angiogenesis. Inadequate tissue perfusion results in hypoxia, acidosis, nutrient scarcity, and rising NAD^+/NADH ratio (reflecting reduced catabolism of metabolic substrates). These phenomena are detected by an array of molecular sensors (a small selection of which are illustrated), including hypoxia inducible factors (HIFs), AMP-dependent protein kinase (AMPK), NAD^+-dependent deacetylases (e.g., Sirtuin1 or SIRT1), and Forkhead transcription factors (FoxOs). Cumulatively, these sensors orchestrate a molecular response, including increased VEGF production and signaling, increased Dll4/Notch signaling, and altered endothelial cell (EC) metabolism, which promote angiogenesis, and aim to restore adequate tissue perfusion. Elements of this figure were adapted from images made freely available by Servier Medical Art (http://www.servier.com/Powerpoint-image-bank).

and stimulates catabolic pathways in order to generate ATP (Long and Zierath, 2006); it is also a potent stimulus for VEGF expression (Ouchi et al., 2005). Low cellular nutrient supply is also associated with an increase in the NAD^+/NADH ratio, which is sensed by deacetylating enzymes called Sirtuins, which can augment proangiogenic gene expression and modify the activity of proteins such as Notch (Potente et al., 2007; Phng et al., 2011). Notably, PHD enzymes also require 2-oxoglutarate (an aerobic metabolite of the Kreb's cycle) in order to hydroxylate HIFs, so under nutrient deprivation HIFs are less susceptible to degradation (Aragones et al., 2009). Beyond the scope of this discussion, many other transcription factors regulate EC responsiveness to nutrient deprivation, including peroxisome-proliferator-activated receptors (PPARs) and forkhead box proteins (e.g., FOXOs) (Aragones et al., 2009).

Beyond oxygen and nutrient supply, inflammation is another major modulator of angiogenesis. Inflammation is characterized by tissue invasion of inflammatory cells, such as neutrophils, monocytes, and macrophages, which secrete an array of vasoactive cytokines and growth factors. In acute inflammation, cytokines induce vessel dilation to enhance blood flow and increase vascular permeability, allowing more cells to invade and repair tissue damage (Szade et al., 2015; Muller, 2003). However, in persistent chronic inflammation, EC become activated and undergo a proangiogenic switch, where interleukins and tumor necrosis factor α (TNFα) can stimulate sprouting (Jackson et al., 1997). For example, TNFα induces the transcription factor NFκB, resulting in EC Ang2 expression and initiation of the sprouting process (Pober and Sessa, 2007; Imhof and Aurrand-Lions, 2006). Moreover, macrophages can secrete classical proangiogenic factors, such as VEGF, further driving vascular sprouting, but often in a poorly coordinated manner resulting in abnormal vascular structure and function (Jackson et al., 1997).

Angiogenesis and Vascular Heterogeneity

Beyond the clear differences between arterial, venous, lymphatic, and capillary EC, there is notable heterogeneity across microvascular EC, which reflects the diverse requirements of organ- or tissue-specific vascular beds. Whereas neurovascular EC form a restrictive "blood–brain barrier" to regulate cellular and molecular transit to the brain parenchyma, renal glomerular EC are fenestrated to facilitate blood filtration (Nolan et al., 2013). Besides the heterogeneity of EC in function (e.g., permeability, thermoregulation, or vasomotor tone), EC also differ in shape. For example, arterial EC are longer than venous EC, and possess a higher surface area; when arterial segments were surgically inverted, EC realign with flow within 10 days, demonstrating EC remodeling in response to shear stress (Aird, 2007). Endothelial heterogeneity has been attributed to genetic and (micro) environmental factors, as each EC responds to its local environmental biomechanical and biochemical milieu (Aird, 2007). Thereby, EC function as an organotypic cell niche, secreting angiocrine factors which modify the tissue parenchyma, yet also

responding to parenchymal signals that reciprocally manipulate vascular behavior (Rafii et al., 2016). Hence, even after the process of angiogenesis has formed or extended a vascular bed, this neovasculature must then adapt its form and function to its environment.

Diseases Associated With Abnormal Angiogenesis

While it is beyond the scope of this article to offer a detailed description of angiogenic abnormalities associated with the range of human diseases (Table 1), some key themes emerge by considering a few important examples. In particular, these serve to highlight our evolving and incomplete appreciation of the processes that cause abnormal angiogenesis, but also emphasize the potential value of effective therapeutics.

Inherited Disorders

Many rare genetic disorders interfere with angiogenesis and vascular patterning. While these generally represent the most extreme forms of vascular abnormality, elucidating their mechanisms has transformed our understanding of how blood vessels develop and mature. One important example is hereditary hemorrhagic telangiectasia, which causes diffuse arteriovenous vascular malformations, resulting in problems such as chronic gastrointestinal bleeding and high-output heart failure (Dupuis-Girod et al., 2010). It is caused by mutation of proteins involved in the TGF-β signaling system, which mediates complex endothelial–mural cell interactions during maturation of newly formed vessels (Mahmoud et al., 2011). Another interesting example is cerebral cavernous malformations, which are friable and disordered vascular growths, associated with mutations in a range of genes that have recently been shown to converge on mitogen-activated protein kinase signaling (Zhou et al., 2016). Excessive signaling through this pathway in EC causes abnormal venular dilatation in mice, similar to human malformations, although the precise mechanism of this remodeling remains unclear. These examples illustrate the nuanced regulation of normal vascular development and quiescence, although the contribution of these specific mechanisms to noninherited diseases associated with abnormal angiogenesis is unclear.

Cancer

It has long been recognized that tumors are highly vascular, leading Judah Folkman to make his seminal hypothesis that tumor growth is dependent on angiogenesis, and that this could be therapeutically targeted (Folkman, 1971). Moreover, blood vessels are frequently the conduit through which tumors spread to distant sites, a process called metastasis. Although current antiangiogenic therapies have not achieved dramatic responses in most cancers (discussed later), it remains accepted that angiogenesis is crucial to tumor biology, and continues to be an enticing therapeutic target. However, as our understanding of tumor vasculature evolves, it is becoming clear that reducing the abundance of vessels in tumors may be an overly simplistic therapeutic goal. For example, tumor perfusion is heterogeneous (Hensley et al., 2015), and often impaired as a result of abnormal vascular architecture (Stylianopoulos and Jain, 2013). Moreover, some have even speculated that adverse tumor environments, characterized by ischemia and acidosis caused by inadequate perfusion, result in evolutionary pressure favoring selection of more aggressive tumor clones (Potente et al., 2011). Recent data have also highlighted the possibility of tumor vascular mimicry, a process by which cancer cells may form a perfused network, independent of vascular cells, suggesting antiangiogenic therapies alone may be inadequate (Wagenblast et al., 2015). As discussed later, such observations are leading to the development of entirely novel therapeutic approaches.

Table 1 Examples of diseases associated with abnormal angiogenesis

Organ or tissue	*Diseases associated with "excessive" or abnormal angiogenesis*	*Diseases associated with "impaired" angiogenesis*
Multiorgan	Cancer, infectious diseases, vasculitic, and autoimmune disorders (e.g., systemic sclerosis)	
Cardiovascular	Monogenic vascular disorders (e.g., hereditary hemorrhagic telangiectasia), atherosclerotic plaque	Diabetes (except retinopathy), critical limb ischemia, lymphedema
Nervous system	Multiple sclerosis	Amyotrophic lateral sclerosis, Alzheimer's disease
Gastrointestinal	Inflammatory bowel disease	
Skin	Psoriasis, eczema	
Reproductive system	Endometriosis	Preeclampsia
Ocular	Diabetic retinopathy, wet age-related macular degeneration, retinopathy of prematurity	
Adipose	Obesity	
Musculoskeletal	Rheumatoid arthritis	Fracture nonunion
Respiratory	Asthma, primary pulmonary hypertension	Bronchopulmonary dysplasia

Some disorders may overlap across multiple organs or tissues, and as discussed in the text, it is important to note that the distinction between excessive and impaired angiogenesis is imperfect, for example, in the case of diabetes.

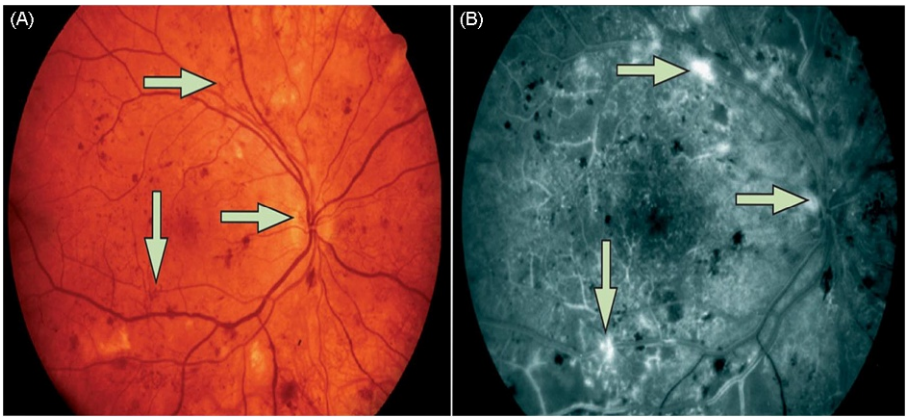

Fig. 4 Diabetic retinopathy. Retinal photomicrograph (A) and corresponding fluorescein angiogram (B) illustrating new vessel formation (*arrows*) around the macula, with associated leakage from abnormal neovessels indicated by intense white signal on angiography. Yellow exudates and red hemorrhages are also seen throughout the retina (A). Adapted from Cheung, N., Mitchell, P. and Wong, T. Y. (2016). Diabetic retinopathy. *Lancet* **376** (9735), 124–136, with permission from Elsevier.

Diabetes

Diabetic retinopathy provides a vivid illustration of the microvascular abnormalities that affect many vascular beds in people with diabetes (Fig. 4). In its early stages, it is associated with a loss of pericytes, which leads to capillary regression, along with the development of microaneurysms and vascular leakage (Hammes et al., 2011). These abnormalities cause retinal ischemia, which promotes angiogenesis, although the resulting neovasculature is of poor quality, frequently resulting in hemorrhage and inadequate retinal perfusion. Ultimately, visual impairment or blindness can ensue, although excellent glycemic and blood pressure management can substantially reduce the risk of microvascular complications (Holman et al., 2008a,b). The renal vasculature is also sensitive to the metabolic disturbances of diabetes, resulting in abnormal thickening and leakage of the glomerular capillary–podocyte interface. This impairs its filtration properties, which can culminate in renal failure (Reidy et al., 2014). Myocardial infarction, stroke, and limb ischemia are the principal causes of morbidity and death in people with diabetes (Booth et al., 2006), and angiogenesis is a crucial part of the adaptive response to these. Diabetes impairs the neovascularization of many ischemic tissues (Cubbon et al., 2012), and this is thought to explain some of the increased morbidity after major cardiovascular events in people with diabetes (e.g., more frequent gangrene after limb ischemia). The diminished angiogenesis in some vascular beds, coexisting with "excessive" angiogenesis in the retina is often described as a paradox. However, while there are clear differences in pathology across vascular beds, it is reasonable to generalize that diabetes causes loss of capillaries, and impairs the capacity to form structurally and functionally normal neovessels.

Age-Related Macular Degeneration (ARMD)

The "wet" form of ARMD is associated with abnormal retinal angiogenesis, and represents one of the major successes of antiangiogenic therapy. Although the pathophysiology remains incompletely understood, wet ARMD is characterized by abnormal neovascularization of the macula by vessels originating from the choroidal layers, as a result of increased local VEGF expression. These new vessels are prone to leakage and hemorrhage, which cause further retinal injury, eventually culminating in central visual field loss (Holz et al., 2014). Various anti-VEGF therapeutics (antibody- or trap-based, as discussed later) have proven able to stabilize or regress visual loss in phase-3 clinical trials, resulting in their widespread therapeutic use (Holz et al., 2014). Nevertheless, these therapies are limited by the need for repeated local injection, and can only address one facet of this multifactorial disease process.

Therapeutic Manipulation and Its Pitfalls

Growth Factor Administration and Gene Therapies to Promote Angiogenesis

Preclinical studies in the 1990s led to great excitement about the potential of recombinant growth factors, or gene therapy to promote angiogenesis in ischemic diseases. The first description of plasmid-based VEGF-A gene therapy in a patient with critical limb ischemia was published 20 years ago by Jeffrey Isner's group (Isner et al., 1996), and was soon followed by promising phase I trial data in patients with critical limb ischemia and myocardial ischemia (Baumgartner et al., 1998; Losordo et al., 1998). However, even these studies began to hint at potential challenges, with some recipients developing transient local edema (Baumgartner et al., 1998), perhaps predictably given that VEGF is known to enhance vascular permeability (Senger et al., 1983). More importantly, subsequent blinded randomized controlled trials have failed to demonstrate meaningful improvements in

relevant outcomes, such as limb salvage (Rajagopalan et al., 2003). Possible reasons for these disappointing data include: the inability of individual growth factors to promote effective angiogenesis; inappropriate magnitude, duration, and distribution of growth factor expression; growth factor resistance associated with disease states; ineffective stimulation of large vessel growth; and the inability of animal models to mimic human disease. Ongoing clinical trials of gene therapy and recombinant growth factors will address some of these issues (Cooke and Losordo, 2015).

Cell Therapies to Promote Angiogenesis

More recently, "cell therapy" has emerged as a potential approach to harness angiogenesis, with transplanted cells either directly forming neovessels or indirectly promoting endogenous vessel growth via paracrine mechanisms. Although early trials of bone marrow mononuclear cells (BMMCs) in the context of ischemic heart disease had aimed to regenerate myocardium, improvements in myocardial perfusion were often more notable than changes in ventricular function (Perin et al., 2004). While it now appears unlikely that BMMCs meaningfully improve ventricular function (Fisher et al., 2015), meta-analysis of early (phase I/II) clinical trials in the context of refractory angina supports their capacity to improve myocardial perfusion (Khan et al., 2016). However, while preclinical models suggest that BMMCs can augment angiogenesis (Kamihata et al., 2001), this may not be the mechanism of increased perfusion in humans (Stempien-Otero et al., 2015). Although many other lineages are being evaluated in clinical trials, perhaps the most relevant to angiogenesis are c-Kit$^+$ cells. In the SCIPIO phase I trial, ex vivo expanded autologous myocardial c-Kit$^+$ cells were infused into the coronary arteries of patients with ischemic cardiomyopathy (Bolli et al., 2011). A substantial improvement in ventricular function was noted using magnetic resonance imaging, in association with alterations in scar properties, which could be due to myocardial regeneration and/or increased local perfusion. Recent preclinical studies have shed important light on this, by showing that tissue-resident c-Kit$^+$ cells frequently act as local vascular endothelial progenitor cells (Liu et al., 2015; Sultana et al., 2015), and can promote substantial angiogenesis after transplantation (Fang et al., 2012). Many other cell lineages also offer potential to promote angiogenesis (e.g., induced pluripotent stem cells), but available evidence remains limited to preclinical studies (Zhang and Xu, 2014).

Antiangiogenic Therapies

As introduced earlier, therapeutic inhibition of angiogenesis is well established in the routine management of some ocular diseases and cancers. Currently available therapies use a number of approaches, including monoclonal antibodies, fusion proteins, and small molecule inhibitors, to interfere with proangiogenic ligands, their receptors, or downstream intracellular signaling cascades. VEGF-A is the principal target of the most established therapies (e.g., Bevacizumab, or Avastin—a humanized monoclonal antibody targeting VEGF-A, used in a limited number of advanced cancers; Ranibizumab or Lucentis—an antibody fragment targeting VEGF-A, used in wet ARMD; Sunitinib or Sutent—a small molecule multiple-target tyrosine kinase inhibitor, used in some advanced cancers). Although it is beyond the scope of this article to discuss these agents in depth (for excellent review see Ye, 2016), it is important to acknowledge their limitations, and significant side effects. The hope that antiangiogenic therapy would offer a step-change in cancer therapy has been tempered by experience with Bevacizumab, which is only licensed for use in a limited set of advanced cancers, and achieves modest increases in overall or progression-free survival. For example, when added to standard chemotherapy regimens, Bevacizumab has been shown to prolong overall survival of women with advanced cervical cancer by 3.7 months (Tewari et al., 2014). Moreover, its detrimental impact on the wider vasculature can cause treatment-limiting hypertension, renal dysfunction, and vascular thrombotic events, emphasizing the important role of VEGF-A in normal physiology. Such issues are common to many cancer therapies, and do not detract from the therapeutic paradigm of manipulating tumor vasculature, but clearly emphasize the need to better understand tumor angiogenesis before developing new therapies. A number of excellent review articles discuss factors that may allow more effective translation of antiangiogenic therapies (Ye, 2016; Potente et al., 2011), and some of these are highlighted in the following section.

Recent Advances

Our understanding of the fundamental processes regulating angiogenesis has advanced substantially over that past decade, and these insights are likely to be important in the development of more effective and less toxic therapies to modulate angiogenesis. It is impossible to discuss fully the breadth and depth of these advances, so we instead summarize some key developments.

Vascular Endothelial Metabolism

Metabolism broadly describes the chemical reactions upon which all life depends, such as generating energy from degrading substrates, or synthesizing proteins and nucleic acids. While the chemical reactions mediating these processes are well understood, their role in regulating cell biology is only beginning to emerge. Recent studies have shown that the vascular endothelium is unusual in the way it utilizes the classical energy substrates, glucose and fatty acids, and these observations are particularly important to angiogenesis.

Firstly, EC use glycolysis as their predominant energy source, in spite of abundantly available oxygen in the vascular lumen; this is a very inefficient way of producing energy (i.e., ATP) from glucose, when compared with oxidation of glucose. When EC glycolysis is partially inhibited, no impact is noted on cell survival, but the motility of cells is substantially reduced, and this is associated with impaired angiogenesis (De Bock et al., 2013). Importantly, this glycolysis appears to occur predominantly in the actively remodeling lamellipodia (or foot processes) required for cell migration, where mitochondria are not in proximity to supply ATP. Furthermore, it was shown that reducing glycolysis could override genetic cues (i.e., reduced Notch signaling) that promote EC tip cell formation, suggesting that metabolism is downstream of growth factor-induced angiogenic sprouting. Indeed, preclinical tumor studies have gone on to show that mice with reduced EC glycolysis have slower tumor growth (Xu et al., 2014), and pharmacological inhibition of glycolysis can reduce other forms of pathological angiogenesis (Schoors et al., 2014).

More recently, it has also been shown that EC rely on metabolism of fatty acids in an unexpected way, with important implications during angiogenesis (Schoors et al., 2015). It is generally assumed that fatty acid oxidation (FAO) is used by cells to produce energy required for homeostatic and anabolic processes. However, in EC it was shown that long-chain fatty acids, such as palmitate, are partially oxidized to provide a carbon source for nucleotide synthesis. Inhibition of FAO does not result in an EC energy deficit, but instead causes a profound reduction in proliferation, and therefore impairs angiogenesis. This use of fatty acids is relatively specific for ECs, and may represent an alternative target for antiangiogenic therapies.

Broader regulation of metabolism has also emerged as an important mechanism by which EC quiescence is maintained, thereby suppressing angiogenesis. The transcription factor FOXO1, which is repressed by many proangiogenic growth factors, appears to impair EC glycolysis and mitochondrial oxidation (Wilhelm et al., 2016). Activation of FOXO1 in ECs impairs their proliferation and reduces angiogenesis in vivo, with inhibition of FOXO1 resulting in the opposite effect. These data suggest the growth factors that modulate EC responses during sprouting angiogenesis do so, at least in part, by modulating metabolism, again highlighting the potential of EC metabolism as a therapeutic target.

Vascular Normalization

In spite of pronounced vascularization, tumors are often poorly perfused, suggesting that their vasculature is structurally and/or functionally abnormal. This can result in local ischemia, acidosis, and increased interstitial pressure, which may drive the formation of aggressive tumor clones; moreover, this is likely to impair delivery of chemotherapy. Electron microscopy of tumor vessels has revealed pronounced irregularity of the luminal surface, with a loss of cell–cell contacts and frequent luminal filopodial protrusions, in stark contrast with the typical organized phalanx pattern of EC in normal vessels (Mazzone et al., 2009) (**Fig. 5**). By reducing the expression of the oxygen sensor PHD2 in EC, effectively promoting hypoxia sensing, it was possible to restore (or "normalize") the typical vessel architecture, although this had no impact on the number of tumor vessels. Crucially, this was associated with marked increases in tumor perfusion, but with corresponding decreases in tumor metastasis. This observation poses a major challenge to prior assumptions about what therapeutic modulation of tumor vasculature should achieve—in other words, perhaps normalization of vessels, rather than destruction of vessels may be a more effective strategy. More recent work has shown that tumor vessel normalization can be achieved in preclinical models by administration of chloroquine (Maes et al., 2014), suggesting that this established malaria and rheumatoid arthritis therapy could be repurposed in cancer clinical trials.

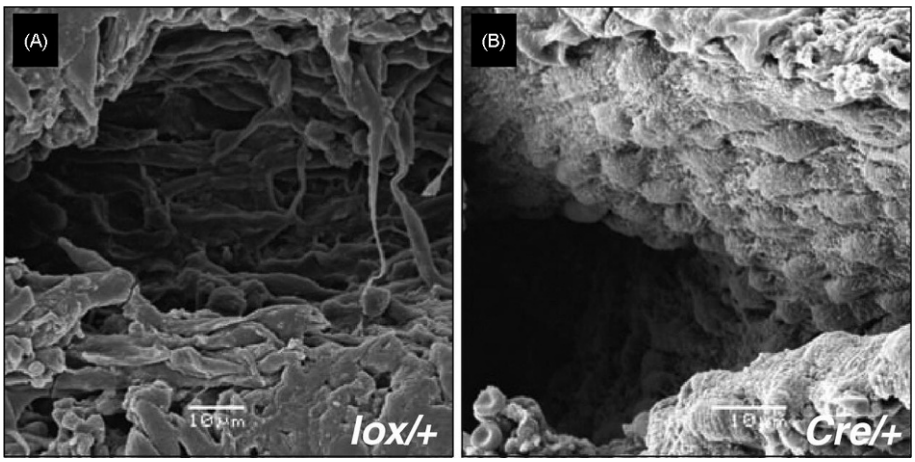

Fig. 5 Endothelial normalization. Scanning electron micrographs of tumor microvessel in control (A) and PHD2$^{\pm}$ (B) mice, illustrating the abnormal irregular endothelial morphology of tumor vessels, which is normalized to an organized phalanx endothelial morphology by PHD2 haploinsufficiency. Adapted from Mazzone, M., et al. (2009). Heterozygous deficiency of PHD2 restores tumor oxygenation and inhibits metastasis via endothelial normalization. *Cell* **136** (5), 839–851, with permission from Elsevier.

Endothelial Shear Sensing in Angiogenesis

The vasculature is continually exposed to the mechanical force of blood flow, which exerts shear stress upon EC. It is well recognized that these mechanical forces influence vascular remodeling and angiogenesis, but the nature of this relationship and its underlying mechanisms are continuing to emerge. Recently, it has been shown that sprouting angiogenesis can be induced and sustained when a threshold of local shear stress is exceeded, and that this may offer a homeostatic mechanism regulating shear forces across a vascular network (Galie et al., 2014). Interestingly, it has also been shown that perception of shear stress regulates EC metabolism by reducing glycolysis and mitochondrial content, which presumably results in cellular quiescence in mature perfused vessels (Doddaballapur et al., 2015). Another important finding from recent research is the role of an ion channel called Piezo1 in EC shear sensing, or mechanotransduction (Li et al., 2014). Piezo1 was previously known as a sensor of noxious mechanical stimuli, but when knocked down in EC, prevents the usual shear stress-induced calcium influx and cellular alignment with blood flow. Moreover, a lethal failure of vascular development occurred in mice with EC deletion of Piezo1, which was associated with marked failure of vascular patterning into arteries, veins, and capillaries. These observations serve to highlight the importance of the physical and biochemical environment encountered during angiogenesis, and may help design additional therapeutic approaches.

Antiangiogenic Splicing of VEGF-A in Disease

Alternative splicing of VEGF-A, resulting in paradoxically antiangiogenic "VEGF-Axxxb" isoforms (Woolard et al., 2009), was described over a decade ago (Woolard et al., 2004). More recent studies have now shown this to be a common and potentially modifiable phenomenon in diseases associated with increased and impaired angiogenesis. For example, it is now apparent that the antiangiogenic VEGF-A165b isoform is increased in the ischemic muscle of patients with peripheral vascular disease (Kikuchi et al., 2014). Preclinical studies suggest inflammation, typical in many patients with peripheral vascular disease, could induce changes in the splicing of VEGF-A, resulting in more antiangiogenic isoforms. Importantly, recombinant antibodies against VEGF-A165b could rescue impaired angiogenesis in these preclinical models, whereas recombinant VEGFA-165b impaired angiogenesis. Increased production of VEGF-A165b has also been implicated in the impaired vascularization of adipose tissue in people with obesity (Ngo et al., 2014), a phenomenon that is thought to promote adipose inflammation, insulin resistance, and diabetes. Conversely, reduced splicing of antiangiogenic VEGF-A isoforms has been noted in human cancer (Woolard et al., 2004). Importantly, it now appears that the splicing factors responsible for determining whether pro- or antiangiogenic VEGF-A isoforms are produced are the serine/threonine protein kinases 1/2 (SPRK1/2), and these could be a therapeutic target in the modulation of pathological angiogenesis (Nowak et al., 2010).

Summary

Remodeling of the vasculature is integral to normal development and physiology, and so has evolved many complex regulatory mechanisms, which when perturbed can contribute to diverse disease processes. It is clear that while our knowledge of angiogenesis has advanced substantially over recent decades, there remains much to be discovered if we are to develop safe and highly effective therapeutic strategies that can improve health by modulating angiogenesis. In particular, it appears likely that these will need to reconsider historic dogma regarding how angiogenesis causes disease, and what a therapeutic strategy should aim to achieve. For example, it may be that restoration of normal tissue-specific vessel quality is more important than manipulating vessel abundance. Given the unique access of the vascular system to most tissues, more effective therapeutic manipulation offers major opportunities to manage disease and improve health.

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Animal Models of Ischemic Heart Disease: From Atherosclerosis and Thrombosis to Myocardial Infarction

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Introduction

Coronary heart disease (CHD) is a complex human pathologic condition that is predominated by atherosclerosis (AS). The development of AS in the vasculature involves increased lipid accumulation in the artery walls as well as chronic inflammation propagated by a variety of cell types. The potentially fatal or debilitating effects of atherosclerosis are due to the rupture of unstable plaques and the subsequent thrombosis of epicardial vessels. This acute thrombosis leads to myocardial ischemia and subsequent myocardial infarction if not treated promptly with reperfusion therapies. Myocardial infarction remains one of the leading causes of heart failure. Each year, over 1 million people in the United States suffer a myocardial infarction (MI) (Mozaffarian et al., 2016). While the majority of patients survive their initial MI, despite optimal medical therapy, more than 300,000 patients each year develop debilitating chronic heart failure due to adverse cardiac remodeling leading to an increased need for hospital-based care.

Although modern medicine has made significant advancements in the ability to lower lipids, inhibit thrombosis, promptly reperfuse the heart, and modify adverse postmyocardial infarction remodeling, CVD remains the leading cause of morbidity and mortality in the developed world. Therefore, understanding mechanisms of atherosclerosis, thrombosis, and myocardial infarction are imperative for the development of new effective therapies. To this end, the use of animal models has proved invaluable to elucidating our understanding of atherosclerosis, thrombosis, myocardial injury, and heart failure. Indeed the premise of the NIH Consortium for preclinicAl assESsment of cARdioprotective therapies (CAESAR) was to address translation from animal models to humans (Lefer and Bolli, 2011a; Jones et al., 2015). However, the reality is that no one animal model recapitulates the complexities of human ischemic heart disease. The goal is to attempt to recapitulate portions of a disease

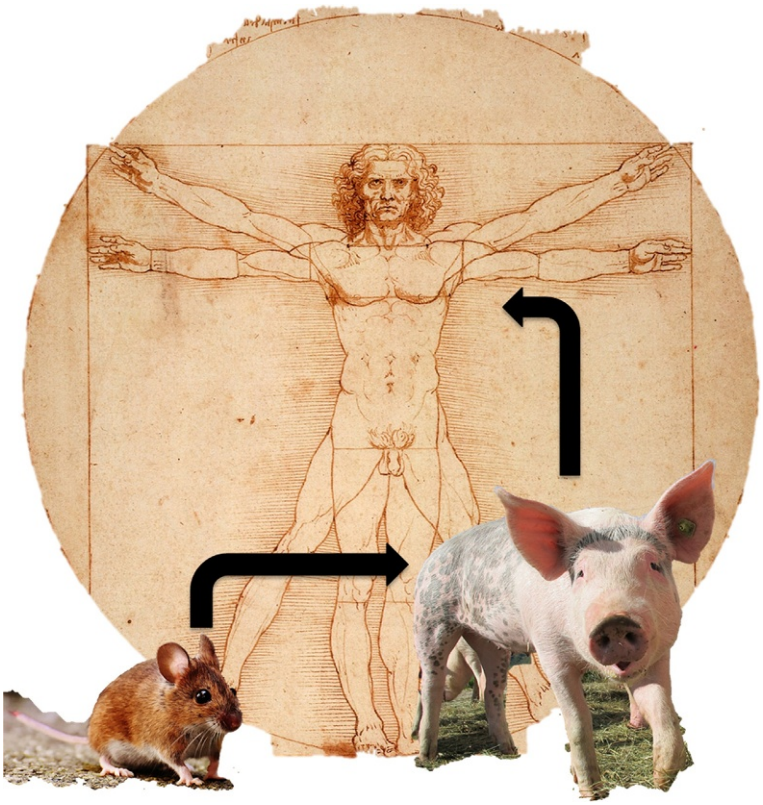

Fig. 1 Modern progression of animal models in the research and discovery for ischemic heart disease. No animal model completely recapitulates the complexity of human cardiovascular disease. Small animal models provide proof-of-concept studies that can be validated in large animal studies to provide the necessary data to proceed to human studies. Images obtained and modified from pixabay.com.

spectrum that will translate from small to large animal studies and then to the treatment of human disease (Fig. 1). The current chapter will review the various animal models that are available to study: (A) atherosclerosis, (B) thrombosis, and (C) myocardial ischemia-reperfusion injury and infarction-induced heart failure.

Atherosclerosis

Background

Initiation of atherosclerosis

Atherosclerosis is one of the most common forms of cardiovascular disease. It typically occurs when lipid accumulates in the subendothelial space of the artery wall. This lipid, most commonly low-density lipoprotein (LDL), can then be modified by oxidation (oxLDL) or other inflammatory stimuli into a form that triggers the migration and extravasation of monocytes to the site of "injury" (reviewed in Glass and Witztum, 2001). These infiltrating monocytes differentiate into macrophages and begin the arduous process of trying to remove the accumulated cholesterol and lipid. If the lipid burden becomes such that macrophages cannot efficiently remove it, the lipid accumulates in the cell, and the macrophages take on a foam-like appearance when observed by microscopy and are therefore called "foam cells." Through their production of chemokines such as MCP-1, they recruit more monocyte/macrophages to the atherosclerotic plaque, and the vicious cycle continues. As the lesion progresses, a necrotic core forms that is filled with foam cells, oxidized lipid, and dead and dying cells. This necrotic core can be contained by a fibrous cap, maintained mainly by collagen production by smooth muscle cells (SMCs). These SMCs can likewise develop into foam cells as they accumulate cholesterol. In addition to SMCs and macrophages, $CD11c^{+}$ dendritic cells (DCs) and T cells are major components of the plaque. Macrophages and DCs present antigen to T cells which can modulate local inflammatory responses, producing interferon-gamma which can inhibit smooth muscle cell production of collagen and therefore compromise plaque stability.

Plaque rupture and thrombosis

It is now commonly accepted that the atherosclerotic plaque size is not necessarily as critical as the stability of the plaque (Finn et al., 2010; Niccoli et al., 2014). Specifically, a plaque that is large but stable might be more desirable than a smaller unstable lesion.

This is mainly due to the predisposition of unstable plaques to rupture and induce a thrombus, the main culprit in myocardial infarction and stroke. Plaque rupture is more likely to occur at the shoulder of the lesion due in part to this being the site of neovascularization and increased expression of adhesion molecules that promote invasion of macrophages, T cells, and mast cells (O'Brien et al., 1996; van der Wal et al., 1998). T cells have been shown in both human and animal-derived plaques to correlate with plaque rupture (Weyand et al., 2008; Watanabe et al., 2007). One possible role of T cells in this process is that their production of interferon-gamma (IFN-γ), an inflammatory cytokine can inhibit SMC production of collagen (Wang et al., 2007; Hansson et al., 1988). The inhibition of SMC function coupled to macrophage production of matrix-degrading enzymes (matrix metalloproteinases or MMPs) and continued apoptosis and necrosis of macrophages and SMCs create the perfect environment for the erosion of the protective fibrous cap and eventual rupture and thrombus formation. The mechanisms of this process are detailed in later sections in this chapter. Here we will discuss the animal models that have been developed to study atherogenesis and plaque rupture with a particular focus on mouse models.

Animal Models of Atherosclerosis

As with any model of human disease, there are many practical and biological considerations when deciding on an appropriate animal model of atherosclerosis for your research questions (Daugherty et al., 2017). Practical considerations may include size, the ability of your institution to house and maintain the animal, the cost of the animal, and cost to house the animal. Also, it is important to consider your endpoint of interest. For example, if you require an animal model that has a lipid profile and an atherosclerotic lesion very similar to humans or if you are conducting preclinical trials for new therapies, then a nonhuman primate model might be most appropriate. However, this would not be the best choice if you have a limited research budget, your institution does not have a primate facility, or you are interested in understanding the mechanisms of atherosclerosis that might require multiple studies and the use of genetically altered animals. In this case, the mouse model would be superior to the nonhuman primate model. Swine are known to have a cardiovascular system of similar size and physiology as humans and therefore, if this is an important parameter for your research, using a porcine model of cardiovascular disease might be most appropriate (Rodrigues et al., 2005). In general, although there are always exceptions, the larger the animals, the more closely it models the human disease whereas smaller animals may have several caveats to consider, they are much easier to house, genetically manipulate and the availability of test reagents is greater, especially for mouse models (**Fig. 2**). In this chapter, we will discuss the pros and cons of common animal models of atherosclerosis. Our primary focus will be on mouse models as these are the most extensively used in the field.

Mouse models of atherosclerosis

For several decades, the mouse has been the primary small animal model for human atherosclerosis. Due to their small size, mice are much easier to handle compared to some of the larger models of atherosclerosis, including rats and rabbits. Also, mice are more affordable to house, with multiple mice usually housed per cage. These practical advantages of mouse models apply to any experimental study of human disease. Unfortunately, wild-type mice are inherently resistant to atherosclerosis (Morrisett et al., 1982). Even when placed on high-fat diets that contain cholic acid (termed the Paigen diet, 15% fat, 1.25% cholesterol, 0.5% cholic acid), the majority of mouse strains develop fatty streak lesions at best (Paigen et al., 1987). Also, mice notoriously do not suffer from spontaneous plaque rupture. This may be due, in part, to the fact that mice carry most of their cholesterol in the HDL fraction with very low levels of LDL, VLDL, or triglycerides in circulation. Also, mice lack CETP (Tanigawa et al., 2007), and their physiology (i.e., blood pressure, heart rate, size, metabolism, etc) is quite different from man. Unlike pigs and nonhuman primates, mice are less similar to humans as it relates to their lipoprotein profiles and susceptibility to cardiovascular disease.

Perhaps the greatest advantage that mice have over larger animals is their ability to be genetically manipulated. Because of this, there have emerged two central genetic models of human atherosclerosis in mice: the apolipoprotein E-deficient mouse (apoE$^{-/-}$) (Zhang et al., 1992) and the LDL receptor-deficient mouse (LDLr$^{-/-}$) (Ishibashi et al., 1993). Both of these genetically modified mouse strains have been experimentally and genetically manipulated in every way imaginable attempt to model the human

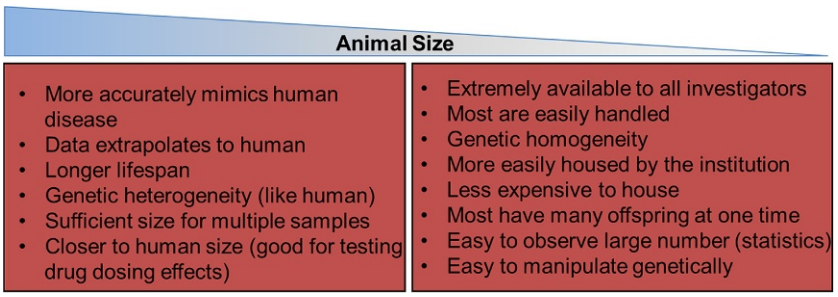

Fig. 2 Advantages of animal models of atherosclerosis based on size. In general, as you move from larger to smaller animal models you lose the close relationship between your model and human physiology, size, and genetic heterogeneity. With smaller animal models, you gain the ability to test several subjects (increase statistical power), minimize financial burden, and gain the ability to easily manipulate genes and protein expression using state-of-the-art genetic tools.

condition more accurately. The contribution of these two mouse strains to our current understanding of human CVD is immense. The remainder of this section will discuss the general features of these mouse models including both advantages and disadvantages of each.

The apoE$^{-/-}$ mouse model of atherosclerosis

Apo E is a protein associated with VLDL, LDL, and some species of HDL (Krieger and Herz, 1994; Mahley and Ji, 1999; Mahley, 1988). Its primary function is to promote uptake LDL as it promotes binding of this lipoprotein to the LDLr. In humans, there are three major isoforms of apoE, apoE2, apoE3, and apoE4 (reviewed in Mahley, 1988). ApoE3 is the most common isoform of apoE and binds the LDLr with high affinity (Mahley, 1988). ApoE3 is associated with protection from cardiovascular outcomes. ApoE4, on the other hand, is less commonly expressed in humans but is more closely associated with adverse cardiovascular outcomes, autoimmunity, and susceptibility to Alzheimer's Disease (Zhao et al., 2017; Mahley, 2016; Huang, 2010). ApoE2 is the least commonly expressed isoform of apoE and is associated with decreased lipoprotein clearance and a human genetic disease termed type III hyperlipoproteinemia (Phillips, 2014).

ApoE$^{-/-}$ mice were first reported in the atherosclerosis literature in 1992 when Nobuyo Maeda, at the University of North Carolina, Chapel Hill, and Jan Breslow, at the Rockefeller University, New York, first described their cardiovascular phenotype (Piedrahita et al., 1992; Plump et al., 1992). The apoE gene was inactivated in mouse embryonic stem cell genome by homologous recombination, and the resulting homozygous animal was devoid of all circulating apoE (Piedrahita et al., 1992). These studies demonstrated that absence of apoE in mice induces spontaneous hypercholesterolemia with an accumulation of cholesterol in mainly the chylomicron and VLDL fractions (**Fig. 3**) (Zhang et al., 1992; Plump et al., 1992). Also, atherosclerosis was evident in the aortic sinus of 8-week old apoE$^{-/-}$ mice without the need for feeding diets rich in fat, cholesterol, or cholate (Zhang et al., 1992). When compared to wild-type mice, apoE$^{-/-}$ animals typically have total cholesterol levels approximately four times higher with HDL levels about half of their normal counterparts (Zhang et al., 1992). Triglycerides were relatively normal, making the apoE$^{-/-}$ mice fairly similar to humans who possess reduced levels of apoE. Also, it was determined that the VLDL particle in apoE$^{-/-}$ mouse circulation was perhaps different than the human counterpart, containing a higher apoB48:apoB100 ratio and more closely resembling a chylomicron fragment than an LDL particle.

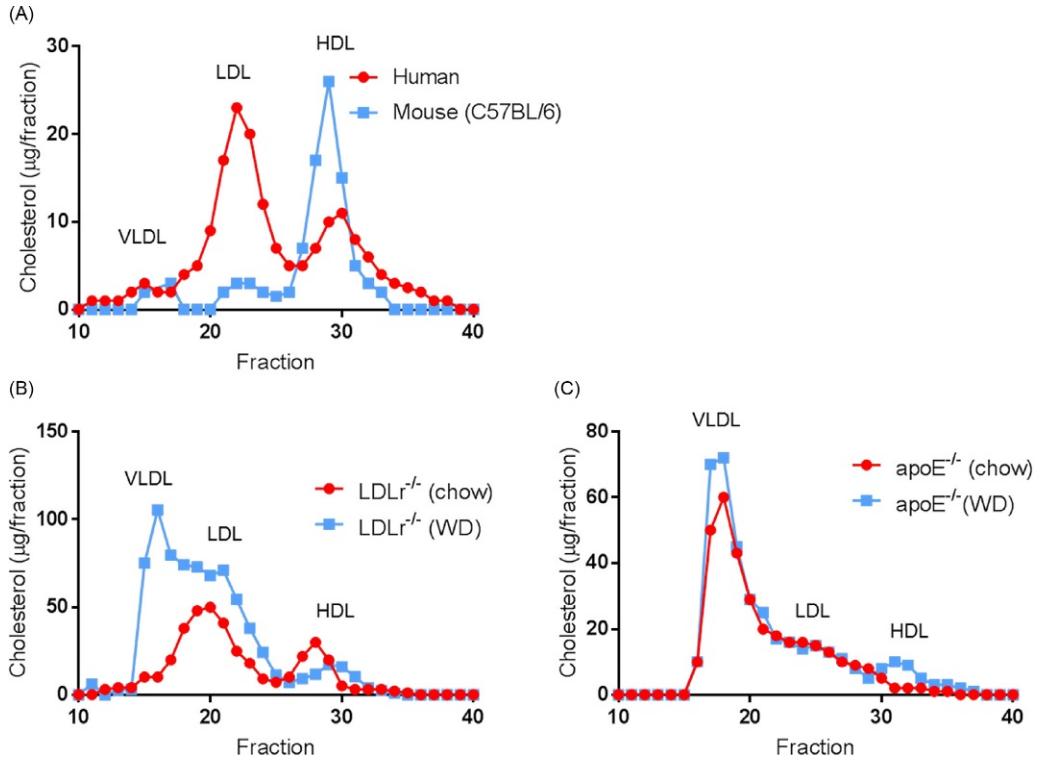

Fig. 3 Representative FPLC plots of human and mouse plasma lipoproteins. (A) Size separation of lipoproteins in plasma of humans (red circles) versus normal C57BL/6 mice (blue squares). (B) Comparison between LDLr$^{-/-}$ mice on chow (red circles) versus Western diet (blue squares). (C) Comparison between apoE$^{-/-}$ mice on chow (red circles) versus Western diet (blue squares). All data are hypothetical but represent what one might observe in a typical FPLC plot of lipoprotein profiles.

Another advantage of the apoE$^{-/-}$ mouse is that, when fed a normal chow diet, their atherosclerotic lesions are similar in complexity to human lesions (Ghiselli et al., 1981; Smith and Breslow, 1997; Nakashima et al., 1994). Lesion composition can be altered by feeding a high-fat diet containing cholesterol (Nakashima et al., 1994; Reddick et al., 1994). In this case, lesions are enriched for foam cells, likely due to the greater than 1000 mg/dl serum cholesterol levels that can be obtained when apoE$^{-/-}$ mice are fed diet. Classic studies by Russell Ross describe lesion progression in apoE$^{-/-}$ with both high fat and normal chow feeding (Nakashima et al., 1994). In this study, Nakashima et al. examined ApoE$^{-/-}$ mice either a chow diet or a Western-type diet and examined mice at ages ranging from 6 to 40 weeks. Lesions in animals fed either chow or Western diet developed lesions in the aortic root and the branch points of the aorta. Lesion progression progresses from the root to the lesser curvature of the aortic arch, the branches of the brachiocephalic artery and right common carotid artery and branch points at the mesentery and renal arteries and iliac bifurcation (Nakashima et al., 1994). If left long enough, apoE$^{-/-}$ mice on both diets eventually develop lesions along the thoracic and abdominal aorta with Western diet-fed mice developing lesions in this area much earlier than normal chow-fed mice. The classic description of lesion histology included adherence of mononuclear cells and formation of early foam cells and fatty streaks and the eventual appearance of fatty streaks that contained both macrophages and smooth muscle cells. Nakashima et al. described severe atherosclerosis in the coronary arteries that eventually led to a significant narrowing of the artery lumen (Nakashima et al., 1994). This occurred without any signs of myocardial infarct. It is now well established that mice are not great models of human myocardial infarct despite significant coronary artery involvement. Models of myocardial infarct are covered in another section of this chapter.

The LDLr$^{-/-}$ mouse model of atherosclerosis

Homozygous deletion of LDL receptor in humans leads to the genetic disorder termed familial hypercholesterolemia or FH (Hobbs et al., 1990). The LDLr is responsible for efficient removal of LDL particles from tissues and circulation. Uptake is facilitated by the apolipoprotein B-100 component of LDL binding with high affinity to the LDLr. It was previously known that defects in the LDLr led to hypercholesterolemia in Watanabe-heritable hyperlipidemic (WHHL) rabbits (reviewed below and in Shiomi et al., 2013). One year after the first description of apoE$^{-/-}$ mice, Joachim Herz's group generated and published a new model of atherosclerosis: the LDLr-deficient mouse (Ishibashi et al., 1993). Mice homozygous for the LDLr deletion require Western diet feeding to obtain extremely elevated levels of circulating cholesterol (Ishibashi et al., 1993). This is likely due to VLDL in mice containing apoE-48 and not apoB-100, the primary proteins associated with VDL and LDL, leading to clearance of these lipoproteins in the liver independent of LDLr. LDLr$^{-/-}$ mice develop early lesions, which similar to the apoE$^{-/-}$ model and in humans are mainly composed of lipids and macrophages with the presence of some T cells; typically lesions contain very few B cells (Roselaar et al., 1996; Zhou and Hansson, 1999). LDLr$^{-/-}$ mice develop fatty streaks when left on a chow diet and more complicated lesions when fed a Western diet (Herijgers et al., 1997; Merat et al., 2000). Necrotic cores with caps composed mainly of macrophage foam cells are evident in these advanced lesions. However, unlike advanced lesions in humans and plaques observed in apoE$^{-/-}$ mice fed a Western diet, lesions in LDLr$^{-/-}$ mice do not form what might be considered a legitimate fibrous cap (Nakashima et al., 1994; Ishibashi et al., 1994).

Although one could argue that apoE$^{-/-}$ mice develop lesions more similar in cellular content to human lesions, LDLr$^{-/-}$ animals have one major advantage over apoE$^{-/-}$, the ability to receive bone marrow from any LDLr-competent animal without compromising atherogenesis. Specifically, it was demonstrated by Fazio et al. that if you rescue lethally irradiated apoE$^{-/-}$ mice with bone marrow from apoE-competent animals, in this case, the apoE produced by the macrophages is sufficient to restore normal serum lipids and protect the animal from atherosclerosis (Fazio et al., 2002). This same group demonstrated that this was not the case for LDLr$^{-/-}$ mice in which restoration of LDLr expression in the hematopoietic compartment via bone marrow transplant had no effect on serum lipids, atherosclerosis progression, or lesion composition (Fazio et al., 1997). Therefore, the LDLr$^{-/-}$ mouse does not require backcrossing to whatever genetic model one wishes to study and may accelerate studies; especially in cases of immunity and atherosclerosis where the entire immune system can be manipulated to study its role in atherosclerosis.

It should be mentioned that neither the apoE$^{-/-}$ nor the LDLr$^{-/-}$ mouse are appropriate models of plaque rupture and resulting myocardial infarction. Regardless of the complexity or size of the plaque, these animals simply do not suffer actual cardiovascular events that would recapitulate the human condition. This gap in the field was alleviated somewhat by the development of the SR-BI, apoE double knockout mouse (Braun et al., 2002, 2003; Trigatti et al., 2000). SR-BI (Scavenger receptor-BI) serves as the receptor for HDL and is critical for normal reverse cholesterol transport and removal of excess cholesterol from tissues and cells and cholesterol handling in the liver (Trigatti et al., 2000). By knocking this receptor out on the apoE$^{-/-}$ mouse, the ability to handle cholesterol in any capacity is essentially lost. SR-BI, apoE double knockout mice develop severe coronary atherosclerosis leading to spontaneous infarction and very early death (Braun et al., 2002, 2003; Trigatti et al., 1999). Therefore, as with any animal model, choosing the right animal to test your hypothesis and understanding the endpoints and readouts are key to making sure you are using the right system to answer your specific question.

Large Animal Models of Atherosclerosis

There are numerous animal models of atherosclerosis (Lee et al., 2017). The choice of model depends on the ultimate goal of your study. Large animal models afford several advantages over smaller models such as mice. For example, studying pharmacokinetics

might be more appropriate in a larger animal where the dose and kinetics would be similar to humans. The metabolic rate of a rodent is much faster than man and therefore is often not translatable (Gordon, 2017). This section will discuss the common large animal models of atherosclerosis and their utility in scientific discovery.

Watanabe heritable hyperlipidemic rabbits

Watanabe Heritable Hyperlipidemic (WHHL) rabbits, described by Yoshio Watanabe (Watanabe, 1980), are a strain of rabbits that suffers from hyperlipidemia and spontaneously develop aortic atherosclerosis in 100% of animals by 5 months and older. At even older ages (older than 15 months), Watanabe described the presence of xanthoma of digital joints in the majority (63%) of rabbits (Watanabe, 1980). The animals have an accumulation of LDL cholesterol that leads to foam cell formation and atherosclerosis. It was later discovered that the defect in these animals is a decrease in LDLr due to genetic deletion (Tanzawa et al., 1980). Homozygous WHHL rabbits may be a better model for the genetically equivalent human disease, Familial Hypercholesterolemia (FH), and its associated atherosclerosis, as macrophage foam cells and human-like cellular composition are present very shortly after fat feeding (Rosenfeld and Ross, 1990; Tsukada et al., 1986; Rosenfeld et al., 1986). Heterozygous WHHL rabbits fed cholesterol-containing high-fat diets develop atherosclerotic lesions similar to that seen in humans (Atkinson et al., 1989). Cholesterol-fed, heterozygous WHHL rabbits gain lipoprotein profiles with cholesterol in the VLDL and LDL size range (Atkinson et al., 1989). This leads to the appearance of atherosclerotic plaques in the thoracic and abdominal aortas by 12 weeks of diet feeding. By 24 weeks of diet feeding, the WHHL rabbits had developed complicated plaques characterized by foam cells, cholesterol clefts, calcification, and necrotic cores covered by a fibrous cap (Atkinson et al., 1989). The WHHL rabbit continues to be a popular animal model of atherosclerosis and can be consistently seen used in current research.

Porcine model

Pigs are considered an appropriate model for numerous reasons. The anatomy and the physiology of the porcine circulatory system, for the most part, closely resemble the human. This may make it more appropriate to translate findings from the pig about wound healing, lipoprotein metabolism, and development of atherosclerosis in human disease. For example, a recent study by Fenning et al (Fenning et al., 2015). demonstrated that pigs develop lesions in the coronary arteries as well as in the abdominal aorta in a site-specific manner similar to human. These studies demonstrate that similar to human cardiovascular disease, lesions in various vascular beds of the pig are not identical and therefore might benefit from different treatment. One example of this is associated phospholipase A_2 (Lp-PLA_2), an enzyme important in atherogenesis as it mediates the formation of oxidized lipoproteins in the artery wall (Fenning et al., 2015; Wilensky et al., 2008). In clinical studies, Lp-PLA_2 levels and activity have been shown to be increased in individuals with cardiovascular disease (Ge et al., 2016; Yang et al., 2017) and in association with other diseases where atherosclerosis is a comorbidity such as hypertension (Kim et al., 2017), psoriasis (Vachatova et al., 2016), and chronic kidney disease (Wang et al., 2016). As these differences in disease in different vascular beds are more difficult to study in smaller animals, one can surmise why studying atherosclerosis in the pig can be a valuable preclinical tool.

In addition to the spontaneous development of fatty steaks, the pig model is also useful for studying diet-induced atherosclerosis in the context of hypercholesterolemia. When fed a high-cholesterol diet for short periods of time, pigs develop fatty streak lesions predominately in the abdominal aorta (Fenning et al., 2015). Not surprising, long-term feeding of pigs with high cholesterol diets resulted in increased atherosclerosis in coronary vessels. In both cases, lesion composition and morphology closely resembles that seen in humans. While the pig is an excellent large animal model to study atherosclerosis, obvious drawbacks include the cost of maintaining and housing the animals, their size, and the difficulties of handling.

Non-human primate models

In 1954, Taylor et al. described the formation of spontaneous atherosclerosis and hyperlipidemia in rhesus monkeys (TAYLOR et al., 1962). This was associated with increased ingestion of dietary fat. Although many studies have demonstrated varied degrees of severity of atherosclerosis in different species of monkeys, the nonhuman primate model has the advantage of having a human-like lipoprotein profile. This is characterized by levels of LDL and HDL comparable to humans, an attribute associated with expression of CETP (an important protein absent in the more commonly used mouse models) (Strong and McGill, 1967; Clarkson et al., 1976; Rudel et al., 1985). Near human lipoprotein profiles have made the nonhuman primate and attractive model to study potential new therapies. However, the expense of working with nonhuman primates becomes one large disadvantage to this model.

Nonhuman primates have been critical in testing new potential therapies. Two of the most recent examples include the microRNA-33 inhibitor and monoclonal antibodies to proprotein convertase subtilisin/kexin type 9 (PCSK9) (Rayner et al., 2011; Chan et al., 2009; Liang et al., 2012). Studies using African green monkeys demonstrated the utility of inhibiting microRNA-33 on improving HDL cholesterol levels and decreasing atherosclerosis burden (Rayner et al., 2011). More recent studies with monoclonal antibodies to PCSK9 in cynomolgus monkeys showed that a single injection reduced serum cholesterol by 80% and this decrease was maintained for at least 10 days (Chan et al., 2009). Similar results were observed by Liang et al. (Liang et al., 2012), where monoclonal antibody-mediated blocking of PSCK9 binding to the LDLr, and thereby inhibiting its degradation, reduced LDL in cynomolgus monkeys with normal or diet-induced hyperlipidemia. When combined with a statin this effect was amplified. While neither of the PCSK9 inhibition studies in nonhuman primates examined atherosclerotic outcomes, they do demonstrate that nonhuman primates are an invaluable first-step model for pharmacokinetic and efficacy testing before moving to phase I clinical trials.

Animal Models of Arterial Thrombosis

Background

In humans, plaque instability can result in rupture of atherosclerotic lesions and subsequent vessel thrombosis (Lusis, 2000; Burke and Virmani, 2007). Without immediate medical attention, coronary artery thrombosis results in myocardial cell death and can lead to diminished cardiac function with hemodynamic instability causing cardiogenic shock and electrical instability causing lethal ventricular arrhythmias (Anderson and Morrow, 2017). Current treatments to restore coronary vessel patency after a thrombotic event involve establishing patency of the vessel via balloon angioplasty and drug-eluting stent placement (Anderson and Morrow, 2017). When done promptly, these corrective procedures reduce the risk of cardiovascular complications and sudden death (Anderson and Morrow, 2017). The use of animal models to study the physiological progression of thrombotic events has proved invaluable in understanding mechanisms of disease and has led to current treatments available in clinical practice.

Acute injury model of thrombosis

As discussed above, the formation of an unstable atherosclerotic lesion in humans precedes plaque rupture and thrombosis (Lusis, 2000; Burke and Virmani, 2007). There are no animal models that effectively recapitulate this pathology; therefore the majority of thrombosis research relies on acute injury to the blood vessel while monitoring thrombus formation via changes in blood flow measured by placing a Doppler flow probe downstream of the site of vessel injury (Li et al., 2016; Bonnard and Hagemeyer, 2015). Also, imaging methods can be employed to visualize and quantify the formation of a thrombus (Falati et al., 2002; Celi et al., 2003). Previously used methods of thrombus initiation include mechanical disruption of the vascular endothelium with wire injury, laser-induced injury, and chemical-induced injury with ferric chloride (Westrick et al., 2007; Cooley, 2012). The most popular animal models used to decipher mechanisms of arterial thrombosis include nonhuman primates (Myers, 2012), pigs (Fuster et al., 1991), dogs (Badylak et al., 1988), rabbits (Abela et al., 1995), rats (Kurz et al., 1990), and mice (Dörffler-Melly et al., 2000), but others used less often include ferrets (Schumacher et al., 1996), sheep (Kim et al., 2010), and zebra fish (Jagadeeswaran, 2005).

Small Animal Models of Thrombosis

Small animal models of thrombosis have the potential for rapid screening and discovery, high availability, low cost, and reproducibility of results, making them ideal for routine use in the laboratory.

Mice

The most widely used small animal model in research and discovery of human arterial thrombosis is the mouse (NIESWANDT et al., 2005). The most common model used in mice is the ferric chloride ($FeCl_3$) model of arterial injury (Kurz et al., 1990) that was initially developed in rats (see below). In brief, the common carotid artery or mesenteric artery is exposed, a flow probe is placed on the artery to monitor blood flow, and $FeCl_3$ is applied to the vessel outer vessel surface at a defined concentration for a specified period, usually 3 min. $FeCl_3$ results in a free radical-induced injury that disrupts the vascular endothelium but with limited collagen exposure in the subendothelial matrix (Eckly et al., 2011). Subsequent work has also suggested that the erythrocyte-mediated platelet adherence to the endothelium is responsible for the initiation of thrombus formation (Barr and Chauhan, 2013). Regardless of the mechanisms, the degree of injury, and therefore the rapidity of thrombosis, can be modulated by the concentration of the ferric chloride and the time of exposure (Wang and Xu, 2005; Li et al., 2013). Furthermore, using transgenic mice treated with $FeCl_3$-induced arterial thrombosis, the role of the platelet fibrinogen receptor, glycoprotein IIb/IIIa (Ni et al., 2000), the platelet ADP receptor, $P2Y_{12}$ (Andre et al., 2003), platelet endothelial adhesion molecule-1 (Falati et al., 2006), inhibitors of factor Xa (Hechler et al., 2011), cyclooxygenase 1/2 (Riehl et al., 2011), and microparticles (Thomas et al., 2009), have been elucidated. Indeed, $FeCl_3$ vascular injury model has demonstrated the antithrombotic roles of clinically approved platelet aggregation inhibitors, including aspirin (WANG et al., 2006), clopidogrel (Wang and Xu, 2005), and ticagrelor (Patil et al., 2010).

In addition to the $FeCl_3$ vascular injury model, laser-guided injury via Rose Bengal (Thacker et al., 2012), and electrolytic-induced vascular injury models of arterial thrombosis (Kusada et al., 2007) have been employed in mice. Both Rose Bengal and electrolytic-induced injury also induce an oxidative stress injury (Cooley, 2012). However, these models can be difficult given the need for lasers for Rose Bengal or the techniques necessary for electrolytic induced injury.

Rats

As mentioned above, the $FeCl_3$ model was initially developed in rats (Kurz et al., 1990), but it has been used ubiquitously. Thrombi resulting from $FeCl_3$ application have been shown to contain activated platelets, fibrin deposition, and erythrocytes (Kurz et al., 1990). In addition to this, the physical crush model (Hill-West et al., 1994), external heating of the carotid artery (Lenfors et al., 1993), and intimal tuck model (Nayak and Deschler, 2005) have been adapted to the rat vasculature. Despite all these methods, the ease and relatively low cost of the $FeCl_3$ model remain the gold standard for investigating thrombosis in rats. An additional factor that strengthens the use of rats in the laboratory is the ability to manipulate expression levels of thrombotic molecules such as tissue factor by retroviral vectors (Hasenstab et al., 2000). Although the number of transgenic rats expressing proteins relevant for cardiovascular disease is low, future development of rats with knockout or overexpression of proteins involved in arterial thrombosis will allow for the application of previously developed techniques such as the $FeCl_3$ model of arterial thrombosis.

Large Animal Models of Thrombosis

In the interest of safeguarding human health, the majority of compounds designed to treat arterial thrombosis must be tested in the largest animal available that most closely resembles human cardiovascular physiology. This is especially important when large conduit blood vessels are the subject of scrutiny. However, cost and availability prevent nonhuman primate models from being used routinely as a method of discovery and research. The following section will review the large animals available to study arterial thrombosis. Similar to small animals, thrombosis research in large animals requires a blood vessel insult that initiates thrombus formation culminating in vessel occlusion.

Canine model

Early studies by Folts et al. pioneered the methodology used in arterial thrombosis which required using a vascular clamp to physically crush the endothelium followed by applying a plastic cylinder around the coronary artery to produce a stenosis (Folts et al., 1976). This method leads to thrombus formation and cyclic flow reduction to near zero with a sudden spontaneous return to near control levels. These landmark studies led to a significant increase in our understanding of thrombus formation (Folts et al., 1982; Folts, 1991). In addition to the Folt's model, electrical vascular injury has also been used to initiate thrombus formation in the coronary arteries of dogs (Romson et al., 1980). Differences in platelet hyperactivity appear to play a more prominent role in the propagation of thrombi in adult mongrel dogs than in humans (Strony et al., 1993). Ethical concerns and availability of dogs have caused them to be a less popular model than the porcine model.

Porcine model

Typically, swine are easier to handle and more affordable than nonhuman primates. The pig's large arteries, while smaller than human and nonhuman primates, are still significantly larger than those of small rodents, therefore providing surgically or percutaneous approaches. Also, the pig's coagulation and fibrinolysis system have a high degree of overlap between pigs and human (Münster et al., 2002). Several models have been used. The initial open-chest porcine model involved physically crushing the aortic vessel leading to a fibrin-rich and platelet-rich thrombi (Lin et al., 2000). Besides physical crush, electrical stimulation of the common carotid artery (Kohda et al., 1999) and balloon injury can be used to generate thrombi in pigs (Morishige et al., 2003). The drawbacks to the porcine model include the higher rate of plasma coagulation (Sondeen et al., 2013) and a lack of fully developed antibody and molecular probes (Münster et al., 2002; Napoli et al., 2006). Therefore, these issues should be kept in mind when proceeding with swine studies.

Non-human primates

The baboon is arguably the ideal model that most resembles human thrombogenic physiology. Initiation of a thrombotic process in baboons may require dual injuries such as forceps crush and thrombin administration (Flameng et al., 1986; Herijgers and Flameng, 1991). This approach has been essential in measuring the effects of monoclonal antibodies and small molecules on the size of thrombi through the arterial wall. Another useful nonhuman primate is the cynomolgus macaque. Studies with these animals have used the Rose Bengal model to target the exposed saphenous artery and activate the intravenously injected chemical Rose Bengal to release reactive oxygen species causing local vascular endothelial injury. This method has been useful in studying the effects of agents that inhibit thrombotic molecules such as plasminogen activator inhibitor-1 (PAI-1) (Izuhara et al., 2010). While the nonhuman primate models have been essential in the preclinical use of antithrombotic compounds, their large size, high cost, the required surgical expertise, and equipment make nonhuman primates an unsuitable model for routine laboratory use.

Models of Myocardial Injury and Ischemic Heart Failure

Background

The choice of the animal model and the model of myocardial injury requires careful consideration. Large animal models more closely approximate the human heart but are more costly, have limited molecular probes, and require specialized experimental setups (Houser et al., 2012; Wheeler et al., 2012; Ishikawa et al., 2014). As outlined in **Fig. 2**, while small animal models are phylogenetically distinct from humans, potentially limiting the translation of findings, they do allow for assessment of gene overexpression and knockout to provide proof-of-principle studies. Here we will focus on two models that are routinely employed to induce myocardial injury, the ischemia-reperfusion injury model, and the coronary artery ligation model. These two models are distinct in their measures and require further discussion. With myocardial ischemia-reperfusion models, a coronary artery is occluded with either a suture (small and larger animal models) or a percutaneous coronary balloon (large animal models). After a designated period of occlusion, the obstruction is removed to restore flow in the vessel (reperfusion). This model has been used extensively to assess how pharmacologic or genetic interventions impact myocardial infarct size. In contrast, coronary artery ligation models of myocardial infarction do not reperfuse, and therefore can be used to examine the response to a completed infarct and the impact on myocardial remodeling. It should be noted that in murine and rat models, the generation of thrombotic occlusion in the coronary arteries of mice is not experimentally possible to date. Furthermore, neither of the myocardial injury models employs thrombus formation; therefore, the contribution of platelet activation is underestimated in these models. The aim of this section is to describe and highlight the features of some commonly used animal models of ischemic myocardial injury.

Small Animal Models

Mouse and Rat models

Mouse models of myocardial injury have been extensively used by investigators to decipher the contribution of specific cells, proteins, and signaling pathways to myocardial injury and remodeling (Salto-Tellez et al., 2004; Gao et al., 2010a). Myocardial ischemia- reperfusion injury is most commonly induced in mice by coronary artery occlusion with a surgical ligature. As an alternate to surgical ligature, cryogenic injury has been used to cause myocardial damage because it can give a more reliable area of injury. However this technique has not been used extensively (van den Bos et al., 2005a,b). We have extensively used the suture method to induce myocardial infarction in mice (Mital et al., 2011; Cai et al., 2011; Zhang et al., 2017; Smith et al., 2016a). To induce myocardial infarction, mice are anesthetized with either isoflurane or Ketamine/Xylazine injection. Following intubation, a left-sided thoracotomy is performed, and the left coronary artery is either temporarily (ischemia-reperfusion) or permanently ligated with a suture. Ischemia is verified by the change in tissue color distal to the suture. It should be noted that while there is variability in murine coronary anatomy (Kumar et al., 2005; Chen et al., 2017), induction of predictably sized infarcts in C57BL/6 mice is possible (Ahn, 2003). Therefore, the investigator should be careful to use in-bred strains such as C57BL/6 to ensure uniformity. After verifying ischemia, the suture is tied for permanent coronary ligation studies, or loosened at a designated time for reperfusion. The surgical incisions of the chest and skin are closed, and mice are recovered. Infarct size can be measured at 24–72 h using tetrazolium staining, and cardiac function can be measured by a variety of methods including echocardiography (Gao et al., 2011), MRI (Buonincontri et al., 2013), or invasive hemodynamic measurements (Pacher et al., 2008; Zhang et al., 2017; Smith et al., 2016b). Long-term studies to assess the impact of inflammation and fibrotic scar tissue formation can be measured accurately to gain insight into the postinfarct remodeling process (Prabhu and Frangogiannis, 2016). Investigators must be aware of several critical issues with these models. First, the time of ischemia affects the infarct size in mice (Redel et al., 2008). Furthermore, the degree of inflammation and fibrosis, and therefore LV remodeling, is directly proportional to the initial infarct size (Christia et al., 2013). Therefore, it is necessary to demonstrate equal infarct size between groups when comparing the subsequent remodeling responses in between these groups of mice.

Another uniqueness of the mouse coronary artery ligation model is the finding that after infarction, mice like humans can suffer cardiac rupture (Gao et al., 2005, 2010b; Fang et al., 2007). Rupture rates for several mouse strains have been reported (Gao et al., 2005). Furthermore, the incidence of cardiac rupture is proportional to the infarct size and the amount of inflammation (Gao et al., 2010b).

Rat models have been a mainstay in cardiovascular research. Temporary and permanent ligation of the left anterior descending artery has been used to generate myocardial injury and heart failure (Bader, 2010). Because the techniques are comparable to those described above for the mouse, we will not review these models here. It should be noted that because genetically modified rats are not as readily available as mice, the use of rat models for proof-of-concept studies on gene expression is limited. However, numerous studies have employed rat models of ischemic cardiac injury to evaluate pharmaceutical approaches (Wayman et al., 2003).

Large Animal Models of Myocardial Injury

The early animal models of myocardial infarction were developed and reported in dogs as early as 1948. Canine studies are complicated by the fact that evaluation of collateral blood flow must be conducted given that dogs have developed collaterals that can be recruited during ischemia (Leong-Poi et al., 2005). Ischemic heart disease has also been studied in nonhuman primate models (baboons and rhesus monkeys) (reviewed Camacho et al., 2016). However, due to ethical and cost considerations, these species are not widely employed. Indeed, the National Heart Lung and Blood Institute Consortium for preclinicAl assESsment of cARdioprotective therapies (CAESAR) uses only mouse, rabbit, and pig to examine the efficacy of cardioprotective modalities (Jones et al., 2015; Lefer and Bolli, 2011b).

Swine

Swine are considered a superior large animal model for studies of acute myocardial infarction and ischemic cardiomyopathy (ICM) (McCall et al., 2012), with heart size, coronary vessel size, and collateral coronary circulation comparable between humans and swine. One of the most widely used models for the induction of myocardial infarction MI in swine is balloon occlusion of the left anterior descending artery. Indeed, we have used this model to demonstrate that with myocardial ischemia-reperfusion injury transgenic swine overexpressing CD39, a vascular nucleotidase, have a significant reduction in myocardial infarct size (Wheeler et al., 2012). To create reproducible large infarct size, a percutaneous transluminal coronary angioplasty dilation catheter was placed just proximal to the first diagonal branch and inflated to occlude distal flow. Myocardial infarct size (Ellenbroek et al., 2016) and myocardial function can be measured by a variety of methods including echocardiography (Ellenbroek et al., 2016; Ballo et al., 2017), CT (Boudoulas et al., 2012), MRI (Arunachalam et al., 2017), or invasive hemodynamic measurements (Ellenbroek et al., 2016).

A caveat of this model is that it requires specialized equipment, dedicated surgical facilities, and skilled personnel, thereby limiting the number of laboratories able to conduct these studies. Another caveat of this model is the fact that there is no thrombotic occlusion. To understand whether platelet activation and thrombus formation contribute additional injury or an altered profile of inflammatory mediators requires further studies. Other models have also been employed to examine ischemic injury including:

(a) surgical ligation of the LAD or LCX coronary artery in the pig (Ishikawa et al., 2014; Gálvez-Montón et al., 2014), (b) gelatin sponge embolization (Sakaguchi et al., 2003), (c) coronary coil deployment (Gálvez-Montón et al., 2014), and (d) intracoronary ethanol administration . Finally, a critical caveat with the porcine model is that dissection of molecular mechanisms is limited due to a less than robust array of molecular probes for swine proteins.

Summary

Atherosclerosis and thrombosis contribute to a significant proportion of morbidity and mortality in the world. No animal model completely recapitulates the complexity of human cardiovascular disease (**Fig. 1**). However, small and large animal models of atherosclerosis, thrombosis, and myocardial injury are invaluable tools through which numerous laboratories have contributed to understanding the intricacies of atherosclerosis, thrombosis, and myocardial infarction.

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Ankle-Brachial Pressure Index and Pulse Wave Velocity in Cardiovascular Risk Assessment

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Introduction

The ideal tool for assessing the risk status for cardiovascular disease is one that is simple to use and applicable to a large number of subjects (Naghavi et al., 2003). For classifying the cardiovascular risk status of individuals, until now, practice guidelines recommend use of the Framingham Risk Score or other similar cardiovascular risk prediction models, which are based on the traditional risk factors for cardiovascular disease (Wilson et al., 1998; National Cholesterol Education Program Expert Panel on Detection, 2001). However, the traditional risk factors alone do not appear to be adequate to determine the risk of cardiovascular disease (Magnus and Beaglehole, 2001; Brindle et al., 2003). Several biochemical markers, such as the serum levels of C-reactive protein and B-type natriuretic peptide, have also been used, but these add only moderate value to the standard risk factors (Wang et al., 2006). Under these circumstances, the usefulness of physiological markers reflecting vascular pathophysiological abnormalities has received attention for cardiovascular risk assessment. Among these markers, the ankle-brachial pressure index (ABI) and pulse wave velocity (PWV) are markers that are simple to measure and applicable to a large number of subjects (Aboyans et al., 2012; Townsend et al., 2015; Vlachopoulos et al., 2015).

Ankle-Brachial Pressure Index

Rationale for Use as a Cardiovascular Risk Marker

The ABI denotes the ratio of the systolic blood pressure measured at the ankle to that measured in the arm. Because atherosclerotic stenosis is rare in the arteries of the upper extremities, this ratio practically reflects the severity of stenosis of the arteries of the lower extremities. A particular caution is that the blood pressure should be measured in both the arms and the higher value of the two should be used for calculating the ABI (Aboyans et al., 2012). An interarm systolic blood pressure difference of >10/15 mmHg implies subclavian artery stenosis, which is one of the high-risk factors for cardiovascular events (Clark et al., 2012).

In the systemic arterial tree, the caliber of the vessels decreases progressively from center to the periphery, and this tapering causes elevation of the systolic blood pressure at the periphery as a peaking phenomenon (**Fig. 1**, upper panel). In addition, tapering and branching of the arteries from the center to the periphery increase the arterial resistance, which generates a reflected pressure wave directed toward the center (Tomiyama and Yamashina, 2010; O'Rourke and Hashimoto, 2007). Summation of the forward pressure

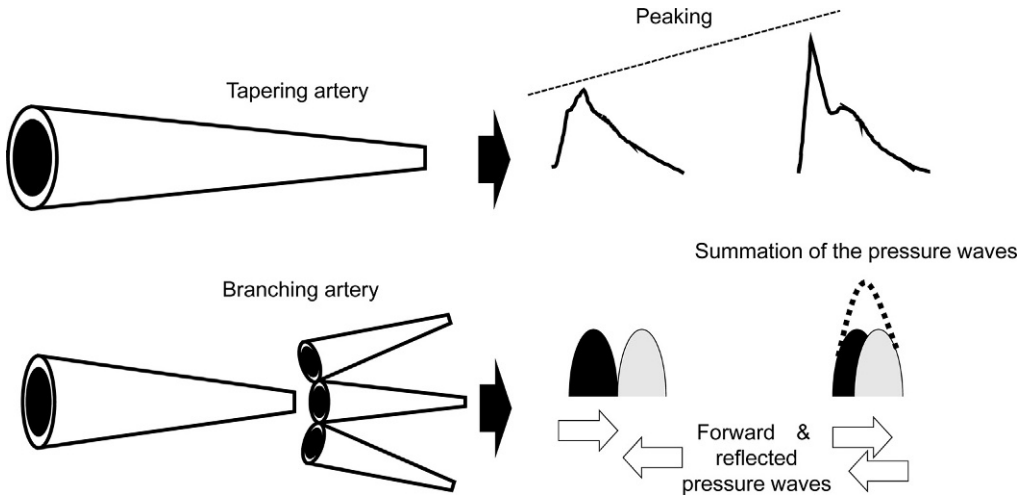

Fig. 1 Mechanisms amplify the systolic blood pressure from the proximal to the distal sites in arteries.

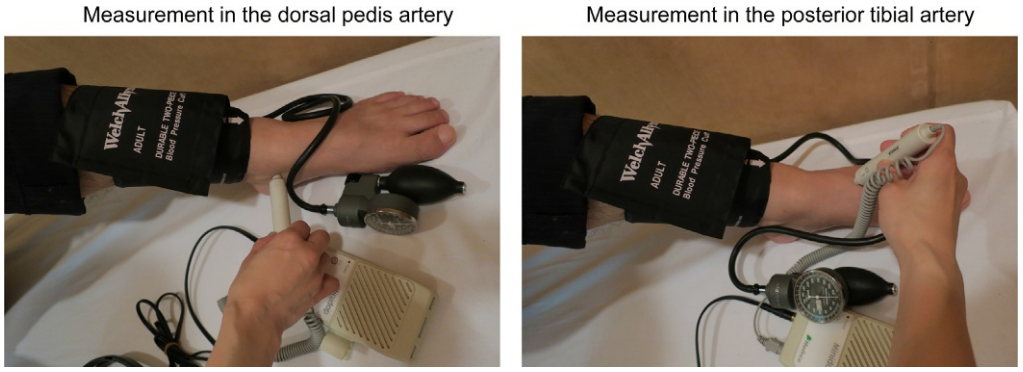

Fig. 2 Ankle-brachial pressure index measurement by the Doppler method.

wave generated by cardiac contraction and the reflected pressure wave also increases the blood pressure at the periphery (Fig. 1, lower panel). These mechanisms amplify the systolic blood pressure from the proximal to the distal sites in arteries.

According to the American Heart Association (AHA) Scientific Statement 2012 (Measurement and interpretation of ABI), values $\leq$0.90 usually signify peripheral arterial disease (PAD) (Aboyans et al., 2012). Previous reports have indicated that both the sensitivity and the specificity of ABI $\leq$0.90, as measured by the Doppler method, to detect >50% stenosis of the arteries of the lower extremities are nearly 90% and 95%, respectively (Carter, 1968; Yao et al., 1969). However, more recent meta-analyses have demonstrated high specificity values (80–99%), but a wide range of sensitivity (20–80%) (Lin et al., 2013; Xu et al., 2010). The ABI value of 0.90 is not a binary cutoff value to identify PAD, and the diagnostic threshold of ABI for peripheral arterial stenosis may vary. For example, Guo et al. (2008) reported that the cutoff value of ABI of 0.95 was associated with 91% sensitivity and 86% specificity to identify significant stenosis of the arteries of the lower extremities in subjects who had undergone conventional digital subtraction angiography as a screening test for PAD. A low ABI value may reflect severe stenosis and be associated with lower walking performance (McDermott et al., 2002), although the association of a low ABI with functional impairment of the lower extremities is less consistent (Aboyans et al., 2012). Despite the presence of lower limb peripheral arterial stenosis, the ABI is high ($\geq$1.40) in some cases; this could be a result of incompressible calcification of the arterial wall. In such cases, the toe-brachial index is useful to identify PAD, and Aboyans et al. (2008) reported that 80% of subjects with high ABI have significant stenosis in lower extremities.

Measurement of the ABI

Several methods are available for measuring the ABI, such as the Doppler method, the oscillometric method, and plethysmography (Aboyans et al., 2012; Vlachopoulos et al., 2015). Most clinical studies till date have used the Doppler method to measure the ABI, and the AHA Scientific Statement 2012 (Measurement and interpretation of ABI) and a position paper from the European Society of Cardiology (ESC) Working Group on peripheral circulation recommend measurement of the ABI by the Doppler method (Aboyans et al., 2012; Vlachopoulos et al., 2015). In the Doppler method, the blood pressure is measured using a Doppler probe in the lower limbs, followed subsequently by measurement in the posterior tibial and dorsal pedis arteries (Fig. 2). On the other hand, in the



Fig. 3 Ankle-brachial pressure index measurement by the oscillometric method.

oscillometric method, which is relatively simpler and requires less training, the blood pressure is measured simultaneously in both the upper and lower extremities by wrapping pressure cuffs (Fig. 3). However, the blood pressure in the posterior tibial artery and dorsal pedis artery cannot be measured separately in the oscillometric method.

Validity, Reproducibility, and Generalizability of ABI

As mentioned earlier, the ABI as measured by the Doppler method has a high specificity for identifying significant stenosis in the arteries of the lower extremities. Similarly, when the ABI was assessed by the oscillometric method, a cutoff value of 0.87 yielded a sensitivity of 84% and specificity of 89% for the detection of 75% stenosis in the lower limb arteries as measured by magnetic resonance angiography (Ichihashi et al., 2014). A review article reported that the average sensitivity and specificity of ABI determined by the oscillometric were $69 \pm 6\%$ and $96 \pm 1\%$, respectively, for the diagnosis of PAD (Verberk et al., 2012). Aboyans et al. (2012) reported that the area under the receiver operator characteristic curve to identify PAD was higher for ABI measured by the Doppler method than for ABI measured by the oscillometric method. However, both the Doppler method and the oscillometric method have been demonstrated to show acceptable reproducibility for measurement of the ABI (Holland-Letz et al., 2007; Richart et al., 2009).

For systolic blood pressure values of <70 mmHg as assessed by the Doppler method, the oscillometric method overestimates the values (Kornø et al., 2009); this finding suggests that the Doppler method may be preferable for measuring the ABI intended at estimating the severity of peripheral arterial stenosis. On the other hand, ABI measurement by the Doppler method needs to be performed by skilled examiners, and both didactic learning and experimental learning are required (Aboyans et al., 2012). In regard to screening for PAD in general practice, Nexøe et al. (2012) suggested that neither the recommendation for use of the Doppler method to measure the ABI nor the measurement of the ABI itself was warranted in general practice due to the low specificity of the assessments. In addition, the time required for the measurement (10–15 min per person) is another issue in general practice (Bendermacher et al., 2012; Davies et al., 2014).

Risk Factors Associated With Abnormal ABI and Recommended Indications for ABI Measurement

Advanced age, gender (male sex), obesity, hypertension, diabetes mellitus, smoking, hypercholesterolemia, and hypertriglyceridemia are risk factors associated with ABI <0.90 (Fowkes et al., 2013). The prevalence rate of ABI <0.90 increases with age in both sexes: <5% in persons aged <50 years, 8–15% in persons aged 50–80 years, and 20% in persons aged >80 years (Fowkes et al., 2013). Selvin and Erlinger (2004) reported that in a cohort with a PAD prevalence rate of 4.4%, the prevalence of persons with versus without hypertension was 6.9% versus 2.2%, the prevalence of smokers versus nonsmokers was 6.8% versus 3.1%, and the prevalence of persons with versus without diabetes was 10.8% versus 3.6%. Accordingly, the clinical recommendations for measurement of the ABI are as follows: persons aged ≥ 18 years with walking impairment or claudication, or nonhealing wounds in the lower extremities, persons aged 50–69 years with a history of smoking or diabetes, and persons aged ≥ 70 years (Olin et al., 2010). In addition, the Trans-Atlantic Inter-Society Consensus (TASC) II proposed measurement of the ABI in subjects with a Framingham risk score between 10% and 20% in 10 years (Norgren et al., 2007). Chronic renal insufficiency is also a risk factor for PAD (Ix et al., 2009) and is an indication for measuring the ABI (Norgren et al., 2007).

Clinical Perspectives

Clinical perspectives of ABIs were summarized in Table 1. Abnormal ABI has two clinical implications: it indicates the existence of PAD and also indicates a high-risk state for cardiovascular disease.

Table 1 Summary of the clinical perspectives of ankle-brachial pressure index and pulse wave velocity

Variable	*Logical mechanism*	*Reproducible*	*Generality and validity*	*Disease-specific*	*Incremental prognostic value*	*Surrogate*
ABIdop	++	++	++	++	++	−
ABIosc	++	++	++	++	++	−
cfPWV	++	++	++	+	++	±
baPWV	++	++	++	+	++	±

Abbreviations: *ABIdop*, ankle-brachial pressure index measured by the Doppler method; *ABIosc*, ankle-brachial pressure index measured by the oscillometric method; *cfPWV*, carotid-femoral pulse wave velocity; *baPWV*, brachial-ankle pulse wave velocity; *logical mechanisms*, logical backgrounds for the measurements; *reproducible*, the reproducibility of the marker; *generalizability and validity*, generalizability of the measurement, and its accuracy; *disease-specific*, reflecting some specific disease or pathophysiological abnormalities; *surrogate*, improved clinical outcomes.
++, good; +, acceptable; ±, limited; −, not acceptable.

Peripheral arterial disease

A low ABI is a risk factor for amputation (Hämäläinen et al., 1999). ABI ≤0.90 has a high specificity for the diagnosis of PAD. However, the Walking and Leg Circulation Study reported that up to a half of the patients with PAD do not exhibit exertional leg pain or atypical leg pain (McDermott et al., 2002), and that PAD is asymptomatic in about 50% of all patients (Olin et al., 2010). Therefore, all subjects with ABI ≤0.90 need evaluation to determine the severity of PAD and specialist consultation for the management of PAD. In contrast, the sensitivity of ABI to identify PAD is only modest. Therefore, in subjects with symptoms suggestive of PAD, but ABI >0.90 (especially ABI 0.90–0.99), postexercise ABI measurement should be considered to screen for the presence of PAD.

High-risk status for cardiovascular disease

In the Reduction of Atherothrombosis for Continued Health (REACH) Registry study, of the patients with symptomatic atherothrombosis, a significant proportion (15.9%) had symptomatic polyvascular disease (Bhatt et al., 2006). Thus, the prevalence rate of cardiovascular disease is high in patients with PAD. On the basis of a review of 18 cohorts studies, the US Preventive Services Task Force reported that ABI ≤0.90 is associated with future coronary artery disease and cardiovascular events, independent of the Framingham risk scores; on the other hand, the significance of ABI >1.30/1.40 as a marker to predict future cardiovascular events is inconsistent (Lin et al., 2013). As compared to aggregate data meta-analysis, meta-analysis of individual participant data collected from all studies is considered as the gold-standard approach to evidence synthesis (Stewart et al., 2015). The Ankle Brachial Index Collaboration Study Group conducted a meta-analysis of individual participant data of 24,955 men and 23,339 women derived from a general population, none of whom had a history of coronary artery disease. They found a reverse J-shaped association of ABI with cardiovascular death. The ABI associated with the lowest cardiovascular risk was 1.11–1.20, and hazard ratio for 10-year cardiovascular mortality associated with ABI ≤0.90 (reference ABI 1.11–1.20) was 2.9 in men and 3.0 in women after adjusting for the Framingham risk scores (Ankle Brachial Index Collaboration, 2008). ABI ≥1.40 was also found to be a significant predictor of cardiovascular events in both men and women. In addition, Emdin et al. (2015) reported that PAD is also a predictor of atrial fibrillation and chronic kidney disease. Irrespective of these findings, it may be difficult to make optimal treatment decisions based on quantitative risk assessment (Aboyans et al., 2012).

ABI 0.09–0.99 is defined as borderline ABI (Rooke et al., 2011), and the Ankle Brachial Index Collaboration Study Group reported that men and women with values of the ABI of 0.91–1.10 also had higher mortality and event rates than those with ABI in the normal range (1.11–1.40). Recent studies have confirmed these findings (Tanaka et al., 2016; Natsuaki et al., 2014). Therefore, it is proposed that ABI in the borderline range be considered as representing a high-risk state for cardiovascular disease.

ABI measurement for cardiovascular risk assessment is feasible. However, the US Preventive Services Task Force concluded that currently available evidence is insufficient to assess the balance between the benefits and risks of ABI measurement as a screening tool for PAD and cardiovascular disease risk assessment (Lin et al., 2013). In **Fig. 4**, the bar graphs represent the number of subjects in each ABI category by the Ankle Brachial Index Collaboration Study Group (Ankle Brachial Index Collaboration, 2008). In this study, while the hazard ratio for death was high for ABI ≤0.90, only 7.4% (1841/24,955 men) and 8.1% (1901/23,339 women) of the study subjects had ABI ≤0.90. On the other hand, the ABI was >0.90 in more than 90% of the study subjects, and 4625 of the 5582 deaths in men (82.8%) and 3480 of the 4342 deaths in women (80.1%) were observed in these subjects. Thus, there are limitations to identifying subjects with a high cardiovascular risk on the basis of the ABI ≤0.90 alone, and the development of some additional approach for screening such high-risk subjects is proposed.

ABI as a Tool for Monitoring Patients During Management of PAD

A report of the American College of Cardiology Foundation (ACCF)/AHA Task Force on Performance Measures, the American College of Radiology, the Society for Cardiac Angiography and Interventions, the Society for Interventional Radiology, the Society for Vascular Medicine, the Society for Vascular Nursing, and the Society for Vascular Surgery 2010 recommend measurement of the ABI at 5-year intervals in adults with PAD (Olin et al., 2010), and the Multi-Ethnic Study of Atherosclerosis study, in which none of the participants had clinical cardiovascular disease at the time of enrollment, reported that over a median follow-up of 9.8 years,

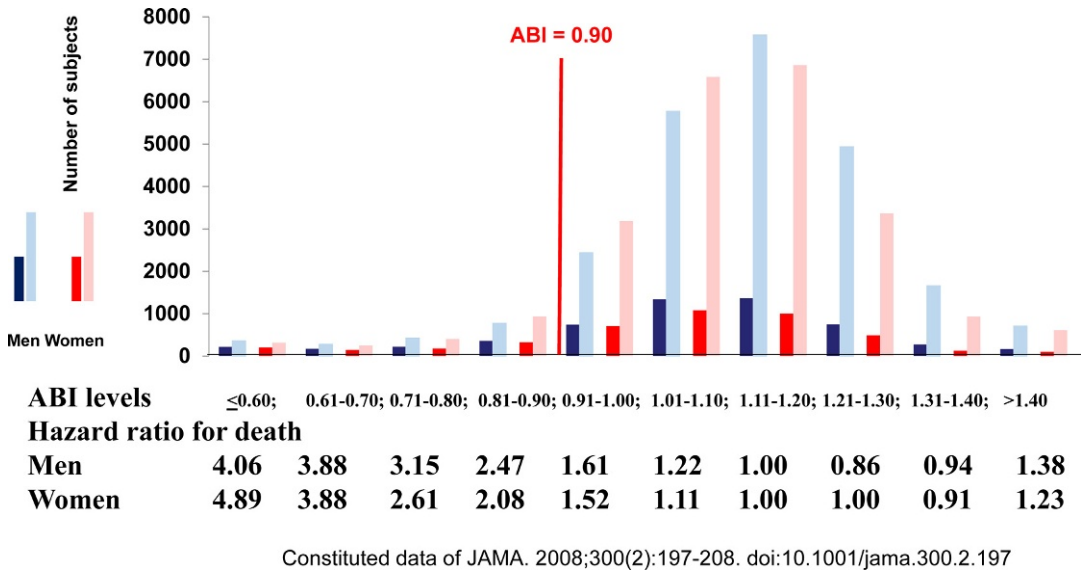

Fig. 4 Relationship between the hazard ratio for death and the number of subjects in each ankle-brachial pressure index level. *Abbreviations*: *dark color bars* represent subjects with cardiovascular events and *light color bars* represent subjects without cardiovascular events. Constituted data of Ankle Brachial Index Collaboration, Fowkes, F. G., Murray, G. D., Butcher, I., et al. (2008). Ankle brachial index combined with Framingham Risk Score to predict cardiovascular events and mortality: A meta-analysis. *JAMA* **300**(2), 197–208. https://doi.org/10.1001/jama.300.2.197.

221 of the 5591 participants (4%) showed decrease of the ABI to ≤0.90, and 89 of the 5591 participants (1.6%) showed increase of the ABI to >1.40 (Garimella et al., 2015).

Cronenwett et al. examined the clinical course of 91 men with mild intermittent claudication. Over a mean follow-up period of 2.5 years, a decrease of the ABI by at least 0.15 was associated with an increased operation rate and symptom progression (Cronenwett et al., 1984). Increase of the ABI by 0.15 following revascularization was predictive of absence of residual stenosis >50% (Decrinis et al., 1994); however, ABI measurement was not useful to predict revascularization failure (Barnes et al., 1989). While exercise therapy or medication (ramipril, an angiotensin-converting enzyme inhibitor) improves the symptoms related to limb ischemia, ABI did not reflect this symptomatic improvement (Parmenter et al., 2015; Kurklinsky and Levy, 2013). Thus, in patients with PAD, ABI monitoring might be applicable to monitor the progression of the disease state, but it might have some limitations in relation to assessment of the effects of PAD treatment.

Summary

ABI is used as a diagnostic tool for PAD and for cardiovascular risk assessment. For assessing the severity of PAD and the progression of the disease, especially in patients with severe PAD, ABI measurement by the Doppler method may be more suitable. For cardiovascular risk assessment, on the other hand, both the Doppler method and oscillometric method are applicable, although the oscillometric method may have the advantage of being applicable to a large number of subjects.

Arterial Stiffness

Rationale for Use as a Cardiovascular Risk Marker

Atherosclerosis, arteriosclerosis, and arterial stiffness

Atherosclerosis and arteriosclerosis are phenotypes of atherosclerotic vascular damage. The arterial wall is composed of three layers (i.e., the intima, including the endothelium, media, and adventitia); atherosclerosis begins from the intima, while the pathological alterations of arteriosclerosis are mostly observed in the media (Tomiyama and Yamashina, 2010; O'Rourke and Hashimoto, 2007; Najjar et al., 2005; O'Rourke, 2015; Safar, 2007). The structure of the arterial wall differs from the proximal sites to the periphery; the arterial tree can be simply classified as being composed of three categories of vessels: the elastic arteries, muscular arteries, and arterioles. The components of the media layer differ between the elastic and muscular arteries. A high content of elastic fibers is observed in the medial layer of the elastic arteries, while the vascular smooth muscle cell layer is dominant in the medial layer of the muscular arteries (Tomiyama and Yamashina, 2010; O'Rourke and Hashimoto, 2007; Najjar et al., 2005; O'Rourke, 2015; Safar, 2007). Age and elevated blood pressure (pulsatile stress) are major factors associated with the degeneration of elastic fibers, increase of the collagen content, and proliferation of the vascular smooth muscle. Elevated blood pressure is also associated with vascular smooth muscle cell hypertrophy (Tomiyama and Yamashina, 2010; O'Rourke and Hashimoto, 2007; Najjar et al., 2005; O'Rourke, 2015; Safar, 2007). Then, arteriosclerosis is a diffuse, but segmental abnormality (elastic/muscular arteries).

Arteries are reported to have the function of efficiently delivering blood to the peripheral organs and of maintaining this delivery function (Tomiyama et al., 2016). Thus, the most important functions of the arterial tree are its function as a conduit for blood (delivery of blood to organs) and its cushioning (dampening) effect (to generate continuous blood flow/reduce exposure of the arterial tree to the cardiac pulsatile energy) (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama and Yamashina, 2010; O'Rourke and Hashimoto, 2007; Najjar et al., 2005; O'Rourke, 2015; Safar, 2007; Tomiyama et al., 2016; Laurent and Boutouyrie, 2007; O'Rourke et al., 2010). Atherosclerosis, a focal abnormality in the arterial tree, causes organ ischemia via arterial stenosis/obstruction. On the other hand, arteriosclerosis is thought to contribute to the initiation/progression of cardiovascular disease via different mechanisms (i.e., impaired cushioning function). The elastic arteries play a dominant role in cushioning against and dampening the pressure oscillations that result from intermittent ventricular ejection (i.e., the Windkessel effect) and transforming the pulsatile flow into a steady flow for supplying oxygen to the tissues (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama and Yamashina, 2010; O'Rourke and Hashimoto, 2007; Najjar et al., 2005; O'Rourke, 2015; Safar, 2007; Tomiyama et al., 2016; Laurent and Boutouyrie, 2007; O'Rourke et al., 2010). This elasticity has beneficial effects on the cardiovascular system, such as facilitating organ blood supply during diastole, especially in the heart, reduction of the cardiac afterload, and/or protection of the arterial wall and microvasculature from the mechanical stresses generated by cardiac contractions (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama and Yamashina, 2010; O'Rourke and Hashimoto, 2007; Najjar et al., 2005; O'Rourke, 2015; Safar, 2007; Tomiyama et al., 2016; Laurent and Boutouyrie, 2007; O'Rourke et al., 2010). The structural changes of the arterial wall associated with arteriosclerosis increase the arterial stiffness; furthermore, elevated blood pressure and/or increase of the vascular tone can also functionally increase the arterial stiffness. Thus, not only structural stiffness but also functional stiffness exerts harmful effects on the cardiovascular systems.

How does arterial stiffness serve as a cardiovascular risk factor?

Increased arterial stiffness directly increases the cardiac afterload because of the elevated cardiac contractile pressure needed for ejection of blood into the aorta. In the arterial tree, the arteries branch and taper toward the periphery, associated with increase of the arterial resistance. Reflection of the pulse wave (from the periphery to the heart) occurs at sites of abrupt increase of the arterial resistance, such as at sites of arterial branching. Increased arterial stiffness increases the travel speed of the reflected pressure wave, thereby augmenting the summation of the forward pressure wave (from the heart to the periphery) and reflected pressure wave in the aorta during the cardiac systolic phase (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama and Yamashina, 2010; O'Rourke and Hashimoto, 2007; Najjar et al., 2005; O'Rourke, 2015; Safar, 2007; Laurent and Boutouyrie, 2007; O'Rourke et al., 2010), which further increases the cardiac afterload (**Fig. 5**).

Increased arterial stiffness affects the coronary blood supply via two mechanisms. First, the cushioning effect of the elastic aorta involves its expansion during systole to store some amount of the blood ejected from the heart, and then delivery of this stored blood to the tissues during diastole. Since the coronary arteries are mostly perfused during diastole, the amount of this stored blood is an important determinant of the coronary blood flow (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama and Yamashina, 2010; O'Rourke and Hashimoto, 2007; Najjar et al., 2005; O'Rourke, 2015; Safar, 2007; Tomiyama et al., 2016; Laurent and Boutouyrie, 2007; O'Rourke et al., 2010). With increased arterial stiffness, the amount of stored blood in the aorta during systole is decreased, which is associated with a reduced coronary arterial blood supply (**Fig. 6**). In regard to the second mechanism, in the healthy condition of the arterial tree, since the speeds of propagation of the forward pulse wave (heart to periphery) and the reflected pulse wave (periphery to the heart) are not so high, the reflected wave returns to the central aorta during diastole (**Fig. 5**, left figure) (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama and Yamashina, 2010;

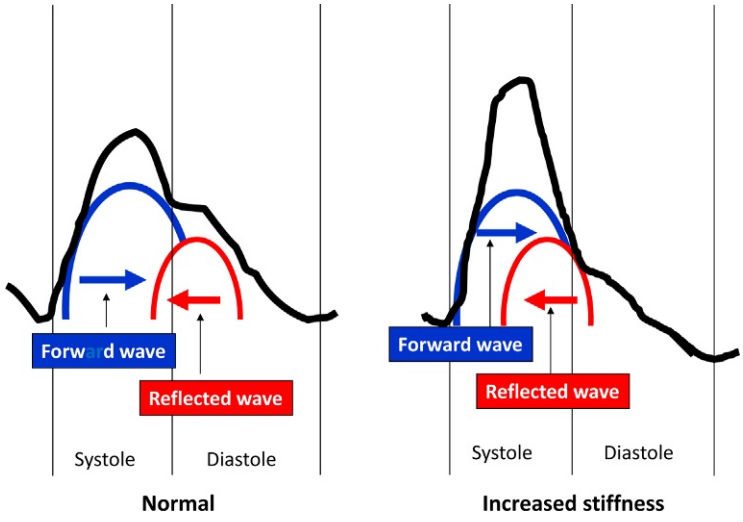

Fig. 5 Central hemodynamics in cases with/without increased arterial stiffness.

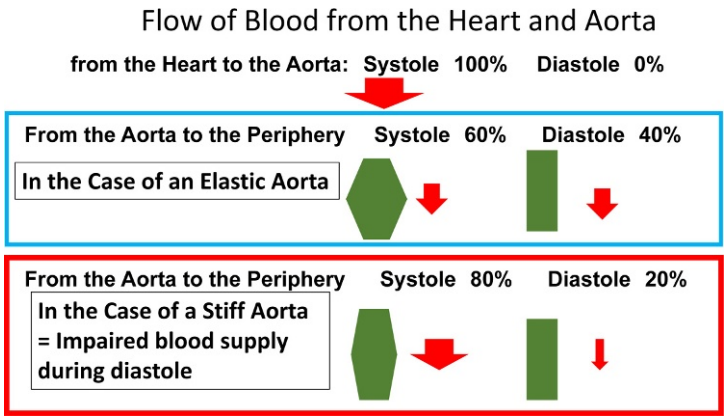

Fig. 6 Conduit function of the large arteries in the presence/absence of increased arterial stiffness.

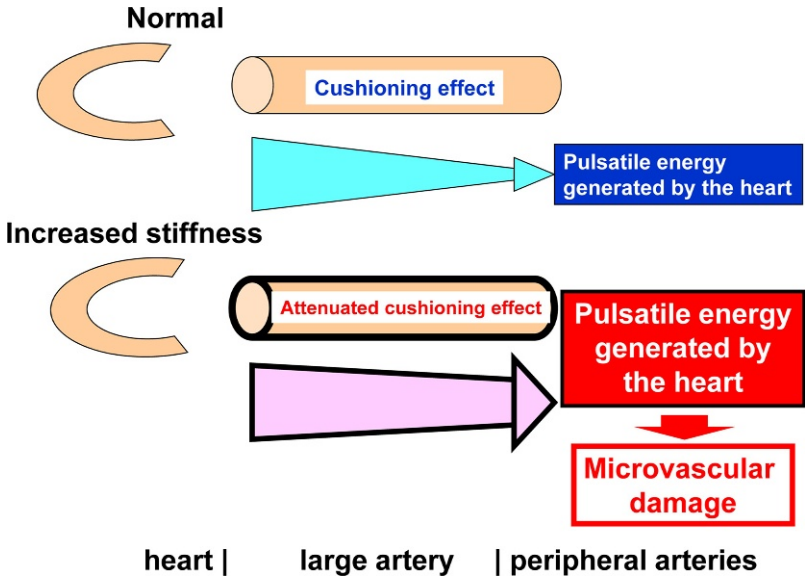

Fig. 7 Cushioning effect of the large arteries on the pulsatile energy generated by the heart. When the cushioning effect is attenuated, the pulsatile energy causes peripheral vascular damage.

O'Rourke and Hashimoto, 2007; Najjar et al., 2005; O'Rourke, 2015; Safar, 2007; Laurent and Boutouyrie, 2007; O'Rourke et al., 2010). This phenomenon increases the diastolic blood pressure and contributes to the maintenance of the coronary blood supply. When the arterial stiffness is increased, elevation of the diastolic pressure by the reflected pulse wave is reduced, resulting in impairment of the coronary blood supply, because the reflected pulse wave returns during systole as a result of increase of the pulse wave propagation speed (Fig. 5, right figure).

Increased arterial stiffness attenuates the cushioning effect of the elastic arteries, and this attenuation amplifies the pulsatility and increases the transmission of pulsatile energy to the peripheral microcirculation (Fig. 7). The specific gravity of mercury is 13, and therefore, a cardiac contraction energy of 140 mmHg is equivalent to the pressure needed for ejection of water to a height of 182 cm ($13 \times 140/10$). Exposure of small vessels to highly pulsatile flow causes microvascular damage, especially, in high-blood flow organs such as the brain and kidney (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama and Yamashina, 2010; O'Rourke and Hashimoto, 2007; Najjar et al., 2005; O'Rourke, 2015; Safar, 2007; Tomiyama et al., 2016; Laurent and Boutouyrie, 2007; O'Rourke et al., 2010), the so-called pulsatile nephropathy and encephalopathy (O'Rourke et al., 2010).

Measurement of the PWV

PWV reflects segmental arterial elasticity. Contraction of the left ventricle generates a pulse wave which is propagated throughout the arterial tree. PWV is calculated as the distance traveled by the pulse wave divided by the time taken to travel the distance (path length) (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama et al., 2016; McDonald, 1968). Increase of the arterial stiffness is associated with increase of the propagation speed of the pulse wave in the arteries. PWV can be measured in any arterial segment

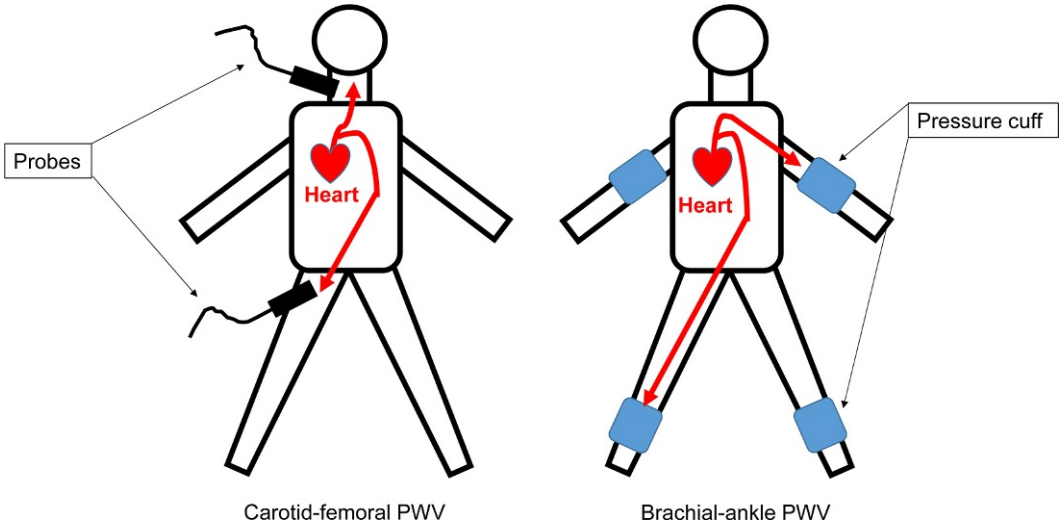

Fig. 8 Schema of carotid-femoral pulse wave velocity and brachial-ankle pulse wave velocity. *Abbreviation*: *arrows*, directions of the pulse wave traveling from heart.

between two pulse-wave palpable regions (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama et al., 2016; McDonald, 1968). In biomechanics, the Moens–Korteweg equation describes the relationship of the PWV with factors related to the blood vessels; this equation is $PWV^2 = E \cdot h / r \cdot p$, where E is the elastic modulus, h is the vessel wall thickness, r is the vessel radius, and p is the blood density. According to this equation, the increase in the vessel wall thickness and/or the decrease in the vessel diameter would result in an increase of the PWV. In addition, age and blood pressure also exert a strong influence on the PWV (Weber et al., 2009).

Conventionally, carotid-femoral PWV is used for assessment of the central arterial stiffness (**Fig. 8**) (Townsend et al., 2015; Vlachopoulos et al., 2015), and recently, mainly in Asia, the brachial-ankle PWV has been measured (**Fig. 8**). In addition, a single-point estimation of the PWV has recently become available, although it is not yet fully established (Weber et al., 2015). In the measurement of the carotid-femoral PWV, the pulse wave is detected by tonometry, Doppler, or oscillometry, and the path length is measured superficially (Townsend et al., 2015; Vlachopoulos et al., 2015). In the measurement of the brachial-ankle PWV, the pressure wave is detected by oscillometry after tying pressure cuffs around the four extremities, and the path length is calculated from the height (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama et al., 2016). Basically, PWV is calculated as the travel speed of the pulse wave between two points on the same artery. However, in the aforementioned procedures, the carotid-femoral PWV is obtained by subtracting the heart-carotid artery PWV from the heart-femoral artery PWV, and the brachial-ankle PWV is calculated by subtracting the heart-brachial artery PWV from the heart-ankle PWV (**Fig. 8**) (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama et al., 2016). These issues are pointed as methodological limitations.

Validity, Reproducibility, and Generalizability of PWV Measurements

The PWVs (carotid-femoral PWV and brachial-ankle PWV) have been demonstrated to show a close correlation to the central arterial stiffness measured by the gold-standard invasive method (Weber et al., 2009; Yamashina et al., 2002). Acceptable reproducibility of these measurements has been reported (i.e., the interobserver Pearson's correlation coefficient r was 0.97 for the carotid-femoral PWV and 0.98 for brachial-ankle PWV) (Yamashina et al., 2002; Mitchell et al., 2004). Usually, skilled personnel and exposure of the inguinal region of the subject are required for carotid-femoral PWV measurement. Therefore, application of this method is mostly limited to research institutes (Mancia et al., 2013). A simple oscillometric method to measure the carotid-femoral PWV has also become available recently (Townsend et al., 2015; Müller et al., 2013). On the other hand, the brachial-ankle PWV can be measured simply by just wrapping a pressure cuff around each of the four extremities (**Fig. 8**) (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama et al., 2016; Yamashina et al., 2002). One of the limitations of measurement of the brachial-ankle PWV is that this parameter reflects not only elastic arterial stiffness but also muscular arterial stiffness (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama et al., 2016; Yamashina et al., 2002). Even so, the brachial-ankle PWV has been demonstrated to show a close correlation to the carotid-femoral PWV (Tanaka et al., 2009). In addition, the ABI can also be measured simultaneously when this oscillometric method is used for measuring the brachial-ankle PWV.

Risk Factors Associated With Increased PWV and Recommended Indications for PWV Measurement

In a systematic review, the contribution of age, blood pressure, and other conventional risk factors for cardiovascular disease to the carotid-femoral PWV was examined, and it was found that the contribution of risk factors other than the age and blood pressure was

small or insignificant (Cecelja and Chowienczyk, 2009). Elevated blood pressure causes functional increase of the arterial stiffness via increasing the arterial wall tension (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama and Yamashina, 2010; O'Rourke and Hashimoto, 2007; Najjar et al., 2005; O'Rourke, 2015; Safar, 2007; Laurent and Boutouyrie, 2007; O'Rourke et al., 2010), and also structural increase of the arterial stiffness via causing structural changes of the arterial wall. Abnormal glucose metabolism and/or metabolic syndrome also increase arterial stiffness (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama et al., 2016). Furthermore, increase of the PWV was observed in subjects with disease states often associated with cardiovascular disease, such as renal dysfunction, pulmonary dysfunction, osteoporosis, and sarcopenia (Tomiyama et al., 2005a; Kimoto et al., 2006; Hirose et al., 2003; Ochi et al., 2010).

The ACCF/AHA guidelines for the assessment of cardiovascular risk in asymptomatic adults do not recommend measurement of the PWV for assessing cardiovascular risk (Greenland et al., 2010). However, based on the evidence accumulated after the publication of this guideline, the ESC guideline recommends measurement of the carotid-femoral PWV in subjects at intermediate risk (Mancia et al., 2013); in addition, a position paper of the European Society of Cardiology Working Group on peripheral circulation proposes measurement of the carotid-femoral PWV in subjects with hypertension and/or diabetes mellitus (Vlachopoulos et al., 2015). For the case of the brachial-ankle PWV, measurement is recommended in subjects with hypertension, diabetes mellitus, chronic kidney disease (stage III–V), and/or coronary artery disease without impaired systolic cardiac function (Tomiyama et al., 2016).

Clinical Perspectives

Clinical perspectives of PWVs were summarized in **Table 1**. Several longitudinal studies conducted in the general population have demonstrated elevated PWV as a predictor of future cardiovascular events, independent of conventional markers such as the Framingham risk scores (Vlachopoulos et al., 2010, 2012; Ben-Shlomo et al., 2014). Furthermore, PWV is valuable as a predictor of the prognosis in patients with renal insufficiency, acute coronary syndrome, stroke, and heart failure (Levisianou et al., 2013; Sung et al., 2011; G□secki et al., 2012; Tomiyama et al., 2005b; Kitahara et al., 2005; Meguro et al., 2009). Aggregate data meta-analysis and independent participant data meta-analysis have been conducted for the carotid-femoral PWV. Vlachopoulos et al. conducted aggregate data meta-analysis in 17 longitudinal studies (6 studies were community-based/general population and 4 studies were subjects with end-stage renal disease) with 15.877 participants. Then, carotid-femoral PWV by 1 m s^{-1} increase corresponded to increase the risk 14% for cardiovascular events, 15% for cardiovascular death, and 15% for all-cause mortality (Vlachopoulos et al., 2010). Ben-Shlomo et al. conducted individual participant data meta-analysis for carotid-femoral PWV in 16 longitudinal studies (8 studies were community-based/general population) with 17,635 participants. Then, a1-standard deviation of carotid-femoral PWV increase corresponded to increase the risk 45% for cardiovascular events, 41% for cardiovascular death and 22% for all-cause mortality (Ben-Shlomo et al., 2014). Vlachopoulos also conducted aggregate data meta-analysis for the brachial-ankle PWV in 18 longitudinal studies (3 studies were community-based/general population and 5 studies were in subjects with end-stage renal disease) in 8169 participants. In their study, increase of the brachial-ankle PWV by 1 m s^{-1} corresponded to a 12% increase of the risk for cardiovascular events, 13% increase in the risk for cardiovascular death, and a 6% increase of the all-cause mortality (Vlachopoulos et al., 2012). The cutoff values of the carotid-femoral PWV and brachial-ankle PWV for predicting the cardiovascular risk status are 10 and 18 m s^{-1} (Townsend et al., 2015; Vlachopoulos et al., 2015; Tomiyama et al., 2016).

Increased arterial stiffness is also a risk factor for the progression of organ damage. Increased PWV has been identified as a predictor of the development of hypertension, progression of renal function decline, and/or cognitive decline (Kaess et al., 2012; Yambe et al., 2007; Chen et al., 2011; Zeki Al Hazzouri et al., 2013). These findings support the concept of pulsatile encephalopathy/nephropathy (O'Rourke et al., 2010).

Monitoring for the Managements

PWV as a tool for monitoring patients during treatment?

The 2013 European Society of Hypertension/ESC Guidelines for the management of arterial hypertension reported that the sensitivity to detect treatment-induced changes, time taken for change, and the prognostic value of change of the carotid intima-media thickness and carotid plaque thickness assessed by carotid ultrasound examination are limited (Mancia et al., 2013). Therapeutic lifestyle modifications (exercise, smoking cessation, weight reduction, salt restriction) have been shown to reduce the PWV (Tanaka and Safar, 2005; Petersen et al., 2015; Tomiyama et al., 2010; Jablonski et al., 2013). Medications for hypertension, diabetes mellitus, and/or dyslipidemia have also been shown to reduce the PWVs (Wu et al., 2015; Rizos et al., 2010; Adji et al., 2011; Tomiyama et al., 2011; Hongo et al., 2008), although the differences in the effects among the drug classes used in the treatment of each of these risk factors have not yet been fully clarified. Guerin et al. reported that improvement of the carotid-femoral PWV by an angiotensin-converting enzyme inhibitor was associated with a better prognosis in patients with end-stage renal disease (Guerin et al., 2001). Orlova et al. (2010) reported that an improvement of the brachial-ankle PWV obtained after 6 months of conventional therapy is predictive of a more favorable prognosis in patients with coronary artery disease. Thus, improvement of the PWV has the potential to be a surrogate endpoint reflecting improvement of the prognosis in patients receiving treatment, but further evidence is needed.

Summary

Increased arterial stiffness, as reflected by the PWV, is an independent risk factor not only for cardiovascular disease but also for progression of organ damage. As compared to atherosclerosis, which contributes to the occurrence of cardiovascular events via causing arterial obstruction/stenosis, arterial stiffness contributes to the occurrence of cardiovascular disease via harmful pathophysiological consequences, such as increased cardiac afterload, impaired coronary blood flow supply, and/or microvascular damage. Lifestyle modification and/or treatment of risk factors for cardiovascular disease improve the PWV. Thus, PWV has the potential to be a management marker (i.e., reflecting not only the prognosis but also the effect of treatment on the prognosis).

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Aortic Aneurysm

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Introduction

Management of aneurysms of the aorta has progressed significantly since the pioneering efforts of Debakey and Cooley over 50 years ago (Cooley, 2001). The traditional surgical techniques have been refined, and the focus has moved toward gaining a fuller understanding of aortic pathology. Disease prevention, refinement of patient selection and technique, together with advances in technology are changing our armamentarium. This article reviews current insights into research and clinical aspects of aortic aneurysm disease.

Definitions

Aneurysms (Greek: aneurysma "to dilate") (Aneurysm, 2016) are localized pathological dilatations of arteries. Although left ventricular aneurysms also exist, the term is usually restricted to *blood vessels* that exceed particular size thresholds. These thresholds may be defined by absolute diameters (adjusted for normal age, sex, and body size variations), or stipulated relative to the diameter

of unaffected adjacent segments. A more general definition designates any dilatation greater than 150% of the normal diameter of the affected artery to be aneurysmal (Goldfinger et al., 2014). The focal dilatation characteristic of aneurysms contrasts with the generalized, modest dilatation and elongation observed with normal aging (termed ectasia) (Elefteriades et al., 2017).

Aortic dissection is a disease process in which blood penetrates into the wall of the aorta through a discrete tear in the inner layer of the artery. Under pressure, this blood separates the layers, creating a false lumen that can propagate longitudinally. The antegrade or retrograde extension of this false lumen can cause complications, including aortic rupture. Dissection is often an acute event superimposed on a chronically degenerated aorta (Elefteriades et al., 2012), which may (usually) or may not (rarely) be aneurysmal. Variants of aortic dissection include penetrating aortic ulcer (which resembles a duodenal ulcer of the aorta) and intramural hematoma (a concentric accumulation of blood in the wall of the aorta, without an intimal tear) (**Fig. 1**; Elefteriades, 2008).

Aortic rupture is a complete, transmural breach of the vessel wall, with blood penetrating all the way through the adventitia. This can occur in the context of aneurysm or dissection, as well as from trauma (aortic transection). Rupture may occur into the pericardium (causing tamponade), thoracic cavity, peritoneal cavity, or retroperitoneal space. Hemorrhage may be restrained by the fibrous tissue and structures surrounding the aorta ("contained" rupture). In the absence of this, "free" rupture usually results in exsanguination. These often-confused terms—dissection, rupture, and transection—are shown schematically in **Fig. 2** (Elefteriades et al., 2012).

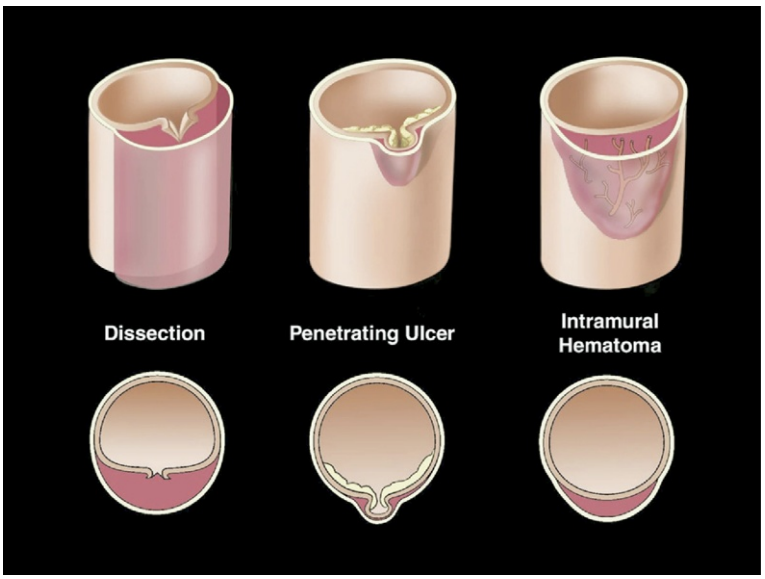

Fig. 1 Aortic dissection and its variants. Reprinted with permission from Elefteriades, J. A. (2008). Thoracic aortic aneurysm: reading the enemy's playbook. *World Journal of Surgery* **32**, 366–374.

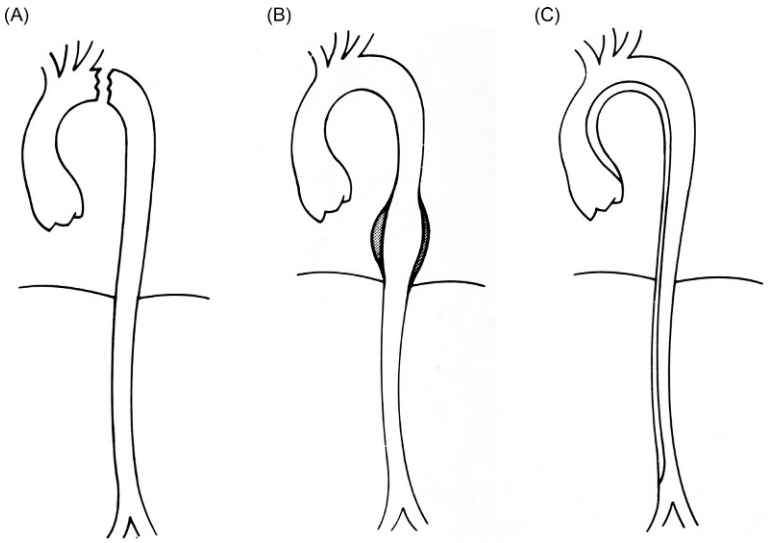

Fig. 2 Three often-confused pathologies (A) aortic transection, (B) "atherosclerotic" descending aortic aneurysm, and (C) aortic dissection. Reprinted with permission from Elefteriades, J. A., Geha, A. S., Tribble, C., Siegel, M. and Cohen, L. S. (2012). *The House Officer's Guide to ICU Care: fundamentals of management of the heart and lungs.* Minneapolis, MN: Cardiotext.

Classification

Aortic aneurysms can be classified by location within the arterial tree, by morphology, as well as by etiology. The aorta can be anatomically divided according to body segment (**Fig. 3**; Isselbacher, 2005). The part of the aorta superior to the diaphragm is known as the thoracic aorta, while that below is the abdominal aorta. The thoracic aorta is further subdivided into ascending and descending parts, with an intervening aortic arch. The abdominal aorta tends to be divided into segments demarcated by the renal arteries, namely, suprarenal and infrarenal. Any of these segments may be subject to aneurysmal dilatation, but thoracic aortic aneurysm and abdominal aortic aneurysm (AAA) are considered distinct disease entities. Indeed, aneurysms of the aorta may be so extensive as to cover more than one of these named parts (e.g., thoracoabdominal aneurysms).

Aneurysms are also distinguished by morphology, specifically by their shape and the nature of their walls. The wall of a true aneurysm maintains the normal trilaminar structure of an artery (intima, media, adventitia), whereas a false aneurysm does not (even the adventitia is violated). False aneurysms (also known as pseudoaneurysms) represent small ruptures of the artery that are contained by the surrounding tissues. In terms of shape, dilatation may affect some or all of the circumference of the artery. Hence saccular aneurysms are outpouchings of an arc of the vessel wall, whereas fusiform aneurysms involve the entire vessel circumference.

Finally, aortic aneurysm is a predominantly an acquired disease. Aneurysms can occur de novo or develop in the context of prior aortic dissection. Moreover, particular congenital lesions predispose to aneurysm formation, the tendency toward which is strongly inherited (i.e., bicuspid aortic valve—see later).

Histology and Embryology

The aortic wall, like that of all elastic arteries, has a trilaminar structure consisting of a tunica intima, media, and adventitia. Most of the mechanical properties of the aorta can be attributed to the media (with the strong collagen component and the stretchable elastin component), although the adventitia is also recognized as a strong layer that maintains aortic diameter and holds stitches when performing surgery on the vessel (Sanz et al., 2007). The media comprises cellular elements (including smooth muscle cells) and structural proteins (notably elastin and collagen) that form the extracellular matrix (ECM). These components are arranged into lamellar units—concentric layers of elastin with interspersed cells and collagen (Wolinsky and Glagov, 1967). Some have likened the layered nature of the aortic wall to the structure of lasagna. The number of lamellar units in the aortic wall is generally

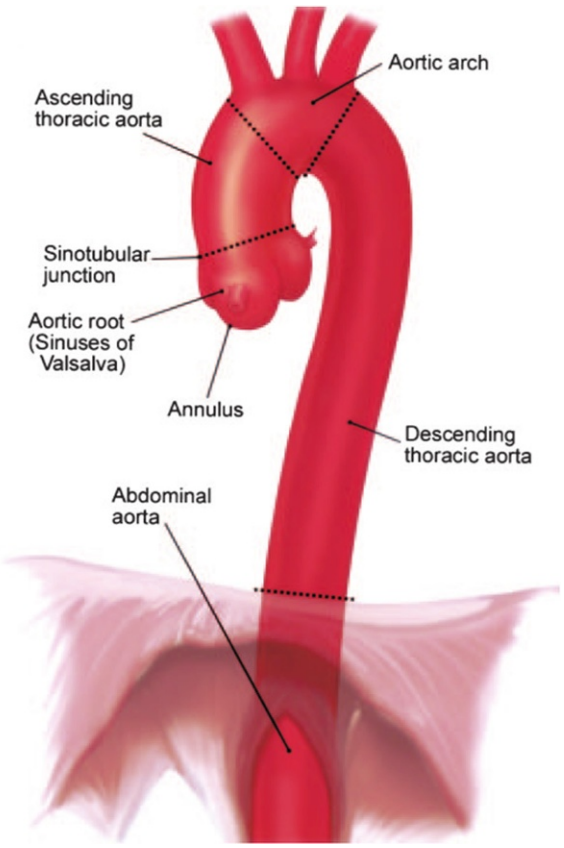

Fig. 3 The parts of the aorta (NB infrarenal aorta not shown). Reprinted with permission from the Massachusetts General Hospital Thoracic Aortic Center.

proportional to the luminal pressure and wall stress that the vessel must withstand, but the number of lamellae is conspicuously low in the infrarenal human aorta (Wolinsky and Glagov, 1969). Elastin, by virtue of its great distensibility, absorbs systolic pressure and contributes to vessel recoil that maintains diastolic forward flow (the "Windkessel effect") (Parker, 2009). Collagen, with its greater tensile strength, is responsible for the aorta's resistance to rupture and deformation (Sanz et al., 2007).

Embryologically, different anatomic portions of the aorta have distinct origins with characteristic structural properties. The ascending aorta and arch are derived from the neural crest, while the descending aorta (distal to the ligamentum arteriosum) originates from mesodermal somites, and the abdominal aorta from splanchnic mesoderm. The thoracic aorta is more distensible, with a vascular outer media, in contradistinction to the abdominal aorta, which is less distensible and has an avascular media. Quantitative differences in the ECM components also exist. These differences influence the susceptibility of the sections of the aorta to various pathological processes.

Pathophysiology

The development of aortic aneurysms can be understood from a number of vantage points. We now have insights into this disease from the molecular biological level up to the engineering level. Ultimately, aneurysm formation results from the combined effects of inherited, degenerative, mechanical, and hemodynamic factors (Elefteriades et al., 2017). Interestingly, aortic aneurysms tend to segregate into two distinct patterns that align with the embryology of the vessel. Aneurysms of the ascending aorta and arch are characterized by cystic medial degeneration (see later), whereas descending and abdominal aneurysms tend to be "arteriosclerotic" (**Fig. 4**; Elefteriades and Farkas, 2010).

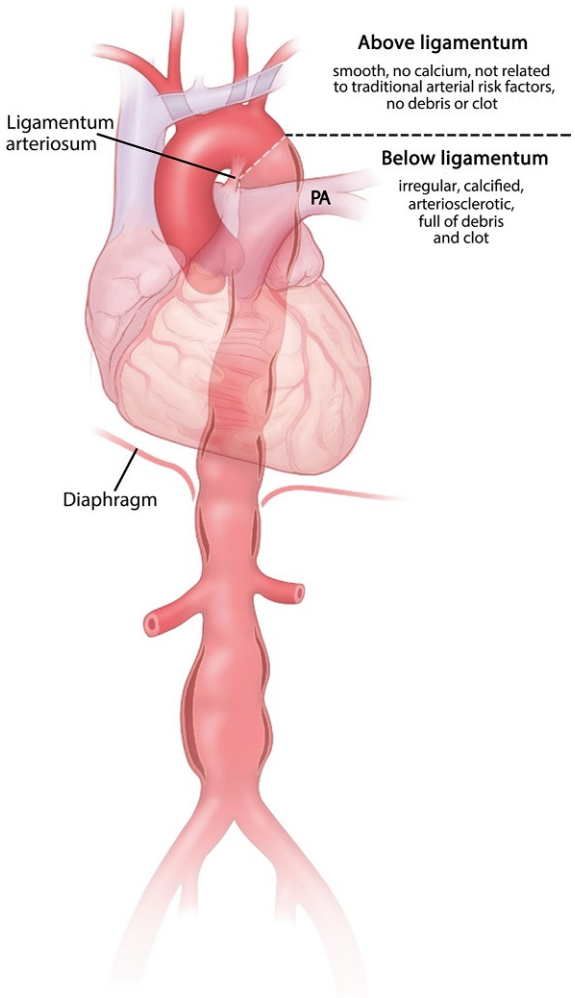

Fig. 4 Distinct patterns of aortic aneurysm disease. Reprinted with permission from Elefteriades, J. A. and Farkas, E. A. (2010). Thoracic aortic aneurysm. Clinically pertinent controversies and uncertainties. *Journal of the American College of Cardiology* **55**, 841–857.

Histological analysis of tissue from thoracic aneurysms often reveals a well-defined pattern known as cystic medial degeneration (previously termed cystic medial necrosis (CMD)). CMD is characterized by the triad of elastic fiber fragmentation, loss of vascular smooth muscle cells (VSMCs), and proteoglycan deposition (Goldfinger et al., 2014) (filling the degenerative voids and providing the "cystic" appearance). A mild form of CMD seems to accompany normal aging; however CMD appears to be accelerated by hypertension and connective tissue diseases such as Marfan syndrome (see later).

A number of overlapping processes are involved in the pathogenesis of aneurysms, namely, ECM destruction, chronic inflammation, cytokine activation, and dysfunction of VSMCs (Wassef et al., 2001; **Fig. 5**; Hackmann et al., 2007). The aortic wall exists in a state of dynamic equilibrium between formation and degradation of ECM components. VSMCs are responsible for collagen and elastin production in addition to serving a contractile function. Matrix metalloproteinases (MMPs) mediate proteolysis and are naturally inhibited by tissue inhibitors of metalloproteinases (TIMPs). An altered ratio of MMP to TIMP activity, together with quantitative (or qualitative—see later) defects in VSMCs, may result in a net proteolytic state that compromises structural integrity of the aortic wall, paving the way for vessel dilatation. Excessive MMP activity has been demonstrated in cases of thoracic and AAA, in addition to altered TIMP levels (Thompson, 1996; Koullias et al., 2004; Kuivaniemi et al., 2015).

Of note, the role of VSMCs is controversial, as these cells have been shown to play seemingly contradictory roles in vascular remodeling. Increased VSMC apoptosis has been implicated in AAA formation, thereby suggesting a (deficient) protective role (Yamanouchi et al., 2010). Conversely, the ability of VSMCs to induce inflammation and MMP production hints at a destructive influence on the ECM (Airhart et al., 2014; Kim and Weintraub, 2016). Moreover, defects in VSMC contractile function have also been suggested as a mechanism contributing to thoracic aortic aneurysm formation (Milewicz et al., 2017).

Chronic inflammation and the role of inflammatory cytokines have also been implicated in the loss of structural integrity of the aortic wall. Inflammatory infiltrates have been observed in both thoracic and aortic aneurysms (Botta et al., 2007), and levels of certain inflammatory cytokines are elevated in some aneurysms (although not consistently so) (Peshkova et al., 2016). Cytokines such as interleukin-1β and tumor necrosis factor-alpha also appear to influence MMP expression (Yoshimura et al., 2006). Transforming growth factor beta (TGF-β) deserves particular attention. This cytokine is expressed by VSMCs and modulates MMP activity. Dysregulated TGF signaling plays a major role in the Marfan syndrome (Matt et al., 2009), while mutations in the TGF-β receptor gene result in the Loeys–Dietz syndrome (see later) (Loeys et al., 2006).

Posttranscriptional regulation of the genes involved in the processes outlined above has also been connected to aneurysm formation. Intracellular micro-RNAs (miRs) are involved in this regulation and have been implicated in the development of many cardiovascular diseases (Raffort et al., 2016). Alterations in the levels of particular miRs in smooth muscle cells from human abdominal aortic aneurysmal tissue have been associated with reduced gene or protein expression of collagen (Cheuk and Cheng, 2014). Animal models of thoracic aortic aneurysms have shown the beneficial effect of miR blockade in the prevention of aneurysm formation (Merk et al., 2012). Nevertheless, the role of miRs in the pathogenesis of human aortic aneurysm remains unclear.

The pathophysiology of the descending and abdominal aorta seems to be distinct from ascending and arch disease, especially as relates to atherosclerosis. However, overlapping pathophysiology exists regarding inflammation, ECM degradation and VSMC apoptosis (Wassef et al., 2001; Kuivaniemi et al., 2015). It has been noted clinically that patients with ascending aortic aneurysms have relatively limited systemic atherosclerosis. This finding was corroborated by evidence from imaging studies that found degree of calcification and intima-media thickness (both markers of (late and early) atherosclerosis) to be reduced in ascending thoracic aneurysm patients (Achneck et al., 2005; Hung et al., 2012). Moreover, the incidence of myocardial infarction was also low in these

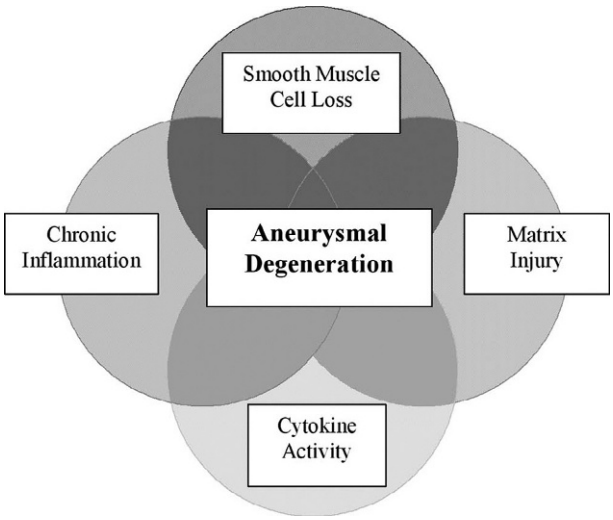

Fig. 5 The overlapping processes involved in the pathogenesis of aortic aneurysm. Reprinted with permission from Hackmann, A. E., Thompson, R. W. and LeMaire, S. A. (2007). In: Elefteriades, J. A. (ed.) *Acute aortic disease*, pp. 309–330. London: Informa Healthcare.

patients (Chau and Elefteriades, 2014). A causal link remains unclear although we speculate that higher MMP activity and altered hemodynamics of ascending aortic disease may play a role in this peculiarity (Chau et al., 2014).

Biomechanical Perspectives

From the mechanical perspective, aneurysm rupture occurs when the mechanical stresses acting on the aortic wall exceeds the maximum wall strength (Vorp and Vande Geest, 2005). The law of Laplace is often used to describe the forces acting on the walls of aneurysms. It states that the wall tension is proportional to luminal pressure and the radius of the vessel ($t = pr$) (Valentinuzzi and Kohen, 2011). However, this is an oversimplification as it assumes an idealized cylindrical shape; in reality aneurysms have complex geometries with irregular distributions of wall tension (Lasheras and The, 2007). The law of Laplace does not allow for local failure in complex geometric forms, which is often the inciting event in aortic ruptures (Gasser, 2016). Moreover, dissections are known to occur at small aortic dimensions (Pape et al., 2007). In vivo studies with epiaortic echocardiography demonstrate that ascending aortic distensibility diminishes, and wall stress increases, as the vessel diameter increases (Koullias et al., 2005). Engineering analyses using biaxial mechanical testing provide further evidence of aortic stiffening with dilatation; the resultant rise in wall stress increases risk of aneurysm rupture and dissection (Martin et al., 2013).

Etiology

Thoracic aortic aneurysms can be broadly classified into syndromic and nonsyndromic categories, with the former manifesting systemic features and the latter manifesting aortic disease alone. The etiology of AAAs is similar to thoracic in some cases. However, it must be stressed that most cases of AAAs do not presently have a clearly defined specific etiology.

Syndromic Thoracic Aortic Aneurysms

Thoracic aortic aneurysm is a feature of a number of well-described syndromes, the presence of which permits directed screening to aid diagnosis and therapy. These syndromes are often described as connective tissue disorders, the most prominent being Marfan syndrome.

Marfan syndrome

Marfan syndrome occurs due to an autosomal dominant mutation in the FBN1 gene, resulting in synthesis of defective fibrillin-1. Among other functions, fibrillin-1 modulates TFG-β signaling. Marfan patients exhibit elevated TGF-β signaling, which upregulates MMP production, resulting in increased ECM degradation. Histologically, this manifests as CMD. With a prevalence of 2-3 per 10,000 people (Judge and Dietz, 2005), Marfan patients have characteristic features, chiefly dolichostenomelia (long, thin extremities), ligamentous redundancy or laxity, ectopia lentis, aortic or mitral valve incompetence, and ascending aortic dilatation (Loeys et al., 2010). The ascending aortic dilatation has a typical morphology—annuloaortic ectasia (see later)—and the aortas of these patients have a strong propensity for rupture and dissection (Milewicz et al., 2005).

Ehlers–Danlos syndrome

The vascular form of Ehlers–Danlos syndrome (also designated "type IV") predisposes to aortic aneurysm and dissection due to defective type III collagen secondary to mutations in COL3A1 (Pepin et al., 2000).

Loeys–Dietz syndrome

Aneurysm formation (and arterial tortuosity) forms one of a triad of features characteristic of Loeys–Dietz syndrome. This syndrome is caused by mutations in TGF-β receptor 1 or 2. Other features include hypertelorism (widely-spaced eyes) and bifid uvula or cleft palate. These patients tend to have a particularly aggressive form of aortic aneurysm, as evidenced by early onset and rapid growth rate, as well as a high rate of premature aortic dissection (Loeys et al., 2006; Loeys et al., 2005).

Nonsyndromic Thoracic Aortic Aneurysms

The majority of nonsyndromic thoracic aneurysms are sporadic, often attributed to hypertension or atherosclerosis or simply labeled idiopathic. Nevertheless, the etiology is becoming clear in certain cases, as outlined later.

Bicuspid aortic valve

Bicuspid aortic valve refers to the presence of two leaflets instead of the normal tricuspid aortic valve morphology. This represents the most common congenital malformation of the heart, affecting 1.3% of the population (Michelena et al., 2015). Also associated with aortic coarctation, this inherited condition predisposes to aneurysm formation due to a *bona fide* "aortopathy" and not simply the result of poststenotic dilatation, as was once believed (Fedak et al., 2016). Aortic dilatation occurs at an accelerated rate,

especially in the presence with valvular dysfunction (stenosis, regurgitation). Aneurysms seem to result due to a complex interplay between genetic predisposition and hemodynamic consequences of the abnormal valve (Fedak et al., 2016; Padang et al., 2013).

Familial thoracic aneurysm and dissection (TAAD) syndrome

The strong heritability of thoracic aortic aneurysm suggests a familial syndrome without specific identifiable genetic defects. Family history studies have revealed that up to 20% of probands with ascending thoracic aortic aneurysms have a family member with known aneurysm disease (Biddinger et al., 1997; Albornoz et al., 2006), casting new light on cases previously attributed to other causes (or considered idiopathic). Multiple inheritance patterns exist, with autosomal dominance and reduced penetrance being most common. Multiple gene mutations have been found to cause familial TAAD (Brownstein et al., 2017; Ziganshin et al., 2015a).

Other causes

Rare causes of aneurysm include infection as well as particular inflammatory diseases. Infective (mycotic) aneurysms present mostly as localized, saccular dilatations. Syphilitic (luetic) aneurysms are a distinct subset of infective aneurysms, now almost of historical interest in developed countries. Inflammatory aneurysms often develop in the ascending aorta or arch as the result of an aortitis, in the context of Takayasu's disease, Behcet's disease, or giant cell arteritis (among others) (Goldfinger et al., 2014; Elefteriades et al., 2017).

Abdominal Aortic Aneurysms

Although AAAs can also develop in the context of connective tissue disorders, they are predominantly a degenerative phenomenon classically attributed to atherosclerosis (Isselbacher, 2005). However this assertion has become controversial in light of evidence showing conflicting associations between AAA and the traditional atherosclerotic risk factors (Lederle et al., 2003; Lederle et al., 1997). It has been suggested that immunologically mediated mechanisms may reconcile this discrepancy (Peshkova et al., 2016). Family history remains an important risk factor, suggesting an underlying genetic component, although not as powerfully or consistently as in thoracic aortic aneurysm disease.

Genetics of Thoracic Aneurysms

The genetic basis of thoracic aortic aneurysm formation has begun to be elucidated, and a number of candidate genes associated with this disease are now known. These genes are involved in ECM matrix regulation, smooth muscle function, and TGF-B signaling pathways. Furthermore, the MYLK (Wang et al., 2010) and ACTA2 (Guo et al., 2007) genes are specifically implicated in aortic dissection. The presence of mutations in these genes has the potential to influence the natural history of aneurysm disease, and hence its management on an individualized basis. Moreover, the prospect of routine whole-exome sequencing may enable prophylactic surgery (Brownstein et al., 2017; Ziganshin et al., 2015a).

Risk Factors

Despite many advances in our understanding of the mechanisms of aneurysm formation, the majority of aortic aneurysm disease does not have a clear pathogenesis. Various risk factors have been associated with an increased incidence of the disease—including family history of aneurysm disease, age, male sex, cigarette smoking, coronary artery disease, cerebrovascular disease, and hypercholesterolemia (Kent, 2014; Ahmed et al., 2016).

Natural History

Studies of large patient databases have characterized the behavior of aortic aneurysms, allowing evidence-based decisions regarding frequency of surveillance and timing of referral for surgical intervention before dissection or rupture can occur. Aneurysm diameter emerges as the major criterion for decision-making. It should be noted that *symptomatic* aneurysms affecting any portion of the aorta mandate surgery regardless of aortic size, as this generally heralds impending rupture. This point, that "symptoms trump size," is often overlooked and deserve strong emphasis.

In terms of disease progression, thoracic aortic aneurysm is an indolent disease with an average growth rate of 1 mm per year. Growth of aneurysms increases as a function of size (**Fig. 6**) and varies by aneurysm location (Elefteriades et al., 2015a). Although the abdominal aorta may sometimes dilate quickly, this is unusual for the thoracic aorta; a report of putative rapid growth of the thoracic aorta is usually explained by nonmeaningful oblique measurements across the aortic arch (Elefteriades and Farkas, 2010).

The importance of size is reflected by data showing that the risk of complications—rupture, dissection, or death—increases with aneurysm diameter (**Fig. 7**; Elefteriades and Farkas, 2010). Furthermore, the cumulative lifetime incidence of complications (dissection or rupture) increases sharply at particular aortic diameters (6 cm ascending, 7 cm descending—**Fig. 8**; Coady et al., 1997). Thus, the major criterion for surgical referral is maximum aortic size, with patients ideally operated on well before their

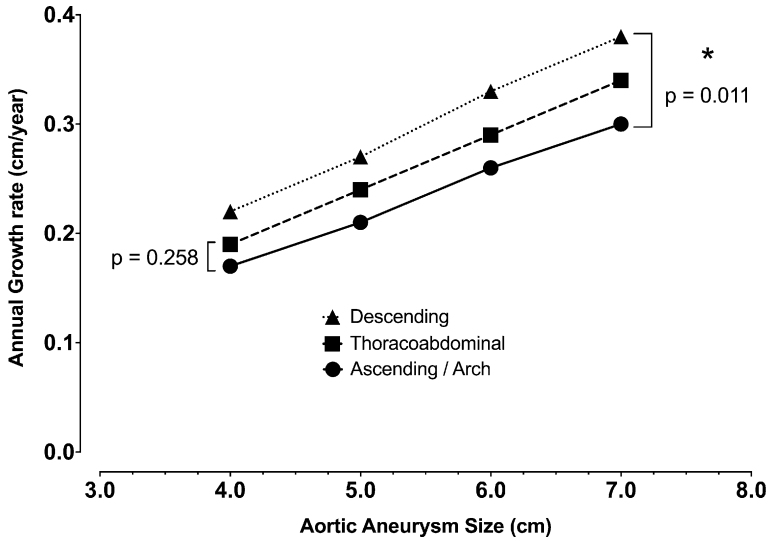

Fig. 6 Annual growth rates of the aorta. Reprinted with permission from Elefteriades, J. A., et al. (2015). Indications and imaging for aortic surgery: size and other matters. *Journal of Thoracic and Cardiovascular Surgery* **149**, S10–S13.

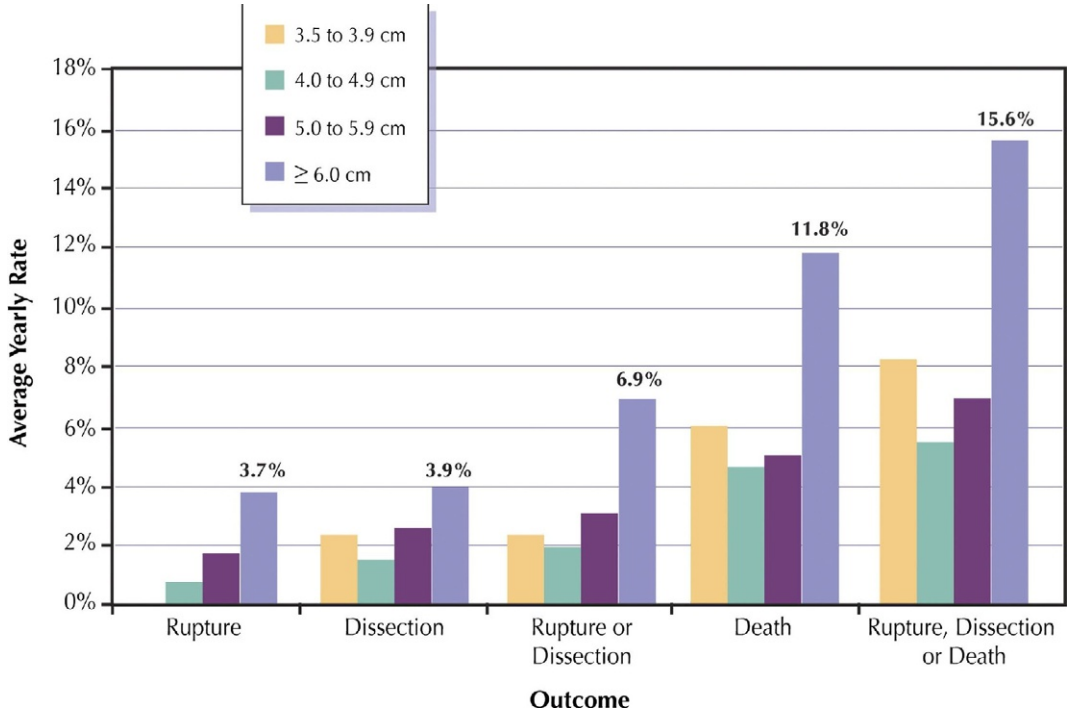

Fig. 7 Average annual rates of rupture, dissection, or death by aneurysm size. Reprinted with permission from Elefteriades, J. A. and Farkas, E. A. (2010). Thoracic aortic aneurysm. Clinically pertinent controversies and uncertainties. *Journal of the American College of Cardiology* **55**, 841–857.

aneurysms reach these dangerous dimensions. Patient size has also been shown to be a useful metric, with aortic size adjusted for body surface area correlating well with risk of complications (Davies et al., 2006). The so-called aortic body size index is most useful at the extremes of size—for very small or very large patients.

The etiology of disease also influences management decisions. Patients with syndromic aneurysms (Marfan syndrome, Ehlers–Danlos syndrome, Loeys–Dietz syndrome), bicuspid aortic valve, or familial cases may be at risk of complications at relatively small aortic diameters. Such patients are therefore often managed more aggressively, with lower size thresholds for surgical referral. Fig. 9 outlines our surgical intervention criteria (Elefteriades, 2008), while Fig. 10 (Brownstein et al., 2017) shows the intervention criteria for specific mutations.

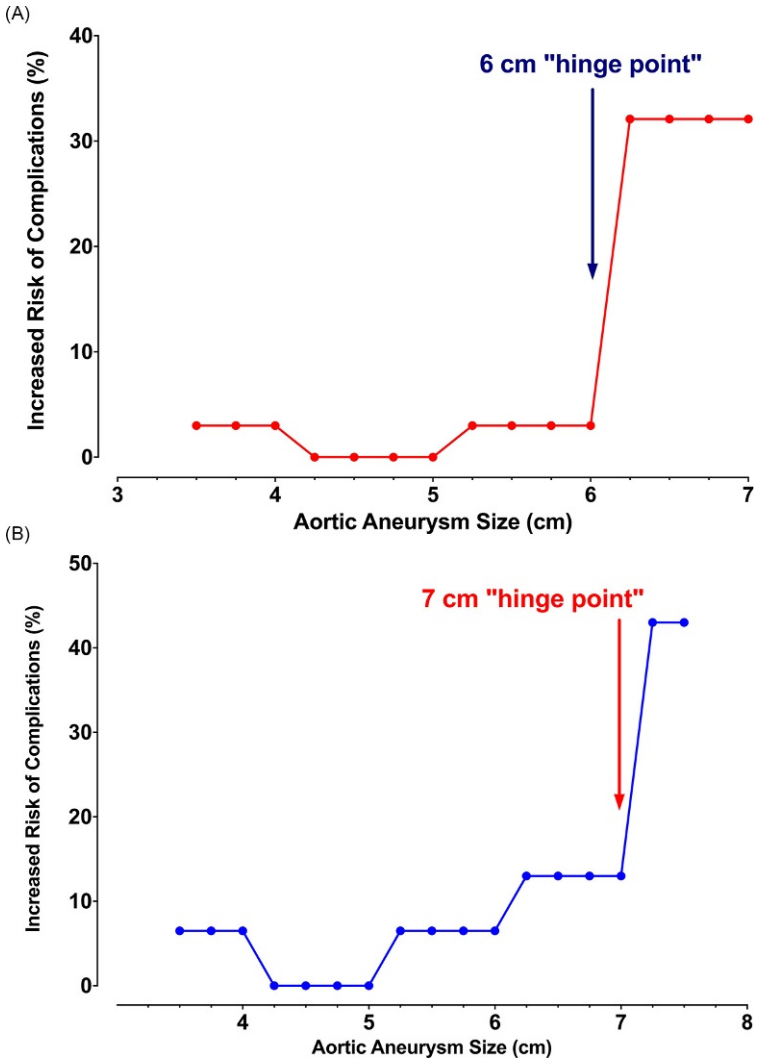

Fig. 8 Percentage increased risk of complications as function of aortic size. Panel A: ascending aorta, Panel B: descending aorta. Modified with permission from Coady, M. A., et al. (1997). What is the appropriate size criterion for resection of thoracic aortic aneurysms? *Journal of Thoracic and Cardiovascular Surgery* **113**, 476–491.

APPENDIX

The Yale Center for Thoracic Aortic Disease Recommended Surgical Intervention Criteria for Thoracic Aortic Aneurysms

1. Rupture
2. Acute aortic dissection
   a. Ascending requires urgent operation
   b. Descending requires a "complication-specific approach"[28]
3. Symptomatic states
   a. Pain consistent with rupture and unexplained by other causes
   b. Compression of adjacent organs, especially trachea, esophagus, or left main stem bronchus
   c. Significant aortic insufficiency in conjunction with ascending aortic aneurysm
4. Documented enlargement
   a. growth ≥1 cm/yr or substantial growth and aneurysm is rapidly approaching absolute size criteria
5. Absolute size (cm)

	Marfan's	Non-Marfan's
Ascending	5.0 cm	5.5 cm
Descending	6.0 cm	6.5 cm

Fig. 9 Yale Aortic Institute guidelines for surgery of thoracic aortic aneurysms. NB Marfan size criteria apply for patients with family history of aneurysm. Reprinted with permission from Coady, M. A., Rizzo, J. A. and Elefteriades, J. A. (1999). Developing surgical intervention criteria for thoracic aortic aneurysms. *Cardiology Clinics* **17**, 827–839.

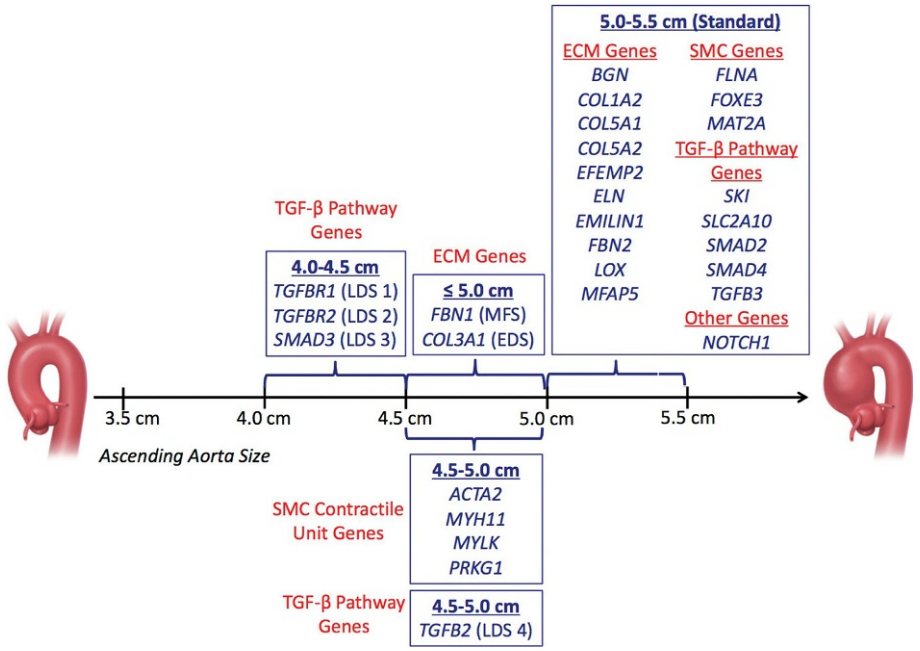

Fig. 10 Simplified schematic illustration of ascending aorta dimensions for prophylactic surgical intervention divided by gene category: ECM genes, SMC contractile unit and metabolism genes, and TGF-β signaling pathway genes. *ECM*, extracellular matrix; *LDS*, Loeys–Dietz syndrome; *MFS*, Marfan syndrome; *SMC*, smooth muscle cell; *EDS*, Ehlers–Danlos syndrome. Reprinted with permission from Brownstein, A. J. et al. (2017). Genes associated with thoracic aortic aneurysm and dissection: an update and clinical implications. *Aorta* (*Stamford*) **5**, 11–20.

AAAs also exhibit growth rates that vary with initial aneurysm diameter. Multiple studies have characterized the natural history of AAA. Smoking has been shown to accelerate aneurysm growth, while diabetes and peripheral arterial disease are associated with reduced growth rates (Brady et al., 2004; Vega de Céniga et al., 2006). The risk of rupture also increases with size (Cronenwett et al., 1985; Nevitt et al., 1989); thus, threshold for intervention is aneurysm diameter of 5.5 cm (Chaikof et al., 2009). Rapid expansion further portends rupture and should also prompt surgical referral (>0.5 cm/year thoracic aorta (Hiratzka et al., 2010), >0.7 cm/year abdominal aorta (Chaikof et al., 2009)).

Precipitation of Aortic Dissection or Rupture

Knowledge of the pathophysiology, natural history, and genetics of aortic aneurysms has enabled us to understand more reliably the onset of their acute complications. In particular, the role of hypertensive surges as the inciting trigger for aortic dissection has been demonstrated in the context of extreme physical exertion. We speculate that this may also account for the curious observation that aortic events occur at times of great emotional stress (Hatzaras et al., 2007). Although a full understanding remains elusive, we propose the following sequence of events (**Fig. 11**; Elefteriades and Farkas, 2010):

1. Genetic predisposition—The patient's genotype defines their propensity for aortic aneurysm formation (or dissection).
2. Medial degeneration—The structural integrity of the aortic wall is compromised by a combination of ECM destruction, cytokine activity, chronic inflammation, and smooth muscle cell loss.
3. Aneurysm formation—The weakened aorta progressively dilates, increasing wall stress.
4. Hypertensive episode—Sudden surges in blood pressure, often at moments of extreme exertion or emotion, result in wall stress exceeding the ultimate tensile strength of the aortic wall.
5. Acute event—The aorta dissects and/or ruptures.

Epidemiology

Data from the Centers for Disease Control and Prevention (CDC), together with statistics from other countries, suggest that aortic aneurysm is being diagnosed at a greater frequency (Acosta et al., 2006; Clouse et al., 1998; Best et al., 2003). However, an accurate knowledge of the epidemiology of aortic aneurysm (particularly thoracic) is difficult to ascertain due to a number of methodological challenges. These include referral center bias, unknown presence of latent cases, misdiagnosis, confusion in terminology, and limitations of administrative databases (Elefteriades and Rizzo, 2007).

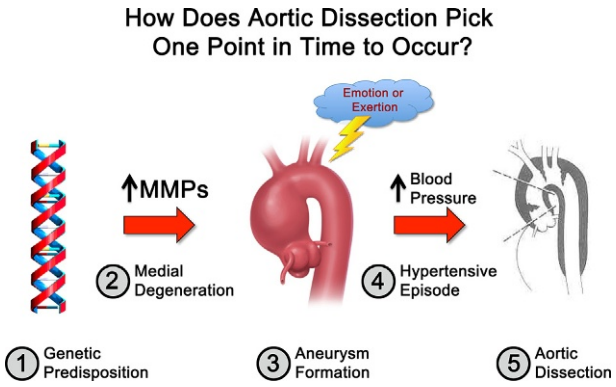

Fig. 11 Proposed series of events that result in aneurysm dissection or rupture. Modified with permission from Hatzaras, I. S., et al. (2007). Role of exertion or emotion as inciting events for acute aortic dissection. *American Journal of Cardiology* **100**, 1470–1472.

Nevertheless, CDC data show that aortic aneurysm accounted for 9,988 deaths in 2015 (National Center for Injury Prevention and Control, 2015). Prevalence of thoracic aortic aneurysm is difficult to obtain for the reasons outlined above. However, the incidence of thoracic aortic aneurysm has been reported as 10.4 per 100,000 population, equally distributed between men and women, although women are 12 years older than men at diagnosis (Clouse et al., 1998). The prevalence of AAA is 5.5% in the over-65 age group and is considerably higher in men (Li et al., 2013).

Reasons for the apparent increase in diagnosis of aortic aneurysms remain unclear although the aging population undoubtedly contributes to this phenomenon. Moreover, there is evidence for a true increase in incidence rather than merely increased detection due to the rising use of 3-D diagnostic imaging (Acosta et al., 2006; Clouse et al., 2004).

With regard to acute complications of aneurysm disease, acute aortic dissection has an incidence of 3.5–6/100,000 (Clouse et al., 2004; Howard et al., 2013), while the incidence of ruptured thoracic aortic aneurysm is 3.5–6/100,000 (Clouse et al., 2004; Johansson et al., 1995). Ruptured AAA has an incidence of 10.6/100,000 equally distributed between the sexes. This rises to 46 per 100,000 men in their 60s, and more than 100 per 100,000 men in their 70s (Acosta et al., 2006).

AAA Screening

Screening enables identification of asymptomatic patients and permits appropriate surveillance or operative management to reduce morbidity and mortality related to AAA rupture. The U.S. Preventive Services Task Force recommends (LeFevre, 2014) one-time screening with ultrasonography for all men aged 65–75 who have ever smoked, with high certainty of overall moderate benefit. If the patient does not have an aneurysm at age 65, he is almost certain never to die from AAA. Selective screening of nonsmokers is recommended on the basis of professional judgment or patient preferences. Insufficient evidence has been found for screening of women, reflecting the male preponderance of the disease. No appropriate general population screening regimens have been established or validated for thoracic aortic aneurysms.

Biomarkers

The silent nature of aneurysm disease and the devastating consequences of its complications warrant the discovery of a biomarker. An ideal biomarker should not only detect aortic aneurysms but also provide some assessment of rupture/dissection risk without the need for actual aortic tissue. (Unlike other organs, the aorta is not amenable to biopsy.) Moreover, the biomarker should help identify at-risk populations.

A better understanding of the pathogenesis of aneurysm formation may guide the search for biomarkers. For example, an appreciation of the role of inflammation in aneurysm formation has led to the investigation of aneurysm-related RNA signatures in circulating leukocytes. The distinct gene expression in peripheral blood cells of thoracic aneurysm patients has shown promise as a marker of thoracic aortic aneurysm in the general population, with a sensitivity and specificity of 72% and 90% (**Fig. 12**) (Wang et al., 2007). A similar philosophy has been applied to cytokines. Advanced glycation end-products (AGEs) have recently been implicated in the formation of thoracic aortic aneurysm (Prasad et al., 2016). The presence of soluble receptors for these AGEs (sRAGE) detectable in the peripheral circulation makes this another candidate for an aneurysm biomarker. miRs, intracellular regulators of gene transcription, have also been discovered in the circulation and present another potential biomarker of aneurysm disease. Studies have shown differential miR expression in the blood of patients with AAA relative to controls (Stather et al., 2015).

A number of other biomarkers are under investigation but sadly most do not detect aneurysm disease before its acute complication. As such they do not fulfill the true clinical need.

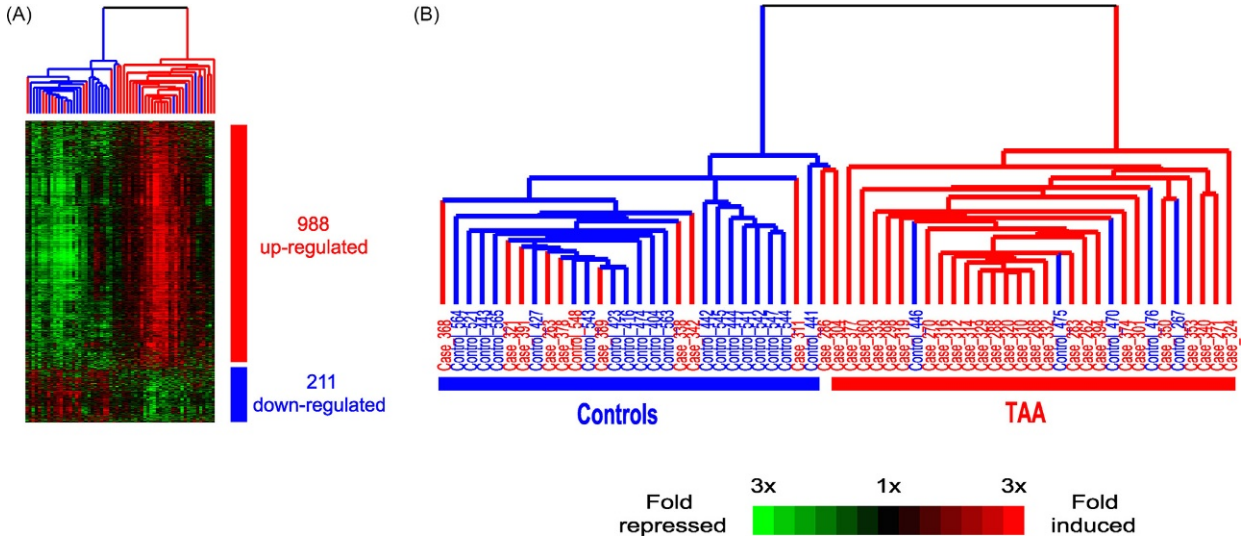

Fig. 12 RNA signature test for thoracic aortic aneurysm. Left: Hierarchical cluster diagram. *Vertical lines* represent individual patients, while *horizontal lines* represent an RNA. *Green* indicated underexpression; *red* indicated overexpression. Right: Experimental "dendrogram" demonstrating clustering of samples into control group (*blue*) and thoracic aortic aneurysm group (*red*). Modified with permission from Wang, Y., et al. (2007). Gene expression signature in peripheral blood detects thoracic aortic aneurysm. PLoS One **2**, e1050.

Clinical Features

Symptoms

The vast majority of aortic aneurysms are asymptomatic. Symptomatic cases (5%–10%) (Elefteriades et al., 2017) present with pain due to stretching of aortic tissue and/or compression of adjacent structures.

The site of the pain provides some indication of aneurysm location. Hence ascending and arch aneurysms present with retrosternal pain, while descending aneurysms present with interscapular back pain. Abdominal aneurysms may cause throbbing abdominal, left flank, or lower back pain.

Compressive symptoms are often associated with arch aneurysms. Impingement on the esophagus may cause dysphagia (the so-called dysphagia lusoria), while airway compression may result in dyspnea and stridor. Compression of the superior vena cava results in facial plethora and edema of the neck and upper limbs. Furthermore, the aneurysm may stretch the recurrent laryngeal nerve causing hoarseness. Descending aneurysms may erode into adjacent lung or esophagus, presenting with hemoptysis and hematemesis, respectively.

In addition to pain, AAAs may compress the duodenum, left iliac vein, left ureter, bladder, and testicular veins. The resultant symptoms include nausea and vomiting, left lower limb swelling, urinary symptoms, and varicocele, respectively.

Signs

Depending on their location, ascending aneurysms may present with the murmur of aortic valve regurgitation due to stretching of the aortic annulus and subsequent poor leaflet coaptation, rendering the valve incompetent. Descending aneurysms generally have no signs. Depending on body habitus, AAAs may be palpated as a pulsatile, expansile mass in the epigastrium.

Acute Complications: Dissection and Rupture

The clinical features of aortic dissection are legion. The possibility of cardiac, neurological, abdominal, and vascular symptoms and signs justifies its "great masquerader" label (Isselbacher, 2007). Aortic dissection classically presents as sudden, severe, tearing chest pain. As with aneurysms, the location of the pain is instructive, and its radiation provides some indication of distal extension. The associated features of hemodynamic instability, dyspnea, syncope, pulse deficit, peripheral neurological deficit, and other signs can be attributed to occlusion of branch vessels, disruption of the aortic valve (with subsequent acute aortic regurgitation), and aortic rupture (**Fig. 13**; Elefteriades et al., 2012).

Aortic rupture presents with features dependent on the location of the event. Rupture into the pericardium manifests features of cardiac tamponade, but most ruptured thoracic aortic aneurysms present with chest pain and hypotension secondary to hemorrhage into the thoracic cavity. Ruptured AAA classically presents as sudden abdominal/flank pain and hypotension in the presence of a pulsatile mass. However, this triad is only present in a third of cases (Brady and Chaikof, 2012). The pain often radiates to the testicles, rectum, or groin. Other features include hematemesis and melena in the setting of rupture into the duodenum, or

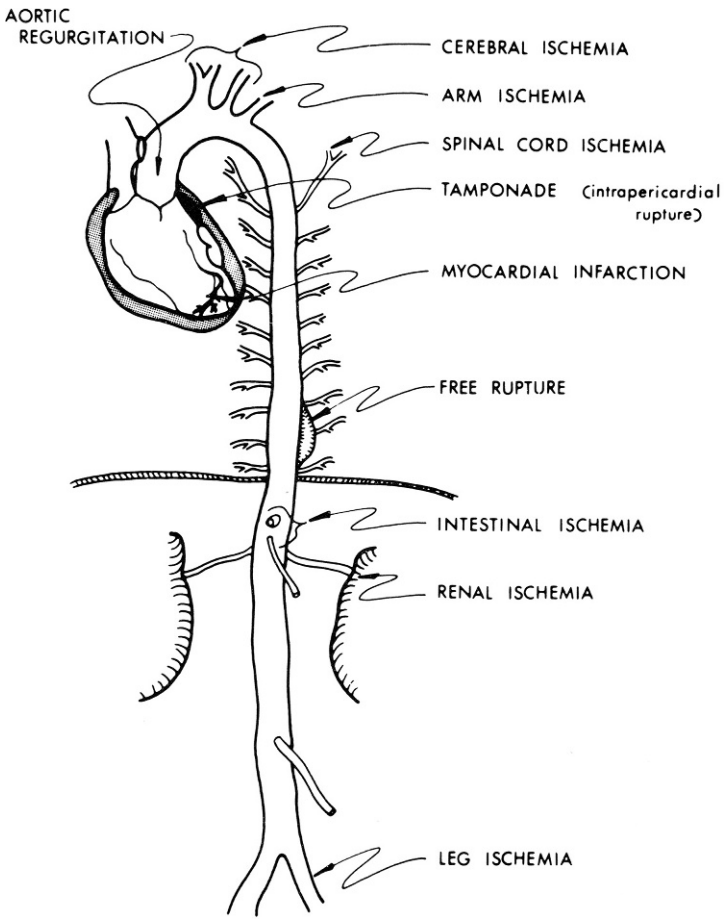

Fig. 13 Complications of acute aortic dissection. Reprinted with permission from Elefteriades, J. A., Geha, A. S., Tribble, C., Siegel, M. and Cohen, L. S. (2012). *The House Officer's Guide to ICU Care: fundamentals of management of the heart and lungs*. Minneapolis, MN: Cardiotext.

congestive heart failure due to rupture into the adjacent vena cava (Silen, 2010). Retroperitoneal rupture may be contained and hence present as back pain without hypotension.

Investigations

There are no laboratory tests that aid in the diagnosis of aortic aneurysm. Imaging remains the cornerstone of diagnosis.

Although quick, inexpensive, and easily available, chest radiography has a limited role in the diagnosis of aortic aneurysm. However, the humble chest radiograph may reveal the contours of the thoracic aorta nicely. Aneurysms may manifest as mediastinal widening on the frontal projection, with a prominent aortic knob and lateral bulging of the normally crisp stripe representing the descending aorta (Danias, 2007).

Echocardiography can clearly image the proximal ascending aorta and can provide information regarding aortic valve morphology and function. Transthoracic echocardiography often reveals aneurysms incidentally while investigating suspected valvular disease. Transesophageal echocardiography (TEE) enables visualization of all parts of the thoracic aorta except the distal ascending and aortic arch. This is due to poor acoustic penetration owing to the intervening trachea and left main bronchus (the "blind spot" of TEE) (Mahajan et al., 2008).

Computed tomography (CT) and CT angiography have become modality of choice for imaging the aorta. Their inherent high resolution, fast image acquisition, ability to visualize the entire aorta, and wide availability make this modality very valuable. Three-dimensional image reconstruction can also enable detailed analysis of aneurysm morphology, while ECG gating minimizes motion artifact (Muhs et al., 2006; Schlösser et al., 2008). However, CT has a number of important limitations that must be borne in mind. The most proximal ascending aorta is suboptimally assessed if the axial planes are too coarse. Dimensions of aortic valve annulus may be spurious due to distortion of the valve plane into a more vertical orientation by enlargement of the ascending aorta. Indeed, aortic tortuosity may result in nonorthogonal slices and therefore yield inaccurate measurements (Elefteriades and Farkas, 2010).

Finally, the effect of cumulative radiation exposure due to multiple examinations, and also the possibility for contrast-induced nephropathy, cannot be ignored. Contrast-enhanced CT is ideal for the detection of aortic dissection due to its excellent ability to detect the inciting intimal tear, extent of the dissection process, and morphology of the true and false lumens (Danias, 2007).

Magnetic resonance imaging provides full, multiplanar imaging of the aorta without utilizing radiation or nephrotoxic contrast agents. However, long image acquisition times and incompatibility with pacemakers, automatic implantable defibrillators, and metallic implants, together with more limited availability, restrict the applicability of this imaging modality (Gaztanaga et al., 2011) to acute aortic dissection patients.

Abdominal ultrasonography is the preferred method for imaging AAAs with near perfect sensitivity and specificity (LaRoy et al., 1989). Although operator dependent, this noninvasive modality is also ideal for surveillance of asymptomatic cases. CT is a more reproducible imaging modality and is useful for preoperative planning as well as postoperative assessment (Brady and Chaikof, 2012).

"Guilt by Association" Paradigm

The silent nature of thoracic aortic aneurysms means this dangerous condition is often only discovered incidentally during imaging studies performed for other diagnostic reasons. However certain clinical and anatomical associates have been identified that may suggest the presence of thoracic aneurysms (Elefteriades et al., 2015b) and encourage diagnostic imaging of the aorta. These so-called Guilt by association factors include intracranial aneurysm, AAA, bovine aortic arch configuration, renal cyst, positive thumb-palm sign, family history, and temporal arteritis (**Fig. 14**; Elefteriades et al., 2015b; Dumfarth et al., 2015; Kuzmik et al., 2010; Ziganshin et al., 2016; Hornick et al., 2012).

Treatment

Medical Therapy

Medical treatment for aortic aneurysms remains limited. The goals of therapy are arrest of aneurysm growth and prevention of complications (dissection or rupture). These goals are approached through aggressive risk factor management, avoidance of strenuous activity, and pharmacological reduction of hemodynamic stress on the aortic wall (Hackmann et al., 2007).

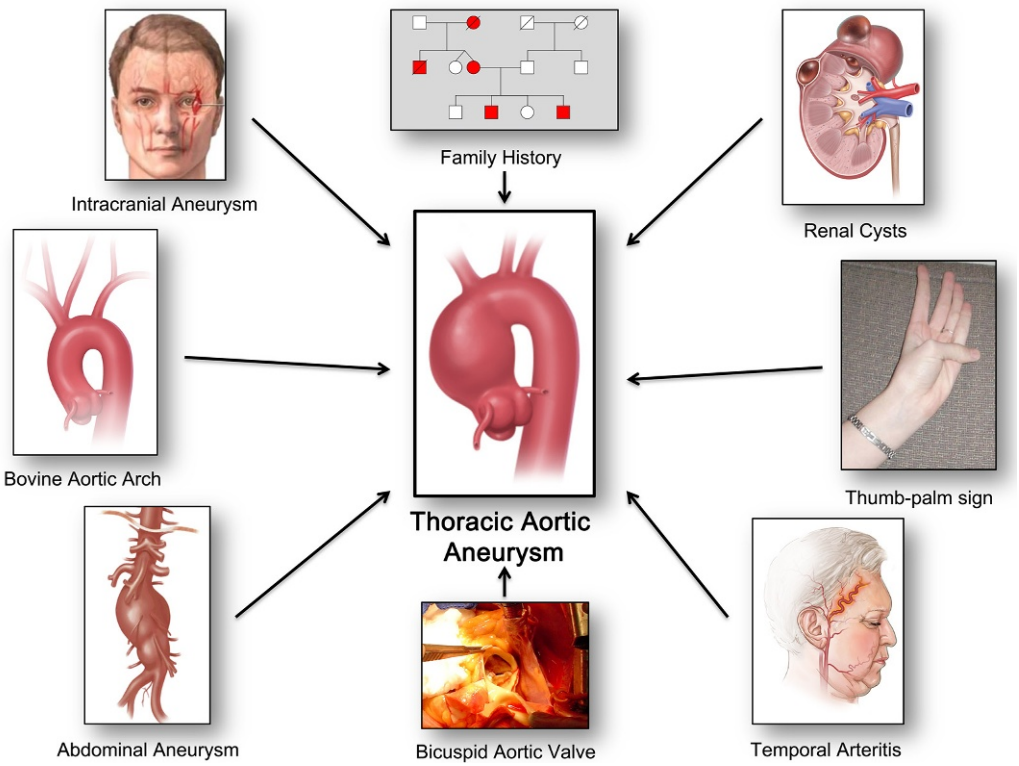

Fig. 14 "Guilt by association" factors linked to thoracic aortic disease. Reprinted with permission from Elefteriades, J. A., Sang, A., Kuzmik, G. and Hornick, M. (2015). Guilt by association: paradigm for detecting a silent killer (thoracic aortic aneurysm). *Open Heart* **2**, e000169.

Thus far, drug therapy has proved to be largely ineffectual. The role of beta adrenergic antagonists remains controversial. These drugs are of theoretical benefit through their reduction of blood pressure, and as anti-impulse therapy to reduce the rate of rise of arterial pressure (dP/dT_{max}) (Sanz et al., 2007). However, there is little robust evidence for their efficacy in slowing the rate of aortic dilatation (**Table 1**; Ziganshin et al., 2015b). Moreover, they are of no benefit in prevention of the clinical end-points of dissection or death (Ziganshin et al., 2015b). The initial success of angiotensin receptor blockers in reducing the rate of aneurysm growth (Brooke et al., 2008) was not borne out in larger clinical trials (Lacro et al., 2014; Forteza et al., 2016). The MMP inhibitor doxycycline also failed to slow the growth of AAA despite promising results in animal models (Meijer et al., 2013). Statins have demonstrated some effectiveness in reducing AAA growth (Takagi et al., 2012), as well as a protective effect in thoracic aortic aneurysms (Stein et al., 2013).

Surgical Therapy

Surgery for aortic aneurysms varies according to the location of the aneurysm. In general, operative management aims to eliminate the risk of rupture or dissection by resection of the aneurysmal segment and its replacement with a synthetic graft. Made of polyethylene terephthalate (Dacron), these grafts are durable and nonthrombogenic, thereby avoiding the need for long-term anticoagulation therapy.

Ascending Aortic Aneurysms

Treatment of ascending aneurysms requires a detailed understanding of the anatomy of the ascending aorta. Extending from the aortic valve annulus to the origin of the innominate (or brachiocephalic) artery, the ascending aorta consists of a proximal sinus portion connected to a tubular portion, with an intervening sinotubular junction. The sinus portion is commonly referred to as the aortic root and contains the three sinuses of Valsalva from which the coronary arteries arise. The involvement of these subcomponents guides the surgical management of ascending aortic aneurysms. Three common patterns exist (**Fig. 15**; Elefteriades et al., 2017)—supracoronary aneurysm, annuloaortic ectasia, and diffuse tubular enlargement.

Supracoronary aneurysms are confined to the tubular portion of the ascending aorta, distal to the coronary artery ostia. These represent the most common form and are treated using a tube graft. Annuloaortic ectasia refers to dilatation of the aortic annulus together with the proximal aorta, giving rise to a "flask-like" morphology. This is typically seen in Marfan syndrome. Treatment involves replacement of the aortic valve and root, often using a composite valve-graft (the modified Bentall procedure). These root replacements have recently been shown to achieve a *normal* life expectancy for affected patients, an outlook identical to an age- and sex-matched population (Mok et al., 2017). Valve-sparing techniques include the David procedure ("reimplantation" technique) and the Yacoub procedure ("remodeling" technique) (Milewicz et al., 2005). These popular methods preserve the patient's own aortic valve. Tubular diffuse enlargement shares features of the other two configurations, with mild dilatation of the aortic annulus and proximal ascending aorta, but with more subtle and uniform ascending aortic dilatation. Treatment is often based on patient age, with composite valve-grafts used in younger patients, as this protects against late dilatation of the proximal aortic root. A more limited approach, utilizing a supracoronary tube graft, may suffice in older patients.

Aortic Arch Aneurysms

Aortic arch surgery is complicated by the need to consider cerebral protection during the operation. Resection of arch aneurysms requires special management of the brachiocephalic vessels that supply the head and arms (innominate, left common carotid, and left subclavian arteries). These arteries are often surgically detached from the native aortic arch as "Carrel patches," whereby two or three vessels are excised *en bloc* with a rim of aortic tissue and subsequently reattached to an aortic graft (**Fig. 16**; Ziganshin et al., 2014). Alternatively, prefabricated branched grafts may be used and the arch vessels anastomosed individually.

Cerebral protection is achieved through the use of deep hypothermic circulatory arrest (DHCA). Hypothermia reduces the metabolic rate of tissues and, in this context, enables a short period of ischemia without complications. At 18°C, cerebral metabolic rate is reduced to 17% of normal (McCullough et al., 1999), enabling approximately 40 minutes of safe circulatory arrest during which the arch can be managed in a bloodless field (Ziganshin et al., 2014). If this time interval is insufficient, direct cerebral perfusion techniques are required to prevent neurological sequealae (Hagl et al., 2001). DHCA and perfusion techniques have been developed to the point that neurologic function is fully and reliably preserved in the vast majority of patients (Elefteriades and Ziganshin, 2015).

Furthermore, arch aneurysms often extend into the descending aorta. The repair of such extensive aneurysms in a single operation is anatomically awkward and prohibitively risky. Hence a staged approach is often taken, with an initial ascending and arch "Elephant trunk" procedure facilitating a subsequent more distal completion procedure (Borst et al., 1983).

Descending and Thoracoabdominal Aneurysms

Repair of these often extensive lesions, particularly thoracoabdominal aneurysms (**Fig. 17**; Elefteriades et al., 2017), requires careful attention to perfusion of the abdominal viscera and spinal cord to prevent ischemic complications, while the aortic cross clamp is applied (Crawford et al., 1986). Spinal cord injury is a particular concern (Griepp and Griepp, 2015). Although the blood supply to

Table 1 Studies on the effectiveness of beta-blockers in patients with Marfan syndrome

			Beta-blocker group			*Control group*			*Study results*	
Author name	*Year of publication*	*Designed to evaluate beta-blocker effect?*	*Number of patients*[a]	*Mean age (years)*	*Number of clinical end-points*[b]	*Number of patients*	*Mean age (years)*	*Number of clinical end-points*[b]	*Do BBs slow aortic dilatation?*	*Do BBs prevent clinical end-points?*[b]
Randomized clinical trial										
Shores et al.	1994	Yes	32	15.4	5/32 (15%)	38	14.5	9/38 (24%)	Yes	No ($P=0.399$)
Observational studies										
Tahernia	1993	Yes	3	10.0 ± 1.0	0/3 (0%)	3	8.3 ± 4.9	0/3 (0%)	Yes	No ($P=1.0$)
Roman et al.	1993	No	79	28 ± 15	18/79 (23%)	34	28 ± 15	3/34 (9%)	N/A	No ($P=0.080$)
Salim et al.	1994	Yes	100	11.1 ± 3.4	5/100 (5%)	13	10.2 ± 4.6	0/13 (0%)	Yes	No ($P=1.0$)
Silverman et al.	1995	No	191	33 ± 14	58/191 (30%)	226	31 ± 17	54/226 (24%)	N/A	No ($P=0.137$)
Legget et al.	1996	No	28	21 (median)	9/28 (32%)	55	21 (median)	8/55 (15%)	N/A	No ($P=0.060$)
Rossi-Foulkes et al.	1999	Yes	15	11.2 ± 5.3	4/15 (27%)	27	8.0 ± 5.2	0/16 (0%)	Yes	No ($P=0.043$)
Tierney et al.	2007	Yes	29	9.2 ± 4.0	1/29 (3%)	34	8.8 ± 4.8	3/34 (9%)	No	No ($P=0.617$)
Ladouceur et al.	2007	Yes	77	6.1 ± 3.2	3/77 (4%)	78	7.4 ± 5.2	8/78 (10%)	Yes	No ($P=0.123$)
Meta-analyses										
Gersony et al.	2007	Includes studies by Tahernia, Roman et al., Shores et al., Salim et al., Silverman et al., Legget et al. *Conclusions*: On the basis of this metaanalysis, there is no evidence that beta-blockade therapy has clinical benefit in patients with Marfan syndrome							N/A	No
Gao et al.	2011	Includes studies by Tahernia, Salim et al., Rossi-Foulkes et al., Tierney et al., Ladouceur et al. *Conclusion*: There is evidence that beta-blockade therapy can slow down the rate of dilatation of the aorta and has clinical benefits on children and adolescents with MFS							Yes[c]	No

[a]Please note that four of the eight studies involved fewer than 30 patients.

[b]Clinical end-points included death or related cardiovascular events.

[c]Although the metaanalysis included the Tierney et al. study, the calculations on aortic growth rates did not include their findings.

N/A, data not available in study.

Reproduced with permission from Ziganshin, B. A., Mukherjee, S. K. and Elefteriades, J. A. (2015). Atenolol versus losartan in Marfan syndrome. *New England Journal of Medicine* **372**, 977–978.

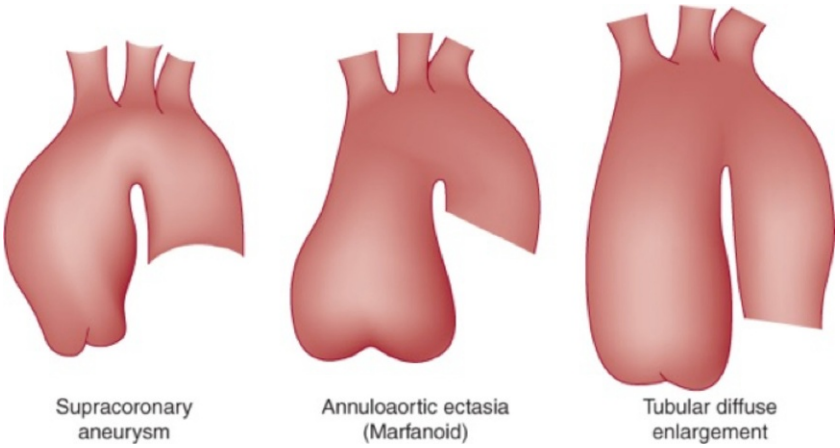

Fig. 15 Morphologies of ascending aortic aneurysms. Reprinted with permission from Elefteriades, J. A., Olin, J. W., Halperin, J. L. and Ziganshin, B. A. (2017). Diseases of the aorta. In: Fuster, V., Walsh, R. A., Harrington, R. A., Narula, J. & Eapen, Z. J. (eds.) *Hurst's the heart* (14th edn.). New York: McGraw-Hill.

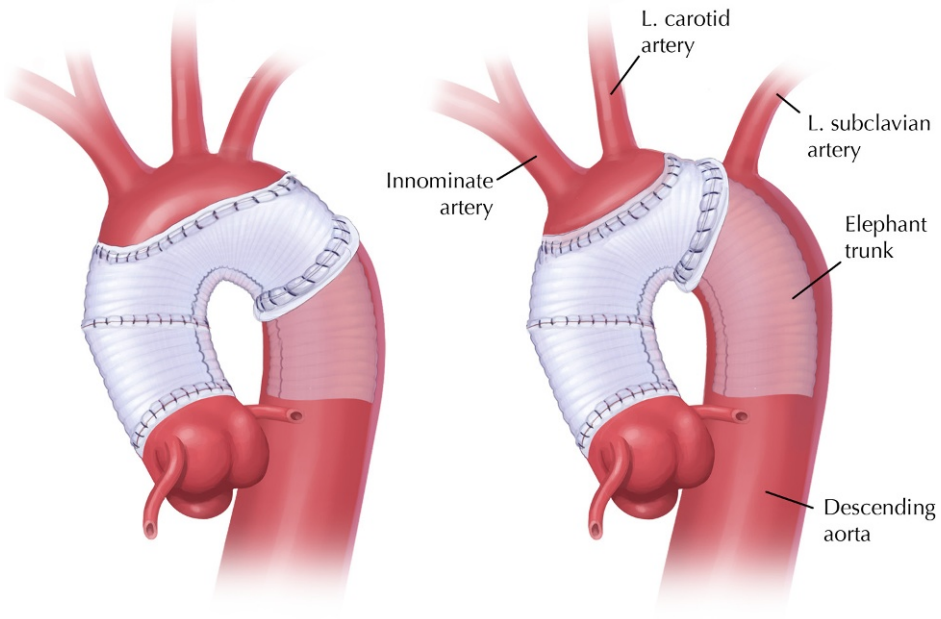

Fig. 16 Techniques for total arch replacement. Reprinted with permission from Ziganshin, B. A., et al. (2014). Straight deep hypothermic circulatory arrest for cerebral protection during aortic arch surgery: safe and effective. *Journal of Thoracic and Cardiovascular Surgery* **148**, 888–900.

the spinal cord is segmental, a significant portion is delivered via the "artery of Adamkiewicz." Its identification with spinal CT angiography enables preoperative planning to prevent its occlusion (Yoshioka et al., 2003).

Protective techniques to reduce the risk of paraplegia include left heart bypass (left atrium to femoral artery), cerebrospinal fluid drainage (Coselli et al., 2002), mild systemic hypothermia (Griepp and Griepp, 2015), and branch vessel attachment (via the inclusion technique) (Kouchoukos et al., 2013). Moreover, spinal cord monitoring allows intraoperative assessment of spinal cord injury (Conrad et al., 2007). Although these techniques have rendered substantial improvements in safety, paraplegia continues to be a dreaded potential complication of extensive thoracoabdominal aortic replacement procedures.

Abdominal Aneurysms

AAAs can be repaired via the traditional open surgical approach or by endovascular means (endovascular aneurysm repair—EVAR). Open surgical repair involves suturing a tube graft within the aneurysm sac, whereas EVAR involves the placement of an "endograft" within the aneurysm. Endografts are polyester or PTFE modular grafts surrounded by a wire frame that are packaged into a small configuration that can be deployed via the femoral or iliac arteries (Brady and Chaikof, 2012). With its lower early mortality rates,

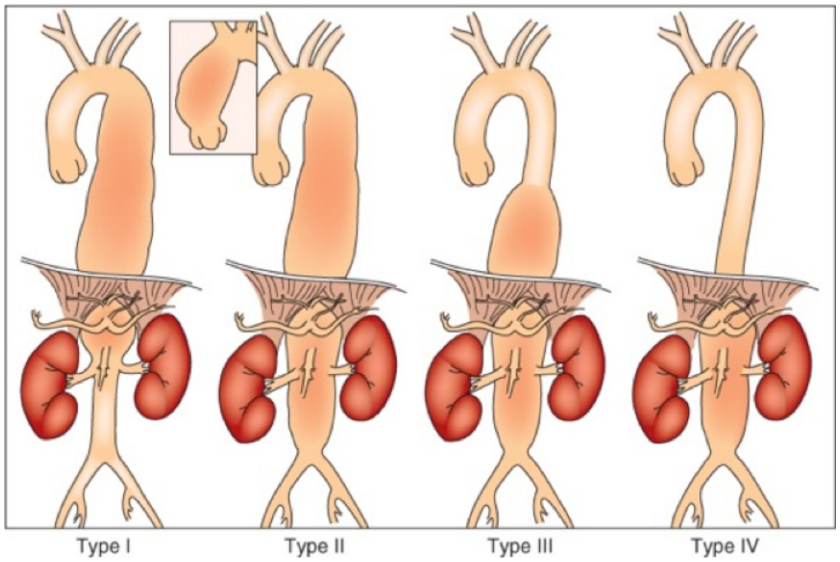

Fig. 17 Crawford classification of thoracoabdominal aortic aneurysms. Reprinted with permission from Elefteriades, J. A., Olin, J. W., Halperin, J. L. and Ziganshin, B. A. (2017). Diseases of the aorta. In Fuster, V., Walsh, R. A., Harrington, R. A., Narula, J. & Eapen, Z. J. (eds.) *Hurst's the heart* (14th edn.). New York: McGraw-Hill.

EVAR has shown benefit in the context of ruptured AAA (Veith et al., 2009). However, its use is contingent on patients having suitable anatomy, as well as various other technical and logistical aspects. EVAR has been used for treatment of descending thoracic aneurysms (thoracic EVAR, TEVAR) (Dake et al., 1994), but its applicability to typical fusiform degenerative aneurysms remains controversial (Elefteriades and Percy, 2007).

EVAR has a number of problematic complications including endoleak (Bashir et al., 2009), graft migration, and occlusion of the iliac limbs of bifurcated grafts. Endoleak is the most common complication and refers to continued flow into the aneurysm sac after deployment of the endograft. Also, EVAR patients require regular follow-up at 6–12 monthly intervals with surveillance CT scans to monitor for adverse events (Brady and Chaikof, 2012).

The benefit of EVAR must be assessed in light of the indolent nature of aortic aneurysms. Given that aneurysms grow slowly, short-term follow-up cannot yet provide solid evidence for its benefit versus surveillance and medical management alone. Medium-term results are required to assess the true effectiveness of this therapy.

Conclusion

Aortic aneurysm is a clinically silent, but highly virulent, disease. Although the role of surgery is well-established, much work remains to be done with regard to diagnosis of latent cases and identification of those patients at high risk of dissection or rupture. Greater understanding of disease pathogenesis may provide better therapeutic targets and reveal a much sought-after diagnostic biomarker. Whole-exome sequencing promises to identify causative mutations, while improved imaging and engineering analyses have the potential to refine decision-making beyond merely size criteria. Moreover, evolution of endovascular techniques will expand our management options and hopefully yield improved future outcomes. These advances will pave the way for management of this disease on a truly individual basis.

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Further Reading

Coady MA, Rizzo JA, and Elefteriades JA (1999) Developing surgical intervention criteria for thoracic aortic aneurysms. *Cardiology Clinics* 17: 827–839.

Aortic Valve Replacement in Patients With Congestive Heart Failure

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Abbreviations

ACC	American College of cardiology
AHA	American Heart Association
AR	Aortic regurgitation
AS	Aortic stenosis
AVA	Aortic valve area
AVR	Aortic valve replacement
CABG	Coronary arteries bypass graft surgery
CAD	Coronary artery disease
CHF	Congestive heart failure
ESC	European Society of Cardiology
LF LG	Low-flow low-gradient
LV	Left ventricle
LVEDD	Left ventricle end diastolic diameter
LVEF	Left ventricular ejection fraction
LVESD	Left ventricle end systolic diameter
NYHA	New York Heart Association
PPM	Patient-prosthesis mismatch
SAVR	Surgical aortic valve replacement
SV	Stroke volume
SVi	Stroke volume index
TAVR	Transcatheter aortic valve replacement

Introduction

Angina-type chest pain, syncope, and congestive heart failure (CHF) are common manifestations of aortic stenosis (AS) with CHF typically being the late, end-stage result that links AS and aortic regurgitation (AR) (Brown et al., 2009a; Iung et al., 2003). In general, CHF in the setting of aortic valve disease carries a dismal prognosis. Patients with AS and CHF have an expected survival of less than 2 years when treated medically (Ross and Braunwald, 1968). More contemporary data demonstrated that the median survival of patients with medically treated AS is 1 year and at 5 years 93.6% of the patients are dead (Monin et al., 2003; Kapadia et al., 2015; Leon et al., 2010; Varadarajan et al., 2006; Brown et al., 2008; Clavel et al., 2008; Pai et al., 2008; Tribouilloy et al., 2009) (**Table 1**).

Table 1 Medical management of severe aortic stenosis: outcomes in patients with congestive heart failure

					Baseline				*Long-term survival*				*Follow-up*
Author	*Year*	*References*	*Subgroups*	*N*	*LVEF (%)*	*Mean gradient (mmHg)*	*AVA (cm^2)*	*NYHA class III–IV (%)*	*1 Year(%)*	*3 Years(%)*	*5 Years(%)*	*10 Years(%)*	*NYHA class III–IV (%)*
Monin	2003	4	Contractile reserve	28	31 (23–35)	27 (22–35)	0.7 (0.6–0.9)	85	70[a]	n/a	20[a]		82
			No contractile reserve	13	30 (27–35)	30 (23–34)	0.8 (0.7–0.9)	83	35[a]	n/a	12[a]		93
Varadarajan	2006	5	ALL	453	52 ± 21	40 ± 16	0.71 ± 0.17	n/a	62		32	18	
			CHF	189					50		20		
			Systolic PAP ≥ 60	83					38		<20		
			LVEF ≤ 40%	159					40		20		
			3–4 + Mitral regurgitation	118					50		25		
Brown	2008	6		90	60 ± 12	n/a	0.9 ± 0.3	n/a	50		15	2	
Clavel	2008	7		57	29 ± 8	21 ± 8	0.92 ± 0.2	47	70[a]	50[a]			
Pai	2008	8	Low LVEF (<35%)	136	24 ± 0.8	33 ± 14	0.67 ± 0.18	n/a	47	n/a	23	n/a	n/a
			Low gradient (<30 mmHg)	121	39 ± 19	24 ± 6	0.76 ± 0.15	n/a	60	40 (2 years)	22	n/a	
Tribouilloy	2009	9		26	27 ± 6	24 ± 6	0.74 ± 0.18	81	35[a]		13 ± 7	n/a	n/a
Leon	2010	10		179	51 ± 14	43 ± 15	0.6 ± 0.2	93	49	35[a]	n/a	n/a	58

[a]Data not provided. Estimated from Kaplan Meier survival curve.

Patients with heart failure symptoms secondary to AR also have a dismal prognosis with only 50% of the patients alive after 3 years (Kamath et al., 2009).

Surgical aortic valve replacement (SAVR) is currently the standard treatment for symptomatic severe AS. In general, it is associated with low peri-procedure morbidity and mortality, symptomatic improvement, and improvement in long-term survival (Brown et al., 2009b; Surgeons TSoT, n.d.). On average, the expected survival after SAVR is approximately 80%–85% at 5 years, 65%–75% at 10 years, and 45%–55% at 15 years (David et al., 2001; Dellgren et al., 2002; Stahle et al., 1997). The functional status, comorbidities, and age of each individual patient have been shown to significantly affect outcomes (Cohen et al., 1999). While patients with normal preoperative left ventricular ejection fraction (LVEF) have an estimated 15-year survival of 62.3%, the presence of left ventricle (LV) dysfunction significantly reduces the long-term survival after aortic valve replacement (AVR). It is 47.6% for those with LVEF 35%–49%, 41% for LVEF 20%–34%, and 36.9% for LVEF <20% (Une et al., 2015).

Many patients with aortic valve disease and CHF are not candidates for surgery given their age, comorbidities, frailty, or the presence of advanced cardiac disease that make their surgical risk unacceptable (Iung et al., 2003; Pellikka et al., 2005). However, with the introduction of transcatheter aortic valve replacement (TAVR), patients who are considered at high or extreme risk for surgery may still be able to experience a benefit similar to those who undergo SAVR. Several studies comparing TAVR to SAVR in extreme, high, and intermediate risk patients have demonstrated similar procedural, short-term, and medial-term outcomes (Mack et al., 2015; Deeb et al., 2016; Leon et al., 2016a,b; Reardon et al., 2016). Despite their high operative risk, most of these patients can benefit from either SAVR or TAVR. Both have been shown to improve their symptoms, cardiac function, and long-term survival when compared to medical management. In this chapter we will review the indications for surgical and transcatheter management of aortic valve disease, with particular attention to the outcomes of patients with advanced heart failure symptoms (NYHA class III–IV) and left ventricular dysfunction (LVEF ≤35%).

Aortic Stenosis and Congestive Heart Failure

AS is mainly considered a disease of the elderly (Nkomo et al., 2006; Lindroos et al., 1993). It is estimated that 2.8% of the population older than 70 years have symptoms related to AS (Nkomo et al., 2006; Lindroos et al., 1993). Of them, 40%–60% have NYHA class III–IV symptoms, and only one third of patients with LVEF ≤35% will eventually proceed to SAVR (Iung et al., 2003; Pai and Varadarajan, 2010; Bach, 2011).

Severe AS is defined a valve area of ≤1 cm^2 with a mean aortic valve gradient of at least 40 mmHg and a peak velocity greater than 4 m/s (Nishimura et al., 2014). The left ventricular outflow obstruction caused by severe AS leads to chronic pressure overload of the LV. To compensate, the LV hypertrophies in order to decrease wall stress. The magnitude and adequacy of that ventricular remodeling and the associated changes in systolic ventricular function determine the clinical presentation, hemodynamic characteristics, response to treatment, and ultimately prognosis (Olson et al., 1987; Carabello, 2006; Brogan et al., 1993; Chukwuemeka et al., 2006).

There are three mechanisms AS can lead to heart failure symptoms: (1) *Diastolic dysfunction*: it is the result of LV hypertrophy, increased wall thickness, and a decreased LV volume to mass ratio. LV end diastolic pressure (LVEDP) increases due to worsening compliance and is not directly from systolic failure (Bonow et al., 2008; Gaasch et al., 1976; Hess et al., 1984; Murakami et al., 1986). Because of the multiple pathophysiologic mechanisms and variable compensatory responses, AS can be viewed as multiple clinical entities based on ejection fraction, stroke volume (SV), and gradient. (2) *Systolic dysfunction secondary to afterload mismatch*: If the hypertrophic process is inadequate to compensate for the increased afterload, wall stress increases and the ejection fraction falls. This condition is called "afterload mismatch" and limits fiber shortening (Olson et al., 1987; Carabello, 2006; Brogan et al., 1993; Chukwuemeka et al., 2006; Bonow et al., 2008). There are two subgroups within this category: (a) preserved SV and therefore elevated transaortic gradients and (b) reduced SV and therefore low transaortic gradient. This last group is difficult to differentiate from the next one. (3) *Systolic dysfunction secondary to intrinsic myocardial dysfunction*: Persistently elevated wall stress, inadequate blood supply, and superimposed ischemia or infarction, myocardial fibrosis, and abnormalities of calcium handling further depress myocardial contractility. As before, these patients have diminished SV and low transvalvular gradients (Carabello, 2006; Bonow et al., 2008). If myocardial dysfunction is secondary to afterload mismatch, SAVR/TAVR is associated with good outcomes. If intrinsic myocardial dysfunction predominates, the response to valve replacement is less favorable with higher operative mortality and less LVEF improvement after SAVR/TAVR. Despite this, these patients have a significantly better prognosis with SAVR/TAVR when compared to medical management alone (Carabello, 2006; Chukwuemeka et al., 2006; Connolly et al., 1997, 2000; Baron et al., 2016; O'Sullivan et al., 2015; Lauten et al., 2014; Conrotto et al., 2016) (**Fig. 1**).

CHF Secondary to AS With Normal Left Ventricular Function and Normal SV

When the LV hypertrophic compensation is adequate, the wall stress normalizes and the left ventricular function is maintained (**Fig. 1**; Nishimura et al., 2014; Olson et al., 1987; Chukwuemeka et al., 2006). These patients have normal left ventricular function and SV as evidenced by a normal or supra-normal ejection fraction. Therefore, the transvalvular gradient is elevated. LVEDP is elevated secondary to decreased compliance from diastolic dysfunction and increased afterload.

These patients respond very well to SAVR and or TAVR. Their surgical risk tends to be low with the risk adjusted operative mortality around 1.6%–2.3%, which has steadily declined over the last 10 years (Brown et al., 2009a; Surgeons TSoT, n.d.).

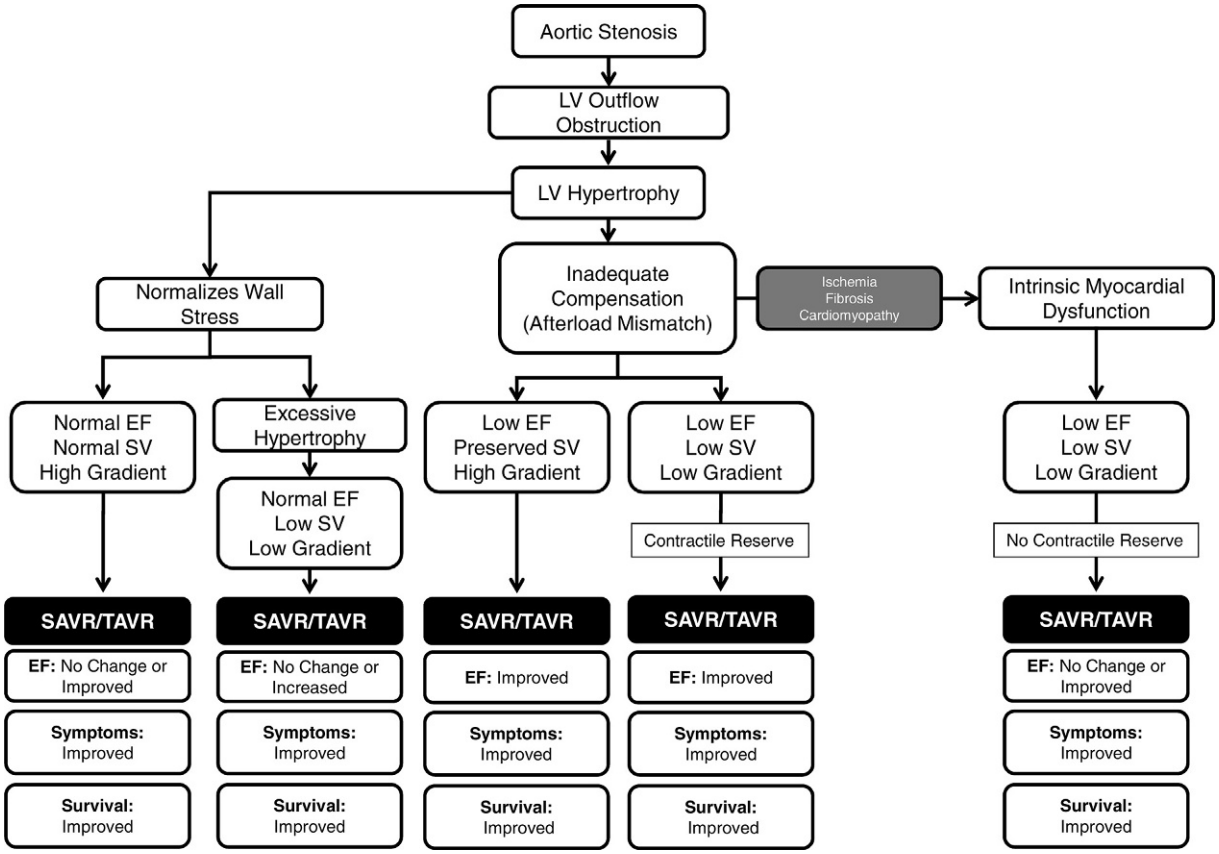

Fig. 1 Mechanisms responsible for heart failure in aortic stenosis and response to aortic valve replacement.

The presence of heart failure symptoms and LV dysfunction increase operative mortality (Brown et al., 2009a; Surgeons TSoT, n.d.). For example, patients with CHF symptoms and a LVEF <30% have an operative mortality of 5.2% (Brown et al., 2009a).

SAVR has been shown to effectively alleviate symptoms and improve quality of life (Vahanian and Otto, 2010). Patients with normal LVEF have a long-term survival similar to that expected for an age- and sex-matched population after AVR (Kvidal et al., 2000). However, there is an excess mortality for patients with NYHA class III–IV symptoms (Vahanian and Otto, 2010; Malaisrie et al., 2010; Kvidal et al., 2000) and for patients with LV dysfunction (Hannan et al., 2009). Even small decrement in LVEF will have negative impact in survival regardless of symptoms (Hannan et al., 2009; Dahl et al., 2015; Mihaljevic et al., 2008).

Valve replacement decreases ventricular afterload. This in turn is associated with improved LVEF and regression of LV hypertrophy and LV mass (Connolly et al., 1997; Pantely et al., 1978; Kennedy et al., 1977). One study demonstrated that LVEF improved by 6.8 EF points after SAVR. The improvement became evident at 6 months and was sustained for up to 10 years after surgery (EF $56 \pm 4\%$ preoperatively, $63 \pm 3\%$ at 0–6 months, $63 \pm 5\%$ at 7–24 months, and 63 ± 4 at 25–120 months) (Sharma et al., 2004). Some studies showed no change in LVEF after AVR in patients with normal LVEF. Compared to the delayed effect of LVEF, LV mass regression was more pronounced in the first six months after surgery. It reached maximal regression at 24 months and also was sustained for up to 10 years (181 ± 26 g/m^2 preoperative vs. 124 ± 27 g/m^2 at 6 months, 117 ± 15 g/m^2 at 24 months, and 113 ± 14 g/m^2 at 120 months after SAVR) (Une et al., 2015; Sharma et al., 2004).

TAVR for AS with normal left ventricular function and normal SV

The PARTNER 1 trial was the first trial of balloon-expandable TAVR in the United States. This trial showed superior survival of TAVR versus medical management improving median survival from 12 to 32 months in inoperable patients (Kapadia et al., 2015). In high-risk patients, long-term survival with TAVR was found equivalent to SAVR (median survival 40 months) (Mack et al., 2015). In the CoreValve US Pivotal trial using a self-expanding valve, high-risk patients undergoing TAVR had lower mortality compared to SAVR at 3 years (32.9% vs. 39.1%)(Deeb et al., 2016). At 5 years, hemodynamic valve performance was stable with no structural valve deterioration; however, there was an increased incidence of moderate to severe AR in the TAVR groups which impacted survival (Kapadia et al., 2015; Mack et al., 2015; Deeb et al., 2016; Daubert et al., 2017; Kodali et al., 2015).

In the TAVR population LV mass regression was observed among survivors at 1-year follow-up (144 ± 42 mm to 131 ± 39 mm) though it was not as significant as previously reported in the SAVR population. Similarly, the EF change in this group also was not significant (Gotzmann et al., 2012). However, there was significant improvement in functional class with the percentage of patients in NYHA III/IV decreasing from 83% preoperatively to 5.5% at 1 year and 7.7% at 3 years (Deeb et al., 2016).

CHF Secondary to AS With Normal Left Ventricular Function and Low Transvalvular Gradient (AVA $\leq$0.8 cm^2, EF $\geq$ 50%, Mean Aortic Valve Gradient<40 mmHg) (Table 2)

This condition is referred as "paradoxical low-flow low-gradient AS" (LFLG). These patients have a disproportionate amount of LV hypertrophy than necessary to compensate for the increased afterload and wall stress leading to thicker ventricles, smaller left ventricular cavities with a restrictive filling pattern (diastolic dysfunction), and intrinsic myocardial dysfunction secondary to myocardial fibrosis (Pai et al., 2008; Nishimura et al., 2014; Hannan et al., 2009; Herrmann et al., 2011; Dumesnil et al., 2010; Pibarot and Dumesnil, 2010). The low transvalvular gradient results from decreased flow across the aortic valve secondary to low SV or prolonged systolic ejection period (**Fig. 1**; Tarantini et al., 2011; Nishimura et al., 2014).

This group represents 9%–35% of patients with severe AS and normal LVEF (Pai et al., 2008; Lauten et al., 2014; Pibarot and Dumesnil, 2011; Herrmann et al., 2011; Tarantini et al., 2011; Hachicha et al., 2007; Le Ven et al., 2013; Parikh et al., 2015). Since they have preserved LVEF and low transvalvular gradient, they are commonly overlooked in clinical practice. The small aortic valve area (AVA) is often attributed to calculation error and the severity of their stenosis is erroneously underestimated (Pibarot and Dumesnil, 2011; Matsumura et al., 2008). Therefore, they are 40%–50% less likely to be referred to surgery (Pibarot and Dumesnil, 2011; Hachicha et al., 2007).

The majority of these patients are in NYHA functional class III–IV at presentation. (Gotzmann et al., 2012; Herrmann et al., 2011; Tarantini et al., 2011). Because they are at a more advanced stage of their disease, they have a worse prognosis than patients with normal EF and high gradient AS (Dumesnil et al., 2010; Hachicha et al., 2007).

While their operative risks may be elevated, several studies have demonstrated that these patients have better survival when treated with SAVR compared to medical management (**Table 2**). The operative mortality is highly variable and ranges between 2.7% and 18%. Given their severe left ventricular hypertrophy, diastolic dysfunction, and decreased systemic arterial compliance, these patients will often have low cardiac output syndrome postoperatively and typically require in addition to meticulous myocardial protection during surgery, aggressive volume resuscitation, vasopressors, and even more invasive hemodynamic cardiopulmonary support (Dumesnil et al., 2010).

Pai studied the outcomes of patients with severe paradoxical LFLG AS (EF $\geq$55%, and a mean transvalvular gradient <30 mmHg) treated by either surgery or medical management (Pai et al., 2008). He compared by propensity score matching 18 patients who underwent SAVR with 14 patients who did not have surgery. One- and five-year survival were 92% and 88% in the SAVR group compared with 82% and 10% in the non-SAVR group. Multiple other series have confirmed these findings and have also found that LVEF and NYHA functional class were improved after surgery (**Table 2**; Tarantini et al., 2011; Hachicha et al., 2007).

The Cleveland Clinic reported recently in a contemporary cohort of SAVR patients with paradoxical LFLG severe AS had significantly worse short- and long-term outcomes as compared to the standard severe AS (EF >50%, high gradient). In addition to this they also found that low stroke volume index (SVi, $\leq$35 mL/m^2) is an independent predictor of worse outcomes (Parikh et al., 2015). Similarly, in a recent meta-analysis, Dayan et al. confirmed both the increased operative mortality with paradoxical LFLG AS compared to other subtypes of severe AS and the beneficial effect of AVR on mortality compared to medical management (Dayan and Vignolo, 2015).

TAVR for AS with normal left ventricular function and low transvalvular gradient (AVA $\leq$0.8 cm^2, EF $\geq$ 50%, mean aortic valve gradient <40 mmHg)

In a subanalysis of the PARTNER 1 trial, Hermann et al. demonstrated that in inoperable patients with SVi $\leq$35 mL/m^2 and preserved LVEF, TAVR reduced 1-year mortality compared with medical management and that there was a trend to benefit at 2 years (66% with medical management vs. 35% with TAVR at 1 year $P=0.02$; 77% with medical management vs. 57% with TAVR at 2 year $P=0.06$). There was no difference in high-risk patients between SAVR and TAVR in 2-year mortality (39.0% vs. 38.3%. P 0.69). There was however an improved 1-year survival with TAVR compared to AVR (Herrmann et al., 2013).

Similar to the findings after SAVR, the outcome of paradoxical LF LG AS after TAVR was worse than in patients with preserved LVEF and high aortic valve gradient. (Gotzmann et al., 2012; Le Ven et al., 2013). Additionally, in all studies this group does better than patients with reduced LVEF and low gradient AS (Gotzmann et al., 2012; Le Ven et al., 2013; Herrmann et al., 2013).

The SVi is an important predictor for all patients with paradoxical LF LG that have a valve replacement procedure, whether SAVR or TAVR. LeVen demonstrated that a low SVi in patients with paradoxical LF LG undergoing TAVR have an increased risk of 30-day mortality (Le Ven et al., 2013). Furthermore, in the PARTNER 1 trial there was a 50% increase in 2-year all-cause mortality in low SVi compared with patients with normal SVi. The impact of low SVi on subsequent mortality was independent in multivariable analysis and was a more powerful predictor of outcome than LVEF or mean transvalvular gradient (Herrmann et al., 2013).

Left ventricular EF did not change after TAVR in this subgroup of AS. LV mass regression was also not significantly higher than patients with high gradients (pre TAVR 155 ± 49 g/m^2 and post TAVR 134 ± 33 g/m^2, $P=0.008$) (Gotzmann et al., 2012).

CHF Secondary to AS With Low LVEF

Low preoperative left ventricular function is the major predictor of outcomes in patients with AS (Connolly et al., 2000; Malaisrie et al., 2010; Kvidal et al., 2000; Hannan et al., 2009; Gotzmann et al., 2012; Moat et al., 2011). The incidence of left ventricular dysfunction in patients with severe AS varies among different studies and with the threshold used. 5.4% of patients in the Society of Thoracic Surgeons database who had isolated SAVR between 1997 and 2006 had LVEF <30% (Brown et al., 2009a). The Euro Heart

Table 2 Severe aortic stenosis with normal left ventricular function and low transvalvular gradient: AVR versus medical management

				Preoperative					*Surgery*		*Long-term survival*				*Postoperative*	
Author	*Year*	*References*	*Subgroups*	*N*	*LVEF (%)*	*Mean gradient (mmHg)*	*AVA (cm^2)*	*NYHA class III–IV (%)*	*CABG (%)*	*30-Day mortality (%)*	*1 Year (%)*	*3 Years (%)*	*5 Years (%)*	*10 Years (%)*	*NYHA class III–IV (%)*	*LVEF (%)*
Aortic valve replacement																
Hachicha	2007	38		80	62 ± 8	32 ± 17	0.76 ± 0.23	n/a	n/a	n/a	>95[a]	93 ± 3	>80[a]		n/a	n/a
Pai	2008	8		18	66 ± 7	26 ± 5	0.77 ± 0.14	n/a	66	n/a	92	88 (2		years)	88	n/a
n/a	n/a															
Tarantini	2011	36		72	61 (56–67)	33 (27–39)	0.9 (0.8–0.99)	35	52	2.7	90[a]		78[a]	15	18	n/a
Herrmann	2011	37		11	61 ± 5	33 ± 7	0.8 ± 0.2	100	n/a	18	n/a	n/a	n/a		100	Improved by 3%
Medical management																
Hachicha	2007	38		91	62 ± 8	32 ± 17	0.76 ± 0.23		–	n/a	81[a]	58 ± 8	<40[a]			
Pai	2008	8		14	66 ± 7	26 ± 5	0.77 ± 0.14		–	n/a	82		10			
Tarantini	2011	36		29	60 (50–66)	33 (27–37)	0.90 (0.75–1.00)	90			80[a]		35[a]			

[a]Data not provided. Estimated from Kaplan Meier survival curve.

Survey of Valvular Heart Disease showed that 2.9% of the patients who underwent SAVR had LVEF <30% and 16.4% had LVEF between 30% and 50% (Iung et al., 2003). In other SAVR series, the incidence ranges from 12% to 21% depending on the LVEF threshold used (Chukwuemeka et al., 2006; Kulik et al., 2006). In a study from an echocardiography database, 26% of patients with severe AS had LVEF ≤35% and 23% had a mean transvalvular gradient ≤30 mmHg (Pai et al., 2008). Only one third of them had AVR (Pai et al., 2008).

The Transcatheter Valve Therapies Registry showed that 7% of the patients undergoing TAVR had an LVEF <30%, while 25% of patients had an LVEF between 30% and 50% (Baron et al., 2016). The prevalence of low LVEF was similar in the U.K. Transcatheter Aortic Valve Implantation Registry (Duncan et al., 2015).

In patients with severe AS and LVEF <50%, the impact of TAVR versus SAVR on LV functional recovery is still controversial (Elmariah et al., 2013; Clavel et al., 2010). Clavel et al. found that TAVR was associated with better LVEF recovery compared with SAVR at 1 year (Clavel et al., 2010). Passeri et al. also found a significant improvement in LV recovery in patients with LV dysfunction (EF <50%) who underwent TAVR. There was an absolute increase in LVEF by 10% or more at 30 days in 48.7% of patients undergoing TAVR (Passeri et al., 2015). Elmariah et al. performed a post hoc analysis of the PARTNER trial and found patients with LV dysfunction had a mean LVEF increase from 35.7 ± 8.5% to 48.6 ± 11.3% ($P < 0.0001$) 1 year after TAVR and from 38.0 ± 8.0% to 50.1 ± 10.8% after SAVR ($P < 0.0001$). They also identified those with a previous permanent pacemaker as a subgroup less likely to have improvement in EF at 30 days and those with higher mean aortic gradients as a subgroup more likely to have improvement. Despite the improvement in EF with TAVR, there was still no difference in the rate or degree of LV functional improvement when comparing TAVR to SAVR in patients with severe AS and LVEF <50% at baseline. They concluded that TAVR was a feasible alternative to SAVR for this population (Elmariah et al., 2013).

CHF secondary to AS with low LVEF and high transvalvular gradients (Table 3)

The subgroup of patients with severe AS and reduced LVEF, *who* are still able to generate trans-aortic gradients ≥40 mmHg, benefit significantly from SAVR (Pai et al., 2008; Connolly et al., 1997; Kulik et al., 2006; Flores-Marin et al., 2010; Powell et al., 2000; Vaquette et al., 2005; Halkos et al., 2009) (**Fig. 1**, **Table 3**). They represent 20% of the patients with severe AS and low LVEF (Kulik et al., 2006).

The 30-day mortality can range from 9% to 19.5%. Significant predictors of operative mortality were preoperative myocardial infarction, coronary artery disease (CAD), and cardiomegaly.

Symptomatic improvement occurred in the majority of patients after SAVR. Most patients were in functional class I or II at late follow-up. LVEF improved early after SAVR and continued to improve at late follow-up (Sharma et al., 2004; Vaquette et al., 2005). The improvement in LVEF was usually more pronounced than in patients with preserved LVEF and severe AS (Sharma et al., 2004). Greater AS severity (smaller AVA and higher mean gradients), better preoperative ejection fraction, less remodeled ventricles, and the absence of CAD or previous myocardial infarction were associated with improvement in LVEF (Connolly et al., 1997; Matsumura et al., 2008; Flores-Marin et al., 2010).

Long-term survival ranged from 77% to >90% at one year and from 58% to 71% at 5 years. In the absence of CAD, survival of patients with severe aortic and reduced left ventricular function with elevated gradients was similar to the expected survival of the overall population (Connolly et al., 1997). Independent predictors of long-term survival by multivariate analysis are listed in **Table 4**.

TAVR

In patients with low LVEF and high gradients treated with TAVR, no difference was found in the procedural and 30-day mortality when compared to patients with LVEF ≥50% and high gradients (Conrotto et al., 2016; Gotzmann et al., 2012; Passeri et al., 2015; Elhmidi et al., 2014). However, at longer follow-up, patients with high gradients and low LVEF did worse than patients with high gradients and preserved LVEF. At 6 months there was a twofold higher mortality among patients with LVEF ≤35% compared to >50% (26.9% vs. 15%, $P = 0.03$) (Elhmidi et al., 2014). But when compared to patients with low gradients, those with high gradients still had better outcomes (Gotzmann et al., 2012).

Improvement in LVEF occurs early (within 48 h) after TAVR in two third of patients with reduced LVEF. This early recovery is associated with improved clinical outcomes (Dauerman et al., 2016). Surprisingly, one study showed that LV dysfunction (LVEF ≤35%) is actually an independent predictor of LV function recovery (Elhmidi et al., 2014). Even if LVEF fails to improve early after TAVR, the LVEF is still expected to increase by more than 10% points in 40% of the patients by 6 months and in 50% of them by 12 months (Dauerman et al., 2016). Significant improvements in LVEF (44 ± 6% to 53 ± 9%, $P < 0.001$) and LV mass regression (171 ± 43 units to 135 ± 33 units, $P = 0.003$) at 1 year have also seen in this subgroup (Gotzmann et al., 2012).

CHF secondary to AS with low LVEF and low transvalvular gradients (Table 5)

This subgroup is typically referred to as "classical low-flow low-gradient" and tend to be the most clinically challenging patients to manage because of the severity from both the CHF and AS. They have more advanced myocardial dysfunction secondary to (a) afterload mismatch and therefore reversible or (b) the combination of afterload mismatch *and* intrinsic myocardial dysfunction that will not reverse with valve replacement (Carabello, 2006; Hwang et al., 1989). While most patients experience some benefit from SAVR, the subgroup that potentially benefits the most are those with reversible myocardial dysfunction. Hence, identifying patients with reversible dysfunction is of paramount importance. Reversible myocardial dysfunction secondary to afterload mismatch is associated with the presence of contractile reserve (increase in the SV ≥20%) with the infusion of low-dose

Table 3 AVR for severe aortic stenosis with left ventricular dysfunction and high transvalvular gradient

					Preoperative				*Surgery*		*Long-term survival*				*Postoperative*	
Author	*Year*	*References*	*Subgroups*	*N*	*LVEF (%)*	*Mean gradient (mmHg)*	*AVA (cm^2)*	*NYHA class III–IV (%)*	*CABG (%)*	*30-Day mortality (%)*	*1 Year (%)*	*3 Years (%)*	*5 Years (%)*	*10 Years (%)*	*NYHA class III–IV (%)*	*LVEF (%)*
Connolly	1997	25		154	27 ± 6	44 ± 18	0.6 ± 0.2	88	51	9	82[a]	60[a]	58		7	39 ± 14
Powell	2000	43		55	22 ± 6	41 ± 14	0.5 ± 0.2	84	55	18	77% and 33% without and with preop MI				Increased by 22 points in 95%	n/a
Vaquette	2005	44		155	25 ± 5	43 ± 13	0.6 ± 0.15	89	13	12	>90[a]		71		3	47
Matsumura	2008	46		90	37 ± 10	42 ± 17	0.7 ± 0.2	n/a	0	n/a	n/a	n/a	n/a	n/a	n/a	57 ± 11
Pai	2008	8	Low LVEF (<35%)	58	26 ± 7	40 ± 16	0.64 ± 0.17	n/a	59	9	80	n/a	58	n/a	n/a	n/a
Flores Marin	2009	42		82	33 ± 6	42 ± 18	0.58 ± 0.2	84	29	19.5	80[a]		70		5	n/a
Halkos	2009	45	LVEF <40	119	<40	n/a	n/a	42	45	10.9	82[a]		62		n/a	n/a
			LVEF 25–40	83	25–40	n/a	n/a	n/a		14.5						
			LVEF<25	36	<25	n/a	n/a	n/a		2.7						

[a]Data not provided. Estimated from Kaplan Meier survival curve.

Table 4 Independent risk factors associated with 30-day mortality, long-term survival, and LVEF improvement after AVR for low LVEF-low gradient aortic stenosis

Independent risk factors associated with 30-day mortality after AVR for low LVEF-low gradient aortic stenosis

Author	*References*	*Factor*	*HR or RR*	*95 CI*	*Association*
Coronary artery disease					
Powell	43	Previous myocardial infarction	14.9	2.4–92.1	Positive
Levy	47	Multivessel coronary artery disease	2.2	1.02–5.02	Positive
Connolly	25		4.6	1.4–15	Positive
Flores Marin	42		2.09	1.261–51	Positive
Tribouilloy	9	Concomitant CABG	9.7	1.9–49.9	Positive
Rothenburger	48		4.12	0.94–18.7	Positive
Myocardial dysfunction					
Tribouilloy	9	Mean aortic valve gradient $\leq$20 mmHg	10	1.2–84.9	Positive
Monin	4		4.7	1.1–21	Positive
Levy	47	Preoperative mean aortic valve gradient	0.89	0.83–0.96	Positive
Levy	47	Absence of contractile reserve	4.4	1.1–17.5	Positive
Monin	4,49		10.9	2.6–43.4	Positive
Rothenburger	48	LVESD $>$54 mm	0.24	0.05–1.05	Positive
Vaquette	44	Cardiothoracic ratio $\geq$0.6	12.2	5.4–27.4	Positive
Flores Marin	42	Preoperative mitral regurgitation	2.37	1.44–80	Positive
Rothenburger	48	NYHA class III or IV	0.14	0.02–1.12	Positive
Comorbidities and other factors					
Halkos	45	Age	1.05	1.01–1.08	Positive
Flores Marin	42	Female gender	2.6	2.2–89	Positive
Rothenburger	48	Creatinine $\geq$1.4	11	2.34–56.82	Positive
Halkos	45	Emergent status	5.9	1.21–28.08	Positive
Halkos	45	Cardiopulmonary bypass time	1.03	1.01–1.03	Positive
Connolly	26	Small prosthesis	n/a		

Independent risk factors associated with long-term survival after AVR for low LVEF-low gradient aortic stenosis

Author	*References*	*Factor*	*HR or RR*	*95 CI*	*Association*
Aortic valve replacement					
Monin	4	AVR	0.3	0.17–0.53	Positive
Pai	8		0.5	0.3–0.87	Positive
Tribouilloy	9		0.16	0.12–3.16	Positive
Pereira	54		0.19	0.09–0.39	Positive
Coronary artery disease					
Levy	47	Multivessel coronary artery disease	1.85	1.05–2.72	Negative
Tribouilloy	9		1.3	1.08–2.07	Negative
Connolly	25		n/a		Negative
Pai	8	Concomitant CABG	n/a		Negative
Myocardial dysfunction					
Monin	4,49	Contractile reserve	0.4	0.23–0.69	Positive
Levy	47	Preoperative mean aortic valve gradient$>$20 mmHg	0.95	0.91–0.99	Positive
Tribouilloy	9	Preoperative mean aortic valve gradient $\leq$20 mmHg	11.25	1.83–14.7	Negative
Connolly 97	25	Preoperative low cardiac output	n/a		Negative
Flores Marin	42	Postoperative low cardiac output	4.4	1.20–15.5	Negative
Tarantini	83	LVESVI $\leq$90 mL/m^2	n/a		Positive
Vaquette	44	Postoperative early increase in EF $\leq$10 units	0.96	0.94–0.97	Negative
Halkos	45	Low preoperative LVEF	0.98	0.97–1.0	Negative
Levy	47	Preoperative atrial fibrillation	1.75	1.07–2.85	Negative
Comorbidities					
Pereira	54	Age	1.05	1.02–1.07	Negative
Halkos	45		1.05	1.03–1.09	Negative
Vaquette	44		2.6	2.1–4.1	Negative
Pereira	54	Creatinine $>$1.5	1.5	(1.2–1.9)	Negative
Pai	8	Renal failure	n/a		Negative
Halkos	45	Peripheral vascular disease	1.86	1.13–3.06	Negative

(*Continued*)

Table 4 Independent risk factors associated with 30-day mortality, long-term survival, and LVEF improvement after AVR for low LVEF-low gradient aortic stenosis (Continued)

Independent risk factors associated with long-term survival after AVR for low LVEF-low gradient aortic stenosis

Author	*References*	*Factor*	*HR or RR*	*95 CI*	*Association*
Halkos	45	Previous stroke	1.94	1.16–3.85	Negative
Halkos	45	Renal failure requiring dialysis	2.95	1.13–7.75	Negative
Levy	47	Euroscore >10	1.13	1.04–1.25	Negative

Independent risk factors associated with LVEF improvement after AVR for low LVEF-low gradient aortic stenosis

Author	*References*	*Factor*	*HR or RR*	*95 CI*	*Association*
Coronary artery disease					
Quere	53	Multivessel coronary artery disease	–0.2		Negative
Connoly	25	Less coronary artery disease	n/a		Positive
Myocardial dysfunction					
Quere	53	Mean aortic valve gradient ≤30 mmHg	−0.5		Negative
Vaquette	44	High mean aortic valve gradient	1.05	1.0–1.1	Positive
Matsumura	46	Preoperative LVEF	n/a		Positive
Matsumura	46	End systolic volume index <48 mL/m^2	n/a		Positive
Matsumura	46	End diastolic sphericity <0.57	n/a		Positive
Vaquette	44	Cardiothoracic ratio <0.6	5.95	3.0–11.6	Negative
Other					
Connolly	26	Female gender	n/a		Positive
Connolly	26	Small preoperative aortic valve area	n/a		Positive
Pereira	54	Preoperative syncope	n/a		Positive
Pereira	54	Systemic hypertension	n/a		Negative

dobutamine while patients without contractile reserve are believed to have intrinsic and therefore irreversible myocardial dysfunction (Monin et al., 2001, 2003; Nishimura et al., 2002, 2014; Steinhauser and Stone, 2007) (**Fig. 2**). The determination of irreversible myocardial dysfunction by the absence of contractile reserve is not perfect since a large number of patients without contractile reserve will still benefit from SAVR (Monin et al., 2003; Tribouilloy et al., 2009; Quere et al., 2006).

The dobutamine challenge also helps to differentiate patients with low cardiac output and true severe AS from those with low cardiac output and mild AS or pseudo AS by examining the changes in AVA, SV, and mean aortic valve gradient (**Fig. 2**). Patients with true severe AS respond to the dobutamine-induced increase in SV with an increase in the mean gradient, while the calculated AVA remains low. Patients with pseudo-severe AS may or may not increase their mean gradient, but the hallmark is that the AVA will actually increase. Patients with pseudo-severe AS do not benefit from SAVR (Monin et al., 2001, 2003; Steinhauser and Stone, 2007; Nishimura et al., 2002).

The presence of contractile reserve has prognostic implications for patients treated either with SAVR or with medical management. It helps predict the operative risk as well as the long-term survival of patients with low EF low gradient AS. Operative mortality in patients with contractile reserve is significantly lower (5%–7%), compared to those without contractile reserve (26%–33%) (Monin et al., 2003; Tribouilloy et al., 2009; Nishimura et al., 2002).

Long-term survival and functional status after SAVR is also influenced by the presence contractile reserve. In a multicenter study by Monin, they demonstrated that patients with contractile reserve had improved 1-year (90% vs. 60%) and a 5-year (74% vs. 37%) survival after SAVR compared to those without contractile reserve. In the same study, NYHA functional class improvement occurred in 84% of patients with contractile reserve versus in 45% of patients without (Monin et al., 2003). In a subsequent substudy, Quere analyzed the outcomes of patients who survived SAVR and showed that contractile reserve did not influence long-term survival suggesting that the contractile reserve is only a primary determinant of surgical risk (Quere et al., 2006).

In spite of the high operative mortality and limited long-term survival associated with SAVR in patients without contractile reserve, AVR still significantly improved their prognosis compared to medical management. Monin demonstrated that 1- and 5-year survival with or without contractile reserve was better for patients treated with SAVR than for patients who received medical therapy (**Tables 1** and **5**; Monin et al., 2003).

Tribouilloy demonstrated on 81 patients with low-flow/low-gradient AS without contractile reserve that SAVR was associated with lower 5-year mortality (13% vs. 54%) when compared to medical therapy, despite the 22% surgical mortality. In addition only 9% of the SAVR patients had heart failure symptoms at follow-up compared with 81% of the medically managed patients (Tribouilloy et al., 2009). Clavel et al. (2008) demonstrated in the same group of patients that SAVR was only associated with improved overall survival compared with medical management in the subset of patients with more severe stenosis (AVA <1.0 cm^2). This lack of improvement was likely due to the high operative mortality (18%) (Clavel et al., 2008). Once operative mortality was

Table 5 AVR for severe aortic stenosis with left ventricular dysfunction and Low transvalvular gradient

					Preoperative				*Surgery*		*Long-term survival*				*Postoperative*	
Author	*Year*	*References*	*Subgroups*	*N*	*LVEF (%)*	*Mean gradient (mmHg)*	*AVA (cm^2)*	*NYHA class III–IV (%)*	*CABG (%)*	*30-Day mortality (%)*	*1 Year (%)*	*3 Years (%)*	*5 Years (%)*	*10 Years (%)*	*NYHA class III–IV (%)*	*LVEF (%)*
Brogan	1993	17		18	n/a	≤30	≤0.4 cm^2/m^2	100		33.0	n/a	n/a	n/a	n/a	11	n/a
Connolly	2000	26		52	26±8	23±3	0.7±0.2	85	62	21.0	65[a]	62	38[a]		23	32±14
Pereira	2002	54		68	22±6	25±4	0.6±0.1	65	60	8.0	82±6		78±7 (4 years)		18	30±12
Rothenburger	2003	48		35	25±4	26±6	0.6±0.18	90	(14 pts)	17.1	72	68 (2 years)	64		24	40±5
Monin	2003	4	ALL	95	30 (24–35)	29 (23–34)	0.7 (0.6–0.8)	80	30	14.0		79			30	n/a
			Contractile reserve	64	31 (23–35)	27 (22–35)	0.7 (0.6–0.9)	85	30	5.0	90[a]		74[a]		16	n/a
			No contractile reserve	31	30 (27–35)	30 (2334)	0.8 (0.7–0.9)	83	26	32.0	65[a]		37[a]		55	n/a
Tarantini	2003	83		52	28±7	29±6	0.57±0.2	n/a	31	8.0	95[a]		89[a]		Improvement in 85%	43±10
Chukwuemeka	2006	18		60	26±4	27±19	0.76±0.37	93	25	3.3	93		77	48		
Quere	2006	53	ALL	66	29±6	31±6	0.68±0.16	89							9	47±11
			Contractile reserve	46	28±6	31±7	0.65±0.16	89		6.0			90±5		7	47±11
			No contractile reserve	20	31±6	32±6	0.74±0.13	90		33.0			92±7		15	48±11
Kulik	2006	41		79	<40% in 72%	29±7	0.8±0.2	53	62	7.5	97±2		83±5	72±8	16	
Clavel	2008	7		44	29±11	26±8	0.9±0.2	52	68	18.0	75[a]	70[a]				Improvement >10%
Levy	2008	47		217	28±6	25±5	0.7±0.2	83	34	16.0	80[a]		49±4		16	41±13
Pai	2008	8	Low gradient (<30 mmHg)	47	45±20	25±4	0.76±0.15	n/a	n/a	n/a	89	82 (2 years)	80			
Tribouilloy	2009	9		55	27±7	29±7	0.74±0.15	29	27	22.0	75[a]		54±7		9	46±10

[a]Data not provided. Estimated from Kaplan Meier survival curve.

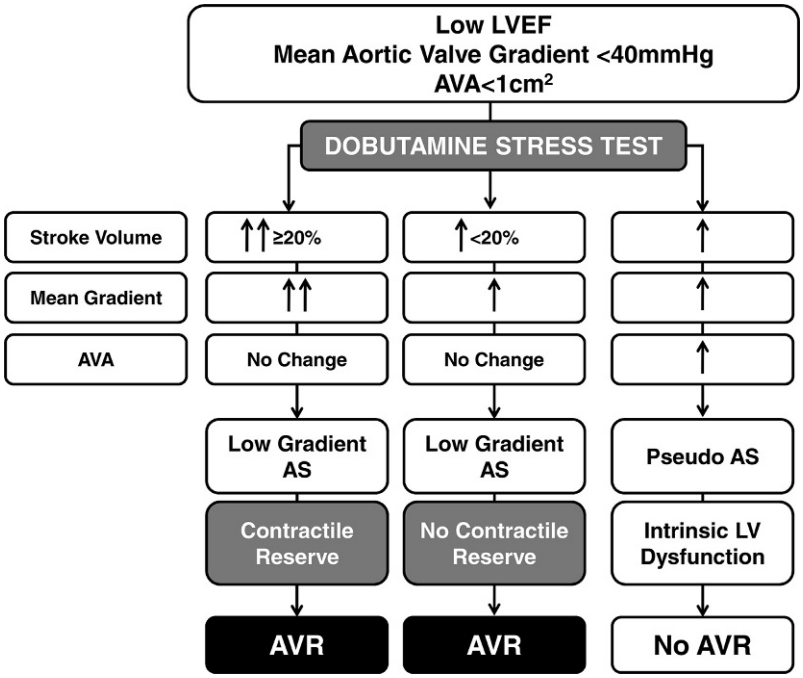

Fig. 2 Dobutamine stress test for the determination of contractile reserve and aortic stenosis severity.

excluded, patients who survived SAVR had excellent late survival compared with patients treated medically (70% vs. 50%) (**Table 5**; Clavel et al., 2008).

Early postoperative improvement in ejection fraction is associated with improved long-term survival and functional status (Quere et al., 2006; Morris et al., 1993). Connoly demonstrated that LVEF improved in 74% of the survivors. LVEF improved $10 \pm 14\%$ units. Positive change in LVEF was associated with female sex and smaller preoperative AVA (Connolly et al., 2000).

Quere demonstrated that LVEF improvement can be observed in patients without contractile reserve after SAVR (Quere et al., 2006). 83% of patients with and 65% of the patients without contractile reserve obtained $\geq 10\%$ improvement in LVEF after SAVR. The magnitude of the improvement was similar regardless of the presence of contractile reserve. Contractile reserve was not a predictor of improvement in LVEF after SAVR. Therefore, absence of contractile reserve does not always predict irreversible myocardial dysfunction. Higher preoperative mean aortic valve gradient and absence of multi-vessel CAD were associated with improvement in LVEF after SAVR (Quere et al., 2006). A small recent study looking specifically at Global LV Longitudinal Strain (GLS) showed a twofold increase in operative mortality with resting GLS <9% compared to GLS >9%. However, beyond 30 days, there was no difference in survival between the groups (Dahou et al., 2015).

TAVR

In the GARY registry, LF LG AS was present in 11.7% of patients undergoing TAVR (Lauten et al., 2014). This cohort experiences a higher 30-day (7.8% vs. 4.9%) and 1-year mortality (32.3% vs. 19.8%) compared to other groups (Lauten et al., 2014; Gotzmann et al., 2012). They also had higher rates of low cardiac output syndrome, cardiopulmonary resuscitation, and mechanical circulatory support during the post TAVR period (Lauten et al., 2014; Schaefer et al., 2015). While the outcomes may be worse compared to other AS subgroups undergoing TAVR, there is evidence to suggest patients should still proceed with TAVR. The PARTNER trial shows that TAVR and SAVR improved 30-day, 1-year and 2-year mortality (25% vs. 7.4% at 30 days, 59% vs. 25% at 1 year, 76% vs. 46% at 2 year) for LF AS compared to medical management (O'Sullivan et al., 2015; Pibarot and Dumesnil, 2011). However, when comparing SAVR to TAVR, the 30-day (5.6% vs. 9.5%), 1-year (25.9% vs. 23.3%), and 2-year mortality (42.9% vs. 37.1%) were similar in this subgroup of patients (Elmariah et al., 2013; Herrmann et al., 2013). Improvement in SV at discharge after TAVR is associated with improved outcomes (Anjan et al., 2016).

After 1 year, the improvement in LVEF was similar after both transcatheter and surgical valve replacement, with most of this improvement occurring within the first 30 days (O'Sullivan et al., 2015; Elmariah et al., 2013). Mean LVEF increased from $35.7 \pm 8.5\%$ to $48.6 \pm 11.3\%$ ($P < 0.0001$) at 1 year after TAVR and from $38.0 \pm 8.0\%$ to $50.1 \pm 10.8\%$ after SVAR($P < 0.0001$). Fifty three percent of TAVR patients and 62.3% of SAVR had normalized their LVEF a year after the procedures ($P = 0.34$) (Elmariah et al., 2013). Other studies suggest LV function improved more in TAVR patients compared to SAVR (Clavel et al., 2010). This discrepancy is likely attributed to the superior hemodynamics of TAVR valves and exclusion of CAD in the PARTNER trial (O'Sullivan et al., 2015; Elmariah et al., 2013). Dauerman et al. showed in the self-expanding CoreValve trials that early LVEF recovery (defined as a LVEF increase of $\geq 10\%$ at 30 days) occurred in 62% patients but it was not associated with improved 1-year

survival (Dauerman et al., 2016). In the PARTNER trial early improvement in LV function was associated with reduced rates of all cause and cardiac mortality in the TAVR group at 1 year, but not in the SAVR group (Elmariah et al., 2013). Higher baseline LVEF, previous permanent pacemaker, previous MI, and low gradient (<40 mmHg) were associated with reduced likelihood of early LVEF improvement (Elmariah et al., 2013; Dauerman et al., 2016). More recently SVi (<35 mL/m^2) and low baseline aortic valve gradient (<40 mmHg) have been identified as more powerful predictors of poor outcomes than LVEF or gradients alone (Le Ven et al., 2013; Herrmann et al., 2013). The presence of CAD or incomplete revascularization has also been associated with increased 1-year mortality in LFLG AS (O'Sullivan et al., 2015).

Predictors of outcomes

Long-term outcomes in severe AS with low LVEF and low transvalvular gradients are not accurately predicted by the presence of contractile reserve alone (Quere et al., 2006).Thus other factors should be considered to risk stratify patients before SAVR. Several studies have identified independent predictors of early mortality, long-term survival, and improvement in LVEF and functional class (**Table 4**).

Predictors of 30-day mortality include CAD (as defined by previous MI, multi-vessel CAD, and concomitant coronary arteries bypass graft surgery (CABG)), which likely indicates the presence of intrinsic and probably irreversible myocardial dysfunction secondary to fibrosis or myocardial infarction (Tribouilloy et al., 2009; Connolly et al., 1997; Flores-Marin et al., 2010; Powell et al., 2000; Levy et al., 2008; Rothenburger et al., 2003). In addition, concomitant CABG increases the operative time and complexity of the surgery. Surrogates for more advanced left ventricular dysfunction are low mean transaortic gradients, absence of contractile reserve, and dilated LV (Monin et al., 2001, 2003; Tribouilloy et al., 2009; Flores-Marin et al., 2010; Vaquette et al., 2005; Levy et al., 2008; Rothenburger et al., 2003). The increased mortality with small prosthesis size may be the result of high residual aortic valve gradient and incomplete relief of the LV outflow obstruction (Connolly et al., 2000). Patients with LV dysfunction tolerate poorly residual aortic gradient and patient prosthesis mismatch (PPM) since they are highly sensitive to increased afterload (Connolly et al., 2000; Kulik et al., 2006; Blais et al., 2003; Ruel et al., 2006). PPM results in decreased survival, lower freedom from heart failure, and incomplete left ventricular mass regression (Kulik et al., 2006; Ruel et al., 2006). PPM should be avoided by implanting prosthesis with superior hemodynamic performance and considering the prosthetic effective orifice area indexed to body surface area at the time of SAVR. Some have advocated the use of stentless valves (Bevilacqua et al., 2002). Transcatheter aortic valves may have an advantage in this group since they have better hemodynamic performance with larger postoperative AVA and lower transvalvular gradient than surgically placed valves (Kulik et al., 2006; Smith et al., 2011).

Other factors associated with early mortality are common predictors of increased surgical risk (age, presence of comorbidities, advanced functional status, emergency surgery, and female gender) (**Table 4**).

SAVR is the main factor associated with long-term survival, LVEF improvement, and improved functional status in patients with low LVEF and low gradient AS (Monin et al., 2003; Pai et al., 2008; Tribouilloy et al., 2009; Pereira et al., 2002). This reinforces the notion that even risky, SAVR provides a significant survival advantage to these patients compared to medical management alone.

Other factor negatively associated with long-term survival was presence of CAD (Pai et al., 2008; Tribouilloy et al., 2009; Connolly et al., 1997; Levy et al., 2008). As in early mortality, CAD indicates intrinsic myocardial dysfunction. Factors associated with ventricular dysfunction (contractile reserve, low preoperative mean gradient, pre- and postoperative low cardiac output, remodeled ventricles, and atrial fibrillation) also negatively affected long-term survival (Monin et al., 2001, 2003; Tribouilloy et al., 2009; Connolly et al., 1997; Tarantini et al., 2011; Flores-Marin et al., 2010; Vaquette et al., 2005; Halkos et al., 2009; Levy et al., 2008). Early improvement in LVEF predicted long-term survival indicating that most of myocardial dysfunction was reversible secondary to afterload mismatch (Vaquette et al., 2005). Advanced age, comorbidities, and elevated EuroSCORE also predicted decreased survival (Pai et al., 2008; Vaquette et al., 2005; Halkos et al., 2009; Levy et al., 2008; Pereira et al., 2002).

Improvement in LVEF is another factor that helps predict early and late survival (Connolly et al., 1997; Elmariah et al., 2013; Matsumura et al., 2008; Powell et al., 2000; Vaquette et al., 2005; Dauerman et al., 2016; Quere et al., 2006; Pereira et al., 2002). In addition, the presence of systemic hypertension was negatively associated with improvement in LVEF. It was presumed that systemic hypertension was associated with myocardial fibrosis. The only independent factor associated with improved functional status at late follow-up was the early improvement in LVEF >10 ejection fraction units (Vaquette et al., 2005).

Many of the variables associated with adverse outcomes are similar to those associated with adverse outcomes in the general population of AS patients. Hannan identified age >60 years, LVEF <50%, CHF, myocardial infarction less than 24 h before surgery, lower body surface area, previous cardiac operation, and several comorbidities as independent predictors of 30-day mortality(-Hannan et al., 2009). Factors associated with increased long-term mortality were concomitant CABG, age >60, small body surface area, emergency status, and comorbidities (Hannan et al., 2009). Mihaljevic identified the following risk factors as associated with early death: older age, LV dilatation, and smaller prosthetic size. Risk factors for late death were older age, greater degree of AS, greater LV mass index, smaller prosthetic size, LV dysfunction, and advanced symptoms. Risk factors associated with advanced symptoms include calcific AS and severe LV dysfunction (Malaisrie et al., 2010).

PPM after SAVR has been shown to have a negative impact on short- and long-term survival (Head and Mokhles, 2012). In high risk surgical candidates with severe AS, PPM occurs more frequently (60% vs. 40%) and is more severe (28% vs. 19.7%) in patients undergoing SAVR compared to TAVR. While there is no randomized trial comparing the incidence and outcomes of PPM after SAVR versus TAVR, there is some data to suggest high-risk patients, who are susceptible to PPM or have a small aortic annulus, should undergo TAVR instead of SAVR (Pibarot and Weissman, 2014).

Percutaneous Aortic Valvuloplasty

Percutaneous balloon aortic valvuloplasty is an alternative for patients with severe acquired AS and LV dysfunction who are not candidates for SAVR or TAVR. Percutaneous aortic valvuloplasty was associated with temporary reduction of transvalvular gradients (from 55 to 29 mmHg), increase in the AVA (mean increase 0.3 cm^2), improvement in left ventricular performance, and symptomatic improvement (Passeri et al., 2015; Anon, 1991). However, it had several disadvantages: (1) it was associated with a 25% risk of complications, (2) the improvement was short lived with recurrence of the symptoms within a few months and (3) there was no survival benefit (Anon, 1991; Otto et al., 1994; Lieberman et al., 1995; Nishimura et al., 1991; Kapadia et al., 2010). With the advent of transcatheter aortic valve implantation, aortic valvuloplasty has reemerged as a procedure for the treatment of AS (Tissot et al., 2011). It is usually utilized in patients with CHF or cardiogenic shock to stabilize them and bridge them to TAVR or to a high-risk AVR (Kapadia et al., 2010; Tissot et al., 2011).

Future of TAVR Technology

As the technology improves and access challenges are overcome this technology will be ubiquitous. At the present time TAVR is FDA approved for extreme risk, high risk, and intermediate risk patients who have severe AS. Outcomes in these trials (Kapadia et al., 2015; Leon et al., 2010; Nkomo et al., 2006; Lindroos et al., 1993; Pai and Varadarajan, 2010; Bach, 2011; Passeri et al., 2015; Dauerman et al., 2016; Hwang et al., 1989; Tissot et al., 2011) as shown above are equivalent to surgery and in some cases even better. Low risk trials are underway and results will be available in the next few years.

As the technology is being deployed in lower risk and younger patients, there are several issues that still require attention particularly as the life expectancy of the lower risk population is significantly longer. New generation devices will address perivalvular regurgitation and post procedure conduction disturbances requiring pacemaker. The questions regarding durability, leaflet thrombosis, and stroke rate will need to be addressed before widespread applicability in younger population (Hamm et al., 2016; Holmes and Mack, 2015). Five-year data from the PARTNER trial demonstrates that valve performance and cardiac hemodynamics are stable after implantation in both TAVR and SAVR in patients alive at 5 years.

Aortic Regurgitation and CHF

Aortic insufficiency leads to both pressure and volume overload on the LV (Nishimura et al., 2014; Chukwuemeka et al., 2006; Bekeredjian and Grayburn, 2005). Volume overload (increased preload) results from the diastolic regurgitant volume. Increased afterload is the result of the increased aortic SV (regurgitant volume plus forward SV) that leads to systolic arterial hypertension. The increase in wall stress leads to compensatory LV dilatation and eccentric hypertrophy. These changes decrease wall stress and preserve ejection fraction. Progressive LV dilatation overcomes those compensatory mechanisms leading to myocardial dysfunction and decreased EF. At this point, LV function will improve after SAVR. Persistent regurgitation and further increase in wall stress lead to further systolic dysfunction secondary to ischemia and myocardial fibrosis (Holmes and Mack, 2015; Bekeredjian and Grayburn, 2005). At this stage, when the ventricle is severely dilated, intrinsic myocardial dysfunction becomes the predominant mechanism responsible for LV dysfunction and SAVR is less likely to improve LV function. However, even without LVEF improvement, SAVR will improve loading conditions and facilitate CHF management (Nishimura et al., 2014).

CHF secondary to severe AR has several commonalities with the one secondary to severe AS. It is common, its medical management results in poor outcomes, and it is undertreated.

In the Euro Heart Survey, AR was the third most common valve pathology after AS and mitral regurgitation. Only one third of those patients were treated surgically. That proportion is even lower in patients with left ventricular dysfunction: only 22% of patients with LVEF between 30% and 50% and 3% of patients with LVEF<30% were treated with AVR (Iung et al., 2003; Kamath et al., 2009; Supino et al., 2006).

A review of the STS database reinforces the notion that AR in CHF and low LVEF is undertreated: 47% of isolated AVR patients had some degree of AR. 52% were in NYHA class III–IV but only 5% had LVEF <30% (Brown et al., 2009a).

The natural history of asymptomatic severe AR with normal LVEF and normal ventricular dimensions is benign (Nishimura et al., 2014; Bekeredjian and Grayburn, 2005; Bonow et al., 1991; Borer et al., 1998). However, once CHF, ventricular dilatation, or LV dysfunction develop, the prognosis of medically treated patients is poor. Survival ranges from 20% to 50% at 5 years and the majority of patients are in NYHA class III–IV (Kamath et al., 2009; Bekeredjian and Grayburn, 2005; Sionis et al., 2010; Khandhar et al., 2009). Thus, surgery is recommended when (a) patients become symptomatic (ACC-AHA guidelines class I and European Society of Cardiology (ESC) guidelines class IB), (b) the LVEF is ≤50% independently of symptoms (ACC-AHA guidelines class I and ESC class IB) or (c) the LV dilates (left ventricle end diastolic diameter (LVEDD) >75 mm or left ventricle end systolic diameter (LVESD) >55 mm, ACC-AHA guidelines class IIa) (Nishimura et al., 2014; Khandhar et al., 2009). The ESC guidelines recommends surgery with lesser degree of LV dilatation (LVEDD >70 mm or LVESD >50 mm, class IIaC) (Khandhar et al., 2009).

The surgical outcomes of patients with advanced CHF (NYHA class III–IV) and severe LV dysfunction (LVEF ≤35%) secondary to chronic AR have only been studied in a few series (**Table 6**).

Table 6 AVR for severe aortic regurgitation with left ventricular dysfunction

					Preoperative				*Surgery*		*Long-term survival*				*Postoperative*	
Author	*Year*	*References*	*Subgroups*	*N*	*LVEF (%)*	*Mean gradient (mmHg)*	*AVA (cm^2)*	*NYHA class III–IV (%)*	*CABG (%)*	*30-Day mortality (%)*	*1 Year (%)*	*3 Years (%)*	*5 Years (%)*	*10 Years (%)*	*NYHA class III–IV (%)*	*LVEF (%)*
Klodas	1997	77		128	49 ± 14	n/a	n/a	100	32	7.8			72 ± 4	45 ± 4		
Chaliki	2002	78	Low LVEF	43	28 ± 5	7.4 ± 0.8	4.1 ± 0.7	58		14	80[a]		60[a]	41 ± 9	25 ± 9	34 ± 14
			Moderate LVEF	134	43 ± 5	7.0 ± 0.8	6.5 ± 0.9	49		6.7	90[a]		78[a]	56 ± 5	17 ± 4	47 ± 12
			Normal LVEF	273	59 ± 6	5.3 ± 0.7	4.2 ± 0.8	29		3.5	95[a]		85[a]	70 ± 3	9 ± 2	56 ± 10
Rothenburger	2003	48		20	24 ± 6	6.7 ± 0.8	5.6 ± 0.3	90	70	0	94	86 (2 years)	74		45	40 ± 5
Chukwuemeka	2006	18		132	28 ± 6	6.6 ± 0.9	5.2 ± 0.8	72	18	1.4	99		81	61		
Kamath	2009	11		53	27 ± 6	7.0 ± 1.0	5.7 ± 1.2	62	32	4	88		70			
Kandhar	2009	75		32	53 ± 16	6.3 ± 1.0	4.3 ± 1.3	87	n/a	n/a	90		62			
Sionis	2010	74		26	36 ± 4	6.9 ± 9	5	100		0	92				12	45

[a]Data not provided. Estimated from Kaplan Meier survival curve.

The operative mortality in this group was high (**Table 6**). Operative mortality was four times higher in low ejection fraction patients than in patients with normal EF (14% vs. 3.7%) (Chaliki et al., 2002). Concomitant procedures and advanced NYHA class increased operative mortality (Chaliki et al., 2002). Klodas demonstrated that patients in NYHA class III–IV had a six time higher operative mortality (7.8% vs. 1.2%) (Klodas et al., 1997).

AVR improves long-term survival compared to medical management. One-year survival ranged from 80% to 99%. Five-year survival ranged from 60% to 80%. The only study that compared medical management with SAVR showed that SAVR improved 1-year survival from 65% to 88% and 5-year survival from 37% to 70% (**Table 6**; Kamath et al., 2009). After adjusting for baseline variables SAVR was associated with a significantly lower hazard of mortality (HR 0.59, 95% CI 0.42–0.98, $P < 0.04$) (Kamath et al., 2009).

Other independent predictors of long-term survival were preoperative LVEF, NYHA class, and age (Chaliki et al., 2002). Five-year survival for patients with LVEF <35% was 60% compared with 85% for patients with normal EF. This survival, although better than with medical management, was 65% lower than the expected survival of an age- and sex-matched population (Chaliki et al., 2002). This suggests that SAVR does not completely reverse the myocardial dysfunction induced by long-standing AR.

Other factor associated with poor long-term survival was dilated LV as identified by indexed LV dimensions. Patients with LVESDi $\geq$20 mm/m^2 and LVEDDi $\geq$30 mm/m^2 had worse long-term survival independently of their preoperative LVEF and NYHA functional class (Brown et al., 2009b). Previous studies have shown that extreme LV dilatation (LVEDD $\geq$80 mm) did not prevent improvement in LV function after SAVR (Klodas et al., 1996).

Even patients with extremely reduced LVEF (LVEF <20%) achieved a survival advantage with SAVR compared to medical management (Kamath et al., 2009). Severe pulmonary hypertension (systolic pulmonary pressure >60 mmHg) and functional mitral regurgitation adversely affect long-term survival (Pai and Varadarajan, 2010; Khandhar et al., 2009). SAVR and mitral valve repair were also associated with a better survival than medical management (Pai and Varadarajan, 2010; Khandhar et al., 2009).

SAVR results in symptomatic improvement. The majority of patients remain free of CHF symptoms after SAVR (Rothenburger et al., 2003; Chaliki et al., 2002).

Similar to AS the impairment in LVEF in AR is related to a combination of (a) afterload mismatch and (b) intrinsic myocardial dysfunction. LVEF improvement after AVR will depend on the relative contribution of each mechanism. SAVR resulted in LVEF improvement in the majority of patients with severe LV dysfunction (Brown et al., 2009b; Chaliki et al., 2002). The improvement is more pronounced in patients with lower preoperative EF (Brown et al., 2009b; Chaliki et al., 2002). The time course of LVEF improvement shows a modest initial decrement in EF followed by a gradual improvement over the course of the next 6 months (Brown et al., 2009b; Senechal et al., 2011). Maximal LV regression after SAVR may take up to 4 years (Une et al., 2015). Late improvement in LVEF was associated with the presence of early LV reverse remodeling defined as a 10% reduction in LVEDD (Senechal et al., 2011). LVEF decreased significantly late after AVR in patients with no early LV reverse remodeling. Preoperative LV SV >97 mL was the best independent predictor of early reverse remodeling (Senechal et al., 2011).

Heart transplantation and mechanical circulatory support should be considered as an alternative treatment in this group of patients (Christie et al., 2010). However, in spite of the high operative mortality most patients with CHF secondary to severe AR with severely reduced LVEF greatly benefit from SAVR (Chaliki et al., 2002). Most patients achieved lasting symptomatic improvement and improvement in their ejection fraction. Long-term survival is better than the one after heart transplantation without the side effects and complications of immunosuppression and rejection.

TAVR

Percutaneous options for treatment of pure AR are undergoing feasibility trials across the world (Roy et al., 2013; Seiffert et al., 2014; Zhu et al., 2015). Absence or minimal calcification of the native aortic cusps in pure AR increases the valve embolization or residual paravalvular regurgitation. Excessive oversizing carries a subsequent hazard for aortic root rupture or incomplete valve expansion. Limited data is available from trials and registries in patients who are not candidates for surgery. The implantation success is greater than 95%; however, there is an increased risk of double valve placements (Roy et al., 2013; Seiffert et al., 2014). Thirty-day mortality and one-year mortality were elevated (Roy et al., 2013; Seiffert et al., 2014).

Conclusions

The prognosis of patients with CHF secondary to aortic valve disease with left ventricular dysfunction is poor. AVR, although risky, is associated with improved long-term survival, improved ventricular function, and functional class compared to medical management. TAVR provides a new alternative for those patients particularly for those who are considered inoperable or at high risk for surgery. The decision to intervene in these patients is challenging. They should be systematically evaluated by the heart valve team where cardiologist, imaging specialist, cardiac surgeons, interventional cardiologist, and nurse coordinators consider the severity of their cardiac disease, surgical risk, comorbidities, frailty, other factors that may limit their life expectancy, and the risk-benefit ratio of different treatment strategies as well as the patient preferences and values (Iung et al., 2003; Otto et al., 2017; Chikwe and Adams, 2010). These factors should be carefully considered to risk stratify and guide the decision to perform surgery or TAVR.

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Arrhythmias in Cancer and Cancer Treatment: A Review

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Introduction

This article provides a comprehensive review of the cardiac arrhythmias that occur in the setting of cancer. The first section reviews chemotherapy-associated arrhythmias and is organized by class of medication. **Table 1** serves as a reference from which readers can quickly identify the scientific literature utilized in the discussion that follows. Next follows a discussion on the QT interval, a time interval acquired from the electrocardiogram (ECG) that is associated with dangerous, potentially lethal ventricular arrhythmias. The utility of using the QT interval as a predictor of ventricular arrhythmias is assessed. Prevention and management of QT interval prolongation is included. The second section reviews cardiac arrhythmias associated with radiation therapy. It begins with a discussion of the risk of arrhythmia in patients receiving radiation. Next follows a discussion of radiation therapy in patients with cardiac implantable electronic devices (CIEDs), which include pacemakers and implantable cardioverter-defibrillators (ICD). Risk of CIED malfunction and a discussion on whether CIEDs should be relocated prior to radiation therapy are included.

Multiple cardiac arrhythmias are reviewed in this article. Cardiac arrhythmias can arise from different regions of the heart and have varying degrees of clinical significance. Atrial arrhythmias include bradycardia, atrial fibrillation, atrial flutter, and supraventricular tachycardia (SVT). Ventricular arrhythmias include ventricular tachycardia (VT) and ventricular fibrillation (VF). Arrhythmias can also originate between the atria and ventricles at the atrioventricular (AV) junction. These are called AV conduction blocks and for the purposes of this article are grouped with atrial arrhythmias. **Table 2** provides definitions for the cardiac arrhythmias discussed in this article. There are many additional arrhythmias that are beyond the scope of this article, which focuses on the most common and significant types.

Chemotherapy-Induced Arrhythmia

Arrhythmia Risk by Class

Alkylating agents

Arrhythmias

- Cyclophosphamide and ifosfamide
  - Atrial fibrillation is rarely documented, but one study did note 11.8% of 34 patients having transient atrial fibrillation (Yusuf et al., 2009).
  - QTc interval prolongation by an average of 21 milliseconds (ms) has been reported.

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Table 1 Arrhythmia risk by type of arrhythmia

Arrhythmia	*Agent*	*References*
Bradycardia		
SA node dysfunction	Platinums	Schlaeffer et al. (1983), Bashir et al. (2007)
	Anthracyclines	Kilickap et al. (2007)
	Antimetabolites	Khan et al. (2012), Talapatra et al. (2007), Stewart et al. (2010), Cil et al. (2007), Perez-Verdia et al. (2005)
	Antimicrotubule agents	Tamargo et al. (2015), Rowinsky et al. (1993), Rowinsky et al. (1991b), Arbuck et al. (1993), Kietpeerakool et al. (2006), Chamberlain and Kormanik (1995), Kanat et al. (2003), Zekri (2011)
	Monoclonal antibodies	Foran et al. (2000), Zhou et al. (2015)
	mTOR inhibitors	Cox et al. (1997)
	Proteasome inhibitors	Enrico et al. (2007)
	Thalidomide and analogs	Palumbo et al. (2008), Hinterseer et al. (2006), Ali et al. (2013), Barlogie et al. (2006), Fahdi et al. (2004), Kaur et al. (2003), Yamaguchi (2008), Rajkumar et al. (2006), Palladini et al. (2005), Rajkumar et al. (2003)
	Topoisomerase inhibitors	Dodds and Rivory (1999), Miya et al. (1998)
	Tyrosine kinase inhibitors	Ou et al. (2016), Ou et al. (2013), Ou et al. (2011), Gallucci et al. (2015)
AV node dysfunction	Alkylating agents	Ando et al. (2000), Agarwal and Burkart (2013), Sculier et al. (1985), Ramireddy et al. (1994)
	Anthracyclines	Kilickap et al. (2007)
	Antimetabolites	Khan et al. (2012), Kosmas et al. (2008), Stewart et al. (2010)
	Antimicrotubule agents	Rowinsky et al. (1991b), McGuire et al. (1989)
	Arsenic trioxide	Huang et al. (1998), Huang et al. (1999), Kathirgamanathan et al. (2013)
	Monoclonal antibodies	Behling et al. (2016)
	Proteasome inhibitors	Enrico et al. (2007), Lee et al. (2011), Dasanu (2011)
	Thalidomide and analogs	Hinterseer et al. (2006)
Atrial tachycardia		
Atrial fibrillation/flutter	Alkylating agents	Kupari et al. (1990), Yusuf et al. (2009)
	Melphalan	Phillips et al. (2004), Sirohi et al. (2000), Moreau et al. (1999), Olivieri et al. (1998)
	Platinums	Canobbio et al. (1986), Tomkowski and Filipecki (1997), Tomkowski et al. (2004), Richards et al. (2006), Bischiniotis et al. (2005), Zakaria et al. (2011)
	Anthracyclines	Kilickap et al. (2007), Oster and Rakowski (1981), Montella et al. (2005)
	Antimetabolites	Aziz et al. (1998), Kosmas et al. (2008), Polk et al. (2016), Santini et al. (2000), Tavil et al. (2007), Ferrari et al. (2006), Ciotti et al. (1999), Peres et al. (2010), Wooten et al. (2000)
	Antimicrotubule agents	Palma et al. (2002), Lombardi et al. (2004), Kaakeh et al. (2012)
	Cytokines	Friess et al. (1989), Odashiro et al. (2002), White et al. (1994), Dutcher et al. (1997)
	Histone deacetylase inhibitors	Sandor et al. (2002), Stadler et al. (2006)
	Monoclonal antibodies	Foran et al. (2000), Lenihan et al. (2004)
	Nonreceptor TK inhibitor	Burger et al. (2015), Byrd et al. (2014), Chanan-Khan et al. (2016), Shanafelt et al. (2016), Leong et al. (2016), Lee et al. (2016a), Wang et al. (2016), Gustine et al. (2016), Thompson et al. (2016), Yun et al. (2017), Vrontikis et al. (2016), McMullen et al. (2014)
	Proteasome inhibitors	Shah et al. (2004), Enrico et al. (2007)
	Thalidomide and analogs	Gonzalez Rodriguez (2011)
	Topoisomerase inhibitors	Stinchcombe et al. (2008), Poulsen et al. (2009), Kridis et al. (2013)
	Tyrosine kinase inhibitors	Petrini et al. (2012), Mego et al. (2007)
Supraventricular tachycardia	Alkylating agents	Kupari et al. (1990), Ando et al. (2000), Muller et al. (2004)
	Melphalan	Feliz et al. (2011), Mileshkin et al. (2005)
	Platinums	Raja et al. (2013), Lara et al. (2005)
	Anthracyclines	Steinberg et al. (1987)
	Antimetabolites	Peres et al. (2010), Tayer-Shifman et al. (2009), Aziz et al. (1998)
	Antimicrotubule agents	Rowinsky et al. (1991b), Kamineni et al. (2003)
	Cytokines	Martino et al. (1987), Lee et al. (1989), Margolin et al. (1989), Atkins et al. (1999), White et al. (1994), Tsimberidou et al. (2005)
	Histone deacetylase inhibitors	Stadler et al. (2006), Piekarz et al. (2009)
	Monoclonal antibodies	Foran et al. (2000), Coiffier et al. (2002)
	mTOR inhibitors	Kim et al. (2013), Duzova et al. (2008)
	Proteasome inhibitors	Berenson et al. (2005)
Ventricular tachycardia		
QT prolongation	Alkylating agents	Kuittinen et al. (2010), Kupari et al. (1990)
	Anthracyclines	Tamargo et al. (2015), Kupari et al. (1990), Iwata et al. (1984), Sarubbi et al. (1997), Nousiainen et al. (1999), Galetta et al. (2005)

(*Continued*)

Table 1 (Continued)

Arrhythmia	*Agent*	*References*
	Antimetabolites	Tamargo et al. (2015), Guglin et al. (2009), Stewart et al. (2010)
	Antimicrotubule agents	Shetty and Gupta (2014), Lesimple et al. (2013)
	Arsenic trioxide	Huang et al. (1998), Ohnishi et al. (2002), Ohnishi et al. (2000), Barbey et al. (2003), Soignet et al. (2001), Raghu et al. (2009)
	Histone deacetylase inhibitors	Piekarz et al. (2006), Shah et al. (2006), Stadler et al. (2006), Lynch et al. (2012), Giles et al. (2006)
	mTOR inhibitors	Ikitimur et al. (2015), Akers et al. (2004), Minematsu et al. (1999), Ozkanlar et al. (2005), Gerhardt et al. (2001)
	Nonreceptor TK inhibitors	Infante et al. (2016)
	Thalidomide and analogs	Hamlin et al. (2004)
	Topoisomerase inhibitors	Shinar and Hasin (1984)
	Tyrosine kinase inhibitors	Ghatalia et al. (2015), Shah and Morganroth (2015), Shah et al. (2013), Kazandjian et al. (2014), Brave et al. (2008), Kloth et al. (2015), Kantarjian et al. (2010), Kantarjian et al. (2007), Kantarjian et al. (2006), Tolcher et al. (2011), Bello et al. (2009), Zang et al. (2012), Liu et al. (2015), Leboulleux et al. (2012), Lee et al. (2016b), Arnold et al. (2007), Miller et al. (2005), Tamura et al. (2006), Holden et al. (2005), Heymach et al. (2007), Kiura et al. (2008), Wells et al. (2012), Heath et al. (2013), Kim et al. (2012), Saglio et al. (2010), Kovacs et al. (2006), Cooper et al. (2014), Pro (2015), Goss et al. (2016)
Monomorphic ventricular tachycardia	Alkylating agents	Ando et al. (2000), Quezado et al. (1993)
	Platinums	Tomkowski and Filipecki (1997), Tomkowski et al. (2004), Richards et al. (2006), Bischiniotis et al. (2005)
	Anthracyclines	Schimmel et al. (2004), Rudzinski et al. (2007), Lipshultz et al. (2013), Steinberg et al. (1987)
	Antimetabolites	Kosmas et al. (2008), Fidan et al. (2011), Hrovatin et al. (2006), Yilmaz et al. (2007), Khan et al. (2012), Keefe et al. (1993), Zwitter et al. (2005), Sauer-Heilborn et al. (1999)
	Antimicrotubule agents	Rowinsky et al. (1991b), Rowinsky et al. (1991a)
	Arsenic trioxide	Yamazaki et al. (2006), Ohnishi et al. (2002), Ohnishi et al. (2000), Shigeno et al. (2005), Unnikrishnan et al. (2004), Beer et al. (2006)
	Cytokines	Ravaud et al. (2002), Sonnenblick and Rosin (1991), Margolin et al. (1989), White et al. (1994), Oleksowicz et al. (2000)
	Histone deacetylase inhibitors	Piekarz et al. (2009), Shah et al. (2006)
	Monoclonal antibodies	Arai et al. (2005), Ferguson et al. (2006), Lenihan et al. (2004), Gibson et al. (2016)
	mTOR inhibitors	Pulzer et al. (2013)
	Nonreceptor TK inhibitors	Wallace et al. (2016)
	Thalidomide and analogs	Ballanti et al. (2007)
	Topoisomerase inhibitors	Tomonaga et al. (2008)
Polymorphic VT, ventricular fibrillation	Anthracyclines	Wortman et al. (1979), Couch et al. (1981), Arbel et al. (2007)
	Antimetabolites	Shah et al. (2012), Lai et al. (2015), Kido et al. (2016), De Gennaro et al. (2016), Ng et al. (2005), Stewart et al. (2010), Fradley et al. (2013), Ray et al. (2016)
	Arsenic trioxide	Unnikrishnan et al. (2001), Naito et al. (2006), Westervelt et al. (2001)
	Cytokines	Budd et al. (1984)
	Histone deacetylase inhibitors	Lynch et al. (2012)
	Monoclonal antibodies	Poterucha et al. (2010)
	mTOR inhibitors	Hodak et al. (1998), Johnson et al. (1992)
	Topoisomerase inhibitors	Von Hoff et al. (1980), Foldes et al. (1982), McLaughlin et al. (1983), Riela et al. (1981), Legha et al. (1979), Weiss et al. (1986)
	Tyrosine kinase inhibitors	Loffi et al. (2015)

  - AV block was documented in 4 of 39 breast cancer patients receiving cyclophosphamide, but they also received anthracyclines (Ando et al., 2000).
  - SVT is limited to rare case reports.
- Melphalan
  - Melphalan is given prior to autologous peripheral blood stem cell transplant for non-Hodgkin's lymphoma and multiple myeloma.
  - Atrial fibrillation occurs in 8%–22.5% of patients receiving melphalan.
  - One study reported SVT in 3% of patients receiving melphalan.
- Cisplatin and carboplatin
  - Cisplatin is associated with transient atrial fibrillation when it is administered into the intrapericardial or intrapleural space for metastatic cancer with malignant effusion with rates ranging from 12% to 32%.

Table 2 Definitions of arrhythmia types

Cardiac origin	*Arrhythmia type*	*Definition*
Atria	SA node dysfunction Sinus bradycardia	A slow heart rhythm originating from the SA node with heart rate <60 bpm
	AV node dysfunction AV block	An aberration in electric conduction between the atria and ventricles that often presents with a slow heart rhythm. The dysfunction can localize at various points between the SA node and ventricles with differing clinical significance
	Atrial fibrillation	Disorganized electric activity throughout the atria that disrupts SA to AV node conduction and presents with irregularity of both heart rate and rhythm
	Atrial flutter	A closed-loop electric circuit within an atrium that causes reentrant, often rapid, conduction through the AV node producing an arrhythmia with regular rhythm
	SVT	An arrhythmia with heart rate >100 bpm and not caused by atrial fibrillation or flutter. Etiologies include ectopic atrial focus and reentrant conduction loops between atria and ventricles
Ventricles	QT prolongation	A manifestation of delayed electric repolarization following depolarization of the cardiac conduction system that is measured from the ECG
	Monomorphic VT	At least three consecutive beats originating from a ventricular focus with heart rate >120 bpm. Monomorphic VT is termed "nonsustained" if the rhythm lasts less than 30 s and "sustained" if it lasts ≥30 s
	Polymorphic VT	A rapid heart rhythm (heart rate >100 bpm) originating from the ventricles with continuously varying QRS interval and morphology seen on electrocardiography. Polymorphic VT carries a risk of degeneration to VF. In patients with congenital long QT syndrome, this arrhythmia is referred to as torsades de pointes
	VF	Disorganized electric activity throughout the ventricles that prevents coordinated ventricular contraction. The arrhythmia causes cardiac arrest and can be fatal if not rapidly reversed

SA, sinoatrial; *AV*, atrioventricular; *bpm*, beats per minute.

- When delivered into intrapericardial or intrapleural space, nonsustained monomorphic VT occurs in 8% of patients.
- SVT has been documented when used alongside etoposide, and atrial fibrillation has been documented when used alongside a cyclin-dependent kinase inhibitor, but these were single occurrences.
- Bradycardia has been reported in a single case report.

Mechanism of action Alkylating agents prevent cell division by cross-linking DNA strands, generating abnormal base pairing, and inducing DNA strand breaks. They are known to cause acute perimyocarditis and heart failure syndromes, but arrhythmias can also result (Guglin et al., 2009). Arrhythmias are thought to arise from direct cardiotoxicity to myocytes and ischemic damage from coronary vasospasms and intracapillary microthrombi (Tamargo et al., 2015; Kupari et al., 1990).

Anthracyclines

Arrhythmias

- QT prolongation occurs in 11.5%–14.2% of patients with an average prolongation of 14–25 ms.
- Eleven cases of polymorphic VT have been reported, and sudden cardiac death has resulted.
- In one study, 15.2% of patients receiving doxorubicin developed SVT.
- In one study, 12.1% of patients receiving doxorubicin developed nonsustained monomorphic VT.
- In one study, 10.3% of patients receiving doxorubicin developed paroxysmal atrial fibrillation.
- In one study, 3.4% of patients receiving doxorubicin developed bradycardia.
- There is a case report of complete heart block requiring a pacemaker following doxorubicin therapy.

Mechanism of action Anthracyclines inhibit DNA and RNA synthesis by intercalating between base pairs on nucleic acid chains and via topoisomerase binding, thereby preventing cell growth and division (Geisberg et al., 2012). In vitro doxorubicin administration causes arrhythmias in neonatal rat cardiomyocyte cultures (Lampidis et al., 1980), a phenomenon that is blocked by esmolol pretreatment, suggesting that anthracyclines impact beta-adrenoceptor-mediated changes in cardiomyocyte calcium gradients (Gorelik et al., 2003). Further, doxorubicin increases peak current through L-type calcium channels in rat cardiomyocytes (Keung et al., 1991) and inhibits sarcoplasmic reticulum function in rabbit cardiomyocytes (Earm et al., 1994). Action potential prolongation following doxorubicin administration has been demonstrated in vitro (Binah et al., 1983) and may be mediated by sarcoplasmic reticulum calcium leak and inhibition of rectifier potassium currents causing a delayed rise but increased peak intracellular calcium concentrations (Wang and Korth, 1995). Sarcoplasmic calcium-ATPase function is potently inhibited by doxorubicinol, a doxorubicin metabolite, in canine cardiac cells (Boucek et al., 1987).

Antimetabolites

Arrhythmias

- 5-Fluorouracil
  - QT prolongation can occur, but malignant ventricular arrhythmias are rare and often occur in the setting of coronary ischemia or coronary vasospasm rather than directly mediated through the chemotherapeutic agent.
  - SVT occurred in 8.8% of patients in one study.
  - Sinus bradycardia occurs in 2.9%–12%.
  - Nonsustained monomorphic VT occurs in 3.7%–7.4% of patients.
  - Sustained monomorphic VT has been documented in a case report.
  - Atrial fibrillation has been documented in case reports, and incidence may approximate 2.9%.
  - AV block is limited to rare case reports.
- Capecitabine and gemcitabine
  - There was a case of QTc prolongation from 413 to 492 ms followed by polymorphic VT.
  - There was a case of VF felt to be secondary to coronary vasospasm.
  - Atrial fibrillation/flutter was documented in 1.1% of patients treated with capecitabine and in occasional case reports following gemcitabine.
- Fludarabine
  - Atrial fibrillation occurred in 6.1%, and atrial flutter occurred in 2.2% of patients who received fludarabine in preparation for hematopoietic stem cell transplantation.
- Methotrexate
  - There have been isolated case reports of atrial fibrillation, bradycardia, and nonsustained monomorphic VT.

Mechanism of action Antimetabolites are associated with coronary vasospasm mediated through either endothelial nitric oxide synthase-associated direct toxic effects on vascular endothelium or endothelium-independent protein kinase C-mediated vasoconstrictive pathway (Alter et al., 2006; Porta et al., 1998). Other proposed mechanisms include direct endothelial damage causing microvascular thrombus formation and interruption of myocardial cellular metabolism (Becker et al., 1999). The myocardial supply–demand mismatch theory is consistent with ECG findings of ST segment deviation or inversion, pathological Q waves, and decrease in QRS amplitude (de Forni et al., 1992; Tamargo et al., 2015).

Antimicrotubule agents

Arrhythmias

- Taxanes are associated with asymptomatic bradycardia in 2.9%–30%.
- Nonsustained monomorphic VT has been reported with rates as high as 9.1%.
- Sustained monomorphic VT, SVT, AV block, and atrial fibrillation are limited to rare case reports.
- QT prolongation of 2–9 ms is associated with eribulin.

Mechanism of action Antimicrotubule agents prevent cell division by directly interacting with tubulin to stabilize microtubules. They produce cardiotoxicity by reducing coronary flow via vasoconstriction, likely causing the observed conduction abnormalities (Alloatti et al., 1998). They also induce histamine release, which causes bradycardia and AV conduction delays (Rowinsky et al., 1993; Tamargo et al., 2015).

Arsenic trioxide

Arrhythmias

- QT prolongation occurs in 38%–100% and often exceeds a 60 ms increase.
- Nonsustained monomorphic VT occurs in 17%–50% of patients.
- There are at least five reported cases of polymorphic VT.

Mechanism of action Arsenic trioxide increases cardiac calcium currents and reduces trafficking of the human ether-a-go-go-related gene (hERG)-mediated rapid (I_{kr}) and slow (I_{ks}) delayed rectifier potassium channels from the endoplasmic reticulum to the cell membrane (Ficker et al., 2004; Chiang et al., 2002; Drolet et al., 2004; Cubeddu, 2009).

Cytokines

Arrhythmias

- Interferons
  - Eight percent of patients receiving interferon alpha developed SVT, and 12% developed atrial fibrillation.
  - Nonsustained monomorphic VT occurred in 35% of patients receiving interferon gamma.
  - Sustained monomorphic VT and polymorphic VT are limited to case reports.
- Interleukins
  - 9.9%–21.5% of patients receiving IL-2 developed arrhythmia, almost exclusively SVT.
  - Atrial fibrillation and ventricular arrhythmias are limited to rare case reports.

Histone deacetylase inhibitors

Arrhythmias

- QTc prolongation of an average of 14.4 ms is reported.
- Ventricular arrhythmias, atrial fibrillation, and SVT are limited to rare case reports.

Mechanism of action Histone deacetylases modulate histone acetylation, which regulates DNA compaction and ultimately gene expression. Histone deacetylase inhibitors interfere with this process, thereby inducing cell cycle arrest, redifferentiation, and apoptosis (Cuni et al., 2017). The mechanism by which they cause arrhythmias is unclear.

Monoclonal antibodies

Arrhythmias

- Rituximab
  - 2.3% of patients developed bradycardia, and 1.5% developed atrial fibrillation.
  - Nonsustained monomorphic VT is limited to rare case reports.
  - Polymorphic VT occurred in one patient.
- Trastuzumab
  - Sinus bradycardia is reported in 15% of patients, and syncope has resulted.
- Alemtuzumab and nivolumab
  - Atrial fibrillation, sustained monomorphic VT, and AV block are limited to rare case reports.

mTOR inhibitors

Arrhythmias

- There is a case report of bradycardia in a liver transplant patient.
- In adults, SVT is limited to one patient receiving tacrolimus. In the pediatric population, 22.7% of patients receiving tacrolimus had SVT, but these patients also received either azathioprine or mycophenolate mofetil.
- QT prolongation has been demonstrated in multiple animal models, and there are case reports from transplant patients using mTOR inhibitors for immunosuppression.
- Sustained monomorphic VT and polymorphic VT are limited to rare case reports.

Mechanism of action Tacrolimus is a calcineurin inhibition, while sirolimus blocks IL-2 receptor-dependent signal transduction. Both bind FK-binding protein 12 (FKBP12). FKBP12 binds ryanodine receptors in the sarcoplasmic membrane, and this interaction stabilizes calcium currents in cardiomyocytes. Gene knockout or mutation of FKBP12.6 or the ryanodine receptor in mouse models exhibits diastolic sarcoplasmic calcium leaking, atrial fibrillation, and catecholamine polymorphic VT (Li et al., 2012; Sood et al., 2008; Jiang et al., 2010). Overexpression or stabilization of FKP12.6 protects against catecholamine polymorphic VT in mice (Vinet et al., 2012; Gellen et al., 2008; Lehnart et al., 2006). Impaired calcium trafficking may cause action potential prolongation and early after depolarizations (Fauconnier et al., 2005). The arrhythmogenic effects may also be caused by dysregulation of cardiomyocyte sodium currents (Maruyama et al., 2011).

Nonreceptor tyrosine kinase inhibitors

Arrhythmias

- Ibrutinib
  - Atrial fibrillation ranges in incidence from 5.1% to 10.7%. Often, the atrial fibrillation persists despite medical treatment.
  - Ibrutinib also causes platelet dysfunction, which complicates thromboembolic prophylaxis of atrial fibrillation (Yun et al., 2017; Vrontikis et al., 2016).
- Ribociclib
  - QTc prolongation occurred in 9% of patients at 600 mg/day dosing and 33% of patients at >600 mg/day dosing.

Mechanism of action The increased risk of atrial fibrillation in ibrutinib use is thought to be due to decreased phosphoinositide 3-kinase (PI3K)-Akt activity as a result of Bruton tyrosine kinase and tec protein tyrosine kinase inhibition (McMullen et al., 2014). Ribociclib is a cyclin-dependent kinase 4/6 inhibitor (Infante et al., 2016).

Proteasome inhibitors

Arrhythmias

- Bradycardia was reported in 2.9% of patients.
- Atrial fibrillation was reported in 2.9% of patients.
- AV block, atrial fibrillation, and SVT are limited to rare case reports.

Mechanism of action Bortezomib is a 26S proteasome inhibitor used to treat multiple myeloma. By upregulating the adrenergic GRK2, bortezomib may be protective against malignant ventricular tachyarrhythmias in the setting of myocardial ischemia (Yu

et al., 2005; Huang et al., 2008). However, in the absence of myocardial ischemia, this same mechanism may predispose to bradyarrhythmias and AV conduction abnormalities (Enrico et al., 2007).

Thalidomide and analogs

Arrhythmias

- Bradycardia occurs in 12%–53% of patients with several requiring pacemaker implantation.
- QT prolongation and AV block are limited to rare case reports.

Mechanism of action Sinus bradycardia is likely mediated by inhibition of dorsal motor neurons by reduced TNF-α levels (Palumbo et al., 2008; Hinterseer et al., 2006). Coronary artery spasm is a less frequently proposed mechanism (Ali et al., 2013).

Topoisomerase inhibitors

Arrhythmias

- Topoisomerase I inhibitors
  - Atrial fibrillation was reported in 9.5% of patients receiving irinotecan, but they also received carboplatin and paclitaxel.
  - Sustained monomorphic VT and bradycardia are limited to rare case reports.
- Topoisomerase II inhibitors
  - QT prolongation of an average of 64 ms has been reported but resolved within 24 h of infusion.
  - There are 31 cases of ventricular arrhythmia-associated cardiopulmonary arrest occurring within four hours of amsacrine (AMSA) infusion, 14 of which being fatal.
  - The majority of cases of VF occurred in the setting of profound hypokalemia.

Mechanism of action Irinotecan may cause bradycardia by transient noncompetitive inhibition of acetylcholinesterase (Dodds and Rivory, 1999; Miya et al., 1998). Though amsacrine may not directly reduce potassium levels, it does cause acute hypomagnesemia (Seymour, 1993) and blocks cardiac hERG-mediated rectifier potassium currents (Thomas et al., 2004).

Tyrosine kinase inhibitors

Arrhythmias

- QT prolongation has been associated with cabozantinib, ceritinib, crizotinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, osimertinib, sorafenib, sunitinib, vandetanib, and vemurafenib.
- The most pronounced QT prolongation has been associated with vandetanib (36.4 ms), nilotinib (25.8 ms), lapatinib (23.4 ms), and sunitinib (22.4 ms).
- VF has been associated in a case report with vandetanib.
- Bradycardia is common following crizotinib with incidence reaching 42%–69%. The average decrease in heart rate is approximately 25 bpm, and patients can have severe bradycardia with heart rates less than 45 bpm.

Mechanism of action Tyrosine kinase inhibitors reduce hERG phosphorylation and in turn inhibit rectifier potassium currents (Zhang et al., 2003, 2008, 2012; Barros et al., 1998; Davis et al., 2001). However, downregulation of PI3K also cause decreased L-type calcium current, decreased peak sodium ion current, and increased persistent sodium current (Lu et al., 2012).

QT Monitoring

The QT interval is used as a surrogate for risk of polymorphic VT, and drugs that prolong the QT interval are felt to increase risk. There is not a consensus definition of QTc prolongation, but the most common definition is QTc >450 ms in men, >470 ms in women, or an increase of 60 ms from baseline (Yusuf et al., 2008). Polymorphic VT almost always occur in patients with a QTc of ≥500 ms, but the true association between QTc duration and risk of malignant arrhythmia is unknown (Piekarz et al., 2006; Bednar et al., 2002) as there are no trials assessing the correlation between QTc interval and patient outcomes (Strevel et al., 2007). Further, there are medications associated with polymorphic VT that do not significantly lengthen the QT interval (Morganroth, 1993). Drug-induced QT prolongation is almost always caused by blockade of hERG association rectifier potassium outflow channels, but not all medications that block hERG potassium channels cause significant QT prolongation (Sanguinetti and Mitcheson, 2005).

Prior to testing for QTc prolongation in clinical trials, several preclinical experiments are performed using animal models and in vitro assays using nonhuman cells transfected with hERG (Sharma et al., 2017). The latest innovations in development involve human-induced pluripotent stem cells. Patient-specific hiPSCs can be induced to differentiate into cardiomyocytes, endothelial cells, and cardiac fibroblasts (**Fig. 1**).

These cells can be used for in vitro studies on chemotherapy-associated cardiomyopathy and high-throughput cell contractility assessments. The results from these analyses can then be used to develop cardiac safety indexes for chemotherapeutic agents (Sharma et al., 2017).

The 2005 International Conference on Harmonization E14 guideline advised clinical QT prolongation evaluation of all nonantiarrhythmic drugs seeking approval at up to maximally tolerated dosing (Food and Drug Administration HHS, 2005; Shah, 2005). The participants for these trials were intended to be healthy volunteers with normal baseline ECG, but given the

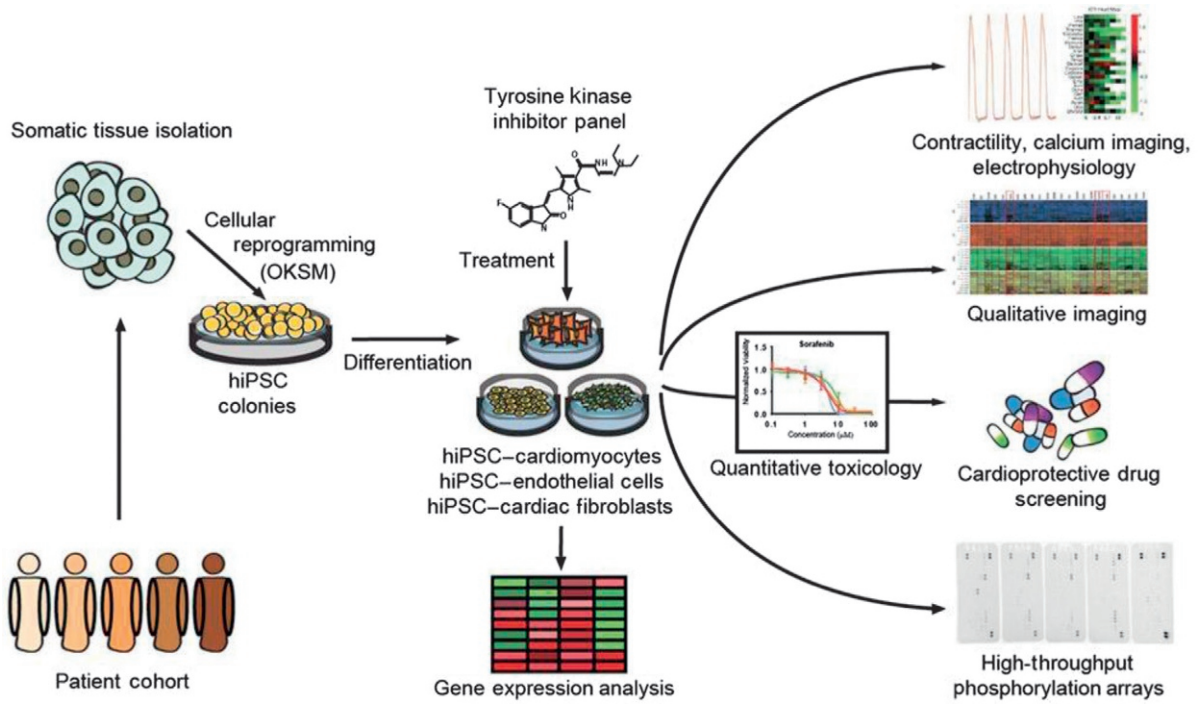

Fig. 1 Using hiPSC-CMs made from somatic tissue samples. hiPSCs were differentiated into hiPSC-CMs, hiPSC-ECs, and hiPSC-CFs. Purified cardiomyocytes were treated with TKIs and examined for alterations in cell viability, contractility, cellular signaling, and gene expression. Reproduced from Sharma, A., Burridge, P. W., Mckeithan, W. L., Serrano, R., Shukla, P., Sayed, N., Churko, J. M., Kitani, T., Wu, H., Holmstrom, A., Matsa, E., Zhang, Y., Kumar, A., Fan, A. C., Del Alamo, J. C., Wu, S. M., Moslehi, J. J., Mercola, M. and Wu, J. C. (2017). High-throughput screening of tyrosine kinase inhibitor cardiotoxicity with human induced pluripotent stem cells. *Science Translational Medicine* **9**, 377.

adverse effects of chemotherapy, it would not be ethical to carry out such studies on cancer medications in healthy patients. Instead, clinical trials are used to evaluate for drug-induced QTc prolongation in chemotherapies (Shah et al., 2013; Rock et al., 2009; Morganroth et al., 2010; Shah and Morganroth, 2012). Individuals with cancer have higher incidence of baseline QT prolongation (Varterasian et al., 2003, 2004; Cuni et al., 2017; Naing et al., 2012), receive additional QT-prolonging medications (Yusuf et al., 2008; Piekarz et al., 2006), and frequently have electrolyte abnormalities. Treatment regimens often include multiple chemotherapeutic agents at a time, making it difficult to determine which agent is responsible for a given outcome or effect (Tamargo et al., 2015). Thus, evaluating the propensity for chemotherapeutic agents to prolong the QTc interval is less straightforward than evaluating in other clinical settings.

The clinical trials used to assess QT prolongation in chemotherapies likely underestimate rare adverse events due to limited sample size and short follow-up times. Postmarketing safety reporting can improve awareness of rare adverse events, but there is no ICD-10 code for QT interval prolongation (Schwartz and Woosley, 2016). Further, only symptomatic cases are reported, and such reports do not include total number of patients receiving medications, so frequency estimations are impossible (Cuni et al., 2017; Tuccori et al., 2015).

Prevention and Management of QT Prolongation

There are multiple mechanisms in place to counteract repolarization abnormalities; thus, QT prolongation is only thought to occur when compensatory mechanisms are impaired. Prevention of QT prolongation centers around minimization of factors that deplete this repolarization reserve (Cuni et al., 2017). Careful family history can suggest congenital long QT syndromes and cardiac risk factors. Laboratory evaluation identifies electrolyte abnormalities that should be corrected, especially hypokalemia and hypomagnesemia. Renal and hepatic insufficiencies are important considerations when considering the pharmacokinetics of a given chemotherapy. Medication reconciliation must be performed to identify concomitate medications that predispose to electrolyte disturbances (such as diuretics) or cause QT prolongation (antiarrhythmics, antibiotics, antifungals, antidepressants, antipsychotics, antiemetics, and antihistamines) (Cuni et al., 2017). The QT interval should be monitored while administering medications with QT prolonging potential, and these medications should be infused slowly. Uvelin et al. and Tisdale have developed algorithm for management of patients with prolonged QT, which is adapted and presented in **Table 3** (Uvelin et al., 2017; Tisdale, 2016). When polymorphic VT does occur, the algorithm in **Table 4** should be implemented promptly to terminate the arrhythmias and prevent degeneration into VF.

Table 3 Prevention of QT prolongation and ventricular arrhythmias

1. Assess the risk for arrhythmia occurrence on admission
   - Measure the QT interval on ECG using Bazett's formula or QT nomogram
   - Corrected QTc >500 ms carries significant risk of polymorphic VT
   - If there is a prolonged QTc, what is the likely cause of this? Look at
     - Patient characteristics
     - Usage of medication prolonging the QT interval
2. Assess the risk for arrhythmia occurrence daily in patients with known risk factors
   - Compare QTc on admission to subsequent days
   - Compare QTc before and after administration of drugs that potentially prolong QT interval
3. Avoid coadministration of two or more medications that prolong QTc
4. Avoid the use of QTc interval-prolonging drugs in patients with QTc >450 ms
5. Avoid the use of QTc-prolonging drugs in patients with heart failure and left ventricular ejection fraction <20%
6. Adjust doses of renally eliminated QTc-prolonging drugs in patients with acute kidney injury or chronic kidney disease
7. Avoid the use of QTc-prolonging drugs in patients with congenital long QT syndromes
8. Reduce dose or discontinue QTc-prolonging drugs if the QTc interval increases to ≥60 ms or to ≥500 ms. Closely monitor QT interval with serial ECGs until the QTc normalizes
9. Beware of the presence of more than one risk factor for polymorphic VT at the same time—for example, electrolyte imbalances and administration of QT-prolonging medication
10. Slow infusion and continuous ECG monitoring mandatory when administering QT-prolonging medication intravenously
11. Correction of electrolyte disturbances
    - Give daily potassium and magnesium supplementation in patients on diuretics
    - Maintain potassium levels at >4.0 mmol/L
12. Patients with known congenital long QT syndrome—same guidelines. These patients should be on a regular β1 adrenergic receptor antagonist

Source: Uvelin A, Pekakovic J, Mijatovic V. J Anesth. 2017 Feb 22; Tisdale J. Can Pharm J (Ott). 2016 May; 149(3): 139–52.

Table 4 Therapeutic options for polymorphic VT

1. Identify and stop the offending agent immediately
2. Call for help
3. If hemodynamically unstable, defibrillate patient
4. If hemodynamically stable, administer magnesium sulfate
   - 1–2 g intravenously administered over 15 min with continuous ECG monitoring
   - Can repeat dosing twice
   - Maintain magnesium levels >1 mmol/L
5. Consider lidocaine administration
   - Bolus 1–1.5 mg/kg body weight
   - Follow with 2 mg/h continuous intravenous infusion
6. Increase heart rate if bradycardia is present
   - Isoproterenol 2–10 mcg/min continuous intravenous infusion or temporary overdrive electric pacing
   - In congenital long QT interval syndrome, use temporary overdrive electric pacing
7. If no bradycardia and persistent polymorphic VT following magnesium, defibrillate patient

Source: in A, Pekakovic J, Mijatovic V. J Anesth. 2017 Feb 22; Tisdale J. Can Pharm J (Ott). 2016 May; 149(3): 139-52.

Radiation-Induced Arrhythmia

Risk of Arrhythmia

Radiation therapy has become more precise over time allowing for lower doses and reduced risk for long-term adverse effects (Specht et al., 2014). Despite this, arrhythmias are still a known late adverse effect of mediastinal radiation therapy. In 703 patients with Hodgkin's lymphoma and a mean follow-up of 9 years, 16% had an arrhythmia. Most of these patients, however, also received anthracyclines, vinca alkaloids, or both (Maraldo et al., 2015). In cancers with worse prognosis, higher radiation dose can be used as risk of late adverse effects is less of a concern. In 112 patients receiving radiation therapy for stage III non-small-cell lung cancer, atrial fibrillation/flutter occurred in 8.9% patients. This population received a high heart dose (mean 20 Gy) deemed acceptable due

to expected prognosis of less than 2 years (Wang et al., 2017). There are multiple studies evaluating the association between heart radiation dose and cardiovascular adverse effects, but these center more on common sequelae such as myocardial infarction, heart failure, pericarditis, or valvular heart disease than on arrhythmias (Hancock et al., 1993; Hooning et al., 2007; van Nimwegen et al., 2015).

Radiation and CIEDs

Radiation can cause CIED malfunction via direct irradiation to the device (Hurkmans et al., 2005a,b; Mouton et al., 2002; Rodriguez et al., 1991; Brambatti et al., 2015), indirectly due to scattered radiation of neutrons produced by the linear accelerator (Zecchin et al., 2016), or electromagnetic interference from the linear accelerator and betatrons (Brambatti et al., 2015; Makkar et al., 2012; Solan et al., 2004; Marbach et al., 1978; Yerra and Reddy, 2007). The degree of device malfunction varies from temporary changing to interference or safety mode during the radiation therapy session, persistent change to interference or safety mode requiring device reprogramming, to total device failure with cessation of pulse generation (Souliman and Christie, 1994).

Radiation therapy causes device malfunction both in vitro (Zaremba et al., 2014) and in porcine model (Zaremba et al., 2013). In a study of 560 patients with CIED undergoing radiation, Zaremba et al. reported the annual rate of radiation treatment in this population increasing by 199% between 2003 and 2012. In device evaluations after radiation, pacemaker malfunction was 2.5%, while ICD malfunction was 6.8% with the majority of malfunctions being electric resets. There were no life-threatening events or device removals (Zaremba et al., 2015). Brambatti et al. evaluated 261 patients with CIEDs receiving radiation and reported 14.6% requiring device reprogramming and 3.4% undergoing device repositioning to the contralateral side. Three patients experienced inappropriate ventricular pacing, and one patient had a power-on reset of the device (Brambatti et al., 2015). In 69 patients with CIEDs receiving radiation, Makkar et al. reported two patients with resets of ICD with loss of historic diagnostic data, but there were no cases of device malfunction or premature battery depletion over the 6-month follow-up (Makkar et al., 2012). Predictors of CIED malfunction include high beam energy ($\geq$15 MV), neutron-producing radiation, and abdominal/pelvic radiation, while CIED radiation dose does not predict device malfunction (Grant et al., 2015; Zaremba et al., 2015). Device malfunction risks during radiotherapy are summarized in **Table 5** (Viganego et al., 2016).

Due to perceived association of CIED radiation dose and increased risk for device malfunction, there are institution-based standard guidelines currently used to determine when a device should be relocated (Brambatti et al., 2015; Makkar et al., 2012). International guidelines express similar concern about this potential link (in particular when receiving 10 Gy radiation to the CIED or when there is high pacing dependence) and advocate for consideration of device relocation (Hurkmans et al., 2012; Salerno et al., 2016). However, there are trials reporting no association between incident CIED radiation dose and device malfunction and advocate against device relocation (Grant et al., 2015). Further, various guidelines recommended safe radiation dose limits that differ significantly and thus are unreliable (Hudson et al., 2010). With limited available data and the lack of consensus between different guidelines, the decision on whether to relocate a CIED should be multidisciplinary – including cardiologists and oncologists combined input – and on a patient-specific basis (Carrillo, 2012; Zaremba et al., 2016). Manufacturer-based recommendations on RT in patients with a CIED are summarized in **Table 6** (Viganego et al., 2016), and a patient-specific recommendation based on risk stratification is summarized in **Table 7** (Viganego et al., 2016).

Table 5 Cardiac implantable electronic device complications due to RT

Type of CIED failure	*Frequency*
Permanent damage	Rare
Early battery depletion	Rare
Temporary loss of sensing	Uncommon
Transient oversensing or EMI	Uncommon
Temporary pacing inhibition	Uncommon
Temporary loss of capture	Rare
Temporary increase in pacing thresholds	Uncommon
Very rapid pacing	Very rare
Temporary increased sensor rate or change in pacing rate	Common
Power-on reset or revision to safety mode	Common, especially if neutron-producing[a]
Increase battery charge time, decreased shock energy	Rare, described at radiation dose above recommended tolerance
Complete CIED failure (the loss of output/sensing)	Rare, described at radiation dose above recommended tolerance

RT, radiotherapy; *EMI*, electromagnetic interference; *CIED*, cardiac implantable electronic device.

[a]High-energy beam (>10 MV photons).

Source: Viganego F, Singh R, Fradley MG. Curr Cardiol Rep. 2016 Jun; 18(6): 52.

Table 6 Primary CIED manufacturer recommendations on device management during RT

Manufacturer				
Recommendation for RT		*Boston Scientific (Scientific, 2012)*	*Medtronic (Medtronic, 2013)*	*St. Jude Medical (Medical, 2013)*
Estimate radiation dose		Yes	Yes	Yes
Cumulative radiation dose limit	PPM	None	All models, 5 Gy	None[a]
	ICD	None	1 Gy; recent models, 3–5 Gy	None[a]
	CRT-D	None	All CRT-D models, 5 Gy	None[a]
Shielding		Yes	No protection against neutrons	Not mentioned
Position CIED outside RT beam		Yes	Yes	Yes
Level of monitoring		Patient-specific	Evaluate marker channel during initial RT session	ECG monitoring advised
Programming considerations	PPM	Use device-based telemetry. Consider asynchronous pacing if PPM-dependent	Consider asynchronous pacing if PPM-dependent	Consider asynchronous pacing if PPM-dependent. Turn sensor to OFF or PASSIVE for rate adaptive PPMs
	ICD/CRT-D	Deactivate tachy therapy or place magnet on CIED	Deactivate tachy therapy or place magnet on CIED	Deactivate tachy therapy or place magnet on CIED
Assessment of CIED after RT is completed		Yes, detailed CIED evaluation	Yes, check for device reset[b]	Yes, detailed CIED evaluation
Remote monitoring		Yes, LATITUDE® when available	Not mentioned	Not mentioned

CIED, cardiac implantable electronic device; *RT*, radiotherapy; *PPM*, permanent pacemaker; *ICD*, implantable cardiac defibrillator; *CRT-D*, cardiac resynchronization therapy defibrillator; *Gy*, gray; *ECG*, electrocardiography.

[a]Manufacturer reports that current devices have been tested up to 30 Gy without adverse effects, but testing was not performed with high-dose sources such as linear accelerators.

[b]For ICD or CRT-D patients, if alert tone is heard after placing a magnet over CIED, electric reset has occurred, and device should be checked. For PPM patients, if pacing rate is 65 bpm after placing a magnet over CIED, electric reset has occurred.

Source: Viganego F, Singh R, Fradley MG. Curr Cardiol Rep. 2016 Jun; 18(6): 52.

Table 7 Recommendations on CIED management in patients undergoing RT

Recommendations based on risk level of patient					
	General recommendations	*Low-risk patient*	*Medium-risk patient*	*High-risk patients*	*References*
General	– Avoid CIED in direct RT beam – RT dose to PPM $\leq$2 Gy – RT dose to ICD $\leq$0.5 Gy – Inform pt of risks – Review manufacturer records – Avoid high-energy (>10 MV) beam – Avoid neutron-producing beam				Grant et al. (2015), Marbach et al. (1994), Frizzell (2009), Makkar et al. (2012)
Definition		CIED dose <2 Gy, not PPM-dependent[a]	CIED dose 2–10 Gy or <2 Gy and PPM-dependent[a]	CIED dose >10 Gy	
Before RT initiation	– Consult cardiology, interrogate CIED, evaluate PPM dependency – Turn on audible alerts – Estimate RT dose to CIED			Consider CIED relocation, adapt dose or alternative to RT	Hurkmans et al. (2012), Makkar et al. (2012)
During RT			– ACLS/BLS trained staff and	ECG monitoring during each session	Crossley et al. (2011), Hurkmans et al. (2012),

Table 7 (Continued)

Recommendations based on risk level of patient

	General recommendations	*Low-risk patient*	*Medium-risk patient*	*High-risk patients*	*References*
	– Train staff on signs/ management of CIED malfunctions – Audiovisual monitoring of all pts – Continuous ECG during first RT – Consult cardiology if CIED malfunction – Deactivate ICD antitachy therapy		equipment present – Pacing equipment or cardiology nearby – Pulse-check method if PPM-dependent	with trained personnel	Brambatti et al. (2015), Frizzell (2009)
After RT	Remote CIED monitoring for all pts	CIED check at 1 week and 1, 3, and 6 months of final RT fraction	Weekly CIED interrogation	CIED check within 24 h of each RT fraction	Hurkmans et al. (2012), Brambatti et al. (2015)

CIED, cardiac implantable electronic device; *RT*, radiotherapy; *Gy*, gray; *pt*, patient; *ACLS*, advanced cardiac life support; *BLS*, basic life support.

[a]The lack of intrinsic rhythm with pacemaker inhibition <40 bpm.

Source: Viganego, F., Singh, R., Fradley, M. G. 2016. *Curr. Cardiol. Rep.* 18(6), 52.

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Arrhythmogenic Right Ventricular Cardiomyopathy

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Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC), also known as arrhythmogenic right ventricular dysplasia, is a heritable heart-muscle disorder that predominantly affects the right ventricle (Corrado et al., 2017). Macroscopically, there is a scarred appearance with fibrous or fibrofatty replacement of myocardium. These pathologic changes primarily involve the inflow tract, outflow tract, and/or apex of the right ventricular (RV) (Basso et al., 1996). However, more recent data has noted involvement of the posterolateral LV with sparing of the RV apex early in the disease (Protonotarios and Tsatsopoulou, 2004). The RV myocardial scarring initially produces typical regional wall motion abnormalities but later may involve the free wall and become global, producing RV dilation and systolic dysfunction. Tissue replacement can also involve areas of the LV with relative sparing of the septum. In addition, electron microscopy has revealed gap junction abnormalities of desmosomes in the RV myocardium, consistent with mutations in genes encoding desmosomal proteins responsible for ARVC/dysplasia (ARVC/D) in most patients (Crossman et al., 2015).

Clinical perspectives of ARVC/D initially developed from experience with patients who presented with arrhythmias of RV origin and/or sudden death (Hulot et al., 2004). However, additional information regarding the clinical features and course of subjects has developed based on electrocardiographic (EKG) abnormalities and by cascade family screening (Baranchuk et al., 2015; Zhang et al., 2014). Presentation is most common between the ages of 10 and 50 years, with a mean age at diagnosis of approximately 30 years. The disease is virtually never diagnosed in infants or toddlers and is rarely identified before the age of 10 (Philips and Cheng, 2016).

Historical Background

ARVC/D was described by Giovanni Maria Lancisi in 1736 in his book *De Motu Cordis et Aneurysmatibus* (Taylor, 2009). He reported a family with disease recurrence in four generations: the affected members presented with palpitations, heart failure, dilation and aneurysms of the RV, and sudden death. Doctors Marcus, Nava, and Thiene reported the first clinical and pathologic series of

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patients with ARVC/D in the 1980s (Taylor, 2009; Basso et al., 2009). Marcus et al. in 1982 reported the disease in adults, first emphasizing the origin of arrhythmias from the RV and the histopathological substrate consisting of fibrofatty replacement of the RV free wall, accounting for epsilon wave and ventricular arrhythmias of RV origin with left bundle branch block (LBBB) morphology (Basso et al., 2009). Nava et al. first proved familial occurrence with an autosomal dominant pattern of inheritance and variable penetrance in 1987–88. In 1988, Thiene et al. observed an impressive series of sudden deaths in the young (≤35 years), with pathology consisting of ARVC/D, most occurring during effort, and all characterized by inverted T waves in the right precordial leads on EKG and apparently benign ventricular arrhythmias of LBBB morphology (Basso et al., 2009). They accounted for 20% of all sudden deaths in the young and for the first time acknowledged that ARVC/D is another important cause of sudden death in that age group (Basso et al., 2009). Diagnostic imaging was then implemented to visualize the RV, either noninvasively through echocardiogram or invasively through angiography. Signal-averaged EKG proved to be a sensitive tool to detect delay in the electrical impulse transmission in the RV myocardium (Gallo et al., 2016; Bae et al., 2014; Kamath et al., 2011). Improvements in diagnostic procedures led to the proposal of diagnostic criteria, whether major or minor, based upon RV dysfunction or structural alterations at imaging, tissue characterization at biopsy, repolarization or depolarization abnormalities and arrhythmias on the EKG, as well as family history of sudden death (**Table 1**) (Calkins, 2015). ARVC/D was included among cardiomyopathies in the revised World Health Organization (WHO) classification in 1995 (McKenna et al., 1994). The first gene defect was discovered in the recessive variant of the disease from the Naxos island consisting of a cardiocutaneous syndrome of ARVC/D, palmoplantar keratosis, and woolly hair. A deletion was detected in the gene encoding plakoglobin, a cell junction protein (Coonar et al., 1998; McKoy et al., 2000). Thereafter, other genes encoding cell junction proteins were found defective in the dominant, classical form of ARVC/D: desmoplakin, plakophilin-2, desmoglein-2, and desmocollin-2. Other variants of the disease were explained by mutation of the ryanodyne 2 receptor and transforming growth factor β 3 genes. The discovery of these gene mutations allowed preliminary genotype–phenotype correlations to be made. The implantable cardioverter defibrillator (ICD) represented a major advance in therapy (Calkins, 2015). Finally, electroanatomic mapping proved to be a sensitive tool for identifying areas of fibrofatty replacement with low-amplitude electrical activity (Mussigbrodt et al., 2017; Orgeron and Calkins, 2016; Pathak and Garcia, 2017; Yin et al., 2017).

Table 1 Task force criteria for the diagnosis of ARVD published in 2010

I. Global and/or regional dysfunction and structural alterations	Major	Two-dimensional echocardiogram: regional RV akinesia, dyskinesia, or aneurysm AND 1 of the following (end-diastole): PLAX RVOT ≥32 mm (corrected for body size [PLAX/BSA] ≥19 mm/m^2) PSAX RVOT ≥36 mm (corrected for body size [PSAX/BSA] ≥21 mm/m^2) Fractional area change ≤33% MRI: regional RV akinesia or dyskinesia or dyssynchronous RV contraction and 1 of the following: Ratio of RV end-diastolic volume to BSA ≥110 mL/m^2 (male) or ≥100 mL/m^2 (female) RV ejection fraction ≤40% RV angiography: regional RV akinesia, dyskinesia, or aneurysm
	Minor	Two-dimensional echocardiogram: regional RV akinesia or dyskinesia AND 1 of the following (end-diastole): PLAX RVOT ≥29 to <32 mm (corrected for body size [PLAX/BSA] ≥16 to <19 mm/m^2) PSAX RVOT ≥32 to <36 mm (corrected for body size [PSAX/BSA] ≥18 to <21 mm/m^2) Fractional area change >33% to ≤40% MRI: regional RV akinesia or dyskinesia or dyssynchronous RV contraction AND 1 of the following: Ratio of RV end-diastolic volume to BSA ≥100 to <110 mL/m^2 (male) or ≥90 to <100 mL/m^2 (female) RV ejection fraction >40% to ≤45%
II. Tissue characterization of the wall	Major	Residual myocytes <60% by morphometric analysis (or <50% if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy

(*Continued*)

Table 1 (Continued)

	Minor	Residual myocytes 60%–75% by morphometric analysis (or 50%–65% if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy
III. Repolarization abnormalities	Major	Inverted T waves in right precordial leads (V1, V2, and V3) or beyond in individuals >14 years of age (in the absence of complete RBBB QRS≥120 ms)
	Minor	Inverted T waves in leads V1 and V2 in individuals >14 years of age (in the absence of complete RBBB) or in V4, V5, or V6 Inverted T waves in leads V1, V2, V3, and V4 in individuals >14 years of age in the presence of complete RBBB
IV. Depolarization/conduction abnormalities	Major	Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of the T wave) in the right precordial leads (V1 to V3)
	Minor	Late potentials by SAECG in ≥1 of 3 parameters in the absence of a QRS duration of ≥110 ms on the standard ECG: Filtered QRS duration (fQRS) ≥114 ms Duration of terminal QRS <40 μV (low-amplitude signal duration) ≥38 ms Root-mean-square voltage of terminal 40 ms 20 μ Terminal activation duration of QRS ≥55 ms measured from the nadir of the S wave to the end of the QRS, including R′, in V1, V2, or V3, in the absence of complete RBBB
V. Arrhythmias	Major	Nonsustained or sustained ventricular tachycardia of left bundle branch morphology with superior axis (negative or indeterminate QRS in leads II, III, and aVF and positive in lead aVL)
	Minor	Nonsustained or sustained ventricular tachycardia of RVOT configuration, LBBB morphology with inferior axis (positive QRS in leads II, III, and aVF and negative in lead aVL) or of unknown axis >500 ventricular extrasystoles per 24 h (Holter)
VI. Family history	Major	ARVC/D confirmed in a first-degree relative who meets current Task Force criteria ARVC/D confirmed pathologically at autopsy or surgery in a first-degree relative Identification of a pathogenic mutation categorized as associated or probably associated with ARVC/D in the patient under evaluation
	Minor	History of ARVC/D in a first-degree relative in whom it is not possible or practical to determine whether the family member meets current Task Force criteria Premature sudden death (<35 years of age) due to suspected ARVC/D in a first-degree relative ARVC/D confirmed pathologically or by current Task Force Criteria in second-degree relative

Inheritance Patterns

Two patterns of inheritance have been described in ARVC/D: (1) autosomal dominant form, which is most common, and (2) autosomal recessive form (e.g., Naxos disease, Carvajal syndrome) in which ARVC/D is part of a cardiocutaneous syndrome including hyperkeratosis of the palms and soles and woolly hair. Patients with multiple gene mutations tend to present earlier and with more frequent ventricular tachyarrhythmias, heart failure, and LV dysfunction. Clinical features associated with mutations in each of the associated genes are discussed separately (Campuzano et al., 2013; Hershberger et al., 2009; Lodder and Bezzina, 2013).

Clinical Presentation

Symptoms

The majority of patients ultimately diagnosed with ARVC/D are symptomatic on presentation. The principle symptoms associated with ARVC/D are dizziness, palpitations, and syncope. However, as many as 40% of persons diagnosed with ARVC/D are asymptomatic, coming to attention following screening triggered by a definite diagnosis in a family member. In one of the largest reported series of 130 patients at a tertiary referral center, palpitation was the most common symptoms (67%) followed by syncope (32%) and atypical chest pain (27%) (Hulot et al., 2004).

Sudden Cardiac Death

Sudden cardiac death (SCD) occurs in patients with ARVC/D and can be the first presentation of the disease. In a review, from France, of forensic autopsies (mean age 34 years, males and females equally represented), 200 (10.4%) were associated with ARVC/D. Three-quarters of episodes occurred during routine daily activities, 10% during the perioperative period, and only 3.5% while participating in sports (Tabib et al., 2003).

Ventricular Arrhythmias

Arrhythmogenic cardiomyopathy is distinguished from dilated cardiomyopathy by a higher propensity toward ventricular arrhythmia exceeding the degree of ventricular dysfunction. Ventricular arrhythmias can range from frequent ventricular premature beats (PVC) to sustained ventricular tachycardia (VT) (Nava et al., 2000). The most common ventricular arrhythmia is sustained or nonsustained monomorphic VT that originates in the RV producing a LBBB pattern (Pathak and Garcia, 2017). VT may originate from the RV inflow tract, RV apex, or RV outflow tract. When VT arises from the RV outflow tract, it may be difficult to distinguish ARVC/D from idiopathic RV outflow tract tachycardia. The frequency of various ventricular arrhythmias in ARVC/D differs along with the severity of the disease and varies from 80% to 95%. The incidence of PVCs in patients with Naxos disease is as high as 92%, as well as a higher incidence of sustained ventricular arrhythmias and SCD. Although most of these arrhythmias appear to arise from the RV, histopathologic abnormalities (fibrosis and/or adipose tissue) in the LV and His bundle and its branches were commonly observed at autopsy in a series of patients with ARVC/D and SCD (McKenna et al., 1994).

Supraventricular Tachyarrhythmia

Supraventricular tachyarrhythmia (SVT) is present in up to 25% of patients with ARVC/D referred to for the treatment of ventricular arrhythmias; less often, SVT may be the only arrhythmia present. In decreasing order of frequency, supraventricular arrhythmias in these patients include atrial fibrillation, atrial tachycardia, and atrial flutter (Camm et al., 2013; Tonet et al., 1991).

Left-Dominant Arrhythmogenic Cardiomyopathy

The pathologic process in classic ARVC predominantly involves the RV and sometimes extends to the LV. In contrast, patients with left-dominant arrhythmogenic cardiomyopathy (also known as left-sided ARVC or arrhythmogenic left ventricular cardiomyopathy) have pathological changes that predominantly involve the LV (Di Bella et al., 2009; Sen-Chowdhry et al., 2008).

Asymptomatic Presentation

Approximately 40% of patients are asymptomatic when ARVC/D is identified as part of screening of affected family members. ARVC/D is often suspected due to the presence of nonspecific EKG or echocardiographic abnormalities, or ventricular arrhythmias on Holter or exercise testing in the context of a positive family history.

Diagnostics Criteria

An international task force proposed guidelines in the form of a qualitative scoring system with major and minor criteria in 1994 (McKenna et al., 1994). The task force revised the guidelines in 2010 to improve diagnostic sensitivity, mostly for the clinical screening of family members, by providing quantitative criteria for diagnosing RV abnormalities and adding molecular genetic criteria (**Table 1**) (Marcus et al., 2010a). However, the diagnosis remains problematic because of the low specificity of EKG abnormalities, multiple causes of RV arrhythmias, difficulties in the use of imaging to assess RV structure and function, and the sometimes puzzling results of genetic testing.

Electrocardiogram

Abnormalities of EKG are both related to depolarization and repolarization. The pathognomonic abnormality of repolarization is epsilon wave. The repolarization abnormalities are T wave inversion (**Fig. 1** and **Table 1**).

Signal-Averaged Electrocardiogram

Signal-averaged electrocardiogram (SAECG) is an example of high-resolution EKG. The SAECG is a computerized technique for detecting subtle abnormalities in the surface EKG that are not visible to the naked eye. It is calculated by computing the arithmetic mean of multiple EKG complexes. SAECG records ventricular signals of very low magnitude. These are also called ventricular late potentials. The purpose of signal averaging is to improve the signal-to-noise ratio to facilitate the detection of low-amplitude bioelectric potentials. Averaging multiple QRS complexes (or p waves) "reinforces" the consistent ventricular component while diminishing the inconsistent noise components, thus improving the signal-to-noise ratio (**Fig. 2**).

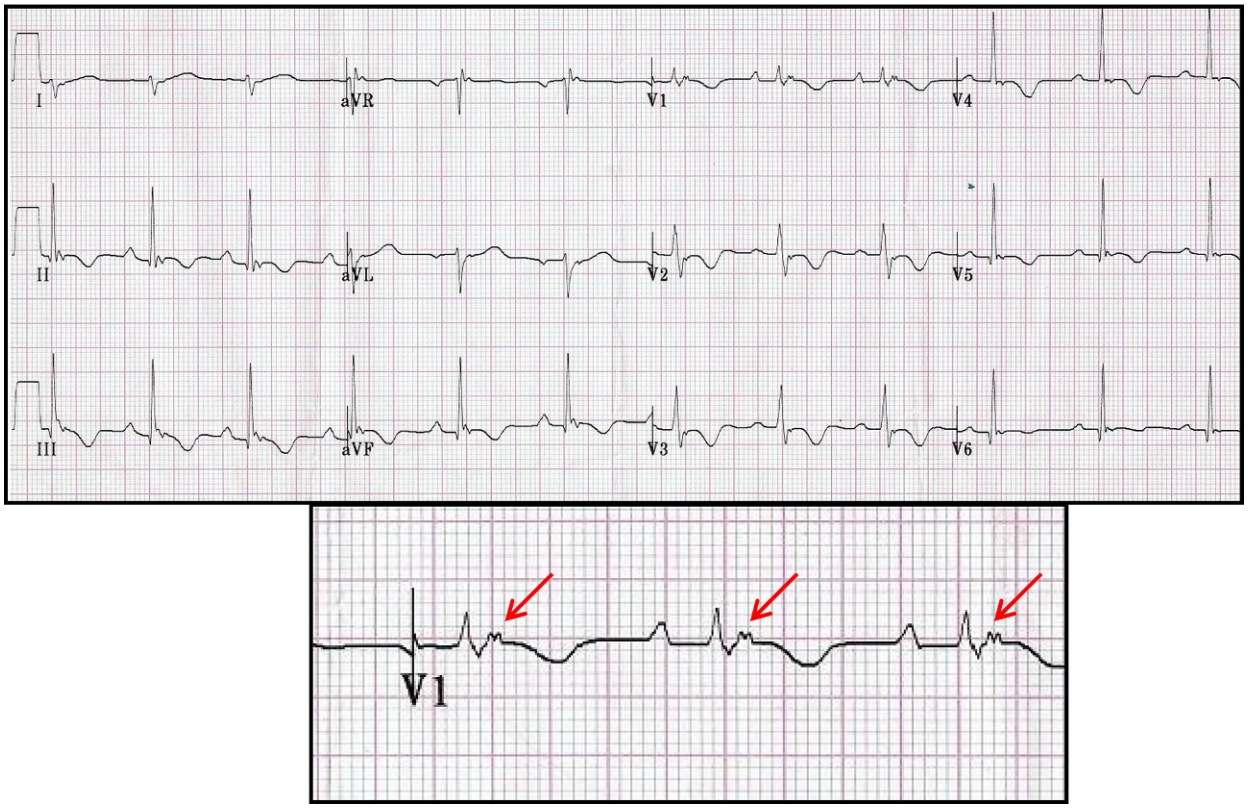

Fig. 1 An EKG showing Epsilon waves and T wave inversions. *Arrows* are pointing at Epislon waves.

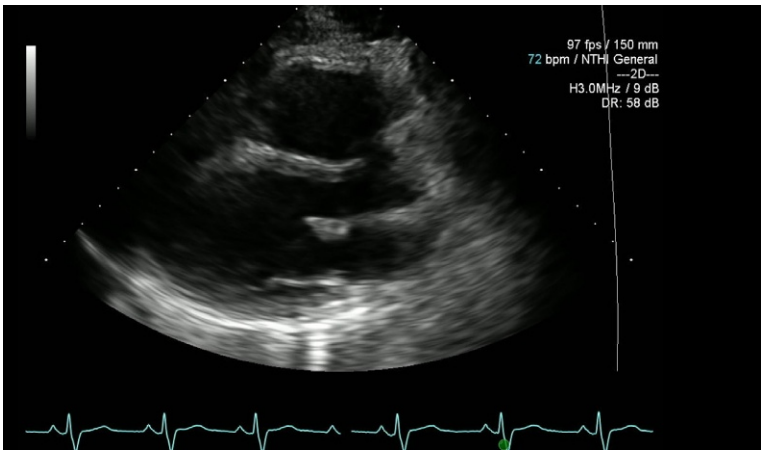

Fig. 2 A two-dimensional transthoracic echocardiogram image showing enlarged RVOT diameter.

Cardiac Magnetic Resonance for Diagnosis of ARVC/D

Cardiac magnetic resonance (CMR) imaging is a critical tool for evaluating functional and structural cardiac abnormalities in subjects being examined for ARVD/C. According to the CMR imaging criteria described by the 2010 TFC, the presence of regional RV akinesia, dyskinesia, or dyssynchronous RV contraction is essential to meet criteria for ARVC/D diagnosis. Subsequently, based on different degrees of RV end-diastolic volume index (RVEDVI) enlargement and quantitative assessment of RV ejection fraction (RVEF), major or minor criteria are fulfilled (Marcus et al., 2010b). Some MRI studies suggest that RV fatty infiltration and late gadolinium enhancement are common in these patients; however, reliance on subjective assessment of RV fibrofatty infiltration by CMR has been proven to be problematic and for this reason these pathologic findings are not included in the current task force criteria (Fig. 3; Rastegar et al., 2016).

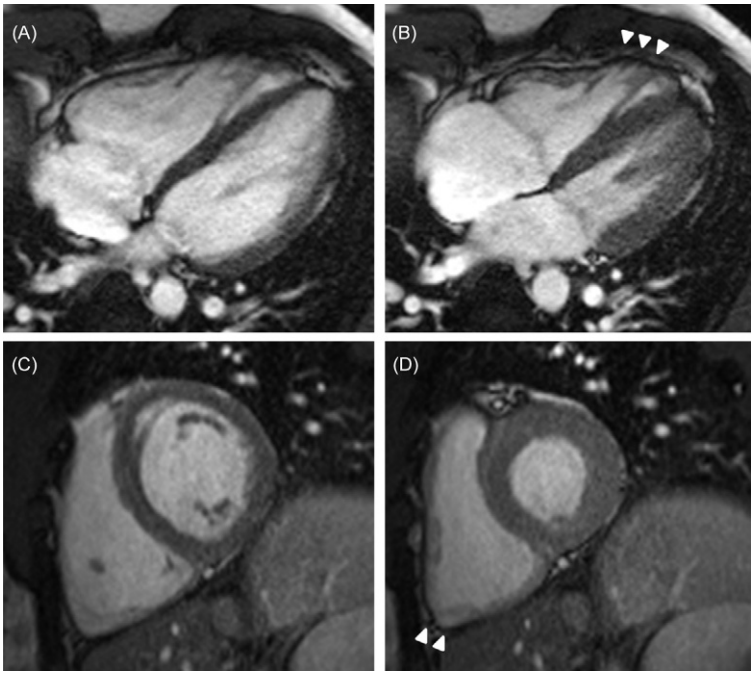

Fig. 3 Four-chamber (top panels) and short-axis (bottom panels) bright blood images in a male subject presenting with syncope. End-diastolic images are shown in the left panels, while end-systolic images in the right panels. The right ventricle is dilated (RVEDVI = 115 mL/m^2) with mild systolic dysfunction (RVEF = 41%). Evidence of apical dyskinesia (*white arrowheads* in panel B). Small outpouching area is also noted in the systolic short-axis image (*white arrowheads* in panel D). Overall these findings meet major MRI criteria for ARVC.

Misdiagnosis of ARVC/D Using CMR

Misdiagnosis of ARVC/D is a well-recognized problem. In many cases, CMR misinterpretation is the cause of overdiagnosis of ARVC/D. It is important to realize that, although CMR may be regarded the standard of reference for evaluation of RV morphology and function, the use of CMR alone is not the "gold standard" for ARVC/D diagnosis. Rather, the TFC prescribe the use of multiple diagnostic tests. Great caution must be employed when the only abnormality in a presumed ARVC/D patient is found on CMR, as it is uncommon for ARVC/D patients to have a normal EKG and Holter monitor but an abnormal CMR.

Genetics of ARVC/D and Family History

Several desmosomal gene mutations have been found in patients with ARVC/D. The estimated overall rate of successful genotyping among patients meeting the diagnostic criteria for ARVC established by the international task force is approximately 50%. The most commonly affected gene is plakophilin 2 (PKP2) (in 10%–45% of patients), followed by desmoplakin (DSP) (10%–15%), desmoglein 2 (DSG2) (7%–10%), and desmocollin-2 (DSC2) (2%). Screening of nondesmosomal genes only marginally affects the detection rate for mutations. Genotyping is most often used to identify a mutation that is considered likely to be causal in a proband who fulfills phenotypic diagnostic criteria, and thus to identify gene carriers among family members by means of mutation-specific genetic testing. Genotyping to confirm the diagnosis in an isolated patient with a borderline or questionable phenotype is not indicated on a routine basis. The true prevalence of disease-causing mutations has yet to be determined. Therefore, a negative genetic test does not exclude the possibility that the phenotype is due to a mutation of an unknown disease-causing gene. On the other hand, the interpretation of an apparently positive genetic test for ARVC is made more challenging by the difficulty in differentiating causative mutations, especially missense mutations, from nonpathogenetic variants and polymorphisms, mainly because of the lack of functional or biologic studies that confirm the pathogenetic effects of gene variants. The prognostic value of genotyping also remains to be elucidated. Patients with multiple desmosomal gene mutations are likely to have a more severe phenotype and may have an increased lifetime risk of malignant arrhythmias and SCD.

Genetic counseling should be sought for interpretation of the commercially available genetic tests for ARVC. Otherwise, critical information regarding the complexity of genetic data and their limitations for clinical diagnosis and prognosis may not be made clear when the results of such tests are reported.

Clinical Management

Risk Stratification

The estimated overall mortality varies among studies, ranging from 0.08% to 3.6% per year (Corrado et al., 2017). The clinical course of ARVC is characterized by the occurrence of arrhythmic events, which can cause sudden death, and the impairment of biventricular systolic function, which can lead to death from heart failure. Recent studies have shown that annual mortality in the index patients and family member is less than 1% (Zorzi et al., 2016; Groeneweg et al., 2015). The mortality was initially overestimated because it was based on studies at tertiary referral centers, which predominantly included high-risk patients.

Prognosis

The prognosis for patients with ARVC depends largely on the severity of arrhythmias and ventricular dysfunction (Priori et al., 2015). Prior cardiac arrest due to ventricular fibrillation and sustained VT are the most important predictors of life-threatening arrhythmic events during a follow-up. Major risk factors include unexplained syncope, nonsustained VT on ambulatory monitoring or exercise testing, and severe systolic dysfunction of the right ventricle, left ventricle, or both. Although intracardiac electrophysiological (EP) testing has traditionally been used to assess the risk of ventricular arrhythmias, the prognostic value of VT or ventricular fibrillation induced by programmed ventricular stimulation in patients with asymptomatic ARVC remains unclear.

Sports Restrictions

The aims of clinical management of ARVC are to reduce the risk of SCD and improve the quality of life by alleviating arrhythmic and heart failure symptoms. Restriction from intense sports activity is regarded as an important preventive tool for both healthy gene carriers and clinically affected persons in order to protect them from the risk of exercise-related malignant arrhythmic events and disease development or progression. The available evidence indicates that family members with a negative phenotype (either healthy gene carriers or those with an unknown genotype) do not need any specific treatment other than sports restriction; however, lifelong clinical assessment with the use of noninvasive tests at least every 2 years is warranted because of the age-related penetrance and progressive nature of ARVC. The risk of SCD during exercise may be due in part to increased stress on the RV. The association with exercise and RV stress and the frequent induction of arrhythmia by isoproterenol infusion suggest a role for catecholamines. Data obtained from exercise questionnaires and interviews indicate a strong association of endurance training with disease development and arrhythmic risk, supporting three decades of anecdotal observations that endurance sport is particularly dangerous in ARVC/D and should be avoided. Studies have also shown that patients with a desmosomal mutation require far less exercise to develop ARVC/D than patients who are mutation and family history negative. Based on this data, patients with ARVC/D should avoid competitive and endurance exercise. Only low intensity activity (e.g., walking, golf) should be advised. Although a specific cut-off for what level of exercise is safe is not well defined, any activity that causes symptoms of palpitations, presyncope, or syncope should be avoided (Maron et al., 2015).

Pharmacotherapy

Despite limited supportive data, beta-blockers are currently recommended for all clinically affected persons, for both prevention of arrhythmias and reduction of RV wall stress. In patients with ventricular arrhythmias, antiarrhythmic drug therapy offers the potential to ameliorate symptoms, although there is no proof that it confers protection against SCD. Amiodarone, alone or in association with beta-blockers, and sotalol are the most effective drugs, combining the synergistic effects of class III antiarrhythmic properties and beta-adrenergic blockade. The potential for serious cumulative toxic effects precludes long-term therapy with amiodarone, especially in younger patients. Patients in whom right or left heart failure develops are treated with standard pharmacologic therapy, including angiotensin-converting-enzyme inhibitors, angiotensin II receptor blockers, beta-blockers, and diuretics. Therapy with oral anticoagulants is reserved for patients with atrial fibrillation or thromboembolic complications (Hulot et al., 2004).

Catheter Ablation

Catheter ablation is a therapeutic option for patients who have episodes of sustained, monomorphic VT. However, it should be regarded as a palliative rather than curative therapeutic approach because of the high frequency of subsequent recurrences of VT and the unproven efficacy of ablation as a means of preventing SCD (Marchlinski et al., 2004). The poor long-term outcome has been attributed to the progressive nature of ARVC, which leads to the development of multiple arrhythmogenic foci over time. The epicardial location of some VT reentry circuits, which reflects the propensity of ARVC lesions to originate and progress from the epicardium, may also explain the failure of conventional endocardial mapping and catheter ablation (Thiene et al., 1988). Several studies have shown the feasibility and efficacy of epicardial catheter ablation for patients in whom one or more endocardial procedures have been unsuccessful. Radiofrequency ablation and medical therapy with antiarrhythmic agents are usually reserved to prevent or reduce ICD shocks.

Defibrillator Therapy

Although randomized trials of defibrillator therapy have not been performed, data from observational studies have consistently shown that it is effective and safe. Patients who benefit most from defibrillators are those who have had an episode of ventricular fibrillation or sustained VT (Groeneweg et al., 2015). It remains uncertain whether defibrillator therapy is appropriate for primary prevention of SCD among patients with one or more risk factors and no prior major arrhythmic events. In asymptomatic patients with no risk factors and in healthy gene carriers, there is generally no indication for prophylactic defibrillator implantation because of the low risk of arrhythmias and the significant risk of device- and electrode-related complications during long-term follow-up (estimated rate, 3.7% per year) (Wichter et al., 2004).

Therapies on the Horizon

Current therapeutic approaches to ARVC are palliative and partially alleviate symptoms and the risk of SCD but do not prevent the development or progression of the disease process. A definitive curative treatment will require a deeper knowledge of the biologic mechanisms and environmental factors involved in the pathogenesis of ARVC. A recent observation concerns a small molecule designated SB216763, which is an activator of the Wnt signaling pathway. This molecule has been shown to prevent or reverse phenotypic manifestations of ARVC induced by overexpression of defective plakoglobin in a zebrafish model, as well as in rat cardiac myocytes (Asimaki et al., 2014). Although this drug is of interest as a potential mechanism-based therapy of ARVC, it has not yet been studied in humans.

Cardiac Transplantation

Cardiac transplantation is the ultimate therapy for patients with untreatable arrhythmias (e.g., incessant storms of VT or fibrillation) or congestive heart failure that is refractory to pharmacologic and nonpharmacologic therapies (Tedford et al., 2012).

Pregnancy Counseling

Genetic counseling should be performed in ARVC/D patients and their partners when planning a pregnancy, delineating the dominant inheritance mode with variable penetrance in ARVC/D (Agir et al., 2014; Murray et al., 2013; Iriyama et al., 2013; Hodes et al., 2016; Herrey, 2014). In women with ARVC/D, increased circulatory demand during pregnancy and delivery may in theory precipitate disease progression and trigger symptoms, that is, ventricular arrhythmias. Larger studies of pregnancy in women with ARVC/D are lacking, but case reports have indicated that pregnancy in ARVC/D is generally well tolerated. In severe ARVC/D cases, a moderate risk of disease progression and arrhythmia has been observed from the last trimester until 2–3 months postpartum. In women with RV failure including significant LV involvement, larger studies are lacking, but pregnancy may impose additional risks that need to be addressed at prepregnancy counseling. The influence of repeated pregnancies on RV size and function as well as worsening of symptoms is not known. Pregnancy in women with ARVC/D needs to be addressed by a multidisciplinary team involving cardiologists, obstetricians, and anesthesiologists at a tertiary center. During pregnancy we advocate clinical workup at 3 and 7 months of gestation, as well as at 2–3 months postpartum, including 12-lead EKG, 24-h Holter monitoring and echocardiography, and additional visits if symptoms occur. Antiarrhythmic medication should be continued throughout pregnancy, but may be altered if there are contraindications. Beta-blocker medication is generally well tolerated, and flecainide may also be maintained during pregnancy. Amiodarone is usually regarded as contraindicated during pregnancy due to teratogenicity and high rates of abnormal thyroid function in the newborn. Ideally, indications for ICD should be reassessed before pregnancy. Arrhythmias during pregnancy may warrant ICD implantation. ICD discharge and external electrical cardioversion in case of VT are generally well tolerated by the fetus. In female ARVC/D patients without arrhythmias or abnormal contractile function, vaginal delivery is preferred. In cases with symptomatic ARVC/D, a cesarean section during epidural anesthesia is advocated. Telemetry monitoring during delivery should be continued in 24 h postpartum. In cases with maternal antiarrhythmic medication, the child should be transferred to the neonatal unit for 24-h observation.

Summary

ARVC/D is a genetically determined and heritable form of cardiomyopathy. It is characterized pathologically by the replacement of myocytes by adipose and fibrous tissue and leads to RV failure, arrhythmias, and SCD. The estimated prevalence of ARVC/D in the general population ranges from 1 in 2000 to 1 in 5000. Men are affected more frequently than women, with an approximate ratio of 3:1. ARVC/D can be inherited as an autosomal dominant disease with reduced penetrance and variable expression. Autosomal recessive inheritance is also described. Twelve genes have been identified which are linked to ARVC/D, encoding several components of the cardiac desmosome. Dysfunctional desmosomes resulting in defective cell adhesion proteins, such as plakoglobin (JUP), desmoplakin (DSP), plakophilin-2 (PKP-2), and desmoglein-2 (DSG-2), consequently cause loss of electrical coupling between cardiac myocytes, leading to myocyte cell death, fibrofatty replacement, and arrhythmias. Diagnosis is based on a combination of characteristic abnormalities in family history, electrocardiography, cardiac imaging, as well as endomyocardial

biopsy. Therapeutic options remain limited due to the progressive nature of ARVC/D. Competitive athletics should be avoided. Individuals with ARVC/D and a history of resuscitation from SCD, syncope, the very young, and those with marked RV involvement are at the highest risk for arrhythmic death. The presence of left ventricular (LV) involvement is also a risk factor. Patients who meet the Task Force criteria for ARVC/D are at high risk for SCD and should be considered for ICD placement for primary or secondary prevention, regardless of EP testing results. The role of the EP study and catheter ablation in ARVC/D remains poorly defined, and is frequently used as a palliative measure for patients with refractory VT. Sotalol has been shown to be effective in patients with ARVC/D for inducible as well as noninducible spontaneous VT. Heart transplantation is considered in patients with progressive heart failure and intractable recurrent ventricular arrhythmias.

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Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy

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Introduction

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is an inherited cardiomyopathy characterized pathologically by replacement of the cardiac myocytes with fibrofatty tissue (Fig. 1); this predisposes patients to ventricular arrhythmias (VA), right ventricular failure, and increased risk of sudden cardiac death (SCD). Although structural involvement of the right ventricle predominates, a left dominant form is also seen. In most patients in the United States, structural abnormalities of the right ventricle predominate.

Since ARVD/C was first described in 1982 (Marcus et al., 1982), significant advances have been made in the understanding of the different aspects of this disease. The revision of the task force criteria (TFC) for the diagnosis of ARVD/C in 2010 (Marcus et al., 2010) improved diagnostic sensitivity by taking into account new magnetic resonance imaging (MRI) technology to evaluate cardiac structural abnormalities and new knowledge about genetic testing and electrocardiographic abnormalities.

The treatment of ARVD/C is aimed at properly risk stratifying patients that would benefit from an implantable cardioverter defibrillator (ICD) for the prevention of SCD. Antiarrhythmic drugs and catheter ablation are also commonly used to improve quality of life by preventing VA. Another important consideration in patients with ARVD/C is exercise restriction, since there is increasing evidence supporting the role of exercise as key environmental factor for the development and progression of this disease.

Although there is a lack of rigorous evidence on how to treat and risk stratify patients with ARVD/C, research efforts from many expert groups around the world have helped to a better understanding of the disease and its management.

The purpose of this article is to review the current knowledge of the natural history, clinical presentation, diagnosis, and management of patients with ARVD/C.

Clinical Presentation and Natural History

ARVD/C is a rare inherited cardiomyopathy, with an estimated prevalence of 1 per 5000 individuals (Peters et al., 2004). Patients usually present during the second to fifth decade of life with symptoms that can be attributed to the presence of VA, such as presyncope, syncope, and palpitations with progression to heart failure being uncommon (Groeneweg et al., 2015; Dalal et al., 2005).

Although ARVD/C is predominantly a disease of the right ventricle (Jain et al., 2008; Rastegar et al., 2014, 2015; Bhonsale et al., 2015), involvement of the left ventricle (LV) may occur. Left-dominant arrhythmogenic cardiomyopathy is usually present with arrhythmias of LV origin and the absence of significant right ventricular (RV) systolic dysfunction (Bhonsale et al., 2015; Sen-Chowdhry et al., 2008).

The presentation, clinical course, and survival outcomes of a cohort of 439 index patients who met 2010 TFC for ARVD/C have also been reported (Groeneweg et al., 2015). The mean age at presentation was 36 ± 14 years, and mutations were identified in 63% ($n = 276$). Most patients were symptomatic at presentation ($n = 419$). Forty-eight (11%) presented with cardiac arrest, 25 were resuscitated, and 23 died, and the diagnosis was established at autopsy. An additional 220 (50%) patients presented with a sustained ventricular arrhythmia. During follow-up, 139/171 and 212/245 patients received an ICD for primary and secondary prevention of SCD, respectively. Close to half (48%) of the patients that did not have an ICD implanted ($n = 65$) had sustained VA during follow-up. Recurrent sustained VA was seen in 72% ($n = 301$) index patients at follow-up. After a median follow-up of 7 years, 10 (3%) of patients with an ICD died (3 with heart failure, 2 SCD, 2 of a combination of arrhythmias and heart failure, and

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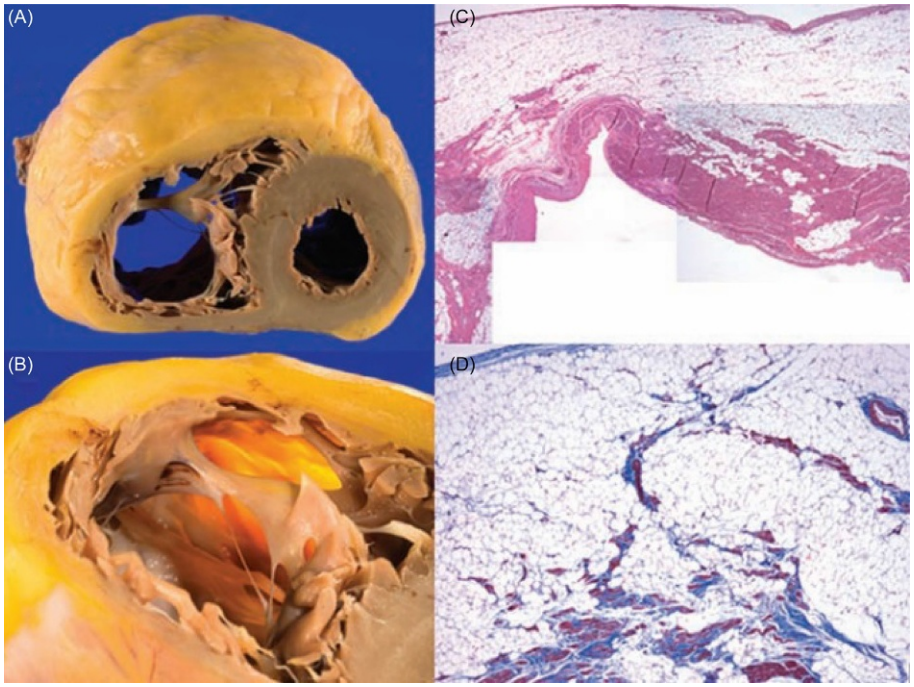

Fig. 1 (A—upper left) Gross specimen of an explanted heart showing a massive right ventricular (RV) with extensive fat that replaced entire RV epicardium leaving a thin rim of fibrotic endocardium. Also note the relative sparing of the left ventricle and the interventricular septum. (B—lower left) Near total fatty replacement of the RV wall with loss of myocytes renders the RV translucent. (C—upper right) Transmural low-resolution histopathologic image shows the fat replacement extending from epicardium to the endocardium. (D—lower right) Trichrome staining reveals fibrous tissue interspersed with fat tissue characteristic of this disease. Reproduced with permission from Tandri, H., Asimaki, A., and Dalal, D. et al. (2008). Gap junction remodeling in a case of arrhythmogenic right ventricular dysplasia due to plakophilin-2 mutation. *Journal of Cardiovascular Electrophysiology* **19**(11), 1212–1214.

3 of noncardiac causes). Eleven (17%) patients without an ICD died (10 SCD and 1 with heart failure). During long-term follow-up, 54 patients (13%) developed symptomatic heart failure, and 18 (4%) underwent cardiac transplantation with 4 dying during follow-up. Overall, 391/416 index patients (94%) who presented alive were alive at the last follow-up. In conclusion, long-term outcome of patients with ARVD/C was favorable in those that were diagnosed and treated appropriately with progression to heart failure being uncommon. ICD implantation reduced the incidence of SCD during follow-up (**Fig. 2**).

Dalal et al. (2005) studied 100 patients diagnosed with ARVD/C with a total of 47 patients received an ICD with 62% ($n=29$) receiving an appropriate ICD therapy at follow-up. Twenty-three patients died at presentation (21 SCD and 2 noncardiac deaths) and 11 died during follow-up (10 SCD and 1 biventricular heart failure). After a median follow-up of 6 years, the majority of patients ($n=66$) were alive (44 had an ICD, 5 had signs of heart failure, 2 had heart transplant, and 18 were on drug therapy), showing that once diagnosis of ARVD/C is established and patients undergo ICD implantation, the mortality is low.

Te Riele et al. (2015) compared a cohort of 75 pediatric-onset cases of ARVD/C (mean age at presentation 15.3 ± 2.4 years) with 427 adult patients (mean age at presentation 38.6 ± 13.4). The authors observed that pediatric patients were more likely to present with SCD ($p=0.003$) or resuscitated cardiac arrest, compared with adults who presented most commonly with sustained ventricular tachycardia (VT) ($p=0.359$). However, once ARVD/C is diagnosed, survival free from sustained ventricular tachycardia, cardiac transplantation, and death was similar between the two groups after more than 8 years of follow-up.

Pathophysiology and Genetics

The genetics of ARVD/C supports the hypothesis that this is a disease of desmosomal dysfunction. Although the pathogenesis of ARVD/C is not fully understood, it is believed that abnormalities in the desmosomal proteins may lead to impaired mechanical coupling between individual cells, which then causes myocyte uncoupling resulting in inflammation, fibrosis, and adipocytosis. This is especially true in situations that increase myocardial strain, such as exercise (Orgeron and Calkins, 2016). This remains an active area of investigation.

ARVD/C is commonly inherited in an autosomal dominant manner with incomplete penetrance and variable expressivity. Genetic testing of probands and family members has become a very important part of the diagnosis and screening of ARVD/C since desmosomal mutations can be found in up to 60% of affected individuals (Groeneweg et al., 2015; Den Haan et al., 2009). Thus, the lack of an identifiable mutation does not exclude the disease.

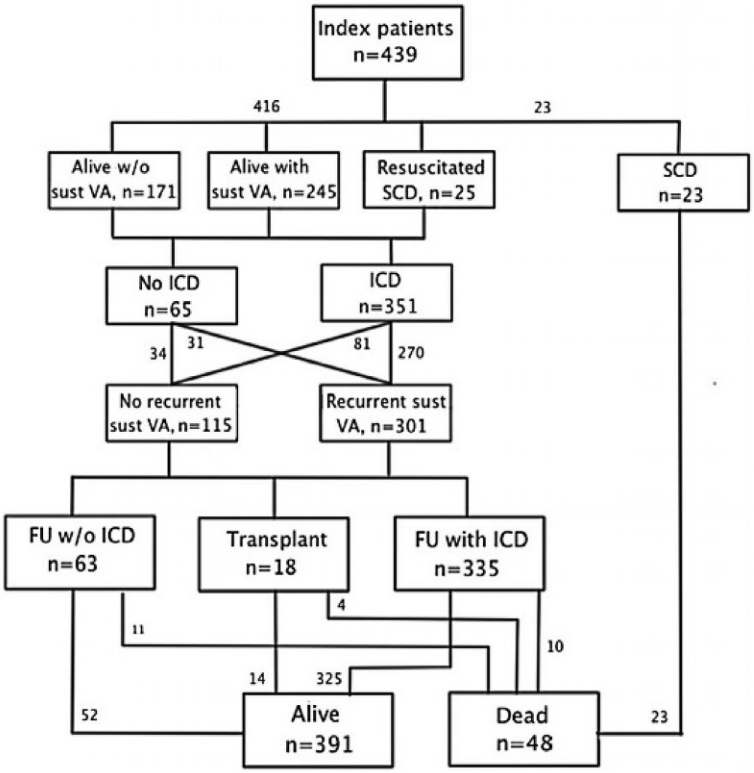

Fig. 2 Schematic representation of the presentation, clinical course, and outcome in arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) index patients. The majority presented with sustained ventricular arrhythmias (VA) and received an implantable cardioverter defibrillator (ICD) during follow-up. Reproduced with permission from Groeneweg, J., Bhonsale, A., and James, C. et al. (2015). Clinical presentation, long-term follow-up, and outcomes of 1001 arrhythmogenic right ventricular dysplasia/cardiomyopathy patients and family members. *Circulation Cardiovascular Genetics*, **8**(3), 437–446.

In a minority of cases, ARVD/C can also be inherited in an autosomal recessive pattern as part of a cardiocutaneous syndrome such as Naxos disease or Carvajal syndrome (Protonotarios and Tsatsopoulou, 2004). The identification of a plakoglobin (JUP) mutation that affects a key component of desmosomes in Naxos syndrome led to several studies investigating their role in other arrhythmogenic cardiomyopathies. Posteriorly, several ARVD/C-associated mutations were identified: plakophilin-2 (PKP2), desmoplakin (DSP), desmoglein-2 (DSG2), and desmocollin-2 (DSC2) (Bhonsale et al., 2015; Den Haan et al., 2009). Mutations in several nondesmosomal genes such as cardiac ryanodine receptor (RyR2), phospholamban (PLN), transmembrane protein 43 (TMEM43), titin, and transforming growth factor β3 (TGFβ3) have also been observed in atypical forms of ARVD/C.

The genetic findings of 577 patients from 241 unrelated families, selected based on the presence of an ARVD/C-related mutation, have been reported (Bhonsale et al., 2015). The majority of participants in the study, 80% ($n=463$), were carriers of a PKP2 mutation, with fewer heterozygous carriers for other mutations associated with ARVD/C (31 DSG2, 31 PLN, 19 DSP, 8 DSC2, 2 JUP, and 1 TMEM43). Four percent ($n=22$) of participants carried two or more pathogenic mutations. The authors observed that patients presenting with SCD/ventricular fibrillation (VF) were younger (median 23 vs. 36 years) than those presenting with sustained monomorphic VT. They also noticed that patients with more than one mutation had earlier occurrence of sustained VT/VF, lower VT/VF free survival and more frequent left ventricular dysfunction, heart failure, and cardiac transplantation than those with only one mutation. Lastly, men were more likely to be symptomatic probands, and they had earlier and more severe arrhythmic expression.

Diagnosis of ARVD/C

The diagnosis of ARVD/C is made through a scoring system with major and minor criteria, based on demonstrating a combination of ventricular dysfunction and structural alterations, tissue characterization, electrocardiographic repolarization/depolarization abnormalities, VA, family history, and genetic testing. A definite diagnosis is made if a patient has "4 points", with a major criterion equal to 2 points and a minor criterion equal to "1 point". A borderline diagnosis is made with "3 points" and a possible diagnosis with "2 points." The original 1994 TFC were updated and published in 2010; their aim was to increase diagnostic sensitivity by integrating new knowledge and technology (Marcus et al., 2010).

It is important to note that there is no gold-standard diagnostic test for ARVD/C. On initial evaluation, all patients should have a physical exam and comprehensive clinical history that includes information about family history of sudden death and arrhythmias; an ECG, 24 h Holter monitoring, signal-averaged ECG, and comprehensive imaging of both ventricles should also be obtained. If the initial workup is suggestive but not diagnostic of ARVD/C, an electrophysiological study should be considered to establish the diagnosis. Right ventriculography and endomyocardial biopsy were also used to aid in the diagnosis of ARVD/C in the past, but they are rarely used currently (Orgeron and Calkins, 2016).

ECG abnormalities are extremely frequent in patients with ARVD/C (Nasir et al., 2004; Te Riele et al., 2013a; Jain et al., 2009). T-wave inversion (TWI) in the right precordial leads (V1–V3) is a major diagnostic criterion, and it is the most common ECG manifestation of ARVD/C. TWI in leads V1 and V2 is a minor criterion. The presence of incomplete or complete right bundle branch block (RBBB) makes the interpretation of known depolarization abnormalities difficult, and it is commonly found in patients with severe cardiac structural abnormalities (Jain et al., 2009). TWIs through V3 still appear to be the feature with the optimal sensitivity and specificity. However, in patients with complete RBBB, R/S ratio <1 in V1 seems to provide the optimal sensitivity and specificity in diagnosing ARVD/C (Jain et al., 2009). T-wave inversion beyond V3 in patients with complete RBBB is also a feature of ARVD/C. Epsilon waves, which are distinct low-frequency deflections in the ECG that occur following the QRS and before the T wave, are uncommon and a marker of advanced ARVD/C. The new diagnostic criteria for ARVD/C include terminal activation delay, which is defined as a prolonged duration of time (>55 ms) from the S wave to the termination of the QRS complex as a minor criterion for ARVD (Marcus et al., 2010) (**Fig. 3**).

A signal-averaged ECG (SAECG) is considered abnormal if one or more of the following criteria are present: (1) filtered QRS duration (fQRS) >114 ms. (2) Terminal low-amplitude (<40 μV) signal duration ≥ 38 ms. (3) Root-mean-square voltage of the terminal 40 ms of the filtered QRS complex (RMS40) <20 μV. A positive SAECG is considered a minor criterion (Marcus et al., 2010).

Holter monitoring is commonly used for the evaluation of frequent premature ventricular contractions (PVCs) and sustained/nonsustained ventricular tachycardia. A left bundle branch block (LBBB) morphology is consistent with the diagnosis of ARVD/C. The presence of 500 PVCs or more per 24 h is considered a minor criterion (Marcus et al., 2010), and it is frequently found in patients with ARVD/C. Bhonsale et al. (2011) reported that patients with higher PVC burden were more likely to receive an appropriate ICD intervention (**Fig. 4**). Other options such as implantable loop recorders, exercise testing to induce VT, or extending the duration of Holter monitoring can be used in the event that a Holter monitor does not capture any arrhythmia.

Echocardiography is the most widely available tool for the assessment of ventricular structure and function. Global or segmental wall motion abnormalities with cavity dilation, hypertrophic RV trabeculation, and systolic dysfunction are some of the findings on a transthoracic echocardiogram that are suggestive of ARVD/C. Right ventricular outflow tract (RVOT) dilation (diameter >30 mm) has been reported to have the highest sensitivity and specificity of echocardiographic parameters in diagnosing ARVD (Marcus et al., 2010). Cardiac MRI (CMR) imaging is the imaging modality of choice in evaluating the right ventricle in ARVD/C (Jain et al., 2008; Rastegar et al., 2014, 2015; Bhonsale et al., 2015; Te Riele et al., 2013b, 2014; Vigneault et al., 2016). CMR has the benefit of providing tissue characterization and identification of intramyocardial fat and fibrosis in addition to assessment of ventricular structure and function (**Fig. 5**). Although MRI is a very important diagnostic tool, it can also be a common cause for misdiagnosis (Bomma et al., 2004). The major and minor criteria for ARVD/C on echocardiogram and CMR are listed in **Table 1**.

Endomyocardial biopsies have been reported to have diagnostic value in the past (Asimaki et al., 2009) especially when the diagnosis is unclear or there is need to rule out a competing diagnosis. However, the test appears to have low diagnostic sensitivity

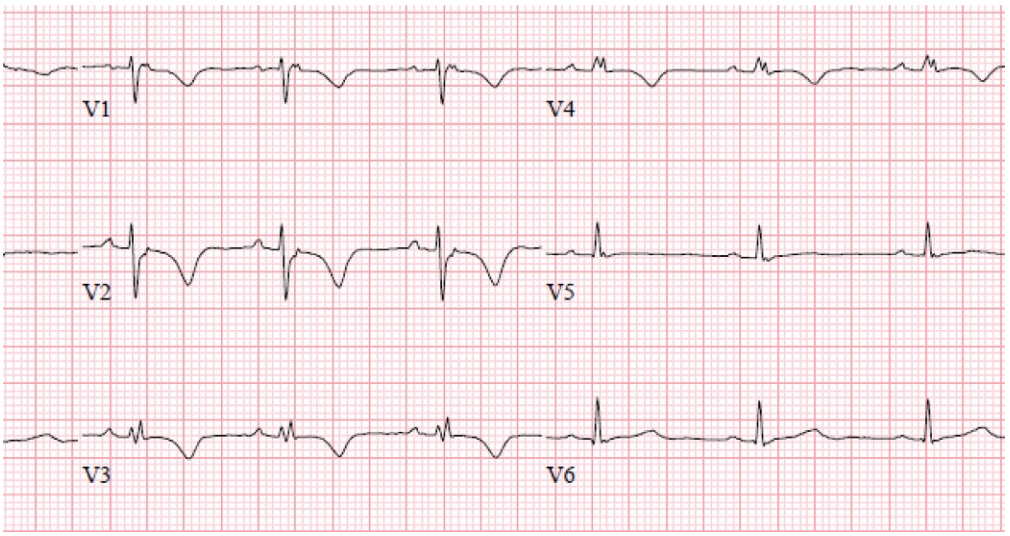

Fig. 3 ECG of a patient with ARVD/C that shows T-wave inversions from V1 to V4 and the presence of an epsilon wave in V1–V2 and terminal activation delay.

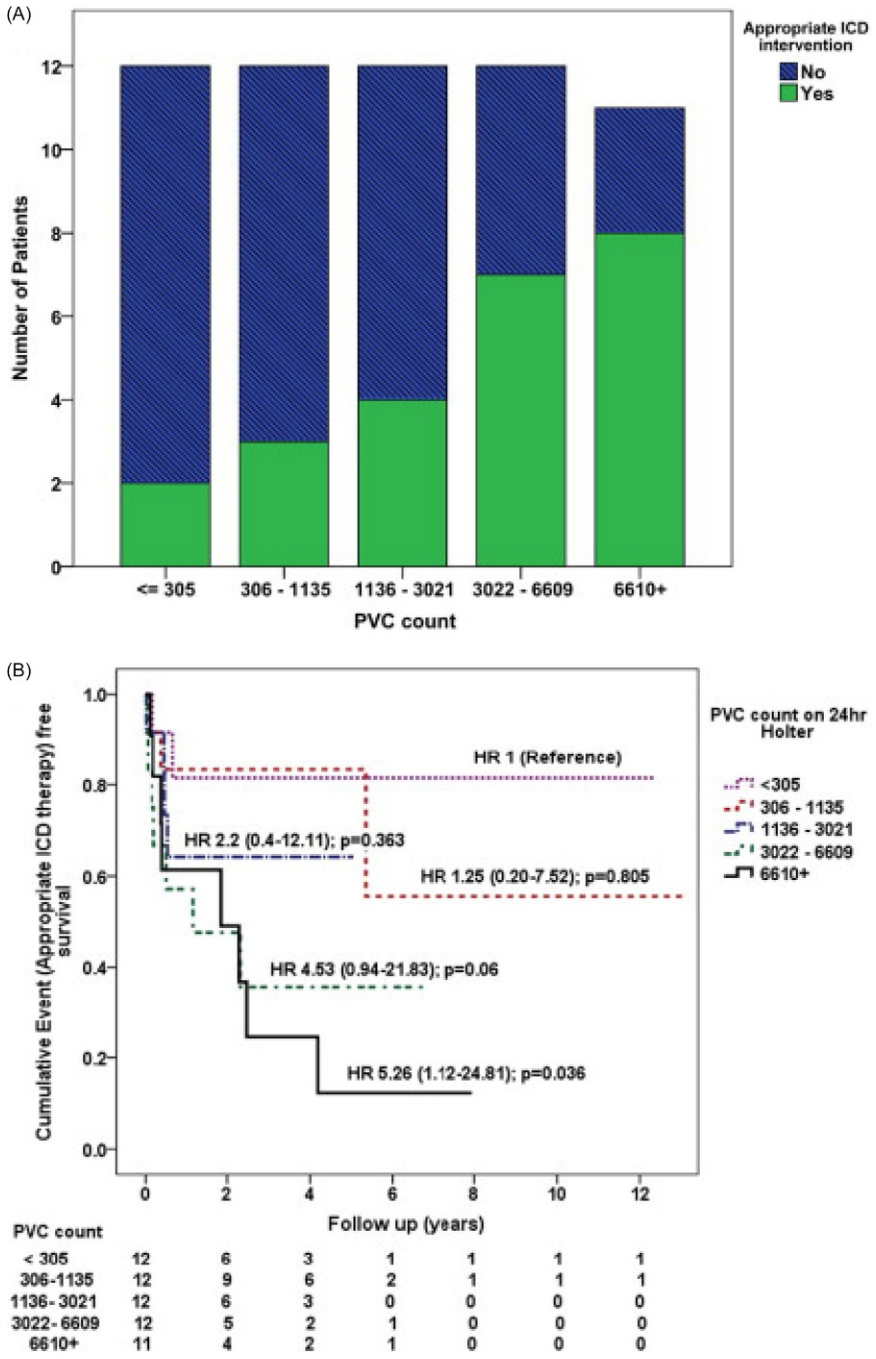

Fig. 4 (A) Relationship between patients experiencing appropriate implantable cardioverter-defibrillator (ICD) therapy and patient groups stratified according to increasing premature ventricular complex (PVC) count. (B) Cumulative survival free of appropriate ICD therapy among patient groups (pentiles) stratified according to increasing PVC count and unadjusted hazard ratio for these patient groups demonstrating progressively increasing risk with worsening PVC burden. Reproduced with permission from Bhonsale, A., James, C. A., and Tichnell, C. et al. (2011). Incidence and predictors of implantable cardioverter-defibrillator therapy in patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy undergoing implantable cardioverter-defibrillator implantation for primary prevention. *Journal of the American College of Cardiology*, **58**, 1485–1496.

for several reasons including patchy distribution of the disease and the presence of similar patterns to that observed in ARVD/C in other diseases such as sarcoidosis and giant cell myocarditis. For these reasons, they are rarely performed nowadays.

The role of genetic testing in the diagnosis of ARVD/C is now well established and is considered a major diagnostic criterion (Marcus et al., 2010). More than 60% of ARVD/C probands are found to have a mutation (Groeneweg et al., 2015; Den Haan et al., 2009). The most important utility of this test is in the cascade screening of family members. Asymptomatic gene carriers are likely to

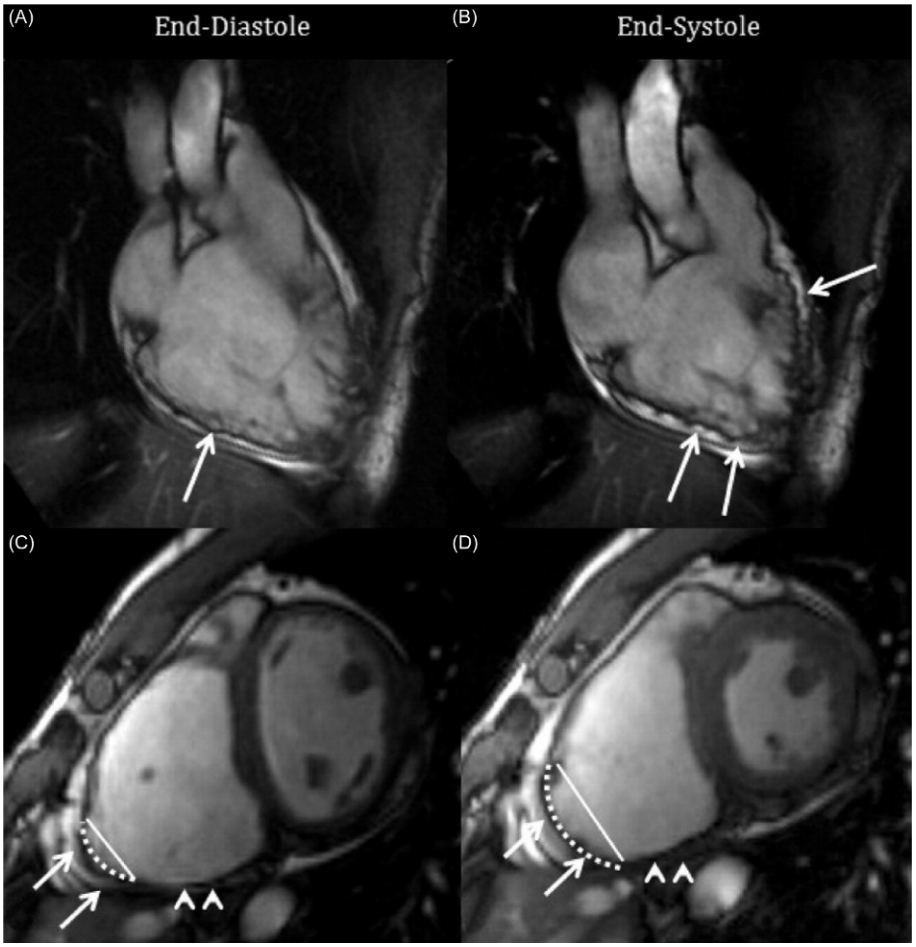

Fig. 5 Cardiac MRI of a patient with ARVD/C. Bright blood images in the right ventricular outflow tract (RVOT) plane obtained in end-diastole (A) and end-systole (B) show microaneurysms *(arrows)* in the RV free wall with persistent bulge in both phases. Short-axis bright blood images obtained in end-diastole (C) and end-systole (D) demonstrate dyskinesis at the acute angle of the RV and hypokinesis in the inferior wall *(arrowheads)*. Reproduced with permission from Calkins, H. (2015). Arrhythmogenic right ventricular dysplasia/cardiomyopathy-three decades of progress, *Circulation Journal*, **79**(5), 901–913.

require life-long monitoring due to age-dependent penetrance, while noncarriers are unlikely to have the disease and serial screening is generally not required (Calkins, 2015).

The diagnosis of ARVD/C should be considered in individuals without known heart disease, who present with frequent PVCs or symptomatic ventricular tachycardia, especially if there is LBBB morphology with superior axis, if the VT occurs in a patient with T-wave inversions in the right precordial leads, or if the LBBB VT ECG pattern is not typical of idiopathic RVOT VT (Calkins, 2013).

The differential diagnosis for ARVD/C should include cardiac sarcoidosis, idiopathic RVOT VT, and VT arising from the aortic root. For patients that present with symptoms of RV systolic heart failure, RV infarct or pulmonary hypertension should be considered. Dilated cardiomyopathy should also be kept in mind when there is evidence of biventricular heart failure and biventricular arrhythmogenic cardiomyopathy when there is significant ventricular ectopy (Calkins, 2013).

Management

The management of patients with ARVD/C has four components. The first step is making sure that ARVD/C is indeed the correct diagnosis. The second step involves accurate risk stratification of patients and to establish the need to have an ICD implanted. The third step consists in minimizing ICD therapies with either medications, catheter ablation, or both. The fourth step is preventing progression of the disease, mostly through exercise restriction. Each of these will be examined in detail:

Establishing an Accurate Diagnosis

The first step is to establish an accurate diagnosis of ARVD/C. Misdiagnosis of ARVD/C is common due to the lack of awareness of the 2010 TFC and misinterpretation of MR imaging (Bomma et al., 2004).

Table 1 2010 TFC for the diagnosis of arrhythmogenic right ventricular cardiomyopathy and other cardiomyopathy/dysplasia

1. Global or regional dysfunction and structural alterations
   - Major
   - Second echo criteria
   - Regional RV akinesia, dyskinesia, or aneurysm and one of the following measured at end-diastole
   - PLAX RVOT $\geq$32 mm or
   - PSAX RVOT $\geq$36
   - Fractional area change $\leq$33%
   - MRI criteria
   - Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and one of the following
   - Ratio of RV end-diastolic volume to BSA >=110 mL/m^2 (male) or $\geq$100 mL/m^2
   - RV ejection fraction <=40%
   - RV angiography criteria
   - Regional RV akinesia, dyskinesia, or aneurysm
   - Minor
   - Second echo criteria
   - Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and one of the following measured at end-diastole
   - PLAX RVOT $\geq$29 <32 mm or
   - PSAX RVOT $\geq$32 <36
   - Fractional area change >33% $\leq$40%
   - MRI criteria
   - Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and one of the following
   - Ratio of RV end-diastolic volume to BSA >= 100 to <110mL/m^2 (male) or >=90 to <100 mL/m^2 (female)
   - RV ejection fraction >40% to <=45%
2. Tissue characterization of wall
   - Major
   - Residual myocytes <60% by morphometric analysis (or <50% if estimated), with fibrous replacement of the RV free-wall myocardium in $\geq$1 sample
   - With or without fatty replacement of tissue on endomyocardial biopsy
   - Minor
   - Residual myocytes 60–75% by morphometric analysis (or 50–65% if estimated), with fibrous replacement of the RV free-wall myocardium in $\geq$1sample
   - With or without fatty replacement of tissue on endomyocardial biopsy
3. Repolarization abnormalities
   - Major
   - Inverted T waves in right precordial leads (V1, V2, and V3) or beyond in individuals >14 years of age (in the absence of complete RBBB QRS $\geq$120 ms)
   - Minor
   - Inverted T waves in V1 and V2 in individuals >14 years of age (in the absence of complete RBBB) or in V4, V5, and V6
   - Inverted T waves in leads V1, V2, V3, and V4 in individuals >14 years of age in the presence of a complete RBBB
4. Depolarization/conduction abnormalities
   - Major
   - Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of T wave) in the right precordial leads (V1–V3)
   - Minor
   - Late potentials by SAECG in $\geq$1 of 3 parameters in the absence of a QRSd $\geq$110 ms on standard ECG
   - Filtered QRS duration (fQRS) $\geq$114 ms
   - Duration of terminal QRS <40 microV $\geq$38 ms
   - Root-mean-square voltage of terminal 40 ms $\leq$ microV
   - Terminal activation duration $\geq$55 ms measured from the nadir of the end of the QRS, including R′, in V1, V2, or V3 in the absence of complete RBBB
5. Arrhythmias
   - Major
   - Nonsustained or sustained VT of LBBB morphology with superior axis
   - Minor
   - Nonsustained or sustained VT of RVOT configuration, LBBB morphology with inferior axis or of unknown axis
   - >500 PVCs per 24 h (Holter)
6. Family history
   - Major
   - ARVD/C in first-degree relative who meets TFC
   - ARVD/C confirmed pathologically at autopsy or surgery in first-degree relative
   - Identification of pathogenic mutation categorized as associated or probably associated with ARVD/C in the patient under evaluation
   - Minor
   - History of ARVD/C in first-degree relative in whom it is not possible to determine whether the family member meets TFC
   - Premature sudden death (<35 years of age) due to suspected ARVD/C in a first-degree relative
   - ARVD/C confirmed pathologically or by current TFC in second-degree relative

ARVD/C, arrhythmogenic right ventricular dysplasia/cardiomyopathy; BSA, body surface area; LBBB, left bundle branch block; PVC, premature ventricular contraction; RBBB, right bundle branch block; RVOT, right ventricular outflow tract; SAECG, signal-averaged ECG; VT, ventricular tachycardia.

Adapted with permission from Marcus, F. I., McKenna, W. J., and Sherrill, D. et al. (2010). Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: Proposed modification of the task force criteria. *European Heart Journal*, **31**(7), 806–814.

Risk Stratification and Decision to Implant an ICD

The second step is the most important goal of management, and it consists in the prevention of SCD. Studies have shown that more than 50% of patients with ARVD/C have received an ICD and have appropriate device interventions during follow-up (Bhonsale et al., 2011; Piccini et al., 2005).

Bhonsale et al. (2011) evaluated 84 patients who received the ICD for primary prevention over a mean follow-up of 4.7 ± 3.4 years. ICD therapy was appropriate in 40 (48%) of 84 patients. Predictors of appropriate therapy included being the proband, demonstrating VT inducibility at electrophysiological study (EPS), having NSVT, and having >1000 PVCs in 24 h. Inducibility at EPS and Non sustained ventricular tachycardia (NSVT) remained significant predictors on multivariable analysis. They also demonstrated that more frequent ventricular ectopy was associated with progressively more frequent ICD therapy. Lastly, the incremental risk of VA and ICD therapy was observed in patients with multiple risk factors.

The management of ARVD/C poses a clinical challenge. Until more rigorous evidence is available, experts from all over the world have developed a consensus statement (Philips et al., 2013) to serve as a clinical guide for the management of this complex pathology. In this document, the authors propose risk stratifying the patients in high, intermediate, and low risk to decide whether the implantation of an ICD is necessary.

Implantation of an ICD is recommended in the high-risk group, which consists of those individuals with history of aborted SCD due to VF, sustained ventricular tachycardia, severe RV (RV EF ≤35% of RV fractional area change (FAC) of ≤17%), severe LV dysfunction (LV EF ≤35%), or both (Corrado et al., 2015).

The intermediate group is divided in two categories. In the first category, an ICD should be considered in patients with ≥1 major risk factor: syncope, NSVT, moderate RV dysfunction (RV EF between 40% and 36% or FAC between 24% and 17%), and moderate LV dysfunction (LV EF between 45% and 36%). Meanwhile, in the second category, an ICD may be considered in the presence of ≥1 minor risk factor: proband status, young age at diagnosis, male gender, compound and digenic heterozygosity of desmosomal mutations, inducibility on programmed ventricular stimulation, extent of T-wave inversion in precordial and inferior leads, amount of electroanatomic scar and electroanatomic scar-related fractionated electrograms, low QRS amplitude, and QRS fragmentation (Corrado et al., 2015).

Lastly, ICDs are not indicated in patients that are healthy gene carriers but do not have any other risk factors (Corrado et al., 2015) (**Fig. 6**).

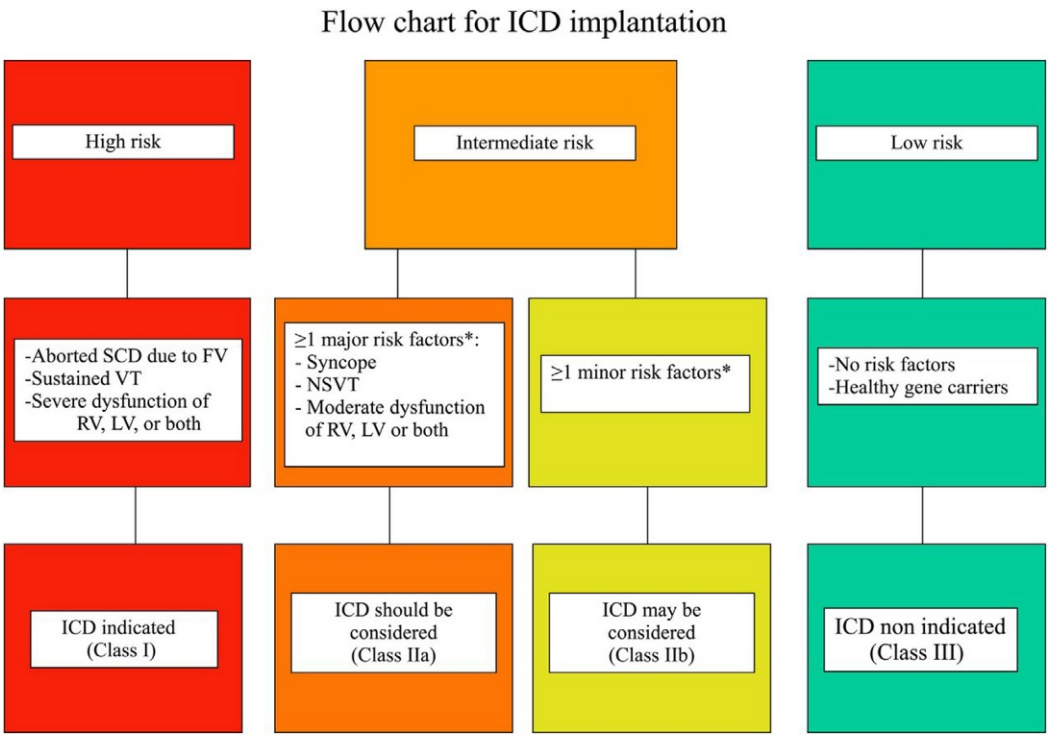

Fig. 6 Flow chart of risk stratification and indications to ICD implantation in ARVD/C. Based on the available data on annual mortality rates associated to specific risk factors, the estimated risk of major arrhythmic events in the high-risk category is 0.10% per year, in the intermediate ranges from 1 to 10% per year, and in the low-risk category is.1% per year. *SCD*, sudden cardiac death; *VF*, ventricular fibrillation; *VT*, ventricular tachycardia; *RV*, right ventricle; *LV*, left ventricle. *See the risk stratification and decision to implant an ICD section for the distinction between major and minor risk factors. Reproduced with permission from Corrado, D., Wichter, T., and Link, M.S. et al. (2015). Treatment of arrhythmogenic right ventricular cardiomyopathy/dysplasia: An International Task Force Consensus Statement. *European Heart Journal*, **36**(46), 3227–3237.

Minimizing Symptoms and Preventing ICD Therapies

Pharmacological therapy

Beta-blockers. Few studies have been done to establish the efficacy and safety of pharmacological therapy in ARVD/C. Beta-blockers are used for almost all patients with the disease based on the fact that they reduce risk of SCD in patients without ARVD/C. Furthermore, high-dose isoproterenol can trigger VT in patients with the disease (Philips et al., 2013; Denis et al., 2014) and most of the arrhythmias and SCD are triggered by exercise.

In summary, beta-blocker therapy is recommended in ARVD/C patients with recurrent VT, appropriate ICD therapies, or inappropriate ICD interventions resulting from sinus tachycardia, supraventricular tachycardia, or atrial fibrillation/flutter with high ventricular rate. Beta-blocker therapy should be considered in all patients with ARVD/C irrespective of arrhythmias (Corrado et al., 2015).

Afterload reducers. ACE inhibitors are commonly used as afterload reducers in patients with ARVD/C. Even though there are no published trials in patients with ARVD/C, there are enough data supporting the use of these medications in a broader population of patients with cardiomyopathy (Calkins, 2013, 2015). Additionally, these medications should be used in patients that develop right- and/or left-sided heart failure as part of heart failure management.

Antiarrhythmic drugs. When it comes to antiarrhythmic agents, the goal is to improve quality of life by preventing symptomatic VA and ICD therapies. Although, there are no randomized control trials to compare the different antiarrhythmic drugs available, amiodarone and sotalol are commonly used.

These medications should be considered as an adjunct therapy to ICDs in patients receiving frequent appropriate discharges and to improve symptoms caused by NSVT and frequent PVCs (Corrado et al., 2015).

Antiarrhythmic drugs may also be considered as an adjunct therapy to catheter ablation in those patients with recurrent ventricular tachycardia (Corrado et al., 2015).

Catheter ablation

Catheter ablation is an option for treatment of patients with ARVD/C who have VT. However, it is important to recognize that its role is not curative but improves quality of life by decreasing the frequency of episodes of VT.

The outcomes of catheter ablation were evaluated on a cohort of 24 patients with ARVD/C who underwent 48 VT ablation procedures (Dalal et al., 2007). The study showed that there was a high rate of recurrence (40/48) of VT in ARVD/C patients undergoing radiofrequency catheter ablation (RFA). The cumulative VT-free survival rates were 75%, 50%, and 25% and at 1.5, 5, and 14 months, respectively.

Philips et al. (2012) assessed the recurrence of VT following RFA and the effect of it in VT burden. The study consisted of 87 patients who underwent a total of 175 VT ablations. The rates of freedom from VT were 47% at 1 year, 21% at 5 years, and 15% at 10 years, over a mean follow-up of 88.3 ± 66 months. After epicardial ablation, the rates of cumulative freedom of VT were significantly longer than endocardial RFV ($p = 0.02$), with 64% and 45% at 1 and 5 years, respectively. VT burden was reduced irrespectively from the type of ablation ($p < 0.001$).

Most recently, a single center experience on 30 ARVD/C patients who underwent epicardial catheter ablation of VT showed a significant reduction ($p < 0.001$) of VT burden (Philips et al., 2015). Twenty seven percent ($n = 8$) of patients had VT recurrence after epicardial RFA. Most of the VT recurrences occurred during exercise, in the first year after the procedure, had fast cycle lengths and required an ICD intervention for termination.

The current recommendations for catheter ablation are for patients with incessant VT or frequent appropriate ICD therapies despite maximal pharmacological therapy. An epicardial approach is for those who fail one or more attempts of endocardial VT ablation. Catheter ablation should also be considered in patients with incessant VT or frequent ICD therapies who have failed pharmacological therapy. A combined approach (endocardial/epicardial ablation) can be considered depending on the operator's experience. Catheter ablation may also be an alternative in those patients who do not want to be treated with pharmacological therapy. (Corrado et al., 2015)

Preventing Progression

The hypothesis that exercise is an environmental factor in the pathogenesis of this disease began with the observation that there are a disproportionate number of athletes among patients with ARVD/C although there are no studies prospectively examining the rate of progression of ARVD/C. Exercise restriction is presently considered the only effective strategy for preventing disease progression.

Evidence of the role of exercise in ARVD/C

One of the first studies to highlight the relationship between exercise and ARVD/C was done by Corrado et al. (2003)) in the region of Veneto, Italy. They found that young athletes had a fivefold risk of dying of ARVD/C compared with nonathletes.

Since then, many studies followed, and their aim has been to determine the role of exercise as a disease modifier in ARVD/C.

La Gerche et al. (2012) were one of the pioneer groups to observe that the ARVD/C phenotype can be acquired through intense exercise. They studied 47 athletes with definite or probable ARVD/C. The majority ($n = 41$) practiced endurance sports (cyclists 72%, distance runners 6%, triathletes 6%, and kayakers 2%). They identified desmosomal mutations in only six athletes, and only two patients in the group had a family history of ARVD/C. They found that the group that performed the most exercise had lower rates of

desmosomal mutations, yet many of them met diagnostic criteria for definite or probable ARVD/C. Based on these results, they concluded that high-intensity endurance exercise may be sufficient to evoke an ARVD/C phenotype, even in mutation-negative individuals.

Saberniak et al. (2014) investigated the impact of exercise on myocardial function. The study included 110 patients, 65 with ARVD/C and 45 mutation positive family members. Exercise participation was measured by metabolic equivalent (MET) minutes per week. Athletes were classified as those participating in vigorous ($\geq$6 METs) for $\geq$4 h/week. The authors found that athletes were more likely to meet diagnostic criteria; they had lower biventricular function and earlier onset of life-threatening VA when compared with nonathletes.

Ruwald et al. (2015) studied 108 probands that met TFC for ARVD/C diagnosis. They assessed the effects of competitive and recreational sports on age of onset and risk of VA or death. Patients self-reported the age at which they initiated the sport and the commonly practiced sport via a questionnaire. They found that the patients engaged in competitive exercise had an earlier presentation of the disease and also had a twofold increase in the risk of life-threatening arrhythmias and death when compared with inactive patients and those practicing recreational sports.

Posteriorly, three important manuscripts were published better defining the link between exercise in ARVD patients with and without mutations and examining the important question of a safe exercise threshold.

The first paper (James et al., 2013) studied the role of exercise in ARVD/C patients with a pathogenic desmosomal mutation. For this purpose, 87 probands and family members were interviewed to determine their exercise used for leisure/recreation, work, and transportation. Participants were classified as an endurance athlete if they performed 50 h/year or more of vigorous intensity sports with a high dynamic demand as defined by the 36th Bethesda Conference Classification of Sports (Task Force 8) (Maron and Zipes, 2005). Our study had several key findings. ARVD/C patients who were endurance athletes became symptomatic at an earlier age. Furthermore, endurance exercise and higher duration of exercise participation were both associated with increasing likelihood of developing manifest ARVD/C. Endurance exercise was also associated with worse survival from a ventricular arrhythmia and heart failure. Lastly, those individuals after presentation who continued to participate in the top quartile for hours of annual exercise had worse survival from first ventricular arrhythmia compared with individuals who reduced their exercise after presentation.

Subsequently, the role of exercise in ARVD/C patients without mutations was reported (Sawant et al., 2014). The duration and intensity of exercise participation in 43 probands without a genetic desmosomal defect were compared with that of 39 probands with desmosomal mutations all of whom met the 2010 TFC. The results show that among nondesmosomal patients, those participating in the highest quartile of MET-hours/year of exercise presented at a significantly earlier age had worse structural abnormalities on CMR and significantly poorer survival from a ventricular arrhythmia during follow-up. Also, exercise participation stratified by mutation status and family history was analyzed. It was observed that nondesmosomal ARVD/C patients had done a similar duration of annual exercise as the desmosomal carriers, but exercise in the nondesmosomal patients was more intense, and more subjects participated in endurance sports and expended greater MET-hours/year of exercise compared with desmosomal patients. Lastly, ARVD/C nondesmosomal patients with no family history performed the highest MET-hours/year exercise compared with nondesmosomal patients with a family history and desmosomal mutation carriers (Fig. 7). Since the nondesmosomal patients

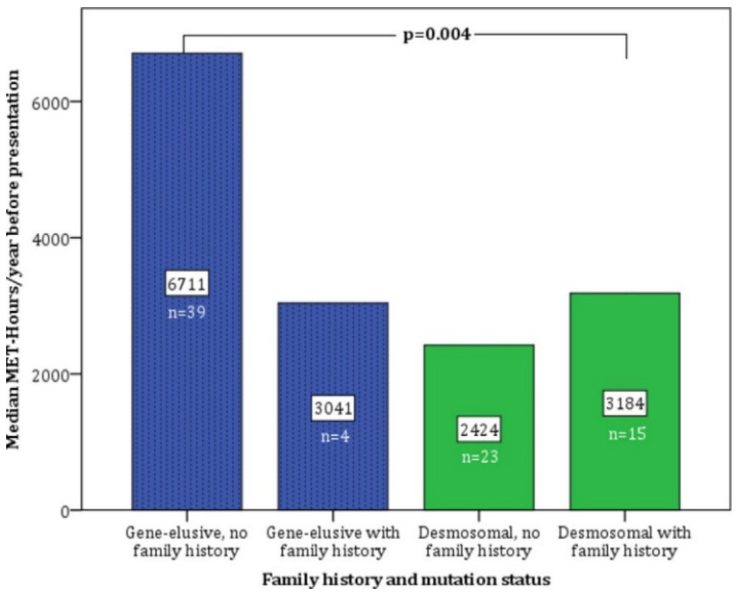

Fig. 7 Exercise intensity among patients stratified by genotype and 2010 family history task force criteria (TFC). Gene-elusive, nonfamilial patients participated in significantly higher-intensity exercise than those with family history or desmosomal mutations ($P = 0.004$, Kruskal–Wallis one-way analysis of variance). MET-hours indicate metabolic equivalent hours. Reproduced with permission from Sawant, A. C., Bhonsale, A., and Te Riele, A. S. et al. (2014). Exercise has a disproportionate role in the pathogenesis of arrhythmogenic right ventricular dysplasia/cardiomyopathy in patients without desmosomal mutations. *Journal of the American Heart Association*, **3**(6), e001471.

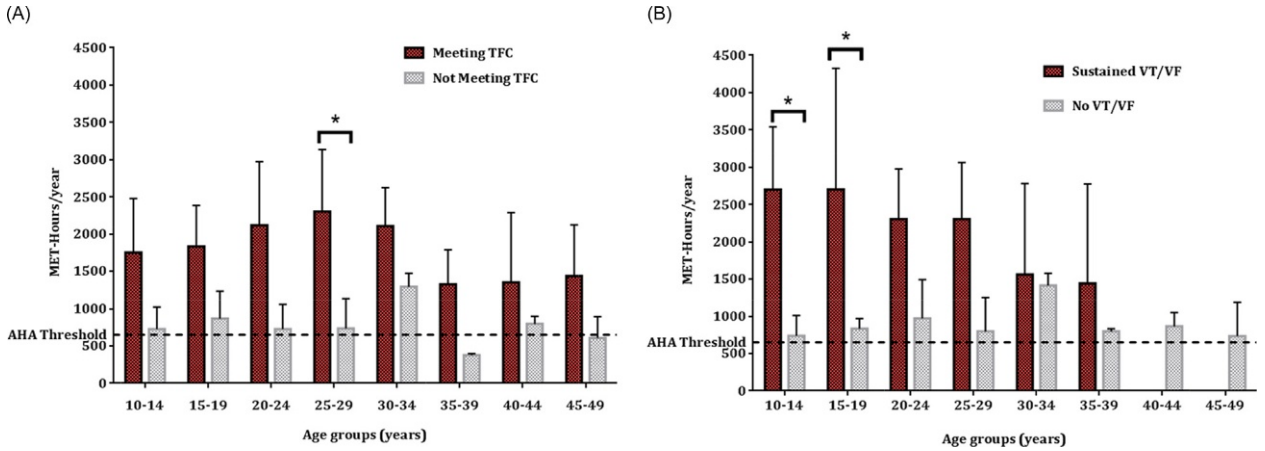

Fig. 8 Median exercise intensity (MET-hour/year) before diagnosis in 5-year age increments compared with the AHA-recommended minimum stratified by diagnosis by TFC (A) and VT/VF history (B). *Dotted black line* represents the upper bound of the American Heart Association (AHA)-recommended minimum exercise (650 MET-hour/year). Error bars depict interquartile range. *Statistically significant $P < .05$. *MET-hr*, metabolic equivalent hours; TFC 2010 task force criteria; *VT/VF*, ventricular tachycardia/ventricular fibrillation. Reproduced with permission from Sawant, A. C., Te Riele, A. S., and Tichnell, C. et al. (2015). Safety of American Heart Association-recommended minimum exercise for desmosomal mutation carriers. *Heart Rhythm*, **13**(1), 199–207.

without family history did the highest intensity exercise, they concluded that exercise may be the key environmental factor driving disease pathogenesis in that subgroup. Interestingly, when we compared annual prepresentation MET-hours and endurance exercise participation during youth (age ≤25), it was found that higher-intensity exercise during youth was associated with earlier age of presentation in both desmosomal and nondesmosomal ARVD/C patients.

The safety of the American Heart Association (AHA) minimum exercise recommendations for overall health (390–690 MET-h/year), for healthy desmosomal mutation carriers, has also been assessed (Sawant et al., 2015). Twenty-eight family members of 10 probands with a PKP2 mutation were interviewed. Probands had done more exercise than family members, and the family members that were athletic had worse outcomes. However, there was no life-threatening VA in the healthy carriers that restricted exercise at or below the upper bound of the AHA minimum exercise recommendations. Furthermore, at the time of ARVD/C diagnosis and their first ventricular arrhythmia, patients had accumulated 2.8-fold and a 3.5-fold greater MET-hour of exercise than the AHA recommendations (Fig. 8). The authors concluded that unaffected PKP2 mutation carriers should refrain from endurance and high-intensity exercise but that they may be safe practicing the minimum exercise for healthy adults recommended by the AHA, although this continues to be an area of active research.

The link between exercise and ARVD has also been demonstrated using transgenic animal models (Kirchhof et al., 2006; Cruz et al., 2015; Chelko et al., 2014; Martherus et al., 2015). These data together show that there is need for more research to evaluate what threshold of exercise triggers the onset of ARVD/C. We also need to understand the interaction of genotype and exercise and other environmental factors with the potential of triggering the disease.

In summary, exercise restriction is a key component in the management of ARVD/C. Recently, an update to the 36th Bethesda Conference Eligibility Recommendations for Competitive Athletes with Cardiovascular Abnormalities has been published by the AHA and the American College of Cardiology (Maron et al., 2015). The current recommendation is avoidance of all competitive activities for patients with probable, borderline, or definite diagnosis of ARVD/C except for sports with a low cardiovascular demand (class III and level of evidence C).

The ARVD/C treatment consensus statement, published by Corrado et al. (2015), concluded that patients with a definite diagnosis of ARVD/C should not participate in competitive or endurance sports with the possible exception of recreational low-intensity sports. Restriction from competitive sports may be considered in ARVD/C family members that are healthy gene carriers and those that have an unknown genotype.

Cardiac transplantation

ARVD/C patients rarely require cardiac transplantation (Tedford et al., 2012), but among 18 ARVD/C transplants, the age of first symptoms averaged 24 ± 13 years, and the age at cardiac transplantation averaged 40 ± 14 years, suggesting early onset of disease in those ultimately requiring transplantation.

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Athlete's Heart

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Introduction

Physical exercise is dependent on the ability of the cardiovascular system to provide oxygenated blood to the critical organs and to remove deoxygenated blood from tissues and the lungs ability to clear carbon dioxide. With exercise a greater proportion of the oxygen is required in the tissue and is extracted from the blood. Athlete's heart or athletic heart syndrome refers to cardiac adaptations that occur from repetitive strenuous physical exercise.

Assessment of gas exchange at rest and with exercise can be performed with cardiopulmonary stress testing. With exercise, there is increasing oxygen demand and oxygen uptake by the tissues. In time physiologic adaptations seen with exercise include a lower blood pressure, lower heart rate, enhanced ventricular contraction, and relaxation. Cardiac output, the product of stroke volume and heart rate, increases with exercise in which a majority this augmentation is due to the increase in heart rate. Other physiologic changes such as peripheral vascular resistance vary depending on the type of exercise performed.

The hemodynamic effects of strenuous training cause differing cardiac adaptations depending on the type of exercise, so-called sport-specific cardiac remodeling. Morganroth and colleagues were first to classify the hemodynamic effects of exercise into two main groups resulting from either endurance (dynamic) and strength (static) exercise (Morganroth et al., 1975).

Isotonic exercise as seen with endurance athletes leads to sustained elevations in cardiac output with a normal to reduced peripheral vascular resistance. This sustained elevation in cardiac output causes a volume challenge to all heart chambers and the aorta which may lead to dilatation. Examples of isotonic exercises include long distance running and swimming. Individuals exposed to endurance training demonstrate left ventricular (LV) enlargement with balanced increases in LV cavity size and wall thickness (Gilbert et al., 1977; Utomi et al., 2013).

Conversely, isometric exercise as seen with strength training or short burst exercises causes a brief but potentially marked elevation in systolic blood pressure and LV afterload. This predominant pressure elevation may lead to cardiac remodeling including concentric LV remodeling and hypertrophy. Examples of isometric exercises include weight lifting and track and field throwing.

Morganroth's original hypothesis did not address cardiac remodeling that may be influenced by ethnicity, age, gender, duration of training and genetics (Maron, 1986). When training, athletes usually focus on exercises that will strengthen specific muscles related to their sport and a combination of both isometric and isotonic exercises is common. Frequently combined isometric and isotonic training (cycling and rowing) result in both volume and pressure load to the heart. This may make it difficult to differentiate pathology from cardiac adaptation to strenuous exercise. Pathologic states such as hypertrophic cardiomyopathy (HCM) usually have asymmetric hypertrophy.

Most amateur athletes have normal chamber dimensions; however LV dilation may occur (**Figs. 1, 2**). In a large series of athletes, LV end diastolic diameter exceeded normal in 40% of patients but only 14% of highly trained athletes had LV chamber dimensions of >60 mm (Pelliccia et al., 1999). Chamber enlargement can be accompanied by a relatively mild increase in absolute LV wall thickness (from 13 to 15 mm) (Pelliccia et al., 1991) and should reverse after cessation of exercise. Hypertrophy greater than 16 mm should raise concern for HCM. LV systolic function in athletes remains normal or low normal with no wall motion abnormalities and preservation right ventricular (RV) contractile function with exercise.

Remodeling of the left atrium (LA) is a physiological adaptation present in highly trained endurance athletes (Pelliccia et al., 2005). Increased anterior–posterior LA dimension (≥ 40 mm) occurs in 20% of athletes where larger dimensions (≥ 45 mm) occur in approximately 2% of athletes (Maron and Pelliccia, 2006). Although there is a quantitative increase in LA size, the overall proportion of the LA should remain proportional to the LV cavity size. Atrial arrhythmias such as atrial fibrillation are also increased in endurance athletes (Mont et al., 2009; Furlanello et al., 1998).

Understanding the aforementioned cardiac adaptations to training is important as these findings will help differentiate cardiac adaptations from pathology. Exercising with an underlying cardiomyopathy may increase the risk of adverse events including arrhythmia and sudden cardiac death. The United States National Registry analyzed 842 athletes over 31 years with autopsy-confirmed cardiovascular diagnoses after sudden cardiac death and found that men had a 6.5 fold higher incidence of disease compared to women and adverse events were also more frequent in nonwhites (Maron et al., 2016). In that series, HCM was the leading cause of sudden cardiac death in athletes.

HCM is a well recognized cause of exercise-related sudden cardiac death among athletes. The prevalence of the disease is approximately 1 in 500 and over 1500 mutations have been associated with the disease (Maron et al., 1995; Maron et al., 2012). Individuals with HCM have characteristic myofibrillar disarray on histologic inspection and gross analysis of the ventricle

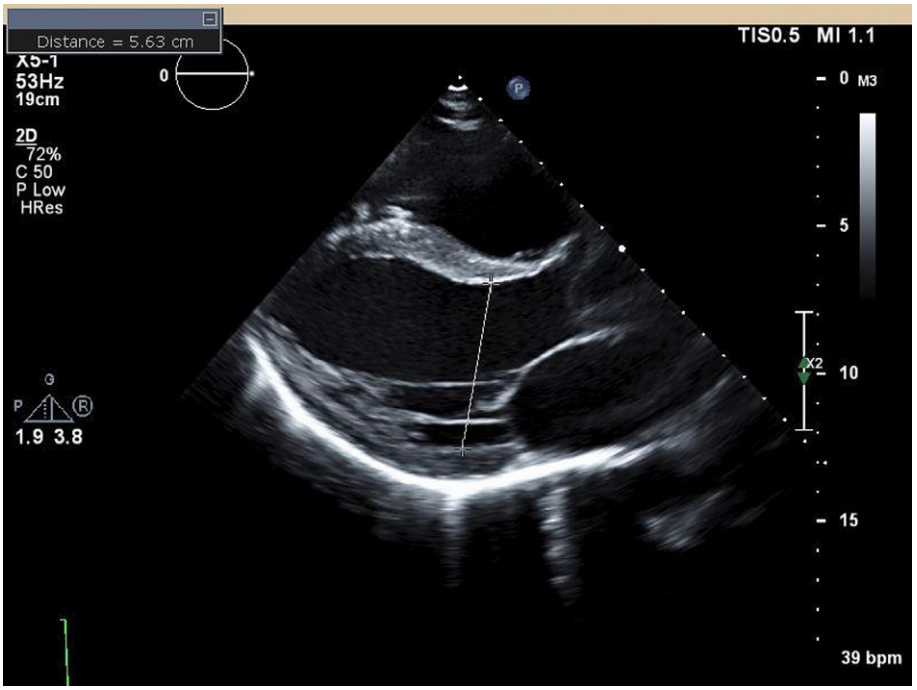

Fig. 1 Transthoracic echocardiographic imaging of an elite athlete showing left ventricular chamber dilation.

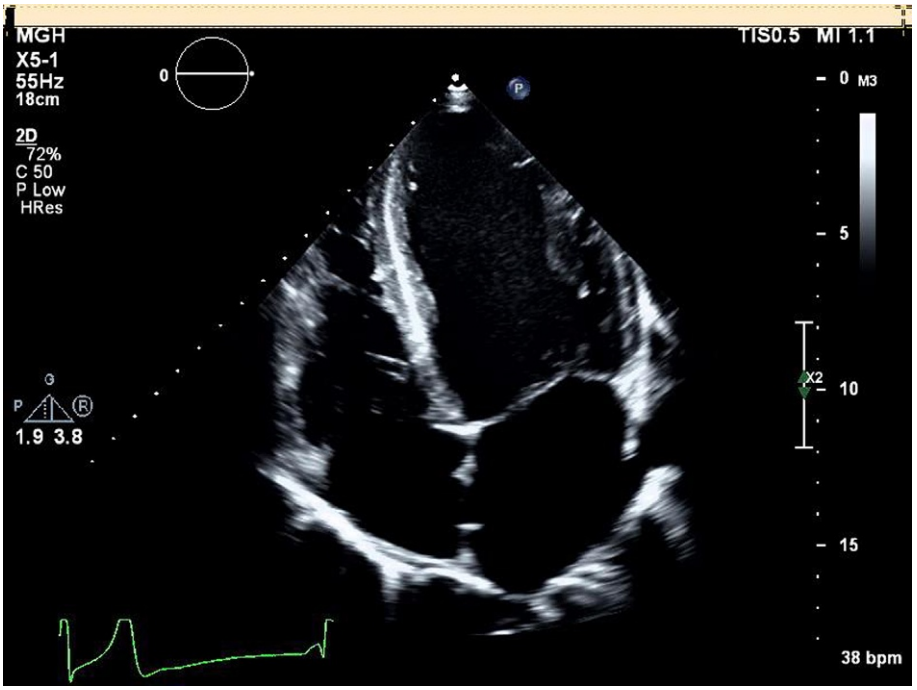

Fig. 2 Transthoracic echocardiographic imaging of an elite athlete showing left ventricular and left atrial enlargement but preservation of overall chamber ratios.

demonstrates LV hypertrophy with increased ventricular wall thickness in either symmetric or asymmetric distribution (Fig. 3). This myocardial hypertrophy can cause LV outflow obstruction and systolic anterior motion (SAM) of the anterior mitral valve leaflet leading to mitral regurgitation (Figs. 4, 5).

The presence of underlying HCM may trigger exercise-induced arrhythmias and patients may present with syncope, lightheadedness with exertion, palpitations or even with sudden cardiac death. The underlying substrate for exercise-induced arrhythmias is unpredictable and athletes with clinical expression of HCM should limit athletic participation to low-intensity

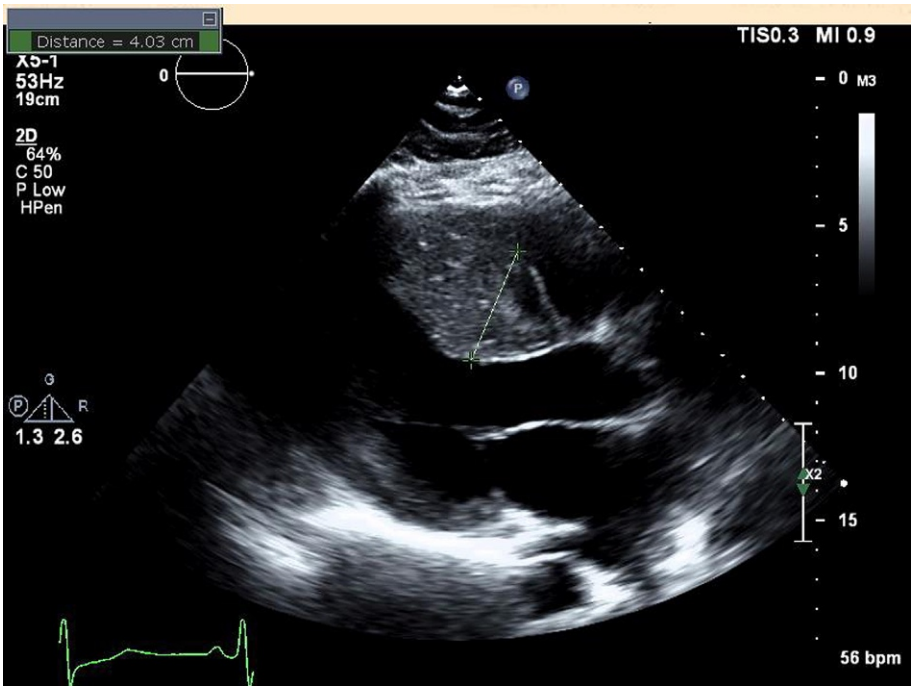

Fig. 3 Transthoracic echocardiographic imaging of a patient with hypertrophic cardiomyopathy showing asymmetric septal hypertrophy.

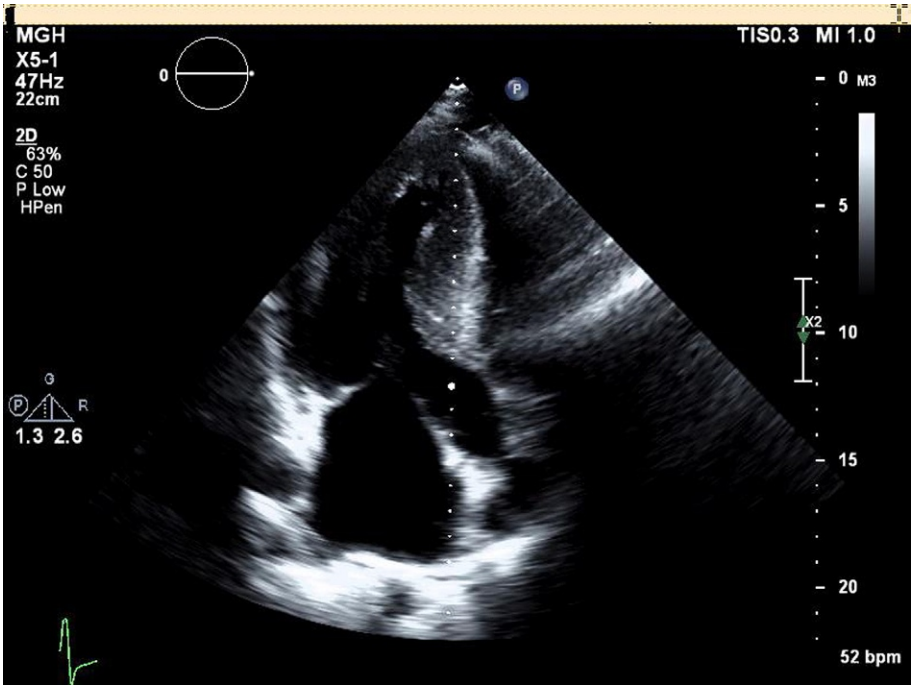

Fig. 4 Transthoracic echocardiographic imaging of a patient with hypertrophic cardiomyopathy showing asymmetric septal hypertrophy and systolic anterior motion of the anterior mitral valve leaflet.

sports (Levine et al., 2015). For patients who are genotype positive but have no phenotypic expression of the disease, participation in competitive athletics is reasonable.

Echocardiography is the preferred initial imaging modality for the diagnosis of HCM. An LV wall thickness of greater than 16 mm is suggestive of HCM. Differentiating LV hypertrophy secondary to exercise remodeling from HCM may be challenging, especially in those individuals whose LV myocardium measures 13–15 mm. Pelliccia and colleagues performed echocardiographic assessments of LV wall thicknesses in 947 elite athletes and found that 1.7% of athletes have LV wall thickness greater than 13 mm

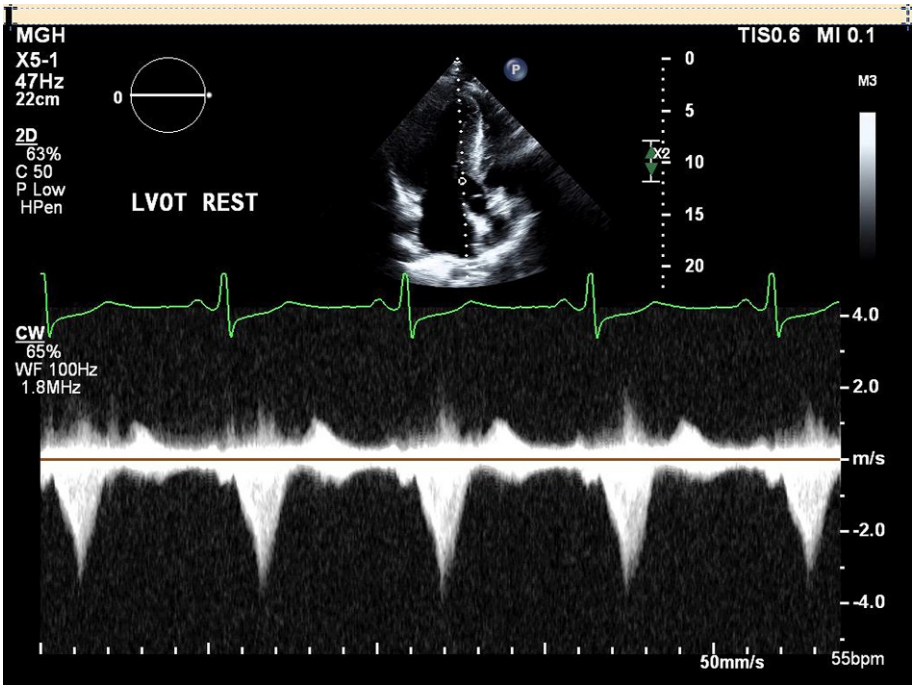

Fig. 5 Transthoracic echocardiographic imaging of a patient with hypertrophic cardiomyopathy showing left ventricular outflow gradient at rest.

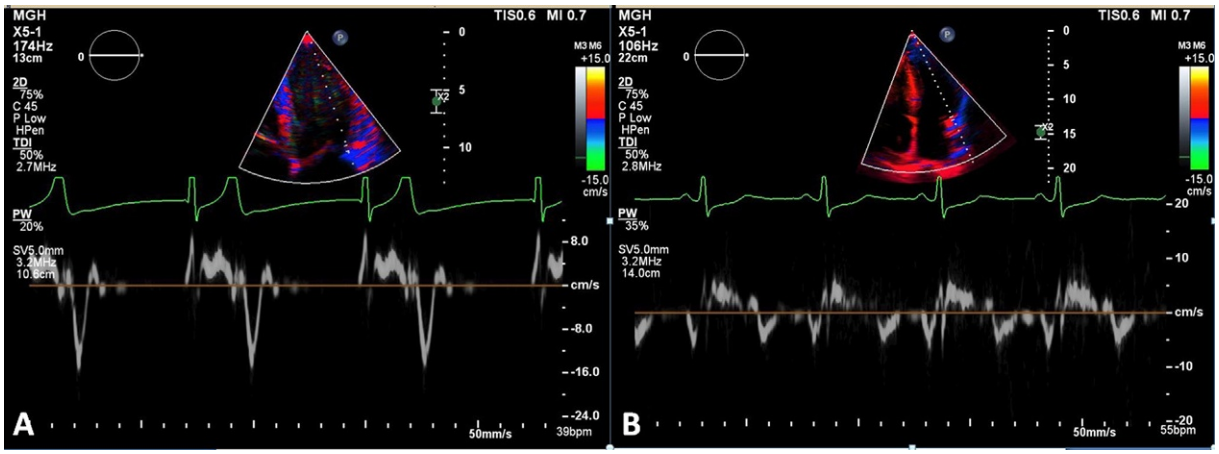

Fig. 6 Echocardiographic tissue Doppler velocities of an elite athlete (A) and a patient with hypertrophic cardiomyopathy (B) showing increased tissue Doppler in the athlete and decreased velocities in the patient with hypertrophic cardiomyopathy.

and all those patients had concomitant LV cavity dilation, a combination not typically found among individuals with HCM (Pelliccia et al., 1991).

Diastolic function of the LV can also be helpful in differentiating between the two diagnoses. The diastolic pulsed tissue Doppler velocities of athletes are usually normal or increased whereas usually reduced in patients with HCM (**Fig. 6**) (Caselli et al., 2014; Cardim et al., 2003). In athletes and transmitral *E*/*A* ratios are often >2 with low *A* velocities. This differs from HCM patients where *E*/*A* is <1 and the *E* velocity deceleration time is increased (D'Andrea et al., 2015).

Left ventricular noncompaction (LVNC) is a rare cardiomyopathy thought to occur due to failure of myocardial compaction during embryological development. LVNC is characterized by increased LV trabeculations, intertrabecular recesses often referred to as "crypts," and thin layer of compacted myocardium. Usually the recesses are located at the LV apex, mid-inferior and lateral walls of the left ventricle and less likely to be seen on the septal wall. Coronary circulation is usually normal although global ventricular function may be depressed and thrombi may form within the crypts. Familial and sporadic cases have been identified.

The natural history of LVNC has not been well defined. Patient presentation of LVNC varies from asymptomatic to presentation with heart failure, thromboembolic events, arrhythmias, or sudden cardiac death. Patient's are managed medically with a focus on associated complications of LVNC and may include arrhythmia ablation procedures, ICD placement, or cardiac transplant in advanced disease.

To help guide the diagnosis, along with a through history and physical, imaging is usually ordered. Echocardiography is usually the first imaging modality ordered to evaluate for LVNC for both the index patient and family members. 2D-echocardiography can identify increased trabeculations in the left ventricle (**Figs. 7,8**). Also, color Doppler and echocardiographic contrast can be used to show blood flow into the crypts thereby decreasing the likelihood of thrombi. A ratio of noncompacted myocardium to compacted myocardium of 2:1 on echocardiography was suggested to help guide the diagnosis of LVNC (Jenni et al., 2001; Ritter et al., 1997). Cardiac magnetic resonance (CMR) is better able to distinguish the border of compacted and noncompacted myocardium. A ratio of noncompacted to compacted myocardium on CMR of >2.3 was suggested for diagnosis of LVNC (Patersen et al., 2005). CMR is also able to comment on the presence of fibrosis and to evaluate ventricular function.

In athletes, the LV remodels and dilates causing the appearance of increased trabeculations in the left ventricle. Caution should be made to not diagnose LVNC in those athletes whose ventricles have remodeled and do not necessarily carry the disease. The presence of increased trabeculations has also been reported in African–American athletes (Gati et al., 2013). Patients with confirmed LVNC with LV systolic dysfunction or tachyarrhythmias should not participate in competitive sports.

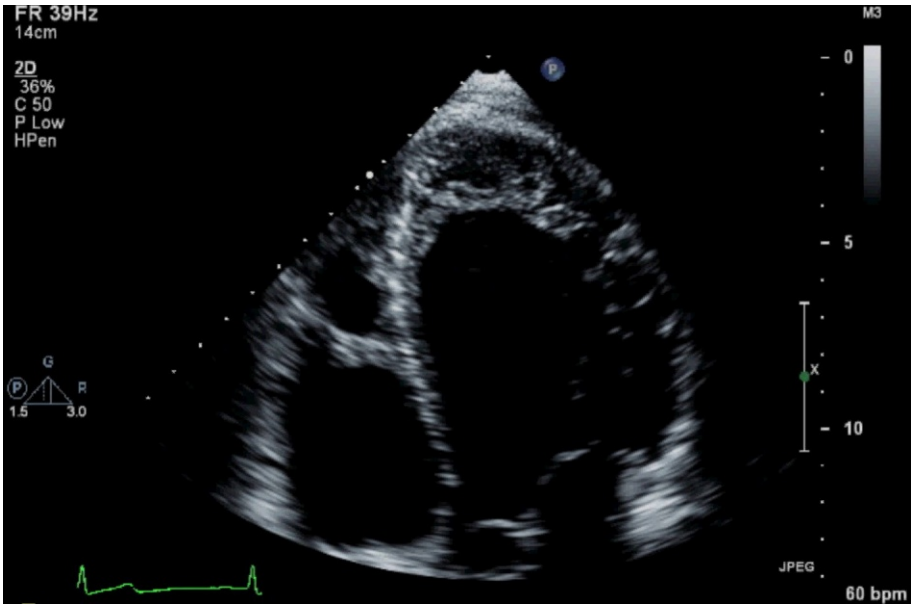

Fig. 7 Echocardiographic image of the left ventricle showing increased left ventricular trabeculations.

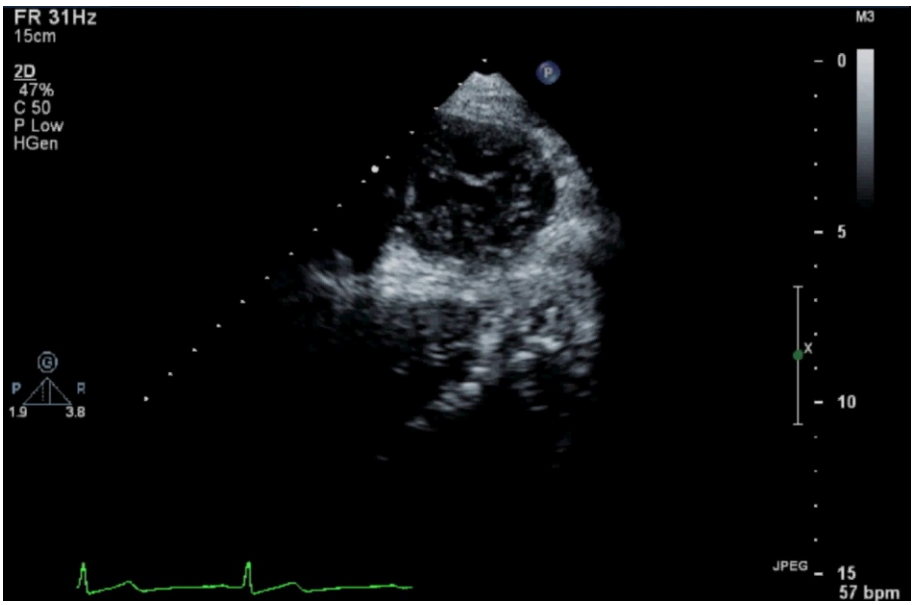

Fig. 8 A zoomed-in echocardiographic image of the left ventricular apex showing increased trabeculations in a patient with LVNC.

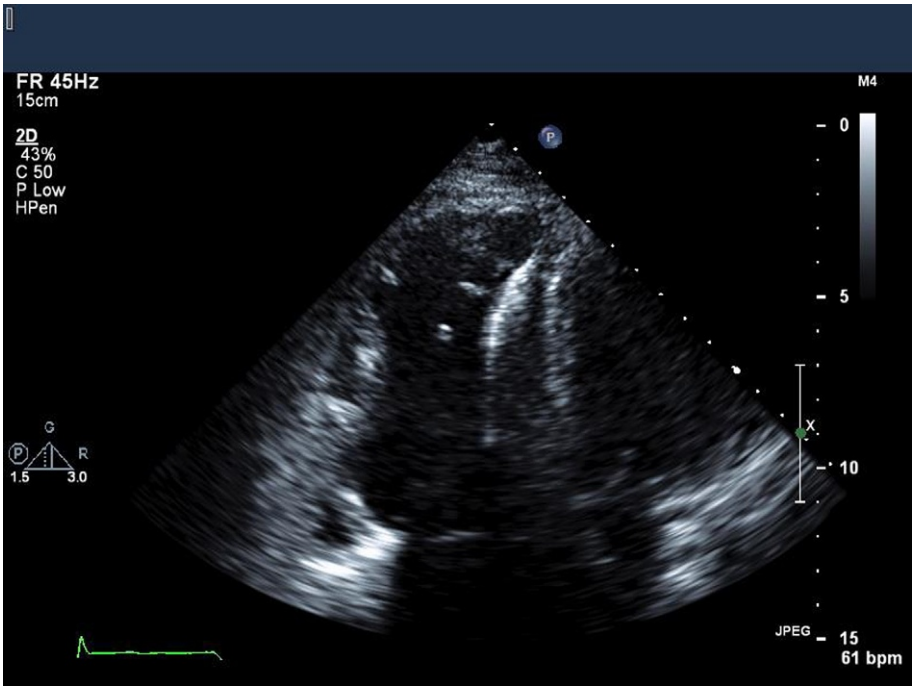

Fig. 9 RV focused view on a patient with ARVD showing a dilated right ventricle with thinned walls. Also seen is an ICD wire in the RV cavity.

Arrhythmogenic right ventricular cardiomyopathy (ARVC), also known as arrhythmogenic right ventricular dysplasia (ARVD), is a rare cardiomyopathy characterized by progressive replacement of the RV myocardium with fibrofatty tissue. The RV outflow tract, RV apex, and infundibulum are usual places of disease involvement. ARVC is an autosomal dominant disorder with variable penetrance. The genetic defect leads to desmosome dysfunction (which work with cell–cell adhesion) causing a "defective glue." The natural history of ARVC includes progressive RV dysfunction and electrical instability (Hubot et al., 2004). Most patients usually present before the age of 40 with right heart arrhythmias although presentation with right-sided heart failure is not uncommon.

Imaging modalities used for the assessment and diagnoses include electrocardiogram, echocardiography and CMR. On electrocardiogram, T wave inversions in precordial leads (V1–V3), epsilon waves, and tachyarrhythmias with left bundle branch pattern may be seen. On echocardiography, the right ventricle is dilated, hypokinetic, with possible aneurysm formation (**Fig. 9**). A thickened or hyperreflective moderator band has also been reported (Bauce et al., 2010). Quantitative measures of RV function such as RV fractional change may be decreased. Fatty infiltration of the RV myocardium can be seen with CMR. A thinned and dilated right ventricle may be seen with computed tomography (**Fig. 10**). The gold standard for diagnosis of ARVD is an endomyocardial biopsy that shows fibrofatty tissue replacement of the RV myocytes and a thinned RV wall.

In endurance athletes, RV dilation may occur (Baggish et al., 2008). This cardiac remodeling and ventricular dilatation in athletes may make the normal trabeculations of the RV more prominent in appearance and difficult to differentiate from pathology. If arrhythmia is found, specifically paroxysmal ventricular tachycardia originating from the right heart, in an otherwise healthy athlete, ARVD should be excluded. Patients with ARVC should avoid competitive sports with the exception of low-intensity class 1A sports.

Screening and Athletic Competition

A comprehensive history and physical examination is recommended prior to athletic competition in order to detect congenital cardiovascular abnormalities. Currently, routine ECG screening for athletes is not recommended by the American Heart Association however may be considered in small cohorts along with the history and physical with awareness that false positives may occur.

Conclusions

Athletic heart syndrome refers to the cardiac remodeling that occurs in response to the hemodynamic challenges of repetitive strenuous exercise. Sport-specific remodeling occurs and it is important to be able to recognize these changes and differentiate them from pathology.

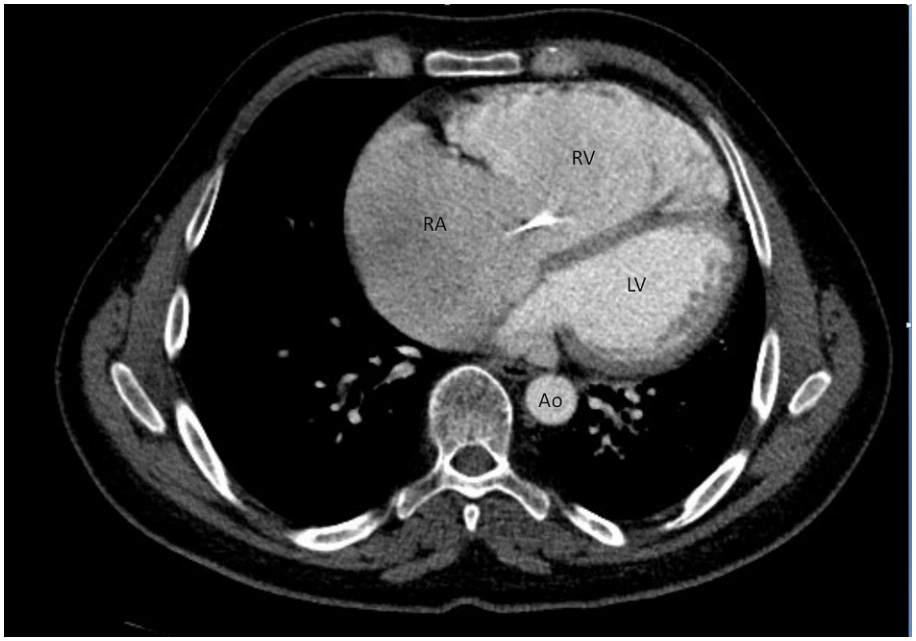

Fig. 10 Contrast computed tomography of a patient with ARVD showing dilated right heart chambers and a thinned right ventricular wall. *RA*: right atrium. *RV*: right ventricle. *LV*: left ventricle. *Ao*: aorta.

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Atrial Tachycardia—Mechanisms and Management

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Introduction

Atrial tachycardias represent a subset of the supraventricular tachycardias. They are characterized by their need only for atrial tissue, not requiring the sinus node, presence of an accessory pathway, or ventricular tissue, for their initiation and perpetuation. They can be further divided into subcategories based on the underlying mechanism of arrhythmia, including atrial flutter—typical and atypical, focal atrial tachycardia, sinus node reentry, and multifocal atrial tachycardia (MAT). We will consider each subcategory separately.

Atrial Flutter

Background

Atrial flutters represent a class of macroreentrant tachycardias within one or both atria. They share features with atrial fibrillation, particularly in terms of anticoagulation and morbidity, but, in many cases, are extremely treatable via catheter-based approaches. They fall under two large categories: the more common typical or isthmus-dependent flutters, and the less common, and often more difficult to treat, atypical or nonisthmus-dependent flutters.

Definition

Unfortunately, the definition of atrial flutter can be confusing. There is an electrocardiographic one which describes flutter as an organized atrial rhythm usually between 250 and 300 beats/min. There is also a mechanistic one which defines flutter as a macroreentrant rhythm, with less concern about rate. As the mechanistic definition makes understanding of the physiology, pathophysiology, and therapeutic alternatives easier to grasp, it is the one that most electrophysiologists prefer.

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Isthmus-dependent atrial flutter is one that uses the cavotricuspid isthmus as an essential part of its circuit, regardless of whether it travels through the isthmus in a clockwise or counterclockwise fashion. Any other macroreentrant tachycardia is termed an atypical flutter (i.e., not dependent on the cavotricuspid isthmus for its perpetuation).

Typical and atypical flutters share the same general characteristics with the major difference being that the former are easy targets of ablation, while the latter can be quite challenging. The following discussion will discuss both of them as a unit except where they differ in significant fashion.

Incidence

It has been difficult to determine the incidence of atrial flutter as it typically has been grouped with atrial fibrillation, or often miscategorized as atrial fibrillation or atrial tachycardia. The Marshfield Epidemiological Study Area reported an overall incidence of 88/100,000 person-years. Incidence rates ranged from 5/100,000 in those <50 years old to 587/100,000 in subjects older than 80. Atrial flutter was 2.5 times more common in men ($P < 0.001$). The risk of developing atrial flutter increased 3.5 times ($P < 0.001$) in subjects with heart failure and 1.9 times ($P < 0.001$) for subjects with chronic obstructive pulmonary disease. Among those with atrial flutter 16% were attributable to heart failure and 12% to chronic obstructive lung disease (Granada et al., 2000). This translates, assuming one can extrapolate to whole of the US population, to an incidence of 200,000 cases of atrial flutter per year. It accounts for about 15% of supraventricular arrhythmias.

Characteristics

Typical flutter

The right atrium is a very complex structure composed of venous orifices, embryological remnants, ridges, and trabeculations. The anterolateral portion, which is trabeculated and includes the right atrial appendage, is separated from the smooth portion of the right atrium which originated embryologically from the sinus venosus. These embryologically distinct portions are separated by the crista terminalis laterally and the Eustachian ridge inferiorly. The crista terminalis is an electrophysiologically important structure as it acts as a line of block to conduction. It runs from just below the origin of the superior vena cava down to just above of the inferior vena cava, where it is extended by the Eustachian ridge along the floor of the right atrium to the os of the coronary sinus. These structures provide anatomical barriers which allow atrial flutter to exist in a protected circuit, which is necessary for perpetuation of reentry. The bottleneck for the circuit is the cavotricuspid isthmus, which is the portion of right atrium between the inferior portion of the tricuspid annulus and the orifice of the inferior vena cava. Because of its dependency on this protected isthmus, the electrocardiographic characteristics are very distinctive and reproducible between individuals.

Typical atrial flutter, which may be referred to as type I flutter by electrocardiographers, has atrial rates that are usually 250–350 bpm, with 300 bpm being the most common. Since the AV node in most individuals cannot conduct atrial rates that high in 1:1 fashion, there is usually 2:1 conduction, but it may be slower. In situations where antiarrhythmic drugs are used which result in slowing of the flutter rate, in very large atria where the circuit length is increased or in very young individuals or individuals with accessory pathways, atrial flutter can conduct 1:1. This latter situation is usually very poorly tolerated and can result in hemodynamic collapse. It may also result in misdiagnosis of the rhythm as a supraventricular tachycardia or a ventricular tachycardia, particularly as the QRS may be widened at these rates in the setting of aberrancy, preexcitation, pseudowidening of the QRS by superimposition of the QRS on the flutter wave or antiarrhythmic drug effect.

On ECG, the flutter waves are regular, recurring, and identical resulting in the classical "saw tooth pattern" with continuous atrial activation (i.e., lack of an isoelectric portion on the ECG). This pattern is most often demonstrated in the inferior leads (II, III, aVf). If the QRS is widened for any reason, it may be difficult to spot these saw tooth waves when 2:1 conduction or faster is present. In these situations, Valsalva maneuver, adenosine, or carotid sinus massage may help in establishing the diagnosis by slowing AV-node conduction, decreasing ventricular rate, and thus unmasking typical flutter waves. These waves are negative in the inferior leads in the case of counterclockwise flutter (referring to how the circuit travels relative to the tricuspid valve in the left anterior oblique projection), but will be positive in the less common clockwise atrial flutter(Bochoeyer et al., 2003) (**Fig. 1**).

Flutter may be paroxysmal, particularly in individuals with structurally normal hearts. In those with larger atria, it may be sustained and very stable, often presenting as tachycardia-induced cardiomyopathy. Although often a primary arrhythmia, it may accompany thoracic surgery, pericarditis, myocarditis, myocardial ischemia, or exacerbations of pulmonary disease.

Clinical presentation is very variable and depends on rate, underlying heart disease and age. Range of presentations include asymptomatic individuals in which it is discovered as an incidental finding, palpitations, fatigue, exercise intolerance, lightheadedness, dyspnea, and in the cases of 1:1 conduction, may present as syncope or hemodynamic collapse. Untreated flutter is a predictor of several poor outcomes, as evidenced by the Framingham Heart Study(Rahman et al., 2016).

Atypical flutter

Atypical flutters can occur anywhere within the atria and as such, do not have a typical electrocardiographic appearance unlike typical flutters(Bochoeyer et al., 2003; Gerstenfeld et al., 2007). They are defined as having the same range of rates as with typical flutter (250–350) and also share the characteristic lack of isoelectric segment on the ECG. Unlike typical flutters, they tend to not occur in structurally normal hearts as they usually require an anatomical focus for reentry, such as an atriotomy scar or prior

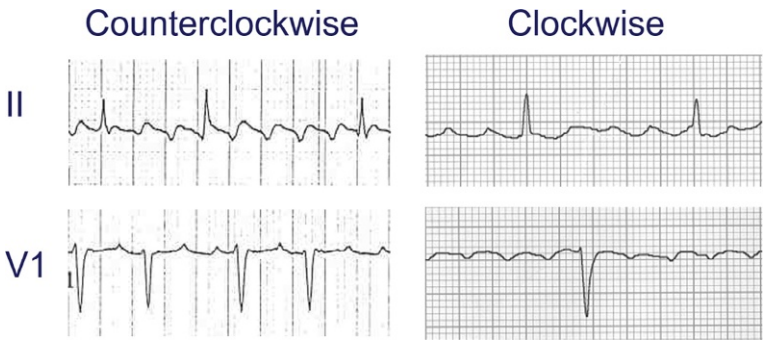

Fig. 1 Representative leads II and V1 demonstrating common appearance of flutter waves for each of counterclockwise and clockwise atrial flutter. With counterclockwise atrial flutter, classical "saw tooth pattern" is seen in lead II and positive flutter waves in lead V1. For clockwise atrial flutter, positive flutter waves are seen in lead II and negative waves in lead V1.

surgeries or ablations. That having been said, they can occur due to functional reentry in the setting of favorable pulmonary vein anatomy or as a proarrhythmic response to antiarrhythmic drugs, most commonly Ic agents such as flecainide and propafenone. The circuits may be very large, or relatively small. In the latter situation, the line between flutters with small reentrant circuits and atrial tachycardia becomes blurred.

Clinical presentations are as variable as with atrial flutter, but they tend to be more sustained.

Mechanisms

Typical atrial flutter represents the quintessential macroreentrant rhythm. In order to have reentry, several elements need to be present:

- A protected isthmus (in this case the cavotricuspid isthmus) which can only be entered on either end (like an insulated electrical cable).
- Unidirectional block in one limb of the circuit. If this is not present, the circuit will terminate as wave fronts coming from opposite directions in the isthmus will collide. A premature atrial contraction will enter the isthmus and block in one direction but conduct in the other. However, the conduction needs to be slow enough to allow recovery of the tissue from the previous depolarization, otherwise, the wave front will encounter refractory tissue and terminate. This brings up the final requirement for reentry:
- Slow conduction required for both initiation and perpetuation. This can be due to medications, particularly sodium channel blockers, which cause slowing of wave front propagation: a classic proarrhythmia of this class of drugs. More frequent causes are enlarged atria which, while not directly causing slow conduction, allow a larger circuit and therefore longer transit time. Fibrosis in the annulus, from pacemaker of ICD leads, prior ablations, valve surgeries, or inflammatory conditions are another predisposing factor(Waldo, 1995; Waldo et al., 1997).

The concept of anisotropic conduction also needs to be understood to understand the mechanisms of reentry. Anisotropic conduction occurs due to increased longitudinal distribution of gap junctions, as opposed to transverse. Therefore, longitudinal conduction can occur much more rapidly than transverse conduction. This is the case with the crista terminalis, for example, which thus creates an effective barrier to conduction in one direction. The crista terminalis linked on either end to the ostia of the vena cavae offer a posterior barrier to reentry with the tricuspid annulus providing the anterior barrier. This anisotropic conduction partially explains why counterclockwise flutter is more common than the clockwise variant as fiber geometry in the annulus appears to favor unidirectional block in one direction over another(Waldo, 1995; Waki et al., 2000).

Many textbooks illustrate typical flutter as a two-dimensional circle around the tricuspid valve. In actual fact, a better way to visualize the circuit is as an upside-down basket where the handle represents the isthmus between the tricuspid annulus and the inferior vena cava. The handle then fans out to the basket proper representing the right atrial septum, the roof of the right atrium and the lateral wall joining up again on the other side. The left atrium is activated passively via the coronary sinus and Bachman's bundle in the roof of the interatrial septum (Fig. 2).

Management

Rate control

Beta-blockers and nondihydropyridine calcium channel blockers can be used for rate control. Digoxin can be added as an adjunctive agent, but is very unlikely to provide adequate rate control on its own. Digoxin used for rate control for atrial flutter should be done cautiously due to the often high doses required to achieve rate control. Because digoxin shortens the atrial action potentials, it may result in conversion of atrial flutter to atrial fibrillation. While this generally results in improved rate control, digitalis toxicity should

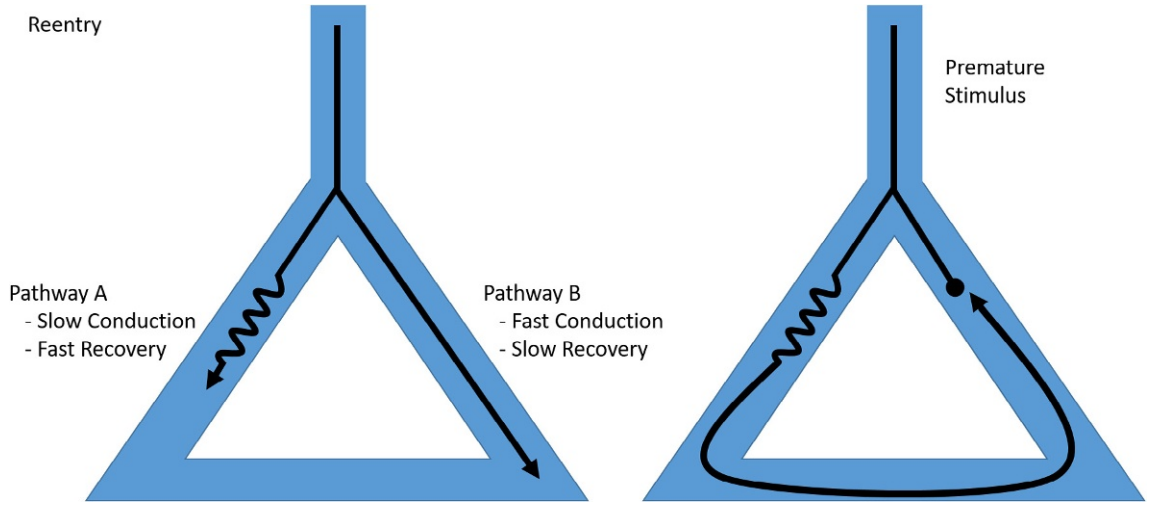

Fig. 2 Concept of reentry is depicted here. In the left panel, a stimulus that comes in at a normal timing interval will conduct down pathway B faster than pathway A. However, if an appropriately timed, premature stimulus is delivered (right panel), the stimulus will find pathway B refractory and conduct only down pathway A. As this conduction is slow, it will arrive back at pathway B, and find that it has recovered, then conduct in a retrograde fashion up pathway B. In similar fashion, pathway A has now recovered and reentry then ensues.

be suspected when this is observed. Nondihydropyridine calcium channel blockers should be avoided in patients with decreased ejection fractions due to their powerful negative inotropic effects. Atrial flutter is usually more difficult to rate control than atrial fibrillation; the reason for this is that conduction in flutter is usually 2:1, 3:1, 4:1, etc. For an atrial rate of 300 bpm, this amounts to ventricular rates of 150 bpm, 100 bpm, 75 bpm, etc., as opposed to the more linear dose-to-ventricular response curve that is possible in atrial fibrillation. Thus, the amount of medication required to slow a ventricular response in atrial flutter from 150 to 100 bpm tends to be much higher than for an equivalent decrease in ventricular rate in atrial fibrillation. Even when rate control is accomplished, the high dose of medications required usually causes side effects, such as fatigue, decreased exercise tolerance and lightheadedness as well other side effects specific to the medications used. Patients in flutter typically have very little heart rate variability which can result in chronotropic incompetence when rate control is excessive.

Adenosine will cause transient atrioventricular block and can be useful to unmask the flutter waves when there is 2:1 conduction and the QRS is wider, therefore making the flutter waves difficult to see. However, because flutter is not AV-node dependent, adenosine will not terminate the rhythm. Judiciously applied carotid sinus massage can accomplish the same effect as adenosine administration.

Because of the difficulties and limitations of the rate control strategy, it should not be considered a great long-term strategy in most cases. It should be seen as a bridge to more definitive care, such as a cardioversion or ablation (**Fig. 3**).

Amiodarone can also be used as a rate control agent in patients who do not tolerate beta-blockade or calcium channel blockers due to hypotension. Intravenous amiodarone will have a hypotensive effect due to the diluents, so has to be administered cautiously. Intravenous amiodarone functions predominantly as a noncompetitive calcium channel- and beta-blocker, but gets metabolized by the liver to *N*-desethylamiodarone, which has sodium and potassium channel-blocking properties. This metabolite starts having significant effect 24–36 h after continuous infusion, so conversion to sinus rhythm is possible. Therefore, care must be taken to ensure that risk of embolization is minimized in the event of conversion.

Anticoagulation

Due to the presence of organized atrial activity on the ECG, it was long felt that atrial flutter presented a lower stroke risk than atrial fibrillation. Due to the fact that atrial fibrillation often coexists in these patients, in addition to most large population studies failing to separate the two rhythms, the actual incidence of stroke prevention in atrial flutter is not truly known. In the Canadian Registry of Atrial Fibrillation database, there was no difference in stroke risk between atrial flutter and atrial fibrillation(Lelorier et al., 2004). Currently, guidelines recommend no distinction between atrial fibrillation and flutter In terms of long-term anticoagulation and periprocedurally for cardioversions(Page et al., 2016). More problematic is how long to continue anticoagulation after the flutter is ablated since many such patients go on to develop atrial fibrillation, which is often asymptomatic after a successful flutter ablation(Chinitz et al., 2007). A definite answer is not available at this time and, like all decisions regarding anticoagulation, will necessitate joint decision-making with an informed patient.

Cardioversion

Due to the difficulties of rate-controlling atrial flutter, cardioversion is an attractive early option. Just like in atrial fibrillation, if the patient is not chronically anticoagulated and the duration of the flutter cannot be firmly established to be less than 24 h, then a



Fig. 3 Analagous representation of right atrial flutter circuit is demonstrated with an upside-down basket. The handle represents the critical isthmus between the inferior vena cava and tricuspid annulus. The basket proper represents the right atrium, with activation propagating out from the isthmus, joining on the other side anterior to the crista terminalis.

transesophageal echocardiogram needs to be performed prior to restoring sinus rhythm to exclude the presence of a left atrial appendage thrombus.

Cardioversion can be performed with a synchronized shock. Generally, low energies are required, in the order of 50–100 J. In individuals with large body habitus, these lower energies may induce atrial fibrillation, which would then require higher energy levels to cardiovert. This is the preferred method because it is expeditious, safe, and not associated with proarrhythmia. However, deep sedation is often required as energy delivery is painful. The inability to give sedation, most commonly due to patient not being in a fasting state, may require pharmacological means for cardioversion.

In the United States, antiarrhythmic drugs that are available intravenously are limited. The most commonly used for atrial flutter would be ibutilide and procainamide. Ibutilide is a potassium channel blocker that has an approximately 60%–90% conversion rate for atrial flutter after one dose. Because the drug can cause QT prolongation and torsades, it needs to be administered in a setting where continuous monitoring, defibrillation and, occasionally, back-up pacing can be delivered. Procainamide has a slower rate of administration and longer half-life and tends to cause more hypotension during its administration usually limiting its administration to ICU-type settings.

Pace termination

Because flutter is a macroreentrant rhythm, introducing a competing wave front of atrial depolarization that can enter the circuit, collide with the flutter wave front and terminate the rhythm is a possible treatment. However, the only time this is a practical solution is when the patient has an atrial lead from an implantable cardiac device, or temporary epicardial pacing wires after cardiac surgery. Placement of an esophageal pacing lead to stimulate the left atrium is rarely used because it is cumbersome and uncomfortable for the patient. Rapid atrial pacing through any mechanism can sometimes terminate flutter, but, more commonly, results in transforming the rhythm to atrial fibrillation, which is often better tolerated and easier to rate control, so this is not an undesirable outcome.

Antiarrhythmic drugs

Unless there is a well-delineated reversible condition (such as pericarditis, postcardiac surgery, thyrotoxicosis), the recurrence rate of atrial flutter is very high. Therefore, recurrence should be expected. One way to reduce the recurrence rate is to administer chronic antiarrhythmic drugs. Class III drugs are generally preferred, such as sotalol, dofetilide, dronedarone, or amiodarone. Class I drugs, particularly the Ic drugs such as flecainide and propafenone can slow the atrial flutter rate sufficiently to allow 1:1 conduction in the AV node. These rhythms are typically over 200 bpm and poorly tolerated. Therefore, good AV nodal blockade should be ensured when using antiarrhythmic medications for atrial flutter, other than amiodarone and sotalol which have intrinsic AV nodal-blocking properties. It should be noted that typical atrial flutter can be cured with a high degree of success and low complication rate with a procedure that is relatively easy to perform for adequately trained physicians. Because of this, there is little reason to subject patients to long-term antiarrhythmic drugs for this condition, unless ablation risk or complexity would be prohibitive due to anatomical or comorbid conditions.

Atypical flutters are usually difficult to treat with antiarrhythmics but can present more of a challenge as the target for ablation is more variable and can be difficult to identify. Because of this, long-term therapy with antiarrhythmic drugs is a more viable long-term strategy than with typical flutters.

Ablation

Ablation of typical atrial flutter is usually straightforward. The critical isthmus between the tricuspid annulus and the inferior vena cava is very easy to reach with a femoral access. The ablation catheter is advanced into the right atrium and flexed into the proximal right ventricle. It is then pulled back from the point where one first sees atrial electrograms to the inferior vena cava, at which point atrial electrograms will disappear. Termination of the atrial flutter proves that the flutter was isthmus-dependent, but the end-point of the procedure is bidirectional block. The radiofrequency lesions result in thermal injury which causes edema initially and is followed by scar formation. As scar consists of collagen, it does not support electrical conduction. Bidirectional block is proven when electrical impulses are no longer able to travel along the isthmus in either direction as determined by pacing from either end of the isthmus. The procedure has low morbidity and low incidence of complications. While acute success is virtually 100%, recurrences can occur months or years later(Waldo, 1995; Morady, 2004) (**Figs. 4–6**).

Ablation of atypical flutter is generally more challenging as the circuits may be due to functional reentry, rather than anatomical, and can occur in either or both atria as well as structures such as the superior vena cava, pulmonary veins, atrial appendages, and coronary sinus. When it is due to a clear anatomical substrate, such as an atriotomy scar, success rates can be quite high. However, when they result from extensive ablations of the atria, such as from ablations or surgeries for atrial fibrillation, the reentrant circuits can be quite complex and targeting a vulnerable isthmus may be difficult. In addition, high recurrence rates make these among the most challenging arrhythmias that the electrophysiologist must contend with.

Focal Atrial Tachycardia

Definition

Focal atrial tachycardia is a specific type of supraventricular tachycardia, characterized by the presence of discrete P waves on surface EKG. Atrial activation begins at a localized site, with subsequent atrial activation spreading centrifugally outward to the remainder of atrial tissue. There is usually an isoelectric segment between P waves, in distinction to atrial flutter, where at any point in time, a portion of the atrial tissue will be depolarized. An analogy would be to consider the wave front generated by dropping a pebble into a pool of standing water.

Incidence

Focal atrial tachycardias are among the least common of the paroxysmal supraventricular tachycardias. The overall prevalence is difficult to establish. However, data from EKG review in both symptomatic and asymptomatic young men has suggested an overall prevalence of 0.34%–0.46% (Poutiainen et al., 1999). Data from the Marshfield study epidemiologic study area demonstrated an overall prevalence of paroxysmal supraventricular tachycardia to be 0.23%(Orejarena et al., 1998). Among patients referred for

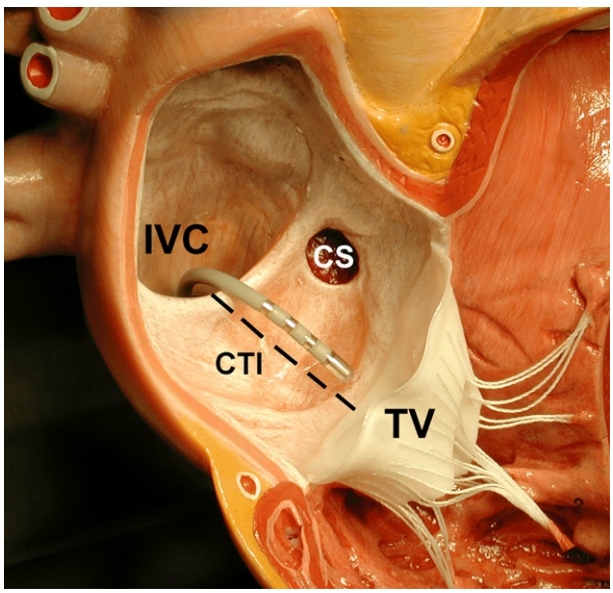

Fig. 4 Depiction of positioning of ablation catheter along the cavotricuspid isthmus in a 3D heart model (IVC = Inferior vena cava, CTI = cavotricuspid isthmus, TV = tricuspid valve, CS = coronary sinus).

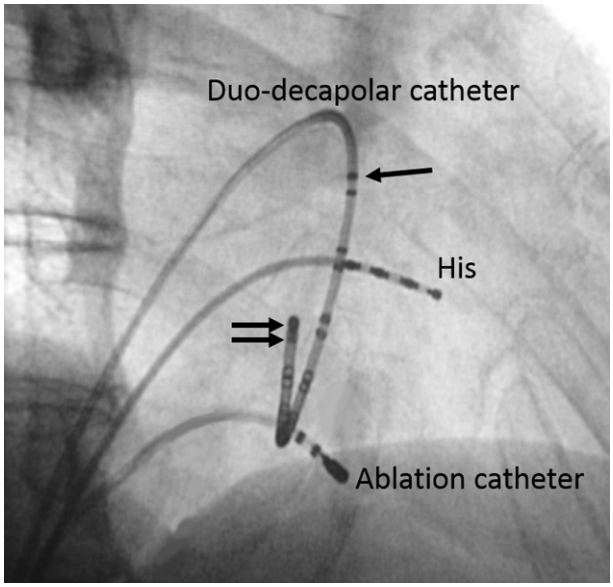

Fig. 5 Fluoroscopic image of catheter positioning during ablation of the cavotricuspid isthmus for typical atrial flutter. The image is in the right-anterior oblique (RAO) projection, such that right ventricle is toward the right of the image and the coronary sinus is in a plane perpendicular to the image. The duodecapolar catheter is positioned such that the distal end is in the coronary sinus (*double arrows*) and the proximal end is near the roof of the right atrium (*single arrow*). A catheter is positioned at the region of the His bundle for reference. The ablation catheter is positioned along the cavotricuspid isthmus, near the tricuspid annulus.

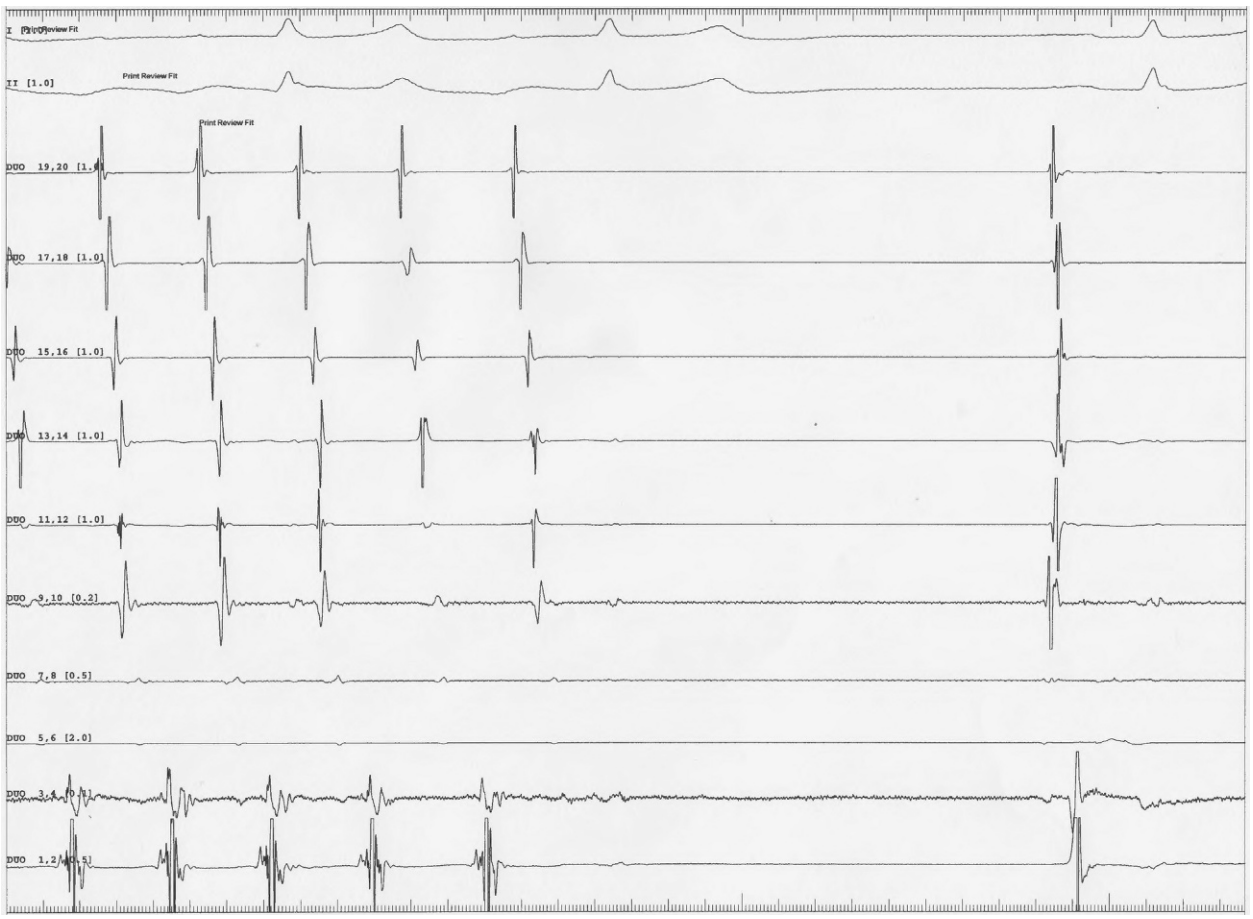

Fig. 6 Intracardiac electrogram during radiofrequency ablation of the cavotricuspid isthmus is shown. There is counterclockwise activation of the right atrium, in that the first signal observed is at the superior aspect of the right atrium (Duo 19,20) with subsequent activation inferiorly (Duo 19,20 to Duo 9,10) and then into the left atrium (Duo 3,4 to 1,2). Poles 7 and 8 are positioned along the isthmus. The first five beats represent atrial flutter. At this point, the flutter terminates as no atrial signal is observed in the coronary sinus. Thereafter, a pause is seen, followed by a junctional escape beat.

electrophysiology study for paroxysmal SVT, the incidence of focal tachycardia is between 3% and 17%(Pedrote et al., 2016; Porter et al., 2004). These data also suggest that patients with atrial tachycardia more often tend to be women.

Characteristics

The rate of atrial tachycardia is usually between 100 and 250 bpm. Episodes may be nonsustained or sustained. Atrial tachycardia may demonstrate a "warm-up" period, in which the tachycardia rate may gradually increase at the onset of tachycardia and "warm-down" at termination. Clinical symptoms can include the sensation of a rapid heart rate, accompanied by dyspnea, chest pressure, lightheadedness, or syncope. In patients with a structurally normal heart, this diagnosis usually carries a benign prognosis. Frequent episodes of atrial tachycardia may be associated with developing atrial fibrillation in the future. There is little evidence to suggest treatment of atrial tachycardia will change the long-term outcome, however.

Surface EKG can provide valuable clues to distinguish atrial tachycardia from other supraventricular tachycardias. Usually, focal atrial tachycardias fall under the category of "long RP" tachycardia—that is the segment from the R wave to onset of P wave is longer in duration than the PR interval. This is neither a sensitive, nor a specific finding, however. Although a tachycardia may demonstrate a short RP interval, this may still represent a focal atrial tachycardia, in that the P wave is conducting to the subsequent R wave, rather than relating to the preceding one.

It is estimated that approximately 75% of focal atrial tachycardia arise from the right atrium. The most common sites of origination in the right atrium include the crista terminalis (31%), tricuspid annulus (22%), perinodal/para-Hisian region (11%), and ostium of the coronary sinus (8%). The most common site in the left atrium is from within the pulmonary veins (19%). Other less common sites of origination include the intraatrial septum, body of the coronary sinus, mitral annulus, and the right and left atrial appendages(Kistler et al., 2006).

The P wave morphology may help with localization, with lead V_1 being the most useful in helping to differentiate a right from a left atrial origin. A negative or biphasic (+/−) P wave in lead V_1 is highly specific for right atrial tachycardia, while a positive or biphasic (−/+) is more suggestive of left atrial tachycardia. A P wave that is positive in the inferior leads (II, III, aVF) suggests a superior location of the tachycardia. Algorithms have been proposed to focally predict and identify origin with some success, however, there is significant overlap and therefore limited sensitivity and specificity. For example, a tachycardia that arises from the right upper pulmonary vein, which anatomically is positioned posterior to the right atrium and the sinus node, may appear similar to a sinus P wave(Kistler et al., 2006).

Focal atrial tachycardia, if arising from near the sinus node, in the region of the superior crista terminalis, or posteriorly from the right upper pulmonary vein, must be distinguished from arrhythmias related to the sinus node, including sinus tachycardia, sinus node reentry, and inappropriate sinus tachycardia. Sinus tachycardia may be appropriate, if there is an underlying physiologic cause, or inappropriate, if there is not. Usually, sinus tachycardia demonstrates a gradual increase in heart rate from normal, which can be discerned on cardiac ambulatory monitoring. While focal atrial tachycardia may demonstrate a "warm-up" period over a few beats, onset is usually abrupt. Identification of P wave morphology may help to distinguish from a focal AT (**Figs. 7–12**).

Sinus node reentry is a less common form of focal atrial tachycardia. It involves microreentry within the sinus node that results in a P wave morphology identical to that of the sinus P wave. Onset is usually abrupt, but rates rarely exceed 150 bpm. An electrophysiologic study is necessary to make this diagnosis and differentiate from focal AT originating from the crista terminalis.

Lastly, inappropriate sinus tachycardia is a condition which is characterized by sinus tachycardia without an apparent, underlying physiological explanation. Periods of sinus tachycardia are observed at rest or with minimal exertion. A constellation of other symptoms, including profound weakness and palpitations may be present. There is a loosely understood relationship and overlap with autonomic dysfunction and postural orthostatic tachycardia syndrome. All secondary causes of sinus tachycardia must be excluded(Page et al., 2016).

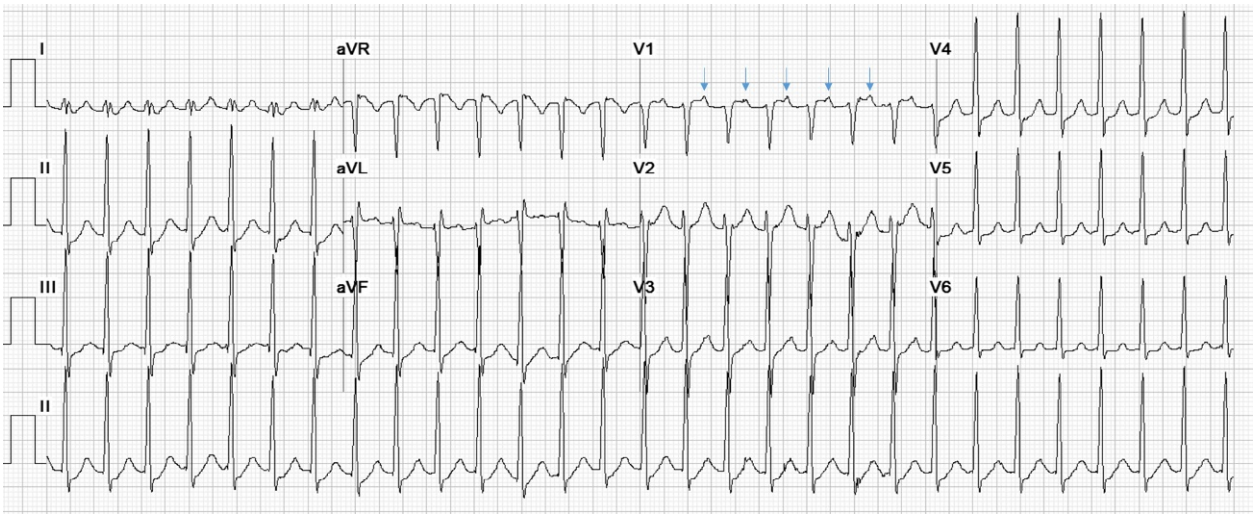

Fig. 7 EKG of focal atrial tachycardia, which was later mapped to the right lower pulmonary vein, where radiofrequency ablation was performed focally, with termination of the arrhythmia. P waves are denoted with *arrows*.

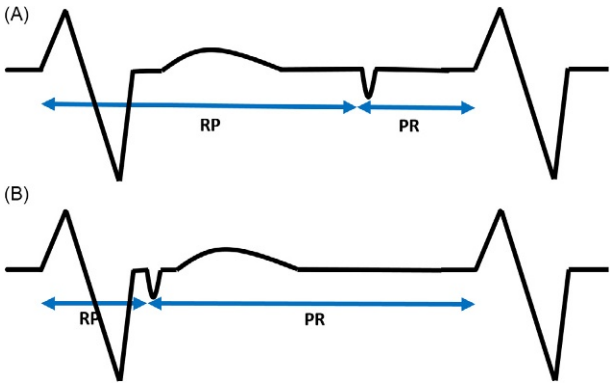

Fig. 8 In the top panel, A, a long RP rhythm is shown (RP segment >PR). Conversely, in the bottom panel, B, the RP relationship is shorter than the PR. Both may occur with atrial tachycardia.

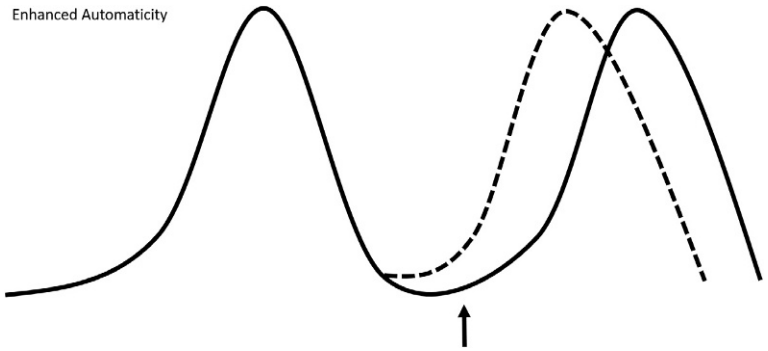

Fig. 9 The *arrow* denotes phase 4 depolarization. The *dotted line* shows an increased slope of phase 4 depolarization, possibly due to decreased I_{k1} or enhanced I_f currents. This will lead to a faster rate of automaticity.

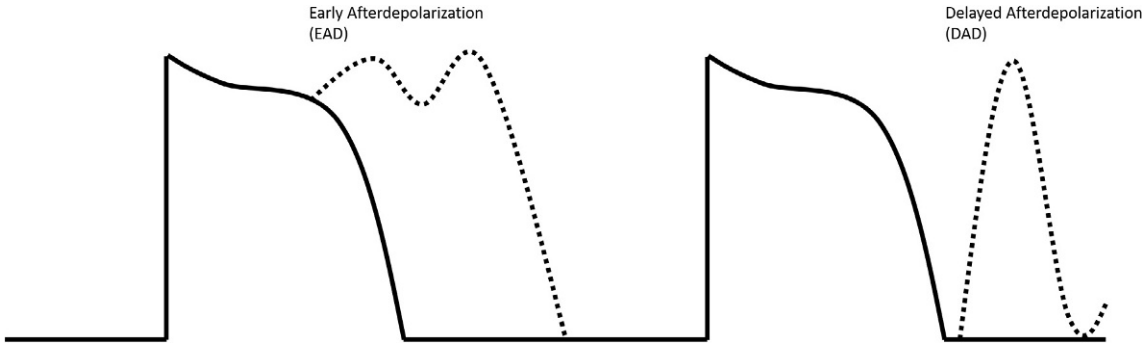

Fig. 10 Early afterdepolarizations occur during phase 2 or phase 3 of the cardiac action potential (left), due to I_{ca} reactivation that may occur in the setting of prolonged repolarization. Delayed afterdepolarizations (right) occur during phase 4, due to Ca^{2+} overload.

Focal atrial tachycardia may contribute to a tachycardia-mediated cardiomyopathy. The overall incidence of this is 10%. Incessant or highly frequent tachycardia is a risk factor for this phenomenon. Abolition of the tachycardia, frequently with catheter ablation, will restore sinus rhythm, with likely improvement in systolic function(Medi et al., 2009).

Mechanisms

The mechanism of focal atrial tachycardia can be due to enhanced automaticity, triggered activity, or microreentry. Understanding these mechanisms will help to understand treatment strategies and use of various diagnostic maneuvers to identify etiologies of undifferentiated narrow complex tachycardias.

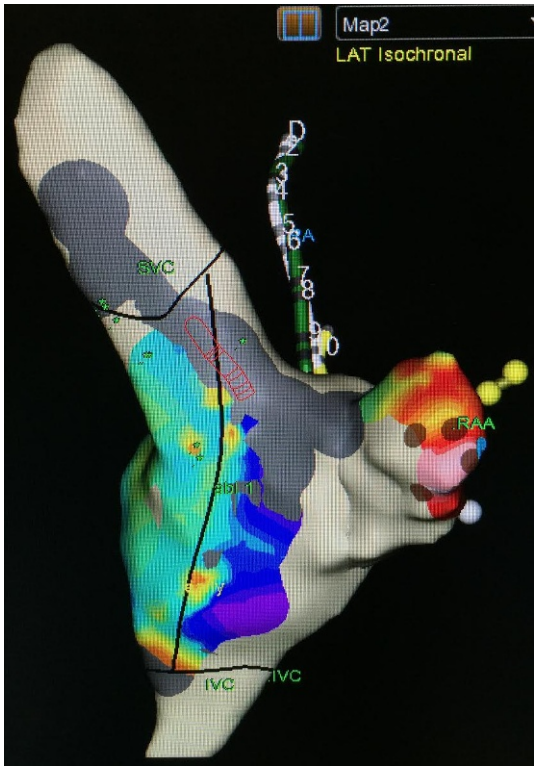

Fig. 11 A 3D electroanatomic activation map is created during focal atrial tachycardia. The *red* area corresponds to earliest activation within the right atrial appendage. Areas of *blue* and *purple* represent later areas of activation. Radiofrequency ablation was carefully performed within the right atrial appendage, without complication, and with termination of the tachycardia.

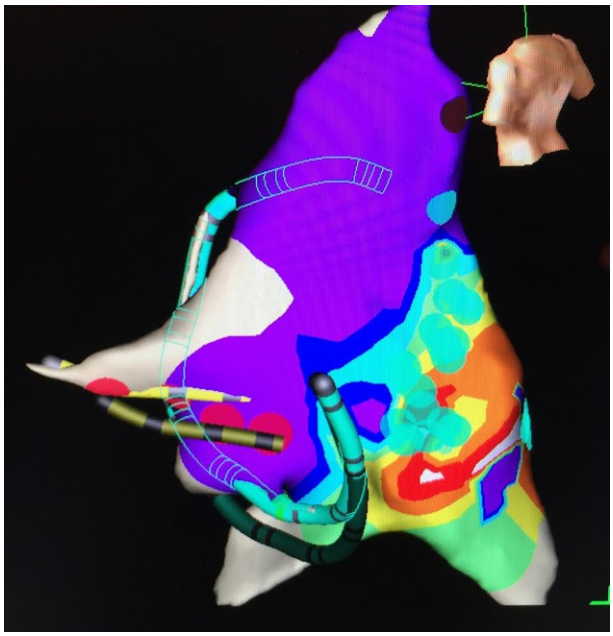

Fig. 12 Similarly, a 3D electroanatomic activation map, created during focal atrial tachycardia, is shown with earliest areas mapped to the interatrial septum in the right atrium (*red* and *white* areas).

- The concept of reentry has previously been discussed in the section discussing atrial flutter. *Microreentry* refers to the same concept applied to a smaller circuit—some have defined this as <2 cm and others as an area that is smaller than the spatial resolution afforded by current 3D mapping systems.
- *Enhanced automaticity* will occur with increased slope of phase 4 depolarization, resulting in an increased firing rate. This is usually fastest in the sinus node, but if abnormal automaticity occurs in other tissue, ectopic atrial activity will be seen. Focal atrial tachycardia, when secondary to enhanced automaticity, may demonstrate a "warm-up" period.
- *Triggered activity* refers to impulse formation that results in afterdepolarizations that occurs during or after the repolarization phases of the cardiac action potential. An early afterdepolarization occurs in phase 3 while a delayed afterdepolarization (DAD) occurs after repolarization has completed (phase 4). Atrial tachycardia with AV block may be seen with digoxin toxicity. The likely mechanism is related to triggered activity, secondary to DADs that may occur in the SA node(Gaztanaga et al., 2012).
- *Macro reentrant* atrial tachycardia refers to atrial flutter, both typical and atypical, which was previously discussed.

Pacing maneuvers during an electrophysiology study can be used to define the mechanism of arrhythmia.

Bedside maneuvers and administration of certain drugs can also be useful in distinguishing the underlying mechanism and distinguishing focal AT from atrial flutter. Adenosine decreases L-type calcium currents via inhibition of intracellular cAMP generation. Via this effect, adenosine may terminate focal ATs that arise due to triggered activity. Additionally, adenosine may activate adenosine-sensitive potassium currents (I_{kAdo}), leading to hyperpolarization and suppress enhanced automaticity. Adenosine does not have an effect on atrial flutter or microreentrant AT(Markowitz et al., 2007). Valsalva maneuvers may terminate a small subset of focal atrial tachycardias that are related to triggered activity. However, the vast majority of focal atrial tachycardias cannot be terminated in this manner. With Valsalva, usually transient AV block is noted, with continuation of atrial activity without conduction to the ventricle.

Acute management

In the acute setting, as for any hemodynamically unstable rhythm, direct current cardioversion may be necessary. This is considered a temporizing measure and, unless a clear precipitant of the arrhythmia is identified and corrected, should not be considered the ultimate therapy.

If the patient is stable, administration of intravenous medications or vagal maneuvers can be useful. Intravenous beta-blockers, diltiazem, or verapamil are generally considered first-line therapy in the treatment of focal atrial tachycardia in a hemodynamically stable patient. Intravenous amiodarone or ibutilide can also be considered in a hemodynamically stable patient(Page et al., 2016). There is no prospective clinical data on the utility of each of these agents.

As discussed previously, adenosine may be useful for termination if the underlying mechanism of the focal atrial tachycardia is triggered activity. If the underlying mechanism is enhanced automaticity, transient suppression of the arrhythmia may be seen. Adenosine may be useful as a diagnostic maneuver: if AV block is achieved with continuation of atrial activity, this eliminates supraventricular tachycardias that are dependent on the AV node for maintenance, such as AV node reentry and atrioventricular reentry (discussed elsewhere). Vagal maneuvers, including carotid sinus massage or Valsalva, may result in the same effect as adenosine and may help in diagnosis of the etiology the supraventricular tachycardia.

Long-Term Management

Pharmacologic

Options for long-term pharmacotherapy include oral beta-blockers, diltiazem, or verapamil. Class IC antiarrhythmic drugs (flecainide or propafenone) as well as the class III agents, sotalol or amiodarone, can also be effective in suppressing focal atrial tachycardia. However, in a symptomatic patient, pharmacotherapy is considered second-line after catheter ablation.

Ablation

Over the past 20 years, the safety and success of catheter ablation for focal atrial tachycardia has markedly improved. The hallmark of electrophysiology study first involves induction of the tachycardia, which, depending on the underlying mechanism of the arrhythmia, can be accomplished with incremental atrial pacing, or programmed stimulation, with or without the use of a beta-agonist, such as isoproteronol. Mapping can be performed either with a contact or noncontact mapping catheter. With contact mapping, a roving catheter is manipulated across the surface of the atrium, attempting to find an early site of activation relative to a fixed reference, usually the surface P wave. This can be done in conjunction with an impedance-based navigation system, which can provide visualization of the catheter within a 3D electroanatomic model of the chamber. A noncontact mapping catheter uses a multielectrode catheter positioned within the chamber, not actually in contact with tissue, and generating a map of earliest activation. This form of mapping may be useful in cases where the arrhythmia is frequently changing, is nonsustained, or is not clinically tolerated by the patient. 3D mapping systems help to significantly increase precision of catheter movement and increase success rates for ablation procedures. If performed in experienced centers, success rates are in excess of 85% with complications rates of ~2%(Pedrote et al., 2016). Major complications occur in 0.3% of cases, which include cardiac perforation, tamponade, or AV block, depending on the location of the tachycardia.

Anticoagulation

In the absence of atrial flutter or fibrillation, there is low thromboembolic risk. Therefore, oral anticoagulation is not indicated.

Multifocal Atrial Tachycardia

MAT is characterized by a rapid (>100 bpm), irregular rhythm with at least three P wave morphologies. MAT is associated with medical comorbidities, including chronic obstructive pulmonary disease, pulmonary hypertension, and valvular heart disease. In general, management of this arrhythmia is focused on treating the underlying medical conditions. Nondihydropyridine calcium channel blockers can be used to slow conduction in the AV node. Verapamil is usually preferred but diltiazem can also be used. In the acute setting, there may some benefit to using intravenous magnesium. Intravenous verapamil is safe and effective for controlling heart rate. Beta-blockers should be used with caution with concurrent pulmonary disease. Amiodarone can be considered. Cardioversion is not useful for MAT, as the rhythm will almost certainly resume. Catheter ablation is also not a treatment option for this arrhythmia(Page et al., 2016).

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Atrioventricular Node Reentrant Tachycardia

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Introduction

Atrioventricular node reentrant tachycardia (AVNRT) is the most common regular supraventricular tachycardia observed in clinical practice. The reentrant circuit, located in the atrioventricular (AV) node, consists of at least two pathways with different electrophysiological properties, called fast and SPs in allusion to their conduction times. In this article we review the mechanisms that lead to the different types of AVNRT, as well as, their clinical and varies electrocardiographic presentations. We provide a simplified algorithm that may allow readers to quickly identify this arrhythmia. Also, we discuss the electrophysiological mechanisms and maneuvers that enable distinguishing AVNRT from other supraventricular tachycardias. Finally, we highlight its acute and long-term management with particular emphasis in current transcatheter mapping and ablation techniques.

Pathophysiology

The Atrioventricular Node

Background and function

The AV node is an integral part of the AV conduction axis within the heart. The atria and the ventricles are separated by a fibrous insulating plane. At the most basic level, the AV node is the link between the conduction system of the atria and the subnodal His-Purkinje conduction system of the intraventricular septum and the ventricles. It allows impulses generated at the sinoatrial (SA) node to propagate from the atria to the ventricles each cardiac cycle. The AV node also accounts for the largest component of the delay in the AV transmission. The delay of the impulses arriving from the atria is fundamental in the overall cardiac cycle, allowing sufficient time in diastole for the ventricles to fill. This atrio-His (AH) delay is broad during normal sinus rhythm with an AH range between 60 and 125 ms (Josephson, 2008). This is largely attributed to the sensitivity of the AV node conduction velocity and refractoriness to changes in autonomic nervous system afferents.

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Apart from its role in the regular cardiac cycle, the AV node has an important survival mechanism which allows impulses to be delayed during periods of rapid atrial activity. A phenomenon known as decremental conduction ensures that as the time intervals between stimulation of the AV node from the atria decrease, the refractoriness of the AV node increases (Patterson and Scherlag, 2002). Decremental conduction is of paramount importance in protecting the ventricles from rapid rates of atrial arrhythmias.

Although it serves as an important protective mechanism for the ventricles during periods of rapid atrial activity, the heterogeneity within the AV node can lead to the generation of arrhythmia on its own. Discussed later are the important anatomic and electrophysiologic properties of the AV node, and their relation to mechanisms of arrhythmia.

AV node anatomy

Tawara was the first to prove the physical existence of the AV node in the early 1900s (Tawara, 1905). Until then, it had been hypothesized that there was an anatomic unit responsible for the connection between the atria and the ventricles and of importance in the conduction and modulation of impulses between these two units. Further histopathological and electrophysiological evaluations of the AV node reveal that it is far from a homogenous structure. In fact, it is remarkably heterogenous, both anatomically and electrically.

An important anatomical landmark when discussing the anatomy of the AV node is the Triangle of Koch (**Fig. 1**). The Triangle of Koch accounts for a significant component of the septal portion of the right atrium on the endocardial surface. It is delimited anteriorly by the insertion of the septal leaflet of the tricuspid valve. The most posterior border is the fibrous strand continuation of the valve of the inferior cava vein: the tendon of Todaro. At the apex of the triangle of Koch, just below the right atrial endocardium, lies the compact AV node. Here, the AV conduction system penetrates the insulating plane at the AV junction. Located superiorly and anteriorly to this site lies the bundle of His (HB) (Anderson and Cook, 2007).

Histopathology and electrophysiologic properties

The AV node is made up of different types of cells that can be broadly categorized based on the region within the AV node that they arise. The compact zone is more homogeneous and less similar to the surrounding myocytes. It is enveloped by the transitional zone, made up of transitional cells. This is a more histologically heterogeneous group of cells that is the result of the merging of surrounding myocytes to the AV node proper. Functionally, action potential characteristics recorded using microelectrodes recordings in the rabbit AV junction have been used to classify the AV node into distinct electrophysiological "regions": atrionodal (AN), nodal (N), and nodal-His (NH). These distinct regions have both unique histopathologic and electrophysiologic characteristics (Anderson and Cook, 2007).

AN region

The AN region corresponds anatomically to the area called the transitional zone. It is the less compact and most inferior portion of the AV node. Atrial cells group into bands and gradually merge into the more compact AV node. The cells are histologically distinct from atrial myocytes and cells in the compact AV node. Two AV node inputs can be observed forming bridges from atrial tissue to

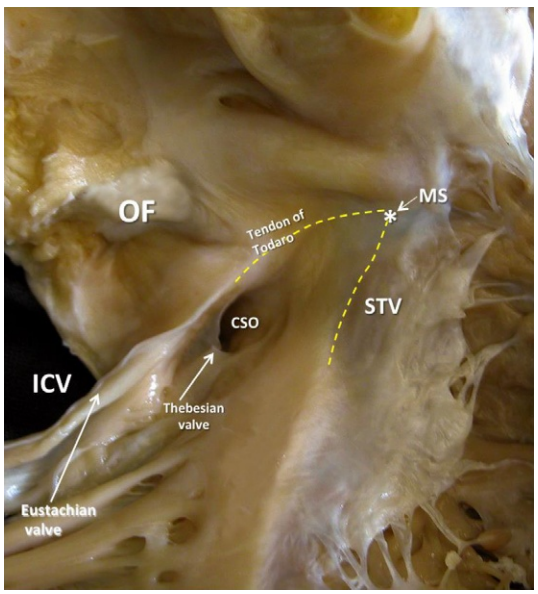

Fig. 1 Simulated right anterior oblique view of the right atrium showing the anatomical landmarks of the triangle of Koch. The apex of the triangle points superiorly whereas the coronary sinus ostium (CSO) is located inferior. The posterior border is given by the tendon of Todaro, and the anterior border by the septal leaflet of the tricuspid valve (STV). The AV node is located in the superior margin of the triangle between the tendon of Todaro, the membranous septum (MS), and the hinge of the septal leaflet of the tricuspid valve. *OF*, oval fossa; *ICV*, inferior cava vein. Courtesy Dr Damian Sanchez-Quintana.

the AV node. The anterior (superior) approach connects the anterior limbus of the fossa ovalis to the AV node close to the triangle of Koch. The posterior (inferior) approach connects the coronary sinus ostium to the AV node close to the interior septal RA. An additional approach has more recently been described connecting the AV node to the septum and the left atrium (Lockwood et al., 2009; Racker and Kadish, 2000; Mazgalev, 2009; Basso et al., 2008).

N region

The N region represents the compact AV node. The compact node is 5–7 mm length, 3–4 mm width (Mani and Pavri, 2014). Here, the transitional cells merge with the mid-nodal cells. The N cells are responsible for the majority of conduction delay within the AV node. This is due to several factors relating to the histopathology of the N cells. The depolarization rates are slower due to a paucity of sodium channels and the action potential in this region is largely mediated by inward L-type calcium channels. Additionally, there are fewer gap junctions in the area, which translates into less intracellular connections and slower propagation of depolarization waves (Tawara, 1905; Mani and Pavri, 2014).

NH region

The NH region corresponds to the cells merging with the HB, which penetrates the insulating AV plane in a leftward direction, away from the right atrial endocardium and toward the ventricular septum. These are closer in appearance to the rapidly conducting cells of the HB.

When traced inferiorly, toward the base of the triangle of Koch, the compact AV node area separates into two extensions. These extensions bifurcate toward the CS ostium and tricuspid annulus (right posterior extension) and the mitral annulus (left posterior extension). The right posterior nodal extension has been classically marked as the SP involved in the AVNRT circuit. The fast pathway (FP) is less well defined from an anatomical point of view and has been classically described in an anterior (superior) location in the Koch's triangle. Nevertheless, the concept of the AV node as having anterior (superior) and posterior (inferior) inputs is a simple theoretical framework that allows the clinician to confront most cases (Issa et al., 2012). Reentry occurring along these pathways is the core mechanism for various subtypes of AVNRT (slow–fast and fast–slow).

In patients with this anatomic characteristic, the depolarizing wave-fronts from the atria arrive at the compact AV node via those two distinct pathways. The anteriorly located pathway conducts impulses faster but has a long refractory period. In contrast, the posteriorly located pathway conducts more slowly but has a short refractory period. This has led to specific observations regarding the electrophysiologic characteristics of the AV node known as the "dual AV nodal pathways physiology." This can be observed under normal physiologic states, but is of great important in abnormal states, such as nodal-dependent arrhythmias. The definition of dual pathways in the cardiac electrophysiology lab (EP lab) depends on the response of the AV node to programmed premature atrial stimuli that demonstrates discontinuous propagation of anterograde impulses through the AV node.

The dual nodal physiology, as well as the phenomenon of decremental conduction, has been demonstrated in both animal (Zhuang et al., 2002) and human (Schauerte et al., 2001) models using programmed atrial stimulation. At normal heart rates, conduction occurs via the FP, so the normal PR interval lasts between 120 and 200 milliseconds (ms). During programmed atrial stimulation, a train of atrial impulses is delivered to the AV node at a fixed cycle length (CL) followed by a single atrial premature stimulus. As the prematurity of the extrastimulus is increased, the conduction time of the earlier atrial impulse to the His signal is prolonged (decremental conduction). The AH prolongation is progressive, adding no more than 50 ms to the previous AH values at each 10 ms decrease in the extrastimulus CL, until the impulse block in the AV node (AV node effective refractory period (ERP)) (**Fig. 2**). The so-called dual nodal physiology consists of an abrupt AH prolongation more than 50 ms. This abrupt prolongation reflects the FP ERP and the "jump" on the impulse conduction from one pathway to the other. Now the impulse is conducted through the SP, named "slow" as it takes at least 50 ms more than the previous impulse to arrive at the HB (Moe et al., 1956). In summary, an AH jump is defined as an increase in the length of the AH interval $\geq$50 ms, caused by a decrease in the coupling interval of an atrial extrastimulus (A1A2) of 10 ms.

Another manifestation of a dual physiology are (1) the presence of two different PR intervals during sinus rhythm in the same patient; (2) a PR interval longer than the RR interval during rapid atrial pacing; (3) two QRS for only one P wave or a "double ventricular response" (Josephson, 2008).

Tachycardia Circuit

Dual AV nodal physiology is the underlying substrate for AVNRT. In other words, AVNRT only occurs in patients with dual AVN physiology; however, this physiology is present in almost half of the population, and only a few of them will develop AVNRT. As in any reentrant tachycardia, the substrate is essential, but other conditions are required for the creation and maintenance of a reentrant arrhythmia (Josephson, 2008):

1. Two anatomically or functionally different pathways that connect proximally and distally forming a closed-loop system.
2. Unidirectional block in one of these pathways (longer ERP) due to a premature beat.
3. Slow conduction down the unblocked pathway (shorter ERP) allowing the previously blocked pathway sufficient time to recover excitability. In other words, the conduction time down the unblocked pathway must exceed the refractory period of the pathway initially blocked to allow a closed-loop conduction system to form. Because the unblocked pathway has a shorter refractory period and recovers faster, it will be ready to receive the incoming activation, maintaining the tachycardia.

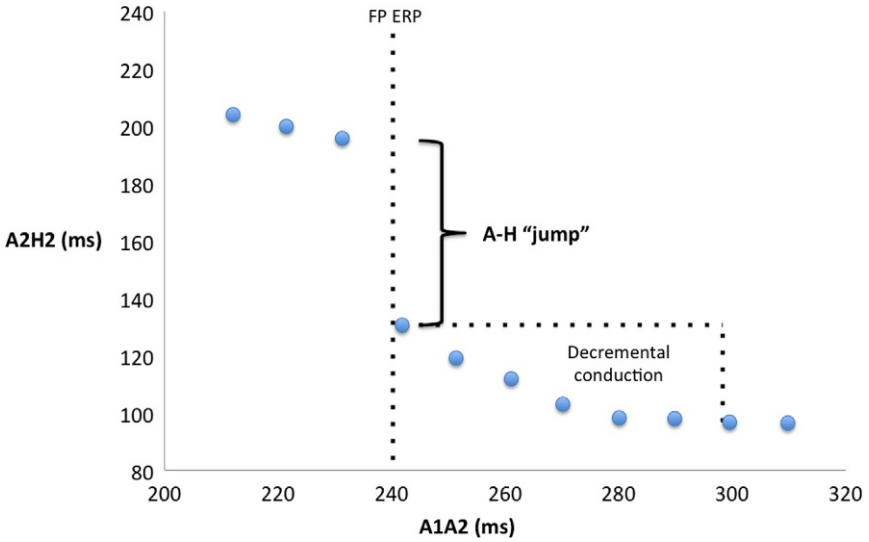

Fig. 2 The model of dual AV nodal pathways on the antegrade conduction curve is shown. The *X* axis represents the time interval between the last beat of the drive train and the premature atrial impulse. The *Y* axis represents the time interval between the premature atrial impulse and the resulting His bundle. With increasingly premature atrial complexes (decreasing A1A2), the effective refractory period of the fast pathway is reached (FP ERP), and the impulse can now only conduct down the slow pathway. At this level, an abrupt increase in the length of the AH interval is seen ($\geq$50 ms); this is called "AH jump."

The AVNRT circuit is limited to the compact AV node and does not involve the ventricles. Whether the circuit involves atrial myocardium (perinodal) is controversial. Other controversial concepts are the presence of upper and lower common pathways. These concepts are referred to additional AV nodal tissue extrinsic to the tachycardia circuit to explain various electrophysiological phenomena observed during AVNRT (Miller et al., 1987). There is real evidence that the distal junction of the slow and fast pathways is located in the AV node, with the existence of a region of AV tissue extending between the distal junction of the two pathways and the HB (the lower common pathway), at least in some patients. The concept of a lower common pathway has been used to explain some unusual situations as the presence of AV block without recording of a His signal during AVNRT (Otomo et al., 2006).

Current evidence supports the role of perinodal tissue and suggests that the fast and slow pathways involved in the reentrant circuit of AVNRT represent conduction over different atrio-nodal connections, thus making at least a small amount of atrial tissue a necessary part of the reentrant circuit (Issa et al., 2012).

In AVNRT, the pathway that has a faster conduction time takes longer to recover to the point where it can accept an incoming activation (longer refractory period). The slower pathway recovers quicker (shorter refractory period) and can accept a new signal earlier than the pathway that conducts faster. If a premature beat occurs in the time period when the FP is not ready to receive a new signal, but the SP is, then the early signal will travel down the SP only prolonging the AH interval and PR interval. When the early signal reaches the lower connection between both pathways, it is possible that the faster is now ready to conduct. If this happens, the early signal will split with part of it traveling down into the ventricles along the normal conduction system. The other part of the signal will return backward, or retrograde, up the FP to the atrium. As the FP conducts faster than the SP, makes the upward conduction so fast that the ventricles and atria are activated almost at the same time. This first beat of reentry, when a retrograde P wave is visualized very close to the QRS, is called echo beat. When this signal reaches the top of the node where the upper connection between the two pathways is located, if the SP is recovered, the impulse will not only activate the atria but also it will travel down the SP. The circuit is now in a loop that will continue until the electrophysiology properties in some of the pathways changes, and the circuit is interrupted (**Fig. 3**).

Anatomic and physiologic relationships mentioned above are related to the typical slow–fast AVNRT, where the activation travels anterogradely using the SP and retrogradely using the already recovered FP. This type accounts for 90% of all AVNRTs encountered in clinical practice, but in some cases, this circuit could be inverted with the first anterograde activation arriving fast usually at different levels in the coronary sinus before reaching back the atrium through the SP (type fast–slow). Also, multiple SPs have been described in up to 14% of the patients with AVNRT (Issa et al., 2012). These pathways would be involved in more rare forms of AVNRT with similar slow conduction between both pathways (type slow–slow).

Epidemiology

AVNRT represents the most common form of paroxysmal supraventricular tachycardia (PSVT) in humans, accounting for more than 50% of all PSVT cases in adults (Josephson and Wellens, 1997).

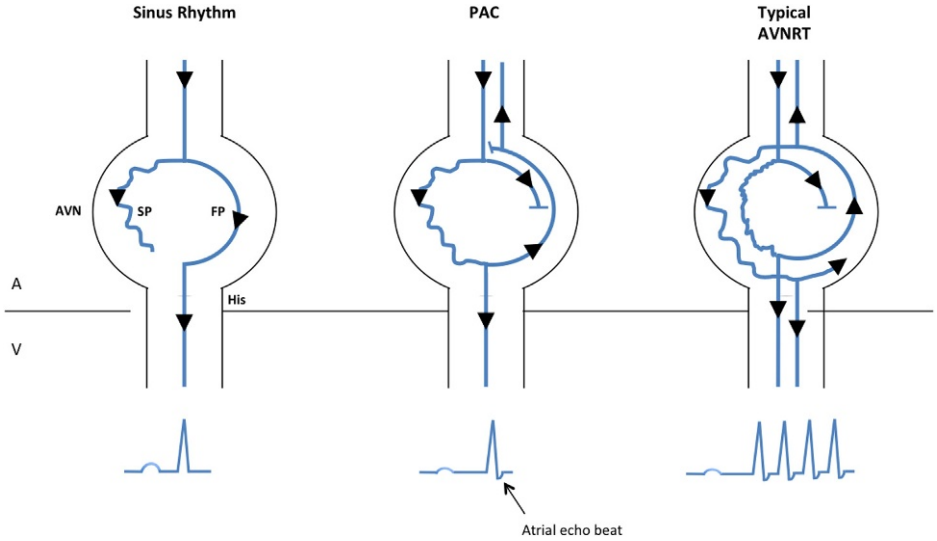

Fig. 3 Prototype of dual AV nodal pathways physiology in sinus rhythm, with a premature atrial complex (PAC) which trigger a typical "slow–fast" AVNRT. See text for details. *SP*, slow pathway; *FP*, fast pathway.

The number of patients with AVNRT increases with age. The mean age of onset is about 30 years old and is unusual in children less than five years old. In contrast, atrioventricular reentrant tachycardia (AVRT) manifests earlier. This pattern may be related to the natural evolution of AV nodal physiology over the first two decades of life, as well as age-related changes in atrial and nodal physiology observed in later decades. AVNRT is more common in women with a 2:1 predominance (Porter et al., 2004; Scheinman and Huang, 2000). The reason may be related to gender differences in the anterograde and retrograde electrophysiological properties (Suenari et al., 2010).

Clinical Presentation

The clinical presentation depends on heart rate, but the most common symptoms are palpitations of abrupt onset and termination. The faster the heart rate and/or the older the patient, palpitations are poorly tolerated, and other hypoperfusion symptoms may be present as dizziness, dyspnea, angina, or syncope.

In some cases and especially in a young woman without ECG documentation, recurrent episodes can be misdiagnosed as a panic attack.

Due to the atria and ventricles contracting at the same time during typical AVNRT, in about half of the patients, palpitations are felt in the neck. Canon A-waves in the jugular venous waveform result from the simultaneous contraction of the atria and ventricles against closed AV valves, causing a reflux of blood into the neck veins. The characteristic flapping or bulging appearance of the neck veins is also described as the "frog sign" (Contreras-Valdes and Josephson, 2016). Although neck palpitations it can be present during AVRT, it is less common. Moreover, neck palpitations have been postulated as the only clinical predictor of AVNRT when positive ECG findings were lacking (González-Torrecilla et al., 2009).

In summary, a woman with palpitations of abrupt onset and termination felt at the neck and first episode after 20 years of age are variables more related with AVNRT than another type of PSVT.

ECG Identification

AVNRT is typically a narrow complex tachycardia (QRS duration <120 ms), with the same QRS morphology as in sinus rhythm. The QRS duration could be prolonged if aberrant conduction (most likely right bundle-branch type) or previous conduction defect exists (Lockwood et al., 2009). The rates of AVNRT vary widely, ranging from 100 to 280 beats per minute (bpm) with an average of 170 bpm. Slower rates use to be seen in older patients (Josephson, 2008).

In typical (slow–fast) AVNRT, the RP interval is very short (RP/RR <0.5) (usually RP <80 ms) and abnormal P-waves are indiscernible or very close to the QRS complex because of the simultaneous atrial and ventricular activation. Thus, the P-wave can distort the initial portion of the QRS ("pseudo" q-wave in inferior leads), it can be masked by the QRS, or distort the terminal part of the QRS ("pseudo" s-wave in inferior leads and "pseudo" r'-wave in lead V1). In atypical AVNRT (fast–slow and slow–slow), P-waves are visible after the QRS (RP/RR >0.5) (usually RP >80 ms) (Issa et al., 2012). When visible, the retrograde atrial activation is seen as negative P-waves in leads II, III, AVF and positive in lead V1 (Fig. 4).

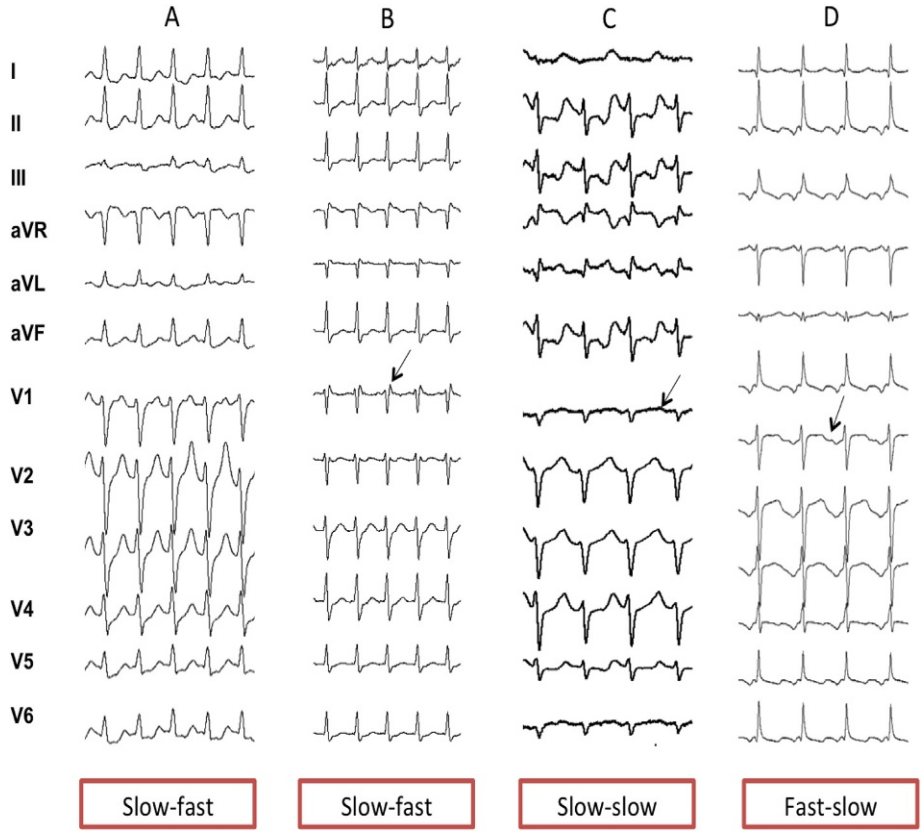

Fig. 4 Different types of AVNRT showing different surface ECG presentation. In all types of AVNRT, the P wave is relatively narrow, negative in inferior leads, and positive in V1. In typical (slow–fast) AVNRT, the retrograde P wave may lie within the QRS (A) or deform the terminal portion of the QRS, "pseudo" r wave in V1 (B). In slow–slow AVNRT the P-waves lies in the ST-T segment (C). In fast–slow AVNRT, the P wave presents before the QRS with a long RP interval (D). *Arrows* mark the positive P-waves in lead V1.

Usually, the P/QRS ratio during AVNRT is 1, however, 2:1 AV block can be present if the level of block occurs below the reentry circuit (in the lower common pathway or below HB). If so, retrograde P-waves are inscribed exactly between QRS complexes.

Electrocardiographic Differential Diagnosis of Narrow-Complex Tachycardia

Narrow-complex tachycardias denote all tachyarrhythmias that originate from the supraventricular tissue. In an acute setting, the quick and correct interpretation of the ECG is paramount since the management may differ between some arrhythmias (Fig. 5).

The proposed algorithm from Queen's University is a simplification of the multiple possibilities for the diagnosis of narrow-complex tachycardias in order to facilitate its clinical use. It is intended to help the physician in the rapid recognition of the most common forms seen in adults (Nadeau-Routhier and Baranchuk, 2016).

During a narrow-complex tachycardia, the first step is to classify the arrhythmia according to whether there is a regular or irregular ventricular rate. An irregular RR interval suggests atrial fibrillation (most likely), multifocal atrial tachycardia (AT) or atrial flutter with variable conduction. If the atrial wave is clearly identified and the rate is greater than the ventricular rate, then atrial flutter ("saw-tooth pattern") or AT is usually present.

If the tachycardia is regular, the next step is to identify the location of the P-waves. If no P-waves are visible, then typical AVNRT (slow–fast) is the most likely diagnose. If the P-waves are visible (negative) after the QRS, analysis of the RP interval is paramount. The differential diagnosis of "short RP" tachycardias includes typical AVNRT and orthodromic AVRT. In "long RP" tachycardias the most likely diagnoses are atypical AVNRT ("fast–slow") and ATs originating in the lower aspect of the atria (i.e., ostium of the coronary sinus).

If the P-wave is visible just before the QRS, then we should review its morphology in the inferior leads. The main differential diagnoses includes sinus tachycardia (positive P-waves), low AT (negative P-waves), or atrial flutter ("saw-tooth" P-waves appearance).

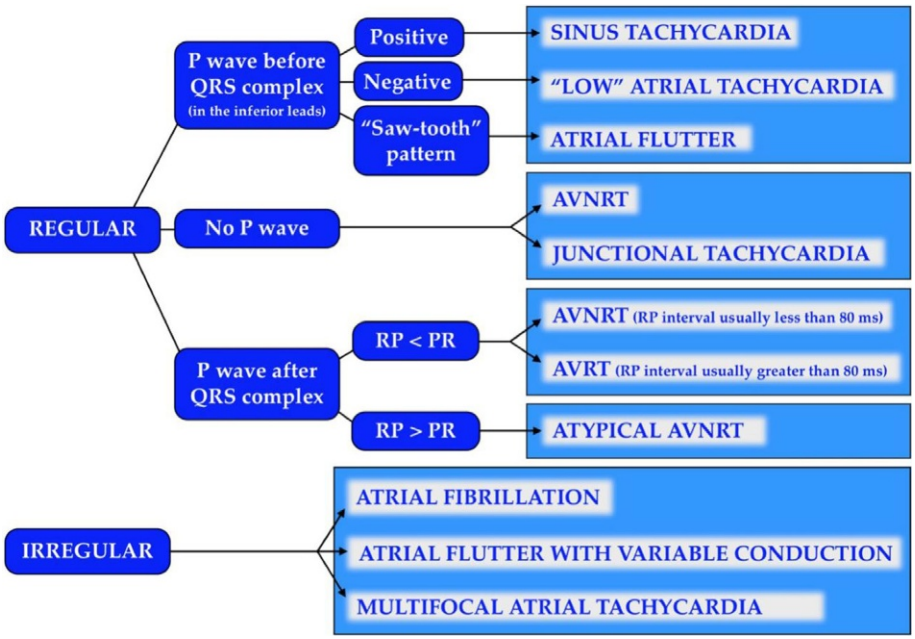

Fig. 5 Electrocardiographic approach to the most common tachycardias with narrow-complex QRS in adults. From Nadeau-Routhier, C. and Baranchuk, A. (2016). In Nadeau-Routhier, C. and Baranchuk, A. (eds.) Electrocardiography in practice: What to do? pp 123–140. Accessed October 2016: https://itunes.apple.com/us/book/electrocardiography-in-practice/id1120119530?mt=13.

Electrophysiological Classification of AVNRT

Depending on which type of pathway the stimulus utilizes as anterograde and retrograde limb of the circuit, three major types of AVNRT exist: typical slow–fast AVNRT, atypical fast–slow, and slow–slow AVNRT. Different methodologies have been used by the investigator during the electrophysiology studies to classify between typical and atypical AVNRT: pattern of earliest retrograde atrial activation (EARA), absolute values of Atrial-His (AH) and His-Atrial (HA) intervals, AH/HA ratios (Heidbüchel and Jackman, 2004; Willems et al., 2004; Otomo et al., 2007; Yamabe et al., 2007; Fujiki et al., 2008).

The facts that AVNRT may present with atypical retrograde activation, and different forms of AVNRT may be seen in the same patient have made the diagnosis intricated (Heinroth et al., 2002). Therefore, appropriate classification is necessary to avoid misdiagnosis and subsequently prolonged procedures during catheter ablation.

Typical AVNRT (Slow–Fast)

Typical slow–fast AVNRT is the most common type of AVNRT (>90%) and the circuit uses the SP anterogradely and the FP retrogradely. In this type of AVNRT, induction is dependent on achieving a critical AH interval. It means that the antegrade conduction down the SP should be slow enough that allows for recovery of the FP to conduct retrogradely (**Fig. 3**). In the EP lab, this can be achieved with atrial extrastimuli testing or atrial burst pacing near the Wenckebach CL (**Fig. 6**). If this cannot be achieved, stimulation under infusion with isoproterenol may be required.

During tachycardia, the EARA (via FP) is usually localized in the anterior septum which is recorded by the electrodes at the HB. However, posterior or even left sided EARA may occur in up to 8% of the patients (**Fig. 7**) (Chen et al., 2004; Nam et al., 2006).

The EARA appears slightly before, at the onset, or just after the QRS complex thus maintaining AH/HA ratio >1. Most important, the HA interval is typically <70 ms, measured from earliest deflection of the HB activation to the earliest rapid deflection of the atrial activation in the HB electrodes. When the HB cannot be recorded during tachycardia, the VA interval is also a useful criterion. The VA interval, measured from the onset of ventricular activation on surface ECG to the earliest atrial activation on the HB electrodes, is <60 ms (**Fig. 8**).

Spontaneous termination of typical AVNRT occurs because of a block on the slow or fast pathway and appears to be "time-related." When the block takes place on the slow antegrade conduction, there is a prolongation of the AH interval (Mobitz type I block) which occur only after an initial period of stable tachycardia intervals. This mechanism is consistent with an underlying concealed fatigue phenomenon. Interruption of the reentry in the retrograde direction (FP) would also be a "time-related" event but developed suddenly without AH interval prolongation (Mobitz type II block) (Chiale et al., 2015).

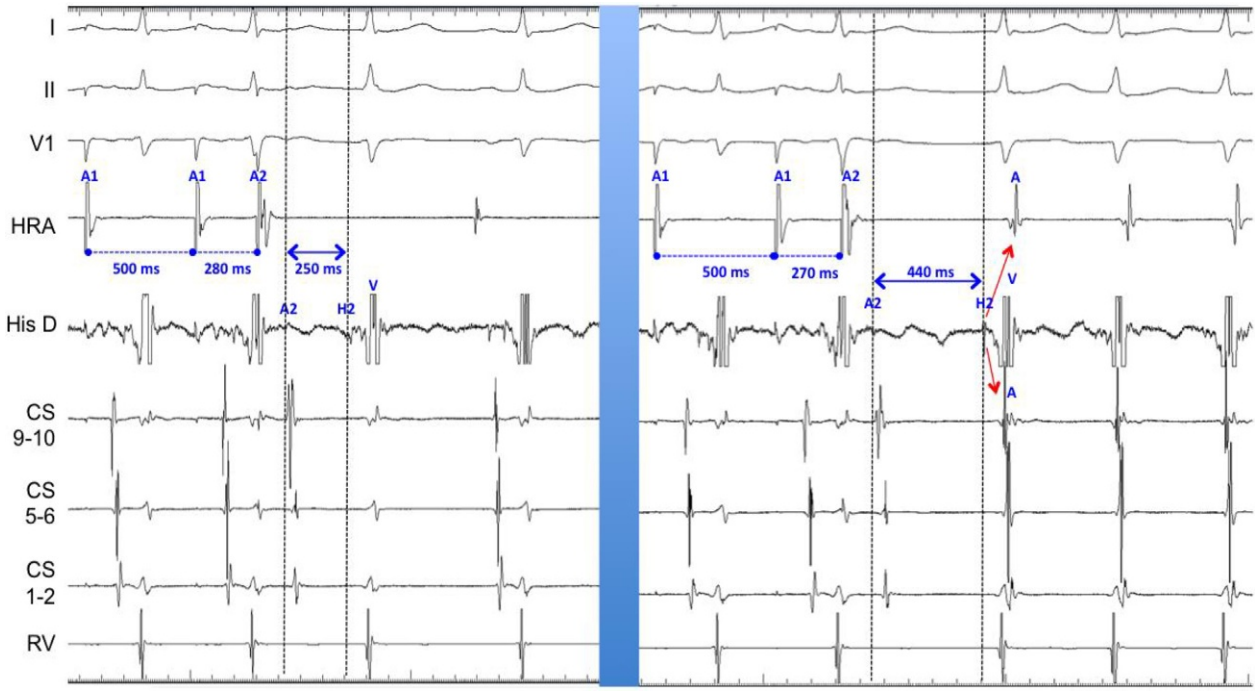

Fig. 6 Demonstration of dual AV nodal physiology and induction of typical AVNRT. Single extrastimulus (A2) of progressively shorter coupling intervals were delivered from the high right atrial (HRA) at a basic drive of 500 ms (A1). A jump or sudden increase of the AH interval from 250 ms (Left) to 440 ms (Right) occurs after shortening of the atrial extrastimulus from 280 to 270 ms. The atrial extrastimulus conducts through the slow AV nodal pathway, followed by retrograde conduction through the fast AV nodal pathway to activate the atrium (*red arrows*) starting a typical AVNRT (Right).

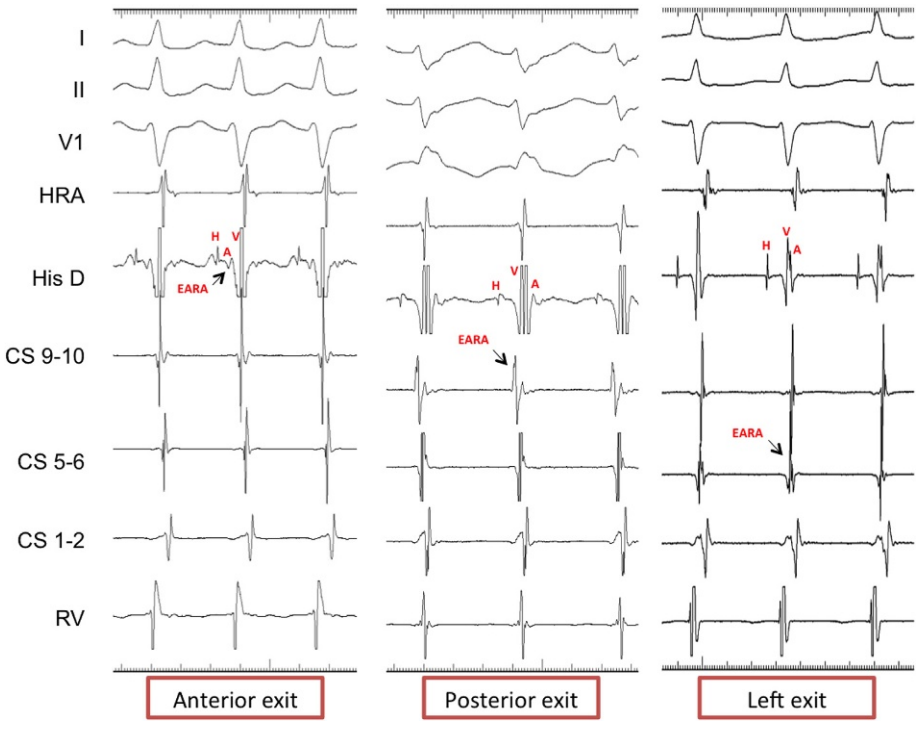

Fig. 7 The figure shows the different location of the earlier retrograde atrial activation (EARA) during typical AVNRT. In the anterior atrial "exit," the ERAA is recorded by the His electrode. In the posterior "exit," ERAA is recorded by the proximal CS electrode. In the left "exit," EARA is recorded in mid or distal CS electrode. *HRA*, high right atrium; *CS*, coronary sinus; *RV*, right ventricle.

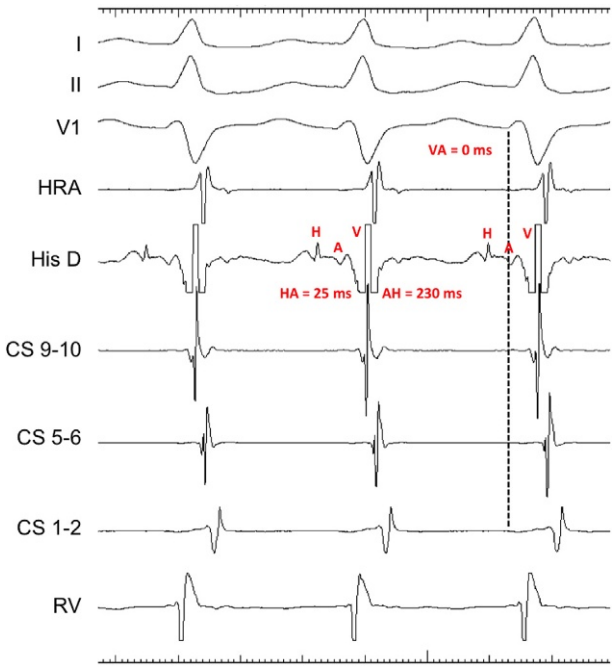

Fig. 8 Intracardiac electrograms in typical AVNRT (AH > HA, HA <70 ms, VA ≤60 ms). Note earliest atrial activation at the His bundle and the simultaneous atrial and ventricular activation. *Dash line* points the VA time. AH, atrial to His interval; HA, His to atrial interval measured from earliest deflection of the His bundle activation to the earliest rapid deflection of the atrial activation in the His bundle electrogram; VA, ventricle to atrial interval measured from the onset of ventricular activation on surface ECG to the earliest deflection of the atrial activation on the His bundle electrograms.

Atypical AVNRT (Fast–Slow and Slow–Slow)

The prevalence of atypical forms of AVNRT has been described around 10%. However, recent studies have reported a lower prevalence 4%–6%. It is possible that the reason for higher prevalence previously reported was due to the characterization as slow–slow atypical AVNRT what were actually typical AVNRT cases (Katritsis and Josephson, 2013). During the tachycardia induction, a critical AH interval is less obvious and sometimes antegrade dual AV nodal physiology is not demonstrable. Both types, fast–slow and slow–slow, can be induced by atrial or ventricular stimulation and frequently required the administration of isoproterenol. Conversely to typical AVNRT, in some patients tachycardia is induced only by ventricular stimulation (Heidbüchel and Jackman, 2004).

In the fast–slow variant, the reentrant circuit occurs in the opposite way than the typical AVNRT: the FP conducts anterogradely and the SP retrogradely. Although it is thought of using the same circuit as typical AVNRT in the reverse direction, the fast–slow form of AVNRT may do not utilize the same limb for FP conduction (Katritsis et al., 2015a,b). Since the retrograde conduction is slower than the anterograde conduction, the AH/HA ratio is <1. Also, as the retrograde activation uses the SP, the retrograde atrial electrograms are recorded after the ventricular activation. The HA interval is ≥70 ms, and the VA interval is ≥60 ms (**Fig. 9**).

In the slow–slow variant, the reentrant circuit uses a slow or an intermediate pathway anterogradely and a second SP retrogradely. This type has the same HA and VA interval cutoff than the fast–slow type, but the AH/HA ratio is usually ≥1.

In both types of atypical AVNRT, the EARA uses an SP and it was traditionally reported in the posterior septum, near the coronary sinus ostium. Several authors have indicated that EARA can also occur at the anterior septum (His bundle electrode) and the left atrium (distal CS electrode) (Chen et al., 2004; Hwang et al., 1997). Therefore, EARA would not be useful to elucidate between the different types of AVNRT (Katritsis and Josephson, 2013).

There are some tachycardias with a relatively prolonged AH interval but an AH/HA ratio <1 that cannot be classified as either fast–slow or slow–slow. Therefore, some authors propose only the HA interval (or VA interval) as a criterion for the diagnosis of typical or atypical AVNRT (**Fig. 10**). Further classification into fast–slow or slow–slow forms would be arbitrary and would not been yet validated (Katritsis et al., 2015a,b).

Electrophysiological Study: Exclusion of Other Mechanisms of Tachycardia

The diagnosis of AVNRT requires the exclusion of other mechanisms as AVRT using a septal retrograde accessory pathway, AT, and junctional tachycardia (JT). Due to the risk of AV block associated with catheter ablation of AVNRT, a correct diagnosis is desired. Even more, successful ablation of AT or AVRT using a septal accessory pathway requires mapping to the earliest atrial electrogram, while in AVNRT the SP is targeted.

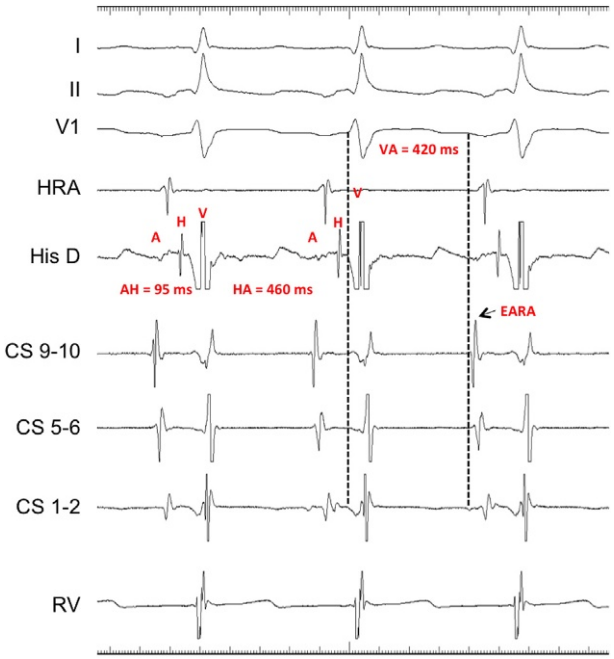

Fig. 9 Intracardiac electrograms in atypical AVNRT. The form is defined as type "fast–slow": AH 70 ms, VA >60 ms. The earliest atrial activation (EARA) is recorded at the proximal CS electrode. *Dash lines* point the VA time.

	HA	VA	AH/HA	Expected ERAA
Typical AVNRT	≤70 ms	≤60 ms	>1	His
Atypical AVNRT	>70 ms	>60 ms	Variable*	Cs ostium

Fig. 10 Electrophysiological classification of AVNRT. *In atypical "fast–slow" type the AH/HA ratio is commonly 1, and in "slow–slow" >1.

It is also noteworthy that there is no electrophysiologic finding that "per se" is diagnostic of AVNRT, often the correct diagnosis is performed after the observation of some tachycardia features like presence of dual AV nodal physiology, EARA, AH–HA–VA intervals, tachycardia induction-termination; in combination with pacing maneuvers. Fig. 11 depicts a simplified algorithm for rapid diagnosis of PSVT used in our EP lab.

In typical AVNRT, by far the most frequent presentation is with a concentric atrial retrograde activation and a VA time ≤60 ms. If this is the case, we should consider two differential diagnoses: septal AT or JT. AVRT is ruled out as the VA time is always longer than 60 ms.

Overdrive pacing from the right ventricle at a CL 10–40 ms shorter than the tachycardia CL is our first tool to rule out AT. To use this maneuver, it is required that the atrial CL be accelerated to the pacing CL and the tachycardia continues after pacing is discontinued. If the postpacing returning activation sequence is atrial–atrial–ventricular ("AAV") rules in AT, whiles an atrial–ventricular ("AV") response indicates that a reentrant mechanism is implied, ruling out AT (Fig. 12) (Knight et al., 1999). Importantly, the last atrial electrogram accelerated to the pacing CL is the first atrial electrogram counted in the interpretation of this response.

Differentiation of JT from slow–fast AVNRT may be a challenge since they share a short HA conduction time with EARA near the apex of the triangle of Koch (His electrode). JT can be differentiated from AVNRT using the maneuver described by Padanilam et al., which consists of analyzing the response to premature atrial contractions (PACs) introduced during or before HB refractoriness (Padanilam et al., 2008). A PAC introduced during HB refractoriness cannot penetrate to the automatic focus and therefore cannot alter the tachycardia. By contrast, in AVNRT, the PAC may advance the next beat of tachycardia by conduction in the SP (Fig. 13). Early PACs introduced before His refractoriness may advance the following beat and continue a JT but should terminate AVNRT because of collision of the antegrade PAC wavefront and retrograde tachycardia wavefront in the FP.

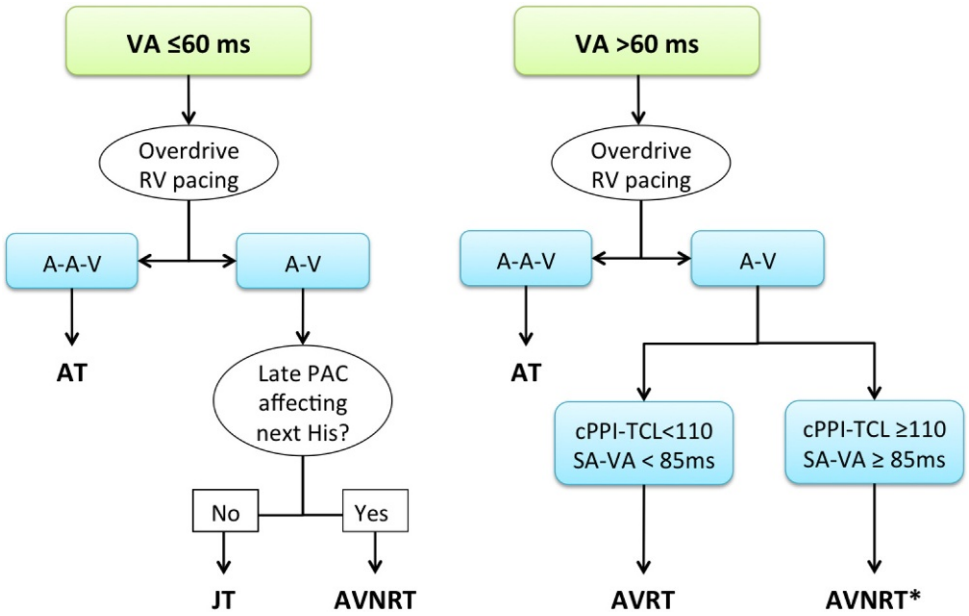

Fig. 11 Algorithm employed in the EP lab for rapid diagnosis of sustained PSVT with 1:1 AV relationship. See text for details. *Even though AVNRT is the most likely diagnosis, AVRT with a decremental pathway should be investigated.

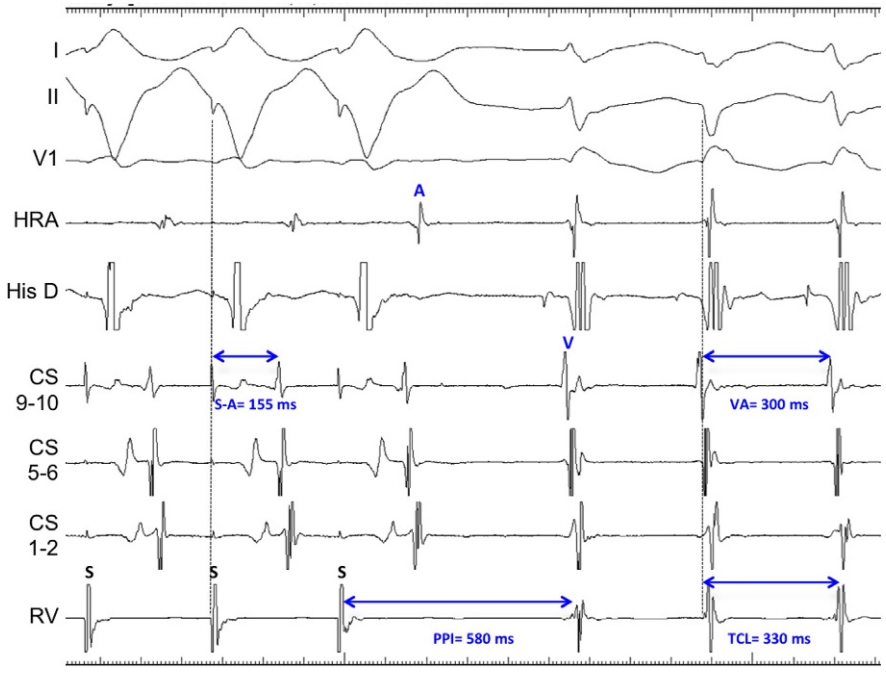

Fig. 12 Response to cessation of overdrive ventricular pacing (310 ms) in a typical AVNRT (CL 330). The atrial CL was accelerated to the ventricular CL and the response after pacing (S) is stopped is A–V, ruling out AT. The PPI–TCL difference is 145 ms (300–155 ms) and the SA–VA interval difference is 250 ms (580–330 ms), both measurements rule out AVRT. The PPI is measured from the pacing stimulus to the first return ventricular electrogram recorded by the pacing channel. The SA interval is measured from the pacing stimulus to the earliest atrial electrogram. The VA interval is measured from the beginning of the earliest QRS complex in SVT to the same atrial electrogram that was used to measure the SA interval.

In atypical AVNRT the VA time is >60 ms or the HA >70 ms with a most common EARA at proximal CS. JT is ruled out since the HA is <70 ms and the EARA occurs at the His electrode. Therefore, we should consider two differential diagnoses: AT and AVRT. Again, overdrive RV pacing is our first maneuver of choice to rule out AT after an "AV" response is obtained. Using the same maneuver, we can also differentiate AVNRT from AVRT. In response to ventricular overdrive pacing, orthodromic AVRT also produces an "AV" response; however, the differences in VA times between pacing and tachycardia are <85 ms (Michaud et al., 2001). During AVNRT the ventricle and atrium are activated in parallel, while during entrainment of AVNRT by ventricular

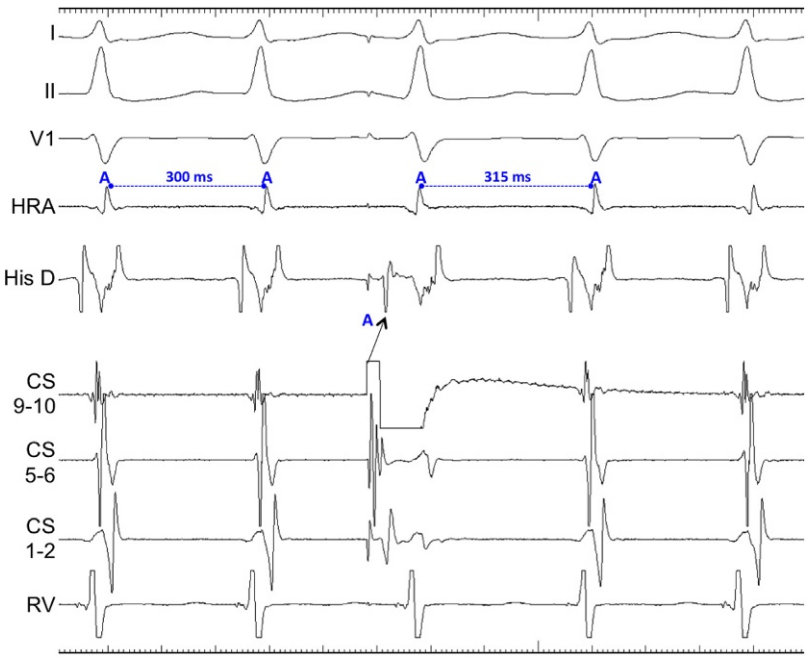

Fig. 13 Late PAC response in AVNRT. During supraventricular tachycardia with a cycle length of 300 ms and simultaneous atrial and ventricular activation, a PAC is delivered by electrodes at the ostium of the coronary sinus (CS 9–10) timed to His refractoriness. The *arrow points* to the local atrial activation at the His channel. Delay of the subsequent atrial activation by 15 ms indicates a diagnosis AVNRT where the PAC prematurely activated the slow pathway, delaying the subsequent beat due to decremental conduction.

overdrive pacing, their activation is in series. In contrast, during orthodromic AVRT and entrainment of orthodromic AVRT, the ventricle and atrium are activated in series. Accordingly, the difference between the VA interval during entrainment and SVT should be longer for AVNRT than for AVRT (**Fig. 12**).

Keeping in mind the same concept, in AVRT the postpacing interval—tachycardia CL difference (PPI–TCL) is <115 ms. A long return AH interval may invalidate this maneuver unless a correction is applied. PPI will increase if overdrive pacing (rate dependent) causes decremental conduction in the AV node. During entrainment of AVRT by RV pacing, the atria are accelerated to the pacing CL. Thus, the input to the AV node is also accelerated and subsequently AV node conduction time will increase (decremental properties) prolonging the AH interval and the PPI. Therefore in AVNRT, the first AH interval after entrainment (last accelerated beat) is often prolonged compared to the AH interval during AVRT. The PPI can be corrected by subtracting the increase in the AH interval on the first return beat compared to the AH interval during spontaneous AVRT from the PPI–TCL difference. A corrected PPI–TCL (cPPI–TCL) <110 ms is diagnostic of AVRT (Gonzalez-Torrecilla et al., 2006).

If overdrive pacing cannot be performed (lack of sustained and regular tachycardia) or interpreted (tachycardia stop during pacing), other maneuvers can be helpful to differentiate AVNRT from AVRT (Hirao et al., 1996; Martinez-Alday et al., 1994). A simple maneuver consists in delivering premature ventricular complexes (PVCs) when the HB is refractory (Josephson, 2008). PVCs that occur during SVT at a time when the stimulated wavefront would be expected to collide with the SVT wavefront in the His-Purkinje network or the ventricular myocardium cannot affect the atrial timing during AVNRT. Therefore, if such PVC affects the atrial timing implies the presence of another pathway (AVRT) connecting the ventricles with the atria (**Fig. 14**) (Veenhuyzen et al., 2012).

Prognosis

AVNRT is not a life-threatening arrhythmia and does not influence survival. In most patients, the episodes are self-terminated or respond acutely to vagal maneuvers or adenosine. Complications as syncope, angina, or heart failure are usually seen in patients with structural heart disease.

Treatment

Acute Management

Any maneuver or drugs that slow AV node conduction can be used to terminate the tachycardia. Vagal maneuvers, including Valsalva or carotid sinus massage, are recommended as the first-line intervention to terminate AVNRT (Class I) (Page et al., 2015).

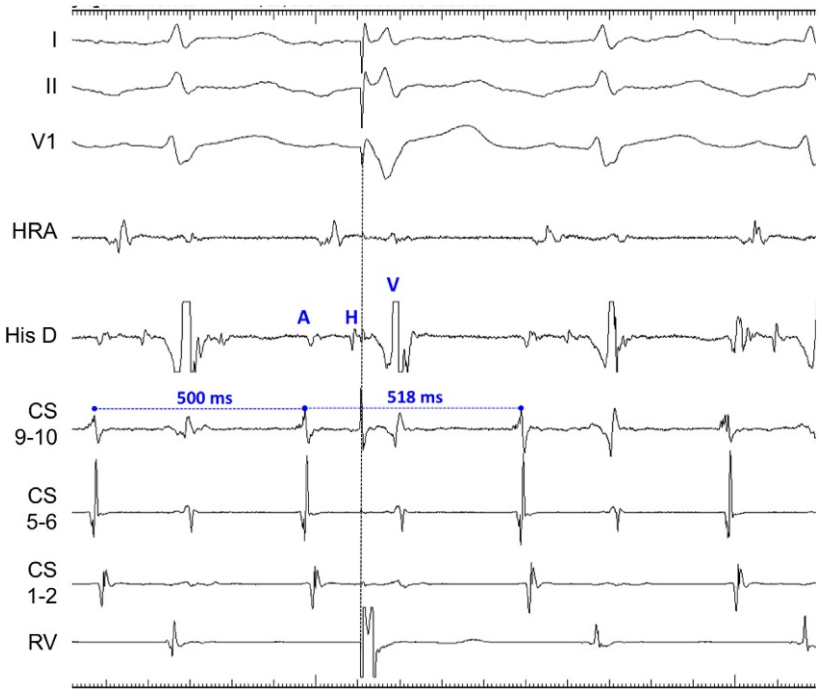

Fig. 14 Delay of atrial activation by a His refractory PVC. During an SVT with a CL of 500 ms and long RP interval (VA >60 ms), a PVC is delivered when the His is refractory and the subsequent atrial activation is delayed by 18 ms. The delayed of atrial activation without a change in the atrial activation sequence indicates that an accessory pathway with decremental conduction is present and participating in orthodromic AVRT.

These maneuvers have shown to be effective in at least one-third of the patients (Lim et al., 1998; Walker and Cutting, 2010). If these interventions fail, termination can be achieved with AV node blocking agents. Adenosine is the drug of choice as it is effective in approximately 95% of cases (Class I) (DiMarco et al., 1990; Cairns and Niemann, 1991; Glatter et al., 1999). Usually starting with 6 mg and going up in increasing dose boluses. Also, intravenous beta blockers, verapamil (10 mg in bolus), or diltiazem (5–25 mg in bolus) can be used (Class IIa) (Amsterdam et al., 1991; Waxman et al., 1981; Dougherty et al., 1992). Importantly, verapamil and diltiazem should be avoided in hemodynamically unstable patients or those with suspected systolic heart failure.

For resistant cases, a second or a third bolus of adenosine, verapamil, or diltiazem is often effective. Finally, in rare occasions that AVNRT cannot be terminated or in hemodynamically unstable patients, synchronized cardioversion with low energy (10–50 J) can always be used (Class I) (Reisinger et al., 2010).

Chronic Management

Because the prognosis of AVNRT is benign, the indication for its treatment relates to the impact on patient's quality of life. Factors to take into consideration during the therapeutic decision include the tolerance of symptoms, the frequency and duration of tachycardia, and the presence of concomitant structural heart disease. In a prospective study of patients who were followed for 15 years, almost half of minimally symptomatic patients who received no therapy became asymptomatic over time (D'Este et al., 2007). Thus, a patient who presents minimally symptomatic episodes of AVNRT that terminate spontaneously or with vagal maneuvers may be followed clinically (Class IIa).

Once we have opted to initiate therapy for preventing recurrences of AVNRT, the question arises whether we should choose catheter ablation or antiarrhythmic drugs.

Transcatheter ablation

Because of its high efficacy (>95%) and low complication risk, catheter ablation is recommended as first-line of therapy in symptomatic patients (Class I) (Jackman et al., 1992; Hindricks, 1996). Usually, the procedure is curative, and chronic pharmacological therapy is not needed. In the past, the target of catheter ablation was the FP located around the anterosuperior aspect of the triangle of Koch ("anterior approach"). However, as the FP is located close to the compact AV node, this approach was linked with high rates of AV block and was consequently abandoned. Nowadays, the target is the SP ("posterior approach") located between the CS ostium and the tricuspid annulus. The "posterior approach" has been proved at least as effective as the "anterior approach" with significantly less rate of AV block (Mitrani et al., 1993).

Radiofrequency ablation technique

The number of vein access and catheters placed for catheter ablation varies center to center. In general, a decapolar catheter is placed in the CS and three quadripolar catheters are placed in the right ventricle, HB, and high right atrium.

Once the diagnosis of AVNRT is confirmed and with the patient in sinus rhythm, the ablation catheter is advanced and positioned anterior to the CS ostium using the right anterior oblique (RAO) view. The angle of RAO should be adjusted so that the His catheter is perpendicular to the fluoroscopic plane. This view is especially helpful because it best displays Koch's triangle (**Fig. 15A**). The initial target zone for SP ablation is the tissue located between the tricuspid valve annulus and the CS ostium. The ablation catheter is advanced into the RV, moved inferiorly, so it lies anterior to the ostium of the CS, and then withdrawn toward the tricuspid annulus until the distal pair of electrodes records a small atrial deflection in addition to a large ventricular deflection (**Fig. 15B**). Clockwise torque will move the catheter toward the septum, confirmed in left anterior oblique view. The optimal A/V ratio in the distal electrode of the ablation catheter may range from 1:2 to 1:5. Also, the atrial electrogram at successful location usually has multiples components (**Fig. 16**). Before delivery energy, the absence of His signal in the distal electrodes has to be confirmed, to prevent FP damage and AV block. As the catheter tends to move with respiration and heartbeats, a long sheath is sometimes needed to enhance catheter stability.

In our laboratory, radiofrequency (RF) energy is delivered using a 3.5 mm nonirrigated catheter, in the temperature mode with a target temperature of 55–60°C for 60 s. During RF delivery, the most reliable marker of effective energy delivery is the continuous monitoring of impedance because a significant drop in impedance indicates better tissue contact compared to tip electrode temperature. We usually delivery RF energy for about 15–20 s waiting for the presence of accelerated junctional rhythm, which suggests effective SP ablation site (**Fig. 16**). If so, ablation is continued until reaching 60 s. In the case of lack of junctional rhythm, the catheter is moved to a new spot by micromovements. In few cases, successful ablation can be achieved in the absence of junctional beats during RF application (Hsieh et al., 1998).

Importantly, energy delivery must be immediately interrupted if fast junctional rhythm or changes in the AV or VA relationship are noticed because they may reflect damage to the compact AVN (**Table 1**) (Hintringer et al., 1995; Tebbenjohanns et al., 1995).

Cryoablation technique

Cryoenergy is an alternative source of energy to RF for catheter ablation procedures. In the recent past, cryoablation has been well studied in patients with AVNRT. This technology has shown a high acute success rate (95%) comparable with RF energy. However, the long-term recurrence rate is higher with cryoablation (9% vs. 4.5%) (Santangeli et al., 2014). The main benefit of cryoablation lies in the low rate of permanent AV block (0% vs. 1%). This is due to at least two specific features of this technology. Firstly, cryomapping makes possible the assessment of functional effects on the AV node before permanent lesion. Secondly, during cryoablation the catheter tip adheres to the tissue because of ice formation. This cytoadherence prevents dislodgement of the catheter avoiding lesions on the AV node.

In summary, cryoablation is particularly useful, as an alternative to RF in high-risk patients. For instance, in those patients where RF is attempted and AVNRT is still inducible. Thus, we need to move the catheter to closer areas in the vicinity of the compact AV node.

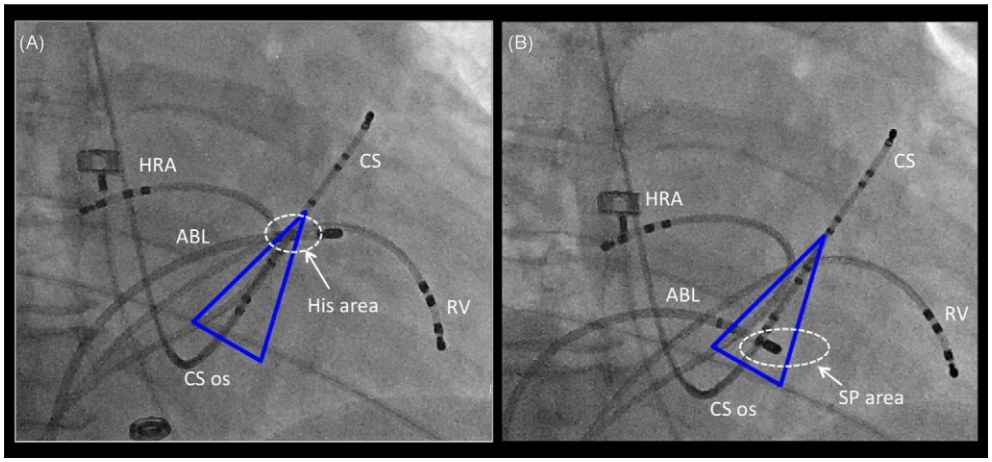

Fig. 15 Right anterior oblique view of catheter positions for anatomically guided slow pathway (SP) ablation. Triangle of Koch is delimited in *blue lines*. The ablation catheter is located close to the His bundle area. AV nodal injury should be expected with ablation at this site (A). Then, the ablation catheter is moved inferiorly and toward the septum to reach the SP area. This is the safest and most effective region for slow pathway ablation (B). *HRA*, high right atrium catheter; *ABL*, ablation catheter; *CS*, coronary sinus catheter; *CS os*, coronary sinus ostium; *RV*, right ventricle catheter.

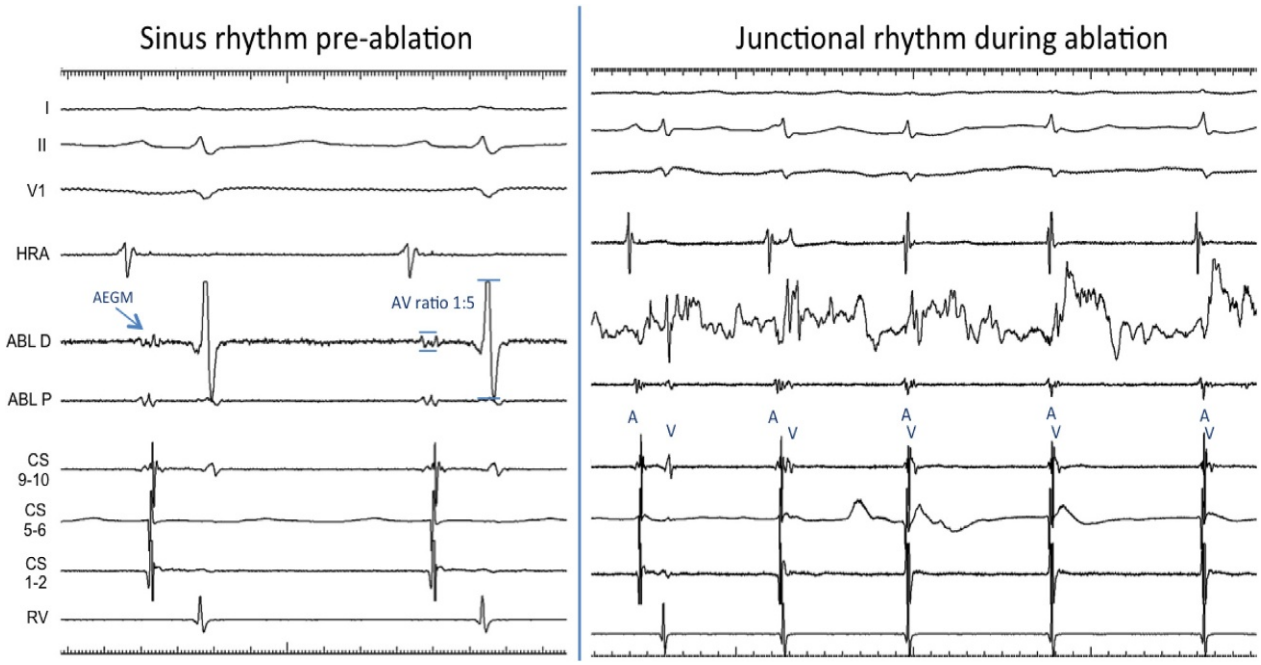

Fig. 16 Ablation catheter at the site of successful slow pathway ablation. The atrial electrograms (AEGM) has multiple components and the A/V ratio is 1:5 (left). At that site, radiofrequency is delivery and junctional rhythm is induced which indicate appropriated slow pathway injury (right).

Table 1 Predictors of AV block during RF ablation

Mapping	His signal in the distal electrode
	Interval <20 ms between the ablation atrial EGM and the His-Atrial EGM
Ablation	Fast junctional rhythm (<350 ms)
	VA block during junctional rhythm
	Non-conducted atrial EGM

The initial part of the procedure is similar to that described above for RF ablation. Cryoablation is performed with either an 8-mm-tip or 6-mm-tip catheter.

There are two commonly used techniques for positioning of the cryoablation catheter in the target tissue. Some operators perform it like RF ablation by first entering the right ventricle, withdrawing the catheter while deflecting the tip caudally. Usually, an A/V ratio of about 1:1 to 1:2 is searched. Other operators start by positioning the tip of the cryoablation catheter one-third of the distance from the level of the CS ostium to the level of the HB catheter in the left anterior oblique view. Once in the target zone, use of cryomapping at −30°C is performed to test safety. If damaging of the AV node is seen, the cryomap is stopped, and the lesion will be reverted. Otherwise, the application is continued to freeze at −70 to −80°C to obtain a permanent lesion. Each application takes between 4 and 5 min, allowing to monitor inducibility during applications. Also, the AH interval is carefully monitored. Sometimes the AH interval progressively increases to the point of complete AV block. Cryoablation is rapidly discontinued when the AH prolongation increases more than 200 ms, or high-grade AV block is achieved. Usually, the AH interval return to normal after the cessation of cryoablation, and permanent AV block is very rare using cryoenergy. In contrast to RF ablation, junctional rhythm is rarely seen during cryoablation. Usually, delivery of several cryoapplications is required to eliminate the SP completely (Wang and Chentg, 2013).

Endpoints of ablation

The "perfect" endpoint for SP ablation is the elimination of dual AVN physiology/SP conduction without impairing the FP (Table 2). Having said that, abolition of all sign of SP conduction is not a sine qua non-requirement for a successful ablation procedure. Therefore, the non-inducibility of AVNRT with residual signs of dual AV node pathways (AH jump) and single AVN echo, is an acceptable endpoint. However, double AV nodal echoes predict inducibility of AVNRT and late recurrence (McElderry and Kay, 2006).

Table 2 End points for catheter ablation

Tachycardia rendered noninducible with/without isoproterenol
Elimination of slow pathway function
● Elimination of 1:1 antegrade conduction over slow pathway
● Elimination of AH interval jumps
Modification of slow pathway function
● AH interval jump with single echoes only (previously inducible)

Antiarrhythmic drugs

Long-term pharmacological therapy is recommended for patients who are not candidates for or prefer not to undergo, catheter ablation. In general, any pharmacological agent that depress the AV node conduction can be used. However, the efficacy preventing recurrences of AVNRT is low (30%–50%) (Lockwood et al., 2009). Both verapamil and diltiazem have been well studied and are an effective alternative to ablation (Mauritson et al., 1982). Evidence for beta blockers is limited. One small randomized clinical trial compared digoxin, verapamil, and propranolol in patients with AVNRT and AVRT. The recurrences and duration of tachyarrhythmia were similar among the three drugs (Winniford et al., 1984). Beta blockers should be chosen over calcium blockers in patients with systolic dysfunction.

If those drugs are ineffective or contraindicated, flecainide or propafenone is the reasonable option in patients without structural heart disease (Class IIa).

Lastly, in patients with infrequent but prolonged episodes of tachycardia refractory to vagal maneuvers, a "pill in the pocket" approach could be considered (Class IIB). This approach consists in the use of a single dose of a drug that has a short onset of action (diltiazem or flecainide) alone or in combination with propranolol to terminate the tachycardia (Yeh et al., 1985).

Conclusions

AVNRT is a common arrhythmia in clinical practice. The full understanding of its electrophysiological mechanisms allows designing the most appropriate therapeutical approach. Adequate ECG interpretation and differential diagnosis with other supraventricular arrhythmias are paramount.

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ATTR Cardiac Amyloidosis: Classification, Diagnosis, and Management

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Introduction

Amyloidoses are a set of systemic diseases whereby misfolded proteins form insoluble aggregates known as amyloid fibrils. These fibrils deposit in the extracellular space of various organs leading to their dysfunction. Cardiac amyloidosis is an infiltrative cardiomyopathy caused by the accumulation of amyloid fibrils in the extracellular space of the myocardium and often leads to diastolic and systolic dysfunction, conduction disease, and death (Ruberg and Berk, 2012; Gertz et al., 2015; Falk et al., 2016). The classification of amyloidosis depends upon which precursor protein leads to the pathologic aggregates; there are dozens reported (Sipe et al., 2016). The predominant causes of cardiac infiltration are immunoglobulin light-chain (AL) amyloidosis and transthyretin amyloidosis (ATTR). This article focuses on the pathophysiology, epidemiology, diagnosis, prognosis, and treatment of ATTR.

Transthyretin (TTR) is a 56-kDa protein, formerly called prealbumin, that is predominantly synthesized in the liver with smaller contributions (<5%) from the choroid plexus and retinal pigment epithelium (Ruberg and Berk, 2012; Gertz et al., 2015). The protein's name is derived from its primary function, *trans*porting *thy*roxine and *retin*ol (Vitamin A) (Gertz et al., 2015; Falk et al., 2016), but it also has a multitude of roles in neuronal growth and neuronal pathways (Vieira and Saraiva, 2014). In its functional form TTR exists as a homotetramer, but monomers are prone to dissociate and misfold into insoluble beta-pleated sheets (Brunjes et al., 2016). In ATTR, it is these misfolded, insoluble proteins that deposit in target organs as amyloid fibrils. ATTR can result from the deposition of normal, or wild-type, TTR (ATTRwt) or from the deposition of genetically abnormal, or mutant, TTR (ATTRm). These distinct processes have varying clinical manifestations and treatments, and both disease entities will be discussed at length in this article.

Pathogenesis

As described earlier, the principle pathophysiologic mechanism uniting all forms of amyloidosis is interstitial deposition of misfolded, insoluble amyloid proteins leading to multiorgan dysfunction and eventually failure.

AL amyloidosis is a disease of the plasma cell, similar but distinct from multiple myeloma, whereby immunoglobulin light chains (lambda and/or kappa) are overproduced. Aggregates of these light chains deposit in target organs causing disease. The kidney is the most commonly affected organ, leading to nephrotic syndrome and eventual chronic kidney disease (Falk et al., 2016).

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The heart is the next most commonly affected organ, but amyloid deposition and clinical sequelae thereof can be found in multiple other organs as well: nerve involvement can result in peripheral neuropathy and postural hypotension, depositions in the tongue can result in macroglossia, microvascular disease can lead to pronounced periorbital bruising, hepatic involvement can lead to a firm and nonpulsatile liver (though usually not frank liver failure) (Falk et al., 2016). There is also some emerging evidence that pathologic circulating amyloid light chains play a role in cardiac dysfunction independent of infiltrative disease (Liao et al., 2001; Brenner et al., 2004).

ATTRwt, formerly called senile cardiac amyloidosis, occurs with the myocardial deposition of misfolded, but genetically normal, TTR. The incidence of ATTRwt increases profoundly with age but the exact mechanism of age-related TTR misfolding and the predilection for clinical disease, especially in men is unclear. Some authors have suggested that age-related biochemical changes to either the native protein or one of its chaperone proteins results in a propensity for TTR to disaggregate and/or impairs the cell's ability to clear the misfolded protein (Ruberg and Berk, 2012; Buxbaum et al., 2012). In contrast, in ATTRm amyloid deposition occurs due to a mutation in the TTR gene that hastens misfolding and aggregation (Ruberg and Berk, 2012). ATTRwt has a significant association with carpal tunnel syndrome (Sekijima et al., 2011) and, depending on the exact mutation, ATTRm often affects peripheral nerves and kidneys in addition to the heart. In both forms of ATTR though, cardiac dysfunction takes the form of an infiltrative and restrictive cardiomyopathy. Amyloid fibrils infiltrate the myocardium and cause increased ventricular wall thickness and impaired lusitropy, resulting in elevated filling pressures and eventually heart failure with preserved ejection fraction (HFpEF). Amyloid infiltration also leads to conduction disease, specifically atrioventricular block and atrial fibrillation or flutter. Progression to systolic dysfunction is a late finding (Gertz et al., 2015).

Epidemiology and Genetics

ATTRwt is sporadic but is overwhelmingly a disease of older, white men (Maurer et al., 2016). Greater than 90% of those diagnosed are older than 60 years, and autopsy studies have shown upward of 25% of all-comers older than 80 years have evidence of cardiac ATTRwt, many of which did not have clinical evidence of heart failure (Ruberg and Berk, 2012; Tanskanen et al., 2008). The true prevalence of ATTRwt is unknown, but there is general consensus that it is under diagnosed and this will bear out in clinical practice with improvements in diagnostic technology (outlined later). A single center study showed that 13% of all patients hospitalized with HFpEF and ventricular wall thickness >12 mm were newly diagnosed with ATTRwt via technetium scintigraphy (González-López et al., 2015).

Epidemiology of ATTRm depends greatly on the specific disease-causing mutation, and there are over 80 disease-causing TTR mutations described today (Benson and Kincaid, 2007). All follow an autosomal-dominant inheritance pattern, but penetrance is variable. The most common pathogenic mutation worldwide and second most common in the United States is V30M (Maurer et al., 2016). The V30M mutation predominantly affects patients with Portuguese, Swedish, Japanese, or Brazilian heritage and has a prevalence around 1:1000 (Ruberg and Berk, 2012; Gertz et al., 2015). The V30M mutation has widely variable clinical penetrance, but peripheral neuropathy is nearly universal (95%), while only 43% have diagnosed cardiomyopathy (Swiecicki et al., 2015). The V122I mutation originates in West Africa and is present in 3%–4% of the US black population, making it the most common ATTR-associated mutation in the United States. The clinical importance of a genetic carrier of V122I is not entirely clear. Studies have shown that mortality is not significantly increased (Quarta et al., 2015), but heart failure outcomes in general are worse for those who carry V122I even in the absence of diagnosed amyloidosis (Buxbaum et al., 2006). The T60A mutation originated in County Donegal in the northwest region of Ireland where it still has a 1% prevalence (Ruberg and Berk, 2012). There is a T60A cadre in the United States as well, where the disease was formerly called Appalachian amyloidosis. In one study, 82% of T60A carriers were diagnosed with cardiomyopathy and 65% were diagnosed with peripheral neuropathy (Swiecicki et al., 2015).

Relative incidence and prevalence data is helpful but not perfectly clear. In the United States the annual incidence of AL amyloidosis is a little more defined than ATTR and has historically been quoted around 1–2 per million patient-years (Kyle et al., 1992). An older (1995) US study of 100 consecutive patients referred for cardiac biopsy for presumed amyloidosis demonstrated that 74% had AL amyloidosis versus 22% ATTRwt and 6% ATTRm (Crotty et al., 1995). A 2010 German study of 101 consecutive patients with previously diagnosed cardiac amyloidosis found higher rates of ATTR, with AL amyloidosis being found in 54% versus 37% ATTRwt and 5% ATTRm (Kieninger et al., 2010). It is also worth remembering that ATTR (particularly ATTRwt) is generally considered to be under diagnosed.

Clinical Features and Diagnosis

Clinical Manifestations

The first clues to establishing the presence of cardiac amyloidosis are clinical. The diagnosis should be considered for any patient being evaluated for heart failure with preserved systolic function (HFpEF), especially when extracardiac manifestations are present. Peripheral neuropathy is common, particularly in ATTRm, and in fact an historical name for heritable forms of ATTR was familial amyloid polyneuropathy. ATTR-associated neuropathy is classically a progressive, symmetric, length-dependent, axonal process. It can affect all nerve types, which can help distinguish this neuropathy from other more common neuropathies (e.g., the small fiber neuropathy associated with diabetes). Neurologic symptoms typically start in the lower limbs and progress to upper limbs.

Autonomic involvement is also common (Gertz et al., 2015). Approximately 95% of V30M, 65% of T60A, and 30% of V122I carriers are diagnosed with peripheral neuropathy. ATTRwt does not typically demonstrate the same discrete polyneuropathy, but approximately 50% of those going on to be diagnosed with ATTRwt will have a preceding diagnosis of carpal tunnel syndrome owing to amyloid infiltration of the connective tissue of the carpal tunnel itself (Sekijima et al., 2011). Nephrotic syndrome is a common presentation for AL amyloidosis (Falk et al., 2016), and renal dysfunction is demonstrated in approximately 15% of those with ATTRwt (probably tracking with age) (Maurer et al., 2016). Prevalence and degree of renal dysfunction in ATTRm depends on the specific mutation (Falk et al., 2016; Maurer et al., 2016)—a study of patients with V30M ATTR showed rates of nephropathy and ESRD approaching 40% and 10%, respectively (Lobato et al., 2004).

The most marked clinical cardiac manifestation of amyloidosis is an infiltrative cardiomyopathy and consequently impaired diastolic function and HFpEF. Symptoms of right-sided heart failure tend to be more common early, but clinical evidence of biventricular failure is not uncommon and frank systolic dysfunction (right or left-sided) is a late finding (Patel et al., 1997).

ECG

Echocardiography reliably shows left ventricular free wall and septal thickening (± right ventricular thickening), but ECG generally fails to show evidence of left ventricular hypertrophy (LVH) (Gertz et al., 2015). The classic association for cardiac amyloidosis is LVH by echocardiography with paradoxically low voltage on ECG; however, previous reports have demonstrated low voltage in only around half of those with AL amyloidosis and 25% of those with ATTR (Rapezzi et al., 2009; Cyrille et al., 2014). A "pseudoinfarct" pattern of pathologic Q-waves, ST-T changes, and/or poor R-wave progression is more a sensitive marker seen in nearly 70% of those with both AL amyloidosis and ATTR (**Fig. 1**).

In addition to these diagnostic ECG clues, frank conduction disturbances are common in cardiac amyloidosis (Maurer et al., 2016; Pinney et al., 2013). In a study of 200 patients with all three forms of cardiac amyloidosis, atrial fibrillation was most common in ATTRwt (38% vs. 17% for ATTRm and 6% for AL) (Cyrille et al., 2014). First degree AV block was common in all groups—45% for ATTRwt, 33% for ATTRm, and 26% for AL—but, second degree AV block was only seen in those with ATTRwt (10% vs. none for ATTRmt and AL). Cardiac biomarkers (troponin I/T and BNP/NT-pro-BNP) are frequently elevated in cardiac amyloidosis and have some role in prognosis, but their utility in diagnosis is limited. Biomarkers will be discussed further in the "**Prognosis**" section of this article. Other simple lab tests crucial to the initial work-up include serum and urine protein electrophoresis (SPEP and UPEP) and specifically immunofixation and a serum-free light-chain assay. This testing should suggest monoclonal gammopathy in patients with AL amyloidosis and should be negative in those with ATTR although the false positive rate approaches 20% in the aged ATTR population (Gillmore et al., 2016).

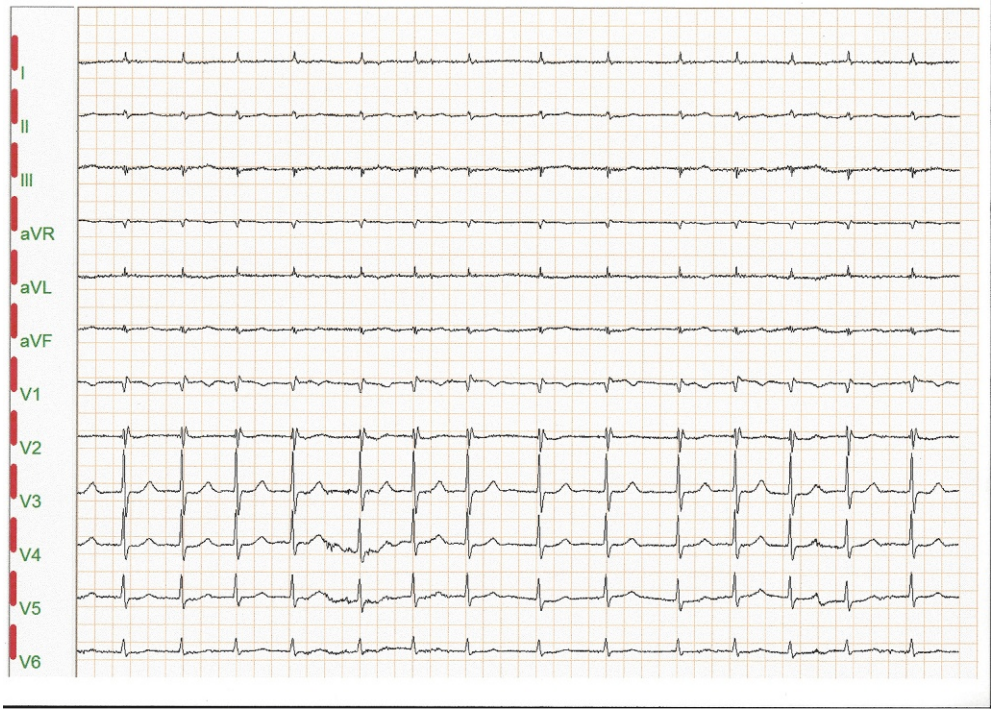

Fig. 1 Twelve lead ECG showing atrial fibrillation and low voltage, especially in the limb leads. Incomplete right bundle branch block and nonspecific STT changes.

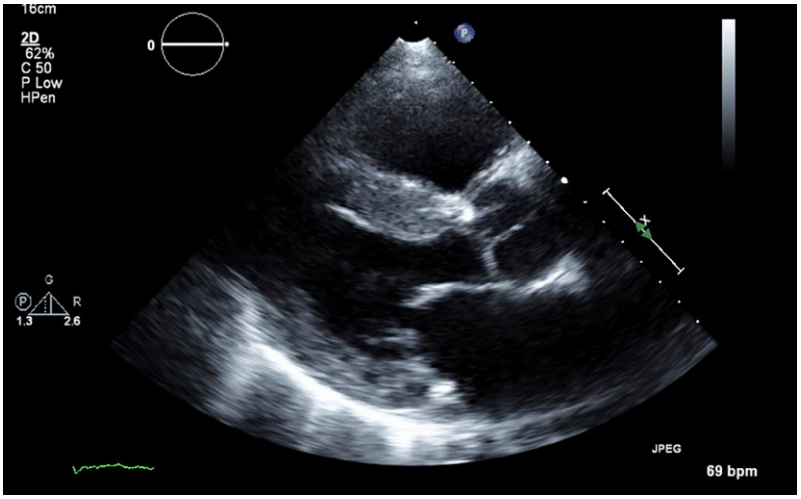

Fig. 2 Parasternal long-axis view demonstrating left ventricular hypertrophy with a "speckled" pattern. Left atrial enlargement is also noted.

Echocardiography

Echocardiography is the most widely available and most well-studied imaging modality for identifying and following patients with cardiac amyloidosis. As has been mentioned, the classic echocardiographic findings include thickened left ventricular wall and septum, small LV chamber volume, and atrial enlargement (Ruberg and Berk, 2012). Ventricular septal thickness is of particular importance. In those with known AL amyloidosis, ventricular septal thickness >12 mm (without concomitant hypertension or aortic valve pathology) defines cardiac involvement of the disease. Degree of ventricular thickness is not, however, uniform across the various cardiac amyloidoses. ATTRwt consistently demonstrates increased LV wall thickness over ATTRm and AL amyloid (Rapezzi et al., 2009; Quarta et al., 2014; Ng et al., 2005), and Cyrille et al. showed a nonsignificant increase in estimated LV mass in those with ATTRwt (355 g) versus ATTRm (333 g) and AL (266 g) (Cyrille et al., 2014). This may reflect a later diagnosis and longer survival of ATTRwt relative ATTRm and AL amyloidosis. At the time of diagnosis, left enlargement (mean 49.5 mm in ATTRwt and 43.1 mm in ATTRm) is characteristic, and pericardial effusion is common (80% in ATTRwt and 59% in ATTRm) (Rapezzi et al., 2009) (**Fig. 2**).

The distinguishing features of an infiltrative cardiomyopathy include findings of LVH by echocardiography without clinical evidence of long-standing hypertension or hypertrophic cardiomyopathy (HCM). While both hypertensive cardiomyopathy and HCM should demonstrate ECG evidence of LVH, there are limitations to overreliance on ECG findings as outlined in the previous paragraph. Other echocardiographic parameters that can be seen in cardiac amyloidosis such as thickening of the atrial septum and/or RV wall should not be seen in hypertensive cardiomyopathy or HCM (Rapezzi et al., 2010; Jacobson et al., 2011). Additionally, speckle tracking and Doppler longitudinal strain can demonstrate even early cardiac amyloid involvement (Koyama and Falk, 2010; Sun et al., 2009) (**Fig. 3**). In a cohort of 45 patients with cardiac AL amyloidosis and 48 with cardiac ATTR, Cappelli et al. showed that LV longitudinal strain was impaired in both AL and ATTR relative to a control group with noncardiac AL amyloidosis (Cappelli et al., 2015). More to the point, Phelan, et al, demonstrated that a longitudinal strain pattern with relative "apical sparing" reliably predicted cardiac amyloidosis relative controls with LVH due to hypertension or aortic stenosis (Phelan et al., 2012). This phenomenon is easily visualized by the "bull's-eye" pattern seen on polar plot diagram (**Fig. 4**).

Cardiac MRI

The advent of cardiac MRI (CMR) has greatly increased the clinician's ability to recognize cardiac amyloidosis and, particularly, to distinguish it from the primary echocardiographic differential of HCM and LVH secondary to hypertension. The distinctive finding for amyloidosis on CMR is global subendocardial late gadolinium enhancement (LGE). Gadolinium is the contrast agent commonly used in MRI. It is an extracellular agent which enters the interstitial space with predictable kinetics allowing for a uniform signal in normal myocardium. With the infiltration of amyloid deposits that characterizes cardiac amyloidosis, the interstitial space is perturbed and gadolinium accumulates in a diffuse pattern (as opposed to a more focal pattern of LGE like that seen in myocardial infarction) (Ruberg and Berk, 2012) (**Figs. 5** and **6**). In a study of 33 consecutive patients with restrictive cardiomyopathy and clinical concern for cardiac amyloidosis sent for both endomyocardial biopsy (EMB) and CMR, Vogelsberg et al. showed that 12 of the 15 patients with biopsy-proven cardiac amyloidosis demonstrated this pattern of LGE (Vogelsberg et al., 2008). This gave a positive predictive value of 92% and a negative predictive value of 85% for global subendocardial LGE in cardiac amyloidosis. There is also some evidence that CMR with more diffuse LGE can differentiate between cardiac amyloidosis and LVH caused by HCM or hypertension where LGE tends to be more focal (Takeda et al., 2013). While it was initially thought that CMR

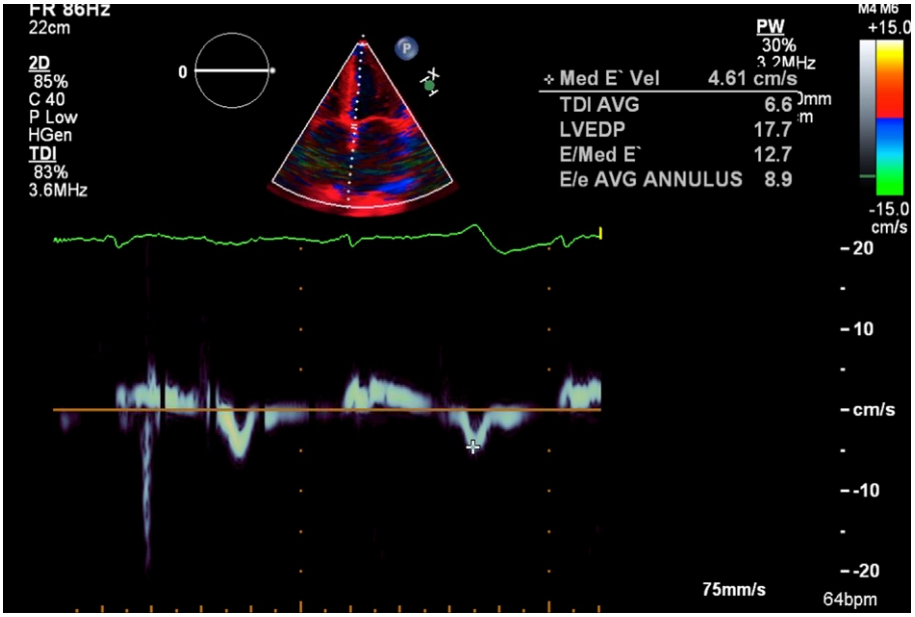

Fig. 3 Tissue Doppler assessment of the septal annulus demonstrates a markedly reduced early diastolic velocity of 4.6 cm/s (+). Normal is greater than 7 cm/s.

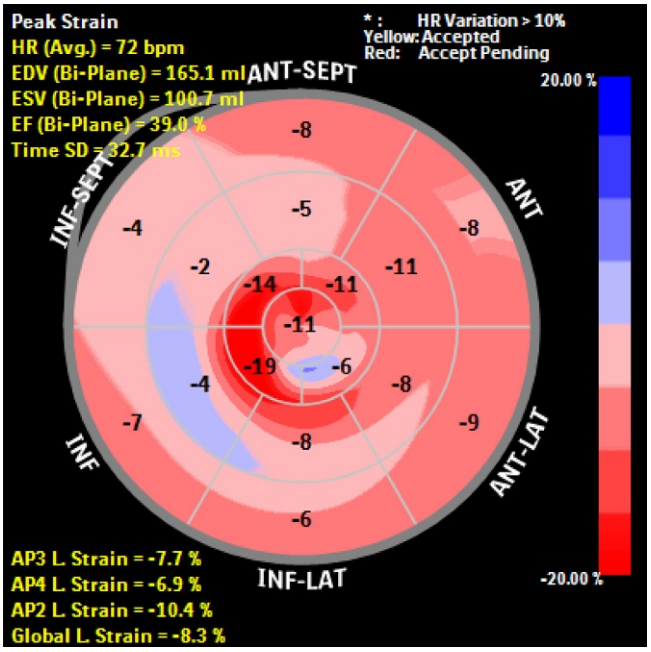

Fig. 4 Typical "bull's-eye" pattern seen on polar plot diagram indicative of longitudinal strain pattern with relative apical sparing.

findings, specifically LGE patterns, were similar between AL amyloidosis and ATTR (Ruberg and Berk, 2012), investigators recently reported that 90% of their patients with ATTR demonstrated transmural LGE as opposed to only 37% of those with AL (Dungu et al., 2014).

Furthermore, difficulty with nulling the images is a typical MRI characteristic in cardiac amyloid (Maceira et al., 2005). It is believed that faster than usual gadolinium washout from the blood and higher myocardial uptake is the cause of this phenomenon.

In addition to LGE, assessment of the extracellular volume fraction through the technique of T1 mapping has also proven successful in identifying cardiac amyloidosis with a high correlation with LGE in these patients (Mongeon et al., 2012). Compared to gadolinium administration, noncontrast T1 mapping technique can be performed throughout all ranges of renal function.

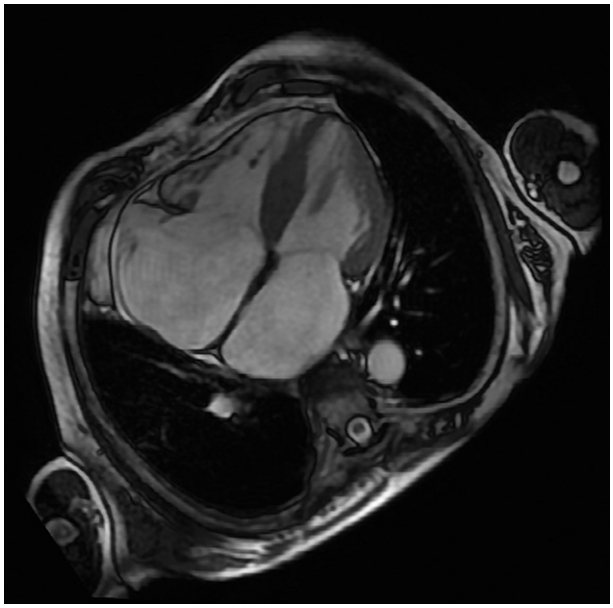

Fig. 5 Four-chamber view showing increased wall thickness and severe biatrial enlargement.

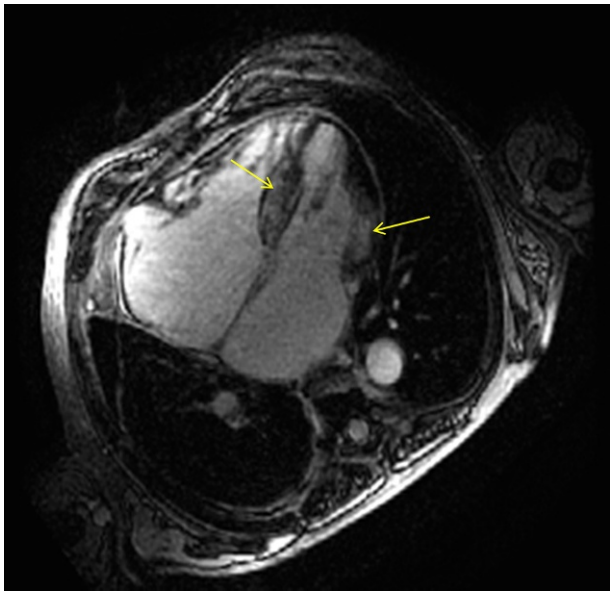

Fig. 6 Corresponding four-chamber view demonstrating late gadolinium enhancement involving the septum and lateral wall.

There is emerging evidence that those with AL cardiac amyloidosis have evidence of strain impairment on CMR even prior to developing LGE (Kuetting et al., 2017). To our knowledge this is yet to be confirmed in ATTR, but this represents yet another promising avenue for early identification of cardiac involvement.

Biopsy

EMB with Congo red staining of amyloid deposits has long been the gold standard for establishing the diagnosis of cardiac amyloidosis. However, with a consistent clinical picture and characteristic imaging findings, a biopsy of affected noncardiac tissue can suffice. AL amyloidosis will often have tongue, skin, or kidney involvement, and these sites are more easily accessible for biopsy, but these organs are rarely involved in ATTR to a degree that biopsy is fruitful (Ruberg and Berk, 2012). For these reasons, diagnosis via noncardiac biopsy has generally been less common in ATTR than AL amyloidosis (Gertz et al., 2015). Fine et al. showed that in 286 patients with ATTR (186 with ATTRm and 100 with ATTRwt), 94% of those with ATTRm were able to be diagnosed without an

EMB versus only 35% of those with ATTRwt (Fine et al., 2014). Nerve biopsy is possible in both AL and ATTR if there is significant neuropathy, though this is rarely the case is ATTRwt. In the Fine et al. study sural nerve biopsy was performed in 29% of the ATTRm subjects and was positive in 83% of those (none were attempted in ATTRwt); rectal biopsy (also looking for nerve involvement) was performed in 28% of those with ATTRm and 4% of ATTRwt and was positive in 81% and 50%, respectively. Abdominal fat aspirate is a simple outpatient or office-based procedure that has been studied in ATTR (Jacobson et al., 2011). In the Fine et al study, 76% of those with ATTRm and 84% of those with ATTRwt underwent abdominal fat aspirate with positive results in 67% of ATTRm versus only 14% of ATTRwt. In a patient with suspected ATTR and no extracardiac symptoms, abdominal fat aspirate is reasonable, but its sensitivity is low in the setting of ATTRwt, and the majority of these patients go on to EMB if tissue diagnosis is required.

Historically, biopsy samples of affected tissue underwent immunohistochemical staining for light chains and TTR. Immunoperoxidase and immunogold stains for TTR have the potential for false positives (Satoskar et al., 2011), but the addition of mass spectroscopy analysis to immunohistochemical staining has been shown to improve diagnostic accuracy from 76% to 94% with regard to differentiating AL amyloidosis from ATTR (Gilbertson et al., 2015). The process of genotyping ATTR can be accomplished via isoelectric focusing (electrophoresis), but more recently polymerase chain reaction and gene sequencing using a simple blood sample has simplified the process and provides more definitive diagnosis.

Radionuclide Imaging

Radionuclide imaging is an evolving modality in the world of cardiac amyloidosis and in some cases establishes a diagnosis without a biopsy at all. Several commonly used tracers bind avidly to TTR (both mutant and wild-type) and have the potential to establish the diagnosis of cardiac ATTR or distinguish ATTR from AL when the diagnosis of amyloidosis is known or strongly suspected. Three of the most commonly used radiotracers are ^{99m}Technetium-3,3-diphosphono-1,2-propanodicarboxylic acid (Tc-DPD), ^{99m}Technetium-pyrophosphate (Tc-PYP), and ^{99m}Technetium-hydroxymethylene diphosphonate (Tc-HMDP). An initial study of Tc-DPD in Italy showed that in 25 patients with cardiac amyloidosis (15 with ATTR and 10 with AL), there was robust cardiac uptake in all of the ATTR patients and none of the AL patients (Perugini et al., 2005). However, a larger follow-up study by the same authors demonstrated 100% uptake in the ATTR group but weak uptake in almost a third of the AL group, thus weakening the specificity for this agent (Rapezzi et al., 2011). Tc-PYP is more widely available in the United States, and a study by Bokhari et al. of 45 patients with cardiac amyloidosis (16 with ATTRwt, 17 with ATTRm, 12 with AL) demonstrated a 97% specificity and 100% sensitivity for this tracer in the detection of ATTR (Bokhari et al., 2013). Tc-HMDP is the newest of these agents and in a study of patients with known TTR amyloidosis, this tracer showed cardiac uptake in all those with echocardiographic evidence of amyloidosis and in 8/19 without echocardiographic evidence of cardiac amyloid infiltration (Glaudemans et al., 2014). This suggests that Tc-HMDP may be even more sensitive than echocardiography in detecting early cardiac involvement in those with ATTR. In a study of 121 patients with suspected cardiac amyloidosis, Tc-HMDP scintigraphy demonstrated uptake in 100% of patients with ATTRwt ($n = 21$), 97% of those with ATTRm ($n = 34$), but 7% of those with AL amyloidosis ($n = 14$) (Galat et al., 2015). In total, these studies suggest that robust cardiac uptake is suggestive of ATTR, while lack of cardiac radiotracer uptake virtually eliminates the possibility of cardiac ATTR.

Genotyping and Role for Nonbiopsy Diagnosis

Given the substantial differences in management among the various types of cardiac amyloidosis, prompt diagnosis with identification of the specific amyloidogenic precursor protein is crucial. As outlined previously, amyloid genotyping used to absolutely require a sample of affected tissue, but now TTR gene sequencing with a peripheral blood sample has greatly simplified the task of genotyping once the diagnosis of ATTR is established. In fact, new data suggests that ATTR can be reliably diagnosed without a biopsy of any kind. In a study of 1217 patients with suspected cardiac amyloidosis referred to specialized amyloid centers in the United States and Europe, the absence of monoclonal gammopathy (on SPEP/UPEP, immunofixation, and serum-free light chains) combined with robust myocardial uptake (grade 2 or 3) on technetium scanning had a positive predictive value of 100% for cardiac ATTR (Gillmore et al., 2016). It is worth noting that the pretest probability for cardiac amyloidosis was extremely high in these patients given their referral to a specialized amyloid center. This will likely result in a substantially lower specificity in actual clinical practice; nonetheless, this study has altered the work up for cardiac amyloidosis, and it has given a clear path for the nonbiopsy diagnosis of ATTR. **Diagram 1** outlines our suggested diagnostic algorithm for those patients in whom suspicion of cardiac amyloidosis is high on the basis of the clinical features and imaging characteristics we have described; in particular it highlights the possibility of a nonbiopsy diagnosis.

Natural History

The natural history for all three forms of cardiac amyloidosis involve worsening heart failure and eventual death from pump failure and/or arrhythmia, but there is considerable heterogeneity in any individual's course, and there are varying contributions from noncardiac manifestations depending upon the specific disease entity. ATTRwt has the most delayed diagnosis and the most indolent course of the three. Patients with ATTRwt are typically older (diagnosed in their mid-70s), have the largest ventricular wall thickness, and are more likely to have systolic dysfunction compared to those with ATTRm and AL amyloidosis (Rapezzi et al., 2009;

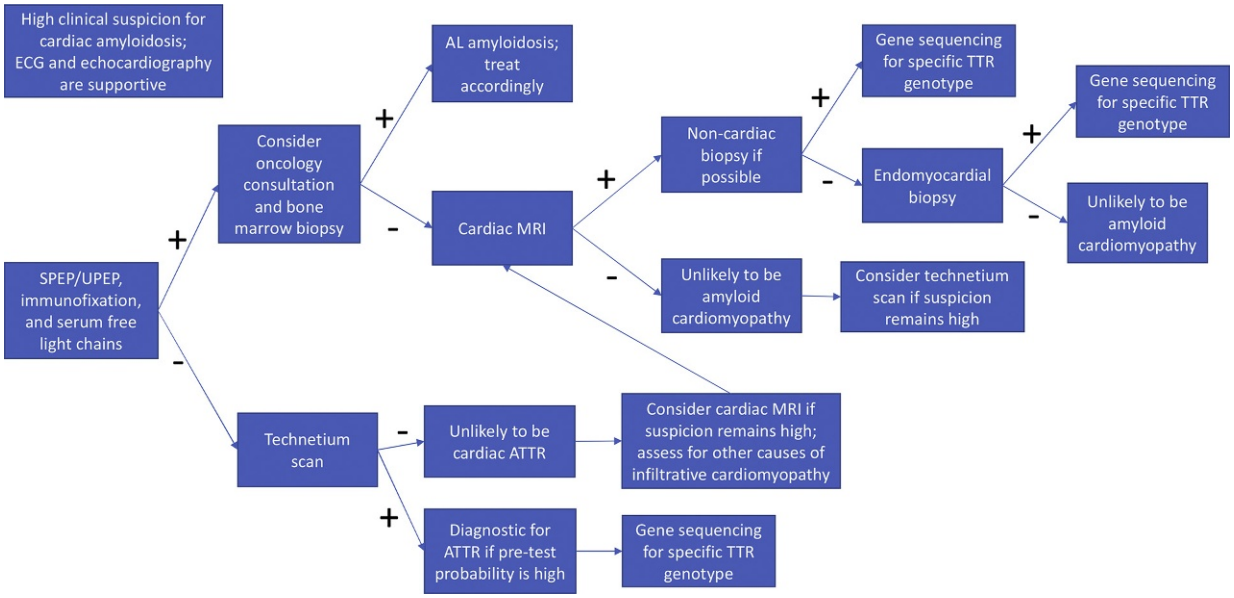

Diagram 1 Diagnostic algorithm for patients with high clinical suspicion for cardiac amyloidosis.

Ng et al., 2005). This likely reflects delayed diagnosis of these patients due to both underrecognition and relative lack of noncardiac manifestations. Despite thicker ventricular walls and advanced age, ATTRwt is associated with a relatively longer survival. A study of 18 consecutive patients diagnosed with ATTRwt compared with 18 randomly selected AL amyloid controls demonstrated median survival of 6 years in ATTRwt versus only 11 months in the AL controls (Ng et al., 2005). Additionally, a 2009 study comparing the clinical course of 233 patients with cardiac amyloidosis (AL $n = 157$; ATTRm $n = 61$; ATTRwt $n = 15$) not only showed that ATTR had prolonged survival over AL, but that ATTRwt specifically had a higher rate of MACE-free survival (77% at 2 years vs. only 51% for AL) (Rapezzi et al., 2009).

As outlined in the "**Pathogenesis**" section of this article, the course for ATTRm is more likely to be complicated by extracardiac manifestations in addition to infiltrative cardiomyopathy, but clinical course varies by mutation and previous therapy (specifically organ transplantation). In a UK/Canadian study of 60 consecutive patients with T60A ATTRm, the median age of symptom onset was 63 years and the median time from diagnosis to death was 41 months (Sattianayagam et al., 2012). For patients with V122I, the commonest ATTR-associated mutation in the United States, two studies of patients with cardiac involvement referred to an amyloid center have demonstrated a median age of 70 years at diagnosis and a median survival of 27 and 25.6 months, respectively (Connors et al., 2009; Ruberg et al., 2012). Only around 40% of carriers of the V30M mutation go on to develop clinically significant cardiomyopathy, but because of the near-universal development of neuropathy, these patients tend to be diagnosed younger (mean age 41 years (Lobato et al., 2004)), and authors have quoted older data demonstrating overall survival around 10-15 years postdiagnosis (Lobato et al., 2004; Andersson, 1976; Ericzon et al., 2015). This mutation also has a particularly high rate of renal dysfunction; around 10% of carriers progress to dialysis-dependent ESRD (Lobato et al., 2004). In one study, these patients had a 2-year survival of only 38% after initiating dialysis.

Taken together, these data suggest that diagnosis is often delayed in those with ATTRwt relative ATTRm (and certainly relative AL amyloidosis), but the course of ATTRwt is generally more indolent and extracardiac manifestations are less common. Among the common mutations associated with ATTRm, those with V30M tend to be diagnosed earliest but die the youngest, owing mostly to the extracardiac manifestations of their disease.

Prognosis

Clinical/Laboratory

There is a robust literature base supporting the link between elevated troponin and BNP or pro-BNP and inferior outcomes in AL amyloidosis (Dispenzieri et al., 2003; Kumar et al., 2012; Palladini et al., 2003). A 2012 study out of the Mayo Clinic using seven different parameters to create and validate an AL severity scoring system showed that only troponin T, NT pro-BNP, and a variable known as free light-chain difference (the difference between involved and uninvolved light chains) were independently predictive of survival (Kumar et al., 2012). The role for biomarkers in ATTR is less definitive but evolving. A Swedish study of 29 patients with ATTRm (mostly V30M) showed low positivity overall for troponin (10% for troponin T and 21% for troponin I), but elevated BNP was found in 76% of these patients and this correlated with ventricular septal thickness and impaired strain pattern on echocardiography (Suhr et al., 2008). In a Japanese study of 27 patients with ATTR (14 with ATTRm and 13 with ATTRwt), the log BNP

correlated with increased left ventricular wall thickness and impaired echocardiographic parameters of diastology (E/e') (Usuku et al., 2013). A 2013 study of 99 patients with ATTRwt demonstrated that NYHA class, need for pacemaker placement, and positive troponin T (but not NT pro-BNP) were all predictive of increased mortality (Pinney et al., 2013). More recently, Grogan and colleagues found that advanced age, presence of pericardial effusion, impaired ejection fraction, and elevations of NT pro-BNP and troponin T were all associated with increased mortality in their cohort of 360 ATTRwt patients (Grogan et al., 2016). Additionally, they developed a staging system based only on threshold elevations of NT pro-BNP (above 3000 pg/mL) and troponin T (above 0.05 ng/mL). Patients negative for both (stage I) or positive for one of the two (stage II) had 4-year survival rates of 57% and 42%, respectively. Those positive for both (stage III) had a 4-year survival of only 18%—a hazard ratio of 3.6 relative to stage I.

Imaging

RV dilatation has been associated with shorter survival in the AL amyloidosis population (Patel et al., 1997). In a cohort of 82 cardiac amyloidosis patients (AL = 26, ATTRm = 37, ATTRwt = 19), NYHA class and tricuspid annular plane systolic excursion less than 14 mm were independent predictors of MACE (Bodez et al., 2016), but not specifically mortality. The diagnostic role for nuclear imaging with technetium was discussed in the last section; however, a recent study of 602 patients with cardiac ATTR (ATTRm = 225 and ATTRwt = 377) failed to demonstrate the prognostic utility of Tc-DPD (Hutt et al., 2017). Tc-PYP demonstrating a quantitative uptake heart to contralateral (H/CL) ratio greater than 1.6 was shown in one study to be associated with poorer 5-year survival in patients with all forms of ATTR (Castano et al., 2016). Serial Tc-PYP scanning as a modality for monitoring disease progression in those with known ATTR was not shown to be beneficial in a recent study of 20 patients (ATTRwt = 10 and ATTRm = 10) (Castaño et al., 2016). While not having a role in diagnosis, assessment of cardiac sympathetic denervation with ^{123}-meta-iodobenzylguanidine (MIBG) scanning has been shown to have prognostic value in ATTRm. In a study of 143 patients with V30M mutation, those with late heart-to-mediastinum uptake ratio below the lower limit of normal (<1.60) were shown to have an increased mortality at 5.5 years with a hazard ratio of 7.19 (Coutinho et al., 2013). Cardiac dysautonomia as assessed by myocardial MIBG uptake and heart rate response to atropine was similarly shown to have prognostic value in ATTRm patient postliver transplantation (Algalarrondo et al., 2016). The diagnostic utility of LGE on CMR was discussed in the previous section, but LGE pattern has prognostic value as well. Raina and colleagues pooled data from seven studies of patients with cardiac amyloidosis undergoing CMR (AL = 275 and ATTR = 146) and found that the presence of LGE was associated with increased mortality at the mean study duration of 25 months with an odds ratio of 4.96 (Raina et al., 2016).

Treatment

Supportive Care

The first and most important step in treating any amyloidosis is to initiate the appropriate supportive care for the sequelae wrought by amyloid deposition. From a cardiac perspective that means addressing symptoms of heart failure and/or arrhythmia. While diuresis is certainly important for control of excessive volume and associated dyspnea and peripheral edema, the restrictive cardiomyopathy characteristic of ATTR often leaves patients in a preload dependent state and those patients do not tolerate aggressive diuresis; loop diuretics should be used with caution (Ruberg and Berk, 2012; Gertz et al., 2015). Spironolactone is often better tolerated (Ruberg and Berk, 2012), and there is some data to suggest a benefit to aldosterone antagonism in HFpEF more broadly (Lewis et al., 2016; Gu et al., 2016; Kosmala et al., 2016; Pfeffer et al., 2015). Older studies have shown less favorable outcomes in AL amyloidosis treated with calcium channel blockers and/or digoxin as these agents can bind to amyloid fibrils and lead to toxicity (Grogan and Dispenzieri, 2015; Rubinow et al., 1981). Cautious afterload reduction with ACE inhibitors or angiotensin receptor blockers has been suggested by some authors as a way to improve forward flow (Ruberg and Berk, 2012) but this has been discouraged by other authors (Gertz et al., 2015; Grogan and Dispenzieri, 2015). Tachycardia is often a compensatory mechanism to maintain cardiac output in these patients, and for this reason beta blockade (or any negative chronotropy) is generally poorly tolerated and considered contraindicated (Ruberg and Berk, 2012; Gertz et al., 2015; Grogan and Dispenzieri, 2015). Left ventricular assist devices (LVAD) have been used in patients with severe cardiomyopathy secondary to ATTR, generally as a bridge to transplantation. As may be expected, mortality is high in these patients that are not able to undergo transplantation (Grupper et al., 2015; Swiecicki et al., 2013). Some authors have suggested that thickened ventricular walls and the relatively small ventricular cavity size characteristic of ATTR poses a technical challenge for VAD over a more traditional dilated cardiomyopathy (Gertz et al., 2015). There are also concerns about high comorbid RV dysfunction in this group and thus inability to tolerate high venous return. Though trials are scarce, there is literature out of the Mayo Clinic suggesting that LVAD placement is feasible as both bridge to transplant and destination therapy in patients with an otherwise poor prognosis and that it prolongs survival over medical therapy alone, even with the bulk of patients having at least moderate RV dysfunction (Grupper et al., 2015; Swiecicki et al., 2013). In 28 total patients followed for approximately 15 months, preoperative LV end diastolic diameter less than 46 mm was associated with increased mortality.

Atrial fibrillation is the most common dysrhythmia associated with ATTR. A study by Cyrille et al. that was previously discussed in this article quoted prevalence of 38% in ATTRwt and 17% in ATTRm (Cyrille et al., 2014). Given the degree of atrial dilatation and amyloid infiltration, cardioversion and other rhythm control strategies are often unsuccessful in this patient group (Ruberg and Berk, 2012). As mentioned, low-dose beta blockade can be attempted for rate control but is often poorly tolerated. Amiodarone has

been used as a rate control agent as well. Though data are relatively sparse, radiofrequency ablation for atrial fibrillation in the setting of cardiac amyloidosis (both AL and ATTR) has been described and has demonstrated symptomatic benefit (Tan et al., 2016). Some authors have suggested that ablation for atrial fibrillation tends to have a lower success rate but is more promising in ATTR patients with a typical flutter circuit (Ruberg and Berk, 2012). In those patients who cannot be rate controlled or cannot tolerate attempts at rate control, there is a role for AV nodal ablation (Tan et al., 2016) and subsequent pacemaker placement. High degree or symptomatic AV block is an indication for pacemaker placement as well (Algalarrondo et al., 2012; Epstein et al., 2008). It is uncertain if dual chamber pacemaker placement with chronic right ventricular pacing in the setting of cardiac amyloidosis poses an additional risk of progressive left ventricular failure. The role of upfront cardiac resynchronization therapy with biventricular pacing in this setting make sense physiologically and has been described in the literature (Zizek et al., 2013), but its benefits have not been studied in a trial setting.

Due to the risk of arrhythmia and sudden cardiac death, prophylactic ICDs have been placed in patients with ATTR, though there is a relative paucity of data supporting their benefit. In one study of ICD placement in 33 patients with AL amyloidosis and 19 with ATTR (10 ATTRwt and 9 ATTRm), ICD firing was much more common in those with AL and appropriate shocks were more common in those patients that had had their ICD placed as secondary rather than primary prevention (Lin et al., 2013). While it seems reasonable to pursue ICD placement in ATTR, particularly for secondary prevention, ultimately no study has shown a survival benefit in this patient population (Lin et al., 2013; Kristen et al., 2008; Varr et al., 2014; Hamon et al., 2016).

Organ Transplantation

Given TTR is primarily synthesized in the liver; orthotopic liver transplantation should be considered for definitive disease-modifying treatment in those with ATTRm. By removing the hepatic source of mutant TTR and replacing this with a source of wild-type TTR there is attenuation of progressive organ dysfunction from continued mutant amyloid deposition. While transplantations are performed all over the world, including the United States, the majority have been done in Europe and Brazil on patients with V30M mutations. Outcomes data for these patients comes from the Familial Amyloid Polyneuropathy World Transplant Registry (FAPWTR), and the 20-year experience was published in 2014 (Ericzon et al., 2015). Greater than 90% of transplanted patients carried the V30M mutation, and 20-year survival for all-comers was 55%. The authors note that this is a substantial improvement to the mean time from diagnosis to death of 10–15 years that is classically attributed to V30M. Independent risk factors for poorer survival included elevated BMI, age at transplant >50 years, time of disease duration prior to transplant, and mutation other than V30M. In fact, patients with V30M transplanted at <50 years had a 10-year survival approaching 80%. Cardiovascular cause of death was reported in 22% of their fatalities which, the authors note, was substantially higher than the 9% rate reported by European Liver Transplant Registry (representing patients with all indications for liver transplantation). Cardiac dysautonomia postliver transplant as assessed by myocardial MIBG uptake and heart rate response to atropine has prognostic value, as was discussed in the previous section (Algalarrondo et al., 2016).

Though liver transplantation has seemingly prolonged survival in patients with ATTRm due to the V30M mutation, liver transplantation is not a panacea for patients with ATTR. A Dutch study of nine patients with ATTRm followed for at least 4 years postliver transplantation showed a decreased health-related quality of life relative a control group of liver transplant recipients (Drent et al., 2009). In particular, six of the nine ATTRm patients reported worse cardiovascular health at 4 years. In fact, worsening cardiac function in ATTRm patients postliver transplant is not uncommon. Several studies have shown progressive infiltrative cardiomyopathy even postliver transplantation (Okamoto et al., 2011; Olofsson et al., 2002). The reason for this seems not to be related to any recurrence of mutant TTR, but rather an accelerated codeposition of wild-type TTR on top of the existing mutant TTR fibrillar matrix. An autopsy study of three patients with ATTRm postliver transplant compared with a control group of seven ATTRm patients who did not undergo liver transplant demonstrated that the relative composition of cardiac amyloid deposition in nontransplanted patients was 60% mutant TTR and 40% wild-type, versus only 25% mutant TTR and 75% wild-type in those who had undergone transplantation (Liepnieks and Benson, 2007). This phenomenon of accelerated deposition of wild-type TTR has also been shown to cause progressive neuropathy in ATTRm patients postliver transplantation (Liepnieks et al., 2010).

This concept of accelerated wild-type TTR deposition worsening cardiomyopathy postliver transplantation has prompted combined heart and liver transplantation, with the premise being that one removes and replaces first the nidus for clinically significant amyloid deposition (the heart) and then the source of amyloidogenic protein (the liver) (Ericzon et al., 2015; Nelson et al., 2013; Sack et al., 2008). In a Danish study of seven patients undergoing heart and liver transplantation due to ATTRm (Leu111Met mutation) and severe cardiomyopathy, two died in the first year while the other five survived to the study's mean follow up of 55 months without recurrence of their infiltrative cardiomyopathy (Nelson et al., 2013). Isolated heart transplant has been reported in ATTR (Davis et al., 2015; Hamour et al., 2008; Thenappan et al., 2014) and seems to be most effective and practical in patients with the V122I mutation who tend to present later in life and have a more indolent course with less frequent extracardiac disease.

Pharmacologic Therapy

There are some disease-modifying pharmacologic treatments available and they are of emerging importance. Pharmacotherapies for ATTR can be divided into stabilizers, degraders, and silencers based on their mechanism of action.

Stabilizers

Diflunisal is an FDA approved nonsteroidal antiinflammatory that has been shown in ATTR patients to impair TTR tetramer dissociation and thus misfolding into amyloid fibrils (Sekijima et al., 2006; Tojo et al., 2006). In an open-label study of 13 patients with compensated heart failure due to ATTR (7 ATTRwt and 6 ATTRm), diflunisal was generally well tolerated but did not impact any cardiac parameters after 1 year of follow-up (Castaño et al., 2012). In an international randomized trial of 130 patients with ATTRm (55% were V30M), diflunisal slowed the progression of neuropathy and improved quality of life; those with heart failure (NYHA <IV) were included in the study but cardiac outcomes were not specifically addressed (Berk et al., 2013). Diflunisal at a dose of 250 mg twice per day is reasonable in patients with ATTR and significant polyneuropathy and/or compensated heart failure and no contraindications to NSAIDs; however, data demonstrating tangible cardiac benefits are lacking.

Tafamidis is another disease-modifying agent that is available in Europe but not currently in the United States. Like diflunisal, tafamidis stabilizes the TTR tetramer and prevents dissociation and resultant fibril formation (Bulawa et al., 2012). Initial studies have shown that tafamidis slows progression of neuropathy in ATTRm patients and that it is well tolerated over a period of years (Coelho et al., 2013a; Coelho and Maia, 2012). In an open label study of 21 patients with non-V30M ATTRm, treatment with tafamidis 20 mg daily for 1 year resulted in biochemical evidence of TTR tetramer stabilization and no deterioration in ventricular wall thickness, left atrial size, or NT pro-BNP (Merlini et al., 2013). In another open label study of 31 patients with ATTRwt and NYHA class <3, tafamidis (20 mg daily for 1 year) was generally well tolerated and resulted in stabilization of both TTR tetramers and NT pro-BNP levels (Maurer et al., 2015). Two patients (6.5%) died and seven (23%) were hospitalized for cardiovascular causes during the study period; 65% showed no worsening of NYHA class throughout the study. There is an ongoing US trial studying the longer-term effects of tafamidis, specifically in those with ATTR cardiomyopathy (NCT 02791230).

Degraders

The tetracycline antibiotic, doxycycline, has been shown in vitro to disrupt existing amyloid fibrils, and tauroursodeoxycholic acid (TUDCA), a bile acid derivative, has been shown to decrease prefibrillary TTR aggregates in a mice (Cardoso et al., 2003). In a mouse model of ATTRm, the combination of these two drugs decreased the overall burden of amyloid deposition (Cardoso et al., 2010). An open label human trial testing doxycline 100 mg twice per day and TUDCA 250 mg three times per day in patients with ATTR and NYHA <4 has been completed and results are forthcoming. Published preliminary data from that trial showed that with 7 patients completing 12 months of therapy and 10 patients completing 6 months of therapy, the treatment was well tolerated and no patients demonstrated progression of their underlying neuropathy or cardiomyopathy (Obici et al., 2012).

Other compounds that have shown promise in halting the progression of cardiac ATTR include green tea extract (specifically epigallocacatechin-3-gallate) and a red wine derivative, resveratrol. In one study, 19 patients with compensated heart failure due to ATTR (10 ATTRm and 9 ATTRwt) were treated with either tablets of 300 mg green-tea extract or the equivalent 1.5–2 L of brewed green tea per day for 1 year. Fourteen patients completed the study and none demonstrated increased ventricular thickness or progression of their cardiomyopathy as assessed by echo and/or CMR (Kristen et al., 2012). Resveratrol has been shown in cardiac myocytes in vitro to both stabilize TTR tetramers and promote association of TTR monomers into tetramers, thus preventing formation of TTR amyloidogenic aggregates (Bourgault et al., 2011). In vivo human studies, though, have focused more on Alzheimer's disease than cardiac ATTR (Pasinetti et al., 2015).

Silencers

The newest disease-modifying therapies being studied are the small molecules—small interfering RNAs (siRNAs) or antisense oligonucleotides. An siRNA is an exogenous RNA molecule that mediates, epigenetically, the suppression of its target mRNA and thus the transcription of its target protein, in this case TTR. Proof-of-concept phase I trial results for two different delivery formulations of a TTR-targeted siRNA (ALN-TTR01 and ALN-TTR02) demonstrated biochemical evidence of reduction in TTR in both patients with ATTR (NYHA <3) and healthy controls (Coelho et al., 2013b). Patisirin (Alnylam Pharmaceuticals) is the trade-name for ALN-TTR02, and it is currently being studied in a phase III trial (NCT01960348), as is another siRNA, revusiran (ALN-TTRsc; Alnylam Pharmaceuticals; NCT02319005). The concept behind antisense oligonucleotides is similar to siRNA, except these molecules are perfect complements that bind directly to their target mRNA mediating its destruction and thus suppression of target protein transcription. IONIS-TTRrx (Ionis Pharmaceuticals) is an antisense oligonucleotide targeting TTR and is currently being studied in a phase III trial (NCT01737398). Interim data from this trial showed a dose-dependent reduction in serum TTR levels (Ackermann et al., 2012). Small molecules are emerging therapies for a variety of diseases, but they are particularly promising for the future pharmacologic treatment of ATTR which thus far has a paucity of convenient, safe, and effective disease-modifying therapies.

Summary

Cardiac ATTR is a progressive and lethal infiltrative cardiomyopathy and is comprised of two distinct disease entities, mutant and wild-type. ATTR, particularly ATTRwt, is more common than previously thought and should be included in the differential diagnosis for any patient with HFpEF. The classic association of thick-walled ventricles on echocardiography and low voltage on

ECG can be a clue for the diagnosis of cardiac amyloidosis, but it is not particularly sensitive. EMB has long been the gold standard for diagnosis of cardiac amyloidosis, and indeed a tissue sample is generally required to diagnose ATTR. However, with CMR and technetium imaging suggestive of ATTR and a positive noncardiac biopsy (generally peripheral nerve or abdominal fat aspirate), the diagnosis of ATTR can be made without an EMB. Once tissue is obtained, isoelectric focusing and/or polymerase chain reaction can differentiate ATTRm from ATTRwt and usually yield a specific mutation. The chief cardiac manifestations of ATTR are HFpEF, atrial fibrillation, and AV block, and supportive care addressing these issues is paramount. Liver transplantation has been considered definitive therapy for those ATTRm patients in whom it is feasible; however, long-term follow-up has shown progression of cardiomyopathy due to acceleration of TTRwt deposition, and this has prompted consideration of combined heart and liver transplantation. Disease-modifying pharmacologic therapies are available as well with diflunisal, tafamidis, and doxycycline/TUFDA, but these are unproven to date. Small-molecule TTR inhibitors—siRNAs and antisense oligonucleotides—are currently being studied and offer promise for halting progression of this lethal disease.

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Behavior Modification and Cardiovascular Disease

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Introduction

Poor diet and insufficient exercise drive cardiovascular disease in the United States and globally. Any serious attempt to cure cardiovascular disease will have to focus on these risk factors at the individual and population level.

The data are sobering. Nearly two-third of Americans are overweight or obese, and half of adults have one preventable, noncommunicable disease like hypertension or diabetes that predisposes them to chronic poor health and early death.

Ischemic heart disease is the cause of death in one in five Americans. Stroke and diabetes follow close behind and are driven by the same risk factors as frank heart disease.

Diet is the largest factor leading to cardiovascular disease and death (Marczak et al., 2016). While diet is a modifiable risk factor, and therefore of particular interest from a public health standpoint, there remains a vast web of food and socioeconomic policies in addition to individual and social behaviors that influence dietary patterns. Lack of exercise also places a tremendous stress on the body and is a modifiable risk factor. Physicians are undertrained to address these risk factors and often feel that they cannot influence patient behavior. Evidence clearly demonstrates that this is false. Appropriate diet and exercise counseling does have a meaningful impact on patient behaviors. It is more useful, on an individual and population basis, than many of the medicines and interventions designed to treat or relieve symptoms of disease caused by poor diet and exercise habits.

The Biochemical Consequence of a High Fat Diet

Poor dietary habits such as excess caloric intake and over consumption of certain nutrients such as saturated fats and carbohydrates found in processed, packaged meats and fast foods contribute to cardiovascular risk (Mozaffarian and Ludwig, 2010).

Increased dietary consumption of saturated fats elevates low-density lipoprotein (LDL) cholesterol secondary to inhibiting LDL receptor activity. Increased carbohydrate intake results in increased carbohydrate storage in the form of triglycerides. These triglycerides are subsequently stored in the liver (Siri-Tarino et al., 2015a). The presence of higher triglycerides in the liver influences the content of lipoproteins that are made in and secreted from the liver. The result is an increase in small LDL particles and a decrease in high-density lipoproteins (HDL) (Siri-Tarino et al., 2010). Small LDL particles are the most atherogenic type that causes coronary artery disease.

Elevated LDL and elevated triglycerides lead to endothelial dysfunction through a multistep process. LDL undergoes oxidation. Oxidized LDL (oxLDL) causes direct damage to vascular endothelial cells. It also activates monocytes and platelets, leading to the formation of lipid laden macrophage foam cells. Foam cells destabilize coronary plaques and predispose them to rupture and subsequently myocardial infarction.

Additionally, the vascular smooth muscle cells (VSMCs) within the vessels are affected by oxidative injury and proinflammatory cytokines secreted by LDL. VSMCs exposed to oxLDL lose some of their contractile and connective properties. When exposed to oxidized LDL the cells become proliferative and migratory. This change allows the cells to migrate to the intima. The interaction of LDL with cells in the innermost layers of the vasculature is the nidus for plaque formation (Helkin et al., 2016).

Saturated fats also dysregulate the dynamics of insulin use. In the setting of increased free fatty acids, by-products of fatty acid metabolism like diacylglycerol (DAG) and ceramide collect within skeletal muscle cells. The presence of DAG and ceramide induces activation of serine/threonine kinases that phosphorylate insulin-receptor substrate (IRS). This results in reduced glucose sensitivity (Snel et al., 2012a). Likewise, accumulation of DAG within hepatocytes activates protein kinase C. This compels a cascade of metabolic dysregulation that results in increased glucose production and decreased glucose uptake by the liver (Snel et al., 2012b).

The macroscopic consequence of these microscopic changes is diabetes, obesity, and cardiovascular disease.

Obesity and sequelae as a consequence of excess calories

Overtime, a positive energy balance secondary to increased caloric intake and decreased physical exercise leads to increase in the number and size of adipocytes (Tchkonia et al., 2013a) as well as deposition of lipids, mostly triglycerides, in adipose tissue throughout the body (Heymsfield and Wadden, 2017a). Adipose tissue is not simply a storage tank; it has immune, mechanical, and endocrine functions. In states of obesity and lipid storage dysregulation, there is increased adipose deposition in ectopic locations, direct toxicity related to excess fat, and activation of the sympathetic nervous system and renin–angiotensin–aldosterone systems. Such changes to the fat mass result in subsequent release of cytokines (adipokines) from adipocytes and macrophages. This leads to a state of systemic inflammation which further propagates metabolic dysregulation and compels fat deposition, (Heymsfield and Wadden, 2017b; Tchkonia et al., 2013b).

Visceral adipose deposition triggers a series of changes that impact systemic health. The most pronounced of these is chronic activation of the sympathetic nervous system. This results in hypertension and dysregulation of multiple endocrine homeostatic mechanisms.

Furthermore, visceral adipose deposition around the kidneys plays a direct role in the genesis of hypertension. The mechanical compression of the kidney from fat stimulates the renal–angiotension–aldosterone system and sympathetic nervous system (SNS). In addition, adipose deposition around the pharynx can lead to obstructive sleep apnea. Sleep apnea is known to be associated with a loss of diurnal variation in blood pressure and the development of heart failure.

Obesity is the ultimate multisystem disease. Health care systems need to attack the roots of this illness from multiple angles. Physician education, patient education, and policy changes are essential to reversing the epidemic of obesity that is driving the vast majority of illness in our country.

What should our patients eat, and how much will it matter?

Reducing saturated fat intake has been the cornerstone of cardiovascular disease prevention. Modern science has revealed that a more nuanced approach is necessary. It is not adequate to simply replace saturated fat; what this nutrient is replaced with will influence the overall cardiovascular benefit of a shift away from saturated fats. Additionally, the source of the saturated fat matters, as dairy can overall have a neutral to beneficial cardiovascular effect, while red meat consumption clearly increases cardiovascular risk.

The concept of a balanced diet has appropriately shifted from an individual nutrient emphasis to an emphasis on whole foods. There is increasing recognition that valuable nutrients are not well absorbed in isolation, or as a "supplement"; the body will extract what it needs most effectively when presented with healthy foods that naturally complex nutrients with molecules like fiber that are further beneficial to health. The most valuable guidance we can give is for patients to eat fruits and vegetables, in addition to fish, nuts, and whole grains, legumes, unsaturated oils, low-fat dairy, and poultry, while limiting highly processed red meats and sugars., (Obbagy and Essery, 2014a; Siri-Tarino et al., 2015b; Part D, 2015a).

Two diet patterns have been shown to reduce cardiovascular risk. Both the Dietary Approaches to Stop Hypertension (DASH) and Mediterranean diet have been shown to decrease cardiovascular risk (Part D, 2015a). The DASH diet emphasizes fruits, vegetables, dairy, whole grains, fish, poultry, beans, seeds, and nuts, while decreasing sodium, sugar, and red meat intake (Heart and Blood Institute, 2015). The Mediterranean diet similarly encourages fruit, nuts, vegetables, cereals, and olive oil (monounsaturated oil); with moderate fish, poultry; and low dairy, red and processed meats, and sugar (Estruch et al., 2013). Other diets such as vegetarianism, lacto-ovo-vegetarian, and veganism have less evidence in support of cardiovascular endpoints such as death from ischemic heart disease, (Obbagy and Essery, 2014b; Part D, 2015b) but have been shown to have some cardiovascular benefit and to be effective in weight reduction (Jensen et al., 2013).

Overall, reduced calorie intake is an essential part of restoring energy balance and reducing weight. This is a challenge due to the profound influence of the processed foods industry, food marketing, and the asymmetrical distribution of healthy food purchase points, particularly in rural and urban areas.

The food environment

If you ask a person, "how many food decisions do you make in a day?" the average answer will be 15. In fact, the average person makes over 220 food-related decisions per day (Wansink and Sobol, 2007). The food environment plays a central role in dietary decisions. To begin at address cardiovascular disease at a population level, physicians and policymakers need to be acquainted with the practical realities of securing healthy food. One example of a challenge is the pervasiveness of food deserts throughout our urban and rural landscapes.

A food desert is a census tract that is both low income and in which 33% of residents live more than one mile (or more than 10 miles in a rural area) from a supermarket or large grocery store (Block and Subramanian, 2015). Disparities in access to nutritious food have contributed to the widespread rates of obesity, seen in rural areas and in neighborhoods that are racially and

socioeconomically clustered (Hilmers et al., 2012). Lower income areas, often with higher populations of African Americans versus White Americans, have fewer supermarkets than higher income areas, which is a barrier for those with limited transportation (Drewnowski et al., 2012). This means that in certain communities, access to fruits, vegetables, whole grains, and dairy products are limited. These neighborhoods often have more convenience stores and smaller grocery stores where nutritious food is not stocked, prices for the limited selection of healthy foods are higher, and there is increased availability of cheap, high calorie, low-nutrition processed food (Krukowski et al., 2010).

In addition to proximity, the price and type of supermarket plays a role in obesity. Shopping at a lower-priced grocery store is associated with a higher likelihood of being obese. Consumers with lower income and less education are more likely to shop at lower-priced grocery stores, even if they are further away from where they live. The food environment of higher-priced supermarkets tends to promote fresh produce; whereas, junk food and nonnutritious food is often cheaper, sold in bulk, and more prominently displayed and marketed at lower-priced grocery stores (Ghosh-Dastidar et al., 2014).

There is an opportunity to erase food deserts. The public health and medicine community are challenged to develop public–private partnerships that eliminate this disparity and connect low income consumers to healthy food at a reasonable price. Projects like the Virtual Supermarket (https://www.baltimarket.org/virtual-supermarket/), in Baltimore, Maryland, and the food prescription projects of Wholesome Wave (www.wholesomewave.org) are crafting creative solutions to this persistent problem.

Additionally, agricultural policies, subsidies, and taxes affect which foods are affordable. Food-marketing policies such as labeling, menu options, and incentive advertising of junk food items affect individual food choices. Communities can promote zoning for grocery stores and farmer's markets and limit fast food restaurants near and within schools (Healthy food environment, 2017).

The challenge of caloric restriction in already obese individuals

Physicians often hear from patients that they "hardly eat" but are unable to lose weight. Furthermore, if they do lose weight, most patients regain it. Approximately 80% of people regain all the weight they lose through a range of efforts. This occurs primarily because of adaptive thermogenesis. Effectively, the body gets more efficient, resulting in the need for fewer calories. More specifically, the body gets more efficient during nonresting energy expenditure (exercise). There is a 20% increase in skeletal muscle efficiency and a decreased sympathetic tone. This results in a relatively hypometabolic state. A 10% reduction in body weight results in a 20%–25% decline in 24 h energy expenditure. As a result, a formerly obese person will need 300–400 fewer calories per day to maintain the same body weight as a person who was never obese.

As physicians, our primary counsel should be that calorie restriction is paramount to weight loss, and that the caloric restriction is the lynchpin of keeping weight off (Keranen et al., 2009). Keeping even 5%–10% of weight loss over an extended period of time is a great achievement (LeCheminant et al., 2005). Exercise is an important component of weight maintenance, but patients should not expect to lose substantial weight with just exercise alone.

Exercise and cardiovascular disease risk reduction

The beneficial effects of physical fitness on cardiovascular health have been well established in multiple observational and randomized controlled trials. There are direct links between increased exercise and reduced risk of cardiovascular disease. It is well known that people who exercise are 50% less likely to develop hypertension. Furthermore, people with hypertension who exercise have a 40%–60% lower risk of dying than hypertensive patients who do not exercise. Exercise has also been linked to increased HDL levels and lower triglyceride levels. Exercise has a neutral effect on LDL levels. These impacts are larger than many pharmacologic interventions, but only 13% of doctors routinely give any advice regarding exercise. The PACE/INSURE studies demonstrate that physician counseling can result in 7%–10% of patients beginning to exercise (Physical Activity and Health, 1996).

In the United States, less than 5% of the total population participates in more than 30 min of exercise daily. Many people quote a lack of time, other commitments, lack of means, or simply a general disinterest in pursuing physical activity. Those who are less educated and have lower incomes are generally less likely to exercise. Women are generally less physically active than men. Minorities in the United States are less likely to exercise. There is even a disparity geographically, with adults in the north-central and western United States more likely to be physically active than those in the south and northeast.

In discussing the impacts of exercise on the cardiovascular system, a delineation between physical *activity* and physical *fitness* must be made. Physical activity, in basic terms, is any bodily movement made by skeletal muscles that causes energy expenditure. Physical fitness, in relation, is defined as set of attributes, unique to an individual, that includes muscle endurance, muscle strength, body composition, and flexibility. These are achieved through regular and regimented physical activity (Physical Activity Guidelines Advisory Committee, 2008).

Broadly, there are two main kinds of exercise: aerobic and anaerobic. Aerobic exercise is a type of physical activity that promotes increased heart rate and promotes increased oxygen uptake from the cardiorespiratory system and trains the skeletal muscles in more efficient oxygen uptake. Aerobic exercise includes activities such as long-distance running, walking, cycling, and other so-called endurance activities. The ultimate goal of aerobic exercise is to develop the cardiovascular system to better and more efficiently regulate oxygen uptake and distribution to the muscle.

In contrast, anaerobic training is a static type of exercise in which muscle metabolism occurs at a rate that overcomes cardiovascular effort, leading to an oxygen debt. This typically occurs in strength exercise training, which typically uses resistance, such as heavy weights, to build overall maximum strength of a muscle and increase muscle size.

Physiologically, physical activity that targets aerobic development increases the body's demand for oxygen and increases the cardiovascular system's ability to provide it. This ability is termed the ventilatory oxygen uptake, or VO_2. VO_2 is derived from the Fick equation and can generally be understood as the product of cardiac output multiplied by the difference of arterial minus venous oxygen content. Ultimately, the purpose of developing aerobic fitness is to increase the stroke volume (thus increasing cardiac output) and also increase the maximal difference between arterial and venous O2 content, meaning that the muscles extract more oxygen. This "maximal" exercise capacity is known as the VO_2 max. As VO_2 max improves with aerobic exercise the body is able to do more physical work with less demand on the cardiovascular system.

Aerobic exercise has been proven to reduce mortality by approximately 30% (Thompson et al., 2003). There is a general decrease in cardiovascular event risk with a specified exercise time of 40 min per session 3 to 4 times per week. The association between *anaerobic* exercise and decreased risk factors is not as well established. There does not appear to be consensus in metaanalyses for anaerobic exercising causing a decrease in blood pressure, though some studies do show that blood pressure is in fact also reduced by anaerobic exercise, and regular anaerobic exercise decreases insulin resistance, which can help offset the features of type 2 diabetes mellitus. However, this is not to the same degree that is found in aerobic exercise (Chaudhary et al., 2010 Dec).

Overall, the AHA/ACC strongly endorses aerobic exercise; resistance exercise can be added to an exercise regimen, but aerobic exercise is preferred over anaerobic exercise for cardiovascular disease risk reduction.

Exercise is also beneficial for people with heart failure, coronary disease, and atrial fibrillation. In those with coronary artery disease, exercise training has been proven to increase exercise capacity, reduce ischemia, delay angina pectoris, and improve endothelial function (Bateman et al., 2011). This can be accomplished either through a supervised cardiac rehabilitation program or through encouragement of physical activity similar to the type recommended by the ACC/AHA for primary prevention of coronary artery disease.

Per AHA/ACC official guidelines, patients with coronary disease should be encouraged to perform their exercise at a moderate intensity, related to their level of physical fitness. Goal heart rate at this intensity of exercise should be 70%–85% of the peak heart rate, calculated by the difference of 220 minus age. Patients can use the "talk test" to determine the strenuousness of their exercise—in true "moderate" exercise, the performer should be breathing at a faster rate than at rest or at light exercise, but still able to talk in full sentences.

In the setting of heart failure, the HF-ACTION study, a large, case-controlled study involving patients across the spectrum of heart failure severity, recommended physical exercise in those with stable, systolic heart failure, noting a decrease in all-cause mortality and rehospitalizations. This was achieved with following the AHA/ACC recommendation of at least 30 min of moderate-intensity exercise three to four times a week. This is a departure from the previously established recommendation that recommended rest and avoidance of physical activity (O'Connor, 2009). Ongoing studies continue to demonstrate the exercise is essential for improving outcomes in patients with atrial fibrillation.

Most people are aware that moderate physical activity is good primary prevention for coronary artery disease, and also for reducing the severity of risk factors. There are multiple electronic exercise measuring tools that people use, such as the "Fitbit" device. These devices allow the regular person to keep track of their fitness levels, amount of physical activity, and they can also measure exercise basics such as heart rate and the amount of calories that are being used during exercise. Studies have been done to determine if these tools change outcomes, especially in regard to weight, BMI, fitness, physical activity, and dietary intake.

The IDEA study, which looked at younger adults, indicated that wearable technology do result in some weight loss benefits as far out as 6 months after implementation of this technology but did not result in sustained weight loss at 2 years. Another study which recruited older, obese, and more sedentary patients did show evidence that wearable technology with feedback and tracking abilities did show some utility in enhancing lifestyle changes that promoted weight loss and reduced waist circumference (Shuger et al., 2011).

However, many of the studies that analyze the utility of these technologies are very small, observational studies, and only look at the effects of these interventions on how they affect overall physical activity levels and weight change; these studies are limited in the respect that they do not have results on how digital technologies affect such things as resting heart rate, VO_2 max, or other more specific physiologic measurements. The specific information that is recorded by these exercise technologies is very limited and usually confined to measuring heart rate and amount of steps taken, or miles exercised. There is a substantial error rate with these devices; up to 92% error has been recorded for estimating moderate to vigorous activity, while up to 65% error rates have been recorded on estimates of sedentary behavior (Rosenberger et al., 2016). App-based technologies have improved accuracy when compared to wearables in clinical trials, but there is still substantial variation. Mobile technologies are digital health tools that have a tremendous drop off rate; the majority drop-out of use after 3 months and only 3% of users become long-term users (Helander et al., 2014).

Medical providers can play an instrumental role in helping to get patients increase their physical activity and physical fitness level. Most physicians are aware of the general benefits of moderate exercise in cardiovascular health, mental health, and pain reduction. However, by one study, nearly 47% of physicians do not talk to patients about exercise as part of a treatment plan, and in the same study, only 13% of the patients polled had any recollection of being counseled on exercise as part of their general health (Sallis and Hovell, 1990).Many physicians are not highly skilled in exercise counseling. However, even simply mentioning the topic is useful to patients. It is further impactful for physicians to write an exercise prescription. Exercise prescriptions have been shown to increase long-term patient compliance in an exercise program (Leijon et al., 2010).

The Challenge of Prevention in Modern Medicine

Over 70% of cardiovascular disease is related to poor diet and reduced exercise. Patients are increasingly looking beyond their medical providers to find counsel and guidance on these important issues. Unsustainable healthcare costs related to cardiovascular disease may be what ultimately alters the landscape from a focus on treatment of disease toward upstream solutions that lay diet and exercise as the cornerstones of modern medical treatment.

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Bicuspid Aortic Valve and Sports Activity

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Introduction

Bicuspid aortic valve (BAV) is a common congenital cardiac disease, usually asymptomatic for a long time. The incidence of BAV is around 0.9%–2% in the general population (Fedak et al., 2002) as well in competitive athletes (Stefani et al., 2008) with main prevalence in male (Basso et al., 2004; Stefani et al., 2008). BAV can be often found in association with other minor congenital cardiopathies as mitral valve prolapsed, interventricular septal defect, patent ductus arteriosus (Duran et al., 1995). All these conditions do not normally represent a real obstacle to practice sports activity, also in competitive athlets. An accurate clinical approach is on the contrary necessary when aortic tract dilatation (Keane et al., 2000; Roberts, 1970a) or coarctation (Nistri et al., 1999a) is present and particular attention is necessary when they are potentially evolving in acute dissection (Ward, 2000). Regular athletic training can induce an hemodynamic load during exercise and particularly pressure overload during strength training may also lead to aortic remodeling (Zeppilli et al., 2006).

BAV is commonly studied for the valve dysfunction. It can show a mobility dysfunction often represented by aortic regurgitaion or stenosis, often evident in a mixed form. A mild aortic valve dysfunction is tollerate in competitive sports activity; however the aortic valve insufficiency can induce a left ventricle (LV) systolic and diastolic enlargment, as well aortic stenosis can determine a progressive LV chamber modification. All these conditions are associated with the LV mass increase. In addition the cardiac adaptations to different forms of athletic conditioning tend to produce disparate morphological forms of the athlete's heart. For this reason, more recently, the LV's morphology and structure is an emerging interested aspect in BAV. The myocardial remodeling due to regular sports activity, so-called athlete's heart, can be therefore partially overlapped to the LV mild enlargement as consequence of both the causes. This aspect, studied either in general population as in athletes needs to be considered for a correct interpretation of the natural history of BAV (Beroukim et al., 2006). The impact of different sports activity in athletes with BAV is in fact not yet completely clarified in sports medicine and therefore an appropriate follow-up and surveillance is often mandatory. The progressive effect of sports training could be an additional factor for heart workload and therefore decisive for the eventual evolution of the disease. The echocardiographic exam is in this population of athletes, necessary at least one in a life before to start with a regular training and potentially suggested as a correct follow-up for a long-time surveillance. A more complete echocardiographic assessment during physical ergometric test, called echostress test, is determinant for a aortic valve and LV performance evaluation.

History and General Implications

The first description of BAV has been attributed to Leonardo da Vinci 400 years ago, anyway several other authors (Osler, 1886) have described the presence of BAV during infective endocarditis or aortic dissection findings (Wauchope, 1928).

The possible association with other cardiac congenital disease, the potential complications in BAV, and also their clinical implications are frequently investigated.

Congenital BAV has been described since 1927 to have a ninefold grater risk disease for aortic dissection (Acierno, 1994). This aspect has contributed to highlight the interest to a possible enhancement of sudden death.

Sports eligibility protocol, mainly in the Italian context, but also in Europe has induced the sports physicians to perform an initial and accurate staging of the BAV, taking into account hemodynamic factors, aortic complications, and other significant cardiovascular anomalies.

The progressive development of the echocardiographic imaging has improved the definition and the incidence of the BAV in general population and also in athletes. The use of 2D ecocardiographic traditional and not traditional (strain) parameters has increased the definition of the myocardial performance and the risk of the complications. The rising interest is nowadays due to the large series of ages represented among the subjects with BAV that are engaged in physical activities and in sports at different levels of intensity.

Sports medicine eligibility is now open to a large context of general population where also the master athletes are involved in competitive sports activity. This is an emerging and new field of interest where some other variables like eventual comorbidities association need to be considered

Embriology and Morphology of BAV

The aortic cups derive from the mesenchymal cardiac cushions situated along the ventricular outflow of the primary heart tube. Among the possible etiologies of BAV, the anomalous behavior of the neural crest cells seems to be one of the most reliable etiologies (Fernandez et al., 1996; Roberts, 1970b). Some researchers have verified the frequent association of BAV with others congenital malformations of the aortic arch in addition to the involvement of the neural crest cells during the semilunar valves growth (Sumida et al., 1989). Others researchers have suggested the possible impairment in the extra cellular matrix and deficit of metalloproteases system as causes of abnormal valvulogenesis particularly in the differentiation and the formation of this one (Fedak et al., 2003). The anatomy of BAV shows often an unequal size of the cusps, the raphe usually appears in the middle of the larger cusp as expression of the site of fusion and therefore without valve tissue.

Respect of normal tricuspid aortic valve (TAV), where the asymmetry of the valve is unusual, BAV shows an eccentric closure normally well evaluated with the bidimensional echocardiographic exam in long-axis view.

The two images in short-axis view (obtained by ESAOTE-My lab echocardiography) show the different position of raphe as expression of cusps fusion and diverse valve closure (**Figs. 1** and **2**).

The BAV can be often associated with a mild aortic valve insufficiency, with eccentric jet (**Fig. 3**).

The long-axis color image shows the eccentric aortic valve coresponding to the aortic mild regurgitation.

Literature reports some different morphological patterns of BAV. The most common origin of the BAV is the fusion of the "left and the right" valve cushions (Pattern A), but the exact mechanism triggering remains unclear. The fusion of right with noncoronary leaflet (Pattern B) is not so common as the pattern "A." Pattern B is on the contrary more frequently associated with aortic stenosis and regurgitation mainly in pediatrics series (Stefani et al., 2014a). The least common variant in BAV is the fusion of left and

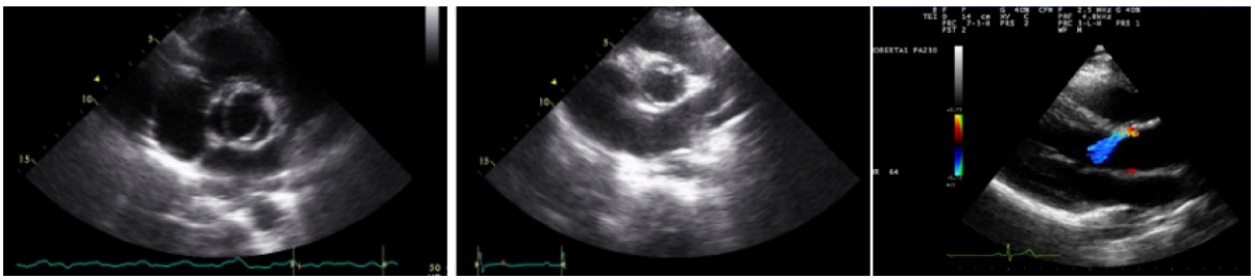

Fig. 1 The two images in short axis view (obtained by ESAOTE-My lab echocardiography) show the different position of raphe as expression of cusps fusion and diverse valve closure. The BAV can be often associated to a mild aortic valve insuficiency, with eccentric jet. The long axis color image shows the eccentric aortic valve corresponding to the aortic mild regurgitation.

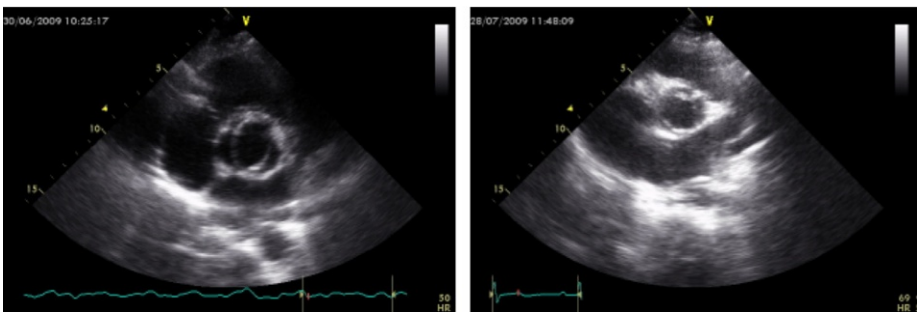

Fig. 2 BAV from 2D short-axis view at echocardiographic exam. Images in short axis view show the different position of raphe as expression of diverse valve closure.

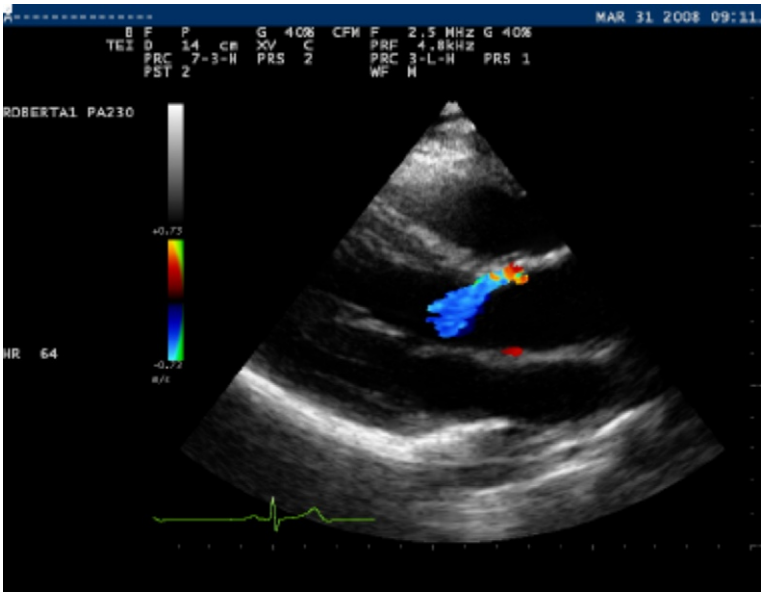

Fig. 3 2D echocardiographic long-axis view of BAV.

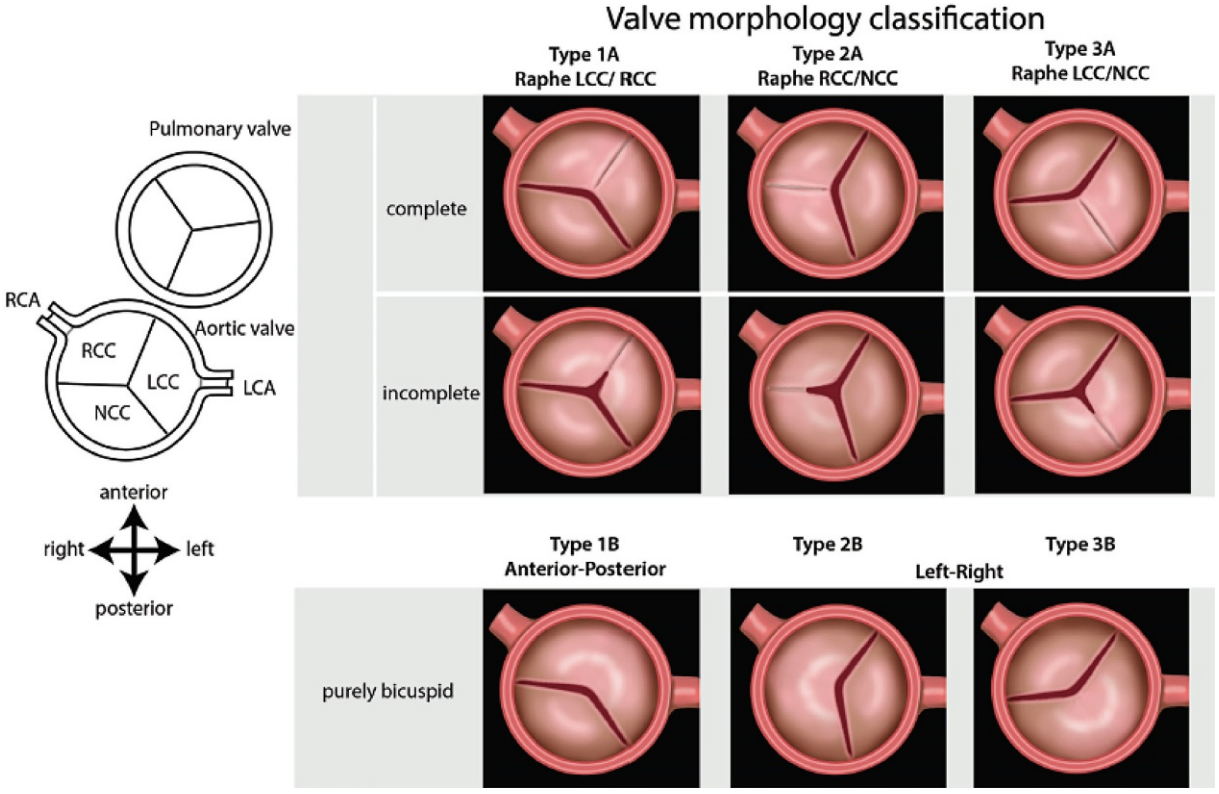

Fig. 4 BAV morphological classification.

noncoronary cusps (Pattern C). These different patterns of fusion determine inevitably a diverse position of the valve commissure and different kind of closure: for example, horizontal, vertical, or oblique with complete and incomplete raphe.

Fig. 4 shows the different morphological pattern and different raphe orientation as consequence of the cusps fusion.

These morphologies of BAV, normally used to classify the type of the valve, have been considered in deep for the correct interpretation of the potential role in the possible various evolution to a different levels of aortic valve dysfunction. It has been also hypothesized a different impact toward the LV involvement and damage, especially in athletes (Stefani et al., 2014a).

The investigation has demonstrated that in any case, the different patterns of BAV do not affect the long-term LV performance in athletes regularly trained, when the valve dysfunction is not severe.

Genetic

Several studies describe BAV as a consequence of an underlying genetic abnormality. A wide group of investigations, conducted among the relatives of subjects affected by BAV, have reported a prevalence of 14.6% in the major of the family studies (Brown et al., 2002; Emmanel et al., 1978). In several familiar clustering the prevalence of BAV is compatible with an high heritability of the disease, while in other studies the number of subjects affected seems to be asymmetric and compatible with an autosomal inheritance patter with reduced penetrance (Huntington et al., 1997). This particular aspect is likely due to mutations in diverse genes with dissimilar inheritance patterns in families. Starting from this background echocardiographic family's screening is recommended, especially for first-degree relatives of subjects affected by BAVs. In addition, considering the frequent involvement of the aortic tract among BAV athletes, a sort of overlapping in genetic pattern (involving the Cr 15), between BAV and ascending aortic aneurysm, has been studied (Glic and Roberts, 1994). Any eventual responsibility of the human gene in the pathogenesis of BAV will need further studies.

BAV and Associated Congenital Cardiovascular Lesions

In the 5% of cases, BAV can be associated with additional cardiovascular malformation.

One of the most common is aortic coarctation that can be present in a "simple" form, when isolated, or "complex" if associated with other intracardiac defects. Literature reports a frequent association of aortic coarctation with BAV where right and left cusps (type A) are fused. In some reports it is present in 15% or 18% of the series analyzed (Goh et al., 2002).

The BAV subjects with associated aortic coarctation are often addressed to a surgical intervention where valve or the vessel is involved. In many cases, subjects submitted to a surgical treatment for the aorta in first line are often reconsidered in second session for the valve replacement (Attenhofer Jost et al., 2002; Coehn et al., 1989; Larson and Edwards, 1984). A particular investigation (Acierno, 1994) in a wide group of athletes involving 2273 competitive and nonelite subjects, evaluated since 1 Jan. 2000 to 31 Dec. 2004, has showed anyway a low prevalence of aortic coarctation in them. In this study only two cases diagnosed among the 356 cases of structural heart disease have been reported. Nonetheless athletes in general do not seem to be less affected by aortic coarctation than general population. However it is relevant to consider that athletes are a selected population, where the preparticipation protocol in order to obtain the eligibility, especially in Italian context, tends to exclude subject with aortic coarctation unless they are operated.

Regarding the other eventual congenital cardiovascular diseases potentially associated to BAV, the most frequently defects found are the patent foramen ovale (PFO), atrial sepal defect, interventricular septal defect, mitral valve prolapsed. They are normally present in same prevalence of general population. All of these cardiac minor congenital cardiac diseases need to be periodically followed, especially in case of regular sports activity. They do not normally represent an obstacle to allow sports activity.

It needs to pay particular attention either in general population or in athletes, to the coexistence of eventual congenital coronary artery anomalies in BAV. The increased incidence of coronary abnormalities origin, described as case report (Fiorella, et al. 2010) and in few cases in BAV, may have clinical relevance, with negative impact during exercise, especially if at maximal exertion up to a sudden death occurrence.

Clinical Symptoms

Although the BAV may remain asymptomatic and without clinical consequences for a lifetime, it may be responsible for severe complications such as aortic stenosis, aortic insufficiency, endocarditis, and aortic dilation/dissection, sometimes requiring surgery. In general population as well a in athletes, the initial diagnosis is often suspected in the presence of "systolic click" and "ejection murmur" at the aortic valve opening.

The murmur is usually noted during the physical examination. The ejection sound is referred to the valve leaflet mobility and it diminishes when the valve becomes more immobile. In young where the valve regurgitation is more frequent than stenosis, this acoustic report is more common. During the clinical examination the "early peck systolic flow murmur" is normally heard after the systolic click sound. In consequence of these few clinical symptoms, the possibility to early discover the presence of BAV is reasonable lower than expected.

Particularly in sports medicine context, considering that in Europe and also in the United States the evaluation to obtain the eligibility does not normally include the echocardiographic exam, the diagnosis of BAV is therefore partially limited.

The finding and morphological definition of the valve depends, therefore principally, on the echoexamination. Ejection fraction (EF) appears normal for several years after the diagnosis. Nevertheless the symptoms related to the eventual heart failure, characterized by a reduced exercise tolerance, as a consequence of the prolonged impact of the aortic regurgitation to the LV chamber plays a relevant role in the clinical features and symptoms. This aspect remains in any case the crucial points in athletes with BAV even if excluded from the eligibility.

BAV athletes need to be early addressed toward a clinical and instrumental approach in order to discover a possible valve and myocardial worsening in them. Many morphological aspects are investigated at rest conditions, but the most important features are evaluated by the physical echostress exam where the valve functioning and its behavior during the exercise are tested.

With respect to the normal aortic valve function, the main parameters observed are the eventual reduction of the opening of the valve during the life and also the eventual worsening of the valve insufficiency during the physical exercise or regular training.

Myocardial contractility is an other relevant aspect tested during stress echo. A progressive increase of the EF without any substantial modifications of the segmental contraction is important characteristics in BAV athletes. The eventual presence of ventricular or supraventricular extrasystolia is also determinant to decide to allow the sports eligibility. The extrasystolia induced by physical exercise can in fact represent a contraindication to allow the sports activity.

Echocardiographic Exam in BAV

The standard 2D echocardiographyc exam is the principal noninvasive tool to ascertain the presence of BAV, to define its morphological characteristics, to verify the presence of other cardiovascular abnormalities eventually associated, and also to follow up the subjects affected by BAV. The echo exam is normally performed following the current AHA guidelines (Cheitilin et al., 2003).

Starting from the long-axis view, the eccentric valve closure and the doming systolic aspect of the cusps are often well evaluated. This feature is confirmed by the presence of two only cusps showed in short-axis view. Sometimes the presence of raphe can make a confusion and address to an incorrect diagnosis in consequence of the possible identification of this one with the anatomical valve commeasure. In this case, the possibility to mistake the trileaflet valve with a bicuspid one could be elevated. Despite the well-known limits due to the sonographer's experience and to the quality of the images obtained, several authors report an high accuracy of the echoexam with a sensibility of 78% and specificity of 96% for the diagnosis (Picano, 2004) in BAV. The apical five chamber view, with the addition of the color and Doppler analysis, is often used to evaluate the degree of the valve failure. The presence of the aortic insufficiency is normally estimated from 0 to 4 degree following a color grade scale from + to ++++, while the aortic flow velocity evaluated by continuous wave (CW) Doppler analysis is directly related to the degree of the valve stenosis. The score of the valve stenosis, BAV in athletes normally accepted to allow the eligibility, is referred to a value not higher than 20 mmHg at rest.

During physical stress test, where the hemodynamical circumstances of the exercise are reproduced, a value of the aortic valve velocity higher than the 3–4 msec and corresponding to a gradient more than 55 mmHg, at the peak of the effort, needs to be evaluated in the context of the specific sport eligibility, kind of sports, and therefore cardiovascular impact. In any case the eventual fast reduction of the aortic valve gradient, in the immediate time after the maximal effort, can be considered as a not pathological event and therefore not an obstacle to practice sports activity. The rate of the clinical examination needs to be established carefully. The aortic valve regurgitation is often found in the adolescent and in young adults, while the aortic stenosis is more frequent in older. Doppler analysis contributes to correctly define the degree of the aortic valve insufficiency. The pressure-half-time measurement (PHT) is the parameter used to classify the degree of the regurgitation as "low, moderate, or severe" (500 ms found at PHT measure is the cut-off value to distinguish moderate from severe). The recognition of a physiological reduction of the aortic valve regurgitation during the physical exercise is the most important aspect to follow up the valve dysfunction.

The complete cardiological assessment of BAV includes the echocardiographic measurement of the aortic tract at four levels (aortic root, valsava sinus, sino tubular junction, proximal ascending aorta).

This is particularly helpful to obtain information regarding the potential progression in dimensions of the first aortic tract, predominantly involving the sinotubular junction of BAV. This aspect is mainly relevant in consideration of the incidence of aortic dissection that in BAV occurs also in the absence of histmic coarctation or hypertension.

The autoptic study reports as this complication is present in approximately in 7%–9% of patients (Coehn et al., 1989).

Especially in young athletes with BAV the aortic root dimension is therefore an important evaluation, as expressly demonstrated in literature (Picano, 2004). The progressive enlargement of the first aortic tract seems to be a peculiar marker of the BAV and therefore independent from the aortic root disease itself. It has been demonstrated as the aortic roots are found larger than normal with the mean rate of progression of 0.5 mm/year at the sinuses of Valsalva and 0.9 mm in the ascending aorta (Gurvitz et al., 2004).

BAV is in fact considered as a congenital defect of the heart or great vessel (Ferencik and Pape, 2003) commonly associated with abnormalities of the ascending aortic tract like dilatation, aneurysm, dissection, and coarctation (Dwards et al., 1978; Niwa et al., 1999). Several echocardiographic and histological studies have suggested an independent association between BAV and aortic dilatation (Hahn et al., 1992; Keane et al., 2000; Nistri et al., 1999b; Pachulski et al., 1991). More recently some of these morphological aspects, evaluated in the general population and also in athletes, have showed concordant and helpful results in terms of management of BAV athletes.

One of the most interesting feature actually emerging is the possibility to find anyway a sort of relationship between BAV and aortic vessel just in particular community or geographic zone (Duran et al., 1995). There is a biologic rationale and data supporting the hypothesis of an intrinsic aortic disease in the presence of BAV. The aortic cusps, the outflow tract, the aortic arc are embryologically linked. They originate from the neural crest and they are often implicated in the arterial dissection of the cervico–cefalic tract.

Among the pathogenetic causes, some specific aspects are recognized as factors inducing this progression.

The NO_2 synthesis defect has been identified to induce a premature apoptosis of the medial layers smooth muscle cells. This deficiency has an high incidence in BAV (42%) respect of controls (Nkomo et al., 2003). Literature has also investigated the role and the contribution of the hemodynamic component as consequence of the severity of the aortic valve failure, in the progressive enlargement of the aortic tract. It has been demonstrated that the proximal aortic dilatation is beyond the implications associated with the valvular lesions (Schmid et al., 2003). Subjects with BAV with associated valve stenosis show an aortic dilatation at all the levels which are not found in subjects with normal TAV and same valvular lesion. This interesting feature has been investigated also in athletes population with BAV and mild aortic regurgitation (Keane et al., 2000; Pachulski et al., 1991), where the poor hemodynamic component of the valve's failure did not represent an obstacle for the regular training or sports activity. The results obtained, during a period of 5 years, support the hypothesis of any additional negative impact of the sport activity on the aortic tract at least in presence of mild regurgitation (Hahn et al., 1992).

Other investigations support this thesis showing that the degree of the aortic dilatation in BAV with stenosis is similar to the subjects with moderate or severe regurgitation and therefore no association between the eventual aortic valve failure and the dilatation of the aortic vessel, as consequence of the BAV presence is actually evident or demonstrated (Galanti et al., 2010).

Several authors have explored the aortic root pathology showing that it consists of a process similar to "cystic medial necrosis," and it is characterized primarily by an extensive loss of elastic elements with the media (Roberts and Ko, 2005a). For this peculiar characteristic composed by an intrinsic abnormality of the proximal aortic tract, the similarities with Marfan syndrome are very strong (McKusick, 1972). However, at present the progression and the involvement of the aortic tract have to be more clearly defined.

The similar enlargement of the first aortic tract dimensions has been described also in athlete's population in particular (Stefani et al., 2008). The yearly enlargement is approximately 0.78 mm/year for the aortic root; 0.61 mm/year for valsalva sinus; 0.81 mm/year sinus tubular junction; 0.98 mm/year proximal ascending per year with the major involvement of the sinus tubular junction tract.

In an other investigation where the possible sport activity impact on aortic tract has been evaluated (Hahn et al., 1992), the results are also in agreement with the data obtained in general population.

In conclusion, nowadays the aortic dimensions in BAV athletes, regularly trained, seem to be in complete overlapping to the general population not skilled (Dwards et al., 1978). No additional negative impact of sports activity in aortic enlargement has been demonstrated.

Echocardiography is consequently considered an helpful tool particularly for athletes where the best decision-making to guarantee the eligibility despite the presence of BAV.

LV Chamber in BAV Athletes

Despite the BAV has been normally considered exclusively an aortic tract disease, more recently a new aspect regarding the possible participation of the intrinsic contractile properties of the LV chamber is growing on.

The standard 2D echo-exam normally includes also the evaluation of the LV chamber calculating end-systolic and end-diastolic dimensions, the interventricular septum and posterior wall thickness and the diastolic parameters derived from the transmitral flow pattern. This approach is normally performed as similar procedure for both, general and athlete population. The attention to a possible development of the LV dimensions in BAV athletes is an additional specific aspect especially in those trained subjects where the eventual overlapping with the athlete's heart modifications can create a confusion for a correct interpretation of the consequence of the regular training. Recent study, during at least a 5-year follow-up (Hahn et al., 1992), has described in BAV athletes an yearly progressive enlargement of the LV chamber that anyway maintains the values within the normal range. This investigation is based on the recent knowledge demonstrating a different expression of the metalloprotease system (MPS) in BAV compared to TAV subjects. The study was conducted to verify any eventual difference in a long period.

The role of this mechanism has been however investigated in the blood vessel tissue but not yet in the myocardial segments (Kornbluth et al., 1999; Le Marie et al., 2005a). The major of research regarding the pathogenesis of the ascending aortic dilatation and based on this hypothesis has in fact focused on aneurysms caused by Marfan syndrome. The molecular mechanism responsible for aneurysm in patients with BAV remains anyway poorly understood. On the other hand, more current information is available on "ascending aortic aneurysms" where BAV disease exhibits an increase of MPS expression respect of TAV (Kornbluth et al., 1999).

In any case, starting from this background and taking care of the progressive enlargement of the LV chamber in BAV respect of controls, often associated with a decrease of the myocardial contractile property during their life, a presence of universal pathological mechanisms underlining the myocardium walls failure has been recently proposed (Le Marie et al., 2005b). This hypothesis is in fact going to be valorized and extended to a more large interpretation and to a new version of the pathogenesis of BAV disease. Authors are now working in evaluating this aspect as a possible cause related with a reduction of the contractile properties of the myocardium described.

Particularly in athletes, where the LV performance is normally evaluated by EF, the necessity to verify the persistence of this parameter during training has to be maximally considered in order to early decide the necessity to avoid any possible further heart impairments due to the additional effects of sport activity in them. However in the absence of currently advanced biological techniques dedicated to a direct and easy evaluation of the intrinsic contractile properties of the myocardial cells, two noninvasive methods are now available in echocardiography: physical echostress and the study of deformation parameters like strain.

The Complementary Information by Speckle Tracking Analysis on LV Performance

In echocardiography the deformation parameters (strain in different components, rotation, and torsion) are often involved to investigate the segmental and global myocardial function. Literature reports several studies in sports medicine to evaluate the LV performance in the presence of cardiac congenital disease or in the presence of valvular disease like aortic stenosis or insufficiency (Lewin and Otto, 2005; Souterland et al., 2004).

Particularly for the longitudinal component of strain LPSS (longitudinal peak systolic strain) it has been shown relevant data with clinical implications. The LPSS plays a recognized predictive role in the initial LV dysfunction of heart valve diseases such as aortic regurgitation or stenosis (Faber et al., 2007; Lewin and Otto, 2005). There is some evidence that, despite normal EF, strain of the LV can be depressed in subjects with aortic valve lesions (Onishi et al., 2007). These lower values of strain of the LV have been used as an indication for early surgery in such patients. In this context particularly in BAV athletes, some studies have been focused focusing to early discover an eventual initial LV failure in them (Poulsen et al., 2007). This kind of investigation has been completed in BAV athletes by using of a more sophisticated program dedicated to the 3D strain evaluation (Stefani et al., 2014b),(Stefani et al., 2009) where the LV contraction and the synergic activation of endocardial and epicardial fibers are more in deep studied. The results in athletes with BAV show values of LPSS within the normal and validated range confirming the normal function of the LV chamber and therefore in agreement with the EF.

From these data it is reasonable to suggest the addition of the deformation parameters in the instrumental evaluation of the BAV athletes in order to obtain a further confirmation of the normal function in them especially in case of high transvalvular gradient. The ST method, now validated, produces results with an high reproducibility and specificity and so it can be proposed, particularly for its easy application, as a routinely employment mainly in subjects where the other parameters are normal. The clinical implication of the gradient alone will need further studies involving a larger group of subjects analyzed: nowadays it cannot be excluded a peculiar utilization in athletes only.

Fig. 5 shows the representation of principal strain direction at the end-systole computed from the deformation at the subendocardial level in three groups of subjects: Control; TAV and BAV, respectively. The strain lines are colored to emphasize the positive angle of endocardial right-handed helical fibers with clockwise movement from base to apex.

Sports Activity in BAV Athletes

The natural history of BAV is usually addressed to the calcification of the cusps in the almost the totally of subjects affected: more than 50% of adults with severe aortic stenosis have a BAV (Köstner and Meinertz, 2007; Roberts and Ko, 2005b).

Nonetheless, one of the most important and crucial points is anyway to verify if BAV is loaded of an incidence of mortality higher respect of normal subjects. Frequent association with complications like endocarditis (Lamas and Eyhyn, 2000), the progression to valve stenosis especially in men (Gaudiani et al., 2004), or worsening of regurgitation requiring the valve replacement in addition to a frequent susceptibility to aneurism formation of aorta (Barndeburg et al., 2004) are not sufficient to clarify the prognostic consequences in terms of mortality. This problem is current not yet completing clarified manly in athletes where additional factors related to the intensity and kind of sports are present. Theoretically the regular training could favorite an early deterioration of the BAV even if the positive general impact of sport activity in men and women are well noted.

In addition to this, in athletes heart is often hard to differentiate the physiological adaptation due to sport from the pathological modification in consequence of valve disease. In case of BAV with preexisting aortic dilatation, intensive exercise, often isometric, can address to an high incidence of aortic rupture. Several recommendations for athletes are therefore referred to the ESC and AHA guidelines (Bonow et al., 1998) but all they do not anyway include the echocardiographic exam in first line. The high prevalence of

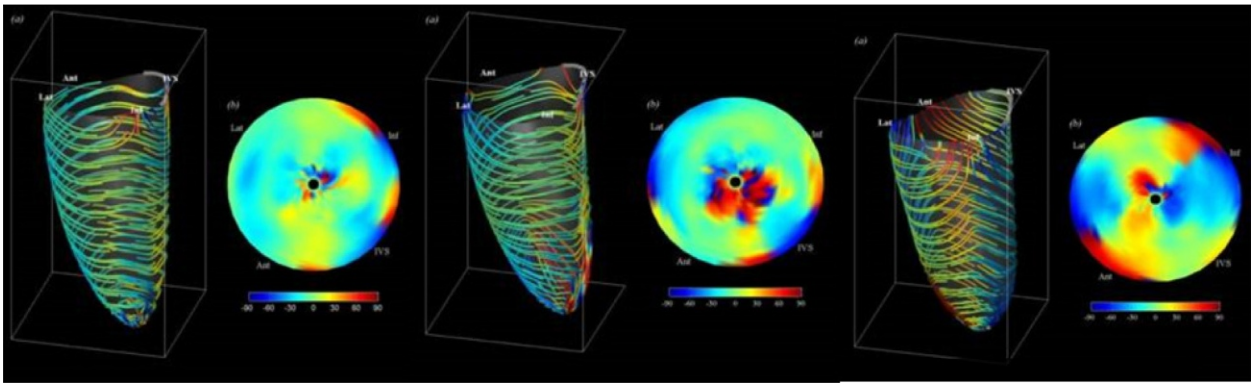

Fig. 5 Representation of principal strain direction at the end- systole computed from the deformation at the sub-endocardial level in three groups of subjects: Control; TAV and BAV respectively. The strain lines are colored to emphasize the positive angle of endocardial right- handed helical fibers with clockwise movement from base to apex.

this congenital cardiac disease and the same prevalence in general population and in athletes are strongly suggestive for a necessity of an early diagnosis potentially helpful. An echo exam at least one in a life could be proposed. This aspect might be considered mainly in the children and adolescent often invited to practice sport after the execution of a general check up or ECG only. For this aspect, both athletes and general population could be considered within in the same investigating model.

Some hemodynamical aspects of the impact of the prolonged physical exercise in athletes have to be considered. Aerobic exercise produces a physiological increase in heart's overloading, involving both the right and the LV; however the chronic intensive LV contraction induces an increase in myocardial performance that can be followed by echocardiographic exam. No substantial differences have been reported in case of resistance exercise with respect to aerobic one especially incase of BAV.

In order to take any decision about sports eligibility, sports physicians should perform an initial accurate staging of the BAV, taking into account hemodynamic factors, aortic complications, and associated significant cardiovascular anomalies.

No peculiar limits for specific sports are evident in literature in case of abnormal BAV function. The eventual contraindications to practice sports are related to the severe aortic valve failure and the LV chamber dysfunction (Köstner and Meinertz, 2007). Physiological adaptations of the heart have to be differentiated from pathological adaptations, and it is important to take into account the adverse consequences that regular training can have on subjects with BAV through different mechanisms. In athletes with BAV and associated aortic regurgitation, a preexisting pathological adaptation caused from a slightly elevated volume load due to the aortic regurgitation, it has to be taken into account and can have negative effect when the athlete is engaged in regular training.

In endurance exercise, the development of eccentric hypertrophy and ventricular dilatation may have consequences on this preexisting pathological adaptation. In athletes involved in mainly static or isometric exercise, the pressure's overload from/to the high systemic arterial pressure found in this type of exercise will cause a chronically elevated aortic wall tension, and consequently aortic dilatation and regurgitation. In the case of BAV with a preexisting aortic dilation, intensive and competitive exercise carries high risk for aortic dissection or rupture, which is even higher in isometric exercise. In fact, during competition, often phases of high intensities occur, inducing relevant increases in arterial blood pressure which would facilitate an aortic rupture. Those rare patients with BAVs associated with an anomalous origin of the coronary arteries have an increased risk of sudden cardiac death (SCD) during exercise. Although seldom recognized and often unappreciated, coronary artery aberrancies and anomalies account for up to 20% of cases of SCD in young athletes. Most types produce few, if any, symptoms, but some pose a much more substantial risk. Anatomical coronary abnormalities can imply a potential risk of coronary artery compression, especially following exercise. These consequences predispose to myocardial ischemia, syncope, or decompensated heart failure, and likely to account for most cases of SCD. Since coronary artery disease is often asymptomatic and most patients have not revealed physical examination, their detection can be quite difficult. Although echocardiography and echostress can be proposed in first line to reduce the risk of SCD in them.

Sports Medicine Indications in BAV athletes

Athletes with BAV who develop valve insufficiency or stenosis may require restrictions from strenuous competitive sports. Particularly subjects with aortic valve insufficiency and aortic dilatation should avoid strenuous sporting activity, as consequence of the eventual increased risk of aortic rupture. A general judgment in athletes with BAV about their ability for competitive sports is not possible, and therefore the eligibility for participation and the ability to continue to practice competitive sports needs to be individualized. It depends on age, severity of lesions, and the type of sport.

The *Guidelines of Bethesda Conference* No. 36 offer the recommendations to continue to participate in sports activity or to avoid (temporarily or permanently) to practice every sports. Some indications are dedicated to the suggest the eventual recreational athletic activities and to support the normal lifestyle for those athletes who are disqualified from competitive sports. However further studies are needed to better understand the genetics of BAV, to know the natural history and the impact of regular training in athletes with BAV especially if competitive.The natural course of BAV is not yet completely known.

In Italy, the competitive sports practice has several legal implications. The sports medicine check up is a complex evaluation based on the complete cardiovascular assessment by ergometric test and it includes also the urine exam. In case of BAV discovery the echocardiographic exam is mandatory. The impairment of the aortic valve and the progressive enlargement of the aortic tract or of the LV diameters may frequently determine the discontinuing of sport activity in BAV. Despite the presence of asymptomatic conditions, a regular yearly clinical and instrumental follow up is generally performed so as to discover as early as possible any signs of myocardial dysfunction.

The current suggestions are to perform a strict echo surveillance in BAV primarily if asymptomatic than symptomatic.

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Bioinformatics Principles for Deciphering Cardiovascular Diseases

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Introduction

Cardiovascular diseases (CVD) are diseases involving the heart and blood vessels, with one of the major pathologies being atherosclerosis, or the narrowing and hardening of blood vessels. Major CVD such as coronary artery disease (CAD), myocardial infarction (MI), and stroke, are the leading causes of mortality worldwide (Mensah et al., 2014), and less deadly CVD such as peripheral vascular disease (PVD) also contribute significantly to the morbidity of CVD. To enable effective prevention and therapy, enormous efforts have been dedicated in the past decades to improve our understanding of the etiology of CVD. These efforts have led to gratifying discoveries on disease mechanisms and subsequently the development of highly effective cholesterol-lowering

medications that have contributed significantly to the recent decreases in the death toll of CVD. However, despite the reductions in death rate, global prevalence of CVD has been increasing and we still face enormous challenges in preventing and reducing the occurrence of CVD and achieving more effective treatments (Mozaffarian et al., 2015; Heidenreich et al., 2011; World Health Organization, 2014).

One of the major hurdles towards eradicating CVD is the lack of comprehensive understanding of the molecular mechanisms. Before the arrival of high throughput technologies, CVD was primarily investigated using classic reductionist approaches that focus on single elements, one at a time, in confined contexts. For instance, if a gene is suspected to play a role in CVD development, perturbation experiments can be conducted in in vivo or in vitro systems to examine and elucidate the function and role of the candidate gene. Such studies have undoubtedly shed light on numerous molecules and regulatory pathways that are involved in CVD. Successful examples of reductionist approaches include the illustration of the cholesterol biosynthetic pathway and the low density lipoprotein receptor pathway, leading to the discovery of numerous lipid lowering drugs including highly effective statins and new classes of PCSK9 inhibitors. However, accumulating evidence, especially that from recent genetic studies, supports that CVD is far more complex than individual genes or pathways at play and the disease complexity is difficult to be fully captured with reductionist approaches (MacLellan et al., 2012). First, CVD is clearly the result of convoluted interaction of numerous genetic and environmental pathogenic factors. Second, CVD involves systems-level changes in diverse cell types, tissues, and organs. Third, multidimensional molecular entities and their interactions determine the complex paths leading to CVD development. Effective prevention and therapeutic strategies can only be achieved through a systems-level holistic view of CVD pathogenesis.

The revolutionary technological advances in the past decade have enabled cost-effective, high throughput profiling of multiomics molecular entities including genomics, epigenomics, transcriptomics, proteomics, metablomics, and microbiomics, paving the way towards a systems-level characterization of CVD pathogenesis (MacLellan et al., 2012; Meng et al., 2013; Civelek and Lusis, 2014). Notably, comprehensive molecular profiles have the capacity to capture the complexity of CVD by reflecting on the initiating genetic and environmental insults and the subsequent multitissue and multidimensional molecular perturbations that lead to the development of CVD. This capacity is further enhanced by the growth of public data repositories such as Gene Expression Omnibus (GEO) (Clough and Barrett, 2016), ArrayExpress (Parkinson et al., 2011), and dbGAP (Tryka et al., 2014), as well as coordinated national data generation efforts such as the Encyclopedia of DNA Elements (ENCODE) (Encode Project Consortium et al., 2007) and the Gene by Tissue Expression (GTEx) projects (G. TEx Consortium, 2013).

In response to the explosion of high throughput data, the field of bioinformatics has also seen significant development. Bioinformatics is a scientific discipline that aims to catalog, store, process, analyze, and interpret the biological significance of a variety of data by combining the tools and techniques of mathematics, statistics, computer science, and biology (Benton, 1996). Scores of bioinformatics tools are now readily available to researchers and are rapidly enabling novel discoveries in all major biological fields. In this review, we first introduce the characteristics of CVD that make big data research a necessity, next we delineate the basic bioinformatics principles applicable to the CVD field and the representative bioinformatics tools and applications. We mainly focus on major omics data types involved in CVD research and the corresponding bioinformatics principles, tools, and applications. We also highlight the importance and fundamentals of systems-level multidimensional data integration that represents the future of bioinformatics investigation on CVD.

Why CVD Requires Data-Rich Approaches?—Multilevel Complexity

A consensus has been reached that CVD are highly complex diseases. The complexity is reflected at several levels: multifactorial risks, multitissue pathologies, and multidimensional molecular perturbations (**Fig. 1**), as detailed below.

Multifactorial Risks

CVD originates from the collective contribution of a complex genetic architecture and its interaction with numerous environmental risk factors (Hunter, 2005; Kathiresan and Srivastava, 2012). CVD are highly heritable diseases, and the heritability estimates are 40–60% for CAD, 16–40% for stroke, and 21–58% for PVD (McPherson and Tybjaerg-Hansen, 2016; Bevan et al., 2012; Leeper et al., 2012), Numerous genetic studies via both candidate approaches and more recently, genome-wide association studies (GWAS), have uncovered hundreds of genetic loci for CVD (Kessler et al., 2016; McPherson and Tybjaerg-Hansen, 2016). In addition to the numerous genetic risks, many environmental factors (the exposome) have also been highlighted as major contributors to CVD. The most notable environmental risks include smoking, alcohol intake, low physical activity, high caloric diets rich in fat, cholesterol, and sugar, infections, environmental chemicals and pollutants, and stress (Cosselman et al., 2015). These environmental risks have been shown to have profound capacity to influence major organ systems critical for the maintenance of vessel and heart functions and likely contribute to the globalization and the soaring prevalence of CVD in the past few decades. Other environmentally linked diseases such as obesity, diabetes, and infectious diseases are also strong risk factors of CVD. Besides the large numbers of individual genetic and environmental risk factors that make CVD complex, growing evidence also supports gene-gene (McPherson and Tybjaerg-Hansen, 2016) and gene-environment interactions (Joseph et al., 2013; Ordovas, 2006; Corella and Ordovas, 2009). For example, interactions between the *COL4A1*/*COL4A2* locus and the *SMAD3* locus have been observed in CAD (Turner et al., 2015), and diet has been shown to modify the association strength of 9p21, one of the strongest genetic predictors for CAD (Schunkert et al., 2008; Samani et al., 2007; Do et al., 2011).

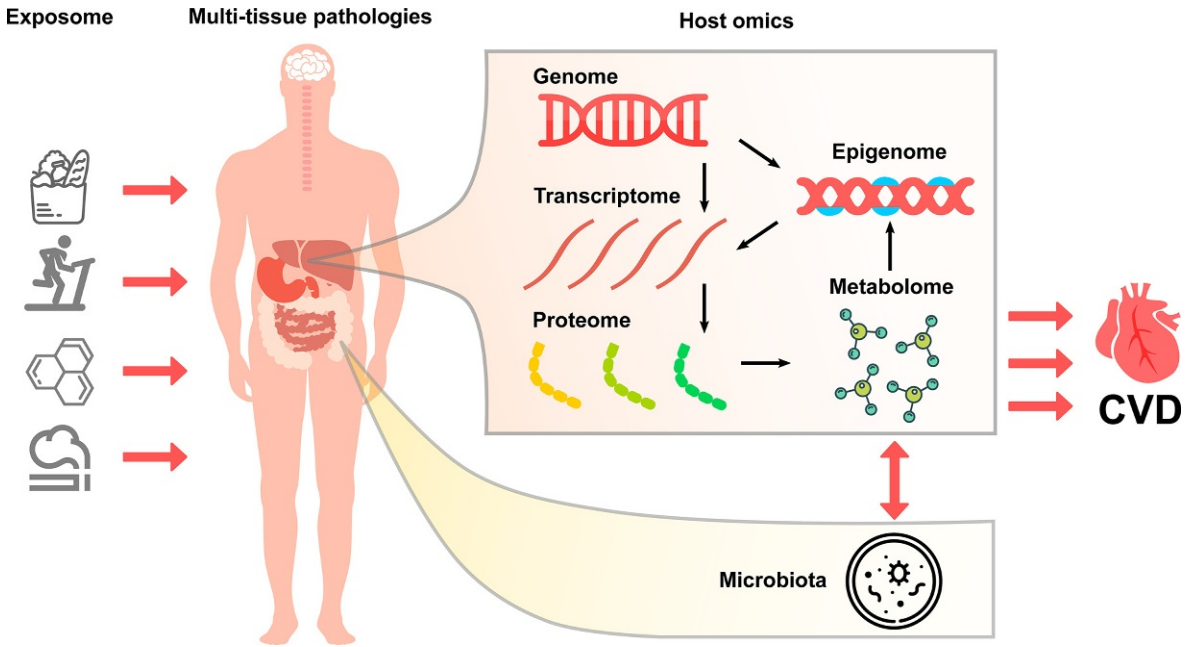

Fig. 1 Multilevel complexity of CVD. The complexity of CVD is reflected as (1) diverse environmental factors related to diet, behaviors, and chemical exposure which interact with genetic determinants to confer risk to CVD; (2) convoluted etiology that involves systemic perturbation in multiple tissues; (3) multidimensional perturbations at different molecular scales and the intricate interactions between the different scales.

Multi-tissue Pathologies

To further complicate the issue, CVD pathogenesis is highly systemic. Although CVD can originate from endogenous defects within the blood vessels or cardiomyocytes, systems-level studies have demonstrated that pathogenic factors from remote sites such as metabolic tissues (liver, adipose tissue), the immune and coagulation systems, and the central nervous system were all implicated in CVD development (Talukdar et al., 2016; Franzen et al., 2016; MacLellan et al., 2012; Malpas, 2010). More importantly, between-tissue communications and interactions are critical for the maintenance and dysregulation of cardiovascular system. Therefore, without a clear understanding of the roles of individual cells, tissues, and organs as well as the interactions among them, it is difficult if not impossible to fully understand CVD.

Multidimensional Molecular Perturbations

Within each cell or tissue involved in CVD, multidimensional molecular events connect and transmit genetic and environmental risk exposures to observable clinical outcomes (Fig. 1). According to the central dogma, genetic variations in the genome control the transcriptome and in turn affect the proteome. On top of this classic information flow, environmental risks factors can interact with the genome and perturb the epigenome such as DNA methylome, histone modifications, and chromatin structure to modulate the transcriptome and proteome. Additionally, the metabolome, either as products downstream of host proteome or products from gut microbiota, can also contribute to epigenomic modifications and subsequently transcriptional and translational alterations. Indeed, studies examining molecular changes in CVD at each of these dimensions have revealed extensive pathogenic perturbations.

In summary, the multilevel complexity described above substantiates the necessity to gather rich data that systematically measures all molecular entities in individual CVD-relevant cells or tissues under diverse risk conditions to describe CVD from a holistic perspective. Such efforts will help better illustrate the complex mechanisms of CVD in a tissue-specific and risk-specific fashion, thus facilitating mechanism-supported disease subtyping and precision medicine.

Bioinformatics Principles and Tools for CVD

With the advances of genomic technologies, the volume of omics-level datasets such as genome sequencing nearly doubles every year (Marx, 2013) and the complexity of data types and structures is also increasing. Consequently, the bottleneck of research output is gradually shifting from data acquisition to data analysis and interpretation. To accommodate the ever-growing size and complexity of the data, bioinformatics has seen dramatic advances in recent years, with new algorithms and tools published every day.

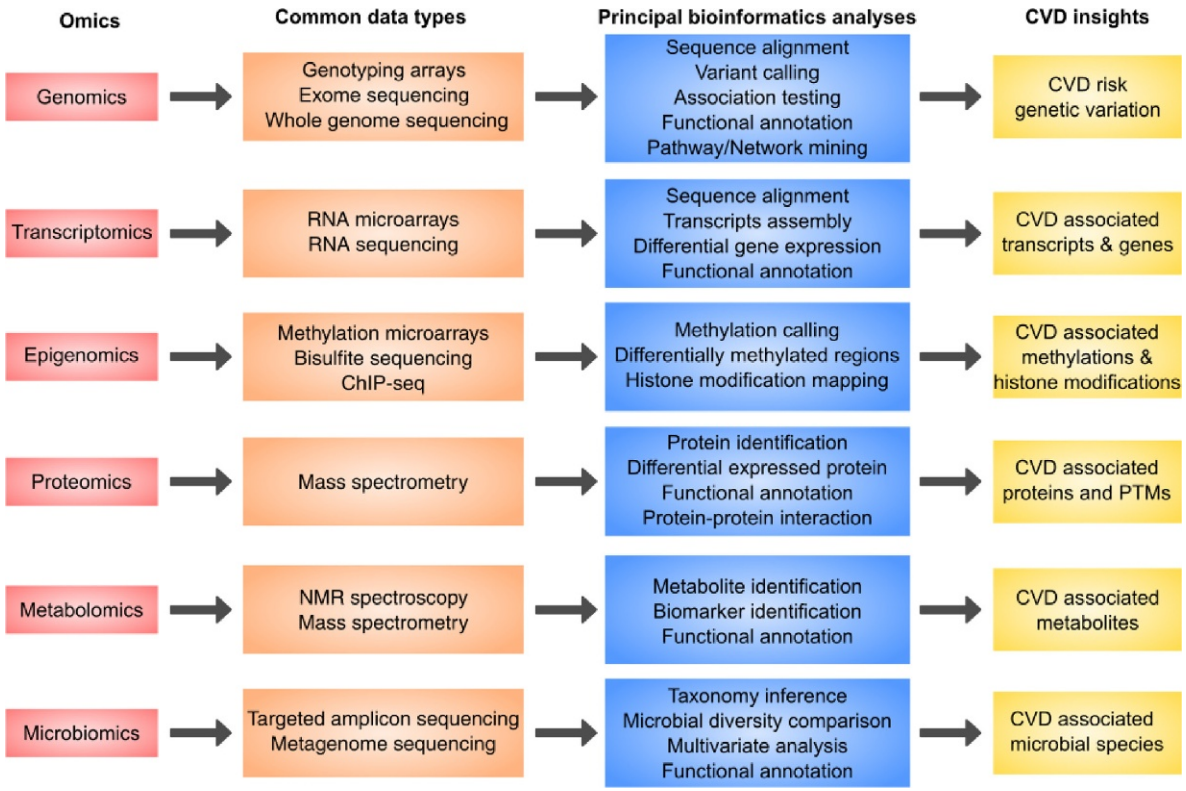

Fig. 2 Principal applications of bioinformatics on omics data for CVD.

Bioinformatics is a highly diversified field encompassing structured data storage, computing platforms, resource databases, and analytical algorithms and tools. We focus the current review on the principles and algorithms of bioinformatics tools for big data analysis, which are usually highly tailored to individual data types. Herein we introduce core bioinformatics principles and tools for commonly encountered data types in CVD studies (Fig. 2), including genomics (genotyping arrays, exome, and whole genome sequencing (WGS)), transcriptomics (microarrays, RNA sequencing), epigenomics (methylation microarrays, bisulfite sequencing, chromatin immunoprecipitation (ChIP-seq)), metabolomics (mass spectrometry (MS)), proteomics (nuclear magnetic resonance (NMR) spectroscopy, MS), and microbiota profiling (16S rDNA sequencing, metagenomics).

Genomics

Genomics measures DNA sequence variations that are heritable. The goal of CVD genomics is to identify heritable genetic risks of CVD, i.e., genetic variants that are associated with and likely causal for CVD development, through genetic association studies either in the form of linkage or genome-wide association analysis. Classic linkage analysis is powerful for Mendelian diseases or traits in which a single genetic locus of strong effect is involved but this methodology has limited power for complex diseases like CVD where a large number of genetic loci of moderate effect size are at play. Therefore, GWAS studies that screen for associations at dense genetic variants at genome scale have become a routine practice. In principle, genomics analysis involves the following steps: variant identification from either array-based genotyping or next-generation DNA sequencing platforms, genome-wide analysis of variant-disease associations, in silico functional annotation of disease-associated variants, and pathway analysis, as detailed below.

Variant identification

Genetic variations manifest in various forms including single nucleotide polymorphisms (SNPs) and structural variations including insertions and deletions (indels), inversions, and copy number variations (CNVs). Detailed catalogs of these variations can be found at bioinformatics databases such as dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) for SNPs and The Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home) for structural variations. Among these, SNPs are the most common form (>10 million in the human genome) and hence the most studied type of DNA variation in GWAS of CVD.

High density genotyping arrays from Affymatrix and Illumina have been the dominant technologies to capture SNPs and CNVs. These arrays utilize the complementary hybridization properties of DNA sequences to design nucleotide probes specific for known SNPs and CNVs. Numerous algorithms and tools have been developed for different genotyping platforms to generate accurate genotype calls, as discussed in detail by LaFramboise (LaFramboise, 2009). For Affymetrix genotyping arrays, each generation has been tailored with specific algorithms (e.g., BirdSeed for the Affymetrix Human SNP Array 6.0), whereas for Illumina arrays, the

algorithm has been relatively stable and is implemented in the Illumina GenomeStudio software. The basic principle underlying the various algorithms and tools is to normalize and model the probe hybridization intensity measures for each SNP or CNV to estimate the most likely call for the allele and genotype of a SNP or the copy number of a CNV for a given sample.

More recently, as genome-scale sequencing has become affordable, whole exome sequencing (WES) that focuses on the protein-coding sequences and WGS that can additionally capture important noncoding regulatory regions like promoters and enhancers (Sims et al., 2014), have become increasingly adopted to detect all forms of genetic variations, both common and rare. The Exome Sequencing Project sponsored by the National Institutes of Health, Heart, Lung, and Blood has generated an invaluable WES resource called the Exome Variant Server (http://evs.gs.washington.edu/EVS/). A large number of variant calling pipelines have been developed, as discussed in detail by Pabinger et al. (Pabinger et al., 2014) and Hwang et al. (Hwang et al., 2015). The basic workflow underlying sequencing-based variant detection is to first align sequencing reads to reference sequences using aligners such as BWA (Li and Durbin, 2009), Bowtie2 (Langmead and Salzberg, 2012), and Novoalign (http://novocraft.com/), followed by variant calling using tools such as the Genome Analysis Tool Kit (DePristo et al., 2011; McKenna et al., 2010), Samtools mpileup (Li et al., 2009), and Freebayes (Garrison and Marth, 2012). The performance of the various pipelines differs by sequencing platforms (e.g., Illumina vs. Ion Torrent) and the types of variants being called (e.g., SNPs vs. indels). Therefore, it is important to follow the best practices recommended for different genotyping or sequencing platforms or to employ and compare multiple tools for variant detection. Following variant calling, various quality control steps are usually taken to detect and remove sex inconsistency, relatedness, batch effects, and population substructure, and to calculate variant and sample call rates to assess quality and remove outliers. These quality control steps can be performed using a robust and popular tool called PLINK (Purcell et al., 2007).

Genetic association testing

To model the association between SNPs or other genetic variations with CVD susceptibility, it is important to first consider the study design (e.g., case-control or population study) and the data type of the phenotype (e.g., categorical or quantitative), because the statistical tests for association analysis are highly dependent on these factors (Cordell and Clayton, 2005). For instance, when comparing CAD cases vs. control individuals, Chi-square test and logistic regression can be used to test the differences in allele or genotype frequencies of individual SNPs between the two populations using the disease status as the categorical endpoint; whereas for population studies of quantitative traits such as cholesterol levels, the parametric linear statistical analysis is one of the most commonly used methods due to its theoretical robustness and ease of implementation using software packages like R, Matlab and SAS. PLINK represents a specialized tool that implements an array of statistical tests suitable for different study designs (Chang et al., 2015; Purcell et al., 2007). Additionally, linear mixed model based tools such as EMMA (Kang et al., 2008) and GEMMA (Zhou and Stephens, 2012) can efficiently account for population structure in genetic association tests.

Association testing of individual variants works well for common variants, but it is severely underpowered for rare variants due to the low frequency of each variant and high abundance of rare variants. To resolve this issue, gene or region-based tests which evaluate the aggregated effect of multiple markers, such as burden tests (Li and Leal, 2008), variance-component tests (Wu et al., 2011), and exponentially combined tests (Chen et al., 2012), have been developed.

Annotation and exploration of functional implications of CVD variants

The most intuitive steps following a GWAS study are to identify the true causal variants and subsequently capture the causal genes. Although the linkage disequilibrium structure helps to significantly reduce the number of candidate variants, there are often tens to hundreds of variants remaining at each risk locus (Yang et al., 2012). Sophisticated statistical framework can be applied to further refine the association signals and quantify the posterior probability of each variant being causal by jointly modeling the association statistics of all variants in a given locus (Hormozdiari et al., 2014). Functional annotation can also be achieved based on knowledge-driven information using tools such as GRAIL (Raychaudhuri et al., 2009) and DEPICT (Pers et al., 2015), as demonstrated in the investigation of CAD loci by (Braenne et al., 2015). In addition, ANNOVAR (Wang et al., 2010), fgwas (Pickrell, 2014), and PAINTOR (Kichaev et al., 2014) stand as popular tools that evaluate the functional importance of variants using functional genomic information derived from resources like ENCODE (Encode Project Consortium et al., 2007; Encode Project Consortium, 2012). Variants that affect the coded amino acids, gene expression, and splicing are more likely to be causal.

Functional genomics studies that identify intermediate quantitative trait loci can also serve as the fundamental basis of functional annotation of genetic variants (Yang, 2012; Meng et al., 2013). Genetics of gene expression studies that identify expression quantitative trait loci (eQTLs) (Schadt et al., 2008) can be especially useful to reveal candidate causal genes for CVD (Folkersen et al., 2010; Braenne et al., 2015; Yang, 2012). The GTEx study also provides an eQTL browser for quick eQTL-gene mapping (Lonsdale et al., 2013). Advanced tools based on Mendelian randomization can also integrate summary-level GWAS data and eQTLs to identify CVD associated genes (Zhu et al., 2016).

Pathway analysis

Complementary to single-variant functional analysis, GWAS-based pathway analysis has been widely used to derive biological pathways that are enriched for disease associated variants (Segre et al., 2010; Shu et al., 2016; Zhong et al., 2010). Popular tools include MAGENTA (Segre et al., 2010), iGSEA4GWAS (Zhang et al., 2010), GSA-SNP (Nam et al., 2010), and GSEA-SNP (Holden et al., 2008). We have also recently implemented a Marker Set Enrichment Analysis that incorporates functional information such as eQTLs into the pathway analysis (Shu et al., 2016). Despite differences in confounder correction and enrichment statistics, the conceptual basis of GWAS-based pathway analysis tools is similar, which is to evaluate the disease relevance of a pathway based on

the overall association strengths of variants assigned to the genes in the given pathway. The primary advantage of such methods is that they can fully utilize the whole spectrum of association information provided by a GWAS, thus substantially improving the statistical power to reveal hidden biology not captured by a few top variants.

Application of genomics in CVD

In recent years, painstaking efforts have been devoted to GWAS to perform genome-wide screening of common variants. Such efforts have identified significant associations of numerous common DNA variations with CVD and related traits (Nikpay et al., 2015; CARDIoGRAMplusC4D Consortium et al., 2013; McPherson and Tybjaerg-Hansen, 2016; Kessler et al., 2016). For instance, the most recent GWAS catalog (https://www.ebi.ac.uk/gwas/; accessed in September 2016) has documents 71 loci for CAD, 13 for MI, 43 for stroke, and 5 for PVD. Several interesting observations have emerged from the genome-scale discovery studies. First, the GWAS loci not only captured well-known risk pathways such as cholesterol and lipid metabolism, immune response, and inflammation, but also unraveled under-appreciated risk processes such as mitochondrial respiration, TGF-beta signaling, and actin networks (Nikpay et al., 2015). Second, the majority of the discovered loci only moderately alter the susceptibility of CVD and are located in noncoding regions. Third, the total heritability explained by the top loci is far below the estimated genetic heritability of individual CVD.

These common-variant-focused GWAS have been recently supplemented by WES and WGS studies that enhance our ability to capture association of rare variants with CVD phenotypes (Galmiche et al., 2011; Ghezzi et al., 2012; McPherson and Tybjaerg-Hansen, 2016; Kessler et al., 2016). For example, recent WES studies have identified rare variants in the coding regions of genes in lipoprotein metabolism to be significantly associated with CAD (Myocardial Infarction Genetics Consortium et al., 2014; Crosby et al., 2014; Do et al., 2015). Successful application of pathway analysis on CVD GWAS has led to novel biological insights that are otherwise difficult to capture (C. ARDIoGRAMplusC4D Consortium et al., 2013; Makinen et al., 2014; Ghosh et al., 2015).

Transcriptomics

Transcriptomics reflects the immediate transcriptional consequences of genetic level perturbations and has the potential to bridge the gap between genetics and other omics layers or CVD traits. Transcriptomic studies on CVD can help identify important transcripts and genes that either contribute to the pathogenesis of CVD or serve as biomarkers of CVD pathology. Gene expression microarrays and high-throughput RNA sequencing (RNA-seq) are the most common technological platforms for transcriptomic profiling. Microarrays are designed based on known transcripts or genes. Compared to microarrays, RNA-seq has higher sensitivity, dynamic range, and the ability to capture novel transcripts or splicing events (Wang et al., 2009). Due to these advantages, RNA-seq has received increased popularity and application. More recently, parallel single cell RNA-seq technologies such as Fluidigm and Drop-seq offer tremendous value in learning the molecular circuits in individual cells of heterogeneous tissues (Hashimshony et al., 2012; Macosko et al., 2015). Here we introduce the fundamental bioinformatics concept and tools for microarrays and RNA-seq analyses.

Analysis of gene expression microarrays data

Microarrays on different RNA species such as mRNA (Hwang et al., 2002), microRNA (van Rooij et al., 2008; Kuwabara et al., 2011) and long-noncoding RNA (Li et al., 2013; Ounzain et al., 2015) have helped identify novel biomarkers and mechanistic insights of CVD, although technically noncoding RNAs belong to the epigenome which we will discuss in the next section. Common platforms are from Affymatrix and Ilumina, although Agilent arrays were also used in earlier studies. Bioinformatics toolkits for microarrays have matured over the past decades, with a wide array of commercial and open-source computational libraries available to effectively handle the workflow of microarray analytical pipelines, including preprocessing data from different platforms to derive measurements of gene or transcript expression levels, data normalization, quality control, differential expression (DE) analysis, and result visualization (www.agilent.com) (Ritchie et al., 2015; Du et al., 2008). Normalization and DE analysis are usually the steps that require the most attention. Technical guidance about the selection of normalization methods has been discussed in detail previously (Quackenbush, 2002; Smyth and Speed, 2003). Although there are numerous variations for DE analysis, *t*-test, and linear model are typically the core underlying elements of different methods (Cui and Churchill, 2003). For Affymatrix microarrays, the affy and limma R Bioconductor packages are widely used. For Illumina microarrays, the GenomeStudio software by Illumina and the lumi R Bioconductor package are popular choices.

Analysis of next-generation RNA sequencing data

Compared to the maturity of microarray analysis methodologies, tools for RNA-seq analysis are currently undergoing rapid development. The basic steps of RNA-seq data analysis include sequence read alignment, read assembly and quantification of gene abundance at gene, transcript, exon levels, DE analysis, and alternative splicing detection. At each analysis step, numerous methods and tools have been developed. For read alignment, the widely used tools are BWA (Li and Durbin, 2009), Bowtie2 (Langmead and Salzberg, 2012), and Novoalign (http://novocraft.com/). More recently HISAT (Kim et al., 2015) has been introduced as a more efficient aligner. For read assembly and transcripts abundance quantification, Cufflinks (Trapnell et al., 2010; Trapnell et al., 2012), StringTie (Pertea et al., 2015) and Ballgown (Frazee et al., 2015) are available. For DE analysis, EdgeR (Robinson et al., 2010), DESeq (Anders and Huber, 2010), Voom (Ritchie et al., 2015), and Ballgown (Frazee et al., 2015) are widely adopted. In terms of alternative splicing identification, popular tools include Cufflinks (Trapnell et al., 2013), GSNAP

(Wu and Nacu, 2010), DEXSeq (Anders et al., 2012), and rMATS (Shen et al., 2014). In addition to these individual methods, computational pipelines that assemble various tools in a sequential manner also exist to facilitate streamlined analysis of RNA-seq data, among which the most notable are TopHat, Cufflinks, and Cuffdiff pipeline (Trapnell et al., 2012), and a more recent HISAT, StringTie, and Ballgown pipeline (Pertea et al., 2016). Importantly, no consensus has yet been reached about the best practices for RNA-seq analysis, and different tools have highly variable behaviors under different study designs, sample sizes, data heterogeneity, as well as the metrics used for performance evaluation (Seyednasrollah et al., 2015).

Functional annotation of gene signature

A transcriptome study typically generates a large number of signature genes that are associated with a disease and it is important to extract biologically meaningful insights from this data. Therefore, categorizing the disease signature genes into molecular pathways can help interpret the transcriptome data. A wide spectrum of pathway analysis tools have been released, each with its own unique advantages and disadvantages (Khatri et al., 2012). One particular web-based tool, DAVID (Huang et al., 2009), offers a user-friendly solution that allows users to flexibly upload lists of genes and easily retrieve the enriched pathways in the gene lists. However, focusing on limited numbers of top signature genes can often lead to the loss of valuable information on genes with subtle but biologically meaningful changes. Tools like gene-set enrichment analysis (GSEA) have been developed to overcome this challenge (Subramanian et al., 2005). The core concept of GSEA is to evaluate the enrichment of differentially expressed genes at the level of gene sets, which can be common biological functions, pathways, chromosomal regions, or regulatory targets. GSEA is able to take advantage of both the expression abundance data and rich knowledge-driven annotation databases like GO (Ashburner et al., 2000), KEGG (Kanehisa and Goto, 2000), and Reactome (Schmidt et al., 2006), to extract gene sets whose member genes show coordinated patterns of up or downregulation without the need to predefine a statistical cutoff to determine the DE of individual genes.

Application of transcriptomics in CVD

Transcriptomics is the most widely used omics approach to study CVD and there are numerous applications of gene expression microarrays and more recently RNA-seq in CVD research. As a result, numerous tissue-specific transcriptomics datasets and signatures have been generated by the CVD community over the past decades and we will not review in detail but only exemplify with a few studies here. In the Framingham Heart Study, (Joehanes et al., 2013) and (Huan et al., 2013; Huan et al., 2015a) have detected blood gene signatures of CVD and blood pressure by comparing the gene expression patterns measured with microarrays. In another example, (Yang et al., 2010) identified aorta gene expression signatures from microarray analysis of atherosclerosis development in both $Apoe^{-/-}$ and $Ldlr^{-/-}$ mouse models. Using RNA-seq, Liu et al. identified gene signatures in different heart failure subtypes and used these signatures to accurately classify heart failure status in a patient population (Liu et al., 2015). In another study, Lee et al. performed a comprehensive characterization of transcriptome in murine hearts by identifying novel exons and transcript clusters (Lee et al., 2011).

Epigenomics

Epigenomics measures various DNA modifications and gene regulatory mechanisms that do not involve DNA sequence changes or genetic variations. The types of epigenetic mechanisms include DNA methylation, histone modifications (methylation, acetylation, phosphorylation, DP-ribosylation, and ubiquitination), chromatin modification, and ncRNAs (microRNAs, long noncoding RNAs or lncRNAs, small interfering RNAs). The epigenome plays a pivotal role in the dynamic regulation of gene expression and is a critical mediator of the influence from environmental factors. Therefore, alterations of the epigenome could help to explain gene by environment interactions that are hypothesized as one of the major contributors to CVD. Indeed, various epigenetic changes have been associated with CVD events and related risk factors (Ordovas and Smith, 2010; Baccarelli et al., 2010; Zhong et al., 2016).

To measure the epigenome, a number of approaches have been developed. Methods for DNA methylome measurements include restriction enzyme-enriched sequencing techniques, affinity-enrichment-based sequencing techniques, DNA methylation arrays, reduced representation bisulfite sequencing, and whole-genome bisulfite sequencing, each with its unique advantages and disadvantages (Heyn and Esteller, 2012; Laird, 2010). For histone modifications and chromatin organization, various sequencing-based methods, such as ChIP-seq, FAIRE-seq, and DNase-seq have become readily available, as reviewed in detail by others (Morozova and Marra, 2008; Telese et al., 2013). For ncRNA profiling, RNA-seq can be used with modifications in the sample processing and RNA extraction steps (Pritchard et al., 2012). Here we introduce the bioinformatics principles and tools for a few commonly used epigenomic platforms.

DNA methylation arrays

Microarray-based methylation profiling is the most commonly used method to retrieve genome-wide methylation profiling, and has contributed to the generation of first-hand evidence linking global methylation perturbations to CVD events (Sharma et al., 2008; Sharma et al., 2014; Zaina et al., 2014; Kim et al., 2010). DNA methylation typically occurs at the cytosine of CpG dinucleotide sites. The Illumina platform is a popular technical solution for methylation profiling at single CpG site resolution and comes in two common sizes which profile 27 k and 450 k CpGs respectively (Bibikova et al., 2011). Additionally, smaller, custom, targeted methylation arrays are available from 48 to 384 loci (that is VeraCode GoldenGate arrays). Similar to gene expression microarrays, mature computation pipelines Methylumi (Davis et al., 2012) and minfi (Aryee et al., 2014)) are available

to handle critical analytical processes of methylation data including quality control, normalization, and differentially methylated region identification. Minfi also provides advanced analytical functions including methylation block finding and subset-quantile within-array normalization that reduce the within- and between-array technical variation (Maksimovic et al., 2012).

Bisulfite sequencing

Bisulfite sequencing is used for profiling of DNA methylation. Bisulfite sequencing leverages the fact that methylated cytosine remains unaffected under bisulfite treatment, whereas unmethylated cytosine will be converted to uracil. Whole genome bisulfite sequencing (WGBS) is enabled by the advance in next-generation sequencing technologies to analyze genome-wide DNA methylation at single-nucleotide resolution. As a variant of WGBS, reduced representation bisulfite sequencing (RRBS) is a method that first enriches for the genome regions with high CpG content using methylation-sensitive restriction enzymes, thus avoiding the high cost associated with WGBS (Meissner et al., 2005). Similar to RNA-seq discussed earlier, the analysis of RRBS and WGBS involves alignment of sequence reads to a reference genome, followed by the estimation of the methylation percentages at individual CpG sites. Common algorithms for WGBS and RRBS reads mapping include "wild card" (allowing either Cs or Ts to map to Cs) and "three letter" aligning (all Cs are converted to Ts), which are supported by numerous tools like BS Seeker (Guo et al., 2013) and Bismark (Krueger and Andrews, 2011). Performance comparison of different mapping tools has been discussed previously (Kunde-Ramamoorthy et al., 2014). Methylation percentages at each cytosine can be computed and compared between groups to detect differentially methylated CpG regions (DMRs) using tools such as methylKit (Akalin et al., 2012), DMAP (Stockwell et al., 2014), MOABS (Sun et al., 2014), and metilene packages (Juhling et al., 2016). The statistics underlying DMR detection can be Fisher's exact test (Akalin et al., 2012), logistic regression (Akalin et al., 2012) or *t*-tests (Hansen et al., 2012).

ChIP-seq

For epigenomics, ChIP-seq has been mainly utilized to characterize histone modifications, DNA methylation, and nucleosome positioning (O'Geen et al., 2011; Furey, 2012; Landt et al., 2012). In ChIP-seq, antibodies are first used to immunoprecipitate different types of chemical modifications or DNA binding proteins along with cross-linked DNA to enrich the genomic DNA sites of interest such as specific types of histone modification sites. The enriched genomic DNA is then sequenced. Technical challenges involved in ChIP-seq experiments include antibody quality, obtaining large numbers of required cells, and previous knowledge of a histone modification or DNA-binding protein location (Furey, 2012). Similar to RNA-seq and RRBS, ChIP-seq reads need to be mapped to the reference genome using aligners, as described previously. Once reads have been aligned, the next step involves determining "peaks" where binding signals are enriched. There are a number of popular strategies for peak calling including MACS (Zhang et al., 2008), PeakSeq (Rozowsky et al., 2009), and ZINBA (Rashid et al., 2011). Among these methodologies, MACS is the most simplistic, fitting the data to a Poisson distribution; whereas both PeakSeq and ZINBA take into account the mappability of genomic regions where significant peaks are determined against a null random background distribution. Once these peaks have been called, individual peaks can be compared between samples using tools that are traditionally used in differential gene expression like EdgeR (Robinson et al., 2010) and DESeq (Anders and Huber, 2010). Both methods have similar underlying methodologies where they call differential peaks by modeling the data as having a negative binomial distribution and use a modified Fisher's exact test (Robinson and Smyth, 2008). Histone modification signals are generally diffused and lack well-defined peaks, posing unique technical challenges to map epigenetic states from ChIP-seq data (Wang et al., 2008). Therefore, new algorithms have been developed based on spatial clustering (Zang et al., 2009) or hidden Markov models (Song and Smith, 2011) to improve the signal-to-noise ratio. The chromatin landscape can be further correlated to transcriptional regulation elements like enhancers to better clarify the whole regulatory circuitry (Wamstad et al., 2012).

Application of epigenomics in CVD

The relationship between CVD and each type of epigenetic marker measured with various technological platforms has been under intensive investigation, and the involvement of all types of epigenomic mechanisms ranging from DNA methylation and histone modification, to noncoding RNAs is strongly supported. For example, RRBS has been used to link global methylation events with CVD (Sharma et al., 2008; Zaina et al., 2014), revealing a hypermethylation pattern in the atherosclerotic lesions that connects to pathways related to endothelial and smooth muscle functions. ChIP-seq has also been used to reveal differential histone methylation profiles associated with heart failure (Movassagh et al., 2011). The role of noncoding RNAs in CVD has been supported by a number of studies of microRNAs and lncRNAs (Wu et al., 2014; Kataoka and Wang, 2014; Uchida and Dimmeler, 2015; Huan et al., 2015b;Klattenhoff et al., 2013).

Proteomics

Proteomics is the large-scale investigation of proteins, the vital functional unit of living organisms. Importantly, despite being the translational product of mRNA, transcriptomic level expression is poorly correlated with protein levels, making it difficult to predict protein expression from transcriptome profiling (McGregor and Dunn, 2006). Proteomics can help comprehensively characterize protein alterations in the process of CVD and capture posttranslation modifications (PTMs), thereby facilitating the identification of protein level biomarkers, regulatory mechanisms, and potential therapeutic targets. A number of proteomics technologies have been developed, including one- and two-dimensional gel electrophoresis (2D-GE), protein chip, high-performance liquid chromatography, and MS (Breker and Schuldiner, 2014). The protein identification step is highly dependent on the technological

platforms and has been discussed in detail by other reviews (Altelaar et al., 2013; Larance and Lamond, 2015). Here we focus on the bioinformatics tools in the differential and functional analyses.

Differential protein expression

Similar to the other omics fields, comparison of protein levels under different conditions can help prioritize important proteins in pathogenesis. Although statistical and computational frameworks discussed for transcriptomics studies may also be applied in proteomics, there are challenges unique to proteomics (Langley et al., 2013). First, label-free protein expression data usually contains missing values for proteins with low abundance, which could break the normality assumption of statistical tests like *t*-test, ANOVA, and linear regression. In addition, most proteomics studies involve small sample sizes due to the relatively low throughput of proteomics technologies and therefore could suffer from high noise-to-signal ratio. For MS, this challenge can be alleviated by methods like (1) normalized spectral index (Griffin et al., 2010) which combines multiple abundance features and eliminates variance between MS measurements, and (2) hybrid-based approach (Wang et al., 2012) that combines the advantages of intensity-based and presence/absence-based protein quantification.

Functional annotation

The previously mentioned pathway and functional gene set databases used for annotating transcriptomic data can also be applied to proteomic data, in combination with gene set enrichment analysis tools like GSEA (Isserlin et al., 2010). In addition, protein-specific annotation resources like Protein Analysis Through Evolutionary Relationships (PANTHER) which performs comprehensive evolutionary and functional classification using a webserver (Mi et al., 2016), are also available. Protein-protein interactions (PPI) are also important determinants of protein functions and are critical for biological processes (Stelzl et al., 2005). There are a number of public PPI repositories that vary by data source and method of construction and organizing interactions, like the Human Protein Reference Database (Prasad et al., 2009) and STRING (Szklarczyk et al., 2015), as detailed elsewhere (Lage, 2014).

Application of proteomics in CVD

Wide-spread proteomic alterations have been captured in CVD (Sharma et al., 2013; Lindsey et al., 2015). In addition, proteomics studies have been carried out in multiple CVD related cell types including arterial smooth muscle cells (Dupont et al., 2005), cardiomyocytes (Stastna et al., 2010), and pro-angiogenic cells (Urbich et al., 2011), as well as subcellular regions such as the mitochondrial proteome (Taylor et al., 2003) and the extracellular matrix (Barallobre-Barreiro et al., 2012). Moreover, these efforts have been pooled into public proteomics data repositories such as the Human Proteome Map (Kim et al., 2014) and the Proteomics Identification Databases (Vizcaino et al., 2013), allowing for efficient protein identification and quantification for future proteomics studies. In CVD, PPI information is usually contextualized in the form of networks that bear additional structural and functional relationships through the topological layouts. The networks can be used to interrogate novel functional complexes and regulatory hubs for CVD (Li et al., 2014a; Ren and Liu, 2013; Magger et al., 2012).

Metabolomics

Metabolomics platforms and tools

Metabolomics focuses on metabolites that are traditionally considered the final stops of biological information flow, reflecting perturbations in the genome, epigenome, transcriptome, and proteome, although metabolites can also feedback to the gene regulation process by modulating the epigenome. Typically, a metabolomics study can take a targeted approach to specifically measure predesignated metabolites involved in certain pathways or biological functions, or an untargeted approach, where no prior knowledge of metabolites selection is needed (Kordalewska and Markuszewski, 2015). Advances in MS and NMR spectroscopy have made it possible to perform rapid identification and quantification of hundreds of metabolites in signaling, receptor binding, translocation, and biochemical reaction pathways (German et al., 2005). Each platform has inherent advantages and disadvantages, such as the high reproducibility but a low sensitivity in NMR-based techniques compared with MS-based techniques (Gibbons et al., 2015; Gika et al., 2014).

Bioinformatics is applied in the data processing and analysis steps in metabolomics studies (Johnson et al., 2016). The data processing workflow typically involves data conversion, feature detection, data set alignment, normalization, metabolite identification, and quality control (Sugimoto et al., 2012). Pipelines like MetaboAnalyst offer step-wise functional modules to complete the whole processing workflow (Xia et al., 2012). The accurate identification of metabolites is further powered by metabolite annotation databases like The Human Metabolome Database (Wishart et al., 2009). In addition, functional analysis can be performed by comparing lists of metabolites with predefined functional groups like KEGG metabolic pathways (Xia et al., 2015; Xia and Wishart, 2010b; Xia and Wishart, 2010a), where metabolites can be related to each other and provide targets for downstream mechanistic studies.

Application of metabolomics in CVD

Metabolomics studies have successfully contributed to the identification of metabolite biomarkers associated with atherosclerosis (Wurtz et al., 2012), CAD (Laborde et al., 2014), MI (Mohamad et al., 2015), and CVD (Wang et al., 2011). The association between the plasma concentration of choline, betaine, and trimethylamine-N-oxide (TMAO) with the increased risk of CVD is probably the most prominent and robustly validated metabolomics discovery in the field (Koeth et al., 2013; Wang et al., 2011;

Tang et al., 2013), and has led to the encouraging establishment of new CVD therapy (Wang et al., 2015). Metabolomics has also been integrated with GWAS to establish functional link between genetic variants, intermediate metabolic traits, and CVD relevant endpoints (Suhre et al., 2011).

Microbiota

The intestinal microbiota consists of trillions of commensal microorganisms that mediate the effect of diet, the primary environmental determinant of CVD. As the major processing station for food, microbiota are closely involved in the homeostasis of metabolites, thus establishing a link with the metabolome (Wikoff et al., 2009). Microbiota can be classified into various taxa including kingdoms, phyla, classes, orders, families, genera, and species based on sequence similarity and phylogenetic information. Here we focus on bioinformatics for microbial DNA assessed by two approaches: (1) targeted amplicon analysis which only focuses on a few selected marker DNA sequences that can differentiate bacterial species and (2) metagenomics in which the whole microbiota DNA sequence is interrogated.

Targeted amplicon profiling

Bacteria carry unique DNA sequences that are absent in eukaryotes and can reflect bacterial speciation. One such bacterial DNA sequence, the 16S rDNA, encodes the 16S ribosomal RNA and is the most widely used amplicon for microbiota profiling because the gene sequence is highly conserved within bacteria of the same genus and species but different between genera and species (Woo et al., 2008; Janda and Abbott, 2007). Briefly, whole DNA from gut microbiota is isolated and 16S rDNA is amplified and then sequenced. The 16S rDNA sequence data is clustered into operational taxonomic units (OTUs, microorganisms grouped by the similarity of their DNA sequences), either with or without reference information of known taxa. Reference based OTU clustering is getting more and more favorable due to the increasing aggregation of microbiota sequences in public repositories (Turnbaugh et al., 2007; Caporaso et al., 2010). Simultaneously, the phylogenetic relationships among sequences can also be inferred using the UniFrac method or phylogenetic diversity measurement (Lozupone and Knight, 2005; Faith and Baker, 2006). In addition to OTUs and phylogenetic trees, microbial diversity can be further dissected and visualized using analyses such as rarefaction curves (for within-community diversity) and principal coordinates analysis (PCoA, for across community diversity) (Kuczynski et al., 2012). The streamlined pipelines QIIME (Caporaso et al., 2010) and Mothur (Schloss et al., 2009) are available to conduct the above mentioned analyses.

Metagenomic profiling

Metagenome of human microbiota consists of a vast number of nonredundant genes with highly diversified functional composition (Li et al., 2014b). Unlike amplicon-based profiling that focuses on the determination of phylogeny from selected genes, metagenomics sequencing has the capacity to produce the whole sequences of all bacteria in a given biological sample and shed light upon the functional potential of the microbial community. However, the overwhelming volume, complexity, and lack of annotation resources of metagenomics data pose unique challenges to bioinformatics (Thomas et al., 2012). First of all, for metagenome assembly, classical reference-based methods are less favorable due to the enormous gap between microbial diversity and the coverage of reference database. As a result, numerous de novo metagenome assembly algorithms have been developed, such as the de Bruijn graph-based approach (Chaisson and Pevzner, 2008) and coabundance based segregation of metagenome (Nielsen et al., 2014).

The next critical step after assembly is to perform functional annotation of metagenomics data, where sequences can be mapped to known orthologous groups with well-documented functional descriptions that are deposited in various large annotation databases such as eggnog, KEGG, and COG (Huerta-Cepas et al., 2016; Kotera et al., 2015; Galperin et al., 2015). Both local software such as SmashCommunity (Arumugam et al., 2010) and webservers such as RAST and IMG/M (Meyer et al., 2008; Markowitz et al., 2012) are available for this task.

Lastly, to maximize clinical relevance, metagenomics studies typically aim to identify difference of features (organisms, clades, OTUs, genes, or functions) between samples or disease states. This can be a daunting task due to the large number of species and functional groups under investigation, and the high intersubject variability of microbial communities. Various advanced statistical models have been developed to address this need. For example, Metastats utilizes an extended *t*-test to detect differential features from the count data (White et al., 2009). On top of that, LEfSe (Linear discriminant analysis effect size) allows for the capture of differential features consistent with biologically meaningful categories (Segata et al., 2011). And MetaPath can further identify finer-level metabolic subnetworks by adopting a statistical framework to aggregate the sequence-level differences into biological pathways (Liu and Pop, 2011).

Application of microbiota profiling in CVD

Mounting evidence has suggested that microbiota is related to a wide spectrum of metabolic, immune response, and signaling events that are fundamental in cardiometabolic disease pathogenesis (Nicholson et al., 2012; Turnbaugh et al., 2006; Vijay-Kumar et al., 2010; Hansen et al., 2015). One exemplary piece of direct evidence connecting gut microbiota with CVD is from the studies on TMAO, which is generated by the microbial metabolism of choline (Griffin et al., 2015). The association of elevated plasma TMAO concentration and increased atherosclerosis and CVD risk has been found to be dependent on gut microbiota (Wang et al., 2011; Koeth et al., 2013). However, direct evidences linking specific microbiota alteration to CVD are still limited, with only a few genera suggested to be involved by small cross-sectional studies (Karlsson et al., 2012; Hansen et al., 2015).

Multiomics Data Integration

The above discussions are mainly focused on the bioinformatics aspects at individual omics levels. However, to fully disentangle the molecular underpinnings of CVD that connect pathogenic perturbations to the ultimate phenotypic manifestation, it is essential to holistically investigate the flow of biological information starting from the genetic perturbations to different intermediate components. We have comprehensively reviewed the principles and applications of systems-level data integration (Meng et al., 2013) and will only briefly summarize the principles here (**Fig. 3A**). First, multiomics integration typically requires coherent collection of various types of omics datasets from a study population under well-controlled conditions. When comprehensive datasets are not available from the same biological samples, it is also possible to integrate multidimensional omics data across studies. Omics data can be partitioned into three broad categories: genetics which carries causal information, intermediate omics (e.g., epigenomics, metabolomics, proteomics), and disease phenotypes. Next, pair-wise relationships within and between data types are analyzed. Within the same omic level, relationships between molecular entities such as protein-protein correlations and gene coexpression patterns are retrieved. This is typically performed using network modeling approaches such as the weighted gene coexpression network analysis (Langfelder and Horvath, 2008) and directional network construction. Such networks form the organizational maps among molecular entities and inform on functional relationship between molecules. Between omics dimensions, pair-wise associations are calculated, such as GWAS between genetic markers and disease phenotypes, genetics of gene expression or eQTLs analysis between genetic markers and gene expression, or correlations between microbiota and metabolites. Thirdly, causality modeling between intermediate markers and a disease phenotype can be performed using genetic markers as the causal anchor to

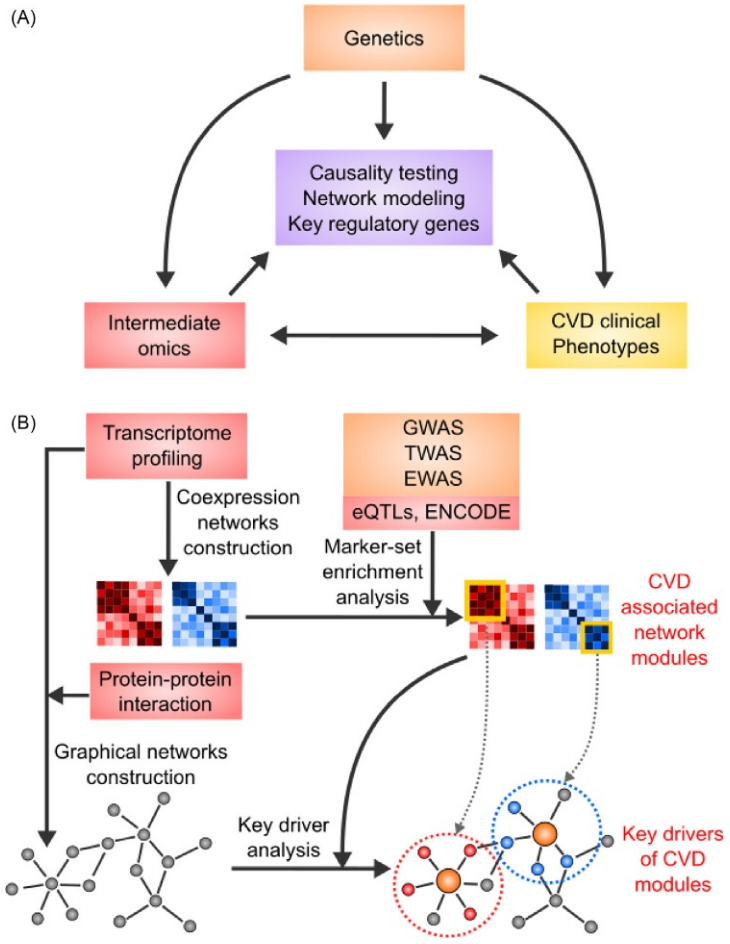

Fig. 3 Fundamentals of multiomics data integration for CVD. (A) A systems biology strategy that integrates different layers of big data relevant to CVD. Genetics causally drives the changes in intermediate omics (e.g., epigenome, microbiome, proteome, etc.) and CVD clinical phenotypes. Pairwise relationship between different data types can be retrieved. This information can further enable systems-level integration through approaches such as causality testing, network modeling, and identification of key regulatory genes. (B) Mergeomics, a multiomics data integration framework to study CVD. First, transcriptomic profiling data is used to construct CVD-related coexpression networks. This can lead to the identification of CVD associated network modules by leveraging different types of omics-wide association, eQTLs, and ENCODE data using the Marker-set Enrichment Analysis. On the other hand, transcripts and protein level interaction data can be used to generate biologically relevant graphical networks. Interrogating networks using the Key Driver Analysis helps reveal key drivers of CVD modules that serve as candidates for downstream investigation. *GWAS*, genome-wide association study; *TWAS*, transcriptome-wide association study; *EWAS*, epigenome-wide association study.

determine which intermediate markers are likely causal for the disease. Lastly, biological pathways and molecular networks that are informed by the multiomics datasets are retrieved and differentiated into causal and reactive pathways and networks based on genetic information and causal inference. The causal pathways and networks can be further analyzed to retrieve key regulatory genes that are central and connect with a large number of disease genes in the network organization. These predicted regulatory genes, once mechanistically tested and validated, may serve as more effective drug targets. The advantage of this integrative framework not only utilizes data patterns but also explicitly considers the intrinsic biological relationships between omics data types.

Multiomics integration tools

Although methods and tools for individual components of the above described workflow have been developed, convenient pipelines or tools for multiomics integration are sparse at present. The limited number of multiomics integration tools mostly target cancer research, such as iCluster. iCluster implements a joint latent variable model to facilitate clustering of different types of data and it has been applied to integrating mutation, CNV, DNA methylation, and gene expression data to determine tumor subtypes in glioblastoma (Shen et al., 2012). This type of tool mainly uses data patterns and does not consider the intrinsic biological relationships between data types and may limit mechanistic discoveries. To facilitate more general applications of multiomics integration in various disease areas and enable mechanistic insights, we have recently developed a flexible and convenient computational pipeline Mergeomics, both as an R package (Shu et al., 2016) and as a user-friendly web server (Arneson et al., 2016), to implement the integration workflow described above and to identify disease networks, pathways, and key regulators that are informed by multiomics data sets (**Fig. 3B**). Mergeomics takes as input (1) summary level marker-disease association information from genome-, transcriptome-, epigenome-, proteome-, and metabolome-wise association analysis, (2) functional relationship or causal information between omics markers and genes, (3) membership of genes in pathways or molecular networks, and (4) molecular network topology information. The outputs include pathways and networks associated with a disease and a list of prioritized potential regulatory genes; when genetic information is given, the genes, pathways, and networks are putatively causal.

Application of multiomics data integration in CVD

A number of pioneering studies published in recent years have exploited the power of multidimensional data integration in understanding the etiology of CVD. Earlier multiomics studies in CVD were mainly conducted in mouse F2 crosses with genomics and transcriptomics as the main data types (Wang et al., 2007; Yang et al., 2010). More recently the data types have been expanded to epigenomics, metabolomics, proteomics, and microbiota in the hybrid mouse diversity panel (HMDP) comprised of ~100 inbred and recombinant inbred mouse strains (Bennett et al., 2010; Parks et al., 2013; Bennett et al., 2015). Multiomics studies in human populations are also emerging (Huan et al., 2015b; Inouye et al., 2010; Inouye et al., 2012; Talukdar et al., 2016; Franzen et al., 2016). For example, taking advantage of genetic and gene expression data from relevant tissue samples of CAD patients, the Björkegren group (Franzen et al., 2016; Talukdar et al., 2016) constructed regulatory gene networks and identified both CAD-causal subnetworks and key drivers. The relationship between key drivers and CAD related phenotypes was further prioritized and validated using HMDP and siRNA knockdown. They also revealed a tissue-specific expression pattern that is dependent on the CAD genetic susceptibility of participants.

As direct access to internal tissues relevant to CVD is not always possible in human studies, integration of multidimensional omics data across studies and species has also been extensively explored over the past years (Makinen et al., 2014; Zhao et al., 2016). For example, using CAD GWAS from the CARDIoGRAM study and tissue-specific transcriptomics, eQTLs, and gene networks from a multitude of studies, Makinen et al. captured an array of CAD associated pathways and networks (Makinen et al., 2014). These pathways/modules are projected onto tissue-specific Bayesian networks to identify novel CAD key regulators. In another study by Zhao et al., CAD GWAS, transcriptomic profiling in CAD patients and controls, and network modeling were used to unravel tissue-specific relations among the CAD candidate genes at top CAD loci (Zhao et al., 2016).

Considerations, Challenges and Remaining Gaps

Special Statistical and Computational Considerations of Omics Data

Multiple testing correction

One key feature common to all omics platforms is the large number of variables measured (e.g., millions of SNPs in genomics and tens of thousands of genes in transcriptomics). Consequently, the number of statistical tests performed within each omics level is large, which dramatically increases the random chance for spurious positive results. This phenomenon is called "multiple testing," and correcting for multiple testing is critical for determining the appropriate statistical cutoffs that minimize false discoveries. A number of statistical methods have been developed for multiple testing correction. Among these, the most convenient approach is Bonferroni correction, which is simply the desired p value cutoff (e.g., $p<0.05$) divided by the number of tests. For instance, $p<5\times10^{-8}$ is usually the cutoff used in GWAS to determine genome-wide significance of genetic loci, which is calculated by 0.05/(1 million tests). However, Bonferroni correction is likely too stringent as it assumes all performed statistical tests are independent, which ignores the intrinsic nonindependent relationships between molecular markers. Popular alternatives include the Benjamini-Hochberg false discovery rate (Benjamini and Hochberg, 1995), q-value (Storey and Tibshirani, 2003) and permutation test.

Data dimension reduction

Another consideration that is related to the large numbers of testable features and the multiple testing issue is to leverage dimension reduction techniques to reduce high dimensional data comprised of hundreds, thousands, or even millions of features into fewer dimensions based on coordinated behaviors of groups of molecular features. Principal component analysis (PCA) is the most commonly used approach for easy visual identification of outliers and batch effects and summarizing group behaviors (Nyamundanda et al., 2010). PCA is conducted by projecting features onto a set of orthogonal components which are arranged in decreasing order of variance explained using an eigenvalue decomposition. However, it can often be difficult to visualize all relevant combinations of principal components as only two dimensions can be visualized at once. The t-distributed stochastic neighbor embedding (*t*-SNE) is another useful dimensionality reduction technique which leverages the Kullback-Leibler divergence when only two dimensions are desired for visualization (van der Maaten and Hinton, 2008). As an alternative to PCA, partial least squares regression (Helland, 1988; Höskuldsson, 1988) is a supervised method based on correlation that fits a regression model to the features which have been projected to a new space and generates weights for each of the features. Other unsupervised learning methods, like hierarchical clustering, can leverage a "bottom up" or "top down" approach to decide whether clusters should be joined or split based on the chosen distance metric to find related features (Johnson, 1967). However, if the goal is not to group related features, random forests method (Breiman, 2001) allows for the most informative features to be selected for a particular attribute of interest. Therefore, these dimension reduction methods can not only detect and partition features that are statistically or functionally related, but can help detect data irregularities and alleviate the multiple testing burden.

Challenges and Remaining Gaps

As of September 2016, searching "CVD" in GEO returns ~350 unique datasets involving more than 30,000 samples and 200 platforms. Despite the rapid advances of bioinformatics, there are still challenges and gaps in tackling the formidable amount and complexity of CVD data. First, due to the imbalance of data availability of different omics types, most mature tools with robust performance were designed for genomics and array-based transcriptomics analyses. For the other data types, the availability and performance of methods and tools are highly variable. Therefore, better performing algorithms and methods are still in great need for newer data types such as RNA-seq. Second, while novel data analytic and mining approaches are being released on a daily basis, few of them adequately take intrinsic biological properties of data into consideration when designing algorithms. This could lead to false discoveries and artifacts even if the technical implementation is valid. Third, current bioinformatics efforts still mainly focus on methodologies specific to individual types of data, and analytical frameworks and tools that could effectively incorporate different data types and perform multiomics data integration are still limited. Fourth, algorithms and tools that can examine between-tissue interactions and conduct dynamic modeling of big data are lacking. Lastly, while the CVD community has collectively generated rich data and numerous published studies, this information is scattered and there is currently no comprehensive bioinformatics database that curates CVD-related multiomics datasets or platforms that summarize the biological findings from bioinformatics studies of CVD to allow queries of the valuable biological insights. More coordinated efforts are needed from the CVD research community to collectively improve the bioinformatics implementation in this promising area.

Conclusions and Future Directions

Owning to the rapid advances of modern molecular technologies, we are now moving towards realizing a systems-level understanding of CVD. Bioinformatics has played an indispensable role in this process. First of all, bioinformatics has proven to be invaluable in quality assessment and preprocessing of large-scale omics data into reliable and analyzable molecular profiles with majority of the quality issues, artifacts, and confounders removed or corrected. In addition, bioinformatics employs sophisticated statistical, mathematical, and computational modeling algorithms and techniques to reduce the complexity of data and retrieve biologically relevant molecular patterns at each omics level. Furthermore, given the systemic nature of CVD, bioinformatics enables sophisticated modeling frameworks that integrate different levels of omics data in a tissue-specific and biologically meaningful manner for comprehensive and novel mechanistic insights. Ultimately, whether researchers can effectively and properly utilize bioinformatics methods will determine how much hidden value can be eventually realized from multimillion dollar datasets.

For the CVD field, a main future direction of bioinformatics lies in facilitating the translation of the basic understanding of CVD into clinically relevant applications to improve the precision of diagnostics and therapeutics. In this regard, bioinformatics can disseminate both mechanistic insights and molecular biomarkers obtained from the omics studies through well-designed databases or query systems. This type of effort can enable more effective utilization of available data and avoid a waste of resources. Bioinformatics can also facilitate the design of diagnostic biomarker panels based on molecular patterns of CVD subtypes to improve diagnostic accuracy and enable personalized medicine. Moreover, bioinformatics can help predict novel drugs or drug targets and reposition existing drugs based on matching molecular patterns or mechanisms between CVD and drugs. Despite these promises, it is important to note that bioinformatics findings can be easily questioned for their robustness and accuracy. Thus it is imperative to push for more in-depth multidisciplinary collaborations between bioinformaticians and researchers on the bench and clinical sides, in order to speed up the validation and translation processes and fully realize the potential of bioinformatics in mechanistic discovery and translational medicine for CVD.

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Biomarkers in Heart Failure, Use of

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Introduction

Heart failure (HF) is defined as a "complex clinical syndrome that results from any structural or functional impairment of ventricular filling or ejection of blood" (Yancy et al., 2013). HF remains a growing epidemic and currently affects greater than 5 million people in the United States alone. Greater than 650,000 cases are diagnosed each year, and the incidence increases with advancing age. Furthermore, mortality remains high with 50% passing away within 5 years of diagnosis (Yancy et al., 2013). HF can be classified as HF with reduced ejection fraction (HFrEF) in patients who have an EF of $\leq$40% and HF with preserved ejection fraction (HFpEF) in those with an EF of $\geq$50%; those with EF values in between are considered to have borderline HFpEF (Yancy et al., 2013). Although HFrEF has multiple, evidence-based treatment options, therapies for HFpEF are still lacking.

Given this significant burden of disease as well as the complexity of the syndrome of HF, instruments to supplement the information ascertained from clinical history and physical exam are warranted. Biomarkers are useful tools to assist with diagnosis, risk stratification, and management of HF, and recently there has been an explosion in the discovery of new biomarkers as well as the volume of biomarker research (Januzzi and Felker, 2013). **Fig. 1** shows various established and emerging biomarkers.

Criteria for what constitutes a useful biomarker have been proposed by Morrow and de Lemos and are as follows: (a) to provide accurate and repeated measurements with short turnaround times, (b) to provide additional information beyond what can be ascertained from a thorough clinical assessment, and (c) to use results to aid in making clinical decisions (Morrow and de Lemos, 2007). Subsequent to these, Januzzi and van Kimmenade recommended the following criteria specific for HF biomarkers: (a) have been evaluated in a wide range of patients characteristic of the diagnosis with rigorous statistical methods; (b) can be easily, quickly, and accurately measured with defined biological variability and low analytical imprecision of the assay; (c) demonstrate an important component of HF pathophysiology, if extracardiac independently useful information for diagnosis, prognosis, disease progression, or therapy should be contributed; and (d) provide clinically useful information for caregivers and patients to assist in diagnosis, risk stratification, and management by adding to what can already be determined by standard clinical and laboratory data (van Kimmenade and Januzzi, 2012).

To date, only the natriuretic peptides (NP) fulfill the aforementioned criteria, and they remain the gold standard HF biomarker; however, there are multiple emerging biomarkers that are also gaining relevance. This article serves to review the use of the NPs as well as other promising biomarkers in the diagnosis, risk stratification, and management in patients with HF.

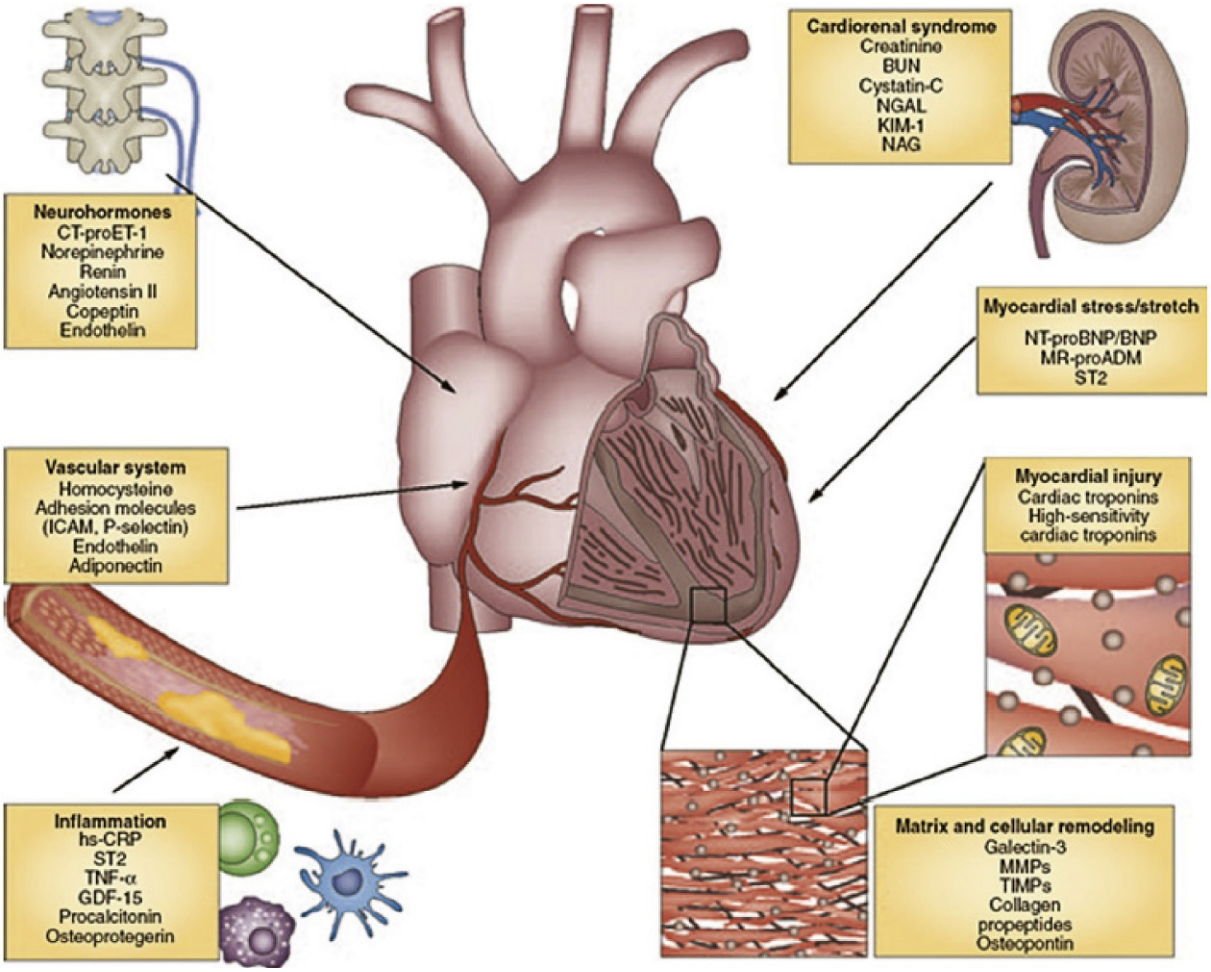

Fig. 1 Pathophysiologic representation of the multiple established and emerging biomarkers in heart failure. Reproduced with permission Gandhi, P. U., Testani, J. M., Ahmad, T. (2015). The current and potential relevance of heart failure biomarkers. *Current Heart Failure Reports* **12**, 318–327.

Natriuretic Peptides

Discovery and Biology

The NPs were first discovered in 1981 when de Bold identified a substance, which was later identified to be atrial natriuretic peptide (ANP), from rat atrial tissue that resulted in an increase in urine volume, excretion of sodium, potassium, and chloride, as well as reduction in arterial blood pressure (de Bold et al., 1981). Subsequently in 1988, B-type natriuretic peptide (BNP) was isolated from porcine neural tissue and was found to have similar physiologic effects (Sudoh et al., 1988). BNP was later discovered in both atrial and ventricular myocytes, identifying the heart as its primary source.

The primary trigger for release of both ANP and BNP is myocardial wall stress, but the hormones differ in how they are stored and secreted. ANP is produced predominantly in atrial cardiomyocytes and is stored as a prohormone that is subsequently cleaved to produce a biologically inert fragment and active ANP. The latter has a very short half-life, and therefore it is difficult to measure clinically (Munagala et al., 2004). Small amounts of BNP are stored in both atrial and ventricular myocytes, but the majority of BNP is produced by ventricular myocytes due to their greater mass. After gene activation, initially a 134 amino acid prepropetide is produced; this prepropetide is cleaved into $proBNP_{1\text{-}108}$ and a 26-amino acid signal peptide. At the time of secretion, $proBNP_{1\text{-}108}$ is subsequently further cleaved by corin into BNP and amino-terminal proBNP (NT-proBNP) (Munagala et al., 2004).

Although synthesized by the same pathway, BNP and NT-proBNP have important differences. Notably, BNP is biologically active, exerting multiple systemic effects such as vasodilation, diuresis, natriuresis, increased renal perfusion, as well as antifibrotic effects on the myocardium, whereas NT-proBNP is biologically inert. Additionally, the half-life of BNP is 22 min, whereas the half-life of NT-proBNP is 120 min (Motiwala and Januzzi, 2013). There are also significant differences in their numerical concentrations with NT-proBNP typically five to eight times higher than BNP, though there is no direct conversion (Heil and Tang, 2015).

Diagnosis

The application of BNP for the diagnosis of acutely decompensated HF (ADHF) was established in the landmark Breathing Not Properly trial. Approximately 1500 patients presenting to the emergency department with dyspnea were included in the study and BNP measurement was obtained upon presentation. The diagnosis of ADHF was subsequently evaluated by two cardiologists, who were blinded to the BNP results; 47% of the patients were determined to have dyspnea secondary to ADHF. Taking into consideration multiple historical and physical exam findings, a BNP concentration of $\geq$100 pg/mL was the most accurate characteristic for diagnosing HF with an odds ratio (OR) of 29.6 ($P<0.001$), sensitivity of 90%, and specificity of 73% (Maisel et al., 2002).

Similarly, the use of NT-proBNP in the assessment of dyspnea in 599 patients was examined in the ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) study. An NT-proBNP concentration <300 pg/mL had a 99% negative predictive value for ruling out the diagnosis of ADHF. Additionally, elevated NT-proBNP concentration was the strongest independent predictor of the diagnosis of HF with a sensitivity of 90%, specificity of 85%, and an OR of 44 ($P<0.0001$) (Januzzi et al., 2005). An NT-proBNP cut-point of 900 pg/mL was comparable to a BNP cut-point of 100 pg/mL, but an age-stratified approach improved the positive predictive value of NT-proBNP; therefore, the following cut-points for NT-proBNP were established for use in ADHF: 450 pg/mL for age <50 years, 900 pg/mL for age 50–75 years, and 1800 pg/mL for age >75 years (Januzzi et al., 2006b). **Table 1** shows the various cut-points for diagnosis of HF using both single and multiple cut-point strategies (Januzzi et al., 2006b; Kim and Januzzi, 2011).

Although the NPs hold strong diagnostic value, use of biomarkers to diagnose HF should always be used in conjunction with clinical findings, similar to application of any other diagnostic tool. A significant pitfall of the NPs is their lack of specificity, as there are several other conditions that can also increase or decrease their concentrations (**Table 2**) (Daniels and Maisel, 2007; Yancy et al., 2013). Many of these conditions are often comorbidities associated with HF, further complicating interpretation. Therefore, it is of utmost importance to use biomarkers as another tool in our armamentarium to supplement clinical information and assist with diagnosis. Concentrations may also fall in between the cut-points for ruling out and ruling in HF or the "gray zone," as shown in **Table 1**. This occurs in approximately 20 percent of patients presenting to the emergency department, and in these patients clinical correlation is even more important to determine the underlying etiology of elevated NP concentration and arrive at the correct diagnosis (van Kimmenade et al., 2008).

Although the NPs are most frequently used to aid in the diagnosis of ADHF, they have also been investigated to assist with diagnosis of chronic HF in the ambulatory setting. In the outpatient setting, their negative predictive value to exclude the diagnosis of HF has been the most widely studied application for both BNP and NT-proBNP. Values to exclude HF are considerably lower in this patient population given that they are not as acutely ill as those with ADHF. In an asymptomatic patient, the cut-point of BNP is <20 pg/mL and in a symptomatic patient the cut-point is <40 pg/mL. For NT-proBNP, the cut-points remain age-stratified, as shown in **Table 1** (Hildebrandt and Collinson, 2008; Rehman and Januzzi, 2008). If concentrations above these cut-points are resulted, a cardiac work-up should be initiated.

Based on these data, the NPs have significant diagnostic value in HF when measured in patients presenting with dyspnea, and they have been assigned a Class I, level of evidence A recommendation by the ACC/AHA for use in the diagnosis of HF (Yancy et al., 2017).

Table 1 Cut-points for B-type natriuretic peptide (BNP) and amino-terminal pro-B-type natriuretic peptide (NT-proBNP) (Hildebrandt and Collinson, 2008; Januzzi et al., 2006; Kim and Januzzi, 2011; Rehman and Januzzi, 2008).

"Rule Out" acutely decompensated heart failure	
BNP	<30–50 pg/mL
NT-proBNP	<300 pg/mL
"Rule In" acutely decompensated heart failure	
Single cut-point strategy	
BNP	<100 pg/mL
NT-proBNP	<900 pg/mL
Multiple cut-point strategy	
BNP, gray zone approach	<100 pg/mL to exclude, 100–400 pg/mL "gray zone," >400 pg/mL to rule in
NT-proBNP, age-stratified approach	<450 pg/mL for age <50 years, <900 pg/mL for age 50–75 years, <1800 pg/mL for age >75 years
Ambulatory care	
BNP	20 pg/mL (asymptomatic) or 40 pg/mL (symptomatic)
NT-proBNP	<125 pg/mL for age <75 years or <450 pg/mL for age >75 years OR <50 pg/mL for age <50 years, <75 pg/mL for age 50–75 years, and <250 pg/mL for age >75 years

Table 2 Causes of elevated and reduced natriuretic peptide concentrations (Daniels and Maisel, 2007; Yancy et al., 2013).

Elevated concentrations	*Reduced concentrations*
Cardiac	
Heart failure, involving right or left ventricle	Heart failure occurring anatomically upstream from the left ventricle
Acute coronary syndrome	Flash pulmonary edema
Atrial fibrillation or flutter	Pericardial constriction
Valvular heart disease	Pericardial tamponade
Myocarditis	
Cardiac surgery	
Cardioversion	
Noncardiac conditions	
High-output conditions	Obesity
Critical illness	
Anemia	
Older age	
Renal Failure	
Pulmonary disease (cor pulmonale, acute respiratory distress syndrome)	
Pulmonary embolism	

Prognosis

In addition to their use in the diagnosis of HF, the NPs possess substantial prognostic value. In patients with ADHF, a BNP concentration of >200 pg/mL was found to be predictive of future HF events and mortality over 90 days when compared to those with a BNP <200 pg/mL (29% versus 9%; $P=0.006$) (Maisel et al., 2004). NT-proBNP is similarly prognostic in those with ADHF. Data from the PRIDE study revealed that an NT-proBNP concentration >986 pg/mL upon presentation to the emergency department was the strongest predictor of mortality at 1 year with a hazard ratio of 2.88 in adjusted analysis (Januzzi et al., 2006a). Gray zone concentrations are also prognostic, and it is important to note that patients with NP concentrations in this range have a worse prognosis than those who have truly negative NP concentrations (van Kimmenade et al., 2008).

Although baseline NP concentrations have established prognostic merit in ADHF, subsequent studies have demonstrated even greater prognostic value for follow-up measurements, particularly those measurements obtained prior to discharge (Kociol et al., 2011). A predischarge BNP concentration of <350 pg/mL was associated with significantly reduced rates of HF readmission or death over 180 days of follow-up (Logeart et al., 2004). Other studies have examined a percent reduction in NT-proBNP over the course of hospitalization and have found that variation in NT-proBNP concentration is a significant predictor of future HF admission and death (Bettencourt et al., 2004). Furthermore, a less than 50% reduction in NT-proBNP concentration is associated with a 57% increase in risk of readmission or death compared with those who have a greater than 50% reduction in a multivariable analysis (Michtalik et al., 2011). The ACC/AHA guidelines have recently assigned a class IIa recommendation to obtain a predischarge NP value to establish post-discharge prognosis (Yancy et al., 2017).

NPs also are considerably prognostic in the chronic HF setting. In a systematic review, each 100 pg/mL increase was associated with a 35% increase in the relative risk of mortality (Doust et al., 2005). There have also been multiple studies that portray the significance of serial measurements over time in patients with chronic HF. In patients with HFrEF who were symptomatic but stable from the Val-HeFT study, changes in BNP concentrations over 12 months revealed that those who had concentrations in the lowest quartile (relative to a median BNP concentration of 97 pg/mL) had the best prognosis, followed closely by those who began with high concentrations that decreased to the low range. Those who either had concentrations in the highest quartile over the entire 12-month period or those who had low concentrations that increased from the lowest to the highest quartile over the 12 months had the worst prognosis (Latini et al., 2006). Similar results were found with NT-proBNP (Masson et al., 2008). Therefore, the ACC/AHA guidelines have given a class I, level of evidence A recommendation for use of NPs in prognostication of HF or to establish disease severity in chronic HF (Yancy et al., 2017).

Management

Treatment of HF is complex and multifaceted including pharmacologic, device-based, and lifestyle-based therapies. The NPs have an established role in the diagnosis and risk stratification of patients with HF, raising the consideration that their concentrations may be useful in managing HF as well. Furthermore, multiple established HF therapies have been shown to reduce NP concentrations with improved effects on clinical characteristics including hemodynamics and filling pressures (Motiwala and Januzzi, 2013).

These therapies include loop diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers, aldosterone antagonists, and even nonpharmacologic therapies such as cardiac resynchronization therapy and exercise (Motiwala and Januzzi, 2013).

In patients with ADHF, the mainstay of therapy is decongestion with use of diuretic therapy. While diuretics result in a decrease in NP concentrations, it is unclear whether using NP concentrations would assist in specifically guiding therapy. Studies have been conducted to evaluate this question (Stienen et al., 2014) but have not yet been published. Therefore, evidence is currently insufficient to recommend use of NP-guided therapy in the inpatient setting (Yancy et al., 2013). However, use of NP concentrations in the diagnosis of HF has been shown to improve triage, reduced cost, and shorten intensive care unit stays (Boldanova et al., 2010; Moe et al., 2007).

Use of NPs to guide therapy in ambulatory HF has been studied dating back to 2000 with the seminal study by Troughton et al. which demonstrated a significant improvement in outcomes when a goal of lowering NP concentrations was used in addition to standard of care compared to standard care alone (Troughton et al., 2000). Since then, approximately 10 studies including a total of ~2500 patients have been conducted to further investigate this concept, but the results have been variable primarily due to heterogeneous study design, differences in follow-up and medication titration between study arms, and variability in both selection and achievement of an NP target. Positive studies were more likely to demonstrate significant treatment differences between study arms and achieve lower NP concentrations when compared to studies that were negative or neutral (Troughton et al., 2014a).

While current evidence is limited due to the aforementioned caveats, the trials that revealed a significant benefit with a biomarker-guided approach have demonstrated important advantages including uptitration of guideline-based neurohormonal therapies (Motiwala and Januzzi, 2013), enhanced quality of life (Bhardwaj et al., 2012), and reduction in left ventricular size with improved left ventricular function (Weiner et al., 2013). This strategy has also been found to be cost-effective (Sanders-van Wijk et al., 2013) and safe (Gandhi et al., 2015b). Furthermore, meta-analyses have been conducted which have shown an approximate mortality benefit of 25%–30% with the application of NP-guided HF management (Felker et al., 2009; Porapakkham et al., 2010; Savarese et al., 2013). More recently, an individual patient meta-analysis was conducted, revealing approximately a 40% reduction in all-cause mortality in those younger than 75 years of age; however, no significant benefit was found in those over the age of 75 (Troughton et al., 2014b). In order to definitively investigate the strategy of NP-guided management of ambulatory HF, a large, multicenter, randomized, controlled trial (Guiding Evidence-Based Therapy Using Biomarker Intensified Treatment in HF, GUIDE-IT, NCT#01685840) was conducted (Felker et al., 2014). However, this study was terminated early due to lack of difference in the primary outcome between treatment arms. The results have not yet been published, but are likely to be informative. Substudy analyses are currently underway. (Chow et al., 2017)

In addition to using NPs to guide therapy in patients with established HF, there has been recent interest in employing NP concentrations to assist with managing patients at risk for HF with the goal of prevention. One study demonstrated a substantial reduction in the development of clinical HF or left ventricular dysfunction with intensified diagnostic testing and treatment in patients with a BNP >50 pg/mL (Ledwidge et al., 2013).

Finally, the concept of using biomarkers to create novel therapies has also been exemplified by the NPs with the development of the medication valsartan/sacubitril (LCZ696), which is a combination of the angiotensin receptor blocker valsartan and a neprilysin inhibitor sacubitril. Neprilysin is a neutral endopeptidase that degrades multiple proteins including the NPs. Inhibition of neprilysin subsequently increases the concentrations of these beneficial proteins, counteracting vasoconstriction, sodium retention, and remodeling induced by other neurohormones (McMurray et al., 2014). The PARADIGM-HF trial demonstrated a reduction in mortality and HF hospitalization in patients with symptomatic ambulatory HFrEF who were receiving LCZ696 compared with those who received the angiotensin-converting enzyme inhibitor enalapril (McMurray et al., 2014).

Preserved EF

Patients with HFpEF tend to have lower NP concentrations compared to patients with HFrEF (van Veldhuisen et al., 2013). Although there have been some data to suggest that the sensitivity may be slightly reduced for both BNP and NT-proBNP to assist with diagnosing HF (Maisel et al., 2003; O'Donoghue et al., 2005), the concentrations do retain similar prognostic value regardless of ejection fraction (van Veldhuisen et al., 2013). Additionally, in patients with an ejection fraction of >45%, NT-proBNP was found to be one of the strongest independent variables for all-cause mortality (Komajda et al., 2011). Use of NPs to guide management in patients with HFpEF remains uncertain, as only a small percentage of patients with HFpEF were included in clinical trials (Troughton et al., 2014b) and guideline-based therapies for HFpEF are yet to be established.

Troponin

Biology

The troponins are proteins that help regulate contraction of both cardiac and skeletal muscle. The cardiac troponin unit is composed of troponin I, troponin T, and troponin C. Troponin I and troponin T are found in two locations in the cardiomyocyte: a small percentage can be found in the cytoplasmic pool, but the majority is located in the contractile apparatus. Troponin is most commonly used to detect cardiomyocte injury in the setting of suspected acute coronary syndrome, for which it is highly sensitive and specific (Thygesen et al., 2012). The sensitivity of troponin assays continues to increase and the most current generation of

high-sensitivity assays are employed in multiple parts of the world, and have recently been approved in the United States. In addition to their application for acute coronary syndrome, elevated troponin concentrations can also be found in HF for which multiple proposed etiologies exist, including cardiomyocyte necrosis due to increased wall stress, epicardial coronary artery disease, or even direct injury from neurohormones and inflammatory markers (Januzzi et al., 2012; Kociol et al., 2010).

Diagnosis

Troponin is not used to diagnose HF, but acutely decompensated HF can be present in the setting of an acute coronary syndrome. If an elevated troponin concentration is found, it is imperative to determine the etiology as it may drastically change management, particularly if the elevated concentration is due to a type I myocardial infarction involving plaque rupture (Januzzi et al., 2012).

Prognosis

Although troponin does not possess a significant role for diagnosis of HF, troponin measurement has established utility for prognosis. The ACC/AHA guidelines have assigned a class I recommendation in acutely decompensated HF and class IIb for chronic HF for the purpose of prognostication (Yancy et al., 2017). In patients hospitalized with acutely decompensated HF from the Acute Decompensated HF National Registry (ADHERE) database, 6.2% were found to have a troponin concentration that was deemed positive by a conventional assay. Those with an elevated concentration had a significantly higher rate of in-hospital mortality compared to those without an elevated concentration (8.0% versus 2.7%, $P < 0.001$) (Peacock et al., 2008). With high-sensitivity assays, nearly all patients with acute HF have measurable concentrations (Pascual-Figal et al., 2012), and higher concentrations are independently associated with worse prognosis, including cardiovascular mortality or death from HF (Felker et al., 2015).

In patients with chronic HF from the Valsartan HF Trial (Val-HeFT), troponin was detected in 10.4% and 92% of the patients using both a conventional and highly sensitive assay, respectively. Elevated concentrations were found to be predictive of mortality and HF hospitalization; moreover, addition of highly sensitive troponin improved prognostication in risk prediction models (Latini et al., 2007). Finally, serial measurements of high-sensitivity troponin were also examined for prognostic value, and increasing concentrations over time were associated with an increase in all-cause mortality (Masson et al., 2012).

Management

To date, no studies have specifically investigated the use of troponin to manage HF. However, troponin concentrations have been measured in various therapy trials and have found increased concentrations with use of omecamtiv mecarbil (Teerlink et al., 2016) and decreased concentrations with serelaxin (Metra et al., 2013) and valsartan/sacubitril (LCZ696) (Packer et al., 2015), but the significance of these changes remains unclear until further investigations are undertaken.

Preserved EF

Similar to the NPs, concentrations of troponin tend to be lower in those with preserved EF compared to those with reduced EF (Santhanakrishnan et al., 2012), and troponin concentrations appear to have similar prognostic value in both types of HF (Sanders-van Wijk et al., 2015). Furthermore, in patients admitted with acutely decompensated HFpEF, troponin elevation was recently found to be independently associated with worse in-hospital and postdischarge outcomes (Pandey et al., 2016). With regard to treatment, valsartan/sacubitril (LCZ696) was also shown to reduce high-sensitivity troponin concentration in patients with HFpEF (Jhund et al., 2014).

ST2 and Galectin-3

Biology

Suppression of tumorigenicity 2 (ST2) and galectin-3 are emerging HF biomarkers. ST2 is a member of the IL-1 receptor family, and it has multiple forms including a transmembrane ligand (ST2L) and a soluble form (sST2) (Weinberg et al., 2002). It is a marker of myocardial strain (Weinberg et al., 2003), fibrosis, and remodeling (Sanada et al., 2007), and expression of the ST2 gene is triggered in response to myocyte and fibroblast strain (Weinberg et al., 2002). Galectin-3 is a member of the β-galactoside-binding lectin family that is found in macrophages and fibroblasts. It is associated with fibrosis of many organs, including the heart (Sharma et al., 2004).

Diagnosis

ST2 and galectin-3 are not useful to assist with diagnosis of HF.

Prognosis

Both sST2 and galetcin-3 have been investigated for risk stratification in acute and chronic HF. In patients presenting with shortness of breath in the PRIDE study, sST2 was significantly elevated in those with acutely decompensated HF compared to those with

shortness of breath from other causes. Additionally, elevated sST2 was associated with an increased risk of mortality in adjusted analyses. Combination of sST2 with NT-proBNP identified subjects with the highest mortality risk (Januzzi et al., 2007). Another study examined multiple biomarkers in over 5000 patients with acutely decompensated HF, and sST2 was found to be among the strongest predictors of mortality when combined with clinical factors of risk prediction (Lassus et al., 2013).

Galectin-3 has also been investigated for prognosis in acutely decompensated HF. In data from a recent pooled analysis of three studies examining patients with acutely decompensated HF, elevated galectin-3 concentrations were associated with increased rehospitalization and mortality at 30, 60, 90, and 120 days after discharge. After adjustment for relevant clinical factors and NP concentrations, galectin-3 remained a useful predictor of HF rehospitalization (Meijers et al., 2014).

The prognostic utility of both sST2 and galectin-3 has also been investigated in patients presenting with chronic HF. Soluble ST2 was investigated in 1,114 patients with ambulatory HF, and patients with sST2 concentrations in the highest tertile had an increased risk of adverse events (adjusted HR 1.9, $P=0.002$) compared to those in the lowest tertile. When combined with NT-proBNP and added to a conventional risk model (Seattle Heart Failure Model), 15% of patients were reclassified into different risk categories (Ky et al., 2011). Serial sST2 concentrations were found to possess even stronger prognostic merit when compared to other markers, including NT-proBNP and high-sensitivity troponin T (Gaggin et al., 2014).

Multiple studies have investigated the utility of galectin-3 for risk stratification in chronic HF. In a substudy of the HF: A Controlled Trial Investigating Outcomes of Exercise TraiNing (HF-ACTION) trial, elevated galectin-3 concentrations were associated with reduced HF hospitalization-free survival, but its prognostic value was not retained when adjusted for other clinical factors, particularly with inclusion of NP concentrations (Felker et al., 2012). Similar results have been found with other studies, and subsequently a head-to-head trial was conducted comparing sST2 with galectin-3 which demonstrated its modest prognostic value when compared to sST2 (Bayes-Genis et al., 2014). However, a recent meta-analysis that investigated patients with both acute and chronic HF revealed that a 1% increase in circulating galectin-3 concentrations was associated with a 28% increase in all-cause mortality and a 59% increase in cardiovascular mortality in models including both renal function and NT-proBNP (Chen et al., 2015). The ACC/AHA guidelines have assigned a class IIb recommendation for measurement in acutely decompensated HF and symptomatic chronic HF to assist with prognosis for both sST2 and galectin-3 (Yancy et al., 2017).

Management

Although there have been no prospective studies examining sST2 or galectin-3 to guide management, there have been a number of important retrospective observations. There appears to be a reduction in sST2 concentrations with use of beta-blocker therapy (Gaggin et al., 2013) as well as with the angiotensin receptor blocker valsartan (Anand et al., 2014). Additionally, another study found an interaction between elevated sST2 concentrations and mineralocorticoid receptor antagonist therapy (Maisel et al., 2014). Similar observations have been found for galectin-3, but interestingly those with lower (rather than elevated) galectin-3 concentrations seem to benefit from treatment with rosuvastatin (Gullestad et al., 2012) or valsartan (Anand et al., 2013). Although experimental models have suggested a potential relationship between galectin-3-mediated fibrosis and aldosterone (Calvier et al., 2013), clinical studies have not found a significant therapeutic interaction (Gandhi et al., 2015a). Finally, targeted anti-galectin therapies are also being investigated in experimental models and have demonstrated attenuation of fibrosis (Yu et al., 2013). At this time, more evidence is needed before sST2 or galectin-3 can be applied to guide HF management.

Preserved EF

Data regarding sST2 in HFpEF appear to be conflicting. One study investigating patients with acutely decompensated HF and preserved EF did not show predictive value with regard to outcomes at 6 months (Frioes et al., 2015), whereas another study showed sST2 was an independent predictor of mortality in patients with acutely decompensated HF regardless of EF (Manzano-Fernandez et al., 2011). Galectin-3 has also been examined in patients with HFpEF, and it was found to be independently prognostic in this patient population for mortality and HF hospitalization (de Boer et al., 2011). Furthermore, the aforementioned pooled analysis included subjects with HFpEF and also showed elevated galectin-3 concentrations were associated with risk of short-term hospitalization in these subjects as well (Meijers et al., 2014).

Future Directions

The number of biomarkers that are being investigated continues to rapidly expand (Januzzi and Felker, 2013) raising questions regarding the optimal method to investigate and apply biomarkers to patient care. To date, the NPs remain the gold standard and have truly exemplified how a biomarker can aid in diagnosis, prognosis, management, and development of novel therapies. Given that each biomarker provides a different window into the pathophysiology of HF, it is possible that a multimarker strategy may be the most optimal approach (Allen and Felker, 2010). One study examined a multimarker panel (BNP, high-sensitivity C-reactive protein, myeloperoxidase, soluble fms-like tyrosine kinase receptor-1, troponin I, soluble toll-like receptor 2, creatinine, and uric acid) incorporating a variety of biological pathways in 1513 patients with HFrEF for risk stratification and found that addition of this panel to the Seattle HF model resulted in a significant improvement in prognostication (Ky et al., 2012). Prior to clinical adaptation of a multimarker strategy, demonstration of incremental benefit beyond existing clinical information and risk prediction models, therapeutic implication, and cost-effectiveness is imperative (Allen and Felker, 2010).

Conclusion

The use of biomarkers in HF is a rapidly expanding area of interest. The NPs remain at the forefront of this field with troponin, sST2, and galectin-3 rapidly approaching. Future studies will assist in clarifying the appropriate use of both established and emerging markers for assisting in diagnosis, risk stratification, and management of this complex condition.

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Biomarkers in Ischemic Heart Disease

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Introduction

Ischemic heart disease (IHD) is one of the most important cardiac diseases and defined by an inadequate oxygen supply of the myocardial cells (Task Force Members et al., 2013). The mismatch of demand and supply can be caused by various conditions including plaque-related obstruction of coronary arteries, microvascular dysfunction or vasospasm. Coronary artery disease (CAD) is one of the most important factors leading to IHD. It is an ongoing chronic process, which is based on accumulation of foam cells in the intima, followed by plaque formation and possible plaque-rupture. Stable IHD is defined by either stable symptoms (angina or dyspnea), which are thought to be related to CAD, or asymptomatic patients with prior-diagnosed CAD. An acute worsening or new onset of symptoms is defined as acute coronary syndrome (ACS). It includes acute myocardial infarction (AMI), which is caused by myocardial ischemia, or unstable angina (Roffi et al., 2016).

Cardiovascular disease and especially IHD remains one of the most important causes of death accounting for up to 30% of all deaths worldwide in 2012 (World Health Organization, 2017). Within the past decades medical treatment with statins, antiplatelet-drugs, and ACE-inhibitors, as well as revascularization have substantially improved the outcome of patients with IHD. The assessment of CAD in the population is difficult, as clinical information and imaging results are required. The prevalence of angina has been reported in the general population and is highly variable, as it ranges from 5% in younger women up to 14% in older men (Task Force Members et al., 2013). Therefore, the individual risk assessment in patients with suspected or diagnosed IHD remains an ongoing challenge. In clinical routine two important goals for patients with IHD include (1) the identification of individuals at high risk for future cardiovascular events and (2) the early diagnosis or rule-out of AMI in the acute setting. For risk prediction, several scores (e.g., Framingham Risk Score or ESC Score) have been established. They can identify high-risk individuals based on classical cardiovascular risk factors (Task Force Members et al., 2016a; D'Agostino et al., 2008). In patients with suspected AMI the use of biomarkers is well established and cardiac troponin is the gold-standard in clinical routine.

In the past years, numerous novel biomarkers were discovered and evaluated with regard to their predictive and diagnostic ability in IHD. This article will provide an overview on established and novel biomarker applications in patients with IHD.

General Considerations

Many biomarkers have been evaluated with regard to their diagnostic and predictive ability in patients with cardiovascular diseases and especially IHD. The most promising biomarkers are summarized in the following (Fig. 1). However, so far only few biomarkers are routinely used in the clinical setting.

When investigating new biomarkers several aspects should be kept in mind by researchers and physicians:

(1) At first, a robust test is required to allow reproducible results with a low variation. This is particularly important, when comparing results from different groups or laboratories.
(2) The new biomarker should be compared to other clinical risk markers or biomarkers and improve risk stratification. As an example, a biomarker might be compared to the Framingham risk score, which is an established and validated model for risk prediction. The improvement could be investigated by comparing the c-statistics or the net reclassification. This approach investigates, whether individuals are more likely to be assigned into the correct risk category when adding the biomarker.
(3) The results should be tested and validated in independent samples.
(4) Finally, the biomarker should ideally be cost-effective and improve patient management and outcome.

Metabolic and Renal Biomarkers

Metabolic processes play a central role in the pathogenesis of atherosclerosis. In particular, dyslipidemia was identified as one of the most important risk factors more than half a century ago. The determination of total cholesterol, low-density-lipoprotein (LDL) cholesterol, and high-density-lipoprotein (HDL) cholesterol has been clinical standard for decades (Task Force Members et al., 2013; Piepoli et al., 2016). These biomarkers are also included in established risk score (e.g., Framingham or ESC Score) and are therefore included as the gold-standard, when comparing novel biomarkers for their ability of risk prediction in statistical analyses. For LDL cholesterol, numerous studies provided strong evidence of a causal role in the pathogenesis of atherosclerotic disease. This is different for HDL cholesterol, which is inversely correlated with IHD, but so far could not be related to disease in a direct causal pathway (Voight et al., 2012). Statin-treatment is one of the most effective strategies in secondary prevention and can reduce the LDL cholesterol substantially. This effect is associated with a strong reduction of the relative risk. Furthermore statins are even able to decrease the diameter of coronary plaques (Baigent et al., 2005; Ballantyne et al., 2008). The use of statin therapy is widely established in patients with relevant IHD nowadays. As a consequence, in well-treated IHD patients LDL concentrations lose their predictive ability for mortality (Ndrepepa et al., 2014). Current ESC guidelines recommend annual measurements of LDL cholesterol and aim for a concentration below 70 mg/dL (or a 50% reduction of the baseline concentration) for patients with prior history of CAD (Task Force Members et al., 2013).

Lipoprotein A (Lp(a)) is a protein, which consists of the apolipoproteins B-100 and A (Malaguarnera et al., 2013). It is similar to LDL cholesterol and involved in the development of atherosclerotic disease. The plasma concentrations of Lp(a) are determined by

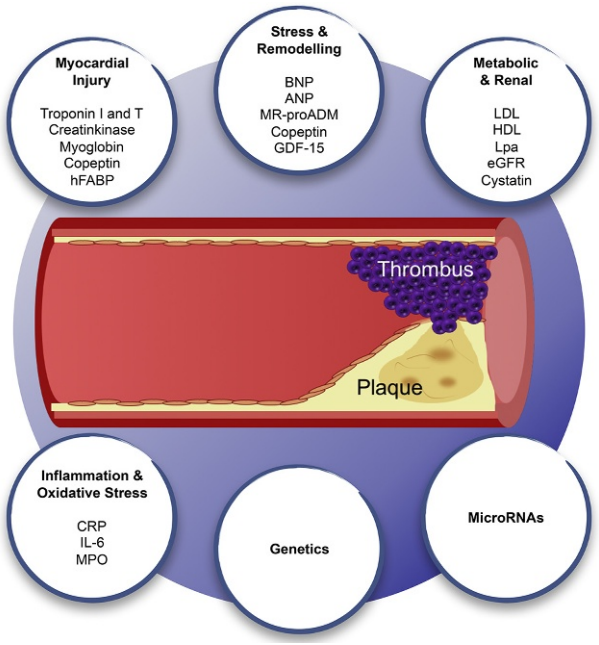

Fig. 1 Overview on selected biomarkers in patients with IHD.

genetic factors and appear to be stable over the lifespan (Nordestgaard et al., 2010). Treatment with niacin or fibrates was able to reduce Lp(a) concentrations, but so far, no clinical benefit was shown for a reduction of Lp(a). Therefore current ESC guidelines do not recommend general testing, while Lp(a) should be measured in patients with high cardiovascular risk or with a strong family history of IHD (Task Force Members et al., 2016b).

Impaired renal function is closely associated with IHD and poor outcome (Go et al., 2004). This is explained by accumulation of risk factors, but also by pathophysiological pathways including increased inflammation and aldosterone activity among others (Mule et al., 2015). In clinical routine the measurement of creatinine and the calculation of the estimated glomerular filtration rate (eGFR) are well established and validated. In a huge population of more than one billion individuals, decreased eGFR was associated with mortality and cardiovascular events (Go et al., 2004). Cystatin C has been investigated as an alternative biomarker for patients with renal impairment. It is thought to be less affected by confounding factors (e.g., age and muscle mass) and might be superior for detection of minor changes in renal function at early stages of renal impairment (Shlipak et al., 2013). In patients with IHD, cystatin C was associated with mortality. The cystatin C-based eGFR appears to be superior compared to the creatinine-based formula (Keller et al., 2009; Waldeyer et al., 2016). However, cystatin C-based IHD risk estimation has not been introduced widely in clinical routine and annual determination of creatinine and eGFR is recommended (Task Force Members et al., 2013).

Biomarkers of Myocardial Injury

Patients with suspected AMI are one of the most important populations, who profit from rapid decision-making based on biomarkers. While patients with ST-elevation AMI can be identified using the electrocardiogram, the diagnosis of non-ST-elevation AMI (NSTEMI) relies on biomarkers and their dynamic changes (Thygesen et al., 2012a). In AMI patients timing is crucial and early diagnostic approaches are needed in the emergency department. On the one hand, high-risk patients with NSTEMI due to obstructive CAD, who receive an early invasive treatment, have an improved outcome, compared to delayed treatment (Mehta et al., 2009; Milosevic et al., 2016). On the other hand, emergency departments are usually crowded and an early discharge of low-risk patients is required. In the past decades, several diagnostic biomarkers and approaches have been evaluated for patients with suspected AMI and are presented in the following. Improved diagnostic accuracy rendered them ideal, also for risk prediction.

Creatine Kinase and Myoglobin

Among the first biomarkers applied in clinical routine for the detection of myocardial necrosis were creatine kinase myocardial band (CK-MB) and myoglobin (Duma and Siegel, 1965; Rosano et al., 1977). Due to a rather slow dynamic change, CK-MB is of secondary importance for acute decision-making. However, an increase of CK-MB remains an important biomarker for patients with diagnosed AMI. Here, the increase of CK-MB is associated with the infarct size (Costa et al., 2008). Furthermore, current ESC guidelines recommend CK-MB as the preferred biomarker for patients who present early after prior AMI, as CK-MB shows a fast decrease after AMI (Roffi et al., 2016). Myoglobin shows an early increase after myocardial necrosis, but is less cardiac-specific and expressed in myocardial as well as skeletal muscle cells. Therefore, myoglobin is not recommended for clinical use anymore (Thygesen et al., 2012b).

Cardiac Troponin

Cardiac troponin is a protein complex, which has a central role in myocyte contraction. The three subcomplexes troponin C, I, and T are bound to tropomyosin and interact with the actin filament (Takeda et al., 2003). Troponin T and I are expressed exclusively in the myocyte and are released after myocardial necrosis (Parmacek and Solaro, 2004). The detection of troponin was first developed in 1987 using a radioimmunoassay (Cummins et al., 1987) and the first clinical application was presented in 1992, as troponin T positive patients with unstable angina had a poor outcome (Hamm et al., 1992). After introduction of troponin in clinical routine, elevated troponin concentrations were used as a diagnostic marker for patients with AMI (Alpert et al., 2000; Task Force Members et al., 2007). Ever since assays detecting troponin T and I underwent a rapid improvement in terms of sensitivity and accuracy. In the past years, numerous newer troponin assays were developed and used in clinical routine. These assays have a much higher sensitivity and can detect troponin concentrations not only in patients with AMI, but even in the general population. Furthermore, the accuracy, measured by the coefficient of variation at the 99th percentile, was improved impressively. Recently, the term of "high-sensitivity" troponin (hs-Tn) assay was introduced for assays with a 10% coefficient of variation, which is below the 99th percentile and that are detectable in at least 50% of the general population. In **Fig. 2** currently available troponin assays together with the specific limit of detection and the percentage detectable in the general population is displayed.

Diagnosis of AMI

Troponin is the best established and most widely used biomarker in patients with suspected AMI. International guidelines for patients with NSTEMI recommended a troponin cutoff concentration at the 99th percentile (determined in the general population) to rule-out AMI (Task Force Members et al., 2007; Amsterdam et al., 2014). Using less sensitive assays at that time, serial sampling was recommended after six or twelve hours in order to assess dynamic changes. After introduction of more sensitive or even high-sensitivity troponin assays a shorter time period of only three hours was suggested and recommended in the 2011 ESC guidelines

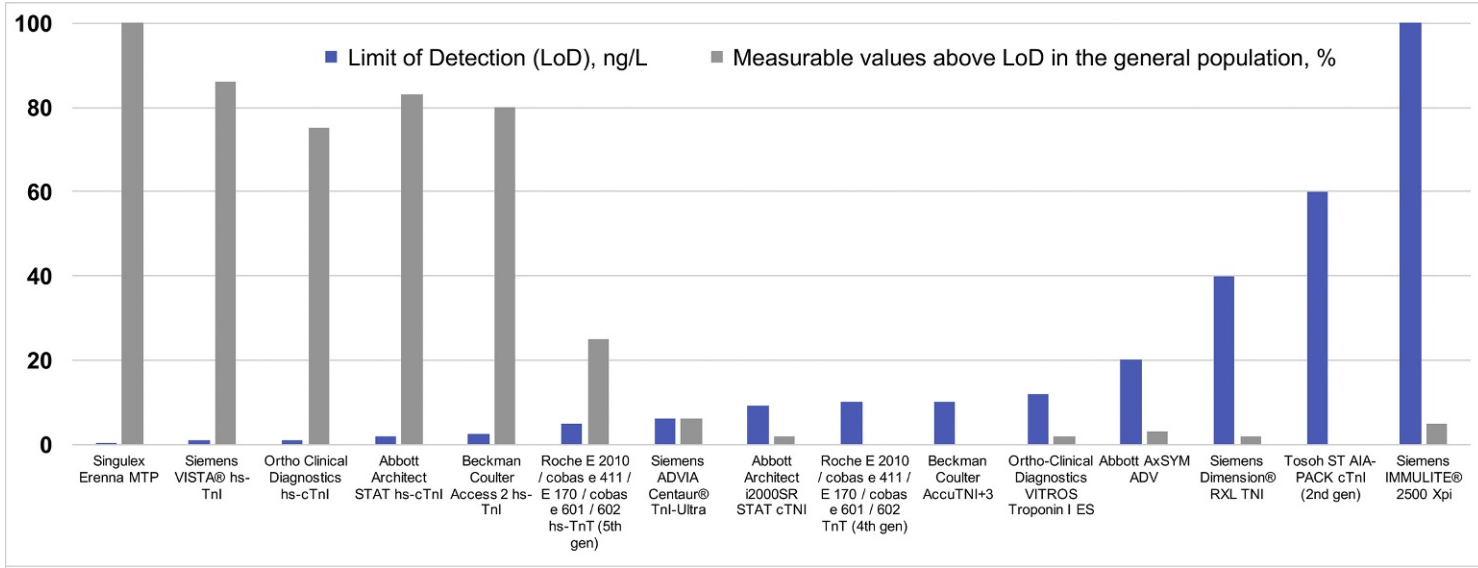

Fig. 2 Overview on selected currently available troponin assays, their limit of detection and rate detectable in the general population. Adapted from Westermann, D., Neumann, J. T., Soerensen, N. and Blankenberg, S. (2017). High-sensitivity assays for troponin in patients with cardiac disease. *Nature Reviews Cardiology*. https://doi.org/10.1038/nrcardio.2017.4.

(Ridker et al., 2008; Reichlin et al., 2009; Keller et al., 2011; Hamm et al., 2011). The ESC working group on Acute Cardiac Care recommended a baseline cutoff concentration at the 99th percentile and a >50% increase after three hours, when the baseline concentration was below the 99th percentile, or a >20% increase of the initial value, when the baseline concentration was above the 99th percentile in order to rule-out or to diagnose AMI. An optional measurement after six hours was recommended.

High-sensitivity assays

Using newer high-sensitivity troponin T and I assays the application of much lower cutoff concentrations became feasible and was investigated in several large cohorts of patients with suspected AMI (e.g., ADAPT, APACE, BACC, High-STEACS, and TRAPID-AMI cohorts). The ADAPT cohort was the first one to reduce the duration of serial sampling to two hours and measured hs-TnI (Than et al., 2012). A combined diagnostic approach based on the 99th hs-TnI percentile, the TIMI risk score, the ECG and history or symptoms, revealed a high diagnostic accuracy to rule-out AMI. A shorter timeframe of only one hour was evaluated in the APACE, BACC, and TRAPID-AMI cohorts (Mueller et al., 2016; Neumann et al., 2016a; Rubini Gimenez et al., 2015). In summary, all cohorts reported a high negative predictive value (NPV) to rule-out AMI based on very low hs-Tn concentrations at baseline (around 3 ng/L for hs-TnT and 2–6 ng/L for hs-TnI) and a low change after one hour (around 12 ng/L for hs-TnT and 5–6 ng/L for hs-TnI). On the other hand, the positive predictive value (PPV) was high, when the baseline concentration was high (52 ng/L for hs-TnT and hs-TnI) or delta change after one hour was high (around 5–6 ng/L for hs-TnT and hs-TnI). Importantly, these concentrations were significantly lower compared to the 99th percentile, which has been described at 27 ng/L (Zeller et al., 2014a).

Baseline troponin concentration

One step further, a single hs-TnI concentration directly at admission was tested to rule-out AMI. The High-STEACS cohort suggested a low cutoff of 5 ng/L and rule-out AMI in 61% of all patients with a NPV of 99.6% (Shah et al., 2015a). The BACC cohort aimed for a NPV of 100%, which was reached at a hs-TnI concentration of 3 ng/L in combination with a low-risk ECG without ischemic signs (Neumann et al., 2016b). This performance was validated independently in the ADAPT and Stenocardia cohorts. Finally, the first US cohort UTROPIA suggested a cutoff concentration at the limit of detection at 1.9 ng/L and described a NPV of 99.6% (Sandoval et al., 2017).

Current clinical practice

The improvements of troponin assay sensitivity enabled earlier decision-making in patients with suspected AMI. The 0/1 h approach has been incorporated in the 2015 ESC guidelines as an alternative to the three hours algorithm (Roffi et al., 2016). The suggested 0/1 h concept is based on assay-specific cutoff concentrations, which are provided in the guidelines. The general concept of early rule-out and rule-in is displayed in **Fig. 3**. Importantly, troponin concentrations are only one part of the decision-making process. Accurate evaluation of symptoms, patient history, physical examination, imaging results, and differential diagnoses is mandatory. Several aspects of the new biomarker algorithms have been discussed controversially (Crea et al., 2016). As an example, it has been argued, if sex-specific cutoff concentrations should be used (Cullen and Mills, 2016; Giannitsis, 2016; Rubini Gimenez et al., 2016; Shah et al., 2015b). Women tend to have lower troponin concentrations in general, which is reflected by a lower 99th percentile. However, currently available data on sex-specific cutoffs is limited and no general recommendation possible. Furthermore, data on patients who present early after symptom onset (e.g., less than three hours) is limited. In these patients, a troponin change might not yet be visible, even using hs-Tn assays. Therefore, the guidelines recommended the baseline rule-out of AMI only for those patients with symptom duration of more than three hours.

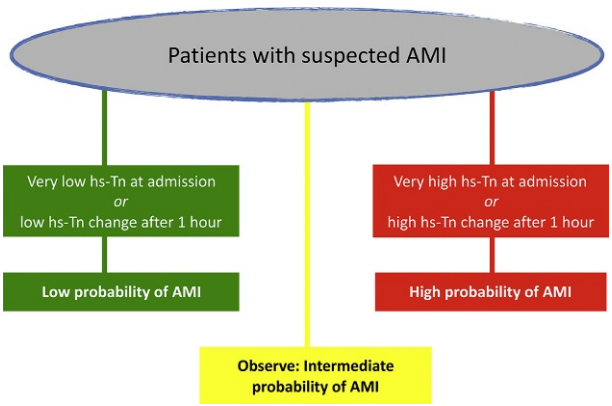

Fig. 3 Summary of the general concept of the ESC guideline 0/1 h approach. The exact cutoff concentrations are assay-specific. The baseline rule-out of AMI is only recommended for patients with symptom duration of more than 3 h.

In the past years hs-Tn assays were used worldwide, excluding the US. Therefore, the current AHA/ACC guidelines still recommend three to six hours troponin serial sampling (Amsterdam et al., 2014). This might change in the future, as the first hs-TnT assay was approved by the FDA in the beginning of 2017 (FDA, 2017). This approval will have major impact on the clinical routine within the US, as application of faster diagnostic protocols will become available.

Risk prediction

The improvement in assay sensitivity opened the venue for a much broader use of the biomarker troponin. Besides acute diagnostic application, the troponin-based risk prediction was investigated in study samples with cardiac disease. In 3679 patients from the PEACE study, which included patients with stable CAD, higher troponin T and I concentrations were associated with mortality and heart failure (Omland et al., 2009, 2013). In the BARI 2D study patients with stable CAD and prior-diagnosed diabetes were included and here hs-TnT concentrations above the 99th percentile were observed in 39% of the study population. Again, troponin concentrations were associated with mortality and future cardiovascular events (Everett et al., 2015a). Serial troponin sampling was performed in the LIPID study, which included patients with a history of AMI or unstable angina in combination with elevated LDL cholesterol (White et al., 2014). Patients were randomized to receive either placebo or pravastatin and followed for at least 1 year. Interestingly, a decrease of troponin was associated with risk reduction for future cardiovascular events and vice versa.

Due to higher sensitivity, hs-Tn concentrations were evaluated for their ability of risk prediction in the community (de Lemos et al., 2010; Saunders et al., 2010). In the FINRISK population, three troponin assays with different sensitivity were compared with regard to their prediction of future cardiovascular events. Interestingly, the most sensitive assay showed the best predictive performance (Neumann et al., 2014). In the large BiomarCaRE consortium hs-TnI was determined in 70,000 individuals from several European cohorts (Blankenberg et al., 2016). Here, hs-TnI concentrations above 5.9 ng/L were strongly associated with incidence of cardiovascular disease within the long-term follow-up period of 20 years. Finally, the use of hs-Tn for risk prediction in primary prevention was evaluated. The JUPITER trial randomized patients with elevated hs-CRP, but without cardiovascular disease, to receive either placebo or rosuvastatin (Everett et al., 2015b). Again hs-TnI was impressively associated with cardiovascular events within the follow-up time of 2.5 years. The WOSCOPS trial included patients with elevated LDL cholesterol, but without cardiovascular disease and randomized them to receive either placebo or statin-treatment (Ford et al., 2016). Patients receiving statins had a high relative risk reduction, especially when their hs-TnI was high. These findings are interesting and promising, but do not qualify for guideline recommendations yet. Further studies are needed to provide more insight to a more personalized medicine. In future troponin will be an important tool for risk assessment of patients with IHD.

Copeptin

Copeptin is a peptide, which is cleaved from the neurohypophysial hormone prepro-vasopressin and secreted in the blood during procession to vasopressin (Keller et al., 2010). It is released during stress and shows a rapid increasing kinetic. Therefore, several studies investigated the use of copeptin as a biomarker in patients with suspected AMI. Due to a low specificity, elevated copeptin concentrations can be observed in various conditions including sepsis, trauma, and shock. On the other hand, a negative copeptin results showed a high NPV for the diagnosis of AMI. When compared to sensitive troponin assays the additional use of a negative copeptin result increased the area under the curve (AUC) (Maisel et al., 2013). However, when using hs-Tn assays this effect does not appear relevant anymore (Mockel et al., 2015).

Heart-Type-Fatty-Acid-Binding-Protein

The heart-type-Fatty-Acid-Binding-Protein (hFABP) is a protein, which is involved in intracellular myocardial transport (Bruins Slot et al., 2010; Reiter et al., 2013). After myocardial necrosis hFABP is rapidly released into the blood stream and was therefore investigated as a biomarker for AMI. However, due to low sensitivity and specificity hFABP has not been proven useful, compared to the diagnostic performance of hs-Tn assays (Bruins Slot et al., 2010; Reiter et al., 2013).

MicroRNAs

In the past years microRNAs have been investigated as potential biomarkers in various diseases (Schulte and Zeller, 2015). MicroRNAs are small, circulating noncoding RNA molecules, which modulate the gene regulation. Up to date more than 2500 microRNAs have been described. MicroRNAs seem to be involved in the development of atherosclerosis and different microRNAs have been found in different stages of coronary plaque stages (Jovanovic et al., 2014). In patients with chest pain a combination set of eight microRNAs (miR-19a, miR-19b, miR-132, miR-140-3p, miR-142-5p, miR-150, miR-186, and miR-210) was able to discriminate patients with unstable angina from other patients (Zeller et al., 2014b). These eight microRNAs were further investigated in a large cohort of patients with CAD (stable and instable) (Karakas et al., 2016). Here, especially miR-132, miR-140-3p, and miR-210 were associated with cardiovascular death within the follow-up of 4 years. In summary, current data on the use of microRNAs is limited and comparison with established biomarkers is needed. In the future, sets of microRNAs might be helpful to improve individual risk stratification.

Biomarkers for Cardiac Stress and Remodeling

B-Type Natriuretic Peptide

Among cardiac biomarkers, the b-type natriuretic peptide (BNP) and the n-terminal fragment (NT-proBNP) are extremely well established. BNP is released from the ventricle during myocardial stress and can be detected in the blood using immunoassays. In the current guidelines for the diagnosis of heart failure, BNP is one of the most important cornerstones to diagnoses or to rule-out heart failure (Ponikowski et al., 2016). There is some substantial overlap of patients with heart failure and IHD, which makes BNP an important biomarker in this population as well.

Already in 2001 BNP was shown to be a strong predictor for mortality in patients with acute coronary syndrome (de Lemos et al., 2001). Furthermore, in patients with suspected CAD undergoing angiography NT-proBNP was a strong predictor for mortality (Kragelund et al., 2005). In stable CAD patients from the PEACE study BNP and NT-proBNP were measured and significantly associated with cardiovascular mortality, heart failure, and stroke (Omland et al., 2007). Importantly, both biomarkers provided incremental value to established risk stratification tools. The HOPE study, which included high-risk individuals, identified NT-proBNP as the most important predictor for cardiovascular events, besides classical risk factors (Blankenberg et al., 2006). Finally, in a multibiomarker approach from the MORGAM project in the general population, NT-proBNP, troponin I, and CRP were the strongest predictors for cardiovascular events and improved the reclassification (Blankenberg et al., 2010). BNP-guided therapy in patients with heart failure has been intensively discussed and might improve patient care. It is currently recommended by the US, but not the ESC guidelines (Ponikowski et al., 2016; Troughton et al., 2014).

Atrial-Natriuretic Peptide and Adrenomedullin

Similar to BNP, the atrial-natriuretic peptide (ANP) or its stable flanking protein MR-proANP, is released from the atrial myocytes during increased myocardial stress or pressure overload (Levin et al., 1998). Adrenomedullin (ADM) is a vasodilatory peptide, which is released in various acute and nonacute conditions. The mid-regional prohormone (MR-proADM) is more stable and can be measured using an immunoassay. It has been studied in patients with heart failure, hypertension, infections, and IHD (Peacock, 2014). Both biomarkers have been investigated in several studies and showed to further improve the risk stratification. In a population of patients with suspected AMI, MR-proANP, and MR-proADM were able to predict mortality or AMI and improved the reclassification, when compared to the GRACE score (Tzikas et al., 2013). Similar results were reported in the PEACE cohort, as both biomarkers were associated with mortality and incident heart failure. In the general population, MR-proADM was associated with subclinical and clinical cardiovascular disease (Neumann et al., 2013). However, the clinical use of these biomarkers is still limited.

Growth-Differentiation Factor 15

The growth-differentiation factor 15 (GDF-15) is a biomarker from the transforming growth factor-beta cytokine family (Wollert et al., 2017). It is increased after tissue damage, apoptosis, inflammation, and oxidative stress. GDF-15 has been evaluated in various cardiovascular disease cohorts where it predicted adverse outcome. In a large sample of patients with IHD, GDF-15 concentrations were associated with mortality, stroke, and AMI (Hagstrom et al., 2017). The AtheroGene study included patients with stabile angina undergoing angiography. In this cohort GDF-15 predicted coronary mortality (Kempf et al., 2009). GDF-15 concentrations were also investigated in the setting of patients with acute coronary syndrome, myocardial infarction, or cardiogenic shock. Here, GDF-15 was also a strong predictor for adverse events (Hagstrom et al., 2016; Fuernau et al., 2014; Eitel et al., 2011). In addition, GDF-15 was a predictor for major bleeding after ACS in the PLATO study (Hagstrom et al., 2016). Finally, the highest GDF-15 concentrations have been observed in patients with acute heart failure (Anand et al., 2010). In the community GDF-15 was a predictor for all-cause mortality (Rohatgi et al., 2012; Wang et al., 2012). These findings render GDF-15 a promising biomarker for risk prediction. However, current data indicates GDF-15 to be less specific for IHD, but rather a more general biomarker.

Biomarkers for Oxidative Stress and Inflammation

Inflammatory processes and oxidative stress are directly involved in development and progress of atherosclerotic disease. Local inflammation is one of the important factors leading to plaque formation, while oxidative stress is a potent inductor of endothelial dysfunction and especially present in the acute setting of plaque disruption (Hansson et al., 2015). Therefore, biomarkers of these pathophysiological pathways have gained attention within the past years.

C-Reactive Protein

The c-reactive protein (CRP) is one of the mostly used biomarkers worldwide to diagnose acute infections. CRP is released from the liver after stimulation by cytokines (e.g., interleukin-6 (IL-6) or tumor-necrosis-factor-alpha) and can be easily measured in blood. CRP concentrations are highly elevated in response to bacterial infections, but are also related to chronic inflammatory processes. Using newer high-sensitivity CRP (hs-CRP) assays lower concentrations can be detected, which makes this a promising biomarker to

identify individuals at risk. In the PEACE study, patients with IHD and elevated hs-CRP above 1 mg/L were at higher risk for future events (mortality, stroke, AMI), even after multiple adjustment (Sabatine et al., 2007). In the ATHEROREMO study the relation of CAD plaque burden and hs-CRP was recently investigated. Interestingly, hs-CRP was associated with plaque burden and incident MACE within the follow-up, but not with thin-cap fibroatheroma (Cheng et al., 2014). These results indicate, that CRP is a more general marker of atherosclerosis and not specific for heart diseases. In contrast to that a large study, which investigated genetic variations resulting in increased CRP concentrations, did not find an association of these polymorphisms with cardiovascular events (Zacho et al., 2008). The JUITER trial was the first study to base treatment decisions on hs-CRP concentrations (Ridker et al., 2008). Patients with hs-CRP above 2 mg/L and LDL cholesterol below 130 mg/dL were randomized to receive placebo or rosuvastatin. The study reported an impressive risk reduction by statin therapy within the complete study population. Interestingly, this effect was most prominent in those individuals with a hs-CRP reduction below 1 mg/L in the follow-up period. In summary, the use of CRP in risk prediction remains an interesting topic, but the clinical implications are not clear yet.

Other Markers

While CRP is the most prominent one, also other biomarkers have been investigated that represent inflammation and oxidative stress. The concentration of IL-6, a cytokine centrally involved in inflammation, is correlated with CRP which is explained by the inflammatory cascade since IL-6 stimulated CRP production and release. It has been shown to be associated with incident AMI or mortality (Fisman et al., 2006). Myeloperoxidase (MPO) is a product of neutrophil granulocytes and related to oxidative stress. With regard to IHD, higher MPO concentrations have been associated with cardiovascular events in patients after ACS (Baldus et al., 2003). In stable patients with CAD, MPO was also a prognostic marker (Tang et al., 2011). Furthermore, the lipoprotein-associated phospholipase A2, the pregnancy-associated plasma protein, and the phospholipase A2 have been discussed (Omland and White, 2017).

Multibiomarker Approach

More recently, a multibiomarker approach has been suggested to improve risk prediction and diagnosis of CAD. The concept of a biomarker-score, which combines the pathological results of different markers, reflects the multifactorial background of atherosclerotic disease. In the Framingham Heart Study, a general population setting, the biomarkers BNP, CRP, urinary albumin-to-creatinine ratio, homocysteine, and renin were associated with a first cardiovascular event (Wang et al., 2006). The combination of 30 cardiovascular biomarkers improved risk assessment for long-term cardiovascular disease incidence (Blankenberg et al., 2010). In another multiplex approach, more than 100 biomarkers were investigated in a cohort of patients undergoing angiography (Ibrahim et al., 2017). The four biomarkers adiponectin, apo C-I, KIM-1, and midkine were selected and combined in a predicting model. These new biomarkers combine the different pathophysiological backgrounds including inflammation (midkine), metabolic status (adiponectin and apo C-1), and renal dysfunction (KIM-1) and resulted in an AUC of 0.87 for the diagnosis of CAD. To date, multimarker approaches have shown that natriuretic peptides and inflammatory biomarkers range among the strongest predictors of CAD. However, the efficiency of multimarker testing in clinical practice has not been proven to be efficient.

Omics and Genetics

Most of the established biomarkers have been identified by classical, hypothesis-driven research. However, over the past years a novel tool for identification of potential biomarkers came up. By the term "omics" the comprehensive measurement evaluation of different biological steps from genome to transcriptome, proteome, and metabolome are summarized. Using mass array measurements huge amounts of data can be generated to detect potential target pathways. This approach requires large and well-characterized samples and novel statistical methods to identify potential biomarkers that may be clinically relevant. Most advanced are genomics with the identification of numerous genetic loci that are associated with disease risk by genome-wide association studies of common genetic polymorphisms and increasingly sequencing information (Abraham et al., 2016; Myocardial Infarction et al., 2016; Nikpay et al., 2015). Within the past 15 years the rapidly evolving field of genome-wide association studies (GWAS) and increasing sequencing data has provided fundamental insights into the genetics of IHD (Pjanic et al., 2016). These large studies aim to discover hitherto unknown pathophysiological pathways of the disease. They are well-suited for this purpose because the approach is largely hypothesis-free and associates specific genetic loci with IHD. With regard to IHD, several large-scale studies have been performed (Samani et al., 2007). In a recently published metaanalysis including 185,000 individuals, 58 loci were identified to be associated with CAD (Nikpay et al., 2015). In the future, these studies will have major impact on our understanding of disease pathophysiology and potential treatment approaches. Genetics remain stable over a lifetime and genetic risk scores improve risk prediction beyond classical risk factors (Abraham et al., 2016). However, clinical use is still limited.

Summary and Outlook

Biomarkers in patients with IHD are well established and used in clinical routine. Cholesterol subfractions are true risk factors as their treatment results in modification of IHD risk. Cardiac troponin is one cornerstone for the diagnosis of AMI that is well established in clinical routine. Development of newer, high-sensitivity troponin assays enabled the detection of much lower concentrations, which are far below the routinely used 99th percentile. This improvement resulted in lower cutoff concentrations and rule-in or rule-out of AMI after only one hour after admission for many patients. Furthermore, high-sensitivity troponin assays are also used for risk prediction in diseased cohorts, e.g., with stable CAD or even the general population. Several other biomarkers are on the horizon and are evaluated in clinical studies. The most promising ones include microRNAs that have been proven to be modifiable and genetics that will help to gain deeper insights into the pathophysiology of IHD development and manifestation.

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Further Reading

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Biomarkers: Heart Failure

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Introduction

Despite significant advances in the field of cardiology and an overall reduction in age-standardized rates of death attributable to cardiovascular disease (Wang et al., 2016), heart failure (HF) is becoming an increasingly prevalent chronic condition. In fact, HF is the most common admitting diagnosis in hospitals located in high-income countries for patients aged 65 years and older, accounting for approximately one million hospital admissions in the United States alone (Braunwald, 2015).

While both medical and device-based therapies for HF have made significant inroads, survival after HF diagnosis still remains poor. The OPTIMIZE-HF (Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure) study demonstrated greater than 8% mortality within 90 days of discharge following a hospitalization for HF exacerbation; the combined endpoint of death or repeat hospitalization was 36% (Fonarow et al., 2008). Some studies estimate survival at 50% at 5 years, and as low as 10% at 10 years following diagnosis of HF (Roger, 2013). These studies illustrate the importance of continued improvements in our understanding of disease progression, management, and disease prevention.

This review will examine the clinical utility of circulating biomarkers in disease management, and potential prevention strategies. Since the introduction of B-type natriuretic peptide (BNP) to guide HF diagnosis, management, and prognosis in 2001 (Maisel, 2009), there has been intense interest in novel biomarker discovery in heart failure, as evidenced by an exponential increase in publications on the topic (van Kimmenade and Januzzi, 2011). However, beyond natriuretic peptides, the clinical utility of biomarkers in HF remains unclear (Wilson Tang et al., 2007). In this review, we will (1) summarize current guidelines for the use of biomarkers in HF; (2) highlight general principles to evaluate clinical utility of novel biomarkers; (3) review existing studies to support the role of select biomarkers to guide prognosis, management, and risk prediction of HF; and (4) identify current knowledge gaps and future directions whereby biomarkers may inform clinical decision-making.

Current Guidelines for Biomarkers in Heart Failure

Many recommendations outlining the use of biomarkers to guide diagnosis and management of HF are available, including practice guidelines from groups such as the American College of Cardiology Foundation (ACCF), American Heart Association (AHA), Heart Failure Society of America (HFSA), and the European Society of Cardiology (ESC). Though these guidelines were developed independently, one commonality has emerged: confirmation of the utility of measuring natriuretic peptides in patients with HF, as summarized in **Table 1**.

All three society guidelines support the use of natriuretic peptides to aid diagnosis of HF in patients suspected of having HF (Ponikowski et al., 2016; America, 2010; Yancy et al., 2013). The ESC guidelines specifically point out the high negative predictive value of natriuretic peptides at specified thresholds and comment on their use to rule out HF but not necessarily to establish the diagnosis (Ponikowski et al., 2016). While useful in symptomatic individuals, the use of natriuretic peptides as a screening test in the absence of symptoms is not recommended by the HFSA guidelines. Once HF diagnosis has been established, ACC/AHA guidelines support a class I recommendation for the use of natriuretic peptides to aid in prognostication, whereas ESC supports a class IIa recommendation for initial assessment in newly diagnosed HF. Beyond aiding in HF diagnosis and prognosis, the ACCF/AHA guidelines also support Class IIa and Class IIb recommendations for the use of natriuretic peptides in guiding medical therapy

Table 1 Summary of major society recommendations for the use of biomarkers in heart failure

Biomarker	*Society**	*Class*	*Setting*
Natriuretic peptides	ACC/AHA	I	Diagnosis: ambulatory, acute
		I	Prognosis: ambulatory, acute
		IIa	Ambulatory HF: achieve guideline-directed medical therapy
		IIb	Acute HF: guide medical therapy
	ESC		Diagnosis: rule out HF
		IIa	Initial assessment in newly diagnosed HF
	HFSA	Recommended	Diagnosis: when HF is suspected
		Not recommended	Routine screening in asymptomatic individuals
Myocardial injury	ACC/AHA	I	Prognosis: ambulatory, acute
Myocardial fibrosis	ACC/AHA	IIb	Additive risk stratification: ambulatory, acute

*Guideline recommendations pertaining to heart failure from ACC/AHA 2013, ESC 2016, and HFSA 2010 documents.

in chronic HF and in acutely decompensated HF, respectively. By contrast, neither the ESC nor the HFSA guidelines remark on the utility of natriuretic peptides in the management of previously diagnosed HF.

Beyond natriuretic peptides, however, the clinical applicability of other biomarkers in routine medical practice is less clear. The ACCF/AHA guidelines also support the use of cardiac troponins in establishing disease severity and prognosis in acute decompensated HF (Class I), and the use of biomarkers of myocardial fibrosis for additive risk stratification, with specific mention of soluble ST2 and galectin-3 (Class IIb). By contrast, the ESC guidelines state that there is no definite evidence supporting the use of other biomarkers in routine clinical practice. Lastly, the HFSA guidelines do not mention additional biomarkers beyond natriuretic peptides. Motivated by these differences, we will examine general principles used to evaluate biomarker studies and summarize key studies supporting current guidelines below.

What Defines Potential Clinical Utility of Biomarkers in HF?

A number of objective criteria have been put forth in order to appraise the potential relevance of biomarkers to clinical practice. The AHA suggests six phases of evaluation for any new biomarker: proof of concept, prospective validation, incremental value, clinical utility, clinical outcomes, and cost-effectiveness (Hlatky et al., 2009). Others have specified three criteria to evaluate the utility of biomarkers: the biomarker must be measurable, informative, and actionable (Morrow and de Lemos, 2007). Specific to the field of HF, the National Academy of Clinical Biochemistry has elucidated similar goals for biomarkers specifically in the setting of HF; in this definition, biomarkers should be able to identify underlying causes of HF, confirm presence of HF syndrome, estimate severity of disease, and predict the risk of disease progression (Wilson Tang et al., 2007).

Beyond assay-specific qualities such as reproducibility, accuracy, and cost, a given biomarker must first associate strongly with the disease in question. The additive value of a biomarker with respect to risk prediction can then be evaluated using metrics of discrimination, reclassification, and calibration. Discrimination indicates the ability of a biomarker to differentiate between an event and a nonevent (Ahmad et al., 2014). Often, the incremental discriminatory value of a biomarker when added to a clinical model is assessed by the change (or improvement) in the C-statistic. Given inherent limitations in the C-statistic, reclassification metrics have also been used (Pencina et al., 2008). The net reclassification improvement (NRI) metric evaluates whether a biomarker is able to assign higher risk to individuals who eventually develop the outcome of interest, while assigning lower risk to those who do not develop events (Pencina et al., 2011). Lastly, calibration is a measure of how frequently a predictive model matches the outcome in reality (Ahmad et al., 2014).

While assessment of discrimination and reclassification help refine our understanding of biomarkers in risk prediction, it is important to note existing limitations. Despite strong associations with HF, the majority of biomarker studies have demonstrated only modest increments in C-statistics and NRI with respect to HF risk prediction (Wang, 2011). It may be that clinical predictors already result in a "high" C-statistic, above which an added improvement with any biomarker is much more difficult to demonstrate (Pencina et al., 2008). It is important to acknowledge that no accepted risk categories exist for HF, making the NRI somewhat more difficult to interpret. Lastly, studies of HF risk prediction have largely combined the categories of HF with preserved ejection fraction and HF with reduced ejection fraction, despite distinct underlying pathophysiology and therapies among the two HF subtypes (Echouffo-Tcheugui et al., 2015; Yancy et al., 2013). This amalgam reduces the specificity of existing risk prediction models.

Selected Biomarkers: What Do We Know?

There has been intense interest in elucidating the role of biomarkers in HF, with studies focused on risk prediction of future HF, as well as prognostication among patients with acute and chronic HF as summarized in **Table 2**. Below we summarize key studies for selected biomarkers.

Table 2 Existing studies examining biomarkers relevant to the ACC/AHA clinical stages of heart failure

Biomarker	*Stage A Risk factors*	*Stage B LV remodeling*	*Stage C/D Symptomatic HF*		
Characteristic	*Prediction of incident HF*		*Diagnosis*	*Prognosis*	*Guide therapy*
Natriuretic peptides	✓✓		✓✓	✓✓	✓
High-sensitivity troponin	✓✓			✓✓	
Soluble ST2	✓			✓✓	
Galectin-3	✓			✓	
Growth differentiation factor-15	✓			✓	
C-reactive protein	✓			✓	
Mid-regional proadrenomedullin	✓			✓	
Urinary albumin to creatinine ratio	✓			✓	
Neutrophil gelatinase-associated lipocalin				✓	

✓ denotes one supporting study or multiple studies with some mixed results.
✓✓ denotes multiple supporting studies.

B-Type Natriuretic Peptide & N-Terminal Pro-B Type Natriuretic Peptide

In the early 1990s, BNP was found to be secreted from the ventricular myocardium in response to pressure overload, in many cases itself a byproduct of volume expansion (Daniels and Maisel, 2007). Downstream effects of the binding of BNP to its receptor include natriuresis, vasodilation, inhibition of the renin–angiotensin–aldosterone system (RAAS), and increased myocardial relaxation (Kim and Januzzi, 2010). Studies focused on tailoring HF management to serum levels of natriuretic peptides began just a few years later, in the late 1990s (Murdoch et al., 1999; Troughton et al., 2000). These studies provided initial evidence that BNP-tailored management may improve clinical outcomes.

Other studies from that time demonstrated that measurement of BNP could rapidly confirm the diagnosis of HF (Dao et al., 2001; Maisel et al., 2002). In a 2002 study known as the Breathing Not Properly Multinational Study, BNP levels alone were found to be more accurate than any other component of the history, physical exam, or additional laboratory values in determining the cause of a patient's dyspnea (Maisel et al., 2002). The PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study, a similar study measuring NT-proBNP levels in the Emergency Department, provided further evidence that natriuretic peptide measurements could facilitate diagnosis of HF (Januzzi et al., 2005).

A 2003 follow-up study to the Breathing Not Properly Multinational Study with 6 months of monitoring revealed that BNP levels were also highly predictive of cardiac events (Harrison et al., 2002). In an analysis of the Framingham Offspring Study, BNP levels were found to be independently predictive of the risk of death, heart failure, stroke, and atrial fibrillation, even after statistical adjustment for traditional risk factors (Wang et al., 2004). Larger, more recent studies have corroborated these early studies; BNP and NT-proBNP have been proven to be strongly associated with cardiovascular events and mortality in both symptomatic and asymptomatic patients (Doust et al., 2005; Di Angelantonio et al., 2009). However, more data are needed from low- and intermediate-risk populations before testing in the general population can be recommended without reservation (Di Angelantonio et al., 2009). There is some evidence that measurement of NT-proBNP levels in a healthy population may not offer reliable predictions regarding cardiovascular outcomes, though prognostic value was added with its measurement in patients with Stage A/B HF, a finding consistent with prior studies (McKie et al., 2010).

Current research in this field is focused on the role of monitoring natriuretic peptide levels both in the inpatient and outpatient settings. During acute HF exacerbations requiring hospitalization, general consensus argues for measurement of BNP levels at time of admission and prior to discharge (Pang et al., 2012). Many studies have shown that a substantial decrease in BNP levels during hospitalization portends good outcomes, while patients with relatively stable or increased BNP levels were more likely to experience hospital readmissions or increased mortality rates (Van Cheng et al., 2001; Bettencourt, 2004; Michtalik et al., 2011). A number of studies have further examined the potential utility of natriuretic peptides in guiding medical therapy in the acute and chronic setting and are summarized under "Future Directions" later in this article. Outpatient monitoring of BNP levels remains controversial (Januzzi and Troughton, 2013; Desai, 2013), in no small part due to the fact that the intraindividual biological variability of BNP and NT-proBNP levels is quite high (O'Hanlon et al., 2007). For this reason, some support the establishment of a baseline or "steady-state" BNP level; recognizing a patient's normal BNP level allows for clinical decision-making based on variation from that mean (Daniels and Maisel, 2007).

Lastly, the utility of BNP in screening approaches for the prevention of HF has also been recently examined and is summarized under "Future Directions." Though there is no single perfect biomarker in HF, the utility of measuring BNP and NT-proBNP has been proven in many different studies, and its current use in establishing diagnosis and risk stratification of patients with HF is widespread. Future studies may further define BNP-guided management strategies and a potential role in screening and HF prevention. Significant value may also be gained from a multimarker approach in the context of several other biomarkers, as described later.

Highly Sensitive Troponin-I (hsTnI) & Highly Sensitive Troponin-T (hsTnT)

The measurement of cardiac proteins troponin-I and troponin-T is a mainstay for diagnosis and evaluation of patients with acute coronary syndrome. However, cardiac troponins have also been found to be elevated in patients with HF (Gaggin and Januzzi, 2013). As biomarker assays become more refined, highly sensitive troponin (hsTn) assays will be increasingly utilized. One benefit of the hsTn assays is that troponin values are measurable in the majority of patients, and therefore elevated levels may represent better discrimination when compared to traditional, low-sensitivity troponin assays.

The measurement of hsTnI in patients with acute decompensated HF has been shown to have prognostic value. This is particularly notable because troponin proteins are often measured in patients with acute decompensated HF on presentation in order to rule out myocardial infarction as a cause of their acute symptoms. In a study of patients hospitalized with decompensated heart failure, Xue et al. (2014) showed that even small changes in troponin values during a HF exacerbation are associated with increased 90-day mortality and hospital readmission; moreover, increasing levels of hsTnI in admitted patients suggest higher mortality than patients in whom hsTnI levels are stable or decreasing. Increased levels of hsTn have also been seen in patients with chronic and symptomatic HF in proportion to the severity of disease (Latini et al., 2007). Notably, the prognostic value of hsTn may improve when evaluated in combination with other markers, such as NT-proBNP and soluble ST2 (Pascual-Figal et al., 2014). Lastly, hsTn concentrations predict incident HF among population-based studies (Wang et al., 2012; deFilippi et al., 2010). As more clinical centers adopt hsTn assays as part of routine medical care, the utility of hsTn measurements in HF will become clearer.

Soluble ST2

ST2, a member of the IL-1 receptor family, is a protein with transmembrane (ST2L) and soluble (sST2) isoforms (Sanada et al., 2007). Soluble ST2 acts as a decoy receptor for the ligand IL-33, which has been shown to be antifibrotic and antihypertrophic (Sanada et al., 2007). Therefore, the cardioprotective effects of the IL-33/ST2L interaction, shown in experimental models to reduce apoptosis and to improve myocardial function, are inhibited by sST2 (Pascual-Figal and Januzzi, 2015).

Similar to BNP and NT-proBNP, ST2 is a protein induced by mechanical strain in cardiac myocytes; it has been found to be significantly higher in patients with severe HF when compared to patients without HF (Weinberg, 2003). Elevations in ST2 indicate a worse prognosis in patients with chronic HF and increases in ST2 levels over a 2-week period have proven to be an independent predictor of mortality or transplantation; notably, dynamic changes in BNP in this study were not shown to predict adverse outcome, suggesting that ST2 may be even more sensitive than natriuretic peptide levels (Weinberg, 2003). A subanalysis of PRIDE, a prospective study of more than 500 patients who presented to the Emergency Department with acute shortness of breath, demonstrated that ST2 levels were as predictive of mortality at 1 year as NT-proBNP (Januzzi et al., 2007). Importantly, elevations of both NT-proBNP and ST2 were associated with the highest 1-year mortality in the cohort, arguing there may be a role for clinical prediction using multiple HF biomarkers simultaneously (Januzzi et al., 2007). This finding has been confirmed in multiple additional studies (Bayes-Genis et al., 2014; Pascual-Figal et al., 2014). Some studies suggest that ST2 may be reliable even in elderly patients with ischemic disease, a population in which natriuretic peptide levels have been shown to be less useful (Broch et al., 2014).

Outpatient monitoring of ST2 is not yet standard of care in clinical practice; however, some studies have demonstrated the utility of its measurement. An analysis of over 3000 patients in the Framingham Heart Study revealed the prognostic value of elevated ST2 levels in a community-based cohort. Patients in the Framingham Heart Study with elevated ST2 levels, only a small percentage of whom had prevalent cardiovascular disease, were more likely to experience adverse outcomes, including incident HF (Wang et al., 2012).

At this point, there does not seem to be utility to serial measurements of ST2 obtained in an effort to guide therapy. However, a single measurement of ST2 might be useful in helping clinicians to make management decisions. One study, though limited by a small sample size, suggests that eplerenone may attenuate left ventricular remodeling in patients with high levels of ST2 following acute myocardial infarction (Weir et al., 2010). The ability of ST2 to reflect ventricular remodeling provides information beyond what can be determined by measuring natriuretic peptides alone. Further, unlike other biomarkers, ST2 levels do not appear to be affected by weight or kidney function, which allows for more standardized measurements across populations (Karayannis et al., 2013).

Galectin-3

Galectin-3 is a β-galactoside-binding lectin that is overexpressed in cardiac macrophages of failure-prone hearts even before overt clinical HF can be diagnosed (Sharma, 2004). Further supporting the idea that galectin-3 is related to cardiac dysfunction is evidence that intrapericardial delivery of exogenous galectin-3 leads to collagen deposition and fibrosis (Sharma, 2004). In fact, murine models showed that the level of galectin-3 associated strongly with the severity of future cardiac fibrosis (Sharma, 2004). It also plays a significant role in fibrosis of other organs, including the liver and kidney (Henderson et al., 2006, 2008).

The aforementioned PRIDE study also identified galectin-3 as a possible biomarker for patients with acute decompensated heart failure. In that study, galectin-3 was found to be a better prognostic marker for 60-day mortality compared to NT-proBNP (Januzzi et al., 2005). Perhaps more importantly, the combination of biomarkers improved prognostic capability (van Kimmenade et al., 2006). A subset of these patients had a longer follow-up period; galectin-3 levels were associated with both 1- and 4-year mortality

(Shah et al., 2014). Further, galectin-3 levels showed an association with echocardiographic markers of LV filling, diastolic function, valvular regurgitation, and RV function (Shah et al., 2014), suggesting that galectin-3 may associate strongly with HF with preserved ejection fraction. Other studies have also shown that the predictive value of galectin-3 may be greater in patients with preserved systolic function (de Boer et al., 2010).

Additional research has demonstrated the value of galectin-3 as a biomarker in patients with chronic heart failure. The DEAL-HF study confirmed galectin-3 as an independent predictor of mortality in patients with moderate-to-advanced chronic HF (Lok et al., 2010). A study of patients with severe HF requiring mechanical circulatory support showed that galectin-3 levels were significantly higher in patients with HF than controls and that HF patients who did not survive ventricular assist device support had higher galectin-3 levels than those that were able to successfully undergo heart transplantation (Milting et al., 2008).

Prospective studies to see whether galectin-3 might be used to guide therapy do not exist. However, multiple studies have shown that increasing galectin-3 levels increase the risk of repeat hospitalization for HF and the risk of death (van der Velde et al., 2013). Other studies have shown that therapies for HF may be more effective in patients with low galectin-3 levels at baseline. Val-HeFT, a study of the effect of valsartan in patients with heart failure demonstrated a reduction in repeat HF hospitalizations for patients with low galectin-3 levels; this finding was not seen in patients with high galectin-3 levels (Anand et al., 2014). Similarly, a *post hoc* analysis of the CORONA (Controlled Rosuvastatin Multinational Trial in Heart Failure) study appeared to show benefit with rosuvastatin therapy in patients with systolic HF and low levels of galectin-3 (Gullestad et al., 2012), despite a lack of evidence for statin therapy for all patients with heart failure.

Lastly, galectin-3 was shown to predict incident HF among community-dwelling adults (Ho et al., 2012). While not yet shown to be a valuable diagnostic marker in heart failure, galectin-3 has been shown to contribute to cardiac fibrosis and ventricular remodeling; its role in the development and progression of HF may lead to significant advancements in disease treatment in the future. At the present time, however, it remains a useful biomarker with prognostic value in both acute and chronic heart failure.

Growth Differentiation Factor-15 (GDF-15)

Growth differentiation factor-15 (GDF-15), a member of the transforming growth factor β (TGF- β) family of cytokines, has been shown to have antiapoptotic, antihypertrophic, and antiinflammatory in cardiovascular disease models (Wollert and Kempf, 2012). Wang et al. (2012) have shown that GDF-15 concentrations were strongly associated with risk of HF and with mortality. A 2007 study showed that GDF-15 levels were significantly increased in patients with HF and that there was a graded relationship between the level of GDF-15 at time of study entry and the risk of death during study follow-up (Kempf et al., 2007). The Val-HeFT study demonstrated similar findings; GDF-15 was again found to be an independent predictor of death. In this study, repeat measurement of GDF-15 12 months after first measurement provided additional information. Changes in GDF-15 levels over time were independent associated with risk of mortality. Further, it was found that the intraindividual variation between GDF-15 levels in patients with stable chronic HF were lower than with other common biomarkers (Anand et al., 2010), suggesting that repeat measurements of GDF-15 may be useful in biomarker-guided therapy.

Future Directions

Biomarker-Guided HF Management

Prior studies examining the use of natriuretic peptides in biomarker-guided care have demonstrated mixed results (Lainchbury et al., 2009; Pfisterer et al., 2009; Januzzi et al., 2011). This heterogeneity in results appears to be related to whether or not the active arm achieved lower natriuretic peptide levels through the trial, in addition to the population being studied, with younger patients and those with heart failure with reduced ejection fraction deriving the most benefit (Yancy et al., 2013). In the Pro-BNP Outpatient Tailored Chronic Heart Failure (PROTECT) study, targeting an NT-proBNP level of 1000 pg/mL or lower resulted in a reduction in cardiovascular events in the active vs standard of care groups (Januzzi et al., 2011). A substudy provided echocardiographic evidence that left ventricular volumes and function improved with lower NT-proBNP levels, irrespective of treatment allocation within the study (Weiner et al., 2014). Multiple other studies have supported improvements in clinical HF outcomes with NT-proBNP-guided therapy (Felker et al., 2009; Porapakkham et al., 2010; Pfisterer et al., 2009). However, the largest prospective, randomized trial to date, the Guiding Evidence-Based Therapy Using Biomarker Intensified Treatment (GUIDE-IT) trial, was recently halted by the Data Safety Monitoring Board 18 months prior to planned study completion in light of an interval analysis demonstrating no difference in the primary outcome between NT-proBNP-guided management and usual care. While the formal publication of GUIDE-IT results is still pending at this time, the future of biomarker-guided therapy remains very much unclear.

Improving Risk Prediction

While single biomarker studies may demonstrate relatively modest effects with respect to discrimination and reclassification metrics, several biomarkers representing distinct physiologic pathways, when combined in a multimarker approach may improve upon current risk prediction models. In the MOCA (Multinational Observational Cohort on Acute heart failure) study, multiple biomarkers showed incremental value above and beyond clinical risk factors in patients with acute decompensated heart failure. The combination of CRP and MR-proADM predicted 30-day mortality, with an NRI of 36.8%, and CRP and soluble ST2 predicted

1-year mortality with an NRI of 20.3% (Mebazaa et al., 2015). Multimarker models have also improved upon risk prediction of future HF in community-based populations. Among Framingham Heart Study participants, the combination of natriuretic peptides, soluble ST2, GDF-15, hsTnI, and hsCRP improved clinical prediction of incident HF with an NRI of 39% (Wang et al., 2012). With the emergence of large-scale mass spectrometry and newer aptamer-based technology (Ganz et al., 2016), the ability to rapidly ascertain a large number of known and novel biomarkers will continue to refine current HF risk prediction models in the future. Lastly, it is important to acknowledge that current HF risk prediction models are focused on overall HF and group together HF with preserved and reduced ejection fraction, despite distinct pathophysiologic drivers and therapies. To this end, future studies examining biomarkers in relation to specific HF subtypes are needed.

Biomarkers in Prevention of Heart Failure

Two recent studies have examined the utility of natriuretic peptides in guiding preventive efforts in HF. The St Vincent's Screening to Prevent Heart Failure (STOP-HF) trial randomized 1374 participants with cardiovascular risk factors free of HF to BNP screening versus usual care. Participants in the BNP screening arm who had elevated BNP levels of 50 pg/mL or higher underwent echocardiography and collaborative subspecialty care. Investigators found that BNP-based screenng reduced incident left ventricular dysfunction and HF and appeared cost-effective (Ledwidge et al., 2013). Similarly, in the PONTIAC (NT-proBNP Selected PreventiOn of cardiac eveNts in a populaTion of dIabetic patients without A history of Cardiac disease) study, 300 patients with elevated NT-proBNP >125 pg/mL were randomized to "intensified" treatment in a cardiac clinic focused on uptitration of medical therapy versus usual care. PONTIAC demonstrated accelerated uptitration of renin angiotensin system antagonists and beta-blockers in the "intensified" group, with a significant reduction in cardiac events after 2 years compared to the usual care group (Huelsmann et al., 2013). These studies suggest that biomarkers may help identify at-risk individuals and have the potential to guide preventive strategies in the future.

Conclusion

Despite the widespread use of natriuretic peptides and hsTn in the diagnosis and management of HF, the clinical utility of the myriad of other cardiovascular biomarkers that have been associated with HF alone or in combination remains unclear. The field of biomarkers in HF is at an exciting inflection point with the emergence of large-scale metabolomic and proteomic platforms now allowing for rapid assessment of existing and novel circulating biomarkers. Future studies will be necessary to refine current risk prediction models using multimarker approaches and HF subtypes, with the ultimate goal of assisting clinicians in risk stratifying and treating their patients with HF.

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Biomarkers: Population Screening and Risk-Stratification

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What is a Biomarker?

A biomarker is a reflection of a biological, physiological, or pathological process or a pharmacologic response to a therapeutic intervention that can be measured objectively, accurately, and reproducibly (adopted from Strimbu and Tavel (2010) and Biomarkers Definitions Working Group (2001)). Thus, a broad spectrum of measures can be considered as biomarkers, including markers measured in various biosamples (e.g., blood, urine, stool, or tissue), imaging tests (e.g., echocardiography, magnetic resonance imaging, or computed tomography), and (electro-)physiological measures (e.g., blood pressure, electrocardiography) (Vasan, 2006). In recent years, also complex molecular markers and marker profiles are being increasingly evaluated as potential cardiovascular biomarkers, including genetic, metabolomic, and proteomic markers (Vasan, 2006; Ussher et al., 2016). In terms of their temporal relation to disease processes and their clinical application, biomarkers can be classified, for example, as markers that identify individuals at high risk of disease, before the disease has developed (antecedent biomarkers), as biomarkers that detect subclinical changes, for example, of the heart or the vascular system (screening biomarkers), or markers that detect clinically overt disease (diagnostic biomarkers) (Biomarkers Definitions Working Group, 2001; Vasan, 2006). Among patients with established disease, biomarkers can inform treatment strategies (e.g., by predicting the response to and efficacy of a given therapy; predictive biomarkers) and help in assessing disease severity (staging biomarkers) and prognosis of patients (e.g., by predicting the future course of disease; prognostic biomarkers) (Biomarkers Definitions Working Group, 2001; Vasan, 2006).

Key Requirements and Features of a Good Biomarker

In order to be applicable in clinical practice, risk stratification, or population screening, biomarkers should meet several requirements, as outlined, for example, by the American Heart Association (AHA) (Hlatky et al., 2009). First, biomarkers need to be accurately and reproducibly measurable (Manolio, 2003). Additionally, one of the most basic requirements for a novel risk marker is a strong statistically significant association with the outcome of interest, independently of other risk factors (Hlatky et al., 2009). Furthermore, the biomarker should explain a reasonable proportion of the interindividual variation of the disease outcome of interest, and improve risk prediction models beyond established risk factors (please see below for

details) (Hlatky et al., 2009; Manolio, 2003). Some characteristics of biomarkers vary with their intended use (e.g., screening, diagnosis, prognosis) (LaBaer, 2005). For example, in clinical settings, biomarker measurements need to be acceptable to the patient, be easy to interpret by clinicians, and should be linked to patient management (Manolio, 2003), in the sense that measuring the biomarker should impact clinical decision making, and ultimately improve patient outcomes. The present article aims to focus on biomarkers in the context of risk stratification and population screening. Important basic concepts for risk stratification and population screening are the estimation of absolute disease risks and the performance of risk prediction models, both of which are detailed in the next paragraph.

Assessment of Absolute Disease Risk and Principles of Risk Prediction

The cardiovascular disease (CVD) risk of a given individual is determined by a complex interplay of multiple risk factors over the life course. To reflect the multifactorial nature of CVD, risk scores have been developed that incorporate information from multiple risk factors to generate estimates of absolute disease risk. Commonly used scores include, for example, the American College of Cardiology (ACC)/AHA 2013 risk score (Goff et al., 2014), the Framingham Risk Score (FRS) (Wilson et al., 1998), the Reynolds Risk Score (Ridker et al., 2007), or the SCORE of the European Society of Cardiology (ESC) (Conroy et al., 2003).

To evaluate the performance of risk scores and prediction models and to assess the incremental value of biomarkers when added to these models, different metrices have been developed, including discrimination, calibration, and reclassification.

Discrimination refers to the ability of a risk prediction model to distinguish individuals who do not develop the outcome of interest over a given follow-up period from those who develop the disease of interest (Greenland et al., 2010). A common measure of discrimination is the C statistic which is equivalent to the area under the receiver operating characteristic (ROC) curve (Hanley and McNeil, 1982). The C statistic incorporates the sensitivity and specificity of a screening or diagnostic test and predicts the probability that in two randomly paired individuals, one with and one without the disease, a given model correctly identifies the one who is diseased from the one who is not. Values for the ROC or C statistic range from 0.5 (uninformative model) to 1.0 (perfect discrimination) (Wang, 2011). As an example, the FRS, which is based on traditional cardiovascular risk factors, yields a C statistic of ~0.75 (Wang et al., 2006).

Calibration quantifies the agreement between predicted and observed risks, that is, the disease risk as predicted by the score on the one hand, and the risk that is actually observed in a given prospective cohort on the other hand (Wang, 2011). It is usually assessed in quantiles of baseline risk and tested using the Hosmer–Lemeshow test. The observed number of events within a given time horizon is compared to the expected number of events, as predicted by the score. Importantly, when transferred to other populations, risk prediction models may have to be recalibrated, in order to account for different mean risk factor levels and differences in the incidence rates of the outcome of interest between populations (Vasan, 2006; Wilson et al., 1998).

A third performance measure, *reclassification*, refers to the ability of a (modified) score to reclassify individuals between defined risk categories. Individuals can be assigned, for example, to low risk, intermediate risk, and high risk, based on their absolute disease risk estimates. When a given score is modified (e.g., by adding a new biomarker to the model), some individuals will shift between risk categories because the predicted risk in the baseline model belonged to a different risk category than their risk based on the modified score. Reclassification refers to the proportion of individuals that is correctly assigned to a new risk category, once additional information is added to the risk prediction model, for example, by including information from a new biomarker (Hlatky et al., 2009). Reclassification of intermediate-risk individuals to the low- or high-risk category might be particularly helpful because for intermediate-risk individuals, treatment decisions are usually less clear as compared to low- and high-risk individuals (Helfand et al., 2009). However, simply quantifying the proportion of individuals that are being reclassified might be misleading, because down-classifying individuals who actually develop an event is inappropriate as is upward-classification of individuals who remain free of CVD. The net reclassification improvement (NRI) (Pencina et al., 2008) subtracts incorrectly reclassified individuals from the number of individuals who are correctly reclassified (in the right direction (upward-classification of individuals with an event during follow-up; downward-classification of individuals free of an event during follow-up). As an orientation, changes in the C statistic of >0.01 and an NRI $>10\%$ indicate meaningful improvement in risk prediction when adding a new biomarker to a traditional model (Folsom, 2013). More recently, Pencina et al. (2011) presented a modified version of the NRI, the continuous or category-free NRI, that counts the direction of change in an extended risk prediction model for every person rather than the crossing of a threshold (Pickering and Endre, 2012). In scenarios where no established categories of risk exist (or risk categories have no clinical/practical consequences), it might be more appropriate to use the continuous NRI (Pencina et al., 2011).

As outlined by the AHA, novel biomarkers should improve the performance of risk prediction models beyond established risk factors (Greenland et al., 2010), including the reclassification of individuals in more appropriate risk strata, and the identification of particularly vulnerable people.

Biomarkers in Population Screening

One of the main purposes of screening biomarkers in the general population is to identify individuals who are at high risk of future CVD but who are currently free of clinical CVD and who might benefit from some sort of intervention (lifestyle modification or pharmacological treatment) to prevent clinically overt disease. In order to serve this purpose, it is important that such biomarkers capture the disease process at an early stage and, for example, detect subclinical changes of the vascular or cardiac system that might

predispose to overt CVD (Vasan, 2006; Naghavi et al., 2003) but that are potentially reversible or whose progression could be slowed down upon appropriate intervention. As an important hallmark, screening markers should have good discriminative abilities. Thus, they should be able to distinguish between individuals with future CVD and individuals who will remain free of CVD. One fundamental basis for such discrimination abilities is a very strong association between the biomarker level and future disease risk (Wald et al., 1999). In this context, it is worth noting that most causal risk factors (including high blood pressure and LDL-cholesterol) do not represent good screening tools because the distribution of risk factor levels in individuals with versus without the disease may substantially overlap leading to a low discrimination of these markers (Wald et al., 1999).

In primary prevention settings, high specificity of a biomarker is particularly important because mislabeling a healthy individual (predicting disease when it is likely to be absent) can cause unnecessary anxiety, medical follow-up examinations (with associated costs), and treatment and may outweigh the consequences of missing a rare condition (Piepoli et al., 2016). However, extensive community screening in people at relatively low risk of CVD is uncertain to be effective in reducing the risk of cardiovascular events. The costs of such screening are high, and it is conceivable that these resources may be more efficiently used in people with higher baseline CVD risk (Piepoli et al., 2016).

Official recommendations: Thus, the ESC does not recommend general systematic CVD risk assessment in men <40 years of age and in women <50 years of age without known risk factors (Piepoli et al., 2016). Still, systematic CVD risk assessment may be considered in adult men >40 years of age and in women >50 years of age or in postmenopausal women without known risk factors. Additionally, the ESC recommends a regular repetition of risk assessment, for example, every 5 years (Piepoli et al., 2016). Similarly, the ACC/AHA guidelines recommend the estimation of absolute 10-year CVD risk beginning at the age of 40 years. The estimation of long-term or lifetime risk is recommended for individuals between 20 and 39 years of age and for those between 40 and 59 years of age who are determined to be at low 10-year risk (<7.5%). For these low-risk persons, the ACC/AHA guidelines recommend a repetition of risk assessment every 4–6 years (Goff et al., 2014).

Risk Stratification

Traditional risk factors form the basis of established prediction models in order to estimate absolute disease risks, which can then be used to stratify a given population, for example, in individuals of low, intermediate, and high CVD risk. Even though the exact cutpoints might differ, this stratification has potential therapeutic/clinical consequences, for example, with respect to the initiation or intensity of risk factor treatment (Greenland et al., 2010).

Biomarkers could potentially help to improve the prediction of absolute disease risk and in identifying those individuals at particularly high risk for CVD (through a more accurate estimation of the absolute disease risk) and through subsequent more accurate reclassification into a more appropriate risk category. Thus, reclassification might be a suitable measure for the usefulness of a new biomarker in improving risk stratification.

Different Types of Biomarkers

Since CVD conditions evolve over the life course, overt CVD is typically preceded by a long period of cumulative exposure to elevated risk factor levels and subclinical alterations of the heart or the vascular system (Gibbons and Dzau, 1994; Cohn et al., 2000). Imaging biomarkers are best suited to detect the presence of subclinical CVD, genetic markers could capture a certain predisposition for a disease of interest, but since most DNA sequence information does not change over the life course, genetic markers are unlikely to capture progressive changes of a disease process or disease activity. Circulating biomarkers could theoretically capture all stages along the disease continuum, including subclinical disease, and changes in circulating biomarker levels could parallel changes in disease activity (Wang, 2011). The following paragraph summarizes recent evidence regarding defined circulating, genetic, and imaging biomarkers.

Circulating Biomarkers

C-reactive protein

C-reactive protein (CRP) is the most established marker of chronic systemic inflammation and has been consistently associated with future CVD in multiple epidemiological studies (Danesh et al., 2004; Buckley et al., 2009). In a large 2012 metaanalysis of 52 prospective studies including 246,669 participants, the addition of CRP to a prognostic model for CVD increased the C statistic only modestly by 0.0039 ($p < 0.001$) and yielded a NRI of 1.52% (Emerging Risk Factors Collaboration et al., 2012). In individual studies ($n = 6502$, $n = 6814$, $n = 3006$, respectively), increments in the C statistic from 0.697 to 0.701 ($p = 0.26$) (Seven et al., 2015), from 0.623 to 0.640 ($p = 0.03$) (Yeboah et al., 2012), and from 0.795 to 0.799 (Wilson et al., 2008) and NRIs of 3.9% (Seven et al., 2015), 7.9% (Yeboah et al., 2012), and 5.6% (Wilson et al., 2008) after adding CRP to FRS risk factors have been reported.

Official recommendations: According to the ACC/AHA guidelines, measurement of CRP for screening purposes may be reasonable for cardiovascular risk assessment in asymptomatic intermediate-risk men ≤50 years of age or women ≤60 years of age (Class IIb recommendation). But CRP measurement is not recommended for risk assessment in asymptomatic high-risk adults, or in low-risk men ≤50 years of age or women ≤60 years of age (Class III recommendation) (Goff et al., 2014; Greenland et al., 2010).

B-type natriuretic peptide

B-type natriuretic peptide (BNP) is synthesized and released from the myocardium as a response to blood pressure and volume overload. It has been recognized as a sensitive marker of acute heart failure (HF) and levels of BNP and of N-terminal prohormone of B-type natriuretic peptide (NT-proBNP), a precursor of BNP, were elevated in individuals with established CVD (Panagopoulou et al., 2013). Furthermore, BNP as well as NT-proBNP levels have also been investigated in community-based samples, and variations within the normal range were found to predict incident CVD in the general population. A metaanalysis of 40 cohort studies reported a summary RR of 2.82 (95% CI: 2.40–3.33) for incident CVD in those with a BNP level in the highest versus lowest tertile. The improvements in risk discrimination (C statistic) were modest, generally ranging from 0.01 to 0.1 (Di Angelantonio et al., 2009). Similarly, in several cohort studies in primary prevention settings, BNP and NT-pro BNP led to relatively minor increments in the C statistic (from 0.01 to 0.03) (Zethelius et al., 2008; Melander et al., 2009; Olsen et al., 2007). In two studies, reclassification was assessed and found to be statistically nonsignificant for cardiovascular events (Melander et al., 2009) or cardiovascular mortality (Zethelius et al., 2008). In another individual-participant-data metaanalysis of 95,716 participants, the addition of NT-proBNP to a model containing conventional risk factors increased the C statistic by 0.012 and 0.019 and yielded NRIs of 2.7% and 2.8%, for two different combinations of endpoints, respectively (Natriuretic Peptides Studies Collaboration et al., 2016). In two United Kingdom-based cohort studies using two different risk scores, NT-proBNP was associated with increased CVD risk and resulted in an increase in the C statistic by 0.017 ($p=0.005$) for one of the risk scores but did not improve the C statistic of the other risk score. However, NT-proBNP yielded NRIs of 5.9% and 4.7% (Welsh et al., 2016). Interestingly, in studies that compared BNP (or NT-proBNP) with CRP, BNP/NT-proBNP have demonstrated consistently stronger associations with vascular events and a higher predictive value for cardiovascular and coronary events than CRP (Zethelius et al., 2008; Olsen et al., 2007; Blankenberg et al., 2006; Wang et al., 2004).

Official recommendations: The ACC/AHA 2010 guidelines note that we do not expect a benefit from measurement of natriuretic peptides for CVD risk assessment in asymptomatic adults at the moment due to uncertain discrimination and reclassification values and thus do not recommend this approach for screening in the general population (Class III recommendation) (Greenland et al., 2010).

Lipoprotein-associated phospholipase A2

A newer biomarker in the field of risk prediction is Lipoprotein-Associated Phospholipase A2 ($LpPLA_2$). This enzyme is expressed by inflammatory cells and is bound to lipoproteins, primarily LDL. $LpPLA_2$ leads to the production of proinflammatory factors (Zalewski and Macphee, 2005). A metaanalysis of 32 prospective studies revealed a linear relation between $LpPLA_2$ activity and mass and vascular risk in individuals with and without CVD (Thompson et al., 2010). However, there are only few data concerning the ability of $LpPLA_2$ to improve risk discrimination, calibration, or reclassification. Two studies reported a significant increment in the area under the ROC curve although the increments were relatively small (0.006 and 0.022, respectively) (Daniels et al., 2008; Folsom et al., 2006). In another study of 765 participants, $LpPLA_2$ resulted also in a moderate statistically nonsignificant increase in the area under the ROC curve (0.02, $p=0.31$) and was associated with a statistically nonsignificant NRI of 9.5% (Tsimikas et al., 2009). However, in a Multiethnic cohort of 1821 female CVD patients and a matched subcohort of 1992 women, the association between $LpPLA_2$ and CVD risk disappeared after adjusting for traditional cardiovascular risk factors. The addition of $LpPLA_2$ to two predictive models improved the C statistic by only 0.005 and 0.006 and resulted in a small NRI of 2.7% and 3.5%, respectively (Cook et al., 2012).

Official recommendations: The ACC/AHA guidelines indicate that $LpPLA_2$ might be reasonable for cardiovascular risk assessment in asymptomatic adults with intermediate risk (Class IIb recommendation) (Greenland et al., 2010).

Troponin

Cardiac troponins are released in response to cardiomyocyte necrosis, reflect myocardial cell damage, and are mainly measured in clinical settings in patients for the diagnosis of acute myocardial infarction (MI) (Cahill et al., 2015). Recently, new techniques have been developed that are sensitive enough for detecting low concentrations of circulating troponin and, thus, are usable to measure troponin in general population samples (Cahill et al., 2015). Within the BiomarCaRE project (Blankenberg et al., 2016), a large consortium of multiple clinical and community-based prospective cohort studies across Europe, troponin I emerged as an independent predictor for CVD, cardiovascular mortality, and total mortality in the general population (total sample size, $n=74{,}738$). In moderate to large individual studies (AGES, $n=5764$; the Scottish Heart Health Extended Cohort, $n=15{,}340$; ARIC, $n=9698$), troponin was consistently associated with CVD, coronary heart disease (CHD), or related endpoints (Thorsteinsdottir et al., 2016; Zeller et al., 2014a; Saunders et al., 2011). Adding troponin I to standard prediction models led to increments in the C-statistic ranging from 0.0044 (Zeller et al., 2014a) to 1.4 (Thorsteinsdottir et al., 2016) and NRIs were between 3.2% (Zeller et al., 2014a) and 4.8% (Blankenberg et al., 2016).

Official recommendations: So far, there are no official recommendations regarding the use of troponin for screening and risk prediction for CVD in asymptomatic individuals or the general population.

In summary, existing circulating biomarkers lead to relatively modest improvements in risk prediction. Their clinical utility, cost-effectiveness, and potential ability to change patient management and outcome need to be further evaluated. Two of the most comprehensively studied circulating biomarkers for CVD risk are CRP and BNP/NT-proBNP. While both biomarkers are clearly positively associated with CVD in various settings, improvements in discrimination and risk reclassification are generally only moderate.

Genetic, Metabolomic, and Proteomic Biomarkers

Genetics

In the past decade, multiple common genetic variants have been identified that are reproducibly associated with clinical CVD events, particularly with coronary artery disease (CAD) (Lieb and Vasan, 2013). By contrast, genome-wide association studies for subclinical disease traits revealed substantially fewer associated SNPs (Vasan et al., 2009). One of the most consistently associated loci is the 9p21 locus, which has been associated with CAD as well as with coronary artery calcification and MI (O'Donnell et al., 2011). Since most of these loci were discovered in large consortia with large samples, effect sizes were typically rather small, ranging from 10% to 30% increased risk per copy of the risk allele, despite the statistically significant associations between genetic variants and MI risk. These effects are expected to result in only modest improvements, if any, in risk discrimination or calibration (Samani et al., 2007; McPherson et al., 2007; Kathiresan et al., 2009). More recently, also low-frequency and rare genetic variants are being evaluated in the context of subclinical and clinical CVD. For example, an exome-wide study revealed modest associations of coronary artery calcium and carotid intima-media thickness with APOB and APOE variants, respectively (Natarajan et al., 2016) and comprehensive genotyping and sequencing efforts revealed rare mutations in ANGPTL4, LPL, and SVEP1 to be associated with CVD risk (Myocardial Infarction Genetics et al., 2016).

Furthermore, combinations of genetic variants (genetic risk scores) have been evaluated in the context of clinical and subclinical CVD. For example, in two observational studies ($n = 1154$ and $n = 4392$), a polygenic risk score was significantly associated with a greater risk of coronary artery calcification and carotid plaque, respectively (Natarajan et al., 2017). And in a joint investigation of five prospective population cohorts, a genomic risk score was associated with incident CHD. In two of these five studies a fixed-effects metaanalysis was performed and revealed that addition of this score to traditional risk factors significantly improved the C statistic (by 0.016) and yielded a NRI of 10% (Abraham et al., 2016). Similarly, in a study using data of nine prospective European cohorts, two different genetic risk scores improved net reclassification over the FRS for coronary risk prediction and one of the scores also improved discrimination (Hughes et al., 2012). A review on the clinical usefulness of genetic tests for atherosclerosis reported additive effects of a high genetic risk score (including 50 genetic variants) and self-reported family history with respect to the prediction of incident CHD (Paynter et al., 2016). Another recent study demonstrated that genetic risk scores and lifestyle factors interact in their association with CAD (Khera et al., 2016). Cross-classifying individuals based on their genetic risk and their adherence to a favorable lifestyle revealed that a more favorable lifestyle was associated with lower risk of coronary events, regardless of the genetic risk category (Khera et al., 2016). Finally, genetic risk scores might help identifying individuals that might benefit from statin therapy in primary prevention settings, in the sense that statin therapy leads to a greater reduction in relative risk in those with a high genetic risk score (Natarajan et al., 2017).

Official recommendations: Because of small odds ratios and small incremental risk information of individual genetic polymorphisms, the ACC/AHA and the ESC do not recommend genotype testing for CHD risk assessment in asymptomatic adults (Class III recommendation) (Greenland et al., 2010; Piepoli et al., 2016).

Metabolomics and proteomics

Metabolites are small biochemicals and end-products of complex regulatory pathways that provide information about metabolic processes. In a prospective study of three population-based cohorts, a panel of 68 lipids and metabolites was evaluated in relation to incident CVD and revealed that, after adjusting for several CVD risk factors, higher serum phenylalanine and monounsaturated fatty acid levels were associated with increased CVD risk, whereas higher omega-6 fatty acids and docosahexaenoic acid levels were associated with lower risk (Wurtz et al., 2015). A risk score including these four biomarkers yielded a NRI of 7.6% and 5.3%, respectively, with the majority of individuals being reclassified belonging to the intermediate risk group (Wurtz et al., 2015). In analyses of metabolite profiles and their association with prevalent and incident atrial fibrillation in 2458 Framingham Heart Study participants (Ko et al., 2016), none of the examined amino acids, organic acids, lipids, and other plasma metabolites was significantly associated with the risk of future atrial fibrillation. In this analysis, previous results that demonstrated a relation between metabolites and atrial fibrillation could not be replicated (Ko et al., 2016). In a mass spectrometry-based nontargeted metabolomics approach, four metabolites (lysophosphatidycholine 18:1, lysophosphatidycholine 18:2, monoglyceride 18:2, sphingomyelin) were associated with incident CHD in multivariable-adjusted models and, in combination, led to moderate improvements in discrimination (C statistic: 0.76 vs. 0.75) and reclassification (NRI: 9.2%) (Ganna et al., 2014). In people with established CVD, a large panel of circulating protein markers ($n = 1130$) has been evaluated and revealed that a set of nine proteins is associated with cardiovascular outcomes and improves discrimination by 0.05–0.09 after addition to the Framingham secondary event risk model (Ganz et al., 2016).

Imaging Biomarkers

Coronary artery calcium

A number of observational studies have reported strong associations between coronary artery calcification (CAC) and the risk of incident cardiovascular events (Helfand et al., 2009; Pletcher et al., 2004; Greenland et al., 2007; Hoffmann et al., 2016). Most reports indicated a RR of coronary events of ≥ 2 for those with any CAC compared with those with no CAC, a RR of >4 for those with CAC scores >100, and a RR of >10 for persons with a score of >1000 (Pletcher et al., 2004; Greenland et al., 2007). Available evidence also suggests that CAC scores may improve discrimination. In the MESA and the Rotterdam Studies, CAC scores

were strong predictors of cardiovascular and coronary events and raised the C statistic from 0.77 (with traditional risk factors) to 0.81 (plus CAC score) and from 0.72 to 0.76, respectively (Elias-Smale et al., 2010; Folsom et al., 2008). There is also increasing evidence that CAC scores improve reclassification, particularly for individuals at intermediate risk. In the MESA cohort, 23% of individuals with events were correctly classified upward and 13% without events were correctly classified downward (Polonsky et al., 2010). Analyses in the Framingham Heart Study (n=3486) revealed similar results, displaying consistent association of CAC with incident major CHD and CVD in multivariable-adjusted statistical models. CAC significantly improved discrimination beyond traditional risk factors for CHD (C statistics, 0.78–0.82), but not for CVD, and provided substantial reclassification for CVD and CHD where two-thirds of the intermediate-risk population were reclassified accurately (Hoffmann et al., 2016).

In the MESA cohort, the impact of 13 negative risk markers has been evaluated and revealed that among all negative CVD risk markers analyzed, a CAC score of 0 resulted in the largest proportion of correctly downward reclassified individuals (Blaha et al., 2016). In a separate report from the MESA cohort, a CAC score >100 had a significant HR of 4.6 for CHD in low lifetime risk individuals. Adding CAC to traditional risk factors increased the area under the ROC curve from 0.775 to 0.806, though this difference was not statistically significant (Joshi et al., 2016).

It has to be kept in mind, though, that CAC scanning goes along with exposure to ionizing radiation. This is particularly relevant when considering CAC scanning for broad applications on the population level. Thus, the health consequences associated with exposure to ionizing radiation have to be weight against the potential benefits with respect to cardiovascular prevention on the population level (Lauer, 2009).

Official recommendations: The ACC and AHA consider the measurement of CAC as reasonable for cardiovascular risk assessment in asymptomatic adults at intermediate risk (10%–20% 10-year risk) (Class IIa recommendation), and it may also be reasonable in persons at low to intermediate risk (6%–10% 10-year risk) (Class IIb recommendation). Due to the radiation exposure and general low prevalence of calcification in individuals with no or few other risk factors, persons at low risk (<6% 10-year risk) should not undergo CAC scan for cardiovascular risk assessment (Class III recommendation) (Goff et al., 2014; Greenland et al., 2010). The ESC released similar recommendations (Piepoli et al., 2016).

Carotid intima-media thickness

Another established technology for assessing subclinical atherosclerosis is ultrasound of the carotid artery (Nezu et al., 2016). Multiple epidemiological studies and a recent metaanalysis have reported an independent association of carotid intima-media thickness (IMT) with cardiovascular events although estimates of effect vary widely. However, Folsom and colleagues reported that carotid IMT led to a small increase in the C statistic (0.77–0.78) for predicting cardiovascular or coronary events (Folsom et al., 2008). In the ARIC study, the addition of information on carotid IMT and plaque led to an improvement in the area under the ROC from 0.742 to 0.755 and to a NRI of ~9.9% among intermediate-risk individuals (10%–20% 10-year risk) (Nambi et al., 2010). A metaanalysis, based on 15 population-based cohorts, reported a consistent association, but only moderate and statistically nonsignificant ($p=0.8$) increments in the C statistics from 0.726 (model with traditional CVD risk factors only) to 0.729 with addition of carotid IMT (van den Oord et al., 2013). In 2965 members of the Framingham Offspring Study cohort, the addition of IMT of the internal carotid artery to the FRS increased the C statistic modestly but statistically significantly by 0.009 and reached a statistically significant NRI of 7.6%, whereas the IMT of the common carotid artery did not significantly improve discrimination and reclassification (Polak et al., 2011). Recently published data from the MESA study indicate that IMT confers incremental information beyond the traditional FRS (increase in the area under the ROC curve from 0.721 to 0.7396 ($p=0.0008$)) and beyond a model including traditional risk factors and CAC (increment in the C statistics from 0.7627 to 0.7714 ($p=0.02$)) (Polak et al., 2017). By contrast, a recent metaanalysis could not show any added value of IMT compared with the FRS for the prediction of future CVD (Den Ruijter et al., 2012).

In epidemiological and clinical trial settings, carotid IMT is usually assessed using highly standardized procedures and specifically trained personnel and centralized reading centers (Greenland et al., 2010). It is uncertain whether a comparable level of standardization can be achieved and whether subtle differences in IMT between individuals with different cardiovascular risk were detectable, if IMT measurements were broadly used as screening tools in the population (Wang, 2011). In this context, the difficulty of standardization of the definition and measurement of IMT, its high variability and low intraindividual reproducibility have raised concerns (Piepoli et al., 2016).

Official recommendations: Thus, the ACC and AHA and the ESC do not recommend the routine measurement and systematic use of carotid ultrasound IMT in clinical practice for risk assessment (Class III recommendation) (Goff et al., 2014; Piepoli et al., 2016).

Flow-mediated dilation

Flow-mediated dilation (FMD) is an important marker of vascular function and can be assessed with the use of ultrasound of the brachial artery. Impaired FMD has been associated with an adverse cardiovascular risk profile, including hypertension and hyperlipidemia, and was found to predict clinical CVD events in the community (Greenland et al., 2010; Brevetti et al., 2003; Yeboah et al., 2009). However, in the MESA cohort, FMD did not improve the C statistic and inappropriately down-reclassified 23% of individuals who experienced events (Yeboah et al., 2009). A study, comparing the predictive value of different risk markers for cardiovascular risk assessment, revealed no association of FMD with incident CHD and FMD led to the lowest improvement in the area under the ROC curve (0.016) compared to other biomarkers and also yielded only a small NRI of 2.4% (Yeboah et al., 2012). In a 2014 published study of 618 subjects, individuals above the median of FMD (>11.3%) had a statistically significant OR of 2.78

for cardiovascular events compared with individuals at or below the median. However, alterations in discrimination and reclassification were not reported in that study (Shechter et al., 2014).

Furthermore, brachial ultrasound might not be suitable for broad application as a screening tool, since its standardized performance requires substantial training (Wang, 2011).

Official recommendations: The ACC and AHA do not recommend peripheral arterial FMD for cardiovascular risk assessment in asymptomatic adults (Class III recommendation) (Greenland et al., 2010).

Ankle–Brachial index

The ankle–brachial index (ABI) is a simple and noninvasive measure for the presence of peripheral arterial disease (PAD; indicated by a value of <0.9) (Greenland et al., 2010). ABI values also show a graded relation with the risk for other CVD events and mortality beyond traditional risk factors, for example, in a large metaanalysis including 24,955 men and 23,399 women free of CHD at baseline (Fowkes et al., 2008). In that analysis, discrimination was improved, with the area under the ROC curve increasing from 0.646 to 0.655 in men and from 0.605 to 0.658 in women, after adding the ABI to the FRS. Reclassification occurred in 19% of men and in 36% of women (Fowkes et al., 2008). Associations with stroke have also been reported (Gronewold et al., 2014). However, in that study (n=4299), the C-statistic increased only slightly and nonsignificantly (from 0.751 to 0.767) and the NRI was only 1.5% (Gronewold et al., 2014).

Official recommendations: The ACC and AHA and the ESC state that the measurement of ABI may be considered for cardiovascular risk assessment in asymptomatic adults at intermediate risk (Class IIa-IIb recommendation) (Goff et al., 2014; Greenland et al., 2010).

Left ventricular structure and function

Alterations in cardiac structure and function are important precursors of CVD, particularly of HF in the community (Greenland et al., 2010). Left ventricular mass (LVM) (Levy et al., 1990) and left ventricular hypertrophy (LVH) (Okwuosa et al., 2015), as well as change in both parameters over time (Lieb et al., 2014; Fox et al., 2015), have consistently been associated with incident CVD events. On a parallel note, slight impairments of cardiac function are intermediate phenotypes predicting incident HF (Wang et al., 2003). Since LVM, LVH, and impaired systolic function represent subclinical changes of the cardiovascular system with clear associations to clinical endpoints, such alterations represent potentially interesting candidate phenotypes for population screening. However, their ability to improve risk prediction models is only modest. In the ARIC cohort (n=14489 participants), electrocardiography (ECG)-determined LVH was associated with CVD and CHD events but did not significantly reclassify or improve the C statistic compared with FRS alone (Okwuosa et al., 2015). In the elderly (aged $\geq$65 years), LVH determined by both echocardiography and ECG was predictive of HF and increased the C statistic equally when added to traditional risk factors (Almahmoud et al., 2015). Similar observations of a comparable association of ECG- and echocardiography-assessed LVH was reported in another analysis with stroke in the same cohort (O'Neal et al., 2015).

Alterations in cardiac structure and function can be assessed by imaging technologies (including ultrasound and MRI); specific ECG changes are also indicative of changes in cardiac structure. Vasan and colleagues tested the hypothesis, whether serum markers of neurohormonal activation correlate with and could potentially be used to screen for alterations in cardiac structure and function in the community. BNP and N-terminal proatrial natriuretic peptide (NT-ANP) were positively associated with elevated LVM and left ventricular systolic dysfunction in the Framingham cohort. However, the area under the ROC curve for both outcomes was at or below 0.75 for both peptides, respectively, and increased only moderately (0.02-0.03) when added to clinical risk factors. Thus, the improvements in discrimination were moderate suggesting suboptimal/limited performance as screening tools as screening tool for elevated LVM and left ventricular systolic dysfunction in the community (Vasan et al., 2002).

Official recommendations: Because the methodology for LVH measurement by echocardiography can vary widely between studies, the ACC and AHA recommend the use of methodology and cut-points as defined by the American Society of Echocardiography (Greenland et al., 2010; Nagueh et al., 2016). Due to the lack of convincing evidence that echocardiography improves cardiovascular risk classification, and because of the logistical challenges in performing it, the ESC does not recommend echocardiography for CVD risk prediction (Class III recommendation) (Piepoli et al., 2016). The ACC and AHA do recommend consideration of echocardiography to detect LVH for cardiovascular risk assessment only in asymptomatic adults with hypertension (Class IIb recommendation), but not for asymptomatic individuals without hypertension (Class III recommendation). According to the ACC and AHA 2010 guidelines, resting ECG is reasonable for cardiovascular risk assessment in asymptomatic adults with hypertension or diabetes (Class IIa recommendation) and may be considered for cardiovascular risk assessment in asymptomatic adults without hypertension or diabetes (Class IIb recommendation) (Greenland et al., 2010).

In summary, several imaging biomarkers display consistent associations with CVD and imaging biomarkers are particularly suitable to detect existing subclinical disease. Given that subclinical changes are important precursors of clinical events and indicate structural or functional alterations of the cardiovascular system, these markers are potentially interesting for screening purposes to identify individuals at high risk of clinical events (Wang, 2011). However, the overall evidence for most imaging biomarkers in terms of improving risk prediction and risk stratification is still uncertain and needs to be further studied. The best predictive performance of imaging biomarkers has been observed for CAC, CAC, which can detect prevalent atherosclerosis at the coronary arteries. However, potential risks associated with a population-wide application of CAC scanning have to be taken into consideration (Wang, 2011).

Combination of Multiple Biomarkers (Risk Scores)

Due to the relatively modest performance of individual biomarkers for risk prediction, multimarker scores, including information from multiple biomarkers, have been generated in order to predict disease risks and patient outcomes more accurately (Wang, 2011; Bodi et al., 2005; Newby et al., 2001; Sabatine et al., 2002). Multimarker approaches can, for example, include either several circulating biomarkers (Wang et al., 2006) or multiple genetic markers (Kathiresan et al., 2008). Some multimarker scores also include imaging markers (Cao et al., 2007). One of the first multimarker paper focused on 10 circulating biomarkers, reflecting different pathways, to predict incident CVD in the Framingham Offspring Study ($n = 3209$ individuals) and revealed that the urinary albumin-to-creatinine ratio and BNP levels displayed the strongest associations with CVD among the markers evaluated. However, the addition of these biomarkers led only to small increases in discrimination when added to conventional risk factors (Wang et al., 2006). In 5067 individuals from the Malmo Diet and Cancer Study, six biomarkers were assessed and tested for association with coronary and cardiovascular events. The C statistic was raised by only ~0.01 and reclassifications did not improve (NRI = 0%) (Melander et al., 2009). In 2010, a panel of 30 biomarkers was evaluated in the MORGAM study. None of the biomarkers alone improved discrimination significantly, however, an optimal combination of NT-proBNP, CRP, and troponin I reached a statistically significant improvement of the C statistic by 0.03. The NRI was 11% (Blankenberg et al., 2010). And in participants with dysglycemia, a set of 15 (out of 237 evaluated) cardiometabolic biomarkers were independent determinants of cardiovascular outcomes or death. The C statistic increased from 0.64 to 0.68 (combined endpoints: MI, stroke, cardiovascular death, HF hospitalization, revascularization), from 0.64 to 0.71 (combined endpoints: MI, stroke, cardiovascular death), and from 0.64 to 0.75 (endpoint: all-cause death) (Gerstein et al., 2015).

The use of imaging markers (Carotid IMT and plaque) combined with circulating biomarkers (CRP) was tested, for example, in the Cardiovascular Health Study (n=5888 elderly individuals; mean age, 73 years). Interestingly, elevated CRP predicted cardiovascular events only in individuals with carotid plaque, whereas carotid plaque predicted events independently from baseline CRP. The C statistic was raised by ~0.02 with the addition of carotid measures to traditional risk factors and by <0.01 with the addition of CRP. Reclassification was not assessed (Cao et al., 2007).

The Malmo Diet and Cancer Study tested a combination of multiple genetic markers. A score including nine lipid-associated SNPs was incorporated into risk models and proved to be a significant predictor of cardiovascular events even after adjustment for measured lipid levels. This score did not improve the C statistic but was associated with a moderate improvement in classification (NRI = 6.2%) (Kathiresan et al., 2008). In 6041 Danes, a constructed genetic risk score consisting of 45 genetic risk variants was tested for its ability to estimate the risk of incident MI and CAD. The genetic risk score was significantly associated with the risk of MI but not with CAD, and it did not improve either discrimination or NRI after addition to the European SCORE (Krarup et al., 2015).

When multimarker scores are used, several factors have to be taken into consideration: Biomarkers that have been measured on the same set of individuals and at the same time or belong to the same pathway are likely to have an inherent correlation, and therefore, with the number of biomarkers in a score increasing the C statistic will not automatically increase equally (Wang, 2011; DeLong et al., 1988). Furthermore, multimarker algorithms may need to weigh individual biomarkers differentially depending on the endpoint being predicted (Morrow and Braunwald, 2003).

Summary and Perspectives

Identifying individuals with elevated risk of incident CVD is an important public health priority. Thus, a range of promising biomarkers for CVD risk prediction have been evaluated in the past decades. Many markers have been reported that display strong and consistent associations with CVD endpoints. However, the added value beyond traditional risk factors of most novel biomarkers is limited in most scenarios. Only a minority of markers is, therefore, recommended for screening purposes or to improve risk stratification in the community. For example, one of these biomarkers is CAC that has been shown to improve discrimination and risk reclassification and is considered reasonable for cardiovascular risk assessment in asymptomatic adults at intermediate risk (Class IIa recommendation).

Novel biomarkers are constantly being studied, for example, within the international BiomarCaRE consortium (Blankenberg et al., 2016; Zeller et al., 2014b). It has to be kept in mind, though, that a broad application of screening biomarkers might require specific training programs for physicians to enable them to adequately interpret the results of complex marker profiles (Vasan, 2006). Furthermore, ethical and regulatory issues, e.g. in the context of genetic biomarkers, need to be addressed appropriately (Vasan, 2006; Hook et al., 2004; Hackett and Gutman, 2005).

Therefore, the value of novel biomarkers for screening and risk prediction issues has to be further analyzed and weight against potential clinical, practical, and ethical disadvantages and challenges.

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Biophysics of Ablation—Radiofrequency and Cryoablation

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Introduction

Cardiac arrhythmias remain a significant clinical problem. Despite advances in pharmacologic management, many patients are left with refractory arrhythmias due to clinical inefficacy, or experience significant cardiac and noncardiac toxicities (such as proarrhythmia, heart failure, or organ toxicity) (Fuster et al., 2006; Roy et al., 2000; Singh et al., 2003; Nademanee et al., 2008; Corley et al., 2004). Given the limitations of pharmacologic therapies, alternate approaches were sought for the treatment of cardiac arrhythmias when drugs have been ineffective or cannot be tolerated. The development and evolution of percutaneous catheter ablation for the treatment of cardiac arrhythmias represents a major accomplishment in the field of cardiology.

The clinical use of catheter ablation for the treatment of cardiac arrhythmias is approximately 35 years old, having been first used clinically in the early 1980s as an alternative to technically difficult surgical ablation (Scheinman et al., 1982; Gallagher et al., 1982; Borggrefe et al., 1989). Employing the use of high- and low-energy direct current shocks, initial studies concentrated the use of this technique on reasonably well-understood focal arrhythmia substrates such as the atrioventricular junction or His-Bundle (i.e., complete or total atrioventricular node ablation), AV reentrant tachyarrhythmias (i.e., AV node reentry or accessory AV pathways), and ischemic ventricular tachycardia (i.e., diastolic pathway or VT exit site) (Scheinman et al., 1982; Gallagher et al., 1982; Borggrefe et al., 1989; Newman et al., 1989; Lemery et al., 1989, 1992). Unfortunately, these early ablation procedures were long (>4–8 h), complicated, and associated with a significant potential for complications (myocardial perforation, unintended AV block, venous thrombosis, myocardial depression, proarrhythmia, and sudden death), which largely limited them to patients with malignant arrhythmias or severe comorbidities.

Technological advancements in the late 1980s led to the development of catheters capable of delivering continuous-wave, unmodulated radiofrequency (RF) energy. More recently, novel catheter designs and ablation energy modalities have been sought in an effort to address the perceived limitations associated with RF ablation. This article will focus on the background and lesion science associated with the two dominant energy modalities: (1) RF and (2) cryothermal ablation. An enhanced understanding of lesion science can result in more optimal ablation procedures and improved patient outcomes.

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RF Ablation

The shift from direct current to RF ablation represented a major advance in the field of invasive cardiac electrophysiology. Compared to direct current shocks, RF ablation offered the advantages of: (1) minimal discomfort during energy delivery (allowing the procedure to be performed in conscious patients), (2) no skeletal- and cardiac-muscle stimulation, (3) relatively discrete ablation lesions without barotrauma, and (4) the potential for the premature termination of ablation in an effort to avoid impending complications (Morady, 1999). As such, RF energy provided a reasonably low-risk treatment option and led to the rapid and widespread adoption of percutaneous catheter ablation for the treatment of supraventricular and ventricular arrhythmias.

RF Lesion Formation

The RF ablation circuit consists of an RF generator, the ablation catheter, and the indifferent electrode (Fig. 1). Current is delivered via the catheter electrode and passes through the tissue (and patient) before returning to the generator by the indifferent electrode. It is important to note that the patient represents an important part of the ablation circuit. As current flows through the rest of the patient there is a loss of approximately 30%–40% of total delivered power, particularly in the area near the indifferent electrode patch.

Lesion formation with RF results from the combination of direct *resistive heating* at the catheter electrode interface, and *conductive heating* to the surrounding myocardium (Fig. 2). Resistive heating is the process of generating heat by passing an electric current through a resistive material, which dissipates power in that material. In the case of RF ablation, the catheter electrode delivers high-frequency (usually ~500 kHz) alternating electrical current between the catheter tip and indifferent electrode. This passage of alternating current from the low electrical resistance catheter electrode to the high electrical resistance body tissue results in acceleration of ions in the cardiac cells generating heat. The rise in tissue temperature interrupts the transmission of myocardial signals and results in a lesion.

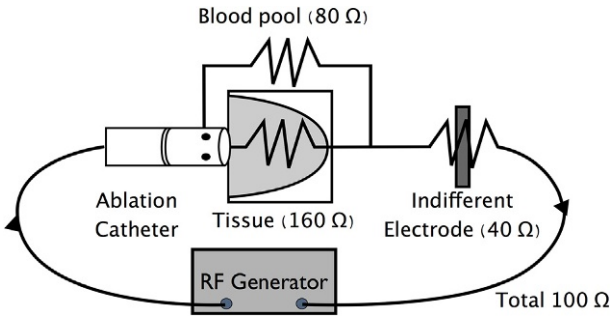

Fig. 1 The RF ablation circuit consists of an RF generator, the ablation catheter, and the indifferent electrode. Current is delivered via the catheter electrode, passes through the tissue (and patient) before returning to the generator by the indifferent electrode. The myocardial tissue and blood pool represent resistance circuits in parallel, with the indifferent electrode and patients body representing resistance circuits in series. In this theoretical example the total resistance is 100 Ω.

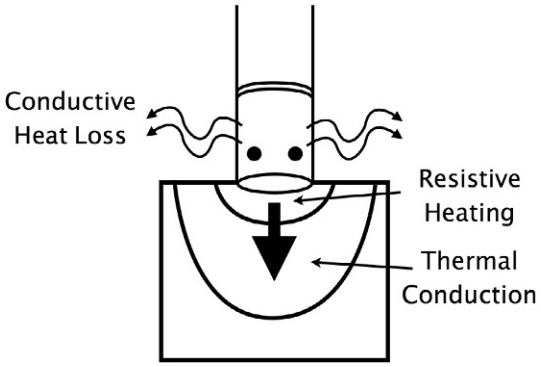

Fig. 2 Lesion formation with RF ablation. Lesion formation with RF results from the combination of direct *resistive heating* at the catheter electrode interface, and *conductive heating* to the surrounding myocardium. In general the effects of RF ablation are reversible up to a tissue temperature of ~50°C. Up to 50°C there is a decreasing action potential duration and amplitude, with progressive cellular depolarisation associated with increase in intracellular calcium. Beyond 50°C there is an irreversible loss in excitability, associated with hyperthermia-induced changes in ionic transport, cytoskeleton collapse, sarcolemmal disruption, and mitochondrial dysfunction.

Recall that current density rapidly decreases with the fourth power of distance from the RF current source. As such the majority (~90%) of delivered power is quickly absorbed into only a small volume of tissue (~2 mm) in close opposition with the catheter electrode tip. The heating of this rim of tissue is quick, with a monoexponential temperature rise to reach steady state in a few seconds (t½ 5–10 s). However, the resistive heating emanates and spreads in a radial fashion to the deeper, cooler tissue layers through microscopic collisions of particles. This transfer of thermal energy (thermal conduction) accounts for the majority of the lesion volume generated by RF ablation. However, the lesion expansion is relatively slow, taking 1–2 min to reach the point of stabilization (i.e., the point where the energy entering the tissue from the thermal source equals the amount of energy that is being dissipated, or "thermal equilibrium"). It is important to note that the extent of lesion penetration will be balanced by conductive heat loss to contiguous tissue as well as convective heat loss to both the circulating blood pool and the intramyocardial circulation.

Temperature and Lesion Formation With RF

RF lesion formation requires achieving tissue temperatures >50°C or higher to result in tissue desiccation, blood coagulation, and protein denaturation. In practice RF lesion size increases linearly with tissue temperature up to the point of plasma coagulation at the catheter–tissue interface (~100°C). These denatured serum proteins form an electrically insulating coagulum along the electrode interface, with the subsequent rise in impedance limiting the penetration of ablation energy into the tissue. Moreover, high tissue temperatures are associated with the buildup of intramural gases with resultant risk of perforation (e.g., carbonization due to excessive heating resulting in a "steam pop") (Nath et al., 1993).

Unfortunately, the relationship between lesion size and electrode tip temperature is variable. This is because the ablation electrode tip temperature (provided to the operator on the RF generator) is not a true a reflection of tissue temperatures (Hogh Petersen et al., 1999). Specifically, the ablation electrode itself does not "heat up" when delivering RF. The temperature rise occurs directly in the tissue and is then conducted back to the tip of the catheter electrode where it is recorded. As such the actual myocardial temperature is usually significantly higher than measured electrode temperature. From a practical perspective this concept is best illustrated by the contrasting lesion dimensions and electrode temperatures observed with nonirrigated RF ablation in a "wedged" state (high electrode temperature with small myocardial lesion) versus irrigated ablation (low electrode temperature with large myocardial lesion). Therefore, the difference in myocardial temperature and electrode temperature must be considered in order to minimize the risk of thrombus formation or the occurrence of a steam pop.

Other Factors Affecting Lesion Formation

While lesion size is directly proportional to tissue heating, the size of an ablation lesion created is ultimately dependent on a multitude of factors including RF power level, duration of RF delivery, convective cooling (e.g., passive blood flow over the electrode–tissue interface, or active cooling with catheter electrode irrigation), electrode size, electrode orientation, and electrode tip/tissue contact.

RF Power, Energy, and Current

Experimental models have demonstrated that RF lesion size increases linearly with RF energy (J), power (W), and current (mA) delivery (**Fig. 3**) (Nath et al., 1993; Hindricks et al., 1989). This is because the use of higher power will result in a combination of: (1) increased source temperature and (2) increasing the radius of heated tissue, which in turn increases the size of the heat source (i.e., zone of resistive heating). However, it is important to realize that in vivo only a fraction of "delivered" energy (or power) is used for lesion formation, with the balance of "useful" energy (i.e., delivered to the tissue and used for lesion created) to "wasted" energy (i.e., in the blood pool and rest of the patient) being determined by a combination of: (1) the degree of electrode surface

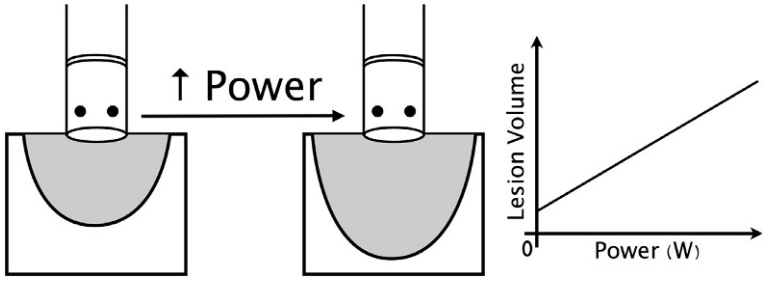

Fig. 3 The effect of increasing power on lesion volume during RF ablation. In general there is a linear relationship between increasing power and lesion volume, with increasing power resulting in deeper penetration of heat. However very high power is limited by emergence of complications such as plasma coagulation at the catheter–tissue interface (which serves as an insulator and prevents heat transfer to the tissue), as well as the buildup of intramural gases (steam pop) (Nath et al., 1993).

contact relative to the target tissue and surrounding blood pool and (2) the relative electrical conductivity of the blood compared to the target tissue (approximately two-fold greater for blood than that of tissue).

Consider the following. In a typical circumstance, approximately 25% of the ablation catheter electrode surface will be in reasonable contact with the target tissue (note: this is highly variable depending on electrode size, orientation, and patient anatomy; however even in ideal circumstances it is highly unlikely that the electrode is ever in >50% contact with the target tissue). As such, with 25% electrode–tissue contact the remaining 75% of the electrode surface will be in contact with blood pool. Thus, when coupled with the two times greater conductivity of blood, this 25% tissue contact will result in a transfer of power to tissue that is roughly six times lower than that delivered to blood pool. Adding in the loss of power to the indifferent electrode (see below) it would be expected that only 8.5% of the total power (or 4.3 W of delivered 50 W) will reach the target endocardial tissue (i.e., 40% loss to patient via indifferent electrode, meaning the remaining 60% of the total power is divided 6:1 between blood and tissue).

While increasing overall power delivery can overcome some of the limitations associated with the power loss to blood pool, excess heating of the electrode tip may result in the formation of thrombus/coagulum which can further impede the flow of electrical current by increasing impedance.

Ablation Circuit Impedance

For unipolar RF ablation the power dissipated throughout the circuit is proportional to the impedance and voltage drop of each component within the circuit. In practice most of the delivered power is shunted through the lower-resistance blood pool (the myocardial tissue and blood pool represent resistive circuits in parallel), with power loss in the return path (the patients body and indifferent electrode patch in series). As such it is important to consider that the return path (patient) plays a significant role in the ablation circuit, accounting for a loss of approximately 30%–40% of the delivered power.

As such, strategies to limit this loss near the indifferent electrode patch, particularly by reducing the indifferent electrode resistance, can result in an increase in lesion size. Returning to our previous example, reducing indifferent electrode resistance from 40% to 20% of the total resistance will result in a significant increase in the power deposited into the myocardial tissue (5.4 W vs. 4.3 W of the 50 W delivered to the catheter). Practically this is accomplished by the positioning of the indifferent electrode closer to the ablation site (i.e., on the trunk rather than the thigh), or using two dispersive electrodes in cases of high baseline impedance.

RF Frequency

RF energy is delivered as alternating electrical current at a frequency of 350–750 kHz in order to accelerate the electrons in the cardiac cells generating heat. While low-frequency alternating current is able to induce similar tissue injury its use is limited by cardiac and skeletal muscle stimulation (resulting in pain and/or arrhythmia induction). Conversely the use of higher RF frequencies (i.e., in the megahertz range) changes the mode of injury from resistive heating to dielectric heating, which is associated with reduced efficiency of heating and a lessened rise in tissue temperature.

Ablation Electrode Material

While platinum is the most widely utilized ablation electrode material, gold electrodes have four times the thermal conductivity resulting in increased effective energy transfer to tissues. In temperature control mode the improved heat dissipation will result in the creation of deeper lesions for a given temperature and impedance (Lewalter et al., 2005).

RF Ablation Duration

The lesions created by RF ablation form over time as a function of heat transfer from the electrode tip to the deeper tissue. This transfer, which is based on the degree of RF energy delivered and rate of thermal conduction, follows a "monoexponential relationship" (Wittkampf et al., 1989) (**Fig. 4**). In other words, the majority of a lesion is created within the first 5–10 s of energy

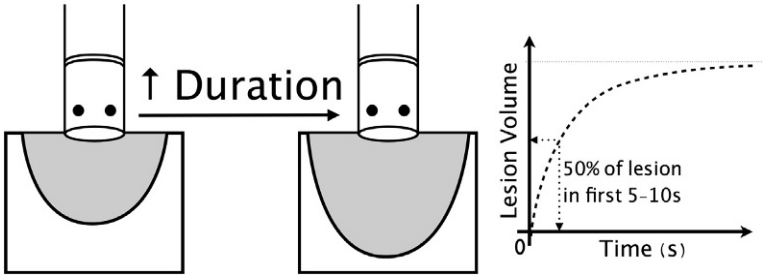

Fig. 4 The effect of increasing ablation duration on lesion volume during RF ablation. For nonirrigated RF ablation the majority of the lesion is created within the first 5–10 s, prior to the lesion volume reaching a plateau at about 45–60 s. For irrigated RF ablation the plateau in lesion volume is delayed to closer to 2 min.

delivery due to the rapidity by which the superficial tissue close to the electrode tip is affected by resistive heating. The remainder of the lesion is generated by the slow thermal energy transfer to deeper tissue layers. This process continues until steady state is reached, meaning that the amount of energy entering the tissue at the thermal source (i.e., heat generated within the lesion) equals the amount of energy being dissipated at the tissue margins beyond the lesion border (i.e., heat transferred away from the lesion). This equilibrium occurs after approximately 60 s for standard RF ablation but can be significantly delayed (i.e., >2 min) for ablation with irrigated RF.

Ablation Mode

Nonirrigated RF ablation is typically performed in power control mode, or temperature control mode. Under power control mode the power output is constant, with a temperature cutoff set only for safety purposes (i.e., the generator will shut off if the temperature limit is reached). This mode is an appropriate for focal ablation in thin myocardium (e.g., focal atrial tachycardia, accessory pathways, and AV nodal substrates); however its use is limited by complications related to the use of high-power settings and/or high target temperatures when performing more extensive ablation in thicker tissues (i.e., the ventricle) or in areas with limited blood flow (e.g., trabeculated tissues with impaired convective cooling). In response, temperature-limited ablation was developed. Under temperature control, RF power delivery is regulated to maintain a constant electrode temperature (typically between 50°C and 60°C). This allows for titration of power (e.g., the delivery of higher power) in order to facilitate creation of the largest possible lesion volume while leveraging the ambient cooling of the electrode by the surrounding blood flow to prevent the electrode–tissue interface temperature from increasing to the point of coagulum formation and/or steam "pop." However, it is important to recall that the tissue temperature may be 10–30°C higher than the electrode temperature, meaning that the risk thrombus formation and/or steam pop may still occur during temperature-limited ablation.

Convective Cooling and Blood Flow

Recall that lesion size increases linearly with tissue temperature, which is a function of the amount of RF energy that is delivered and converted to energy in the tissue. The heat produced by resistive heating is transferred into the deeper tissues by thermal conduction resulting in lesion formation. However, at the same time this generated heat is also conducted back to the electrode tip and the circulating blood pool. Given its characteristics of high heat capacity as well as a relatively high rate of flow the circulating blood pool is a major factor working in opposition of effective heat transfer to the deeper tissue. As such the convective cooling by surrounding blood flow has a significant influence on the amount of RF energy that can be delivered to the tissues and thus lesion formation. This is most relevant in the case of power control mode, where the amount of power delivered is stable and thus an increase in convective cooling will result in a reduction in lesion size by removing heat from the tissue.

However, it is important to recognize that convective cooling can be leveraged to improve lesion formation. In temperature control mode increased convective cooling can facilitate increased lesion size through the delivery of higher power, and thus the achievement of higher tissue temperatures. Conversely, in states of low surrounding blood flow the reduced convective electrode cooling will cause the ablation electrode to reach the target temperature at a lower power level. In these circumstances increasing the electrode temperature to 65°C or 70°C only minimally increases RF power at the expense of a significant increase in the risk of thrombus formation and impedance rise. Passive convective cooling can be facilitated through the use of larger ablation electrodes and electrode orientation.

Catheter Tip Size

In power control mode, the use of a larger ablation catheter electrode results in smaller lesions in comparison to a smaller electrode when ablation is performed at the same power (**Fig. 5**) (Langberg et al., 1990). This is due to the increased surface area associated with the larger electrode size (e.g., an 8-mm electrode has almost twice the surface area of a 4-mm electrode, for a similar radius)

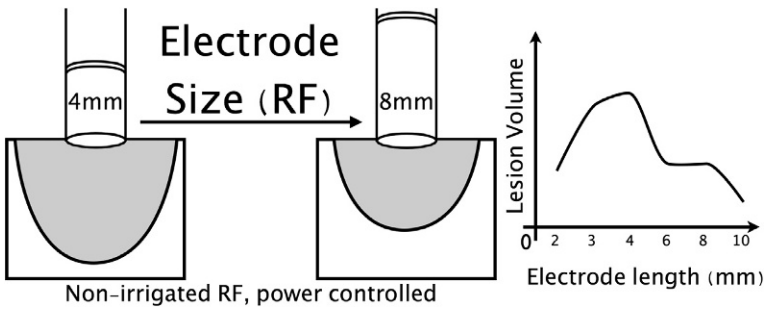

Fig. 5 The effect of increasing electrode size on lesion volume during power controlled RF ablation. The lesion volume increases with electrode tip size up to 4 mm but then decreases substantially as electrode size increases (Langberg et al., 1990).

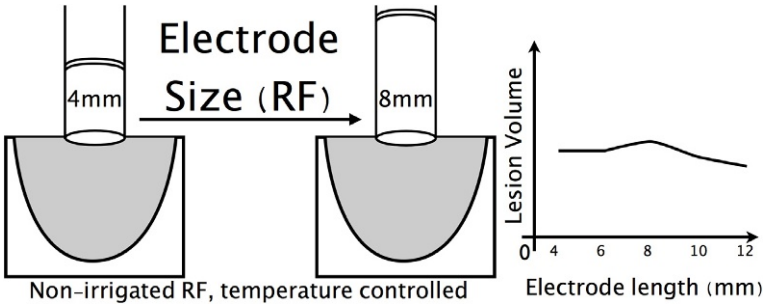

Fig. 6 The effect of increasing electrode size on lesion volume during temperature-controlled RF ablation. With increasing lesion tip size the volume of the lesion is relatively unchanged (Chugh et al., 1999).

resulting in a reduction in the interface impedance between blood and tissue, without altering the impedance through the rest of the patient. As such larger ablation electrodes result in an increasing proportion of current being shunted to blood pool, reducing the efficiency of power transfer, or current density, available to the tissue (Langberg et al., 1990). Specifically, the doubling in electrode tip size (e.g., from 4 to 8 mm) would necessitate a doubling in power (e.g., from 50 to 100 W) to maintain the same density of current provided to tissue. Unfortunately, despite doubling the power the lesion created with a larger ablation tip remains smaller than that with a smaller tip due to the combination of: (1) the increased surface area reducing the interface impedance with blood and tissue (e.g., from 120 to 100 Ω) without altering the impedance through the rest of the patient (e.g., 45 Ω). This further reduces the relative power available near the electrode tip (e.g., 55% with 8 mm vs. 63% with 4 mm, which is then further subdivided between tissue and blood pool based on contact). Lastly, the larger electrode size makes the degree of contact more unpredictable, with variable contact across the electrode resulting in unreliable energy delivery and potential for greater losses of energy transfer to blood pool. As such, due to the reduced efficiency of the larger electrode, a doubling of electrode size may require as much as 1.5–5 times more power to achieve the same tissue temperature and lesion volume (Wittkampf and Nakagawa, 2006).

In temperature control mode, a greater amount of power is delivered as the larger electrode surface area (i.e., increased electrode circumference and length) is more easily cooled by the surrounding blood flow (Chugh et al., 1999). This ambient cooling facilitates the delivery of a greater amount of power for the same degree of blood flow, which results in an increase in lesion volume with the larger ablation electrodes compared to the smaller electrodes (**Fig. 6**).

Catheter Tip Orientation

Catheter electrode–tissue contact surface area is heavily influenced by catheter orientation. With perpendicular orientation and good tissue contact it would be expected that approximately 25% of a 4-mm electrode would be in contact with the target tissue (as the rounded distal end of the electrode represents approximately 25% of total electrode surface). Taking the same 4-mm electrode into a parallel orientation will result in roughly the same amount of the electrode surface being in contact with the tissue (largely owning to limitations with translating significant pressure via the catheter shaft with the electrode in this orientation). As such, the lesion created with the 4 mm tip electrode in both parallel and perpendicular orientation would be expected to be similar when ablation is performed at the same power.

However, the use of larger electrode tips may demonstrate a difference in lesion size between the parallel and perpendicular orientation due to a greater relative difference in electrode–tissue contact (**Fig. 7**). Specifically, the distal end of an 8-mm electrode represents approximately only 12% of the total electrode surface, meaning 88% of the electrode remains in contact with blood pool. This effectively drops the delivered power from 8.5% (or 4.25 W of 50 W) to 3.3% (1.65 W of 50 W) when utilized in power control mode. However, the use of a perpendicular orientation should maintain an approximate 25% contact, which would maintain a power delivery to tissue of approximately 8.5% in power control mode. As such, in power control mode the perpendicular orientation with a larger ablation electrode would result in a smaller lesion owing to proportionately less contact at the electrode–tissue interface.

In temperature control mode a differing result is observed owing to the effect of convective cooling of these larger electrodes (**Fig. 8**) (Chugh et al., 1999). Specifically, a parallel orientation facilitates increased power delivery and results in larger lesions owing to beneficial relationship between tissue contact and convective cooling. While this effect may be observed for smaller electrode sizes (e.g., 4 mm) the relative benefit of parallel electrode orientation on lesion size is more marked for larger electrode tips (e.g., 8 mm).

Catheter Tip Irrigation

The use of active cooling (closed- or open-loop irrigation) enables the creation of larger lesions by providing adequate environmental cooling to facilitate the delivery of more RF energy (**Fig. 9**) (Nakagawa et al., 1995). This active electrode cooling of the results in a lower electrode tip–tissue interface temperature, reducing char and thrombus, as well as facilitating the creation of

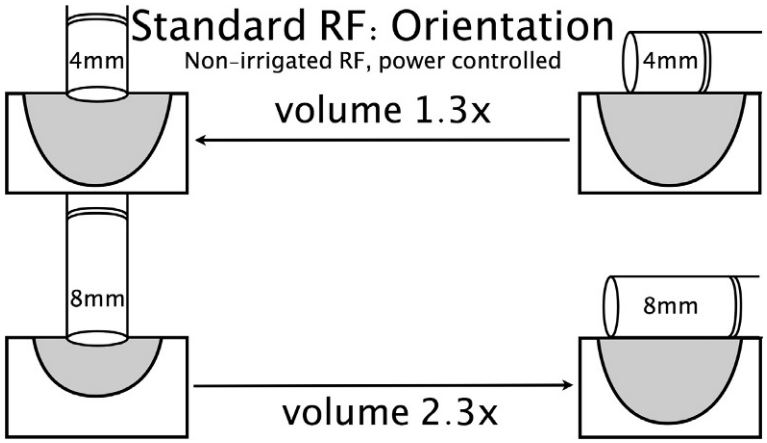

Fig. 7 The effect of catheter orientation on lesion volume during power-controlled RF ablation. For the 4-mm tip catheter the lesion volume is slightly greater in the perpendicular orientation owing to a slight increase in lesion length. For the 8-mm tip catheter the lesion volume is significantly larger in the parallel orientation owing to the reduced current density associated with a perpendicular orientation.

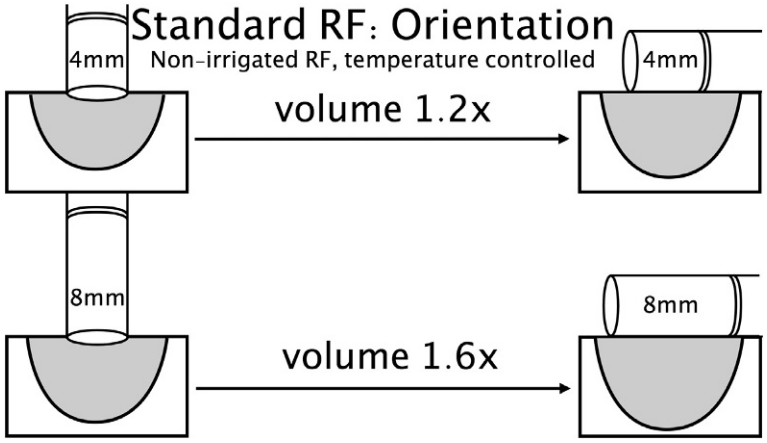

Fig. 8 The effect of catheter orientation on lesion volume during temperature-controlled RF ablation. For both the 4-mm tip catheter and the 8-mm tip catheter the lesion volume is larger in the parallel orientation owing to the ability to deliver increased power although the relative increase in volume is greater with the larger tip catheter (Chugh et al., 1999).

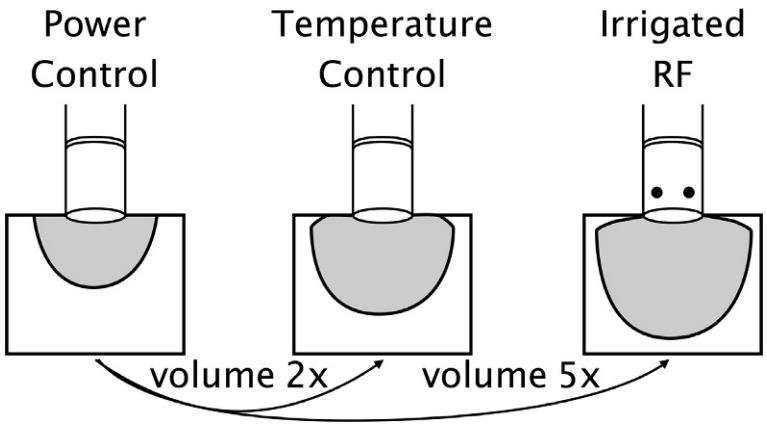

Fig. 9 Comparison of lesion volume generation with power-controlled RF ablation, temperature-controlled RF ablation, and open-irrigated RF ablation (Nakagawa et al., 1995). The use of temperature control and active electrode cooling facilitates larger lesion volume through the delivery of increased RF power, and thus a greater heat transfer to the deeper tissues.

deeper lesions by sustaining adequate RF power even at sites with low blood flow (i.e., the ablation process is no longer limited by an electrode temperature rise). As a result the use of irrigated RF ablation catheters has become the standard of care for ablation in the systemic circulation (such as atrial fibrillation) (Macle et al., 2002) or in thicker myocardial substrates (such as the ventricle) (Reddy et al., 2003).

Closed Versus Open Irrigation

Irrigated RF catheters circulate continuous electrode irrigation fluid either within the electrode (closed loop) or by flushing saline through small openings in the distal ablation electrode (open irrigation). This results in differences in the effect of cooling on the electrode–tissue interface, as well as the lesion itself (**Fig. 10**) (Yokoyama et al., 2006). The closed-loop irrigation focuses the effect of cooling on the interface between the ablation electrode and tissue with minimal effect on the surface area or depth of the lesion. Conversely open irrigation cools the electrode, and it is direct environment (the tissue surface, and the blood pool around the electrode) resulting in a minimally reduced lesion surface area, with the maximum lesion diameter being found slightly deeper into the tissue. In either case it is important to note that irrigation itself does not increase lesion size but merely facilitates the delivery of RF energy in environments limited by temperature.

However, a key difference between open- and closed-loop irrigation is the observation that closed-loop irrigation is associated with a higher electrode–tissue interface temperature despite low electrode temperatures. This electrode–tissue interface temperature frequently exceeded 80°C and was associated with thrombus formation, steam pops, and impedance rises. Conversely open-loop irrigation is associated with lower interface temperatures, and lower risk of thrombus and steam pop (Yokoyama et al., 2006).

Irrigation Flow Rate

For open-irrigated catheters the rate of irrigation flow will affect the size of the lesion produced (**Fig. 11**) (Squara et al., 2014; Gonzalez-Suarez et al., 2016). At the same power level an increase in the irrigation flow rate will result in a smaller lesion due to increased power loss, and increased cooling of the electrode and the adjacent tissue. Conversely a decrease in irrigation flow rate will

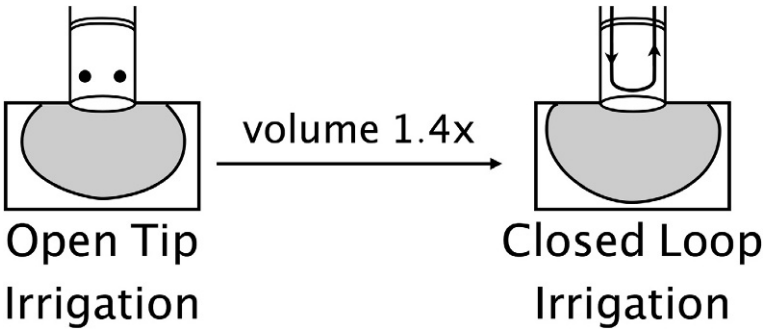

Fig. 10 Comparison of lesion volume generated by open tip and closed-loop saline irrigation (Yokoyama et al., 2006). In general the surface diameter and maximum diameter was slightly smaller with open irrigation, with comparable lesion depths. This led to a slightly smaller overall lesion volume with open tip irrigation compared to closed-loop irrigation. However closed-loop irrigation is associated with higher catheter–tissue interface temperatures, as well as a higher incidence of thrombus formation and "steam pop."

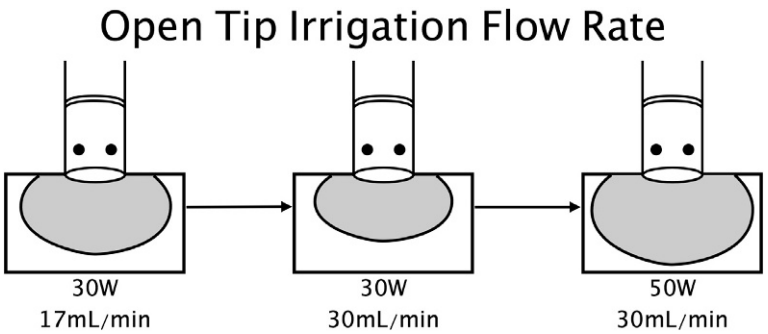

Fig. 11 The effect of irrigation flow rate on lesion volume (Squara et al., 2014; Gonzalez-Suarez et al., 2016). At constant power an increase in irrigation flow rate will result in a smaller lesion volume owing to greater cooling at the tissue interface limiting heat transfer to the deeper tissues. However, in the presence of constant irrigation flow rate increasing power will result in an increased lesion volume.

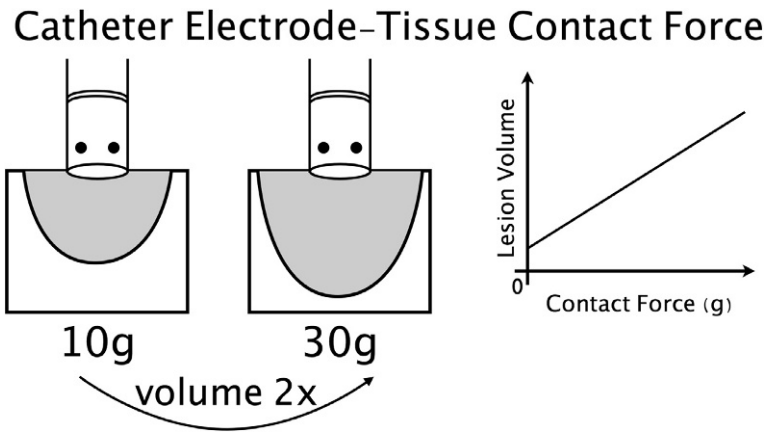

Fig. 12 The effect catheter electrode–tissue contact force on lesion volume. With increasing contact force there is an increase in lesion volume (linear relationship) (Yokoyama et al., 2008).

result in a larger lesion due to less power loss, and less cooling of the electrode and adjacent tissue. As such, the main benefit of an increase in irrigation flow rate is the ability to safely increase power delivery, resulting in increases in lesion size while avoiding complications.

Catheter Tip–Tissue Contact Force

With increasing catheter electrode–tissue contact force there is an increased contact area and more efficient energy transfer (i.e., power delivery) to the myocardium, effectively resulting in lesion creation at a much lower power level (**Fig. 12**) (Yokoyama et al., 2008). Preclinical studies have demonstrated a strong relationship between lesion volume and contact force. Weiss and colleagues performed irrigated-tip ablation using an ovine thigh model using constant power (30 W) but varying contact force (5, 10, 30, and 50 g). They found that lesion width was unaffected by the degree of contact force; however lesion depth and consequently lesion volume did increase with increasing contact force (5.5–9 mm for perpendicular catheter orientation) (Weiss et al., 2002). Similarly, FTI (force time integral, or area under the contact force curve) has been showed to correlate with lesion volume with intermittent contact (characterized by a lower FTI) producing smaller lesions than variable contact with a higher FTI, and with the largest lesions being produced in conditions with the highest FTI (e.g., constant contact) (Shah et al., 2010). Clinically an average CF of <10 g and an average FTI of <500 g/s were associated with higher rates of AF recurrence at 12 months compared to an average CF >20 g (100% vs. 20% recurrence; $P=0.01$) and an average FTI >1000 g/s (75% vs. 31% recurrence; $P=0.03$) (Reddy et al., 2012).

However, it is important to consider that, while increasing contact force is associated with significantly larger lesions it is also associated with a higher incidence of complications, with a clear dose–response relationship that parallels that observed with increasing power (Thiagalingam et al., 2010). As such it has been suggested that "moderate" catheter contact force (20 g) with low power ablation (impedance control and fixed power 20 W) offers the best compromise between safety and efficacy (low risk of popping with a reasonable lesion size, ~ 8.5 mm wide $\times$ 5.5 mm deep). In the event of "light" catheter contact (<20 g) then higher power (30 W) appears to create similar sized lesions compared to lower power ablation with moderate contact; however this is with a small risk of popping.

Cryothermal Ablation

While RF ablation remains the standard of care for the treatment of the majority of arrhythmias, novel ablation energy modalities have been sought in an effort to address some of its limitations. Of these novel energies, cryothermal ablation is the most advanced and thus the most routinely used in clinical practice.

Cryotherapy, or the therapeutic use of freezing temperatures, has a long history of use in medicine. In contrast to RF energy, cryothermal ablation results in cold-induced tissue injury through a combination of direct cellular damage (ice crystal formation during hypothermia) and ischemic cell death (microcirculatory failure and subsequent vascular stasis during thawing). When compared to RF energy, cryoenergy offers the potential advantages of: (1) improved catheter stability, (2) the creation of an ablation lesion that is less arrhythmogenic, (3) reversible tissue suppression (which allows for active electrophysiologic evaluation of potential ablation sites during mapping and/or ablation), (4) a lower risk of damage to collateral structures, and (5) a lesser degree of endothelial disruption and platelet activation (Herrera Siklody et al., 2011; Gaita et al., 2011).

Preceded by a near half-decade of experimentation, the modern era of cryosurgery was initiated by Cooper and Lee in 1961 when vacuum-insulated cryosurgical probes cooled by liquid nitrogen (-196°C) were introduced (Cooper and Lee, 1961). Novel cryoprobes were subsequently developed using thermoelectric cooling (Peltier effect) and later modified for cooling using the

Joule–Thomson effect (i.e., from the expansion of a highly compressed nonideal gas into a region of low pressure). While the thermoelectric method (cooling by the passage a direct current through dissimilar metal junctions) offered limited thermal efficiency, the Joule–Thomson effect was relatively efficacious. Although the core tenets of its design have remained largely unchanged, the transvenous cryoablation catheter has evolved from a 9-French focal cryoablation catheter with slow cooling and a temperature limit of −50°C to the modern 7-French version with rapid cooling and achievable temperatures below −80°C.

Lesion Formation

The lesion is created with cryothermal ablation is through convective cooling, whereby the cryorefrigerant absorbs heat from the surrounding tissue before returning to the console. This results in cold-induced cellular and tissue injury is due to a combination of: (1) direct cellular damage due to the deleterious effects of ice crystal formation during hypothermia and (2) ischemic cell death due to microcirculatory failure and subsequent vascular stasis during thawing (Gage and Baust, 1998). These complex mechanisms occur in the sequence of: (1) freezing, (2) thawing, (3) hemorrhage and inflammation, and (4) replacement fibrosis (Khairy and Dubuc, 2008).

During the first phase, progressive hypothermia results in a slowing of cellular metabolism, whereby ion pumps progressively lose their transport capabilities and the intracellular pH becomes more acidic (Baust et al., 1997). In general, these early effects are transient if the duration of cooling is limited. Thereafter, progressive cooling is associated with the formation of ice crystals, predominantly in the extracellular space (at tissue temperatures below −15°C); however intracellular ice can occur with temperatures below −40°C (Budman et al., 1995). The formation of extracellular ice results in relative hypertonia, with the compensatory egress of water from the intracellular to the extracellular space in an effort to reestablish osmotic equilibrium resulting in cellular shrinkage (Mazur, 1970; Meryman, 1956; Whittaker, 1984). The newly established osmotic gradient precipitates a diffusion gradient between extracellular and intracellular spaces, resulting in biochemical injury (cellular protein damage, enzyme system impairment, and adverse effects on lipoprotein components of the plasma membrane) due to a further reduction in intracellular pH (Whittaker, 1984; Gill et al., 1968).

Upon completion of the freezing phase, the tissue passively returns to body temperature, inducing cellular damage through a combination of: (1) recrystallization and coalescence of intra- and extracellular ice crystals which increase the osmotic damage and generate shear forces, further disrupting tissue architecture (Meryman, 1956; Gage et al., 1985) and (2) restoration of microcirculatory function is associated with a hyperemic vascular response characterized by hemorrhage and inflammation (Baust et al., 1997; Budman et al., 1995; Mazur, 1970). As these blood vessel walls become porous there is increased capillary permeability leading to interstitial edema. This vascular congestion, combined with endothelial injury-induced platelet aggregation and microthrombi formation, culminates in vascular obliteration and ischemic cellular necrosis. As such, while the central region subjected to the coldest freezing temperatures undergoes direct cellular damage, the thawing effect results in the extension of tissue destruction through surrounding microvascular injury.

The final phase of cryoinjury begins concurrent to thawing and is characterized by reactive inflammation, followed by tissue repair and replacement fibrosis. Over the subsequent weeks, these processes culminate in the generation of a mature lesion, which has a distinct, well-circumscribed central region of dense fibrosis surrounded by a narrow border zone of variable cellular death (due to microvascular injury and apoptosis) (Khairy et al., 2003).

Temperature, Duration, Blood Flow, and Lesion Formation With Cryothermy

Factors influencing cryolesion size are fundamentally similar to those observed with RF ablation with some notable exceptions. While tissue temperature and ablation duration are important for both cryothermal ablation and RF ablation, the relationship is slightly more pronounced for cryoablation. Recall that a period of reversible tissue inhibition necessarily precedes irreversible tissue destruction with cryoablation. This factor is more pronounced at the periphery of the lesion where the degree of permanent cellular damage with relatively warmer tissue temperatures (−10°C to −25°C) is directly related to duration of freezing (Khairy and Dubuc, 2008; Gage et al., 1985). In these areas permanent tissue injury requires maintaining a tissue temperature of −20°C for 3–4 min (probe temperature −75 to −80°C). In contrast RF ablation is able to induce permanent tissue injury with shorter ablation durations.

The second major difference in lesion size is the more proportional relationship between electrode size and lesion formation. In contrast to power-controlled RF ablation the use of larger cryoablation catheter tips were associated with significantly larger lesions volumes (approximate doubling in lesion volume with 8 mm catheter compared to 4 mm catheter in perpendicular orientation, but up to 5× increase in volume with parallel orientation—**Figs. 13** and **14**) (Wood et al., 2007, 2009).

For cryoablation the convective warming that accompanies increasing blood flow results in a decrease in lesion volume by approximately 50% (**Fig. 15**). For the 4 mm catheter tip the reduction in lesion volume in high flow states is irrespective of catheter orientation owing to relatively similar contact areas. For the 8 mm tip electrode the parallel orientation is associated with a lesser reduction in lesion volume in high flow states due to relatively reduced convective warming. Specifically, the larger electrode is associated with a greater surface area in contact with the tissue, and thus a smaller portion of the electrode being exposed to blood pool.

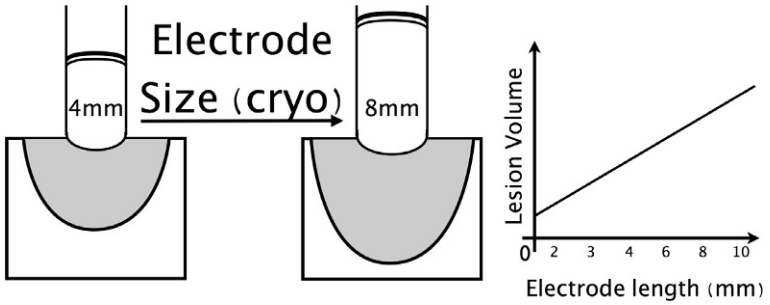

Fig. 13 The effect of increasing electrode size on lesion volume during cryoablation. With cryoablation there is an almost linear increase in lesion volume with increasing electrode tip size (Wood et al., 2007).

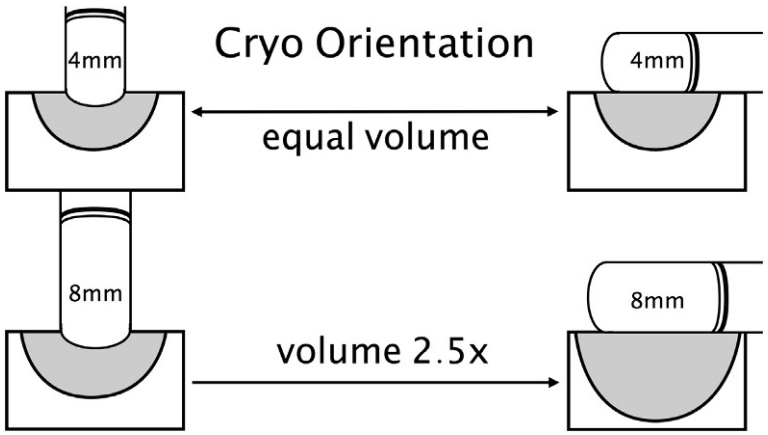

Fig. 14 The effect of catheter orientation on lesion volume during cryoablation. For the 4-mm tip catheter the lesion volume is comparable in parallel and perpendicular orientation. For the 8-mm tip catheter the lesion volume is markedly larger in the parallel orientation (Wood et al., 2007, 2009).

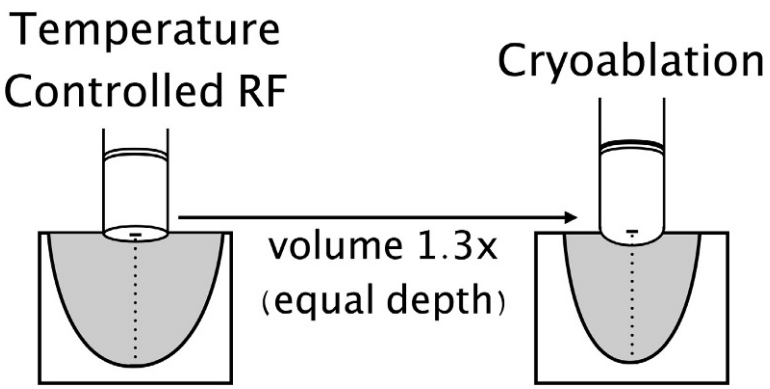

Fig. 15 Comparison of ablation lesions created with a 4-mm tip temperature-controlled radiofrequency (RF) ablation catheter and a 4-mm tip cryoablation catheter. While the depths of the two lesions are comparable, the RF lesion has a greater surface area ($P=0.002$) and thus a greater volume ($P=0.059$). Reproduced with permission from Khairy et al (Khairy et al., 2003).

Comparing Cryoablation to RF

RF energy and cryothermal ablation have both proven to be highly effective in the treatment of arrhythmias with a reasonably low complication rate. However, as the mechanism of cellular injury differs between the two modalities, important relative benefits and drawbacks must be considered.

Reversible Suppression

Both RF ablation and cryothermal ablation are associated with the potential for the premature termination of ablation in an effort to avoid impending complications.

For cryothermal ablation this translates into the ability to dynamically and prospectively assess the safety and efficacy of a potential ablation lesion site. Recall that a period of reversible tissue inhibition obligatorily precedes irreversible tissue destruction as the hypothermic wavefront spreads centrifugally from the catheter tip to the surrounding tissue (Gage and Baust, 1998; Khairy and Dubuc, 2008; Gage et al., 1985). Thus while extreme freezing (i.e., tissue temperatures colder than −50°C) results in near instantaneous permanent tissue injury, the degree of permanent cellular damage with relatively warmer tissue temperatures (−10°C to −25°C) is directly related to duration of freezing (Khairy and Dubuc, 2008; Gage et al., 1985). Thus, the use of milder freezing temperatures and/or shorter ablation times facilitates the assessment of clinical effect at the target lesion site (so-called efficacy mapping), and confirmation that a target site does not result in adverse clinical outcomes (so-called safety mapping).

In the case of RF ablation, the practical benefit of "reversibility" is limited by the inability to assess the effect of heating the tissue at a certain site in the heart without creating an irreversible effect, as well as thermal latency. Recall that the tissue injury occurs as a result of resistive heating and subsequent thermal conduction. After 30–60 s of ablation a state of thermal equilibrium has been reached, in other words the local tissue temperatures have reached their maximum value (i.e., there is a steady flow of heat from the electrode to its environment). If ablation is terminated at the point of thermal equilibrium there will be a direct but gradual drop in tissue temperatures. However, if ablation is terminated prior to the attainment of thermal equilibrium the heat generated from the resistive heating will continue to dissipate to the deeper tissues despite the cessation of RF current flow. This is particularly important during the ablation of structures in close proximity to the AV node, whereby early ablation termination in response to inadvertent affect (i.e., AV block) may still result in further tissue penetration and subsequent injury.

Catheter Adhesion

During RF ablation the catheter remains free-floating during ablation, and active manipulation is required to maintain a stable catheter position at the desired site. Conversely, cryothermal ablation is associated with freeze-mediated catheter adhesion to the target tissue. This catheter–tissue attachment provides several advantages. First, freeze-mediated catheter stability facilitates the efficacious ablation of technically challenging regions, such as sites where contact is difficult to maintain. Second, cryoadhesion eliminates the "brushing effect," whereby RF energy is applied to the target and surrounding nontarget tissue due to cardiac and respiratory motion. It can be expected that increased catheter stability would result in less collateral damage to nearby critical structures, such as the conduction system (Khairy and Dubuc, 2008). Conversely, the lack of brushing effect with cryothermal ablation requires that lesion placement be more precise (especially taking into account the cryocatheter movement prior to adhesion). Lastly, catheter adhesion allows for the dynamic assessment of lesion efficacy. Specifically, the ablation can be performed during ongoing arrhythmia, or programmed electrical stimulation, as there is no risk of catheter dislodgement with arrhythmia termination.

Lesion Characteristics, Endothelial Integrity, and Thrombosis

In comparison to RF, lesions associated with focal cryothermal ablation have a smaller surface area due, in part, to cryoadhesion-induced loss of the brushing effect, with no difference in lesion depth (Khairy et al., 2003). However, when applied to immature myocardium, the rate of expansion of cryoablation lesions with growth is similar to RF (**Fig. 16**) (Khairy et al., 2011).

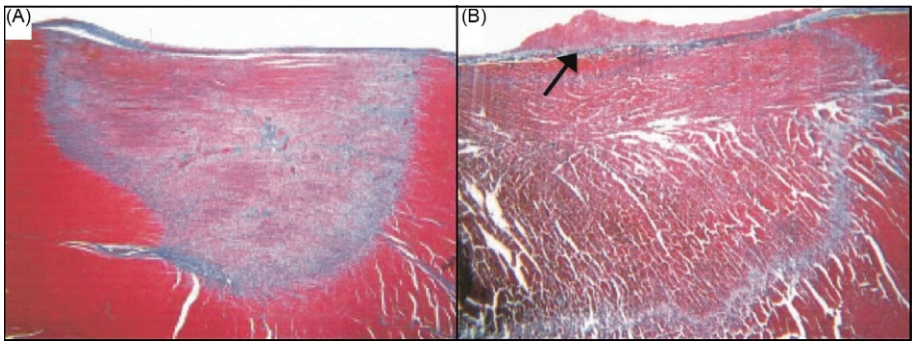

Fig. 16 Histology of lesions generated by cryothermal ablation (A; −75°C x 4 min) and temperature-controlled RF ablation (B; +70°C, 50 W, 60 s). Stained with Masson's trichrome and magnified 16-fold. Note more homogeneous nature of cryolesion, with a smoother, sharper demarcation from normal myocardium (A). In contrast, RF lesion is less well circumscribed, with serrated edges (B). *Arrow* indicates endocardial thrombus formation overlying the ablation site. Reproduced with permission from Khairy et al (Khairy et al., 2003).

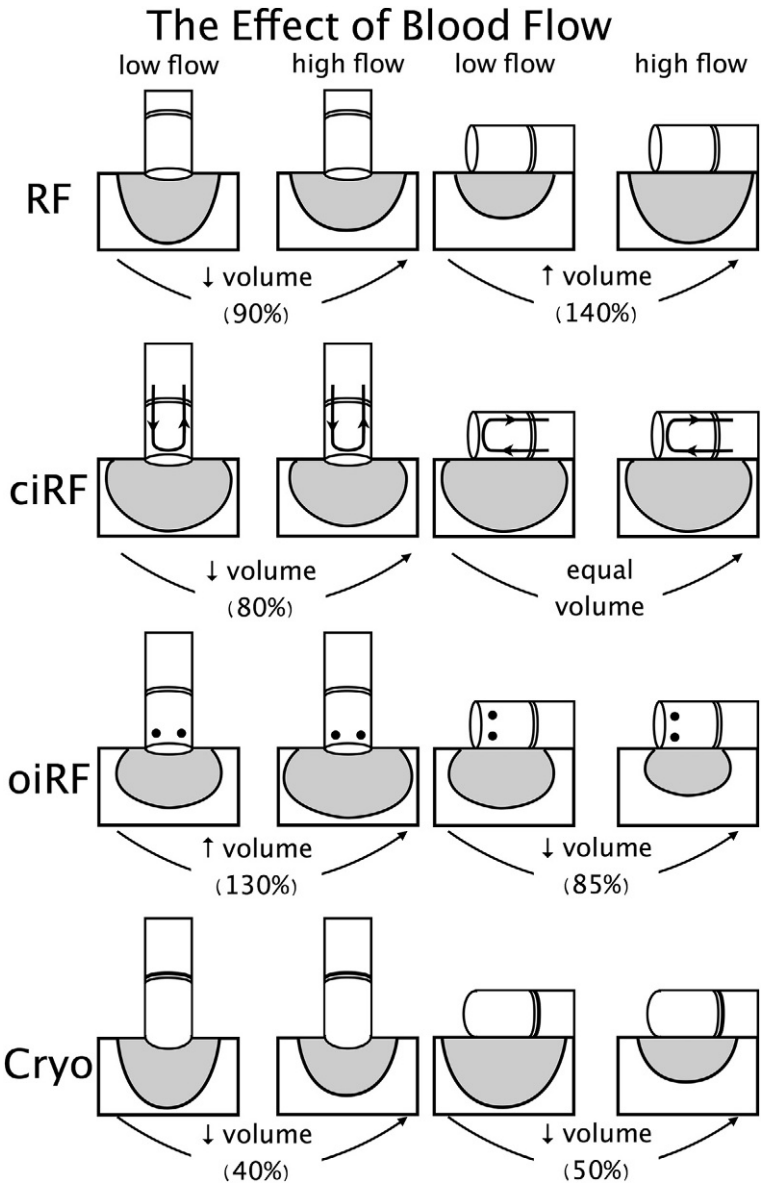

Fig. 17 The effect of ambient blood flow on lesion size, as influenced by catheter orientation (Wood et al., 2007). Low blood flow pictured to the left, and high blood flow pictured to the right. *RF*, temperature-controlled radiofrequency ablation; *ciRF*, closed-loop irrigated RF; *oiRF*, open-irrigated RF; *Cryo*, cryothermal ablation.

Histologically, cryothermy results in a dense, homogenous fibrosis that is well demarcated from normal myocardium (**Fig. 17**) (Gage et al., 1985; Khairy et al., 2003; Klein et al., 1979). In contrast, the hyperthermic injury induced by RF results in diffuse cellular destruction characterized by intralesional hemorrhage and ragged edges less clearly demarcated from underlying normal myocardium (Klein et al., 1979). It may be speculated that lesions produced with cryoablation should be less arrhythmogenic (given that distinct border zones are less susceptible to spontaneous depolarization, **Table 1**).

Although devitalized, cryoablation lesions are associated with preservation of ultrastructural integrity, an observation that is attributed to the remarkable resilience of fibroblasts and collagen fibers to hypothermia (Shepherd and Dawber, 1984). In contrast RF energy denatures the extracellular tissue matrix which has the potential to weaken the ablated tissue, predisposing to myocardial perforation and damage of adjacent structures. With cryoablation the preservation of tissue ultrastructural integrity should theoretically result in a lower incidence of venous or arterial stenosis (given the minimal tissue contraction observed with lesion healing) (Khairy and Dubuc, 2008; Gage et al., 1985; Khairy et al., 2003; Klein et al., 1979; Feld et al., 2003).

Cryothermal ablation lesions are associated with a lesser degree of endothelial surface disruption, as well as a lesser degree of platelet activation and the coagulation cascade activation when compared to RF (Khairy et al., 2003; Hochholzer et al., 2007; Sarabanda et al., 2005). Preclinical studies have demonstrated a significantly lower incidence of overlying thrombus (30.1% vs. 75.8% despite the use of ASA and heparin), and a lesser thrombus volume with cryothermal ablation compared to RF (Khairy et al.,

Table 1 Factors that influence lesion size

Variables	*Modifiers*	*Effect on lesion size*
Tissue	Scar and fat	Smaller
Power	RF	Increases (linearly)
Tissue temperature	RF	Increases (linear: warmer = larger)
	CRYO	Decreases (linear: colder = larger)
Tip temperature	Power control	Increases (variable relationship)
	Temperature control	Increases (variable relationship)
	Irrigated tip	Unrelated
	CRYO	Decreases (linear: colder = larger)
Ablation duration	Power control	Increases (plateau 45–60 s)
	Irrigated-tip	Increases (plateau after ~2 min)
	CRYO	Increases (plateau after ~60 s)
Cooling (convective)	Power control	Smaller
	Temperature control	Larger
	Active (irrigated RF)	Larger (if unrestricted power)
		Smaller (if constant power)
	CRYO	Smaller
Intramyocardial blood flow	RF and CRYO	Smaller
Electrode size (increasing)	Power control	Smaller
	Temperature control	Larger
	CRYO	Larger
Electrode Orientation	Power control	Parallel = larger
	Temperature control	Parallel = smaller
	Active (irrigated RF)	Parallel = smaller
	CRYO	Parallel = larger
Contact force		Increases

2003; Sarabanda et al., 2005). Moreover, in contrast to RF lesions where the extent of hyperthermic tissue injury was positively correlated with thrombus bulk, cryoablation lesion dimensions were not predictive of overlying thrombus volume. Taken together, these effects suggest a possible pathophysiologic mechanism as to why cryoablation is less thrombogenic than RF, which may have important safety implications, particularly for procedures in the systemic circulation (Herrera Siklody et al., 2011; Gaita et al., 2011; Khairy et al., 2003; Hochholzer et al., 2007; Sarabanda et al., 2005).

Conclusion

RF ablation and cryothermal ablation are effective treatment modalities for the invasive management of multiple arrhythmic substrates. Significant tissue injury can be obtained using both technologies in a safe and effective manner. An enhanced understanding of lesion science can result in more optimal ablation procedures and improved patient outcomes.

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Blood Pressure Variability Versus Blood Pressure Level in Risk Stratification

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Introduction

Blood pressure variability has been considered a physiological marker of autonomic nervous system control, with short- and long-term fluctuations from intricate interactions among behavioral, environmental, neural central or reflex influences, along with other potential contributing factors.

Current indexes of blood pressure variability raise methodological issues related to their poor reproducibility, their interdependence, and their association with the level of blood pressure. Besides methodological problems, the progn ostic significance of blood pressure variability remains controversial. Some studies reported association of end-organ damage (Parati et al., 1987a; Tatasciore et al., 2007; Matsui et al., 2011), cardiovascular events (Kikuya et al., 2000; Rothwell et al., 2010a,b; Rothwell, 2010; Webb et al., 2010; Johansson et al., 2012; Shimbo et al., 2012), or mortality (Muntner et al., 2011) with blood pressure variability, whereas others failed to find any association or found variability to be inferior to the level of blood pressure (Pierdomenico et al., 2006; Hansen et al., 2010; Schutte et al., 2012). This review addressed to what extent blood pressure variability and blood pressure level contribute to risk stratification.

Indexes of Blood Pressure Variability

A major problem in many reports is that they assessed target organ damage or the incidence of events as a function of blood pressure variability indexes that are highly dependent on blood pressure level. In the early 1970s, Clement and coworkers (1979) assessed blood pressure variability from the standard deviation (SD) and the coefficient of variation (CV) of blood pressure measurements obtained every 5 min for 3 h in 70 untreated hypertensive patients. Sympathetic activity correlated with level and SD of blood pressure, but not with CV, a measure of variability that is less dependent on level than the SD (Clement et al., 1979). In the 1980s, Mancia and coworker analyzed 24 h continuous intra-arterial recordings and showed that SD correlated positively with blood pressure level and fell with antihypertensive drug treatment, whereas CV was independent of level irrespective of drug intervention (Mancia et al., 1980). Notwithstanding these initial findings, the same group used SD rather than CV to estimate the association of blood pressure variability and target organ damage on the incidence of cardiovascular complications (Parati et al., 1987b; Frattola et al., 1993), and many investigators continue using the SD as the index of blood pressure variability.

Other measures of variability, such as the weighted SD (Bilo et al., 2007), the difference between the maximum minus minimum blood pressure level (MMD), and average real variability (ARV) (Mena et al., 2005) also remain highly dependent on blood pressure level. The weighted SD is the mean of day and night SD values weighted for the number of hours covered by these two periods during ambulatory monitoring. ARV is calculated using the following formula:

$$ARV = \frac{1}{\sum w_k} \sum_{k=1}^{n-1} w_k \times BP_{k+1} - BP_k$$

where n is the number of blood pressure readings, k ranges from 1 to $n-1$, and w_k is the time interval between BP_k and BP_{k+1}. ARV therefore accounts for the order of the measurements and weighs each value according to the measurement interval.

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More recently, Rothwell and colleagues (2010a,b) proposed blood pressure variability independent of the mean (VIM) as a new index. VIM (Rothwell et al., 2010a,b) is the within-subject SD divided by the within-subject mean blood pressure level to the power x and multiplied by the population mean blood pressure level to the power x. The power x is obtained by fitting a curve through a plot of SD against mean blood pressure level, using the model $SD = a \times \text{mean}^x$, where x is derived by nonlinear regression. The correlation of VIM with the other indexes of blood pressure variability is high (Levitan et al., 2013), but VIM does not correlate with the blood pressure level (Rothwell et al., 2010a,b). VIM therefore allows assessing association of outcome with blood pressure variability with little confounding by blood pressure level (Rothwell et al., 2010a,b). However, VIM is tied to the population being examined and cannot be compared across population, because it is derived from the distribution of the blood pressure values in each population.

Recent Evidence on Prognostic Significance

Blood pressure may be recorded over different time intervals: beat-to-beat (very short-term), over 24 h (short-term), and day-by-day or visit-to-visit (long-term). The techniques of measurement include continuous beat-to-beat recordings, usual office blood pressure measurement, 24 h ambulatory blood pressure monitoring, or home blood pressure monitoring.

Beat-to-Beat Blood Pressure Variability

Beat-to-beat recordings allow capturing blood pressure variability, even over short time intervals (Wei et al., 2014). In 256 untreated Chinese patients referred to a hypertension clinic, we assessed the association of target organ damage with VIM, MMD, and ARV, determined from 10 min beat-to-beat, 24 h ambulatory, and 7-day home blood pressure recordings (Wei et al., 2014). Effect sizes (standardized β) were computed using multivariable regression models. In beat-to-beat recordings, left ventricular mass index ($n = 128$) was not ($P \geq 0.18$) associated with systolic blood pressure level but increased with all three systolic variability indexes (+2.97–3.53 g/m^2; $P < 0.04$). The urinary albumin-to-creatinine ratio increased ($P \leq 0.03$) with systolic blood pressure level (+1.14–1.17 mg/mmol, according to the model) and MMD (+1.18 mg/mmol). Aortic pulse wave velocity increased with systolic blood pressure level (+0.69 m/s; $P < 0.001$). In 24-h recordings, all three indexes of organ damage increased ($P < 0.03$) with systolic blood pressure level, whereas the associations with systolic blood pressure variability were nonsignificant ($P \geq 0.15$) except for an increase in aortic pulse wave velocity ($P < 0.05$) with VIM (+0.16 m/s) and MMD (+0.17 m/s). In home blood pressure recordings, the urinary albumin-to-creatinine ratio (+1.27–1.30 mg/mmol) and aortic pulse wave velocity (+0.36–0.40 m/s) increased ($P < 0.05$) with systolic blood pressure level, whereas all associations of target organ damage with the variability indexes were nonsignificant ($P \geq 0.07$). In summary, while accounting for systolic blood pressure level, associations of target organ damage with systolic blood pressure variability were readily detectable in beat-to-beat recordings and least noticeable in home recordings, with 24 h ambulatory monitoring being informative only for aortic pulse wave velocity (Wei et al., 2014).

Reading-to-Reading Blood Pressure Variability

Palatini and associates analyzed 7112 untreated hypertensive participants (43.8% women; mean age, 50.8 years) with daytime and nighttime ambulatory blood pressure enrolled in six prospective cohorts (Palatini et al., 2014). During a median follow-up of 5.5 years, 130 fatal and 455 nonfatal cardiovascular events occurred. In a multivariable-adjusted Cox model, the SD of the nighttime systolic blood pressure predicted cardiovascular mortality (hazard ratio (HR), 1.48; confidence interval (CI), 1.20–1.84) and morbidity (HR, 1.83; CI, 1.17–2.86), whereas SD of daytime systolic blood pressure did not ($P \geq 0.096$). These researchers proposed optimal cutoff limits for the nighttime SD 12.2 mm Hg systolic and 7.9 mm Hg diastolic. Participants above these arbitrary thresholds had a 41–132% higher risk of a composite cardiovascular end point than those below these cutoff limits ($P \leq 0.028$). As outlined in the preceding text, SD as index of blood pressure variability is closely correlated with blood pressure level (Palatini et al., 2014).

Hansen and colleagues (2010) assessed blood pressure variability from the SD and ARV (**Fig. 1**) in 24-h ambulatory recordings in the International Database on Ambulatory blood pressure monitoring in relation to Cardiovascular Outcome (IDACO) population. Higher diastolic ARV in 24 h ambulatory blood pressure recordings predicted ($P \leq 0.03$) total (HR, 1.13; CI, 1.07–1.19) and cardiovascular (HR, 1.21; CI, 1.12–1.31) mortality and all types of fatal combined with nonfatal end points (HR, ≥ 1.07) with the exception of cardiac and coronary events (HR, ≤ 1.02; $P \geq 0.58$). Similarly, higher systolic ARV in 24 h ambulatory recordings predicted ($P < 0.05$) total (HR, 1.11; CI, 1.04–1.18) and cardiovascular (HR, 1.17; CI, 1.07–1.28) mortality and all fatal combined with nonfatal end points (HR, ≥ 1.07), with the exception of cardiac and coronary events (HR, ≤ 1.03; $P \geq 0.54$). SD predicted only total and cardiovascular mortality. The incremental cardiovascular risk explained by adding ARV to models already including 24 h ambulatory blood pressure level and other covariables was less than 1%. This report established that reading-to-reading blood pressure variability is an independent risk factor, significant in a statistical but not in a clinically meaningful manner. It highlighted that the level of the 24 h blood pressure remains the primary blood pressure-related risk factor to account for in clinical practice (Hansen et al., 2010).

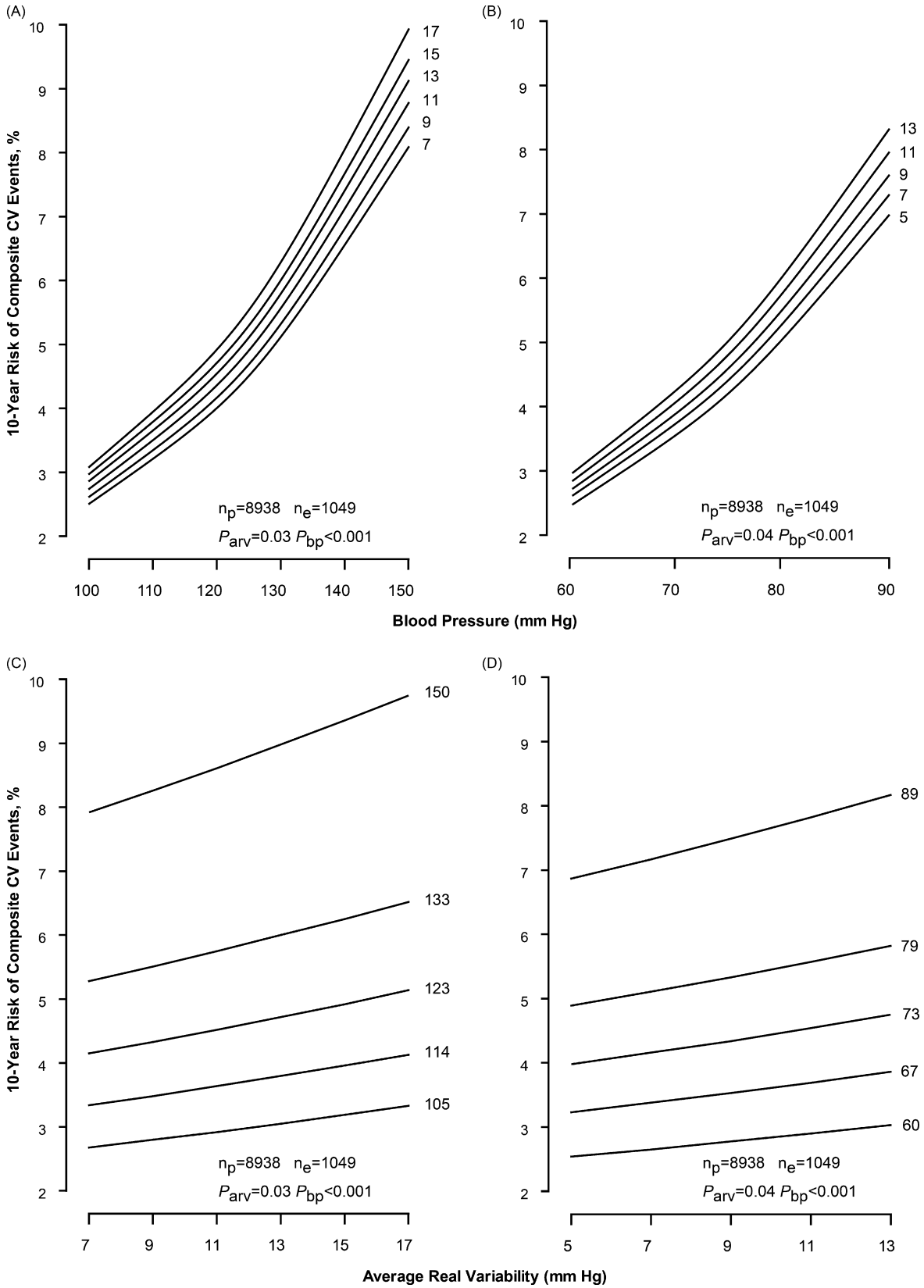

Fig. 1 Ten-year absolute risk of combined cardiovascular events in relation to 24 h blood pressure (A and B) at different levels of systolic and diastolic average real variability (ARV_{24}) and in relation to ARV_{24} (C and D) at different levels of 24 h systolic and diastolic blood pressure. The analyses were standardized to the distributions (mean or ratio) of cohort, sex, age, 24 h heart rate, body mass index, smoking and drinking, serum cholesterol, history of cardiovascular disease, diabetes mellitus, and treatment with antihypertensive drugs. In panels (A) and (B), the risk functions span the 5th–95th percentile interval of the 24 h blood pressure and correspond to the 5th, 25th, 50th, 75th, and 95th percentiles of ARV_{24}. In panels (C) and (D), the risk functions span the 5th–95th percentile interval of ARV_{24} and correspond to the 5th, 25th, 50th, 75th, and 95th percentiles of the 24 h blood pressure. *P*-values are for the independent effect of ARV_{24} (P_{arv}) and 24 h blood pressure (P_{bp}). n_p and n_e indicate the number of participants at risk and the number of events. ARV_{24} denotes average real variability over 24 h. (Reproduced with permission from Hansen, T. W., Thijs, L., Li, Y., Boggia, J., Kikuya, M., Björklund-Bodegård, K., Richart, T., Ohkubo, T., Jeppesen, J., Pedersen, C. T., Dolan, E., Kuznetsova, T., Stolarz-Skrzypek, K., Tikhonoff, V., Malyutina, S., Casiglia, E., Nikitin, Y., Lind, L., Sandoya, E., Kawecka-Jaszcz, K., Imai, Y., Wang, J., Ibsen, H., O'Brien, E., Staessen, J. A., for the International Database on Ambulatory Blood Pressure in Relation to Cardiovascular Outcome Investigators. (2010). Prognostic value of reading-to-reading blood pressure variability over 24 hours in 8938 subjects from 11 populations. *Hypertension* **55**, 1049–1057. Wolters Kluwer Health, Inc.)

Variability of Self-Measured Home Blood Pressure

The prognostic significance of self-measured home blood pressure variability was first assessed in the Ohasama population, using the within-participant SD of the morning systolic blood pressure over 26 days (median) (Kikuya et al., 2008). In multivariable-adjusted Cox models also including blood pressure level, the SD was associated with higher risk of total mortality (HR per 1-SD increase of within-participant SD, 1.18; CI, 1.07–1.31), cardiovascular mortality (HR, 1.20; CI, 1.02–1.40), noncardiovascular mortality (HR, 1.18; CI, 1.04–1.34), stroke mortality (HR, 1.38; CI, 1.12–1.72), but not cardiac mortality (HR, 1.02; CI, 0.89–1.29). The Finn-Home investigators assessed the day-to-day variability of the self-measured systolic blood pressure in the morning. The within-participant SD over 7 days predicted total mortality and cardiovascular events (Johansson et al., 2012). The HRs expressing the incremental risk for a 1-SD increment in variability (3.93 mm Hg) were 1.17 (CI, 1.00–1.30; $P=0.03$) and 1.21 (CI, 1.08–1.40; $P=0.006$), respectively (Johansson et al., 2012). Day-to-day variability in the evening systolic blood pressure was not predictive ($P\geq 0.11$) (Johansson et al., 2012).

Asayama and colleagues (2013) explored whether fatal and nonfatal outcomes were associated with the new indexes of blood pressure variability, VIM, ARV, and MMD, derived from the self-measured home blood pressure. They analyzed mortality and stroke risk in 2421 Ohasama residents after excluding high-risk participants with a history of stroke. Participants were asked to record their self-measured home blood pressure for 4 weeks after at least 2 min of rest in the morning within 1 h after awakening and before taking any blood pressure-lowering drugs and in the evening before going to bed. Over a median follow-up of 12 years, 412 participants died, 139 of cardiovascular causes, and 223 had a stroke. In multivariable-adjusted Cox models including morning systolic pressure, VIM and ARV predicted total and cardiovascular mortality in all participants ($P\leq 0.044$), while VIM predicted cardiovascular mortality in treated ($P=0.014$), but not in untreated ($P=0.23$) participants. Morning MMD did not predict any end point ($P\geq 0.085$). In models already including evening systolic pressure, only VIM predicted cardiovascular mortality in all and in untreated participants ($P\leq 0.046$). When these researchers calculated multivariable-adjusted 10-year risk of cardiovascular mortality and stroke incidence in relation to the mean level and VIM (Fig. 2 in all of the participants), both morning and evening systolic blood pressure were consistent predictors ($P\leq 0.032$) with the exception of cardiovascular mortality in treated participants for morning systolic pressure ($P=0.082$). Being on antihypertensive drug treatment seemed to be the main driver of the significant associations between cardiovascular mortality and blood pressure variability (Asayama et al., 2013), whereas the new indexes of blood pressure variability, VIM derived from self-measured home blood pressure, did not incrementally predict outcome over and beyond mean systolic pressure level.

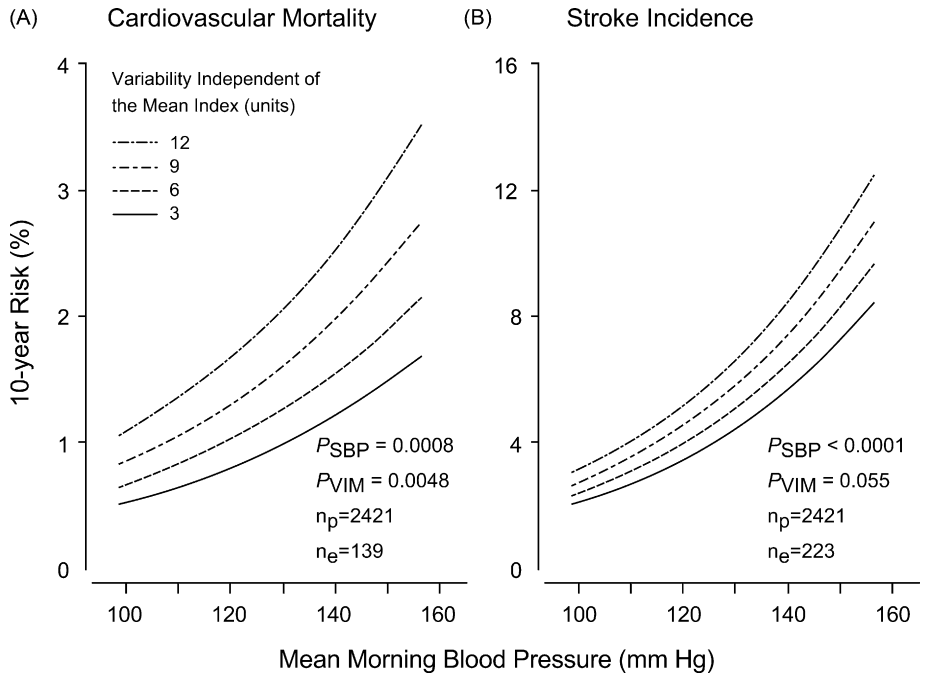

Fig. 2 Absolute 10-year risk of cardiovascular mortality (A) and stroke incidence (B) in relation to the mean level of systolic blood pressure measured at home in the morning in 2421 participants. The analyses were standardized to the distributions (mean or ratio) of sex, age, body mass index, heart rate, smoking and drinking, total cholesterol, diabetes mellitus, history of cardiovascular diseases, and treatment with antihypertensive drugs. Four continuous lines represent the risk independently associated with blood pressure variability independent of the mean (VIM) equal to 3, 6, 9, and 12 units. *P*-values are for the independent effect of SBP (P_{SBP}) and VIM (P_{VIM}). n_p and n_e indicate the number of participants at risk and the number of events. (Reproduced with permission from Asayama, K., Kikuya, M., Schutte, R., Thijs, L., Hosaka, M., Satoh, M., Obara, T., Inoue, R., Metoki, H., Hoshi, H., Ohkubo, T., Staessen, J.A., Imai, Y. (2013). Home blood pressure variability as cardiovascular risk factor in the population of Ohasama. *Hypertension* **61**, 61–69. Wolters Kluwer Health, Inc.)

Within-Visit and Between-Visit Blood Pressure Variability

Results from randomized clinical trials constitute the strongest evidence for the role and reversibility of any cardiovascular risk factor. In the Systolic Hypertension in Europe (Syst-Eur) trial, Hara and colleagues (2014) investigated whether systolic blood pressure variability determines prognosis over and beyond level. Using a double-blind design, 4695 patients ($\geq$60 years) with isolated systolic hypertension (160–219/<95 mm Hg) were randomly allocated to active treatment or matching placebo. Active treatment consisted of nitrendipine (10–40 mg/day) with possible addition of enalapril (5–20 mg/day) and/or hydrochlorothiazide (12.5–25.0 mg/day) (Staessen et al., 1997, 1999). We assessed whether on-treatment systolic blood pressure level, visit-to-visit VIM, or within-visit variability (WVV) predicted total ($n=286$) or cardiovascular ($n=150$) mortality or cardiovascular ($n=347$), cerebrovascular ($n=133$), or cardiac ($n=217$) end points (Hara et al., 2014). At 2 years (median follow-up), in analyses dichotomized by the median, patients with low versus high VIM had similar event rates ($P\geq0.14$). Low versus high WVV was not associated with event rates ($P\geq0.095$), except for total and cardiovascular mortality on active treatment, which were higher with low WVV ($P\leq0.0003$). In multivariable-adjusted Cox models, systolic blood pressure level predicted all end points ($P\leq0.0043$), whereas VIM did not predict any adverse outcome ($P\geq0.058$). Except for an inverse association with total mortality ($P=0.042$), WVV was not predictive ($P\geq0.15$). The double-blind placebo-controlled Syst-Eur trial irrefutably demonstrated that blood pressure-lowering treatment reduces cardiovascular complications by decreasing systolic blood pressure level, but not systolic variability, and that higher systolic blood pressure level predicted risk without material contribution of variability.

In a randomly recruited Flemish population sample ($n=2944$; 50.7% women; mean age, 44.9 years), Schutte and colleagues (2012) computed VIM, MMD, and ARV for WVV, between-visit variability, and overall—within-visit combined with between-visit—variability. Over a median follow-up of 12 years, 401 deaths occurred, and 311 participants experienced a fatal or nonfatal cardiovascular event. Overall (10 readings over two visits), systolic blood pressure variability averaged (SD) 5.45 (2.82) units for VIM, 15.9 (8.4) mm Hg for MMD, and 4.08 (2.05) mm Hg for ARV. In multivariable-adjusted analyses, overall and within- and between-visit blood pressure variability did not predict total or cardiovascular mortality or the composite of any fatal plus nonfatal cardiovascular end point. For instance, the HRs for all cardiovascular events combined in relation to overall variability as captured by VIM, MMD, and ARV were 1.05 (CI, 0.96–1.15), 1.06 (CI, 0.96–1.16), and 1.08 (CI, 0.98–1.19), respectively. By contrast, mean systolic blood pressure level was a significant predictor of all end points under study, independent of blood pressure variability (Schutte et al., 2012). These findings suggest that, in the general population, within-subject blood pressure variability does not have any prognostic significance over and beyond systolic blood pressure level (Schutte et al., 2012).

Conclusion

Methodological issues that might have confounded previous publications on the prognostic value of blood pressure variability as compared with blood pressure level include categorization of continuous variability indexes that are dependent on blood pressure level and the limitation of end points to mortality. While addressing these issues in the aforementioned population studies (Hansen et al., 2010; Schutte et al., 2012), we were never able to identify blood pressure variability as a clinically meaningful cardiovascular risk factor. In particular, our Syst-Eur analysis in line with current recommendations is strong evidence supporting the idea that blood pressure level, not variability, remains centerfold in the primary and secondary prevention of blood pressure-related cardiovascular complications. Although this does not preclude that blood pressure variability remains a target in clinical categorization research, in particular if captured by beat-to-beat recordings (Wei et al., 2014), the current large international population studies and the Syst-Eur randomized clinical trial (Hara et al., 2014) do not support blood pressure variability as prime target in the management of hypertension in clinical practice.

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Brugada Syndrome

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Introduction

Brugada syndrome (BrS) represents one extreme of the clinical manifestation of the J wave syndromes, which are known to be associated with vulnerability to development of polymorphic ventricular tachycardia (VT) and ventricular fibrillation (VF) leading to sudden cardiac death (SCD) in young adults with no apparent structural heart disease (Brugada and Brugada, 1992; Antzelevitch and Yan, 2010; Nam et al., 2008; Haissaguerre et al., 2008) and occasionally to sudden infant death syndrome (Kanter et al., 2012; Antzelevitch, 2001a; Wedekind et al., 2001). The region of the heart most affected in BrS is the anterior right ventricular outflow tract; whereas in other forms of the J wave syndromes such as early repolarization syndrome (ERS), it is the inferior region of the left ventricle (LV) (Haissaguerre et al., 2008; Nagase et al., 2002; Nakagawa et al., 2014; Rosso et al., 2008a; Koncz et al., 2014; Antzelevitch, 2006; Coronel et al., 2005). Accordingly, BrS is characterized by accentuated J waves, appearing as a coved-type ST segment elevation in the right precordial leads, V1–V3, whereas ERS is characterized by J waves, J_o elevation, notch or slur of the terminal part of the QRS and ST segment or J_t elevation in the lateral (type I), infero-lateral (type II) or in infero-lateral + anterior or right ventricular leads (type III) (Antzelevitch and Yan, 2010).

Prevalence

The prevalence of BrS with a type 1 ECG in adults is higher in Asian countries, such as Japan (0.15%–0.27%) (Sakabe et al., 2003; Tsuji et al., 2008), the Philippines (0.18%) (Gervacio-Domingo et al., 2008), as well as among Japanese-Americans in North America (0.15%) (Ito et al., 2006) than in Western countries, including Europe (0%–0.017%) (Letsas et al., 2010; Gallagher et al., 2008; Pecini et al., 2010) or North America (0.005%–0.1%) (Patel et al., 2009; Lee et al., 2005)

Diagnostic Criteria

Fig. 1 illustrates three types of ST segment elevation. Only a Type I coved ST segment elevation is diagnostic of BrS. According to the most recent consensus document (Antzelevitch et al., 2016a,b), BrS is characterized by ST segment elevation ≥ 2 mm (0.2 mV) in ≥ 1 right precordial leads (V1–V3) positioned in the 4th, 3rd, or 2nd intercostal space. When a Type 1 ST segment elevation is unmasked using sodium channel blockers, diagnosis of BrS requires that the patient also presents with one of the following: documented VF or polymorphic ventricular tachycardia (pVT), syncope of probable arrhythmic cause, a family history of sudden cardiac death at <45 years old with negative autopsy, coved-type ECGs in family members, or nocturnal agonal respiration. Inducibility of VT/VF with one or two premature supports the diagnosis of BrS under these circumstances (Sroubek et al., 2016). Table 1 presents the recently proposed scoring system for the diagnosis of BrS.

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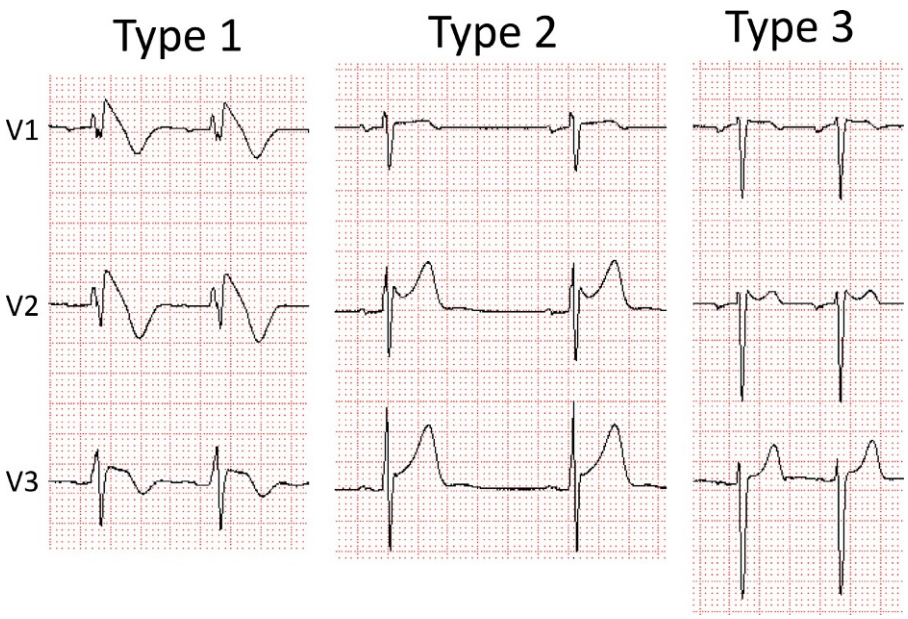

Fig. 1 Three types of ST segment elevation associated with Brugada syndrome. Type I is characterized by a coved-type ST segment elevation of $\geq$2 mm. Only type 1 is diagnostic of BrS. Type 2 is characterized by ST segment elevation of $\geq$0.5 mm (generally $\geq$2 mm in V2) in $\geq$1 right precordial lead (V1–V3), followed by a convex ST. The ST segment is followed by a positive T wave in V2 and variable morphology V1. Type 3 is characterized by either a saddleback or coved appearance with an ST segment elevation of $<$1 mm. Modified from Antzelevitch, C., Yan, G. X., Ackerman, M. J., Borggrefe, M., Corrado, D., Guo, J., Gussak, I., Hasdemir, C., Horie, M., Huikuri, H., Ma, C., Morita, H., Nam, G. B., Sacher, F., Shimizu, W., Viskin, S., Wilde, A. A. (2016). J-Wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. *Heart Rhythm* 32(5), 315–339, with permission.

Table 1 Proposed Shanghai score system for diagnosis of Brugada syndrome

	Points
I. ECG (12-lead/ambulatory)	
(A) Spontaneous type 1 Brugada ECG pattern at nominal or high leads	3.5
(B) Fever-induced type 1 Brugada ECG pattern at nominal or high leads	3
(C) Type 2 or 3 Brugada ECG pattern that converts w/ provocative drug challenge	2
Note: Only award points once for highest score within this category. One item from this category must apply.	
II. Clinical history	
(A) Unexplained cardiac arrest or documented VF/polymorphic VT	3
(B) Nocturnal agonal respirations	2
(C) Suspected arrhythmic syncope	2
(D) Syncope of unclear mechanism/unclear etiology	1
(E) Atrial flutter/fibrillation in patients $<$30 years without alternative etiology	0.5
Note: Only award points once for highest score within this category	
III. Family history	
(A) 1st or 2nd degree relative with definite BrS	2
(B) Suspicious SCD (fever, nocturnal, Brugada aggravating drugs) in a 1st or 2nd degree relative	1
(C) Unexplained SCD $<$45 years in first/second degree relative with negative autopsy	0.5
Note: Only award points once for highest score within this category	
IV. Genetic test result	
(A) Probable pathogenic mutation in BrS-susceptibility gene	0.5
SCORE (requires at least 1 ECG finding)	
$\geq$3.5 points—probable/definite BrS	
2–3 points—possible BrS	
$<$2 points—nondiagnostic	

Reproduced from Antzelevitch, C., Yan, G. X., Ackerman, M. J., Borggrefe, M., Corrado, D., Guo, J., Gussak, I., Hasdemir, C., Horie, M., Huikuri, H., Ma, C., Morita, H., Nam, G. B., Sacher, F., Shimizu, W., Viskin, S., Wilde, A. A. (2016). J-Wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. *Heart Rhythm* 32(5), 315–339, with permission.

Diagnostic Tools

When BrS is suspected in the absence of spontaneous type 1 ST segment elevation, a pharmacological challenge using a sodium channel blocker is recommended. A list of agents used for this purpose are presented in **Table 2** (also see www.brugadadrugs.org). The test is considered positive when a type 1 ST segment elevation is recorded. Drug infusion should be discontinued in case of frequent ventricular extrasystoles or other arrhythmias, or widening of the QRS >130% over the baseline value (Antzelevitch et al., 2005). Alternatively, ECGs can be recorded before and after a large meal, taking advantage of increased vagal tone associated with a "full stomach." The use of in superior positions for V1 and V2 leads increases the sensitivity of recording spontaneous type I ST segment elevation at night or after heavy meals (Shimeno et al., 2009).

Differential Diagnosis

Table 3A presents a list of other causes of ST segment elevation that need to be excluded before establishing the diagnosis of BrS. Conditions that produce a Type 1 Brugada-like ECG include right bundle branch block, pectus excavatum, arrhythmogenic right ventricular cardiomyopathy (ARVC), as well as occlusion of the left anterior descendent artery or the conus branch of the right coronary artery, which supplies the right ventricular outflow tract (RVOT).

Although debate continues as to the extent to which structural abnormalities are present in BrS, most investigators consider BrS to be a channelopathy. Concealed structural abnormalities, such as histologic myocardial fibrosis of the RVOT, which may not become evident using conventional imaging techniques have been proposed to account for or contribute to delayed conduction and ventricular arrhythmias in BrS. MRI and electron beam computed tomography studies of BrS patients consistently show subtle abnormalities, including wall motion abnormalities and reduced contractile function of the right ventricle (RV) and to a lesser extent of the LV, and dilatation of the RVOT (Papavassiliu et al., 2004, 2010; Takagi et al., 2003; Catalano et al., 2009). Antzelevitch

Table 2 Drugs used to unmask the Brugada electrocardiogram

Drug	*Dose*	*Administration*
Ajmaline	1 mg/kg over 10 min	Intravenous
Flecainide	2 mg/kg over 10 min	Intravenous
	200–300 mg	Oral (>1 h)
Procainamide	10 mg/kg over 10 min	Intravenous
Pilsicainide	1 mg/kg over 10 min	Intravenous

Reproduced from Antzelevitch, C., Yan, G. X., Ackerman, M. J., Borggrefe, M., Corrado, D., Guo, J., Gussak, I., Hasdemir, C., Horie, M., Huikuri, H., Ma, C., Morita, H., Nam, G. B., Sacher, F., Shimizu, W., Viskin, S., Wilde, A. A. (2016). J-Wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. *Heart Rhythm* 32(5), 315–339, with permission.

Table 3 Differential diagnosis and modulating factors in Brugada syndrome

A. Differential diagnosis	*B. Modulating factors*
● Atypical right bundle branch block	● Electrolyte abnormalities:
● Ventricular hypertrophy	● Hyperkalemia
● Early repolarization (especially in athletes)	● Hypokalemia
● Acute pericarditis/myocarditis	● Hypercalcemia
● Acute myocardial ischemia or infarction (especially of the right ventricle)	● Hyponatremia
● Pulmonary thromboembolism	● Temperature: hyperthermia (fever), hypothermia
● Prinzmetal angina	● Hypertestosteronemia
● Dissecting aortic aneurism	● Treatment with:
● Central and autonomic nervous systems abnormalities	● Antiarrhythmic drugs: sodium channel blockers (class IC, class IA), calcium antagonists, beta-blockers
● Duchenne's muscular dystrophy	● Antianginal drugs: calcium antagonists, nitrates, potassium channel openers.
● Friedreich's ataxia	● Psychotropic drugs: tricyclic/tetracyclic antidepressants, phenothiazines, selective serotonin reuptake inhibitor, lithium, benzodiazepines
● Spinobulbar muscular atrophy	● Anesthetics/analgesics: propofol, bupivacaine, procaine
● Myotonic dystrophy	● Others: histamine H1 antagonist, alcohol intoxication, cocaine, cannabis, ergonovine
● Arrhythmogenic right ventricular dysplasia	
● Mechanical compression of the right ventricular outflow tract (for instance, pectus excavatum, mediastinal tumor, hemopericardium, etc.)	
● Hypothermia	
● Postdefibrillation ECG	

Modified from Antzelevitch, C., Yan, G. X., Ackerman, M. J., Borggrefe, M., Corrado, D., Guo, J., Gussak, I., Hasdemir, C., Horie, M., Huikuri, H., Ma, C., Morita, H., Nam, G. B., Sacher, F., Shimizu, W., Viskin, S., Wilde, A. A. (2016). J-Wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. *Heart Rhythm* 32(5), 315–339, with permission.

and coworkers have suggested an alternative explanation (Antzelevitch, 2002, 2006; Antzelevitch et al., 1999) pointing out that dilatation of the RVOT may be due to loss of the action potential dome in this region of the heart, which also creates the arrhythmogenic electrical substrate.

In contrast to BrS, imaging techniques in ARVC clearly display morphological and functional changes (e.g., fibro-fatty infiltration, dilatation, bulging/aneurysms, and wall motion abnormalities). ARVC is an inherited cardiac disease resulting from genetically defective desmosomal (DS) proteins (Corrado and Thiene, 2006; Corrado et al., 2011), characterized by fibro-fatty myocardial replacement predisposing to scar-related ventricular arrhythmias which may lead to SCD, mostly in young people and athletes (Thiene et al., 1988). Life-threatening ventricular arrhythmias may occur early, during the "concealed phase" of the disease, prior to overt structural changes (Corrado and Thiene, 2006; Corrado et al., 2011; Delmar and McKenna, 2010). Recent experimental studies demonstrated that loss of expression of DS proteins may induce electrical ventricular instability by causing sodium channel loss of function, predisposing to the development of BrS (Delmar and McKenna, 2010; Delmar and Makita, 2012; Cerrone et al., 2012, 2014; Corrado et al., 2001). These findings support the hypothesis that DS-gene mutations involved in the pathogenesis of ARVC can lead to a decreased depolarization reserve that can manifest as J-wave/BrSs.

Modulating Factors

Sympathetic and parasympathetic influences, hormones, metabolic factors, and pharmacological agents are known to modulate the electrocardiographic and arrhythmic manifestations of BrS (**Table 3B**) (Postema et al., 2009).

Acquired Brugada Pattern

The Brugada ECG is often concealed and can be unmasked with a wide variety of drugs and conditions, including a febrile state, vagotonic agents and maneuvers, α adrenergic agonists, β adrenergic blockers, Class I antiarrhythmic drugs, tricyclic or tetracyclic antidepressants, hyperkalemia, hypokalemia, hypercalcemia, as well as by alcohol and cocaine toxicity (Shimizu, 2005; Brugada et al., 2000a,b; Miyazaki et al., 1996; Babaliaros and Hurst, 2002; Goldgran-Toledano et al., 2002; Tada et al., 2001; Ortega-Carnicer et al., 2001; Nogami et al., 2003; Araki et al., 2003; Pastor et al., 2001). An up-to-date list of agents known to unmask the Brugada ECG and that should be avoided by patients with BrS can be found at www.brugadadrugs.org (Postema et al., 2009).

Genetics

BrS has been associated with variants in 19 different genes (**Table 4**). Genetic defects in *SCN5A* are the most prevalent. To date more than 300 BrS-related variants in *SCN5A* have been described (Antzelevitch, 2012, 2013; Kapplinger et al., 2010; Ackerman et al., 2011). **Fig. 2** shows the overlap between Brugada and other inherited cardiac arrhythmia syndromes attributable to genetic defects in sodium, calcium, and potassium channel defects. The available evidence suggests that the presence of a prominent I_{to} determines whether loss-of-function mutations resulting in a reduction in I_{Na} will manifest as BrS/ERS or as conduction disease (Park et al., 2015; Antzelevitch and Yan, 2015; Szel and Antzelevitch, 2014; Patocskai et al., 2014).

New susceptibility genes proposed and awaiting confirmation include the Transient Receptor Potential Melastatin Protein 4 gene (*TRPM4*) (Liu et al., 2013) and the *KCND2* gene. Variants in *KCNH2*, *KCNE5*, *SEMA3A*, although not causative, have been identified as capable of modulating the substrate for the development of BrS (Verkerk et al., 2005; Wilders and Verkerk, 2010; Ohno et al., 2011; Boczek et al., 2014). Loss-of-function mutations in *HCN4* causing a reduction in pacemaker current, I_f, can unmask BrS by reducing heart rate (Ueda et al., 2004).

Only a small fraction of identified genetic variants in the genes associated with BrS have been examined using functional expression studies to ascertain causality and establish their pathogenicity. Very few have been studied in genetically engineered mouse models, in native cardiac cells, or in induced pluripotent stem cell-derived cardiac myocytes isolated from BrS patients. In silico tools developed to predict the functional consequences of mutations are helpful, but these methods have not been rigorously tested. The lack of functional or biological validation of mutation effects remains the most severe limitation of genetic test interpretation as recently highlighted by Schwartz et al. (2013).

The presence of variants associated with BrS in large public databases of genetic variation such as the 1000 genomes, the National Heart Lung and Blood Institute Exome Sequencing Project (ESP), and the Exome Aggregation Consortium (ExAC) has challenged the view that BrS is a monogenic autosomal dominant inherited syndrome (Refsgaard et al., 2012; Kapa et al., 2009; Andreasen et al., 2013; Alfares et al., 2015; Le Scouarnec et al., 2015; Behr et al., 2015; Veeramah et al., 2014; Risgaard et al., 2013). The absence of genotype–phenotype correlation in some large BrS families with *SCN5A* mutations has also raised concerns (Probst et al., 2009). Collectively, these and other recent studies suggest the possibility that in the individual patient BrS and the susceptibility to VF and SCD may not be due to a single mutation (classical Mendelian view), but rather to inheritance of multiple BrS-susceptibility variants (oligogenic) acting in concert through one or more mechanistic pathways (Bezzina et al., 2013). Moreover, expressivity of the syndrome may be multifactorial in that the genetic predisposition can be modulated by hormonal (testosterone (Matsuo et al., 2003; Antzelevitch, 2003), thyroxine (Korte et al., 2015)), and other environmental factors, as well as

Table 4 Gene defects associated with the Brugada syndrome

Genetic Defects Associated with BrS

	Locus	*Gene/protein*	*Ion channel*	*% of probands*
BrS1	3p21	*SCN5A*, $Na_v1.5$	↓ I_{Na}	11%–28%
BrS2	3p24	*GPD1L*	↓ I_{Na}	Rare
BrS3	12p13.3	*CACNA1C*, $Ca_v1.2$	↓ I_{Ca}	6.6%
BrS4	10p12.33	*CACNB2b*, $Ca_vß2b$	↓ I_{Ca}	4.8%
BrS5	19q13.1	*SCN1B*, $Na_vß1$	↓ I_{Na}	1.1%
BrS6	11q13-14	*KCNE3*, *MiRP2*	↑ I_{to}	Rare
BrS7	11q23.3	*SCN3B*, $Na_vß3$	↓ I_{Na}	Rare
BrS8	12p11.23	*KCNJ8*, *Kir6.1*	↑ $I_{K\text{-}ATP}$	2%
BrS9	7q21.11	*CACNA2D1*, $Ca_v\alpha2\delta1$	↓ I_{Ca}	1.8%
BrS10	1p13.2	*KCND3*, $K_v4.3$	↑ I_{to}	Rare
BrS11	17p13.1	*RANGRF*, *MOG1*	↓ I_{Na}	Rare
BrS12	3p21.2-p14.3	*SLMAP*	↓ I_{Na}	Rare
BrS13	12p12.1	*ABCC9*, *SUR2A*	↑ $I_{K\text{-}ATP}$	Rare
BrS14	11q23	*SCN2B*, $Na_vß2$	↓ I_{Na}	Rare
BrS15	12p11	*PKP2*, *Plakophillin-2*	↓ I_{Na}	Rare
BrS16	3q28	*FGF12*, *FHAF1*	↓ I_{Na}	Rare
BrS17	3p22.2	*SCN10A*, $Na_v1.8$	↓ I_{Na}	5%–16.7%
BrS18	6q	*HEY2* (transcriptional factor)	↑ I_{Na}	Rare
BrS19	1p36.3	*KCNAB2*, *Kvβ2*	↑ I_{to}	Rare

Listed in chronological order of their discovery.

Modified from Antzelevitch, C., Patocskai, B. (2016). Brugada syndrome: Clinical, genetic, molecular, cellular, and ionic aspects. Current Problems in Cardiology 41, 7–57, with permission

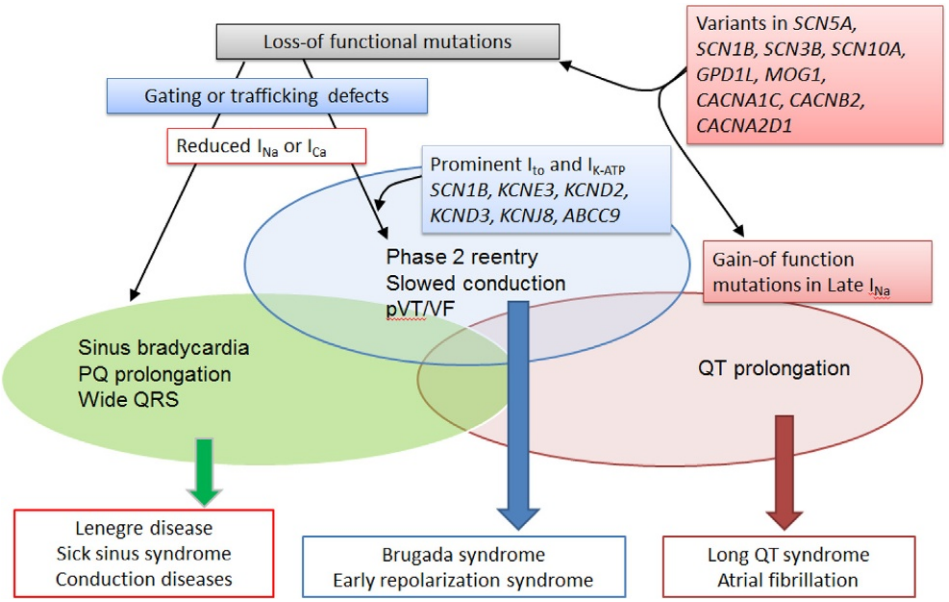

Fig. 2 Schematic showing overlap between Brugada and other inherited cardiac arrhythmia syndromes resulting from genetic defects secondary to loss of function of sodium (I_{Na}) and/or calcium (I_{Ca}) channel current. In the absence of prominent I_{to} or $I_{K\text{-}ATP}$, loss-of-function mutations in the inward currents result in various manifestations of conduction disease. In the presence of prominent I_{to} or $I_{K\text{-}ATP}$, loss-of-function mutations in inward currents cause conduction disease as well as the J wave syndromes (Brugada and Early repolarization syndromes). Early repolarization syndrome is believed to be caused by loss-of-function mutations of inward current in the presence of prominent I_{to} in certain regions of the LV, particularly the inferior wall of the LV. The genetic defects that contribute to BrS and ERS can also contribute to the development of long QT and conduction system disease, in some cases causing multiple expression of these overlap syndromes. In some cases, structural defects contribute to the phenotype. Modified from Antzelevitch, C., Yan, G. X., Ackerman, M. J., Borggrefe, M., Corrado, D., Guo, J., Gussak, I., Hasdemir, C., Horie, M., Huikuri, H., Ma, C., Morita, H., Nam, G. B., Sacher, F., Shimizu, W., Viskin, S., Wilde, A. A. (2016). J-Wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. *Heart Rhythm* 32(5), 315–339, with permission.

morphological changes (fibrosis) (Nademanee et al., 2015). Genetic testing, although helpful in the identification of family members possibly at risk, is generally not used for risk stratification or prognosis purposes (Antzelevitch et al., 2016b; Gray and Behr, 2016).

Ionic and Cellular Mechanisms Underlying BrS

The cellular mechanisms underlying BrS have long been a matter of debate (Wilde et al., 2010; Morita et al., 2009a). Two principal hypotheses have been advanced: (1) The *repolarization hypothesis* (**Fig. 3**) asserts that an outward shift in the balance of currents in right ventricular epicardium can lead to repolarization abnormalities resulting in the development of the substrate for reentrant activity as well as the development of phase 2 reentry, which generates closely coupled premature beats capable of precipitating VT/VF; (2) The *depolarization hypothesis* suggests that slow conduction in the RVOT, secondary to fibrosis, reduced Cx43 leading to

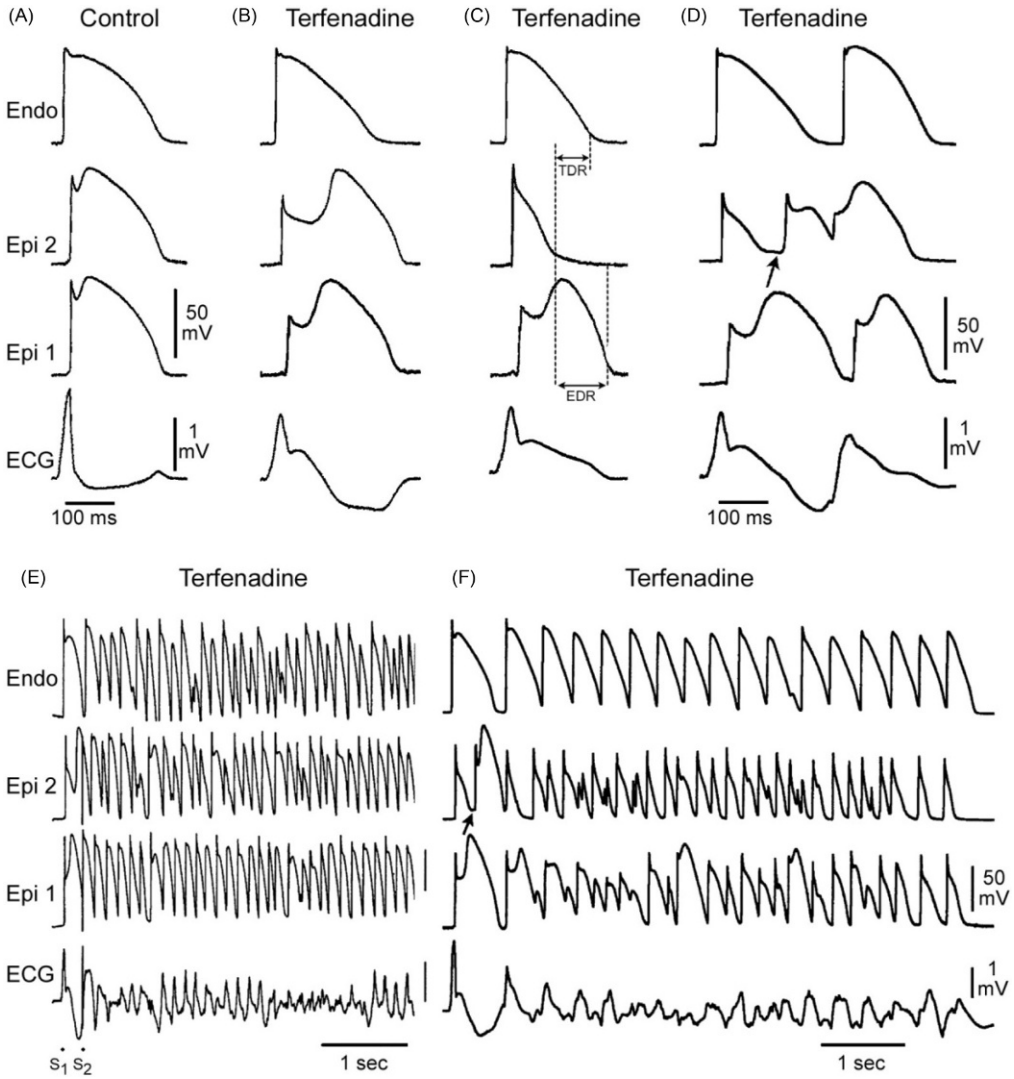

Fig. 3 Cellular basis for electrocardiographic and arrhythmic manifestation of BrS. Each panel shows transmembrane action potentials from one endocardial (top) and two epicardial sites together with a transmural ECG recorded from a canine coronary-perfused right ventricular wedge preparation. (A) Control (Basic cycle length (BCL) 400 ms). (B) Combined sodium and calcium channel block with terfenadine (5 μM) accentuates the epicardial action potential notch creating a transmural voltage gradient that manifests as an ST segment elevation or exaggerated J wave in the ECG. (C) Continued exposure to terfenadine results in all-or-none repolarization at the end of phase 1 at some epicardial sites but not others, creating a local epicardial dispersion of repolarization (EDR) as well as a transmural dispersion of repolarization (TDR). (D) Phase 2 reentry occurs when the epicardial action potential dome propagates from a site where it is maintained to regions where it has been lost giving rise to a closely coupled extrasystole. (E) Extrastimulus (S1–S2 = 250 ms) applied to epicardium triggers a polymorphic VT. (F) Phase 2 reentrant extrasystole triggers a brief episode of polymorphic VT. Modified from reference Fish, J. M, Antzelevitch, C. (2004). Role of sodium and calcium channel block in unmasking the Brugada syndrome. *Heart Rhythm* 1, 210–217, with permission.

discontinuities in conduction and plays a primary role in the development of the electrocardiographic and arrhythmic manifestations of the syndrome. Conduction slowing is not necessarily limited to the RVOT area.

Presumed evidence in support of the depolarization hypothesis was advanced by Nademanee et al. (2011) showing that radiofrequency (RF) ablation of epicardial sites displaying fractionated bipolar electrograms (EGs) and late potentials in the RVOT of patients with BrS suppresses the electrocardiographic and arrhythmic manifestations of BrS. These authors concluded that the late potential (LP) and fractionated electrogram activity are both due to conduction delays within the RVOT (Nademanee et al., 2011; Sacher et al., 2014; Brugada et al., 2015). Similar conclusions were arrived at by Brugada et al. (2015) and Sacher et al. (2014). These authors were of the opinion that ablation of the sites of slow conduction is the basis for the ameliorative effect of ablation therapy. Szel and Antzelevitch (2014) provided a direct test of this hypothesis and showed using an experimental model of BrS that the electrophysiologic manifestation is due to a repolarization defect rather than a depolarization or conduction defect. They showed that low-voltage fractionated electrogram activity similar to that observed in the RVOT of BrS patients develops secondary to heterogeneous appearance of the second action potential upstroke, due to accentuation of the epicardial AP notch and not to slow or delayed conduction (**Fig. 4**). They further showed that high-frequency late potentials develop in RV epicardium due to delayed phase 2 upstroke and to concealed phase 2 reentry. Slow or delayed conduction of the primary beat was never observed in a wide variety of BrS models created by exposing canine right ventricular wedge preparations to drugs mimicking the different genetic defects that have been associated with BrS (Szel and Antzelevitch, 2014). These findings revealed that in the setting of BrS fractionated electrograms and late potentials are due to repolarization rather than conduction defects.

Why then is ablation of regions of the RVOT exhibiting fractionated electrogram activity and late potentials effective in diminishing the ECG and arrhythmic manifestations of BrS? Patocskai et al. tested the hypothesis that ablation ameliorates the BrS phenotype by destroying sites of abnormal conduction. Epicardial regions displaying fractionated electrograms and late

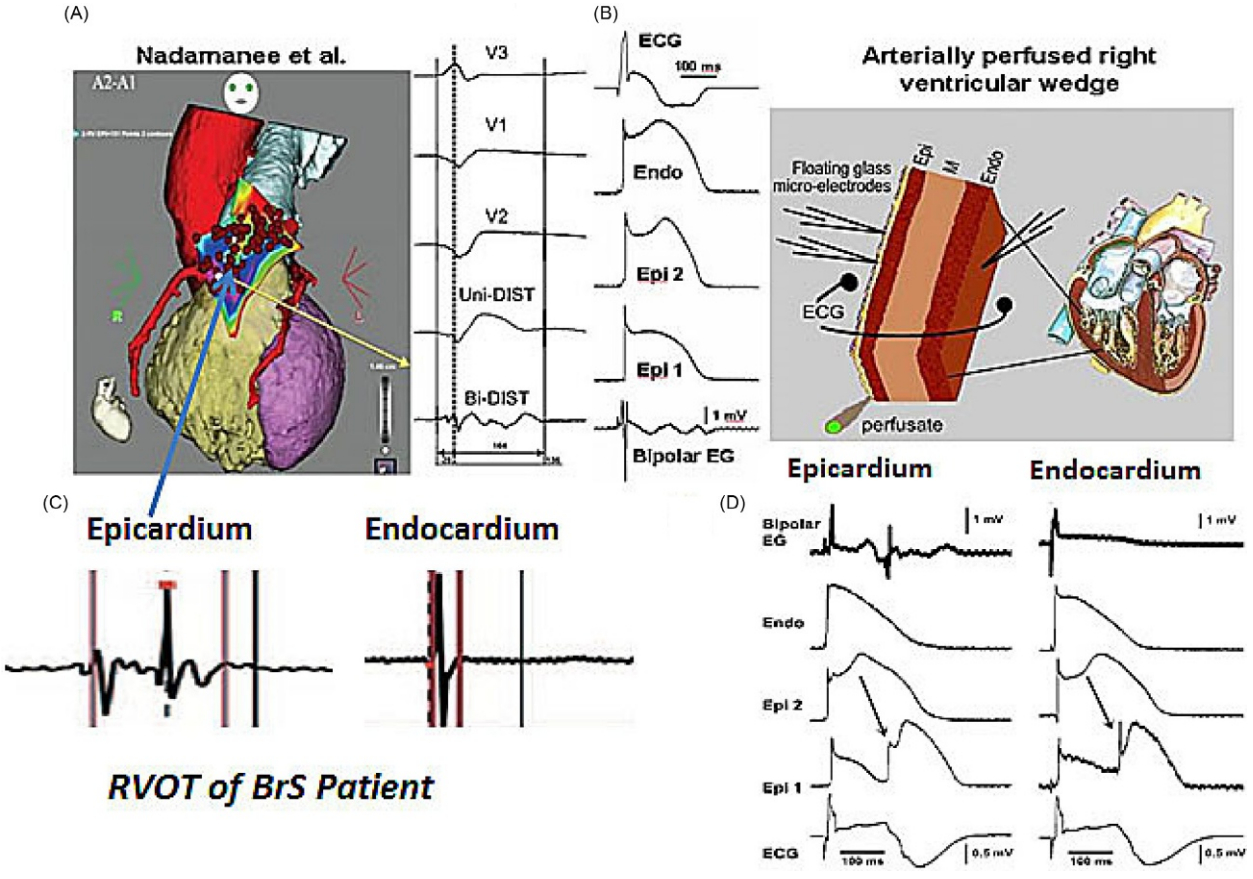

Fig. 4 New interpretation of fractionated electrogram activity and late potentials. Desynchrony in the appearance of the epicardial action potential second upstroke gives rise to fractionated epicardial electrogram (EG) activity and concealed phase 2 reentry gives rise to high-frequency late potentials in the setting of Brugada syndrome (BrS). (A) Shown are right precordial lead recordings, unipolar and bipolar EGs from the right ventricular outflow tract of a BrS patient. (B) ECG, action potentials from endocardium (Endo) and two epicardial (Epi) sites, and a bipolar epicardial EG (Bipolar EG) all simultaneously recorded from a coronary-perfused right ventricular wedge preparation treated with the I_{to} agonist NS5806 (5 μM) and the calcium channel blocker verapamil (2 μM) to induce the Brugada phenotype. Basic cycle length = 1000 ms. (C) Bipolar EGs recorded from the epicardial and endocardial surfaces of the RVOT in a patient with BrS. The epicardial EG displays fractionated electrogram activity as well as a high-frequency late potential late potential (130 ms delay). (D) Bipolar electrograms recorded from the epicardium and endocardium of a coronary-perfused wedge model of BrS, together with AP recordings from an endocardial and two epicardial sites and a transmural ECG. Slow or delayed conduction was never observed (Patocskai and Antzelevitch, 2015b). The clinical data are from Nademanee et al. (2011) and the experimental data are from Szel and Antzelevitch (2014), with permission.

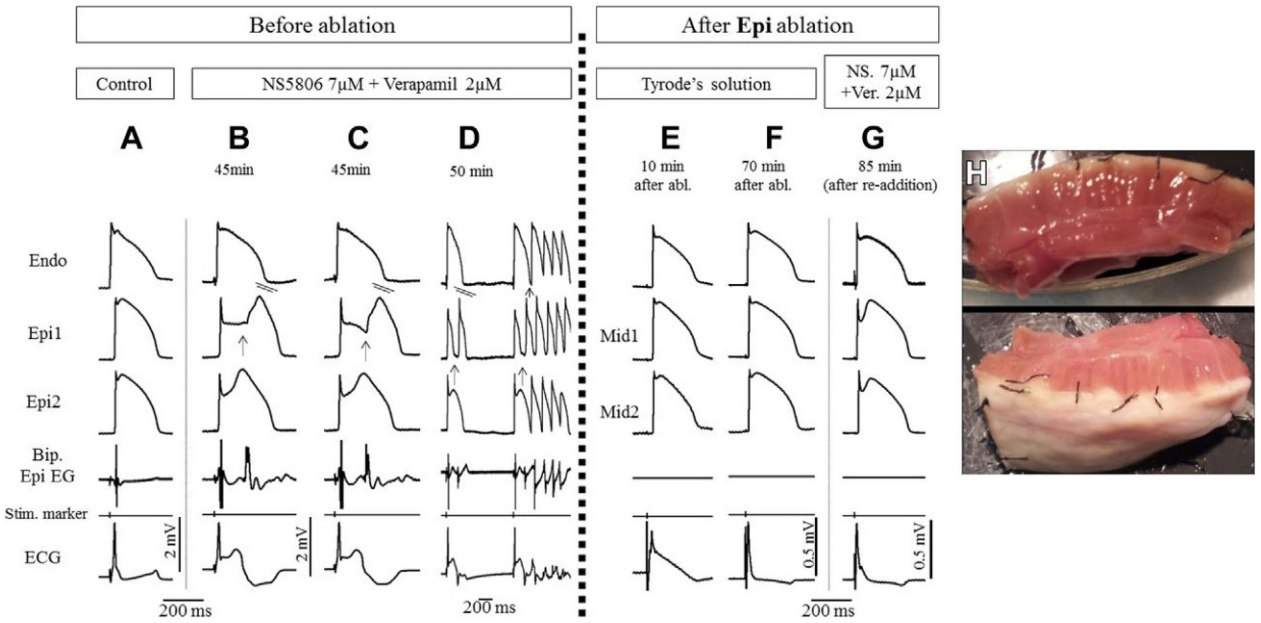

Fig. 5 Radiofrequency ablation of *epicardium* (Epi) suppresses the electrocardiographic and arrhythmic manifestations of Brugada syndrome in coronary-perfused canine RV wedge model generated using a combination of the I_{to} agonist *NS5806* and calcium channel blocker *verapamil*. Each column shows action potentials (AP) simultaneously recorded from an endocardial (Endo) and 2 epicardial (Epi1 and Epi2) sites together with a bipolar epicardial electrogram (Bipol. Epi EG) and an ECG recorded accross the bath. (A) Control. (B, C, D) The addition of NS5806 7 μM and verapamil 2 μM to the perfusate induced pronounced J waves and phase 2 reentry depicting as abnormal electrogram activity when concealed, and giving rise to ventricular fibrillation when it succeeds in propagating out. (E, F) Recovery period of the preparation after epicardial ablation. Note the normalization of ST-elevation after 70 min. Action potential recordings were obtained from midmyocardial (Mid) and subepicardial layers due to inactivation of the epicardium. (G) With superficially ablated epicardium, the readministration of the provocative agents (in the same concentration as before) did not produce pronounced J waves or arrhythmic activity. (H) Photo of wedge preparation after epicardial ablation to a depth of 1–2 mm, taken at the end of the experiment. Reproduced from Patocskai, B., Yoon, N., Antzelevitch C. (2017). Mechanisms underlying epicardial radiofrequency ablation to suppress arrhythmogenesis in experimental models of Brugada syndrome. *JACC: Clinical Electrophysiology*, with permission.

potentials in the experimental models of BrS were ablated. Once again, these electrophysiologic manifestations were shown to be due to repolarization abnormalities and concealed phase 2 reentry rather than conduction abnormalities (Fig. 5). Ablation of the RV epicardium suppressed the manifestation of J waves and ST segment elevation and abolished all arrhythmic activity, a result identical to that obtained by the clinical investigators. These findings provided direct evidence in support of the hypothesis that radiofrequency ablation (RFA) exerts an ameliorative effect by destroying the cells with the most prominent AP notch, thus eliminating the source of repolarization abnormalities responsible for phase 2 reentry and VT/VF (Patocskai et al., 2017).

Further support for the repolarization hypothesis derives from the demonstration that monophasic APs (MAPs) recorded from the endocardial and epicardial surfaces of the RVOT of a patient with BrS were nearly identical to transmembrane APs recorded from the endocardial and epicardial surfaces of the wedge model of BrS (Antzelevitch et al., 2002; Kurita et al., 2002) and from the demonstration that minipigs genetically engineered to heterozygously express a nonsense mutation in SCN5A (E558X) originally identified in a child with BrS (Park et al., 2015) develop conduction defects but not the BrS phenotype. These observations are expected owing to the lack of I_{to} in the pig, which is a prerequisite for the development of the repolarization abnormalities (outward shift imbalance) associated with BrS.

The congruence between BrS and ERS with respect to clinical manifestations and response to therapy provide further support for the repolarization hypothesis. Koncz et al. (2014) developed experimental models of ERS using canine coronary-perfused left ventricular wedge preparations and provided evidence in support of the hypothesis that, similar to the mechanism operative in BrS, an accentuation of transmural gradients in the LV wall is responsible for the repolarization abnormalities underlying ERS. Using ECG Imaging (ECGI), Rudy and coworkers provided additional evidence in support of the repolarization abnormalities by identifying abnormally short activation–recovery intervals (ARI) in the inferior and lateral regions of LV and a marked dispersion of repolarization (Ghosh et al., 2010).

Similarities and Difference Between BrS and ERS

BrS and ERS display several clinical similarities, suggesting similar pathophysiology. Table 5 summarizes the similarities and differences between the two J wave syndromes and mechanisms thought to be involved (Antzelevitch, 2013; Nam, 2012; Yan and Antzelevitch, 1996; Yan and Antzelevitch, 1999; McIntyre et al., 2012).

Table 5 Similarities and differences between Brugada and early repolarization syndromes and possible underlying mechanisms

	BrS	*ERS*	*Possible mechanism(s)*
Similarities between BrS and ERS			
Male predominance	Yes (>75%)	Yes (>80%)	Testosterone modulation of ion currents underlying the epicardial AP notch
Average age of first event	30–50	30–50	
Associated with mutations or rare variants in *KCNJ8*, *CACNA1C*, *CACNB2*, *CACNA2D*, *SCN5A*, *ABCC9*, *SCN10A*	Yes	Yes	Gain of function in outward currents ($I_{K\text{-}ATP}$) or loss of function in inward currents (I_{Ca} or I_{Na})
Relatively short QT intervals in subjects with Ca channel mutations	Yes	Yes	Loss of function of I_{Ca}
Dynamicity of ECG	High	High	Autonomic modulation of ion channel currents underlying early phases of the epicardial AP
VF often occurs during sleep or at a low level of physical activity	Yes	Yes	Higher level of vagal tone and higher levels of I_{to} at the slower heart rates.
VT/VF trigger	Short-coupled PVC	Short-coupled PVC	Phase 2 reentry
Ameliorative response to quinidine and bepridil	Yes	Yes	Inhibition of I_{to} and possible vagolytic effect
Ameliorative response to Isoproterenol denopamine and milrinone	Yes	Yes	Increased I_{Ca} and faster heart rate
Ameliorative response to cilostazol	Yes	Yes	Increased I_{Ca}, reduced I_{to} and faster heart rate
Ameliorative response to pacing	Yes	Yes	Reduced availability of I_{to} due to slow recovery from inactivation
Vagally-mediated accentuation of ECG pattern	Yes	Yes	Direct effect to inhibit I_{Ca} and indirect effect to increase I_{to} (due to slowing of heart rate)
Effect of sodium channel blockers on unipolar epicardial electrogram	Augmented J waves	Augmented J wave	Outward shift of balance of current in the early phases of the epicardial action potential
Fever	Augmented J waves	Augmented J waves (rare)	Accelerated inactivation of I_{Na} and accelerated recovery of I_{to} from inactivation
Hypothermia	Augmented J waves mimicking BrS	Augmented J waves	Slowed activation of I_{Ca}, leaving I_{to} unopposed. Increased phase 2 reentry, but reduced pVT due to prolongation of APD (Morita et al., 2007)
Differences between BrS and ERS			
Region most involved	RVOT	Inferior LV wall	Higher levels of I_{to} and/or differences in conduction
Leads affected	V1–V3	II, IIa, VF, V4, V5, V6; I, aVL, both: infero-lateral	
Regional difference in prevalence			Europe: BrS = ERS Asia: BrS > ERS
Incidence of late potential in SAECG	Higher	Lower	
Prevalence of atrial fibrillation	Higher	Lower	
Effect of sodium channel blockers on the surface ECG	Increased J wave manifestation	Reduced J wave Manifestation	Reduction of J wave in the setting of ER is thought to be due largely to prolongation of QRS. Accentuation of repolarization defects predominates in BrS, whereas accentuation of depolarization defects predominates in ERS
Structural changes—including mild fibrosis and reduced expression of Cx43 in RVOT or fibro-fatty infiltration in cases of ARVC. Imaging studies have also revealed wall motion abnormalities and mild dilatation in the region of the RVOT	Higher in some forms of the syndrome	Unknown	Some have hypothesized that some of these changes may be the result of rather than the cause of the BrS substrate, which may create a hibernation-like state due to loss of contractility in the RVOT secondary to loss of the AP dome

RVOT, right ventricular outflow tract; *AP*, action potential; *PVC*, premature ventricular contraction.

Modified from Antzelevitch, C., Yan, G. X., Ackerman, M. J., Borggrefe, M., Corrado, D., Guo, J., Gussak, I., Hasdemir, C., Horie, M., Huikuri, H., Ma, C., Morita, H., Nam, G. B., Sacher, F., Shimizu, W., Viskin, S., Wilde, A. A. (2016). J-wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. *Heart Rhythm* 32(5), 315–339, with permission.

Risk Stratification

Risk assessment of the factors contributing to development of life-threatening arrhythmias in BrS and ERS has been a high priority in recent years (Brugada and Brugada, 1992; Osher and Wolff, 1953). **Fig. 6** presents a graphic representation of the prevalence and arrhythmic risk associated with the appearance of electrocardiographic J waves and clinical manifestations of BrS and ERS.

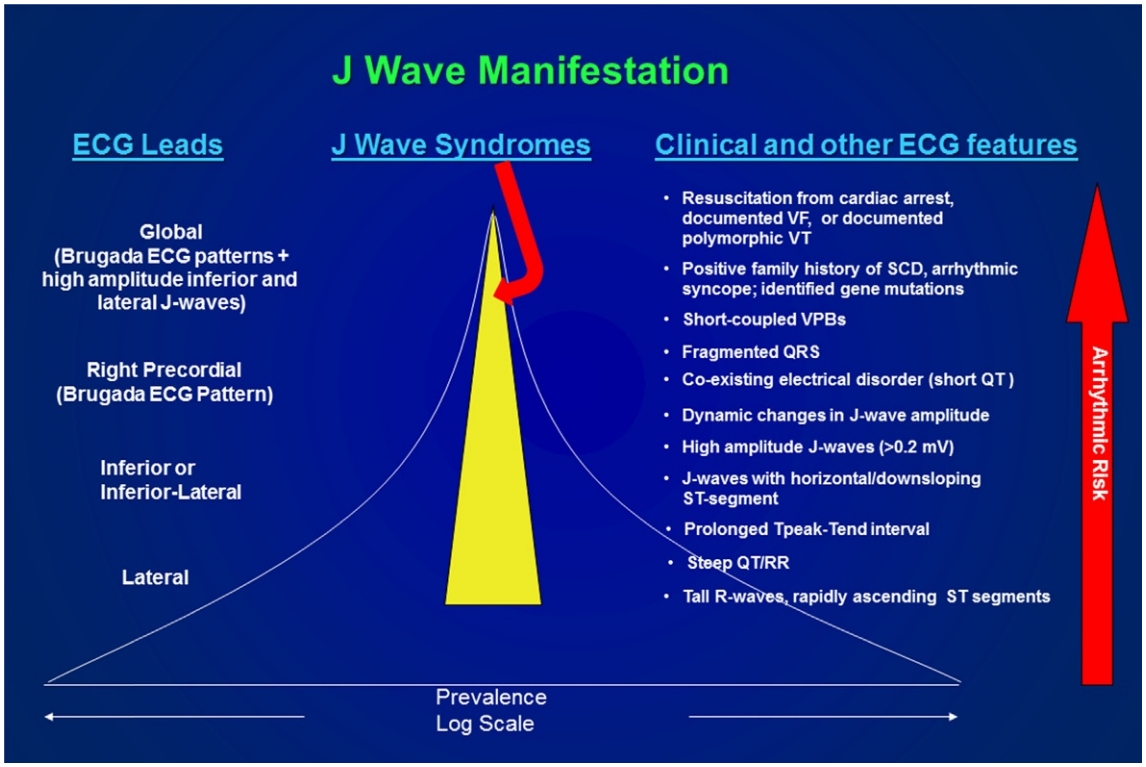

Fig. 6 Prevalence and arrhythmic risk associated with the appearance of ECG J waves and clinical manifestations of Brugada and Early Repolarization syndromes. The yellow highlighted region estimates the prevalence of the J wave syndromes. J waves in the lateral ECG leads have a high prevalence but are associated with a very low arrhythmic risk in a relatively small fraction of the cohort of individuals displaying J waves. On the other extreme, J waves appearing globally in the ECG have a very low prevalence but are associated with a very high level of arrhythmic risk in a large fraction of the cohort presenting with J waves. Likewise, individuals displaying rapidly ascending ST segment elevation have a high prevalence but low risk, whereas subjects resuscitated from cardiac arrest have a very low prevalence by the highest level of arrhythmic risk. Reproduced from Antzelevitch, C., Yan, G. X., Ackerman, M. J., Borggrefe, M., Corrado, D., Guo, J., Gussak, I., Hasdemir, C., Horie, M., Huikuri, H., Ma, C., Morita, H., Nam, G. B., Sacher, F., Shimizu, W., Viskin, S., Wilde, A. A. (2016). J-Wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. *Heart Rhythm* 32(5), 315–339, with permission.

Clinical presentation is the strongest predictor of risk in BrS, overshadowing all other risk factors. The risk of recurrent VF among patients presenting with cardiac arrest is considerable: ≈35% at 4 years (Probst et al., 2010; Kamakura et al., 2009), 44% at 7 years (Conte et al., 2015), and 48% at 10 years (Sacher et al., 2013). One-third of BrS cases present with syncope nowdays (Probst et al., 2010). Their risk of arrhythmic events during follow-up is intermediate: approximately four times higher than the risk of asymptomatic patients (Probst et al., 2010; Priori et al., 2012a,b) but four times lower than that of patients diagnosed postcardiac arrest (Probst et al., 2010), probably due to inclusion of subjects with vagal syncope, which is associated with a relatively good prognosis.

Asymptomatic patients represent approximately 63% of newly diagnosed Brugada patients today (Probst et al., 2010; Kamakura et al., 2009). Their risk of developing symptoms is relatively low (0.5% per year) (Probst et al., 2010; Kamakura et al., 2009). Unfortunately, for most of these patients the first symptom is cardiac arrest or sudden cardiac death. Risk stratification of asymptomatic patients is therefore of utmost importance, and strategies for doing so are discussed later.

Age and gender: The mean age at the time of cardiac arrest in BrS patients is 39–48 (Probst et al., 2010; Kamakura et al., 2009; Conte et al., 2015). BrS in children is rare, but sudden death in this population is reported (Priori et al., 2000; Conte et al., 2014; Probst et al., 2007). Males are at increased risk of displaying a spontaneous type I Brugada ECG and of SCD (Kamakura et al., 2009; Benito et al., 2008).

Spontaneous versus drug-induced type I Brugada pattern: Patients displaying a spontaneous type I ECG at time of diagnosis are at much grater risk of developing arrhythmic events than patients who develop a type I ST segment elevation *only* when challenged with a sodium channel blocker (Probst et al., 2010; Delise et al., 2011) and is an independent predictor of arrhythmic events. Placing the right precordial leads over the 2nd and 3rd intercostal space, in addition to the standard 4th intercostal space, increases the sensitivity for detecting the coved ST segment elevation of the type I Brugada pattern (Miyamoto et al., 2007; Okamura et al., 2015; Govindan et al., 2010).

Electrophysiologic (EP) testing with programmed electrical stimulation: The role of programmed electrical stimulation has long been a matter of intense debate (Brugada et al., 2011; Wilde and Viskin, 2011; Viskin and Rogowski, 2007). The key question is whether the

prognostic information provided by VF inducibility is robust enough for clinical decision-making. Some studies suggest this to be the case (Delise et al., 2011; Brugada et al., 2005; Sieira et al., 2015), whereas others do not (Kamakura et al., 2009; Conte et al., 2015; Priori et al., 2012b). Using a pooled analysis of prospective observational studies of BrS patients undergoing EP study, Sroubek et al. reported that induction with 2 or less extrastimuli was indeed associated with higher risk for development of arrhythmic events and that failure to induce does not necessarily portend low ventricular arrhythmia risk particularly in patients with high-risk clinical features (Sroubek et al., 2016).

Approach to Therapy

Fig. 7 presents recommendations for the management of BrS from the recent expert consensus conference (Antzelevitch et al., 2016b).

ICD and Ablation

The only proven effective therapy for the prevention of SCD in high-risk BrS patients is an implantable cardioverter defibrillator (ICD) (Brugada et al., 2000c; Nademanee et al., 2003). Arrhythmic events and sudden cardiac death in BrS generally occur during sleep or at slow heart rates. These observations notwithstanding a potential therapeutic role for cardiac pacing remains largely unexplored (van Den Berg et al., 2001). A few case reports are available (Bertomeu-Gonzalez et al., 2006; Lee et al., 2000). Nademanee et al. (2011) showed that radiofrequency ablation (RFA) of the epicardium of the RVOT of BrS patients can significantly reduces arrhythmia-vulnerability as well as the ECG-manifestation of the disease and renders VT/VF noninducible. Additional evidence in support of the effectiveness of epicardial substrate ablation was provided by Sacher et al. (2014) and Shah et al. (2011), and Brugada et al. (2015). It is noteworthy that the use of radiofrequency ablation to prevent arrhythmogenesis in the setting of BrS was demonstrated by Morita and coworkers in 2009 using an experimental model of BrS (Morita et al., 2009b).

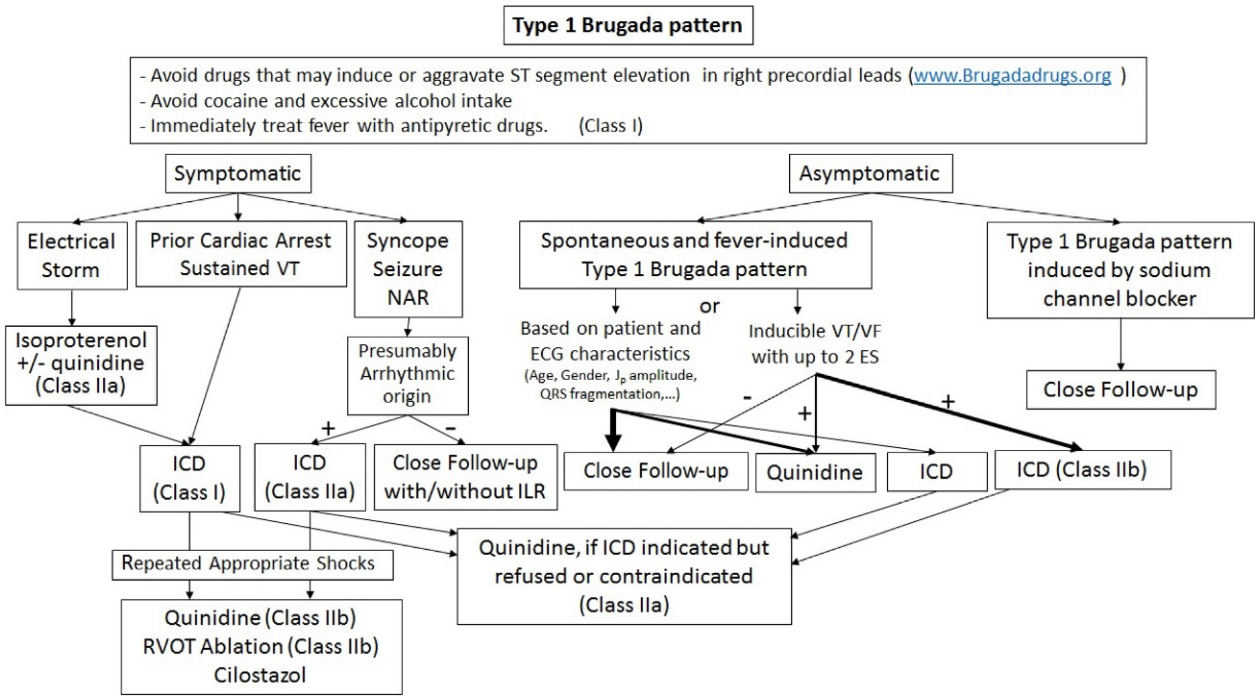

Fig. 7 Indications for therapy of patients with Brugada syndrome. *ES*, extrastimuli at RV apex; *ICD*, implantable cardioverter defibrillator; *ILR*, implantable loop recorder; *NAR*, nocturnal agonal respiration; *pVT*, polymorphic VT; *SCD*, sudden cardiac death; *VT*, ventricular tachycardia. Reproduced from Antzelevitch, C., Yan, G. X., Ackerman, M. J., Borggrefe, M., Corrado, D., Guo, J., Gussak, I., Hasdemir, C., Horie, M., Huikuri, H., Ma, C., Morita, H., Nam, G. B., Sacher, F., Shimizu, W., Viskin, S., Wilde, A. A. (2016). J-Wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. *Heart Rhythm* 32(5), 315–339 with permission.

Pharmacologic Approaches to Therapy

Because the BrS substrate develops as a consequence of an outward shift in the balance of current during the early phases of the epicardial action potential, the principal goal of therapy involves the induction of an inward shift in the balance of current. This can be accomplished either by boosting inward calcium current using β-adrenergic agent or phosphodiesterase III inhibitors or by reducing outward currents including the transient outward current (I_{to}) using agents such as quinidine (Patocskai and Antzelevitch, 2015a).

Because a prominent I_{to} is a prerequisite for the development of both BrS and ERS, partial inhibition of this current is thought to be effective regardless of the ionic or genetic basis for the disease. Unfortunately, cardio-selective and I_{to}-specific blockers are not available. The only agent available in the United States and around the world with significant I_{to} blocking properties is quinidine (Antzelevitch et al., 1999; Yan and Antzelevitch, 1999). Experimental studies have shown that quinidine is effective in restoring the epicardial action potential dome, thus normalizing the ST segment and preventing phase 2 reentry and polymorphic VT in a variety of different experimental models of BrS (Szel and Antzelevitch, 2014; Yan and Antzelevitch, 1999; Minoura et al., 2012; Minoura et al., 2013; Johnson et al., 1960). A recent experimental study suggests that quinidine, owing to its effect to block I_{to}, can also exert a protective effect against hypothermia-induced VT/VF in a J wave syndrome model (Gurabi et al., 2014).

Clinical evidence for the effectiveness of quinidine in normalizing ST segment elevation and or preventing arrhythmic events in patients with BrS has been reported in numerous studies and case reports (Belhassen et al., 2004; Belhassen et al., 1987; Belhassen et al., 2002; Alings et al., 2001; Belhassen and Viskin, 2004; Viskin et al., 2009; Belhassen et al., 2009; Marquez et al., 2013; Pellegrino et al., 2013; Probst et al., 2006; Schweizer et al., 2010; Hasegawa et al., 2014; Marquez et al., 2005; Viskin et al., 2007; Ohgo et al., 2007; Rosso et al., 2008b; Hermida et al., 2004; Mok et al., 2004). A prospective registry of empiric quinidine for asymptomatic BrS can be accessed at http://clinicaltrials.gov/ct2/show/NCT00789165?term_brugada&rank_2. Doses between 600 and 900 mg were recommended, if tolerated (Viskin et al., 2009).

Quinidine may be considered (Class IIb indication) in BrS patients who present with electrical storms and in patients implanted with an ICD who experience repeated appropriate shocks. Quinidine can also be useful in asymptomatic BrS patients displaying a spontaneous Type I ECG, if they qualify for an ICD, and the device is refused or is contraindicated (Class IIa recommendation).

Agents that augment the L-type calcium channel current, including β adrenergic agents like isoproterenol, denopamine, or orciprenaline, have proved usefull (Yan and Antzelevitch, 1999; Schweizer et al., 2010; Ohgo et al., 2007; Antzelevitch, 2001b; Tsuchiya et al., 2002; Kyriazis et al., 2009). Isoproterenol, at times in combination with quinidine, has been utilized successfully to control VF storms and normalizing ST elevation particularly in children (Miyazaki et al., 1996; Belhassen et al., 2002; Alings et al., 2001; Pellegrino et al., 2013; Ohgo et al., 2007; Mok et al., 2004; Maury et al., 2004, 2005; Jongman et al., 2007; Watanabe et al., 2006; Furniss, 2012; Shimizu et al., 2000; Suzuki et al., 2000; Tanaka et al., 2001; Shimizu and Kamakura, 2001; Sharif-Kazemi et al., 2011; Roten et al., 2012; Kaneko et al., 2014). Administration of isoprotereonol is a Class IIa recommendation for BrS patients presenting with electrical storms (Priori et al., 2013).

Phosphodiesterase III inhibitors such as cilostazol (Ohgo et al., 2007; Tsuchiya et al., 2002; Agac et al., 2013) normalize ST segment, elevation in BrS by augmenting calcium current (I_{Ca}) as well as by reducing I_{to} secondary to an increase in cAMP, and heart rate (Kanlop et al., 2011). Milrinone is another phosphodiesterase III inhibitor recently identified in an experimental model of BrS as a more potent alternative to cilostazol in suppressing ST elevation and arrhythmogenesis (Szel and Antzelevitch, 2014; Szel et al., 2013). No clinical reports are available as yet.

Wenxin Keli, a traditional Chinese medicine, has recently been shown to inhibit I_{to} and thus to suppress polymorphic VT in experimental models of BrS when combined with low concentrations of quinidine (5 μM) (Minoura et al., 2013).

Agents that augment peak and late I_{Na}, including bepridil and dimethyl lithospermate B (dmLSB), are suggested to be of value in BrS. Bepridil has been reported to suppress VT/VF in several studies of patients with BrS (Ohgo et al., 2007; Sugao et al., 2005; Murakami et al., 2010; Aizawa et al., 2013). The drug's action is thought to be mediated by: (1) inhibition of I_{to}, (2) augmentation of I_{Na} via upregulation of the sodium channels (Kang et al., 2009), and (3) prolongation of QT interval at slow rates thus increasing the QT/RR slope (Sugao et al., 2005; Aizawa et al., 2013).

Participation in Sports

Because malignant ventricular arrhythmias are infrequent in asymptomatic patients with BrS (Probst et al., 2010) and usually unrelated to physical activity, the presence of these patterns does not contraindicate participation in sports, although insufficient data are currently available to make definitive recommendations for participation in sports.

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C

Cardiac Anatomy, Physiology, and Pathophysiology

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Introduction

The heart is the fundamental blood supply pump of the body, generating the pressure and flow to deliver oxygen and nutrients to all of the body's tissues. It is an intricate four-chambered organ with many unique anatomic and physiological features. Its normal function is dependent upon a myriad of factors including, but not limited to, development, hemodynamics, autonomic nervous system regulation, and hormonal regulation from both the central blood supply and local metabolites. In this chapter, we provide a general overview of cardiac and coronary anatomy, physiology, and pathophysiology.

Cardiac Anatomy

Cardiac Macroscopic Structure and Route of Blood Flow

Cardiac anatomy, coronary blood supply, and cardiac cellular and subcellular structure and function are all critical to cardiac function and physiology. As detailed cardiac anatomy can be found in many anatomical textbooks (Netter, 2010; Moore et al., 2014), we provide here only a brief description of cardiac anatomy to lay the foundation for understanding the physiology of the cardiac cycle. The heart is composed of a series of inflow collecting chambers (atria) and outflow pumping chambers (ventricles) as shown in **Fig. 1**. The atrioventricular valves, the tricuspid and mitral valves, connect the atria to the ventricles and the semilunar valves, the aortic and pulmonary valves, connect the ventricles to the outflow tract (the aorta and pulmonary arteries, respectively). These valves serve to restrict blood flow to one direction under normal circumstances. The heart is separated into a right and left side with the interatrial septum (IAS) and interventricular septum (IVS) separating the right and left atria and right and left ventricles, respectively (**Fig. 1A and B**) (Netter, 2010; Moore et al., 2014).

Deoxygenated red blood cells enter the heart at the right atrium (RA) via the superior vena cava from above and the inferior vena cava from below. The RA is identified by the presence of pectinate muscles, a broad thumb-like right atrial appendage, and a smooth ridge in the superior portion of the RA called the crista terminalis, which divides the right atrial appendage and muscular portion of the RA from the smooth portion of the RA. The wall of the RA is relatively thin and is highly compliant, expanding with blood as it

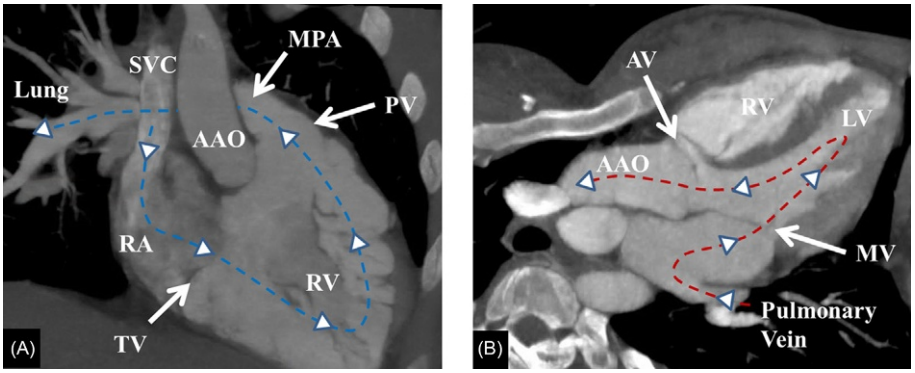

Fig. 1 Cardiac anatomy and route of blood flow tracing the course of (A) systemic deoxygenated blood from the SVC to the lungs (*blue lines*) and (B) oxygenated blood from the lungs to the pulmonary veins to the aorta (*red lines*).

fills during atrial diastole; thus the RA pressure is normally very low (≤5 mmHg, varying with respiration). After filling, the RA pumps deoxygenated blood into the right ventricle (RV) through the tricuspid valve, aided by spontaneous RA contraction at the end of the atrial contraction phase (atrial systole) or ventricular diastole. The tricuspid valve is situated between the RA and the RV and is characterized by its multiple attachments to the ventricular septum by being more apically positioned than the mitral valve. The tricuspid valve has three leaflets: the septal, posterior, and anterior. The tricuspid valve opens during ventricular diastole to allow the RV to fill and closes during ventricular systole to prevent blood from leaking back into the RA from the RV (**Fig. 1A**) (Netter, 2010; Moore et al., 2014).

The RV is identified by its association with the tricuspid valve, its triangular shape, and trabeculated appearance, as well as the moderator band (also known as trabeculae septomarginalis or septomarginal trabeculations), which separates the apical portion of the RV from the rest of the RV. The RV pressure is typically low (systolic ≤25 mmHg and diastolic ≤5 mmHg) and is typically ≤25% of that of the left ventricle (LV). The function of the RV is to pump deoxygenated blood through the pulmonary valve into the pulmonary artery and to the lungs. The pulmonary valve is a semilunar valve situated between the RV and pulmonary artery, has three leaflets, and arises from the infundibulum of the RV. The pulmonary valve is separated from the tricuspid valve by the RV infundibular musculature that forms the right ventricular outflow tract. The pulmonary valve controls blood flow through the pulmonary artery to the lung by opening during RV contraction (systole) and closing during RV filling (diastole), thus preventing regurgitation of blood back into the RV (**Fig. 1A**).

Deoxygenated blood enters the main pulmonary artery and then flows through right and left branch pulmonary arteries on its way to the lung where it passes through a complex network of capillaries and adjacent alveoli to become oxygenated before returning to the left atrium (LA). Like the RV, the pressure in the main pulmonary artery and branch pulmonary arteries are low, typically less than 25% of systemic blood pressure (systolic ≤25 mmHg and diastolic ≤10 mmHg) (Mukharji et al., 1990; Anderson et al., 2004; Partridge and Anderson, 2009; Gavaghan, 1998; Crick et al., 1998). Once oxygenated, blood returns to the LA via four pulmonary veins, which typically enter the posterior aspect of the LA (**Fig. 1A and B**).

The LA is typically identified as lacking significant pectinate muscle and having a thin, finger-like left atrial appendage. Like the RA, the LA has a pulmonary venous component as well as a vestibule and septal surface; unlike the RA, however, the LA has a large body component that connects these other parts together. Similar to its RA counterpart, the wall of the LA is relatively thin and highly compliant, expanding with blood as it fills during atrial diastole. Because of this the LA pressure, though higher than that of the RA, is normally low (≤10 mmHg). The function of the LA is to pump oxygenated blood into the LV through the mitral valve, aided by spontaneous LA contraction at the end of the atrial filling phase (atrial systole) or ventricular diastole.

The mitral valve is situated between the LA and the LV and is identified by its papillary muscle attachments to the free wall of the LV and none to the ventricular septum. The mitral valve is comprised of an anterior leaflet, which is attached to the anterolateral papillary muscle, and a posterior leaflet, which is attached to the posteromedial papillary muscle via fibrous strands of chordae tendineae. During ventricular diastole the mitral valve opens, allowing the LV to fill; during ventricular systole the mitral valve closes, preventing blood from leaking back into the LA from the LV.

The LV is identified by its association with the mitral valve, its cone-like shape, and its numerous dense trabeculations, giving the LV wall a smooth appearance. Similar to its RV counterpart, the LV has an inlet, as well as apical trabecular and outlet components. Unlike the RV, however, the LV chamber is thick-walled, and the pressure generated by the LV at rest is much higher than that of the RV (systolic ≤120 mmHg and diastolic ≤10 mmHg). The function of the LV is to pump oxygenated red blood cells across the aortic valve and through the aorta to bring oxygen to both the upper and lower body via the arterial system. The aortic valve is a semilunar valve with three separate leaflets that is situated between the LV and ascending aorta. The aortic valve controls blood flow by opening during LV contraction (systole), allowing oxygenated blood to enter the ascending aorta on its way to the body, and closing during LV filling (diastole) to prevent regurgitation of blood back into the LV. The pressure in the ascending aorta is typically slightly lower than the LV pressure (systolic ≤120 mmHg and diastolic ≤80 mmHg) at rest, thus allowing forward flow. Oxygenated blood

entering the ascending aorta is then dispersed through a series of arterial branches to supply tissues of the upper and lower body (Holt et al., 1968; Kowalski et al., 1954; Lamers et al., 1992; Paraskevas et al., 2017).

Coronary Anatomy and Supply

The coronary vascular system is comprised of arteries, arterioles, capillaries, venules, and veins and originates as the right and left main coronary arteries just above the AV at the level of the aortic root or sinus of Valsava between the aortic annulus and sinotubular junction (Kini et al., 2007; Schaper et al., 1972). The right coronary ostium is situated at the right (anterior) cusp of the AV and gives rise to the right coronary artery system that feeds the RV. The left coronary ostium is situated at the left (posterior) coronary cusp of the AV and gives rise to the left coronary artery system that feeds the LV. The coronary arteries course over the surface of the heart and divide into smaller branches as they traverse away from the aorta, penetrating the epicardium to supply the remainder of the myocardium. The smaller coronary arteries branch into arterioles, which further branch into large numbers of capillaries, through which the oxygenated blood cells release oxygen to adjacent tissue exchanging it for carbon dioxide. Deoxygenated blood cells then flow back to the RA through the venules, which then coalesce into larger cardiac veins, with blood ultimately returning to the RA through the coronary sinus. The control and function of the coronary circulation has been nicely reviewed (Kern, 2000; van de Hoef et al., 2012, 2015).

The function of the right coronary artery system is to supply nutrient-rich oxygenated blood to the right heart and its electrical conduction system. Shortly after arising from the right coronary cusp, the right coronary artery branches to the conus arteriosus, which supplies the RV outflow tract, and an atrial branch that gives rise to the sinoatrial node artery (in 50%–75% of cases). This runs anteriorly along the RA to the superior vena cava to supply the sinoatrial node. The remainder of the right coronary artery system courses into the right atrioventricular groove posteriorly and downward into the interventricular sulcus and, on its way, gives rise to a variable number of smaller branches into the RA and RV, the most prominent of which is the right marginal coronary artery branch that supplies the right margin of the RV. Further downward on the posterior surface of the RV, 2–3 additional branches arise. The most important of these is the atrioventricular node artery that supplies the atrioventricular node (in 50%–60% of cases), the proximal part of the bundles of His, and portions of the posterior interventricular septum (Netter, 2010; Moore et al., 2014; Kini et al., 2007).

Like the right coronary artery system, the function of the left coronary artery system is to supply nutrient-rich oxygenated blood to the left heart and its electrical conduction system. The main left coronary artery, after arising from the left coronary cusp, courses between the main pulmonary artery trunk and the left atrial appendage, then branches to the left anterior descending and the left circumflex coronary arteries. The left anterior descending artery, which appears to be a continuation of the left coronary artery at the left circumflex branch, then courses along the interventricular groove toward the ventricular apex. It gives rise to the anterior septal perforating arteries that enter the septum of the myocardium and supply blood to two-thirds of the interventricular septum (in 90% of cases), while other branches supply the anterior, lateral, and apical wall of the LV in addition to most of the right and left bundle branches. The left circumflex branch, after arising from the left coronary artery, courses along the left atrioventricular groove and supplies most the LA, the posterior and lateral free walls of the LV, and part of the anterolateral papillary muscle (Netter, 2010; Moore et al., 2014; Kini et al., 2007).

Microscopic Cardiac Anatomy

The macroscopic cardiac anatomy as described earlier is composed of different chambers of the heart, valves, and vessels that make up the cardiovascular system. However, to better understand cardiovascular physiology, one must dive deeper into the microscopic cardiac anatomy. The supporting structure (cardiac skeleton) of the microscopic cardiac anatomy is composed of dense fibrous collagenous connective tissue and the cardiac fibroblasts that are largely responsible for their formation. Within this supporting structure, the cardiac muscle fibers of the atria, ventricles, and heart valves are inserted (Jarvis and Stephenson, 2013).

Cardiac muscle is formed by cells called cardiomyocytes. The most basic unit of cardiomyocytes is the sarcomere, which under microscopy appears striated with alternating dark and light bands. These sarcomeres are connected to the plasma membrane (sarcolemma) by transverse tubules (t-tubules) that facilitate coordinated sarcomeric contraction. The two most important contractile proteins in the sarcomeres are myosin and actin. While myosin forms a thick and flexible filament, its counterpart actin forms a thin more rigid filament. The contractile protein myosin has a long tail and somewhat globular head that binds to actin as well as to adenosine triphosphate (ATP) needed for muscle movement. The contractile protein actin molecules are bound to the Z-disc, forming the borders of the sarcomere. These two contractile proteins (myosin and actin) form myofibrils that are the repeating molecular structures of the sarcomere required for muscle contraction. Contraction occurs when ATP binds to myosin, causing it to separate from the actin component of the myofibril. Cardiac muscle contraction, a complex process which is regulated by calcium influx and stimulus from electrical impulses, will be detailed in the cardiac physiology section (Skwarek-Maruszewska et al., 2009; Severs, 1995, 2000).

Contraction between cardiomyocytes is coordinated by the presence of intercalated discs, which connect individual cardiomyocytes into a functional syncytium. It is this syncytium that supports the rapid spread of action potentials to synchronize the contraction of the myocardium as a whole (Paraskevas et al., 2017). Under light microscopy, intercalated discs appear as thin lines dividing adjacent cardiac muscles and run perpendicular to the direction of the muscle fibers. Adheres junctions (anchor actin filaments to the cytoplasm of cardiomyocytes), gap junctions (connect the cytoplasms of adjacent cells and transmit action

potentials between these cells, and desmosomes (bind adhesion proteins to the cytoskeleton further connecting cells), are all components of the intercalated disc. These components work together within the cardiomyocyte syncytium to coordinate the transmission of action potentials and calcium flux during cardiac muscle contraction (Martini et al., 2012).

Cardiac Physiology

Normal cardiac function is complex and influenced by many factors, including local and systemic hormones, the autonomic nervous system, the inherent load on the cardiac chambers, and the coronary circulation that supplies nutrients and oxygen to this metabolically active tissue. In this section, we describe and integrate these important functions.

The Origin of the Heartbeat–Cardiac Electrophysiology

The electrical firing of "excitable" cells is dictated largely by the electrical membrane potential for each cell type and the ion channels that comprise each cell type. That membrane potential is driven by the influx and efflux of ions (Na^+, K^+, Ca^{2+}); in the heart, specialized cells (pacemaker cells) that reside within the sinoatrial node have "leaky" Na^+ channels (Verheijck et al., 1998; Boyett et al., 2000). These leaky Na^+ channels raise the membrane potential until it reaches threshold (approximately −40 to −30 mV), at which point depolarization occurs (**Fig. 2A**). This rhythmic depolarization is known as the pacemaker potential and is responsible for the automaticity of the heart. Depolarization of pacemaker cells in the sinoatrial node propagates across the left and right atria, causing atrial contraction and activation of the atrioventricular node. Atrioventricular node activation fires the signal down the bundle of His in the interventricular septum so that the ventricles contract from apex to base in a coordinated manner.

Unlike pacemaker cells, cardiomyocytes (nonpacemaker cells) have a resting membrane potential that approximates −90 mV (**Fig. 2B**). Action potentials from neighboring cells can depolarize cardiomyocytes to threshold, triggering a rapid influx of Na^+ through fast Na^+-channels (Phase 0). This initial rapid depolarization is followed by a very brief repolarization caused by the opening of outward K^+ channels (Phase 1). This phase occurs concurrently with the slow influx of Ca^{2+} through L-type Ca^{2+} channels, causing a plateau phase (Phase 2). This plateau phase is thought to be a built-in mechanism to prevent spontaneous firing within the absolute refractory period, or period during which random depolarizations do not occur, and is aided by the continual inactivation of the fast Na^+ channels. As the L-type Ca^{2+} channels close, inward rectifying K^+ channels open (Phase 3), causing a repolarization of the cell back to resting membrane potential (Phase 4). This cycle repeats with every cardiac cycle, initiating from the electrical activities at the sinoatrial node, propagating across the atria to the atrioventricular node, and down the septal bundle of His to contract the myocardium from apex to base.

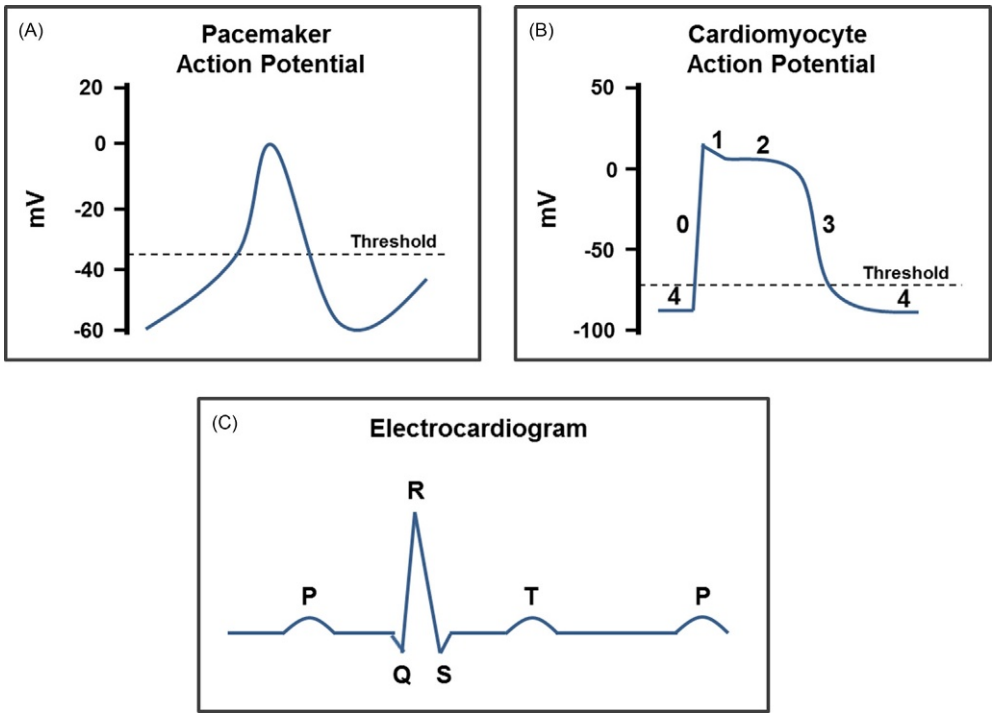

Fig. 2 Action potentials of pacemaker cells at the SA node (A) and cardiomyocytes (B), as well as the resultant normal electrocardiogram (C).

These cyclic alterations in electrical activity can be measured by the electrocardiogram (ECG, Fig. 2C). Atrial depolarization is reflected by the P wave, which is followed by the QRS complex, representing ventricular depolarization. It is important to note that the electrical magnitude of ventricular depolarization at the QRS complex masks atrial repolarization. The T wave represents ventricular repolarization, and the cycle repeats with every cardiac cycle. The duration and shape of the waves in the ECG provide important clues into cardiac arrhythmias, dysfunction, and overt heart failure, which will be discussed in later chapters.

Excitation–Contraction Coupling

The conversion of cardiomyocyte electrical depolarization via the action potential to cellular and syncytial contraction is called excitation–contraction coupling. Critical to the rapid and coordinated conduction of this signal to the interior of each cell is the existence of an intricate system of t-tubules, which are invaginations of the cardiomyocyte membrane designed to efficiently propagate the action potential. As the action potential is transmitted to each cardiomyocyte, Ca^{2+} floods the cytoplasm through L-type calcium channels during Phase 2 of the action potential. Ca^{2+} binds to ryanodine receptors on the sarcoplasmic reticulum to induce the rapid release of calcium stores to bring the intracellular Ca^{2+} concentration to approximately 10^{-7} to 10^{-5} M. Cytosolic Ca^{2+} then binds to troponin-C (TnC) on actin thin filaments to initiate myocyte contraction. Ca^{2+} binding to TnC elicits a conformational change in the troponin complex that dissociates troponin-I (TnI) from the myosin binding site on actin. The myosin head then binds to actin to induce the "power stroke" to shorten the sarcomere, resulting in systolic myocyte contraction. This power stroke uses ATP hydrolysis as its energy source. The release of contraction is caused by movement of cytosolic Ca^{2+} back into the sarcoplasmic reticulum via the sarcoplasmic reticulum calcium ATPase, or SERCA. Calcium is also removed from the cytosol to a lesser extent by the plasma membrane Na^{+}/Ca^{2+} exchange ATPase. The reduction in intracellular Ca^{2+} effectively removes it from TnC to allow rebinding of TnI to the myosin binding site on actin, resulting in the lengthening, or relaxation of the sarcomere back to its basal, noncontracted state.

The Cardiac Cycle: Systolic and Diastolic Function

The net effect of excitation–contraction coupling is the syncytial, coordinated contraction of sarcomeres in myocytes that results in ejection of blood from the heart during systolic contraction and the subsequent refilling of the cardiac chambers during diastolic relaxation. One cardiac cycle is comprised of a diastolic phase and a systolic phase, which can be represented on a pressure–volume loop (Fig. 3). During diastole, left ventricular pressure drops precipitously during isovolumic relaxation to below 5 mmHg of ventricular pressure. This drop in pressure results in passive blood flow from the atria into their respective ventricles through the atrioventricular valves. At the end of diastole, the atria contract, causing the "atrial kick" that accounts for approximately 20% of the end-diastolic volume in the ventricle prior to ventricular contraction. After the atria contract, isovolumic contraction occurs—the initial phase of contraction that continues until ventricular pressure exceeds aortic pressure across the aortic valve (for LV). Ventricular ejection (systole) occurs when the valve opens, causing a rapid reduction in ventricular volume until ventricular pressure drops below aortic pressure. After the valve closes, isovolumic relaxation begins again for the next cardiac cycle.

In addition to the cellular processes that regulate contraction and relaxation mentioned earlier, hemodynamic and neurohormonal forces play a critical role in regulating cardiac function. Central to these processes is the Frank–Starling Law of the Heart, which simplistically states that "within limits, the heart pumps all that it receives." Practically speaking, within limits, cardiac output/stroke volume increases with increased ventricular preload, or venous return (amount of blood that fills the ventricles during diastole) (Katz, 2011). This relationship is thought to be the result of optimal actin–myosin engagement until such point as

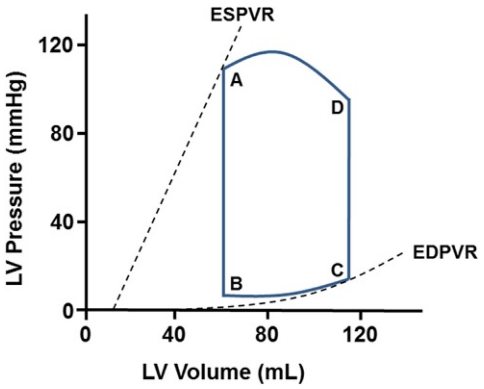

Fig. 3 Pressure–volume relationship in the left ventricle. (A to B) Isovolumic relaxation. (B to C) Passive (LV relaxation) and active (i.e., atrial kick) filling of the LV during diastole. (C to D) Isovolumic contraction—the initial phase of contraction that continues until ventricular pressure exceeds aortic pressure across the aortic valve (at point D). (D to A) Ventricular ejection, or systole, occurs when the valve opens, causing a rapid reduction in ventricular volume until ventricular pressure drops below aortic pressure (point A). After the valve closes, isovolumic relaxation begins again for the next cardiac cycle.

Table 1 Definitions of acronyms

Acronym	*Term*	*Definition*
CO	Cardiac output	The amount of blood pumped by the heart per minute
CI	Cardiac index	Cardiac output normalized to total body surface area
SV	Stroke volume	The amount of blood pumped by the heart per cardiac cycle (heart beat)
HR	Heart rate	The number of cardiac cycles (heart beats) per minute
EDV	End-diastolic volume	The volume of blood in the heart at the end of diastole (filling)
ESV	End-systolic volume	The volume of blood in the heart at the end of systole (contraction)
EF	Ejection fraction	The fraction of blood ejected from the heart with each cardiac cycle (heart beat)

the sarcomeres are stretched at high filling pressures/volumes that these interactions are no longer optimal, thus reducing ejection volume at higher preloads (Katz, 2011). The magnitude of this relationship is not only influenced by preload but also afterload, mainly the arterial pressure against which the heart has to pump blood, and cardiac inotropy, or strength of contraction. Increased afterload and/or decreased inotropy will reduce the overall cardiac output for a given venous return. Decreased afterload and/or increased inotropy will increase the overall cardiac output for a given venous return.

The efficacy of cardiac function can be determined using several physiological concepts and equations. Cardiac output (CO) is the amount of blood flow pumped by the heart per minute and is an overall readout of cardiac function. Many other readouts of cardiac function can be defined by the following equations (defined in **Table 1**):

$$\begin{aligned} CO &= SV \times HR \\ CI &= CO/\text{Body surface area} \\ SV &= EDV - ESV \\ EF &= (EDV - ESV)/EDV \end{aligned}$$

These readouts are just a start to determining how well the heart performs as a pump. There are many other factors not discussed in this section, including cardiac efficiency and cardiac work, which are beyond the scope of this chapter. Further discussion of related cardiac function concepts can be found in other chapters.

Cardiac Pathophysiology

When complex local autoregulatory control, systemic hormonal mechanisms, or both fail to work properly, the consequence is inadequate cardiac output leading to suboptimal nutrient and oxygen delivery to the heart and peripheral organ systems. Failure to maintain adequate cardiac output results in insufficient end-organ perfusion and often end-organ failure. The cardiovascular system can adapt to pathophysiological changes through remodeling in an effort to meet the demand of metabolically active tissues. In some situations, though, these compensatory mechanisms can lead to further cardiac injury, creating a vicious cycle ultimately ending in heart failure. A detailed analysis of the causes and consequences of these pathophysiological changes are outside the scope of this section, so we instead provide a brief overview of some common cardiovascular pathologies.

Congenital Heart Diseases

Congenital heart disease (CHD) (Allen, 2013; Freedom, 2004) is perhaps the most obvious cause of cardiac pathophysiology, as developmental alterations in the cardiac anatomy can affect cardiac output, oxygenation of blood, and both systemic and local blood pressure. More than 40,000 infants are born in the United States each year with CHD (Hoffman and Kaplan, 2002; van der Linde et al., 2011; van der Bom et al., 2012). CHD results in alterations in the cardiac structure in utero and results in a wide range of defects. Some of these are simple and cause minimal impact on cardiac physiology, while others are severe enough to require multiple palliative procedures, often resulting in unique and complex postoperative anatomy with abnormal blood flow. Still, others are incompatible with life, causing fetal or postnatal death, or requiring cardiac transplant either at birth or after failed palliative surgery. Many of the simple defects are small and resolve spontaneously overtime or require straightforward catheter-based interventions or simple surgical repair. Patients with complex constellations of CHD abnormalities will either require multiple cardiac surgical procedures or cardiac transplant. **Table 2** shows an overview of the most common, simple, and complex forms of CHD, which occur in approximately 8 out of every 1000 live births (Hoffman and Kaplan, 2002; van der Linde et al., 2011).

Recent advances in diagnosis and treatment of CHD have dramatically improved patient survival. In the United States alone, there are currently more than 1 million people living with CHD. With increasing numbers of survivors reaching adulthood, many require life-long evaluation for residual anatomic and hemodynamic defects. Of these, approximately 33% have minor defects, with little to no alteration in normal cardiac physiology and generally requiring minimal care. Another 33% have moderate disease, often requiring routine cardiac care, medication, or catheterization or surgical procedures. The last third of these CHD patients develop

Table 2 Overview of congenital heart disease

Septal defects
- Atrial septal defects (primum, secundum, inferior sinus venosus, superior sinus venosus)
- Ventricular septal defects (muscular, perimembranous/outlet, subpulmonary)
- Atrioventricular septal Defects (partial, transitional, intermediate, complete balance, complete unbalance)

Arterial abnormalities
- Patent ductus arteriosus
- Aortopulmonary window
- Congenital anomalies of the coronary artery (anomalous origins from the opposite cusp, anomalous origin from the pulmonary artery and single coronary artery origins)
- Vascular anomalies (vascular rings and slings)
- Aortic arch anomalies (right aortic arch, double aortic arch)
- Transposition of the great arteries
- Congenitally corrected transposition of the great arteries
- Double outlet right ventricle

Atrioventricular valve abnormalities
- Mitral regurgitation
- Mitral stenosis/mitral atresia
- Mitral valve prolapse
- Tricuspid regurgitation
- Tricuspid stenosis/atresia
- Tricuspid valve prolapse
- Ebstein's anomaly of the tricuspid valve

Right-sided congenital heart disease
- Pulmonary stenosis (subvalvar, valvar, and supravalvar)
- Pulmonary atresia with intact ventricular septum
- Pulmonary atresia with ventricular septal defect with multiple aorto-pulmonary collaterals (MAPCAs)
- Tetralogy of fallot/tetralogy of fallot with absent pulmonary valve
- Truncus arteriosus

Left-sided congenital heart disease
- Aortic valve disease (unicuspid and bicuspid aortic valve)
- Aortic stenosis (subvalvar, valvar, and supravalvar)
- Coarctation of the aorta
- Hypoplastic left heart syndrome

Venous abnormalities
- Anomalous pulmonary venous return/connection (partial and total anomalous pulmonary venous return)
- Anomalous system return/connection

Complex cardiac abnormalities
- Univentricular atrioventricular connection
- Double inlet left ventricle
- Congenital Heart disease with heterotaxy syndrome

severe or decompensated heart failure, often without palliative options or with high surgical or cardiac transplant risk, resulting in increased morbidity and early mortality (van der Bom et al., 2012; Wren and O'Sullivan, 2001; Marelli et al., 2007; Warnes et al., 2001; Morris and Menashe, 1991; Zomer et al., 2011).

Abnormal septation between the atria (atrial septal defects), ventricles (ventricular septal defects), both sets of chambers (atrioventricular septal defects), or great vessels (aortopulmonary windows and persistent truncus arteriosus) can result in miscommunication between the right and left heart and ultimately increase volume or pressure load on the right heart. Of these, atrial septal defects are the most common and result in RV volume overload. If left untreated, patients with large defects can develop Eisinmenger syndrome (pulmonary hypertension and right heart failure). Other forms of right heart CHD include tetralogy of Fallot, various forms of pulmonary stenosis, pulmonary atresia, and Ebstein's anomaly. The pathophysiology of these defects often results in increased pressure in the right heart, and in severe cases leads to cyanotic heart disease ("blue" babies) or single ventricular hearts.

CHD affecting the left side of the heart include bicuspid aortic valve, aortic stenosis and complex single ventricular hearts, with the most complex being hypoplastic left heart syndrome, which requires at least three major palliative surgeries and often results in a viable heart albeit with very abnormal anatomy and cardiac pathophysiology. CHD involving the great vessels, and systemic and pulmonary venous anomalies include coarctation of the aorta, various forms of interrupted aortic arch, vascular rings or slings, various forms of transposition of the great arteries, and double outlet right ventricle as well as total anomalous pulmonary venous return. The cardiac pathophysiology and symptoms resulting from these vary but can range from having a minor impact to causing complete alteration of the cardiac physiology. In-depth reading on these various congenital heart disease can be found in this reference (Martini et al., 2012).

Arrhythmias

Arrhythmias can occur as a result of congenital or acquired abnormalities in different ion channels, affecting membrane potential, or defects in the conduction system, such as defects in initiation by pacemaker cells or defects in signal propagation. Arrhythmias range from bradycardia or sinus tachycardia to various levels of heart block and abnormal tachycardia (supraventricular and ventricular tachycardia), and all cause abnormal or uncoordinated contraction.

Arrhythmias have been shown to be secondary to either focal or nonfocal mechanisms. Focal mechanisms include abnormal impulse initiation (normal or abnormal automaticity), triggering from either early or delayed after depolarizations and reflection, whereas the nonfocal mechanisms are various forms of reentry due to circus movement. Examples of automaticity mechanisms of arrhythmias include sinoatrial node dysfunction, enhanced automaticity, overdrive suppression of automaticity, and parasystolic or modulated presystolic activities. Whereas, examples of after depolarization and triggered activities include early after depolarizations and triggered activity, cellular origin of early after depolarizations, ionic mechanisms responsible for early after depolarizations, or delayed after depolarization-induced triggered activity (January et al., 1991; Patterson et al., 2006; Maruyama et al., 2011). The ultimate effect of arrhythmia is poor cardiac output and thus inadequate nutrient and oxygen delivery. Specific mechanisms of arrhythmias will be addressed in later chapters, and many fine comprehensive reviews of the basic mechanisms of cardiac arrhythmia are available (Antzelevitch and Burashnikov, 2011; Tse, 2016; Kanno and Saffitz, 2001; Baruscotti et al., 2005; Cranefield, 1977).

Coronary Heart Disease

The coronary artery system provides oxygen and nutrient-rich blood flow to the metabolically active myocardium and is highly autoregulated (intrinsic ability to maintain blood flow over a wide range of pressures). Coronary heart disease can arise from congenital anomalies, where it is typically diagnosed in childhood, or can be acquired, resulting in narrowing of the coronary arteries, coronary ischemia, and ultimately myocardial infarction. Kawasaki's disease (Newburger et al., 2004), an inflammatory condition thought to be triggered by an autoimmune response, is most commonly seen in children and, left untreated, can lead to coronary aneurysms. Most other acquired coronary heart disease is adult onset.

Although congenital and acquired coronary heart diseases are anatomically different, both result in inadequate blood flow delivered to the region of the myocardium that is affected by the abnormal coronary vasculature. This myocardial injury leads to ventricular dysfunction, arrhythmia, or valve regurgitation depending on the specific areas of the myocardium that is affect. Impaired ventricular function results in a decrease in the ability to generate cardiac output needed to supply nutrients and oxygen to peripheral organs (Mosher et al., 1964).

Congenital coronary anomalies (CCAs) can be isolated but are frequently associated with various forms of CHD. Anatomically and physiologically important CCAs include anomalous left coronary artery arising from the pulmonary artery, resulting in ischemia when the pulmonary vascular resistance drops, decreasing the coronary perfusion pressure. Other CCAs include anomalous origin of the right and left coronary artery from the opposite sinus and can run within the wall of the aorta (intramural) or between the aorta and pulmonary artery (interarterial), myocardial bridge, and, rarely, coronary ostial atresia with collateral flow from the opposite side of the coronary system. The major risk for this group of CCAs is inadequate blood flow delivery to the myocardium that can occur at rest, but worsens with exercise, leading to transient myocardial ischemia, myocardial infarction, or sudden cardiac death. Details of these CCAs, a comprehensive list of all CCAs, and causes and treatment of acquired coronary artery disease can be found in these review articles (Libby and Theroux, 2005; Nabel and Braunwald, 2012; Hauser, 2005; Taylor et al., 1992).

Cardiac Hypertrophy

Normal cardiac development produces an efficient pump that maintains optimal cardiac output and end organ perfusion with minimum wall stress (Grossman et al., 1975). Cardiac hypertrophy occurs as a fundamental process of adaptation to an increased workload due to hemodynamic stress. It can be genetically programmed, as in various forms of hypertrophic cardiomyopathy (Kawano et al., 2016), or it can occur in response to increased hemodynamic load and mechanical stress resulting from arterial hypertension, myocardial infarction, chronic ischemia, inflammation, or valvular disease (Burchfield et al., 2013; Souders et al., 2012; French and Kramer, 2007). The type of hypertrophic remodeling depends on the trigger and concomitant progression of contractile dysfunction. Concentric hypertrophy, in which ventricular walls thicken to reduce capacity, is a common response to increased afterload (such as occurring with valve stenosis or hypertensive heart disease), whereas eccentric hypertrophy, in which the internal diameter of the ventricular wall increases, is often observed in conditions of LV volume overload (such as occurring with valve regurgitation or shunt) (Pfeffer and Braunwald, 1990; Dorn et al., 2003; Kupari et al., 2005; Burchfield et al., 2013). In concentric hypertrophy, sarcomeres are added in parallel to increase wall thickness; in eccentric hypertrophy, sarcomeres are added in series to increase wall diameter. Though cardiac hypertrophy may initially be beneficial (compensatory to maintain cardiac output), as it augments the number of contractile units and reduces ventricular wall stress (Grossman et al., 1975), over time the increased metabolic demand ultimately leads to decompensation with resultant heart failure, associated arrhythmia, and risk of sudden cardiac death (Krishnamoorthy et al., 2011; Pellikka et al., 2005; Haider et al., 1998; Wassilew et al., 2016; Henderson and Anderson, 2009).

The increased mechanical stress in the heart caused by cardiac hypertrophy leads to alterations in cellular signaling and gene expression. In early cardiac hypertrophy, there is a rapid induction of protooncogenes (including c-fos, c-jun, and c-myc) and heat shock proteins (including hsp70) (Kee et al., 2008; Simpson, 1989; Brand et al., 1993). This is followed by both quantitative and qualitative modulation in gene expression and protein synthesis that induce hypertrophy (Komuro et al., 1988; Izumo et al., 1988). Hypertrophy-promoting hormonal factors that are either locally or systemically induced include angiotensin II, endothelin-1, and transforming growth factor-β (van Wamel et al., 2001). Intracellular signaling pathways in cardiomyocytes are activated by mechanical stress via activation of cell surface integrins and their associated cytoskeletal proteins, as well as by interaction with sarcolemmal proteins such as ion channels, ion exchangers, and phospholipases. Mechanical stress also decreases expression of key cardiomyocyte membrane proteins such as SERCA (Anger et al., 1998; Nagai et al., 1989). Subsequent increases in intracellular calcium concentration activate the calcium and calmodulin-dependent phosphatase calcineurin, which is a novel hypertrophic signaling pathway. Interestingly, expression of other key sarcomeric proteins is altered to more fetal, or dedifferentiated, forms, including a switch from cardiac α-actin to skeletal α-actin, and a switch from the α-form of myosin heavy chain to the β-form (Schwartz et al., 1986). It is important to note that many of the mechanisms of cardiac hypertrophy described earlier and in the literature have been observed in cell culture and/or animal models, and it remains uncertain if these same mechanisms lead to cardiac hypertrophy in humans (Maron et al., 2006; Elliott et al., 2008; Sedmera and McQuinn, 2008).

Heart Failure

All of the above-described cardiac pathophysiological states, if left untreated, will lead to heart failure (HF). Thus HF can result from increased hemodynamic overload, ischemia-related dysfunction, ventricular remodeling, excessive neurohormonal stimulation, abnormal myocyte calcium cycling, excessive or inadequate proliferation of the extracellular matrix, accelerated apoptosis, and/or genetic mutations (Dassanayaka and Jones, 2015; Efimov et al., 2010). HF is defined as the inability of the heart to meet the demands of the body due to insufficient force to pump enough blood (systolic heart failure), the inability fill with sufficient blood volume (diastolic heart failure), or a combination of both. Diastolic and systolic HF are highly complex diseases, and both are major contributors to morbidity and mortality. HF can also be classified as predominantly left ventricular, right ventricular, or biventricular and can be chronic or acute depending on the time of onset (Marti et al., 2013). The New York Heart Association's functional classification system, based on activity limitations, is often used by physicians in patient diagnosis and treatment (Guyatt, 1993; Kubo et al., 2004).

Systolic HF is further classified based on ejection fraction and is categorized as either heart failure with preserved ejection fraction (HFpEF) or heart failure with reduced ejection fraction (HFrEF) (Dassanayaka and Jones, 2015; Paulus and Tschope, 2013; Zamani et al., 2015; Maeder et al., 2010). Systolic HF can also be categorized based on cardiac output and is classified as either low-output failure (most common, with a resting cardiac index of 2.5 L/min/m (Moore et al., 2014) and elevated systemic vascular resistance) or high-output failure (resting cardiac index greater than 2.5 L/min/m (Moore et al., 2014) and lowered systemic vascular resistance). In low-output HF there is insufficient cardiac output to meet the body's demand, which worsens during times of increased metabolic demand. Common causes of high-output HF includes severe anemia, vascular shunting, hyperthyroidism, and vitamin B1 deficiency, and high output HF is frequently associated with ventricular dilation.

Diastolic HF, a common form of HFpEF, has increasing prevalence with age, and it has very complex mechanisms and heterogeneous pathophysiology (Basaraba and Barry, 2015; Andersson and Vasan, 2014; Yancy et al., 2013; Kitzman and Upadhya, 2014). Although the major pathology is diastolic dysfunction with impaired ventricular filling, other factors including abnormal ventricular–arterial coupling, systolic dysfunction, pulmonary hypertension, neuroendocrine dysfunction, and chronotropic incompetence contribute to diastolic HF. Diastolic failure is typically seen in patients with hypertensive or valvular heart disease, or with hypertrophic or restrictive cardiomyopathy, but it can also occur in a variety of clinical other disorders. Early recognition and appropriate therapy of diastolic dysfunction may prevent further progression to diastolic HF and death. Identification of diastolic HF requires evidence of signs or symptoms of HF, normal to slightly reduce left ventricular ejection fraction, and evidence of increased diastolic filling pressure. There are limited treatment options, the most important of which is treatment of the underlying disease (Kitzman and Upadhya, 2014).

Summary and Conclusions

The integration of the normal structure–function relationships in the heart is complex, and our understanding of these relationships is continuously evolving. New discoveries through research and clinical practice ultimately guide the best clinical management of these diseases of the heart. The chapters that follow will provide an in-depth glimpse into these new cutting-edge areas of cardiovascular research and medicine, and they will provide further understanding and fodder for debate on the best methods to study and manage cardiovascular diseases.

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Cardiac and Induced Pluripotent Stem Cells

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Introduction

The past ~20 years have been an exciting time for stem cell research and regenerative medicine. The heart long believed to be terminally differentiated organ was discovered to regenerate continuously throughout adult life, albeit at a slow pace (Beltrami et al., 2001; Bergmann et al., 2009, 2015). This paradigm shift led to the discovery and isolation of several populations of cells with stem- and progenitor-like properties, including self-renewal, clonogenicity, and multipotentiality in vitro and in vivo from the adult mammalian heart (Chong et al., 2014). Many of these cells, including KIT^+ cardiac stem cells (Beltrami et al., 2003), $SCA1^+$ cells, Islet-1^+ cells, stage-specific embryonic antigen (SSEA)-1^+, and epicardial progenitors, are identified based on the expression of unique cell surface markers and/or transcription factors, whereas others, such as resident cardiac mesenchymal stem cells (MSCs), side population cells, and cardiospheres, are identified on the basis of specific in-vitro physical properties, such as the ability to form spheres or colony-forming units (Bernal and Galvez, 2013; Faiella and Atoui, 2016; Bollini et al., 2011). Typically, stem cells reside in niches where they are structurally connected to the supporting cells by gap and adherens junctions. The niche constitutes the microenvironment within which stem cells retain their undifferentiated state and receive growth signals from the supporting cells.

Cardiac Stem Cells (CSCs)

KIT Cardiac stem cells (CSCs) have been described in multiple species ranging from rodents to large animals to humans and are likely heterogeneous in nature (Hatzistergos and Hare, 2016). As endogenous resident cells, CSCs are a promising target for additional therapy after cardiomyocyte loss in both the acute and chronic phases of ischemic cardiac disease. Considerable effort has been made to identify specific markers of those stem cells with cardiomyogenic potential. Among the various markers employed to identify resident CSCs, KIT (CD117) has played a prominent role (Torella et al., 2006). KIT (previously known as c-KIT) was first identified in the 1980s as a protooncogene in mammalian cells (Yarden et al., 1987). In 2003, a landmark study showed that KIT identifies rare cardiac-resident adult stem cells, thereby establishing adult cardiac stem cell biology as a field of investigation (Beltrami et al., 2003).

Heterogeneous KIT^+ cardiac cells, some positive for the cardiomyocyte progenitor markers Nkx2-5, GATA4, and Mef2c but negative for hematopoietic lineage markers (LIN^-), are clonogenic, self-renewing, and able to differentiate into cardiomyocytes, smooth muscle cells, and endothelial cells in vitro and are sufficient to stimulate myocardial regeneration following cardiac injury in rats and mice (Ellison et al., 2013). These findings provided the missing link between the identification of small dividing myocytes and the origin of these repopulating cells, laying the ground work for introducing KIT^+ CSCs for treatment of the failing heart. However, analyses of KIT expression during mouse development show that only a small proportion of cardiomyocytes are derived from KIT^+ cardiac progenitor cells (Hatzistergos et al., 2015; van Berlo et al., 2014). These cardiomyocytes form KIT^+ cardiac neural crest cells, an origin that may partly explain their limited prevalence and contribution to the myocardial lineage (Hatzistergos et al., 2015). In contrast, the more prevalent KIT^+ cardiac cells form vascular components and likely originate in the cardiogenic

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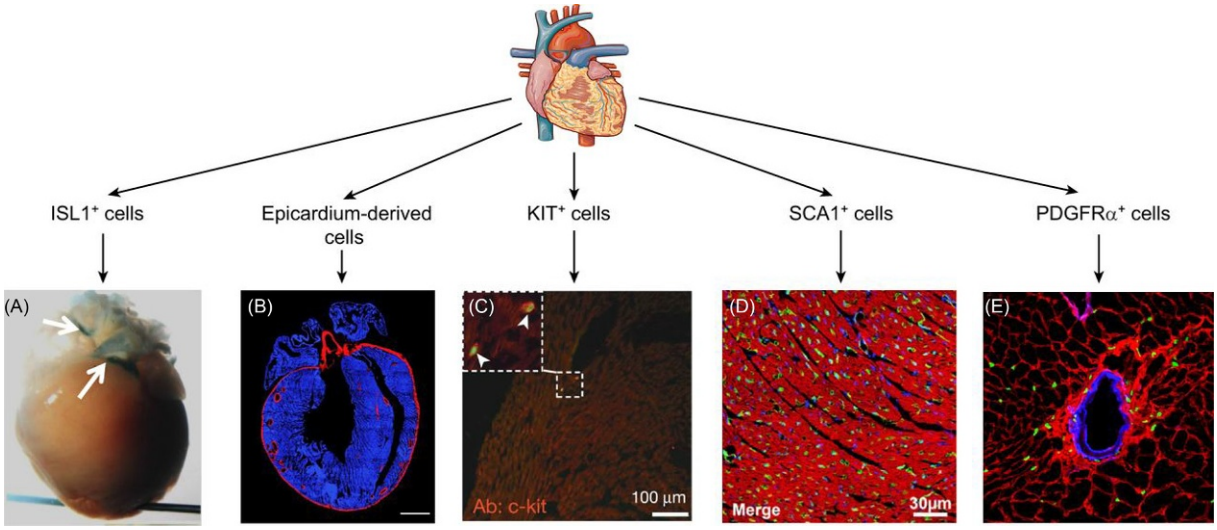

Fig. 1 Stem cell populations in the adult heart. Numerous stem cell populations have been described in the adult heart, many of which are depicted here. (A) A heart from a mouse with an *Isl1*-nuclear *lacZ* knock-in allele. β-Galactosidase stained (*blue*) ISL1$^+$ cells are rare and present in the adult heart only in the conduction system including the sinoatrial node (*arrows*). (B) The epicardium (red perimeter, Note: coronary vessels within the myocardium also stain red) gives rise to perivascular cells and stromal fibroblasts in development, and in the adult this potential is retained, and is activated after injury. Nuclei of cardiac cells are stained with DAPI (*blue*). Scale bar: 500 μm (C) KIT$^+$ cells in the adult mouse heart shown by expression of a *Kit*-Cre allele (*green*) and staining for the endogenous KIT protein (*red*). Inset: two mononuclear KIT-expressing cells (*arrowheads*) (D) SCA1$^+$ cells within the interstitium of the adult heart, marked by GFP (*green*), phalloidin (*red*) showing cardiomyocytes, and DAPI (*blue*) stains nuclei. (E) A heart from a mouse containing a Pdgfra knock-in allele. PDGFRα-expressing cells are present throughout the cardiomyocyte interstitium and within the adventitial zone of coronary arteries. collagen VI (*red*) and smooth muscle cells positive for α-smooth muscle actin (*blue*). Original figure reproduced with permission from Santini et al., 2016.

mesoderm (Hatzistergos and Hare, 2016; Hatzistergos et al., 2015, 2016; Sultana et al., 2015). Despite this skewed developmental origin transplantation of culture-expanded KIT$^+$ CSCs promotes their multilineage differentiation into cardiomyocytes, endothelial and smooth muscle cells in vivo, providing a potentially important cell-based therapeutic strategy for promoting heart regeneration in patients with heart disease (Anversa et al., 2013; Bearzi et al., 2007; Beltrami et al., 2003; Bolli et al., 2011).

These findings led to the SCIPIO trial, the first Phase I clinical trial of KIT$^+$ CSCs in patients with ischemic heart disease (www.clinicaltrials.gov, NCT00474461). In this trial 1 million autologous KIT$^+$ CSCs were administered via intracoronary infusion into patients with ischemic heart failure that were undergoing coronary artery bypass graft (CABG). The 1-year follow-up results from the SCIPIO trial showed that this therapy is safe and feasible and promotes significant improvements in global and regional LV function, decreases scar size, and induces regeneration of viable myocardium (Chugh et al., 2012). Despite their apparent therapeutic utility, there are caveats with respect to the KIT$^+$ cells; they are very rare within the resident myocardium, and their cardiomyocyte derivatives are relatively immature in terms of ultrastructure and Ca^{2+} handling (Bollini et al., 2011).

In addition to KIT$^+$ cells, SCA1$^+$ cells, Islet-1$^+$ cells, the specific side population (SP) cells, cardiospheres (CSs), and cardiosphere-derived cells (CDCs), stage-specific embryonic antigen-1 (SSEA-1$^+$) and epicardial progenitors have been isolated from the heart (**Fig. 1**) (Hierlihy et al., 2002; Messina et al., 2004; Leite et al., 2015; Bollini et al., 2011). This surprisingly high number of CSC subtypes stands in stark contrast with the properties of all other self-renewing organs, in which a single tissue-specific adult stem cell is normally seen (Santini et al., 2016). CSCs promise much in the way of regenerative potential; however, several questions remain unanswered relating to their origin, cell surface markers, degree of overlap of the various subpopulations, and the processes underlying in vivo homeostasis.

CSC Subtypes

SCA1

SCA1 (also known as LY6A) is a cell surface protein often used to identify or enrich for hematopoietic stem cells (Holmes and Stanford, 2007) and is upregulated in a variety of murine cancer stem cells. SCA1 is a convenient marker for stem cell studies because it is expressed in most stem/progenitor cells in a variety of tissues, including the mammary gland (Welm et al., 2002), prostate (Xin et al., 2005), dermis (Toma et al., 2005), skeletal muscle (Gussoni et al., 1999), heart (Rosenblatt-Velin et al., 2005), and liver (Wulf et al., 2003). However, in all cases, it is unclear if the SCA1$^+$ populations are tissue-specific precursor/stem cells or are the hematopoietic, mesenchymal, or endothelial precursor/stem cells associated with these tissues (Holmes and Stanford, 2007).

In the heart, SCA1$^+$ cells are highly heterogeneous and include microvascular endothelial cells, KIT$^+$ progenitor cells, PDGFRα$^+$ cardiac colony-forming units-fibroblast (cCFU-F) and stromal cells, as well other interstitial and perivascular cells. The SCA1$^+$

fraction expresses the cardiac transcription factors GATA4 and Mef2c but lacks other cardiomyocyte lineage markers including Nkx2-5 (Oh et al., 2003). In fact, SCA1$^+$ cells have cardiogenic and vascular differentiation potential (Matsuura et al., 2004; Hsueh et al., 2014) although the multilineage potential of their single-cell progeny is uncertain.

Isl1

The LIM-homeodomain transcription factor, Isl1, binds and controls cis-regulatory elements of the insulin gene (Karlsson et al., 1990). Isl1 is expressed in cardiac neural crest cells and mesodermal progenitors of the second heart field (SHF). Upon differentiation, Isl1 expression is downregulated, suggesting that this transcription factor defines a cardiogenic progenitor cell population (Peng et al., 2013; Prall et al., 2007; Cai et al., 2003). Isl1 cells isolated and expanded from embryonic and postnatal hearts, differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells, among others (Bu et al., 2009; Domian et al., 2009; Moretti et al., 2006; Laugwitz et al., 2005). Hearts of mice lacking Isl1 display severe cardiac defects, including complete absence of the outflow tract and right ventricle and partial loss of the atria. Although Isl1$^+$ CSCs may represent a very attractive source of endogenous cardiac progenitors, their clinical applicability in the adult patient is limited, since the Isl1$^+$ progenitor population rapidly declines after birth. Therefore, the therapeutic potential of Isl1$^+$ cells may depend on the ability to isolate and expand them in vitro or to derive them from human embryonic stem (ES) or induced Pluripotent Stem (iPS) cells prior to cell transplantation.

Side population cells

Side population (SP) cells were first described by Hierlihy et al. and are characterized by their ability to promote the efflux of metabolic markers, such as Hoechst dye, via the Abcg2 protein on their cell surface (Hierlihy et al., 2002). The Abcg2 protein belongs to the family of transporters linked to the ATP cassette (ABC transporters), which early during cardiac development endows these cells with their characteristic phenotype (Di Felice et al., 2009). Under pathological conditions, human *Abcg2* mRNA levels increase, suggesting the possibility of the activation and proliferation of SP cells in response to cardiac disease in an attempt to promote repair (Smith et al., 2008). Increased numbers of Abcg2$^+$ cells have also been detected in the marginal zone of acute infarcted myocardium (Barile et al., 2007). Despite the apparent cardiovascular lineage potential of these populations, there is no definitive proof that cardiac SP cells represent a bona fide stem cell population as defined by clonogenic and self-renewal properties.

Cardiospheres

Cardiospheres (CSs) are outgrowth cells derived from cultured cardiac explants that grow as self-adherent clusters in vitro (Messina et al., 2004). To obtain sufficient numbers of CSs for therapeutic purpose, they can also be cultured and expanded as a monolayer of Cardiosphere-Derived Cells (CDCs) over many passages (Messina et al., 2004). CSs are clonogenic, express stem and endothelial progenitor cell markers, such as KIT, SCA1, CD31, CD34, Nanog, and SOX2, and spontaneously differentiate into cardiomyocytes and vascular cells (Messina et al., 2004; Li et al., 2010). Murine CSs injected into ischemic murine hearts can differentiate into vascular cells and cardiomyocytes (Messina et al., 2004), and CSs derived from the injured myocardium of mice can differentiate into cardiac cells in vivo (Ye et al., 2012). Porcine and human CDCs can also differentiate into endothelial cells and cardiomyocytes in vitro and in vivo (Smith et al., 2007). Together, CSs and CDCs could represent a useful source of CSCs, as they can be easily isolated from a small amount of starting material and can be greatly expanded, without losing differentiation potential. However, a recent report investigating stem cell surface markers showed that CDCs are CD105$^+$ stromal cells of intrinsic cardiac origin that include a variable fraction of CD90 (Thy-1)$^+$ cells and a small minority of KIT$^+$ cardiac progenitors (Cheng et al., 2014). Thus, the majority population is negative for both CD90 and KIT and positive for CD105, suggesting that they are mesenchymal stem cells (MSCs), but their active principle is, as yet, undefined. A clinical trial in which CDCs were transplanted into infarcted areas revealed that the KIT$^+$ cells in cardiospheres are not an important determinant of the therapeutic efficacy of CDCs, whereas their CD90$^+$ cells appear to undermine the overall benefit of CDC therapy (Cheng et al., 2014).

SSEA-1

An SSEA-1$^+$ (stage-specific embryonic antigen-1$^+$) cardiac stem cell population was recently isolated from neonatal and adult rat heart (Bollini et al., 2011). These cells resemble an immature pool of embryonic progenitors and give rise to both myocardial and endocardial cells during normal cardiac development. Pluripotent stem cell-derived SSEA-1$^+$ cells express mesodermal and cardiac lineage marker genes and freshly sorted SSEA-1$^+$ cells expressed brachyury and Flk1, two genes characteristic of early mesoderm (Blin et al., 2010). Furthermore, these cells expressed mesoderm posterior 1 (Mesp1) (Bondue et al., 2008); Tbx6, a mesodermal muscle gene upstream of Mesp1 (Christiaen et al., 2009); Tbx5, a ventricular marker; and Tbx18, a marker of both epicardium (Cai et al., 2008) and the left ventricle (Franco et al., 2006). SSEA-1$^+$ cells also express myocardin (MYOCD), GATA4, Mef2c, and Nkx2-5, key smooth muscle, and cardiac transcription factors of the FHF, as well as Isl1 and Tbx20, the latter more reminiscent of the SHF. A minority of SSEA-1$^+$ population also express Nestin (NES) or PAX3, 2 markers found in neural crest cells (Blin et al., 2010). Under controlled in vitro conditions, SSEA-1$^+$ cells demonstrate cardiomyogenic differentiation potential, forming beating colonies and, when transplanted into an infarcted rat heart, promoting SSEA-1$^+$ myocardial regeneration and functional improvement. It has been suggested that SSEA-1$^+$ CSCs represent a population of embryonic-like primitive cardiac stem cells that can give rise to more committed KIT$^+$, Sca-1$^+$, Abcg2$^+$ cells at later stages of differentiation (Bollini et al., 2011; Ott et al., 2007). These results suggest SSEA-1$^+$ progenitors as candidates for use in cardiac regenerative medicine.

Epicardial cells

Epicardium-derived cells (EPDCs) are a cell population with the potential to restore the myocardium and coronary vasculature. An archetypal example of the paradigm of recapitulating embryonic roles for therapeutic application, the epicardium, and its derivatives play an essential role in the formation of the coronary myocardium and vasculature during development and retain a regenerative potential in the adult (Bollini et al., 2014). The epicardium is derived from the outgrowth of cells from the proepicardial organ, a transient embryonic structure, to overlie the developing heart tube. EPDCs are marked by the transcription factors Wilms tumor 1 (Wt1) (Zhou et al., 2008) and/or Tbx18 (Cai et al., 2008) and can differentiate into smooth muscle, and endothelial cells in all four heart chambers. A rare population of KIT^{+} (Limana et al., 2007) stem cells that express a marker profile identical to those previously described in the myocardium can also be found in the adult human and mouse epicardium. Embryonic EPDCs possess extensive migratory capacities upon stimulation by paracrine factors from the underlying myocardium (Munoz-Chapuli et al., 2002). However, the adult epicardium lies quiescent and requires stimulation by those factors that promote coronary vasculogenesis during development. One such stimulatory factor, Thymosin β4 (Tβ4), restores the quiescent adult epicardium to its pluripotent embryonic state. Tβ4 treatment of infarcted hearts induces significant EPDC proliferation and the formation of a network of perfused, functional vessels that enhance blood flow to the ischemic myocardium. Moreover, Tβ4 facilitates an epicardial contribution of mature de novo cardiomyocytes, structurally and functionally coupled to resident myocardium, which may contribute to the functional improvement of Tβ4-treated hearts post-MI (Smart et al., 2013). Other factors with proven potency in stimulating adult EPDCs are emerging. Activation of the prokineticin receptor (PKR1) results in secretion of its ligand, PK2, by cardiomyocytes. In transgenic mice overexpressing PRK1, PK2 acts on EPDCs, augmenting their proliferation, which subsequently induces neovascularization (Urayama et al., 2008). High-molecular-weight hyaluronan stimulates migration and differentiation of epicardial cells in vitro (Craig et al., 2010). Whether either of these stimuli can be translated into the adult heart for activation of EPDCs remains to be investigated.

Conclusions

Considering these main cell populations, ongoing research is assessing which types of cells are the best candidates for cardiac cell therapy. It is also possible that combining two or more types of stem cells would have additive or synergistic benefits. Studies combining KIT+ CSCs with MSCs indicate that the combination of these two cell types produces greater therapeutic effects than a single cell type in swine models of ischemic cardiomyopathy (Karantalis et al., 2015; Natsumeda et al., 2016; Williams et al., 2013). Based on these combinatorial stem cell approaches, the "Combination of Mesenchymal and C-kit+Cardiac Stem Cells as Regenerative Therapy for Heart Failure" (CONCERT-HF, NCT02501811) trial was recently initiated. The goal of this clinical trial is to assess if administering a combination of autologous CSCs and MSCs improves cardiac structure and function in patients with chronic ischemic cardiomyopathy.

Currently, the extensive cardiac damage resulting from ischemic cardiomyopathy cannot be completely reversed by stem cell therapy, i.e., the regenerative potential of stem cells in damaged tissues is limited (Hatzistergos et al., 2010). Moreover, after a decade of intense research in the field of cardiac cell therapy, there seem to be more questions than answers. Future basic, translational, and clinical research is certainly needed to advance this field further and lead to the development of novel clinical treatments for patients with cardiac failure.

Pluripotent Stem Cells

Introduction

Pluripotent cells can give rise to all cell types, except extraembryonic cells (those that make up placental tissue and extra embryonic membranes) (Ameen et al., 2008; Smith, 2001). The bona fide pluripotent stem cell is the embryonic stem (ES) cell. ES cells are derived from mammalian blastocysts and have the ability to differentiate into cells of all three germ layers (ectoderm, endoderm, and mesoderm). ES cells exhibit robust and infinite growth (self-renewal) while maintaining their normal karyotype. Over the last ~10 years, the advent of induced pluripotent stem cell (iPSC) technology has revolutionized the fields of regenerative medicine, disease modeling, drug discovery/screening, and toxicological testing. In spite of these exciting innovations, there are still major challenges associated with the widespread use of iPSC technology including improving reprogramming efficiency, minimizing genetic and epigenetic instability, and reducing the high costs associated with iPSCs derivatives for high-throughput screening (HTS) applications. There are also problems shared by both ES and iPS cells, including reducing their propensity for tumor formation in vivo and driving maturity of ES- and iPS-derived cells to a more adult phenotype, e.g., iPSC-derived cells such as cardiomyocytes (iPSC-CMs), in terms of structure, calcium cycling, and electrophysiology. iPSCs have yet to enter the clinic for heart-related diseases.

Embryonic Stem Cells

ES cells were first established from mice in 1981 (Evans and Kaufman, 1981), and from humans in 1998 (Thomson et al., 1998). The successful derivation of human ES cell lines extended their great potential to the study of human diseases and allowed us to envisage the future prospect of regenerative medicine. However, there are ethical implications associated with these cells. They are

derived from the inner cell mass of in vitro-fertilized human embryos. Since these embryos could in theory develop into a person, the destruction of these embryos to obtain the cells is, for some, controversial and remains far from settled. Important developments have helped reduce much of the heated debate over ES cell research, in particular the advent of induced pluripotent stem cell (iPSC) technology—fibroblasts (or other somatic cells) genetically engineered to behave like ES cells (Shi et al., 2017).

Induced Pluripotent Stem Cells (iPSCs)

iPSCs hold great promise for cardiac cell therapy (Yoshida and Yamanaka, 2010). Recently, preclinical studies using a variety of cell preparations, including undifferentiated iPSCs, iPSC-derived cardiac progenitors, cardiomyocytes, smooth muscle cells, and endothelial cells, have shown evidence of cardiac repair (Ahmed et al., 2011; Dai et al., 2011; Kawamura et al., 2012; Xiong et al., 2013; Shinozawa et al., 2017; Menasche et al., 2015). The progress in the iPSC field has clearly demonstrated that cellular reprogramming to pluripotency is a fast-evolving technology with great promise for clinical application.

iPSCs are ES-like cells that have been reprogrammed from somatic cells (Mikkelsen et al., 2008; Chin et al., 2010; Guenther et al., 2010). Takahashi and Yamanaka, in 2006, devised an elegant screen for transcription regulator factors within a pool of 24 pluripotency-associated candidate genes that when coexpressed from retroviral vectors in mouse embryonic fibroblasts (MEFs) could activate *Fbxo15*-β-geo (a fusion of the β-galactosidase and neomycin resistance genes) and induce the formation of drug-resistant colonies with characteristic ES cell morphology. None of those factors alone supported cell survival in the presence of antibiotic; however, 22 colonies were formed in the wells that were transduced with all 24 genes. And all these colonies exhibited an ES cell morphology rather than that of the MEFs from which they originated. Next, the authors narrowed down the pool of 24 candidates to a set of reprogramming factors subsequently by identifying the minimally required core set of four genes, comprising octamer-binding protein 3/4 (OCT3/4), SRY-box containing gene 2 (SOX2), Krüppel-like factor 4 (KLF4) and c-MYC, collectively referred to as OSKM factors. The cells reprogrammed by OSKM factors exhibited ES cell-like properties. The resultant cells generated by selection for *Fbxo15* activation, expressed markers of pluripotent stem cells, such as SSEA-1 and Nanog, developed teratomas when injected subcutaneously into immune compromised mice, and contributed to different tissues of developing embryos upon blastocyst injection (Takahashi and Yamanaka, 2006), thereby fulfilling some of the criteria of pluripotency. iPSCs have since been derived from different species—including humans (Takahashi et al., 2007; Yu et al., 2007; Park et al., 2008), mouse (Okita et al., 2007), rats (Li et al., 2009), pig (Wu et al., 2009), and rhesus monkeys (Liu et al., 2008)—by expression of the OSKM factors, demonstrating that fundamental features of the transcriptional network governing pluripotency remain conserved during evolution. These studies opened a new field of stem cell research. The Nobel Prize committee awarded Shinya Yamanaka and Sir John Gurdon the Nobel prize in Physiology or Medicine in 2012 "for the discovery that mature cells can be reprogrammed to become pluripotent."

Advances in iPSC generation

Although successful reprogramming occurred, these "first-generation" iPSCs expressed lower levels of several key pluripotency genes compared with ES cells and appeared to be only partially reprogrammed. Therefore, several laboratories, including Yamanaka's (Okita et al., 2007), attempted to improve the efficiency and quality of iPSC generation. For example, by selecting for the reactivation of the essential pluripotency genes Nanog or Oct4 instead of *Fbxo15*, iPSCs were generated that more closely resemble ES cells molecularly and functionally (Okita et al., 2007; Maherali et al., 2007; Wernig et al., 2007). Furthermore, iPSCs were generated by using only three transcription factors (OCT3/4, KLF4, SOX2, without c-MYC) (Nakagawa et al., 2008). Other methods, including integrating and nonintegrating recombinant viruses (Kaji et al., 2009; Stadtfeld et al., 2008; Zhou and Freed, 2009; Schlaeger et al., 2015), DNA expression vectors (Okita et al., 2008), episomal vectors (Okita et al., 2011), mini-circle vectors (Jia et al., 2010), and liposomal magnetofection (Park et al., 2012) have facilitated the generation of iPSCs. Non-DNA methods involving proteins (Zhou et al., 2009), mRNA molecules (Warren et al., 2010), and various chemical agents (Hou et al., 2013) have also been tested, and a chemical method that produces iPSCs appears to be promising (Hou et al., 2013). iPSCs can be generated in the presence of small molecules (e.g., ascorbic acid and CHIR-99021 and PD-0325901), which may improve reprogramming efficiency (Lin and Wu 2015). A recent report showed that efficiency in iPSC reprogramming can be significantly improved in a microfluidic environment where microliter-volume confinement resulted in a 50-fold increase in efficiency over traditional reprogramming by delivery of synthetic mRNAs encoding transcription factors (TFs) (Luni et al., 2016). High quality and pure human (h)iPSCs were obtained in 2 weeks from initial reprogramming and subsequently differentiated into functional hepatocyte- and cardiomyocyte-like cells in the same platform without additional expansion (Luni et al., 2016). Thus, the microfluidic platform promotes a highly efficient and fast iPSC reprogramming while consistently reducing the required culture media and reagents, ultimately significantly lowering the overall costs. Thus, this technology can provide the opportunity for the affordable generation of high numbers of hiPSCs from a large cohort of patients in an accelerated time frame. More comprehensive knowledge of the reprogramming process is therefore crucial for future clinical applications of iPSCs.

iPSC-derived cardiomyocytes

During gastrulation, cardiomyocytes emerge from the mesodermal layer, in particular from the anterior region of the primitive streak. The balance between inductive signals, mainly fibroblast growth factors (FGFs) and bone morphogenetic proteins (BMPs), members of the transforming growth factor (TGF)β superfamily, and repressive signals, mostly Wingless/INT proteins (WNTs) and ß-catenin, induce the development of cardiac progenitors (Brade et al., 2013; Abu-Issa and Kirby, 2007). Following the

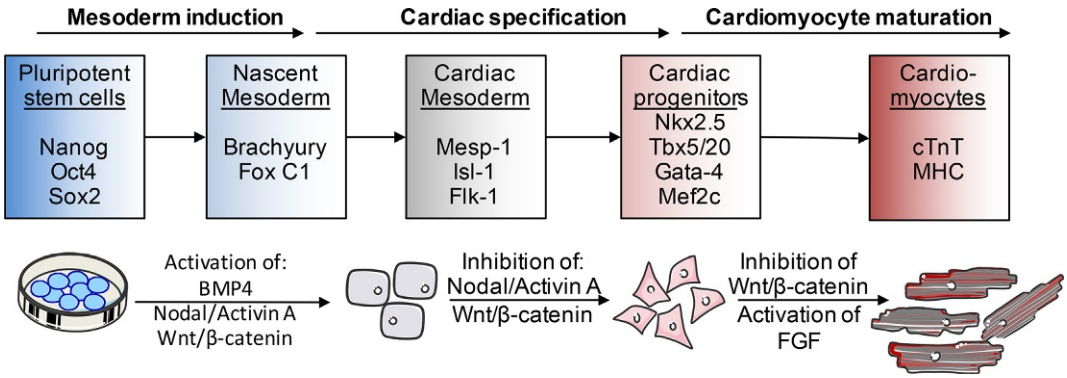

Fig. 2 Sequential stages to obtain cardiomyocytes from pluripotent stem cells. Pluripotent stem cells, differentiate into early mesoderm and subsequently into cardiac mesoderm, committed cardiac progenitors and ultimately into functional beating cardiomyocytes. Transcription factors present at each step are indicated and can be used to monitor the progression of differentiation. The activation or inhibition of biomolecules (growth factors and small molecules) play important regulatory roles in pluripotent stem cell expansion and their differentiation into cardiomyocytes. The figure was composed of original images and images obtained from the Servier Medical Art image bank (www.servier.com/Powerpoint-image-bank).

migration of these cells to the cardiac crescent, the primitive beating heart tube is formed, composed of synchronously beating small myocytes. The contractile impulse begins within the primary pacemaker area in the primitive atrium that will later evolve into the sino-atrial node, where the fast-beating pacemaker myocytes will reside. As development proceeds, CMs in different anatomical regions of the hearts, comprising atria and ventricles, will be generated and linked by the conduction system that includes the sinus node, the internodal pathways, the atrioventricular (AV) node, the AV bundles, and Purkinje fibers (Brade et al., 2013; Barbuti et al., 2016).

Recapitulating natural physiological signaling pathways under in vitro differentiation into cardiac cells is the main concept behind the generation of iPSC-CMs, including the activation or inhibition of signaling pathways. This procedure is dependent on the use of WNT, FGF, and acitvin A, as described earlier. The cardiomyocytes derived from ES/iPS cells consist of several subtypes of cardiomyocytes, including atrial, sinus nodal, and ventricular cardiomyocytes. Under laboratory conditions, the differentiation iPSC-CMs is mostly triggered by the formation of embryoid bodies using the in vitro hanging drop method, monolayer cultures, and inductive cocultures with endoderm-like cells (END2) (Pekkanen-Mattila et al., 2011). Stage-specific markers that mimic developmental processes in embryos have been identified, including mesoderm cells (brachyury), cardiac mesoderm (Mesp1, Flk1, and Isl-1), cardiac progenitors (Nkx2-5, GATA4), and cardiomyocytes (myosin heavy chains). The differentiation of cardiomyocytes with growth factors such as Activin A/Nodal, Bmp4, Cerberus, Wnt3a, and Wnt11 is under investigation (Skalova et al., 2015) (Fig. 2). Although the progress with differentiation protocols has been striking, a closer examination of the resulting cell populations reveals that significant cellular heterogeneity (ventricular-like, atrial-like, and nodal-like cardiomyocytes) and nondifferentiated cells remain, which could adversely affect clinical outcomes. Other hurdles remain in realizing iPSC applications in human heart regeneration, including (a) large-scale cultivation systems required to obtain the billions of required differentiated cells, (b) minimizing residual undifferentiated stem cells and non-CM differentiated cell populations, (c) functional and electrophysiological characterization of purified cardiomyocytes, (d) improved survival of iPSC-CM following transplantation, and e) confirmation of safety and efficacy in large animal models prior to clinical application.

Modeling of genetic diseases of the heart

Human iPSC-CMs offer a unique platform by which human cardiac diseases can be modeled in vitro. Patient-specific iPSCs represent a novel technology that holds great promise for analyzing the molecular basis of heart disease and the outcomes of regenerative medicine (Fig. 3). While the utility of iPSC-based in vitro models will depend upon the nature of the disease, this approach is well suited if a genetic mutation is the causative or contributing factor. iPSC-CMs derived from patient-specific iPSC may reflect the dysfunctional characteristics found in cardiomyocytes in vivo and provides a platform for testing treatments. A few examples of the power of this approach are presented later.

The first disease-specific iPSCs for a pathology affecting the heart were derived from patients with LEOPARD syndrome (LS) (Carvajal-Vergara et al., 2010), an autosomal-dominant developmental disorder with pleomorphic effects on several tissues and organ systems, with hypertrophic cardiomyopathy often being the cause of mortality. Approximately 90% of LS cases are caused by point mutations in the PTPN11 gene that encodes the protein tyrosine phosphatase SHP2. Most cases of LS are due to a single base substitution, either A-to-G at position 386 changing amino acid 279 from Tyrosine to Cysteine (Y279C) or C-to-T at position 1403 (T468M). A minority of cases due to other point mutations (Y279S, A461T, G464A, R498W/L, Q506P, and Q510E/G) were documented subsequently (Conti et al., 2003; Yoshida et al., 2004). Cells were obtained from two patients heterozygous for a T468M substitution mutation in PTPN11, and iPSC lines and subsequently LS-iPSC-CMs were derived (Carvajal-Vergara et al., 2010). These LS-iPSC-CMs had significantly larger surface areas, increased sarcomere assembly, and preferential localization of NFATc4 in the nucleus compared to cardiomyocytes derived from human embryonic stem cells or iPSC-CMs derived from a healthy

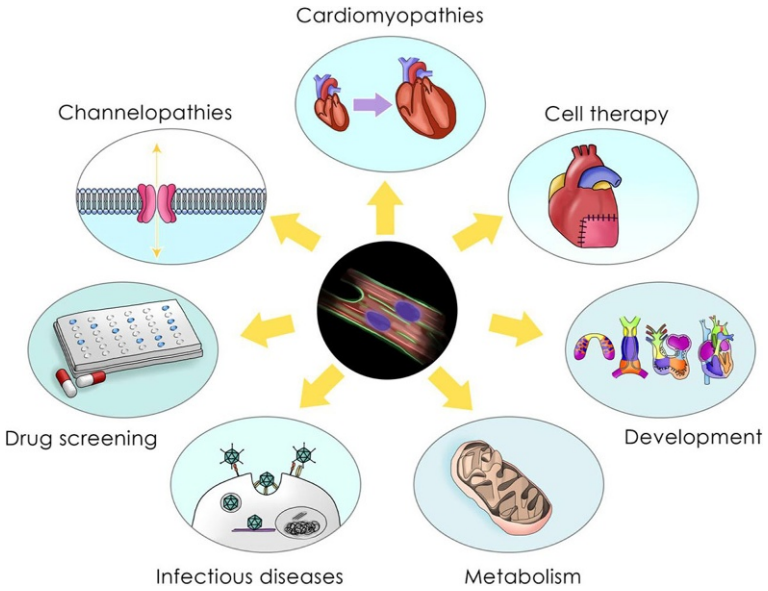

Fig. 3 Current applications of patient-specific induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) technology. iPSC-CMs have been used for disease modeling of inherited cardiomyopathies and channelopathies, regenerative therapies, drug discovery, and cardiotoxicity testing, as well as for studying metabolicabnormalities and cardiac development. Adapted from Karakikes et al., 2015. Used with permission.

brother of one of the LS patients. This LS model demonstrated for the first time that signal transduction (RAS-MAPK) is perturbed in LS, and this effect was seen as early as the pluripotent stem cell stage.

Dilated cardiomyopathy (DCM) is the most prevalent form of cardiomyopathy and is characterized by ventricular dilatation, systolic dysfunction, and progressive heart failure (Harvey and Leinwand, 2011). DCM-iPSC-CMs were generated from a family carrying a point mutation (R173W) in the gene encoding the sarcomeric protein, cardiac troponin T. Compared to control, healthy individuals in the same family cohort, DCM-iPSC-CMs exhibited altered regulation of Ca^{2+}, decreased contractility, and abnormal distribution of sarcomeric α-actinin. When stimulated with a β-adrenergic agonist, DCM iPSC-CMs showed characteristics of cellular stress such as reduced beating rates, compromised contraction, and a greater number of cells with abnormal sarcomeric α-actinin distribution. However, treatment with β-adrenergic blockers or overexpression of the channel protein, Serca2a, improved the function of DCM iPSC-CMs group (Sun et al., 2012). Thus, DCM iPSC-CMs recapitulate in part the morphological and functional phenotypes of DCM.

Familial hypertrophic cardiomyopathy (HCM) is a primary disorder of cardiac muscle and is characterized by a thickened ventricular wall and ventricular septum, increased myocardial fibrosis, disorganized myofibers, and an arrhythmic heart beat (Maron, 2002). Patient-derived HCM-iPSCs carrying a single missense mutation (R442G) in the MYH7 gene were studied. As expected, HCM-iPSC-CMs were larger and had disorganized sarcomere structures and arrhythmic beatings. Additionally, HCM iPSC-CMs exhibited irregular Ca^{2+} handling and ion-channel functions. A large number of genes responsible for "cell proliferation" were increased in HCM-iPSC-CMs compared with control iPSC-CMs. Together these findings suggest a molecular mechanism underlying HCM (Han et al., 2014).

Using a similar approach, additional studies generating patient-specific iPSC-CMs have been applied to model a large number of genetically based arrhythmias, such as long QT syndrome (Moretti et al., 2010), catecholaminergic polymorphic ventricular tachycardia (CPVT) (Itzhaki et al., 2012), arrhythmogenic right ventricular cardiomyopathy (ARVD) (Ma et al., 2013), overlap syndrome (Davis et al., 2012), and others. Furthermore, transplantation of iPSC-CM sheets over the left ventricular surface improves cardiac function in murine and porcine models of chronic myocardial infarction (Kawamura et al., 2012; Higuchi et al., 2015).

While the therapeutic use of human iPSC-CMs is feasible, an autologous approach would be time-consuming, laborious, and cost prohibitive. The use of allogeneic iPSC-CMs might address these issues (**Fig. 4**); however, transplantation of allogeneic iPSC-CMs might induce an immune response and graft rejection. A proof of concept study was recently carried out by Shiba et al. They initially administered $4x10^8$ MHC-mismatched allogeneic iPSC-CMs into immunosuppressed cynomolgus monkeys 2-weeks following infarction, but the cells were rejected within 4 weeks. However, when an equal number of MHC-matched allogeneic iPSC-CMs were similarly injected, the cells survived for 12 weeks with no evidence of immune rejection and the animals exhibited improved cardiac contractile function compared to placebo injected animals. Cell-treated animals did, however, have a significantly higher incidence of ventricular tachycardia (Shiba et al., 2016). This study suggests that allogeneic iPSC-CMs can provide therapeutic efficacy, but that many issues still need to be addressed.

The generation of disease-specific iPSC-CMs represents an important step forward in cardiovascular research and can serve as a useful platform for exploring disease mechanisms and drug screening. This technology can reproduce most peculiarities of a pathological phenotype, including electrophysiological abnormalities, disorganized cellular structure, sensitivity to drugs, and

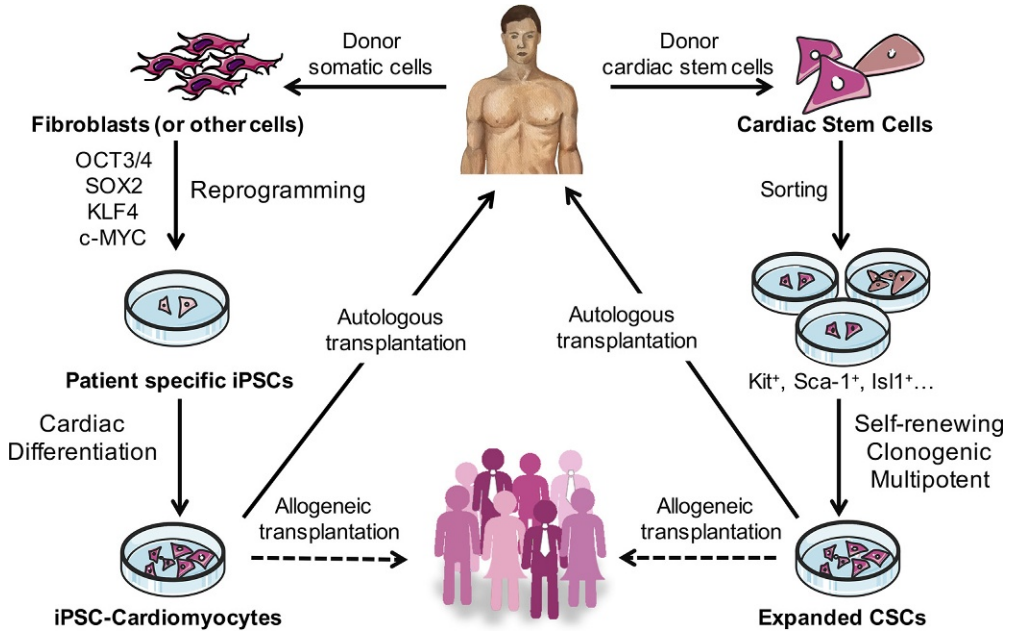

Fig. 4 Cardiac cell therapy strategies using cardiac stem cells (CSC) and induced pluripotent stem cells (iPSCs). CSCs have the potential to proliferate and differentiate into cardiac lineages. CSC subtypes can be sorted by different markers (e.g., Kit, sca-1, and others) each of which may provide characteristic tissue regeneration and repair. iPSCs can be obtained by transducing patient somatic cells (e.g., fibroblasts) with a set of exogenous genes (e.g., Yamanaka factors: OCT3/4, SOX2, KLF4, and c-MYC) or other methodologies. The donor-specific iPSCs can be induced to become iPSC-cardiomyocytes. After expansion, both stem cell populations may be used for autologous transplantation or allogeneic transplantation. The figure was composed of original images and images from the Servier Medical Art image bank (www.servier.com/Powerpoint-image-bank).

other factors. Additionally, the models can be successfully applied to study new methods of genome engineering, such as TALENs and CRISPR/Cas9. However, despite all the excitement associated with iPSCs, there are still several issues that must be solved before this promising technology can be used for clinical purposes.

Conclusions

iPSCs represent an exciting alternative to ES cells, avoiding the ethical issues and providing the opportunity to produce more accurate models for studying human diseases and subsequently more effective therapies. This field is relatively new and substantial progress has already been made, but further intensive investigations into the properties of iPSCs are needed both to increase our understanding of the basic biology of pluripotency and cellular differentiation and to address issues associated with the therapeutic applications of iPSCs. Improvement of the current technologies has been rapid and is ongoing, and the goal of producing clinical-grade human iPSCs and iPSC-derived cells that provide safe and effective therapies appears to be in reach.

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Cardiac Arrhythmia in Heart Failure

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Introduction

A variety of brady- and tachy-arrhythmias can coexist with heart failure (HF) and complicate the care of HF patients. Moreover, an arrhythmia itself may cause or exacerbate HF (e.g., arrhythmia-induced cardiomyopathy (AIC)) and control of these arrhythmias can reverse the cardiomyopathy. The presence of bradyarrhythmias may not only directly increase morbidity in HF patients, but also interfere with titration of atrioventricular (AV) nodal blockers such as beta-blockers and calcium channel blockers used to treat tachyarrhythmias.

This article reviews the diagnosis and management of arrhythmias in patients with HF.

Evaluation of Arrhythmias for Patients With HF

Persistent or frequent cardiac arrhythmias can be readily diagnosed with 12-lead electrocardiogram (ECG). Most arrhythmias, however, are paroxysmal and occur intermittently, requiring further diagnostic testing such as ambulatory monitoring or invasive electrophysiologic study (EPS). The Holter monitor is a 24-h monitor system that is particularly useful in evaluating patients with relatively frequent arrhythmias. It is an indispensable monitoring modality to assess frequency of premature ventricular complex (PVC) and adequacy of rate control in patients with atrial fibrillation (AF). Various types of continuous event recorders are commercially available to provide diagnostic information, usually up to 1 month, for patients with less frequent symptoms suggestive of arrhythmias such as palpitation, dizziness, and syncope. Modern event recorders allow recording of patient-activated events as well as auto-triggered brady- and tachycardiac events and provide timely feedback for physicians and patients (Rosero et al., 2013). Implantable loop recorders (ILRs) are subcutaneously implanted devices that allow for long-term (>3 years) arrhythmia monitoring. The ILRs have been increasingly implanted for the evaluation of unexplained syncope and detection of subclinical AF for patients with cryptogenic stroke (Mittal, 2014; Afzal et al., 2015).

Invasive cardiac electrophysiology study may be indicated in patients with HF to provide the definitive diagnosis of an arrhythmia, establish the etiology of syncope, and assess risk of sudden arrhythmic death. In case of documented tachyarrhythmias, EPS defines accurate underlying mechanism of a specific arrhythmia and localizes cardiac structure responsible for the arrhythmia, which then allows targeted pharmacologic and/or nonpharmacologic (catheter ablation and device) therapies.

Cardiac imaging is an integral part of comprehensive assessment for patients with HF and arrhythmia. In addition to standard imaging modalities such as echocardiography, radionuclide myocardial perfusion imaging, and cardiac CT, cardiovascular magnetic resonance imaging (MRI) has been increasingly utilized in care of patients with HF and arrhythmias. Cardiac MRI provides high spatial resolution and excellent tissue characterization. Increased risk of ventricular arrhythmia and sudden death has been revealed in patients with both ischemic and nonischemic cardiomyopathies who demonstrate more extensive myocardial fibrosis detected

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by late-gadolinium enhancement (LGE) technique (Stirrat and White, 2013; Kallianos et al., 2015). Cardiac MRI is an essential tool in the diagnosis and potential arrhythmic risk assessment of hypertrophic cardiomyopathy (HCM). The extent of LGE-detected myocardial fibrosis has been associated with higher sudden cardiac death (SCD) risk in patients with HCM (Maron, 2015; Weng et al., 2016). The diagnosis of cardiac sarcoidosis frequently relies on CMR, which may demonstrate myocardial inflammation, edema, and fibrosis due to cardiac involvement of the disease (Birnie et al., 2016).

Supraventricular Arrhythmias

Sinus Tachycardia

Sinus tachycardia is common in patients with systolic HF and often correlates with the disease severity. Heart rate reduction can be a therapeutic target in this population. Ivabradine is an inhibitor of the sinoatrial "funny" current (I_f current). In a large randomized control trial, ivabradine has been shown to decrease the composite endpoint of cardiovascular mortality and hospital admission in patients with left ventricular ejection fraction (LVEF) $\leq$35% and heart rate $\geq$ 70 bpm (Swedberg et al., 2010). The use of ivabradine in this population is considered reasonable; however, the guidelines emphasize the initial effort at up-titrating a beta-blocker to a maximum tolerated dose (Yancy et al., 2016).

Atrial Fibrillation

AF is the most common sustained arrhythmia in HF patients (**Fig. 1**). Presence of HF is a risk factor for AF regardless of LVEF, and the diagnosis of AF confers higher mortality in patients with HF (Maisel and Stevenson, 2003; Tsang et al., 2002; Wang et al., 2003). Although clinicians often attempt to restore and maintain sinus rhythm in patients with HF and AF, studies have demonstrated no significant difference in morality between strategies of rate control versus rhythm control in patients with and without HF (Wyse et al., 2002; Roy et al., 2008). Pharmacologic options other than beta-blockers used for rhythm control in systolic HF are generally limited to class III antiarrhythmic drugs (AADs): sotalol, dofetilide, and amiodarone (**Table 1**). Sotalol and dofetilide are contraindicated in patients with prolonged QT interval and/or renal dysfunction. The U.S. Food and Drug Administration mandates a minimum 72-h in-hospital ECG monitoring for the initiation of dofetilide due to the significant risk of QT prolongation and torsades de pointes (TdP). Dronedarone, another class III agent, is contraindicated in patients with symptomatic HF with recent decompensation requiring hospitalization or NYHA class IV failure, and is rarely used in HF population (Kober et al., 2008). Class Ic agents (flecainide and propafenone) are contraindicated in patients with coronary artery disease and/or structural heart disease, limiting its use in HF population in general. Beta-blockers are the mainstay of pharmacotherapy for rate control. Calcium channel blockers should be avoided in patients with impaired LV function due to their negative inotropic effect. AV nodal ablation combined with an

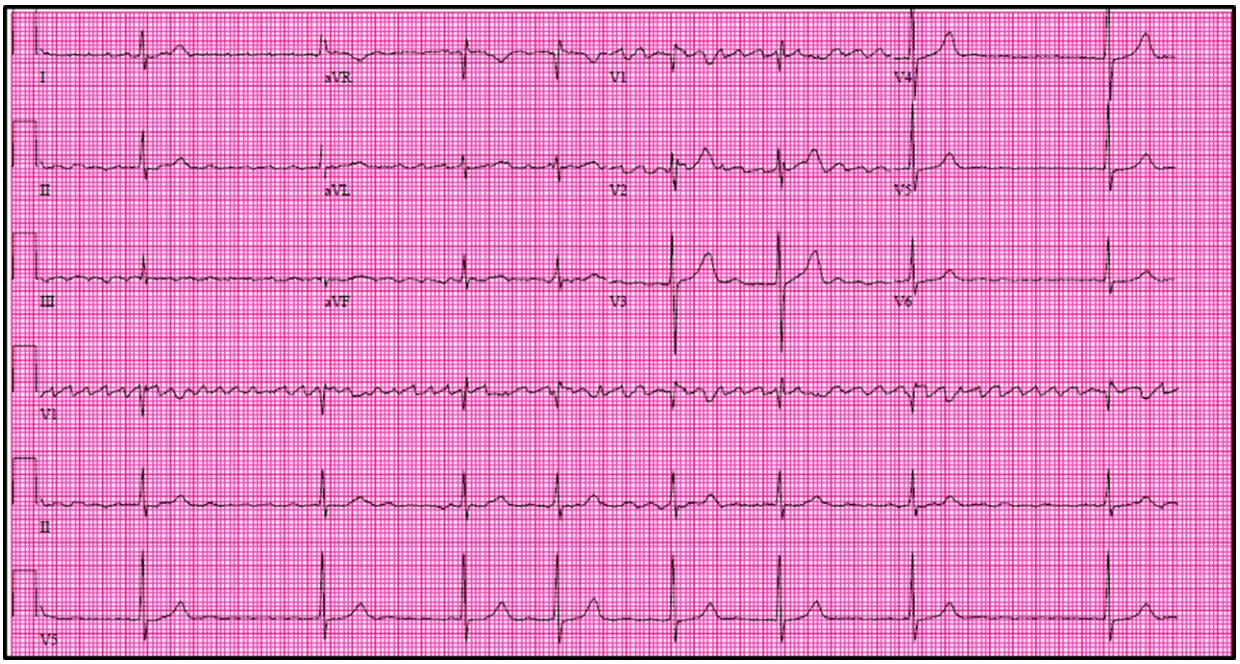

Fig. 1 The 12-lead electrocardiogram shows atrial fibrillation with controlled ventricular response. Fibrillatory waves without discrete P waves and variable intervals between QRS complexes are characteristic.

Table 1 Frequently used antiarrhythmic drugs

Vaughan-Williams classification	*Medication*	*Common use*	*Dose*	*Side effects*	*Comments*
Ia	Procainamide	Preexcited AF (AF associated with WPW), VT	IV: 17 mg/kg at 20–50 mg/min	Hypotension, GI, blood dyscrasias, lupus-like symptoms, TdP	Active metabolite (NAPA) may accumulate if ↓renal function
	Quinidine	VT	PO: Quinidine sulfate 300 mg q8-q12 h Quinidine gluconate 324 mg q8-q12h	GI, TdP, tinnitus, blood dyscrasias	Not commonly used
	Disopyramide	AF, particularly vagally mediated subtype, AF with HCM	PO: 150 mg q6h	Blurry vision, closed-angle glaucoma, urinary retention (anticholinergic effects)	Contraindicated in low EF due to its negative inotropic effect
Ib	Lidocaine	VT	IV: 1 mg/kg bolus; followed by 1–3 mg/min	CNS side effects including seizure and coma	Check blood level to monitor toxicity (therapeutic: 1.5–5 mcg/mL, toxic >9 mg/mL)
	Mexiletine	PVC, VT	PO:150–300 mg q8 h	GI, confusion, tremor, ataxia	Commonly combined with class III drug
Ic	Flecainide	AF, SVT, PVC	PO: 50–150 mg q12h	Proarrhythmic effect	Contraindicated in CAD, significant LVH and low EF. Concomitant AV-nodal blocking agent necessary
	Propafenone	AF, SVT, PVC	PO: 150–300 mg q8h		
II	Metoprolol	AF, SVT, PVC, VT	IV: 5–15 mg q6-8 h PO: Immediate release (tartrate) 25–200 mg q12h Extended (succinate) 25–200 mg daily	Bradycardia, hypotension, bronchospasm, depression	Cardio-selective Metoprolol succinate (extended release) preferred for HF
	Carvedilol		PO: 3.125–25 mg q12h		+ alpha-blockade
	Atenolol		PO: 25–100 mg daily		Cardio-selective
	Esmolol	AF	IV: 0.5–1 mg/kg bolus, followed by 0.05–0.3 mg/kg/min		Cardio-selective
III	Amiodarone	AF, PVC, VT	IV: 150–300 mg bolus, 1 mg/min × 6 h, then 0.5 mg/min × 18 h PO: Loading dose 200–400 mg q8-12 h for 1–2 weeks. Maintenance 200–400 mg daily	Eye, skin discoloration, GI, thyroid (hypo- and hyperthyroid), liver, lung, CNS, neuropathy, tremor, bradycardia, hypotension	Slow onset of effect (days to weeks), and slow elimination (weeks to months)
	Sotalol	AF, PVC, VT	PO: 80–160 mg q12h	TdP, bradycardia, hypotension, HF exacerbation	Mandatory QT monitoring. Avoid hypokalemia and other QT prolonging drugs
	Dofetilide	AF, PVC, VT	PO: 125–500 mcg q 12 h	TdP, AV block	
	Dronedarone	AF	PO: 400 mg q 12 h	HF exacerbation, liver toxicity	Contraindicated in class IV HF, recent decompensated HF, and permanent AF
IV	Verapamil	AF, idiopathic PVC&VT	IV: 2.5–5 mg PO: 120–360 mg daily	Bradycardia, hypotension, constipation	Contraindicated in low EF due to its negative inotropic effect
	Diltiazem	AF, PVC	IV: 0.25 mg/kg bolus; followed by 5–15 mg/h PO: 120–360 mg daily		

AF, atrial fibrillation; *WPW*, Wolff–Parkinson–White syndrome; *VT*, ventricular tachycardia; *TdP*, torsades de pointes; *NAPA*, *N*-acetyl-procainamide; *HCM*, hypertrophic cardiomyopathy; *GI*, gastrointestinal side effect; *PVC*, premature ventricular complex; *CAD*, coronary artery disease; *LVH*, left ventricular hypertrophy; *EF*, ejection fraction; *CNS*, central nervous system; *HF*, heart failure; *SVT*, supraventricular tachycardia.

appropriate device therapy (single-chamber pacemaker, dual-chamber pacemaker, or biventricular pacing system) may be necessary when pharmacologic therapy fails to attain optimal rate control or is poorly tolerated by patients (Kay et al., 1998). Since the AV nodal ablation obligates 100% right ventricular (RV) pacing which can promote further decline in LV function in systolic HF, biventricular pacing system (RV and LV pacing leads) is recommended in patients with LVEF <35%, and should be strongly considered even for patients with less severe LV dysfunction (Epstein et al., 2013a; Leon et al., 2002; Curtis et al., 2013).

Catheter ablation of symptomatic AF is an established treatment option. The efficacy of AF catheter ablation in patients with systolic HF has been evaluated (Anselmino et al., 2016). The long-term success with single ablation procedure is only 40%–50%, and is lower compared to the outcome in general population without HF. The important subset of AF patients in which catheter ablation seems most beneficial is likely those with AIC related to persistent AF (Choi et al., 2010). Significant improvement in LV systolic function is expected after successful ablation and may allow discontinuation of long-term AAD.

Systemic anticoagulation either with warfarin or novel oral anticoagulant is critical for stroke prevention regardless of whether a rhythm or rate control strategy is used.

Atrial Flutter

Atria flutter (AFL) is an organized macroreentrant atrial arrhythmia. Typical AFL involves the cavotricuspid isthmus (the area between the inferior vena cava and the tricuspid annulus), and rotates along the tricuspid valve annulus in a counter-clock direction when seen in the left anterior oblique projection. Twelve-lead ECG of typical AFL is characterized by negative flutter waves in the inferior leads and positive flutter waves in lead V1 (**Fig. 2**). Catheter ablation of the cavotricuspid isthmus is a safe and effective treatment. AF and AFL often coexist in patients with HF in whom only AFL was diagnosed prior to the AFL ablation and the decision to discontinue oral anticoagulant after successful AFL ablation needs to be carefully made after extended ambulatory monitoring and weighed against the risk of thromboembolic complications due to previously undiagnosed AF. Increased left atrial size and AF inducibility with various pacing protocols during AFL ablation have been implicated as risk factors for incidence of AF post-AFL ablation (Ellis et al., 2007; Romero et al., 2017).

Atrial Tachycardia

Atrial tachycardia (AT) is relatively common in patients with HF, and incessant AT may cause or exacerbate systolic HF. With the widespread use of 3D electroanatomic mapping systems, catheter ablation of AT has become highly successful. The P wave morphology of the AT on the 12-lead ECG can assist in localization of the AT origin, and help prepare for the procedure (e.g., need for trans-septal left atrial catheterization and counseling on procedural risk) (Teh et al., 2009). A positive P wave in lead V1 and negative P waves in lateral leads (Leads I and aVL) suggest left atrial origin. Crista terminalis and pulmonary vein ostia are common sites for focal AT in right atrium and left atrium, respectively (Teh et al., 2009).

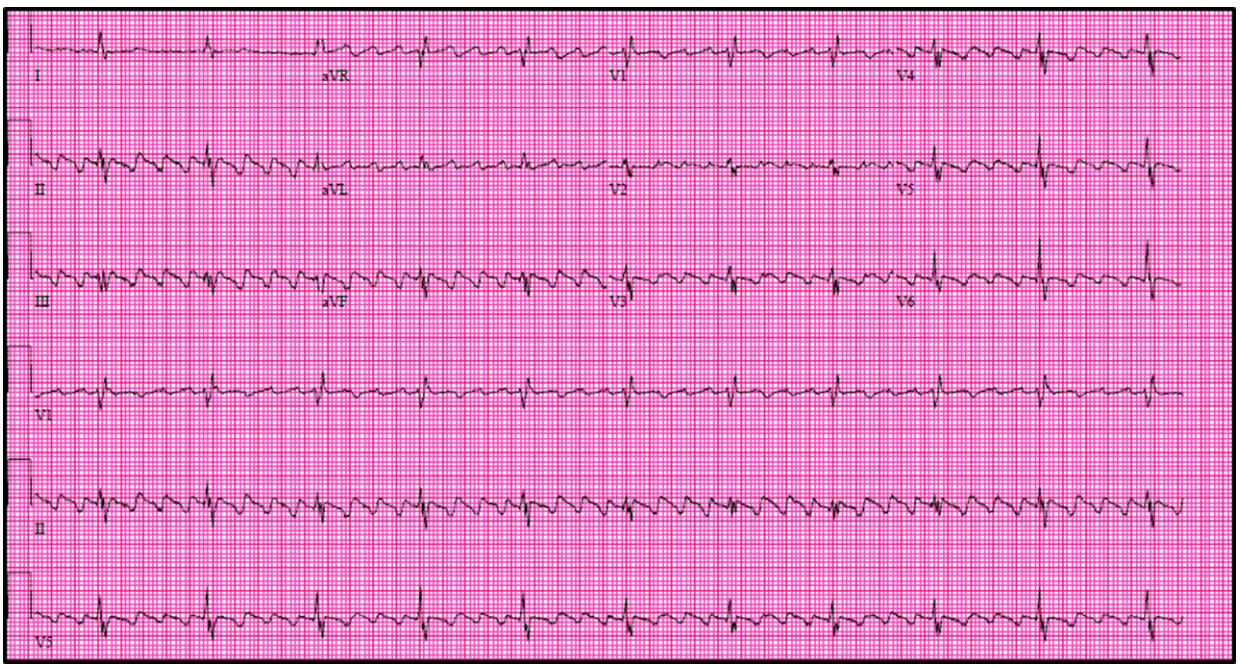

Fig. 2 The 12-lead electrocardiogram shows typical atrial flutter with 4:1 atrioventricular block. Negative flutter waves in the inferior leads (leads II, III, and aVF) and positive flutter waves in the lead V1 are noted.

Other Supraventricular Tachycardias

Catheter ablation is a reasonable first-line therapy for AV nodal reentrant tachycardia (AVNRT) and AV reentrant tachycardia (AVRT) due to its high success rates and low complication risk (**Fig. 3**). Target ablation sites for AVNRT and AVRT are slow pathway and AV accessory pathway, respectively. Although verapamil and diltiazem are reasonable pharmacologic agents for the management of SVT in the general population, the presence of reduced LVEF precludes the use of calcium channel blockers due to their negative inotropic effect. Aggressive treatment of SVT, including catheter ablation, is particularly crucial in patients with implantable cardioverter defibrillator (ICD) to avoid inappropriate shocks for supraventricular tachyarrhythmias (Cardoso et al., 2015).

Ventricular Arrhythmias

Ventricular Tachycardia and Ventricular Fibrillation

Ventricular arrhythmias including PVC, nonsustained VT (NSVT), and sustained VT/VF are more common in patients with structural heart disease, particularly in patients with severely reduced LVEF (**Fig. 4**). SCD is the leading cause of death in industrialized countries and the estimated annual incidence is 300,000–350,000 in the United States (Myerburg et al., 1993; Zheng et al., 2001). Sustained ventricular tachyarrhythmias (VTA) are the most common arrhythmias leading to SCD, and underlying coronary artery disease accounts for a large proportion of SCD in older patients whereas young SCD victims frequently suffer from inherited channelopathies, HCM, dilated cardiomyopathy, and myocarditis (European Heart Rhythm A et al., 2006; Priori et al., 2015). In this section, we discuss the role of pharmacologic and nonpharmacologic therapies in the management of VTA in patients with systolic HF either due to ischemic or nonischemic etiology (Pedersen et al., 2014).

Beta-blockers (bisoprolol, metoprolol XL, carvedilol) are the cornerstone therapy in patients who are at increased risk for VTA and it reduces all-cause mortality and SCD in patients with systolic HF (Anon, 1999a,b). Although there are other available AADs used for patients with frequent VTA to minimize ICD therapy, AAD therapy has not been shown to improve mortality. Ironically, some AADs have been associated with increased mortality. Most notable includes the Vaughan-Williams Class IC drugs, which were studied to suppress PVCs in postmyocardial infarction (MI) patients with LVEF $\leq$40%. The CAST-I and II (Cardiac Arrhythmia Suppression Trials) demonstrated increased mortality associated with flecainide, encainide, and moricizine despite effective PVC suppression (The Cardiac Arrhythmia Suppression Trial (CAST), 1989; Feld et al., 1992).

Other commonly used oral AADs to suppress PVCs, NSVTs, and VTAs include mexiletine, amiodarone, sotalol, and dofetilide (Rose-Jones et al., 2014; Schleifer et al., 2015; **Table 1**). Mexiletine is a Vaughan-Williams Class IB agent and has similar electrophysiological properties to lidocaine. Mexiletine has been used alone and more commonly in conjunction with class III drug such as amiodarone and sotalol (Manolis et al., 1990; Gao et al., 2013). Mexiletine is generally well tolerated and safe; however, it is associated with neurologic (tremor, dizziness) and gastrointestinal (nausea, anorexia) side effects.

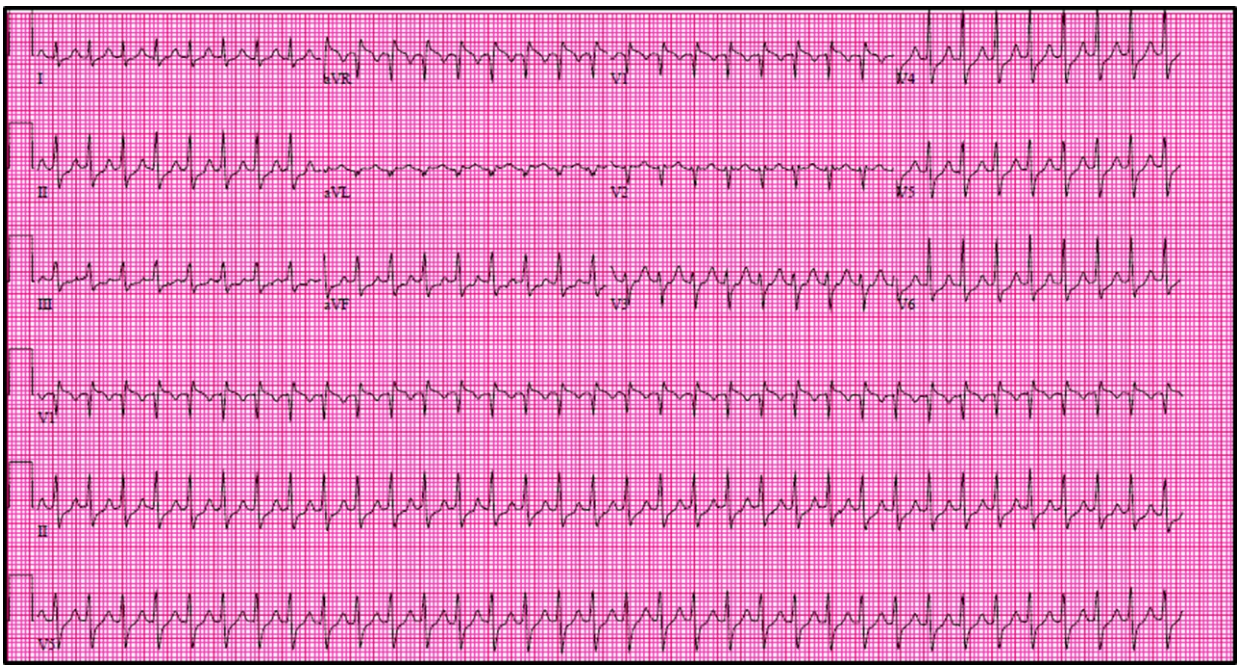

Fig. 3 The 12-lead electrocardiogram shows a narrow complex tachycardia with heart rate of 202 beats per minute. This patient underwent an invasive electrophysiology study, which confirmed the mechanism of the tachycardia to be typical atrioventricular reentrant tachycardia (AVNRT).

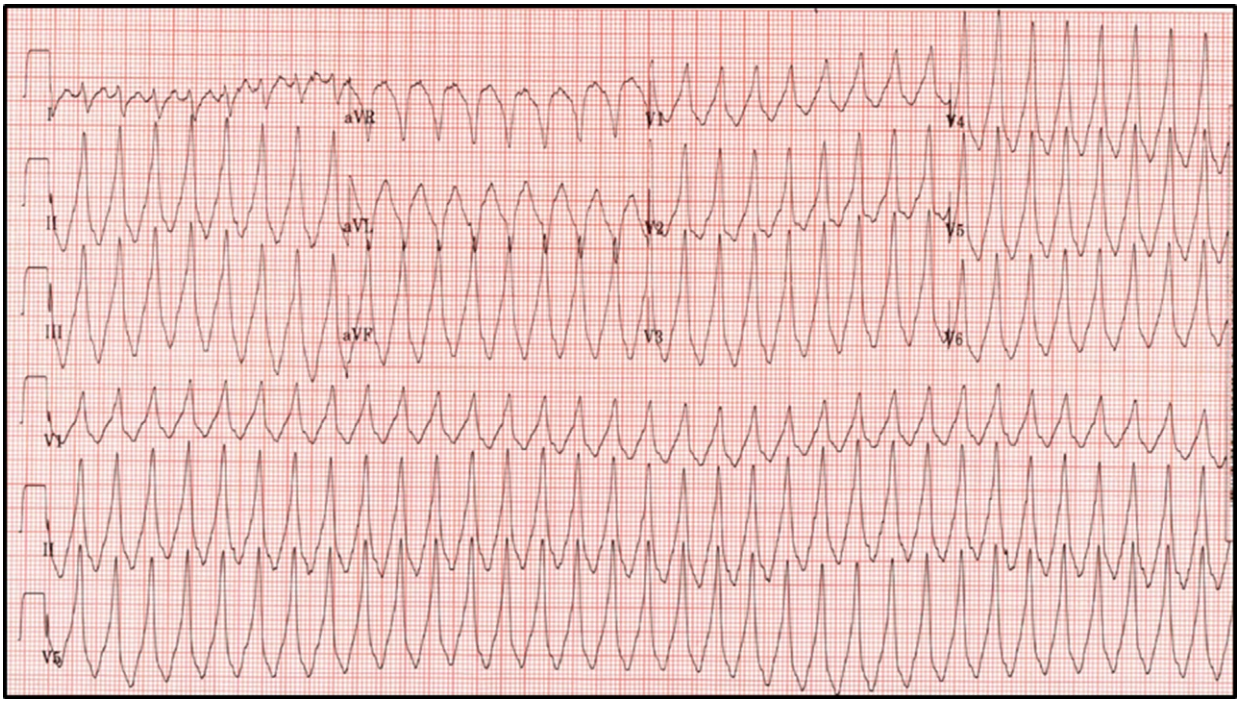

Fig. 4 The 12-lead electrocardiogram of a patient with idiopathic ventricular tachycardia (VT).

Amiodarone is one of the most commonly used AADs for acute therapy of VTAs as well as for chronic suppressive therapy. Although it is classified as a class III AAD, it exhibits various pharmacologic properties including beta-blockade, sodium channel blockade, and potassium channel blockade. Before the routine use of ICDs for SCD prevention, amiodarone was once believed to decrease morality, but the contemporary large-scale clinical trial (SCD-HeFT) showed no survival benefit of amiodarone in patients with NYHA class II–III HF (EF $\leq$35%) as compared with placebo (Bardy et al., 2005). Potential side effects of amiodarone affect thyroid, lung, liver, gastrointestinal tract, skin and eye, and chronic use of the drug calls for close monitoring for organ toxicities including liver function test, thyroid function test, and lung function testing. In the comparative study (OPTIC), amiodarone plus beta-blocker was more effective in reducing the risk of ICD shocks when compared with beta-blocker or sotalol alone, but amiodarone plus beta-blocker was associated with increased risk of drug-related adverse effects (Connolly et al., 2006).

Sotalol is a nonselective beta-blocker that also exhibits class III antiarrhythmic properties. Sotalol (160–320 mg/day) was evaluated as an adjunct therapy to suppress VTA in patients with ICD, and the study showed that sotalol was associated with a lower risk of all-cause mortality or ICD shocks compared to placebo (Pacifico et al., 1999). Sotalol is associated with the risk of TdP and HF exacerbation. Inpatient loading and close monitoring of QT interval is highly recommended. Dofetilide is a class III drug currently approved for the treatment of AF in the United States. Dofetilide appears to be safe in patients with recent MI and systolic HF, as demonstrated in the DIAMOND study. In this trial, 1510 post-MI patients and a low LVEF $\leq$35% were randomized to dofetilide versus placebo. There was no difference in all-cause mortality, but TdP was observed in 7 out of 749 patients in the dofetilide group (Køber et al., 2000). Dofetilide has been evaluated for VTA suppression and has been shown to be as effective as sotalol and may be better tolerated than amiodarone and sotalol. Due to the life-threatening proarrhythmic risk, dofetilide is contraindicated when baseline QTc interval is greater than 440 ms (>500 ms with conduction system abnormalities) and inpatient loading with 3-day ECG monitoring is mandatory for drug initiation. Risk factors for TdP associated with dofetilide include female gender, hypokalemia, and concomitant diuretic use (Shenasa and Shenasa, 2016).

Catheter ablation is an effective adjunctive treatment strategy in patients with systolic HF and sustained monomorphic VT (Yamada and Kay, 2012; Raymond et al., 2009). The survival benefit of the ICD has been well established; however, both appropriate and inappropriate shocks are associated with increased mortality in addition to psychological burden on the ICD recipients (Proietti et al., 2015). Ventricular tachycardia ablation may be the only treatment option in setting of drug-refractory VT storm ($\geq$3 ICD therapies within 24 h) and multiple ICD interventions.

The most common mechanism of VT in patients with systolic HF is a reentrant circuit associated with abnormal myocardial substrate due to previous MI, nonischemic cardiomyopathy, arrhythmogenic RV dysplasia/cardiomyopathy, or other infiltrative myocardial diseases. In post-MI VT, the VT circuit is generally found on the ventricular endocardial surface, and ablation strategies typically target the critical components of the VT circuit identified by mapping procedures during spontaneous or induced VT. This approach requires sustained and hemodynamically stable VT, but it is not always attainable due to VT noninducibility or hemodynamic instability during VT. An alternative approach to VT ablation is to identify abnormal myocardial substrates likely responsible for clinical VT during sinus rhythm or ventricular pacing (substrate mapping), and target them for ablation (Santangeli

and Marchlinski, 2016). Both approaches appear equally effective; however the latter approach generally requires more extensive radiofrequency lesions to achieve the procedural endpoint of VT noninducibility (Kumar et al., 2016). The VT circuit may harbor critical arrhythmogenic substrates in the epicardial myocardium only accessible from the pericardial space, particularly in patients with nonischemic cardiomyopathy. These patients may require epicardial mapping and ablation for controlling VT (Tedrow and Stevenson, 2009; Della Bella et al., 2011). Potential risks of epicardial mapping and ablation include coronary artery damage, pericardial bleeding and cardiac tamponade, phrenic nerve injury, and extra-cardiac organ damage (lung, liver, gastrointestinal tract).

Presently, VT ablation is generally reserved for patients with recurrent VT episodes on at least one AAD or for those intolerant to AADs. The safety and efficacy of "prophylactic" catheter ablation has been evaluated in prospective studies for post-MI patients with spontaneous VTA on no AAD. The SMASH-VT enrolled 128 patients with spontaneous VT and VF (VF = 18%) and showed fewer ICD shocks when compared to a control group (Reddy et al., 2007). The VTACH study is another randomized trial which enrolled 110 patients with VT and LVEF $\leq$50% and demonstrated a higher VTA free survival rate in the ablation group, compared to the control group (no ablation group) (Kuck et al., 2010). In the VANISH trial which recruited patients with recurrent VT despite the use of an AAD, catheter ablation has been shown to be superior to escalation of AAD therapy (increased dose of amiodarone or addition of another AAD) with respect to the composite outcome of death, VT storm, or appropriate shocks. The greatest benefit of catheter ablation was observed in a subgroup of patients who were already receiving amiodarone. There was, however, no mortality difference during a mean follow-up duration of 28 months (Sapp et al., 2016). Recently published long-term analysis of nonrandomized VT ablation trial in patients with ischemic cardiomyopathy showed decreased amiodarone requirement and hospitalization over the 3-year follow-up duration (TERMOCOOL VT trial) (Marchlinski et al., 2016). Whether VT ablation should be performed as a first-line therapy before the trial of AAD in patients with ischemic cardiomyopathy and ICD shocks remains unknown. In contrast to ischemic cardiomyopathy, there have been no prospective randomized studies evaluating the role of VT ablation in patients with nonischemic cardiomyopathy, and long-term outcome of VT ablation in this population has been less well defined. A largest single-center ablation experience in patients with nonischemic cardiomyopathy, including a high rate of adjuvant epicardial ablation (32%), reported a VT-free survival rate of 69% at 60-month follow-up (Muser et al., 2016). Ventricular tachycardia ablation in patients with structural heart disease is rapidly evolving and it is hoped that emerging imaging techniques (cardiac MR, real-time image integration technique), new mapping tools (high-density mapping), and ablation technologies (force sensing catheter, intramural needle ablation) will enhance procedural safety and efficacy (Sapp et al., 2016; Wissner et al., 2012; Yamashita et al., 2016; Viswanathan et al., 2017).

Patients presenting with HF and polymorphic VT or VF warrant thorough evaluation of the underlying etiology, particularly the presence of myocardial ischemia. Polymorphic VT is a ventricular tachycardia with clearly defined QRS complexes that change continuously from beat to beat. TdP is a form of polymorphic VT that occurs in setting of a prolonged QT interval, and is characterized by appearance of QRS complexes twisting around the isoelectric line of the recording (twisting of the points) (Fig. 5). TdP is frequently associated with electrolyte abnormalities (hypokalemia and hypomagnesemia) and the use of QT prolonging drugs (AADs, antibiotics, and psychotropic drugs). Acute treatment of TdP includes external defibrillation, intravenous magnesium sulfate, lidocaine (Class Ib AAD shortens the QT interval), isoproterenol infusion, and temporary overdrive pacing. Overdrive pacing allows shortening of the QT interval by increasing the heart rate >100 bpm. Ventricular fibrillation is a chaotic tachycardia without consistently identifiable QRS complexes and calls for immediate defibrillation (Pedersen et al., 2014).

Premature Ventricular Complex

The presence of frequent PVCs may cause and exacerbate cardiomyopathy and HF (Fig. 6). It may also prevent delivering effective cardiac resynchronization therapy (CRT). PVC-induced cardiomyopathy is an arrhythmia-induced, potentially reversible condition. Postulated mechanisms of PVC-induced cardiomyopathy include ventricular dyssynchrony akin to bundle branch block, abnormal

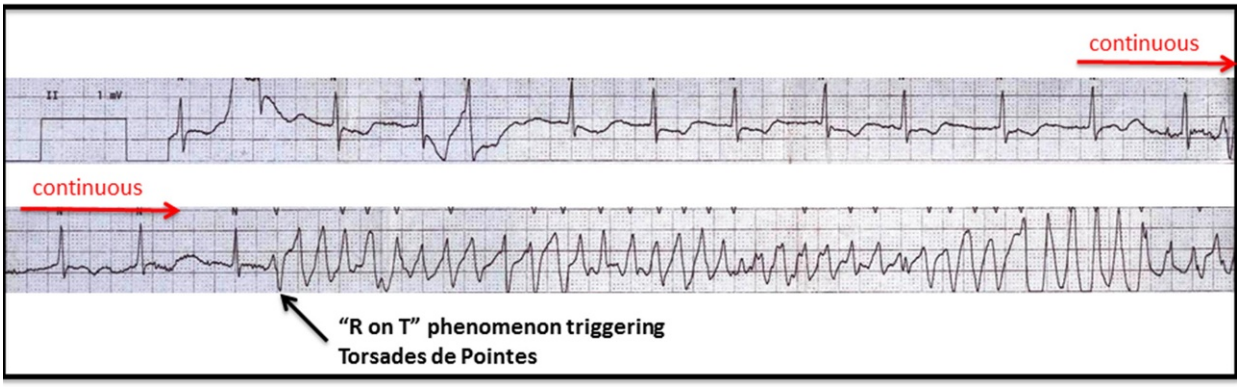

Fig. 5 An example of torsades de pointes (TdP) rhythm. Note prolonged baseline QT interval and initiation of TdP with a premature ventricular complex occurring during the T wave ("R on T" phenomenon). Courtesy of Behzad Pavri MD, Thomas Jefferson University, Philadelphia, PA, USA.

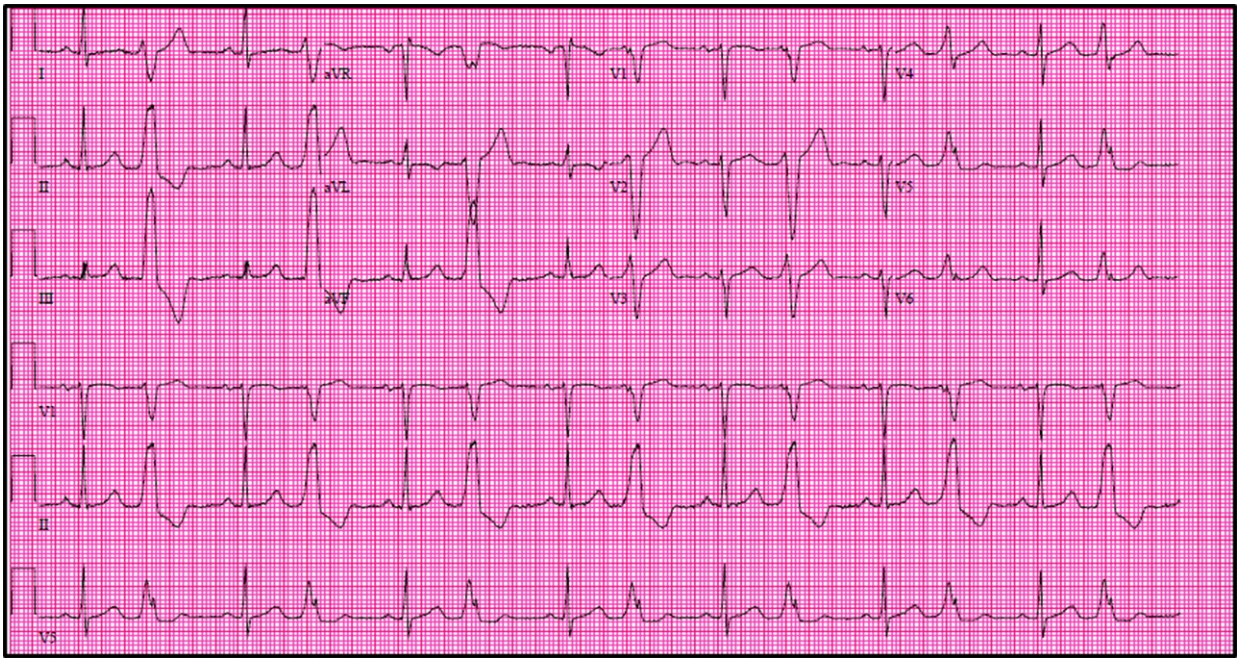

Fig. 6 The 12-lead electrocardiogram shows frequent premature ventricular complexes (PVC). This PVC was successfully ablated in the right ventricular outflow tract.

Ca^{2+} handling, and abnormal ventricular filling from post-PVC pause (Gopinathannair et al., 2015). Although a higher PVC burden (particularly >24%) correlates with the incidence of cardiomyopathy, not every patient with frequent PVC burden develops cardiomyopathy, and PVC-induced cardiomyopathy is rare when a PVC burden is less than 10% (Baman et al., 2010). A beta-blocker is a safe first-line pharmacologic therapy for frequent PVCs in the setting of cardiomyopathy; however, its efficacy for PVC suppression is only modest. Catheter ablation is a reasonable first-line therapy for PVC-induced cardiomyopathy due to frequent monomorphic (unifocal) PVCs. AADs such as mexiletine, sotalol, and amiodarone may be necessary if PVC focus is not amenable to catheter ablation, or multifocal PVCs are encountered. Class Ic AADs such as flecainide and propafenone should be avoided in patients with a reduced LVEF to their proarrhythmic effects. Right and left ventricular outflow tracts are the most common sites for idiopathic PVC. These outflow PVCs are generally associated with benign prognosis, however, rarely cause PVC-induced polymorphic VT and sudden cardiac arrest (Noda et al., 2005).

Device Therapy for SCD Prevention

Rationale for ICD

Today, ICD has an established role in the primary and secondary SCD prevention. Historically, due to the considerable morbidity and mortality associated with surgical implantation (via thoracotomy) of the early generation ICD device, only those who survived cardiac arrest due to VTA qualified for the device implantation in 1980s. Transvenous insertion of the ICD lead and subsequent miniaturization of the pulse generator implanted in the pectoral region eventually led to the widespread use of the ICDs. Survival benefits of secondary prevention ICD placement over chronic AAD therapy were later demonstrated in landmark prospective trials (AVID, CIDS, CASH) (The Antiarrhythmics versus Implantable Defibrillators (AVID) Investigators, 1997; Connolly et al., 2000a; Kuck et al., 2000). A meta-analysis (ICD: $n = 934$ vs. amiodarone: $n = 932$) showed significant reductions in all-cause mortality and in arrhythmic death in patients implanted with ICDs when compared with chronic amiodarone therapy alone (Connolly et al., 2000b).

The proven success of ICD therapy in SCD survivors set the stage for prospective trials for primary prevention ICD therapy for patients at risk for SCD. Specifically, MI survivors with impaired LV systolic function and HF were consistently recognized as conferring the highest risk of cardiac morality, particularly due to sudden arrhythmic death, and targeted for primary prevention trials. A substantial body of clinical evidence emerged from early studies including patients with coronary artery disease and an impaired LVEF (<30–40%), and showed survival benefit in this population (MUSTT, MADIT I, MADIT II) (Buxton et al., 1999; Moss et al., 1996; Moss et al., 2002). In the MUSTT trial, post-MI patients with NSVT and LVEF ≤40% were risk-stratified using invasive electrophysiology study (EPS). Patients with inducible, sustained VTA were randomly assigned to either the EPS-guided therapy group or no AAD therapy. In the EPS-guided therapy group, ICD placement was recommended when VTA was induced on at least one AAD. The primary endpoint of cardiac arrest or arrhythmic death occurred less in the EPS-guided therapy group, and the

benefit was largely driven by the presence of ICD. It should be noted that within the EPS-guided therapy group, assignment to ICD placement (46% in the EPS group) versus AAD therapy was not randomized, and patients were required to fail at least one AAD to receive ICD (Buxton et al., 1999). The findings of MUSTT have been incorporated in the guidelines for primary prevention ICD implantation (Epstein et al., 2013a).

Notably, studies evaluating primary prevention ICD implantation early after MI (30–40 days) did not show mortality benefit (DINAMIT, IRIS). Current device guidelines do not recommend primary prevention ICD placement within 40 days of MI (Epstein et al., 2013a). Likewise, routine ICD placement at the time of coronary artery bypass grafting showed increased infection risk without any survival benefit (CABG-PATCH) (Bigger, 1997). Therefore, it is recommended that LVEF should be reassessed and primary prevention ICD placement should be deferred for at least 3 months after surgical or percutaneous coronary revascularization. A wearable cardioverter defibrillator may be considered for patients perceived to be high risk for SCD during the waiting period (Epstein et al., 2013b; Reek et al., 2016) (**Fig. 7**).

In contrast to the robust evidence of the ICD benefit in patients with ischemic cardiomyopathy, evidence for primary prevention ICD placement in nonischemic cardiomyopathy is not as conclusive. Three prospective studies (AMIOVIRT, CAT, and DEFINITE) failed to demonstrate the benefit of primary prevention ICD in this population (Strickberger et al., 2003; Bansch, 2002; Kadish et al., 2004). The current guideline recommendations regarding the role of primary prevention ICD placement in nonischemic cardiomyopathy derive largely from SCD-HeFT, the largest primary prevention ICD trial to date including 2521 patients with both ischemic and nonischemic patients (Ischemic: 52% vs. nonischemic cardiomyopathy: 48%), NYHA class II/III HF, and LVEF $\leq$35% (Bardy et al., 2005). Patients were randomized to placebo, amiodarone, and ICD arms. The primary prevention ICD therapy was associated with 23% reduction in all-cause mortality, as compared with placebo. Amiodarone showed no survival benefit in this study.

Recently, the utility of primary prevention ICD therapy in nonischemic cardiomyopathy was questioned in a multicenter prospective study in Denmark, in which patients with nonischemic cardiomyopathy and LVEF $\leq$35% were randomly assigned to ICD therapy versus no ICD (DANISH trial) (Kober et al., 2016). Contemporary background pharmacotherapy (beta-blocker, angiotensin-converting enzyme (ACE) inhibitor, or angiotensin receptor blocker (ARB)) was used in >90% of participants, and 58% of patients in both groups received CRT based on the current CRT guidelines. After a median follow-up of 68 months, all-cause mortality was similar in both groups. The DANISH study, however, suggested a mortality benefit of the ICD in younger patients (<68 years of age) while the benefit of ICD to reduce SCD was likely offset by a high nonarrhythmic death rate in older patients with severe cardiomyopathy. Although the study was "neutral" in the total population, it strongly suggests the importance of patient selection for primary prevention ICD for patients with HF due to nonischemic cardiomyopathy. In essence, primary prevention ICD therapy should not be universally withheld for patients with severe nonischemic cardiomyopathy who qualify for the ICD implantation based on the current device guidelines (Epstein et al., 2013a).

Primary prevention ICD trials are summarized in **Table 2**. A complete list of primary and secondary prevention ICD indications (and contraindications) is found elsewhere (Epstein et al., 2013a).

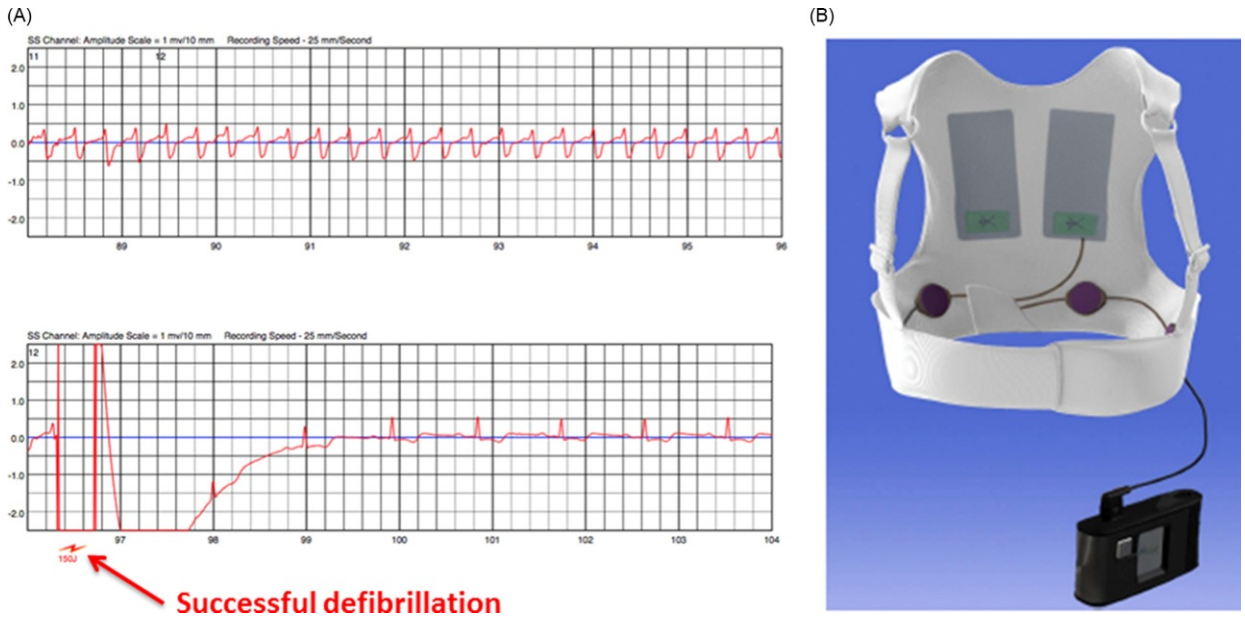

Fig. 7 (A) This is a rhythm strip of a 61-year-old man with recent ST-elevation myocardial infarction and severely depressed left ventricular systolic function (ejection fraction = 25%) who was discharged on a wearable defibrillator. He was found unresponsive due to pulseless VT and successfully resuscitated by the wearable defibrillator (150 J shock) with immediate restoration of sinus rhythm. He subsequently received a secondary prevention implantable defibrillator. (B) A commercially available wearable defibrillator. Image courtesy of ZOLL Medical Corporation, Pittsburgh, PA, USA.

Table 2 Primary prevention ICD trials

ICM versus NICM	*Trial published year*	*EF cutoff*	*Inclusion criteria*	*Study arms*	*Number of patients in ICD arm/control arm*	*Endpoints*	*Results*	*Comments*
ICM	MUSTT 1999	0.4	NSVT. 4 or more days post-MI	EP-guided Rx (AAD or ICD if AAD failed) vs. No Rx	$n=704$, EP-guided Rx:351 (ICD:161), No Rx:353	Primary: cardiac arrest or arrhythmic death Secondary: overall mortality, cardiac death, spontaneous sustained VT	Median f/u 39 mo. Cardiac arrest or arrhythmic death at 5 yrs in EPG:25%, in No Rx:32% (RR 0.73, $P=0.04$), overall mortality at 5 yrs in EPG:42%, in No Rx:48% (RR 1.80, $P=0.06$)	In EPG group, 5-yr rate of cardiac arrest or arrhythmic death was 9% in ICD group and 37% in AAD group ($P=0.001$)
	MADIT I 1996	0.35	MI 3 weeks or more before entry, CHF NYHA I to III, NSVT and inducible and refractory VT/VF, age 25–80	ICD (98 transthoracic and 98 transvenous implants) vs. control	$n=196$, ICD:95, Control:101	Overall mortality	Mean f/u 27 mo, death in ICD:15, in control:39 (HR 0.46, $P=0.009$)	
	MADIT II 2002	0.3	Age >21 (no upper age limit), more than 1 month post-MI	ICD (single chamber or dual chamber) vs. control	$n=1232$, ICD:742, Control:490	Overall mortality	Mean f/u 20 mo, death in ICD:14.2%, in control:19.8% (HR 0.69, $P=0.016$)	Statistically nonsignificant increase in new or worsened HF in ICD group
	CABG-PATCH 1997	0.36	Elective CABG, abnormal SAECG, age <80	Epicardial ICD vs. control	$n=900$, ICD:446, Control:454	Overall mortality	Mean f/u 32 mo, death in ICD:101, in control:95 (HR 1.07, $P=0.64$)	More postoperative infections in ICD group
Implant early after MI	DINAMIT 2004	0.35	6–40 days post-MI, low HRV or elevated HR on Holter monitoring at least 3 days post-MI, age 18–80	Single-chamber ICD vs. control	$n=674$, ICD:332, Control:342	Overall mortality	Mean f/u 30 mo, death in ICD:62, in control:58 (HR 1.08, $P=0.66$)	Statistically significant increase in nonarrhythmic death in ICD group (HR 1.75, $P=0.02$)
	IRIS 2009	0.4	5–31 days post-MI, heart rate >90 bpm on first ECG or NSVT >150 bpm on Holter, age 18–80	ICD (81% single chamber) vs. control	$n=898$, ICD:445, Control:453	Overall mortality	Mean f/u 37 mo, death in ICD:116, in control:117 (HR 1.04, $P=0.78$)	Statistically significant increase in non-SCD (HR 1.92, $P=0.001$). NYHA IV CHF excluded
NICM	AMIOVIRT2003	0.35	Asymptomatic NSVT lasting <30 s, NYHA I–III CHF, age >18	ICD vs. amiodarone	$n=103$, ICD:51, Amiodarone:52	Overall morality	Mean f/u 24 mo, no difference in survival at 1 yr and 3 yrs ($P=0.8$)	
	CAT 2002	0.3	Recent onset NICM <9 mo, NYHA II or III CHF, age 18–70	ICD vs. control	$n=104$, ICD:50, Control:54	Overall mortality	Mean f/u 5.5 yrs, at 4 yrs, cumulative survival in ICD:80% in control:86% ($P=0.554$)	

(*Continued*)

Table 2 (Continued)

ICM versus NICM	*Trial published year*	*EF cutoff*	*Inclusion criteria*	*Study arms*	*Number of patients in ICD arm/control arm*	*Endpoints*	*Results*	*Comments*
	DEFINITE 2004	0.36	Ambient arrhythmias (NSVT or 10 PVCs/h on Holter), NYHA I–III CHF	Single-chamber ICD vs. control	$n=458$, ICD:229, Control:229	Overall mortality	Mean f/u 29 mo, death in ICD:28, in control:40 (HR 0.65, $P=0.08$)	Statistically significant reduction in arrhythmic death in ICD ($P=0.006$)
Both	SCD-HeFT 2005	0.35	NYHA II or III CHF, age >18	Conventional + placebo vs. conventional + amiodarone vs. conventional + shock-only, single-chamber ICD	$n=2521$, Placebo:847,		Amiodarone:845, ICD:829	Overall mortality
Mean f/u	46 mo, death in		placebo:244, in amiodarone:240, in ICD:182. ICD reduced mortality (HR 0.77, $P=0.007$)	Decreased mortality with ICD was seen in NYHA II CHF, not in NYHA III CHF				

ICM, ischemic cardiomyopathy; *NICM*, nonischemic cardiomyopathy; *EF*, ejection fraction; *NSVT*, nonsustained ventricular tachycardia; *MI*, myocardial infarction; *Rx*, treatment/therapy; *AAD*, antiarrhythmic drug; *f/u*, follow-up; *mo*, month; *EPG*, electrophysiological study guided; *RR*, relative risk; *yr*, year; *HR*, hazard ratio; *SAECG*, signal averaged electrocardiography; *HRV*, heart rate variability; *PVC*, premature ventricular complex.

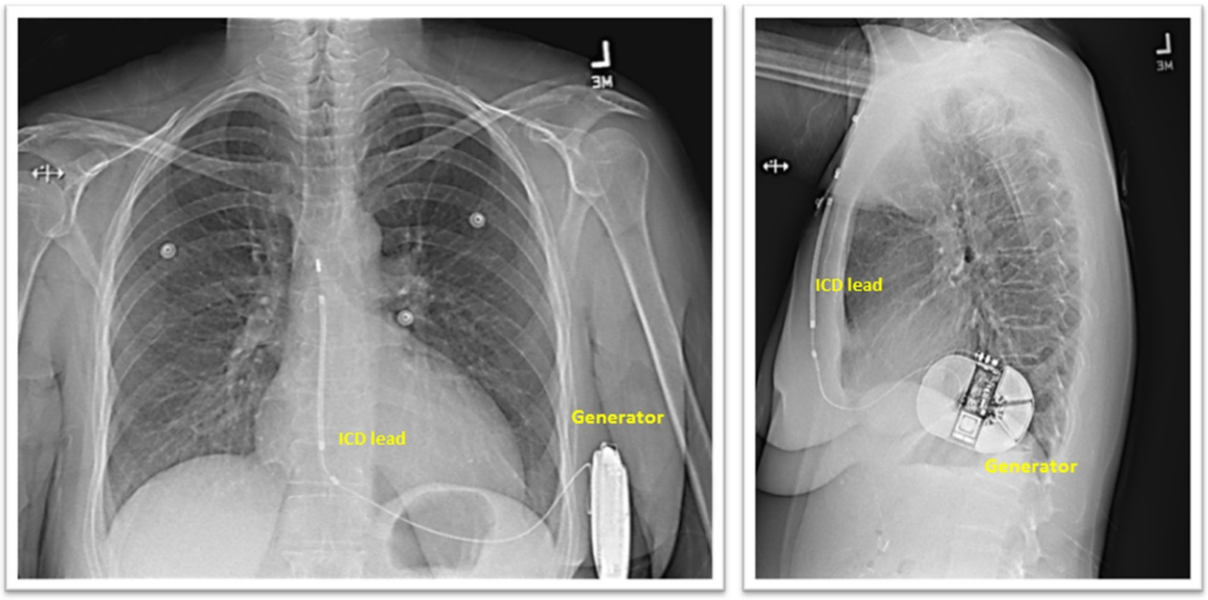

Fig. 8 Posteroanterior (left) and lateral (right) chest X-ray images of subcutaneous defibrillator.

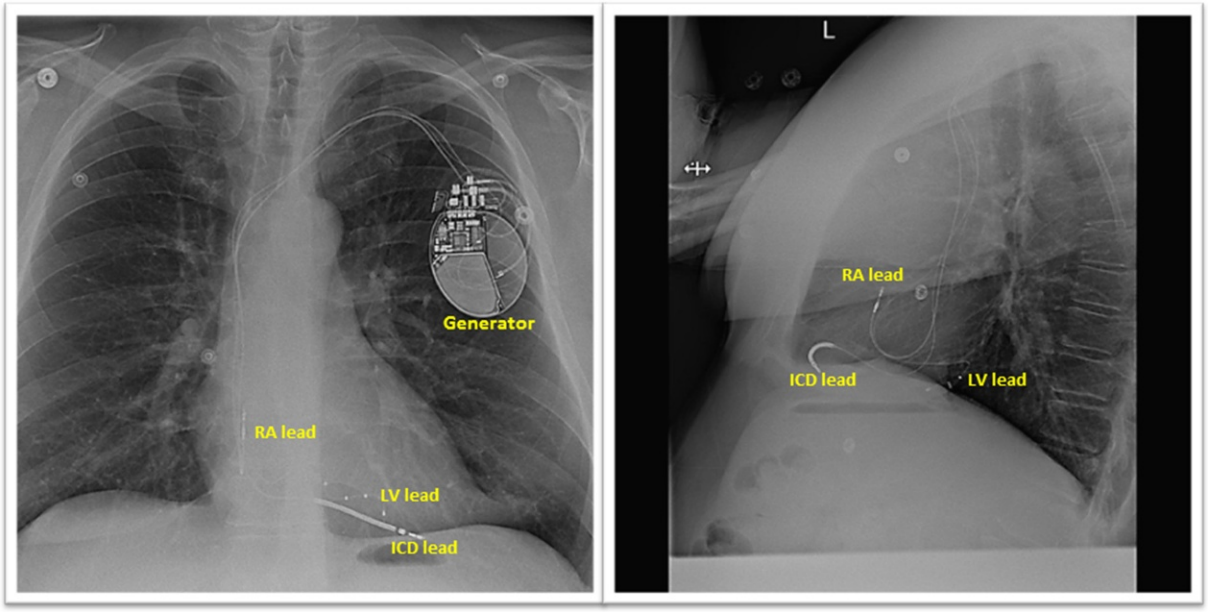

Fig. 9 Posteroanterior (left) and lateral (right) chest X-ray images of biventricular-ICD system. The ICD lead is placed in the right ventricular apex and the LV lead is placed in the posterolateral branch of the coronary vein. *RA*, right atrium; *LV*, left ventricle; *ICD*, implantable cardioverter defibrillator.

Subcutaneous ICD

Contemporary transvenous ICD system consists of a transvenous defibrillation lead and a pectoral pulse generator. Transvenous lead placement is associated with short-term (pneumothorax, hemothorax, cardiac perforation, and tamponade) and long-term complications (tricuspid regurgitation, endocarditis, lead failure, inappropriate shock) (Udo et al., 2012). The totally subcutaneous ICD (SICD) system has been developed to circumvent most of the transvenous lead-associated complications (Lewis and Gold, 2016). The system consists of a pulse generator implanted in the lateral chest wall and a single sensing and defibrillation lead tunneled in the subcutaneous tissue in the anterior chest wall (Fig. 8). Unlike conventional transvenous defibrillation leads, the SICD lead has no central lumen and is believed to be more durable. There is virtually no risk of lead-associated endocarditis or bacteremia owing to the lack of an endovascular component. The SICD is particularly suitable for young patients, patients with limited or no vascular access (congenital heart disease, dialysis, upper body venous occlusion), and patients with previous history of device-related infection or at increased risk for infection. Several limitations of the current SICD system should be noted. First,

patients need to go through preimplant ECG screening to avoid T wave oversensing which can lead to inappropriate ICD shocks. At present, the SICD is not equipped with pacing (other than postdefibrillation temporary demand pacing) or antitachycardia pacing (ATP) capability, and is not suitable for patients with pacing indications (bradycardia and CRT) and with monomorphic VT amenable to ATP. Lastly, although the SICD appears more attractive in certain patient populations as compared with the transvenous ICD system, no head-to-head comparative data is available at present with regard to mortality, efficacy, and complications (Weiss et al., 2013; Lambiase et al., 2014; Burke et al., 2015; Olde Nordkamp et al., 2012).

Risk Stratification

Impaired LV systolic function and the presence of HF are the most powerful predictors of SCD, but the low LVEF alone is neither sensitive nor specific for this purpose. As a result, a substantial number of patients with ICD do not receive appropriate ICD therapy while they are subjected to a myriad of long-term device-related complications including infection, lead malfunction, and inappropriate shocks. Post hoc analysis of MADIT II demonstrated feasibility of simple clinical score to assess relative benefit of the primary prevention ICD in an individual patient. The scoring system consists of five readily available clinical factors, including NYHA class, age >70 years old, blood urea nitrogen >26 mg/dL, QRS duration >120 ms, and the presence of AF, and each factor is assigned 1 point. In this analysis, patients with low (risk = 0) and intermediate risk (risk = 1 or 2) derived the most benefit while patients with a high-risk score (risk ≥ 3) and those with severe renal dysfunction (serum creatinine ≥ 2.5 mg/dL) did not benefit from primary prevention ICD (Goldenberg et al., 2008; Barsheshet et al., 2012).

Likewise, patients with HF with a preserved or mildly depressed LVEF who may be at risk for SCD are automatically excluded from receiving the potentially life-saving device based on the current LVEF-based recommendations for primary prevention ICD (Epstein et al., 2013a; Al-Khatib et al., 2007). There are numerous noninvasive risk markers for SCD including QRS duration, QT interval, QT dispersion, signal-averaged ECG, heart rate variability, and T-wave alternans; however, clinical utility of these markers to guide appropriate patient selection for primary prevention ICD therapy has not been established (Goldberger et al., 2008).

Bundle Branch Block and CRT

Prolonged QRS duration >120 ms is seen in 30%–40% of patients with systolic HF and is associated with increased mortality (Braunschweig et al., 2017). Particularly, left bundle branch block (LBBB) has been associated with higher cardiac event rates in HF patients and is also a risk factor for developing future HF in asymptomatic patients (Baldasseroni et al., 2002; Dhingra et al., 2006). CRT aims to restore interventricular and intraventricular conduction delay by providing nearly simultaneous right and left ventricular pacing. Historically, left ventricular pacing was first provided via epicardial LV lead, but subsequently transvenous LV lead placement via coronary vein cannulation was developed in late 1990s and became the standard implanting technique (Daubert et al., 1998; **Fig. 9**). Survival benefit of CRT was confirmed in two landmark trials (COMPANION, CARE-HF) as well as in a meta-analysis (Bristow et al., 2004; Cleland et al., 2005; Bradley et al., 2003). The COMPANION trial randomized 1520 patients with sinus rhythm, NYHA class III/IV HF, LVEF ≤35%, QRS ≥120 m to optimal medical therapy, CRT pacemaker (CRT-P), or CRT defibrillator (CRT-D). The primary endpoint of death or hospitalization was significantly reduced in both CRT-P and CRT-D groups compared to the optimal medical therapy alone. In this trial, a statistically significant reduction in all-cause mortality was observed only in the CRT-D group, but not in the CRT-P group (Bristow et al., 2004). The mortality benefit of CRT-P device was later demonstrated in the CARE-HF trial, in which 813 patients were randomized to optimal medical therapy alone versus optimal medical therapy and CRT-P (Cleland et al., 2005). These encouraging CRT benefits eventually paved the way for CRT trials for less symptomatic HF patients (REVERSE, MADIT-CRT, RAFT) (Linde et al., 2008; Moss et al., 2009; Tang et al., 2010). The MADIT-CRT enrolled 1820 patients with NYHA class I/II HF, LVEF ≤30%, and QRS ≥130 m and demonstrated decreased primary endpoint of death or HF events in patients with CRT-D, as compared with patients with ICD (Moss et al., 2009).

Although these landmark trials included patients with QRS ≥120 m, subsequent analyses showed that the greatest benefit of CRT device was observed among patients with QRS >150 ms (Sipahi et al., 2011; Stavrakis et al., 2012). Furthermore, CRT benefit was only observed in patients with LBBB morphology, but not in those with non-LBBB QRS morphologies (RBBB, IVCD) (Bilchick et al., 2010; Nery et al., 2011). Currently, U.S. device therapy guidelines provide class I CRT indication to those with sinus rhythm, NYHA class II/III/ambulatory IV HF, LVEF ≤35%, and LBBB QRS ≥150 m. A complete list of CRT indications is found elsewhere (Epstein et al., 2013a).

Presently, U.S. device guidelines do not recommend CRT in patients with QRS <120 ms under any circumstance despite the fact that some HF patients with narrow QRS exhibit mechanical dyssynchrony by imaging assessment (Epstein et al., 2013a; Bleeker et al., 2004; Ghio et al., 2004). In order to evaluate the potential utility of CRT for patients with systolic HF, narrow QRS interval, and mechanical dyssynchrony, the EchoCRT recruited patients with NYHA class III/IV HF, and LVEF ≤35%, QRS <130 m and an echocardiographic evidence of LV dyssynchrony and randomized them to CRT-D versus standard ICD therapy. The study was terminated prematurely for futility after randomizing 809 patients. The primary outcome of death or HF hospitalization was similar in both groups, but more deaths were observed in the CRT-D group (Ruschitzka et al., 2013). Meta-analysis of studies evaluating the benefit of CRT in patients with systolic HF and QRS ≤130 ms suggested increased all-cause mortality compared to nonresynchronized ICD (Shah et al., 2015).

AV Block

When relatively young patients present with AV block, infectious and noninfectious causes need to be thoroughly evaluated. Some disease processes are reversible including myocarditis (e.g., Lyme disease) and myocardial ischemia. Two important noninfectious causes deserve special mention due to the significant risk of ventricular arrhythmias and SCD. Sarcoidosis is a noncaseating, granulomatous disorder of unknown etiology that can affect myocardium and the conduction system of the heart. Presentations of cardiac sarcoidosis include conduction system disease, cardiomyopathy, atrial and VTA, and SCD. Specialized imaging techniques such as cardiac MRI, 18F-Fluorodeoxyglucose PET, and radionuclide imaging are frequently required for the diagnosis when endomyocardial biopsy is either unavailable or nondiagnostic. Implantation of an ICD system should be considered when the patient presents with a clear pacing indication or if impaired LV function is present (LVEF <50%) (Birnie et al., 2016). Patients with familial dilated cardiomyopathy caused by mutations in LMNA gene (lamin A and lamin C) have high risk of progressive AV block, ventricular arrhythmias, and SCD. Prophylactic ICD placement is generally considered appropriate in patients with LMNA-associated cardiomyopathy and a pacing indication even when LVEF is >35% (Hasselberg et al., 2014; van Rijsingen et al., 2012).

Cardiomyopathy Due to Arrhythmia

AIC is a potentially reversible condition due to frequent atrial or ventricular arrhythmias (Gopinathannair et al., 2015). Cardiomyopathy can be partially or completely reversible once arrhythmias are effectively suppressed. Common arrhythmias associated with AIC include AF, AFL, AT, and PVCs. Exact pathophysiology responsible for AIC is incompletely understood, but it likely shares the same underlying mechanisms with cardiomyopathy due to chronic RV pacing, including ventricular dyssynchrony (due to frequent PVC), LV remodeling, neurohormonal activation, and abnormal Ca^{2+} handling. When suspected, AIC should be treated with evidence-based HF regimen (ACE inhibitor/ARB and beta-blocker) and aggressive arrhythmic suppression with either pharmacologic therapy or catheter ablation. It is unknown whether pharmacologic HF regimen should be continued after complete recovery of cardiomyopathy.

Arrhythmias Post Orthotropic Heart Transplantation

Orthotropic heart transplantation (OHT) is an established treatment for patients with end-stage HF. A variety of supraventricular and VTA and successful catheter ablation of these arrhythmias have been reported in the literature. Predisposing factors for these arrhythmias include myocardial ischemia, inflammation, conduction block caused by biatrial anastomosis, parasympathetic denervation, cardiac allograft vasculopathy, and rejection (Thajudeen et al., 2012; Hamon et al., 2014).

Atrial tachyarrhythmias are the most common entities and biatrial anastomosis appears to be associated with higher risk of atrial arrhythmias due to inherent conduction block or slow conduction across the anastomosis lines. Presently, most transplant centers use bicaval donor-to-recipient anastomosis.

Generally, resting heart rate is elevated after OHT due to parasympathetic denervation. However, bradyarrhythmias may be observed after OHT, and usually results from sinus node dysfunction. Biatrial anastomosis is associated with an increased risk of sinus node dysfunction compared to bicaval technique. Sinus bradycardia in the immediate postoperative period may require temporary pacing, but sympathomimetics such as terbutaline and isoproterenol can achieve the target heart rate of 90 bpm. Rejection should be suspected and promptly excluded in case of sustained or late onset bradyarrhythmias. Asystole and pulseless electrical activity are far more frequent than VF as a cause of SCD in OHT recipients, and cardiac allograft vasculopathy (transplant coronary artery disease) is a recognized underlying mechanism for SCD (Vaseghi et al., 2009). A role of primary prevention ICD in OHT recipients who do not meet the conventional criteria is unknown (Tsai et al., 2009).

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Cardiac Biomechanics in Normal Physiology and Disease

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Normal Cardiac Physiology

Three fundamental mechanisms are involved to properly circulate oxygenated blood in conjunction with the pulmonary system (Katz, 1988). These mechanisms are based on mechanics, cell physiology, and molecular biology. The mechanical mechanisms are based on length dependence to change preload and afterload and are theoretically rooted in the sliding-filament theory and the Frank–Starling relationship. The cell physiologic mechanisms involve the cardiac contractile proteins responding to calcium fluxes to mediate excitation–contraction coupling and relaxation. The molecular biological mechanisms revolve around individual protein expression and their sequences, which influences the structure and function of sarcomeres and metabolic processes within individual cells. To understand contractile function and dysfunction of the heart, a description of the organ, cell, and myofilaments is necessary.

Heart Structure and Function

The heart is a biologic pump and serves to circulate blood through the body (Katz, 2010). The heart is composed of myocytes and nonmyocytes. The nonmyocytes include connective tissue, vascular smooth muscle cells, and endothelial cells, among the heterogeneous population of cells, and account for nearly 70% of the heart's cellular population with the remaining 30% as the cardiomyocyte population (Colucci, 2004). The cardiomyocytes are enmeshed in a collagen network and separated longitudinally by intercalated discs, which are specialized cell–cell junctions (Forbes and Sperelakis, 1985). The intercalated discs provide strong mechanical connections between adjacent cells. The normal structure of the heart allows for a sufficient amount of oxygen and nutrients to circulate throughout an organism's entire body.

In humans, the heart is a four-chamber system with two atria (left and right) and two ventricles (left and right). The shape of the human heart is described as an upside-down pear where the atria form the curved top of the heart and the ventricles form a pointed base. The left ventricle contracts with the most force to overcome a blood flow resistance over distance and eject oxygen-enriched blood from the heart into the body compared to the right ventricle that pumps oxygen-depleted blood into the lungs. The left atrium receives oxygenated blood from the lungs, whereas the right atrium receives deoxygenated blood from the body. The left and right side of the mammalian heart is separated by a septum. A valve connects each atrium to the ventricle below it (mitral valve for the left and tricuspid valve for the right). The top of the heart connects to the aorta artery, the pulmonary artery, superior vena cava, and inferior vena cava, which connect to the pulmonary system.

The heart mechanically pumps blood by contracting myofilaments in each cardiomyocyte, which is triggered by electrical stimulation and calcium release, among other elements. The electrical signal cascade begins in the sinoatrial node (SA node), located in the right atrium, with a second cascade triggered in the atrioventricular node (AV node), and located between the atria and ventricles in Koch's triangle. Under normal conditions, electrical activity is spontaneously generated by the SA node, which then travels to the AV node.

The trigger for electrical activity is based in depolarization of the cardiomyocyte in the cardio action potential. The action potential travels to all regions of the cell through the t-tubules that causes opening of voltage-gated calcium channels, influx of calcium into the cytosol, and calcium-induced calcium release from the sarcoplasmic reticulum (SR). When calcium is released from the SR and binds to troponin C, the tropomyosin repositions to reveal the cross-bridge binding site on the actin and the myosin head binds to the actin. The myosin head then binds to the actin and slides the myosin past the actin (Huxley, 1969) with the splitting of adenosine triphosphate (ATP), resulting in a contraction.

The myosin movement across the actin filament is known as a power-stroke cycle (Spudich, 2001). This power-stroke cycle occurs through the hydrolysis of ATP and release of energy (Hill, 1974). Briefly, ATP binds to the myosin head and is broken down into adenosine diphosphate (ADP) and phosphate (P). When the binding site for myosin is revealed on actin, the myosin will weakly bind to actin. The P is then released and empowers the power-stroke of the myosin (Goody, 2003). The myosin disconnects from actin and binds to new ATP, which also creates a reversion to the start of a new cross-bridge cycle and contraction. After a delay, the potassium channels then reopen and cause repolarization to the cardiomyocyte. The sarcomere, and ultimately the heart, will then relax as calcium is pumped back into the SR.

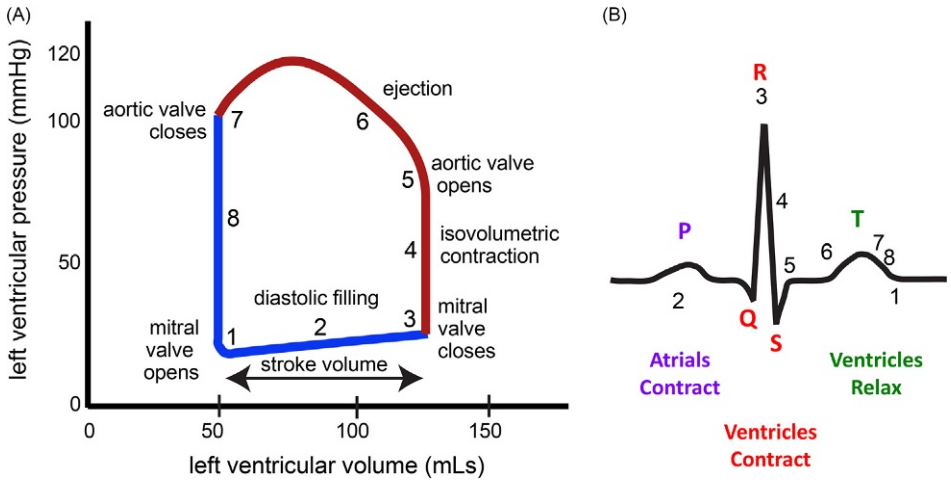

Fig. 1 Pressure–volume relationship of the human heart. (A) The pressure–volume relationship of the left ventricle creates a loop as the heart fills (1–3) and ejects (5–7) blood during a contractile cycle. Systolic pressure (*red*), measured in arteries, increases with inotropy (contractility) of the left ventricle as oxygenated blood is ejected from the heart; diastolic pressure (*blue*), also measured in arteries, occurs between contractility as the left ventricle is empty and filling with blood. Stroke volume is the amount of blood pumped from the left ventricle during a beat. Normal human left ventricular pressure and volume are shown. (B) An electrocardiograph (ECG) trace displays the electrical activity of the heart over a contraction. The numbers correspond to the left ventricle pressure–volume, while the letters represent the normal cardiac electrical deflections toward or away from electromagnetics measuring the electrical activity within the heart, which induces the contraction.

Measuring changes in the left ventricular (LV) volume and the cardiac output (CO) is based on the stroke volume (SV) and heart rate (HR), which is related by:

$$CO = HR^{*}SV$$

The ability of the heart to eject blood, through the SV, at a given afterload and preload describes heart contractility (Katz, 1939). The afterload relates to arterial pressure and affects the end-systolic volume (ESV), and extent of cellular shortening, whereas the preload relates to an end-diastolic volume (EDV). The pressure/volume relationship is described in terms of pressure–volume loop analysis (Fig. 1). The CO is also related to volume as:

$$CO = HR(EDV - ESV)$$

The CO is therefore dependent on the contractility properties of the individual, and aggregate, of the cardiomyocytes. The strength of the systolic contraction is directly proportional to its diastolic expansion as first described by Ernest Starling, stating:

> *The greater the length of the fibre, and therefore the greater amount of surface of its longitudinal contractile elements at the moment when it begins to contract, the greater will be the energy in the form of contractile stress set up in its contraction, and the more extensive will be the chemical changes involved. This relation between the length of the heart fibre and its power of contraction I have called 'the law of the heart'* (Starling, 1920).

Starling essentially stated that the SV of the heart increases in response to an increase in heart's end-diastolic length, which subsequently will increase EDV in a normal heart caused by increase in the blood volume while filling the heart when other factors remain constant. Otto Frank investigated isometric and isotonic contractility of the heart, which was a precursor and contributed to Starling's work.

The Frank–Starling law of the heart is typically graphed with EDV along the X-axis and CO along the Y-axis (Fig. 2). During the filling of the heart with blood, the ventricular walls stretch that leads to a more powerful ejection fraction (EF). The EF is defined as the EDV that is ejected out of the ventricle during a contraction and is mathematically defined as:

$$EF = SV/EDV$$

An increase of blood volume increases the EDV and SV. This length–tension relationship between the fiber length and force produced is typically studied in the laboratory with isolated cardiac cells or myofibrils and can be clinically examined in a number of ways including echocardiogram, magnetic resonance imaging, and computerized tomography.

The amount of active tension developed in cardiac muscle is known to be a function of preload. Active tension is influenced by the number of cross-bridges reacting with actin, which depends on sarcomere length. Changes in preload and sarcomere length also affect active tension by altering sensitivity of troponin C to calcium. The magnitude and rate of active tension is therefore influenced by the preload, which affects inotropy. The changes in inotropy alter the rate of force and pressure development by the ventricle and influence the ejection velocity. The effects of inotropy are described in terms of a force–velocity relationship. The force–velocity relationship describes the relationship between external work and shortening velocity (Katz, 1939; Brutsaert and Sonnenblick,

1969). Essentially, the greater the external load applied on the muscle, the slower the rate of shortening. And likewise, an increase in velocity at any given preload results from increased inotropy and increases force generation by the actin and myosin cross-bridge binding state and turnover.

In review of the structure–function relationship of the heart, it is very evident that the individual cardiomyocytes and myofilaments are the critical components necessary in normal function of the heart.

Cardiomyocyte Structure and Function

Cardiomyocytes are specialized muscle cells with the function of contracting, thus causing the heart to pump blood through the body. Approximately 70% of the proteins within a cardiomyocyte are composed of three types of myofilaments: thick, thin, and elastic (Katz, 2010). The myofilaments are neatly organized within sarcomeres. Sarcomeres are systematically organized in series to form a myofibril and bundles of myofibrils in parallel to create the myocyte. The sarcomere structure is described in terms of lines and bands. The Z-lines (Z-disc) are a dense transverse network that serves as the anchorage site for the thin filaments, as well as the elastic protein titin. Titin extends from the Z-disc, binding to the thick filaments, myosin, to the M-line (middle line). The M-band cross-links myosins and titin. Between the Z-disc and the M-band, and closest to the Z-disc, is the I-band. The I-band (isotropic band) is the zone of thin filaments not interdigitated with thick filaments. The A-band (anisotropic band) is composed of closely packed myosin. Within the A-band is the H-zone, the region where thick filaments are not superimposed by thin filaments. Within the H-zone is the M-line. The thin and thick filament overlap, and their interaction allows for a contraction. During a contraction, the A-bands remain at approximately 1.65 μm in length, and the sarcomere (Z-disc to Z-disc) shortens from approximately 2.2 to 1.7 μm in a beating heart (Katz, 2010) (**Fig. 3**).

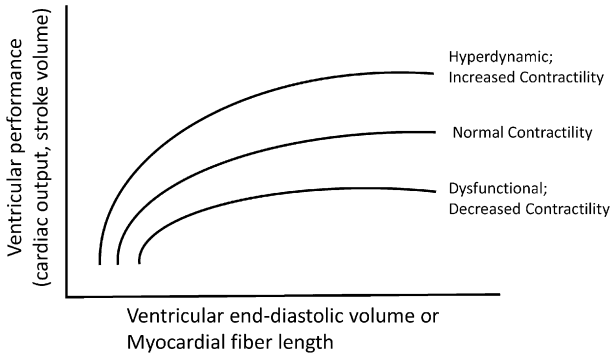

Fig. 2 Frank–Starling law of the heart summarized in graphical representation. As the heart fills with blood and increases the end-diastolic volume, the myofibrils within the cardiomyocyte lengthen and cause a more powerful contraction and ejection fraction.

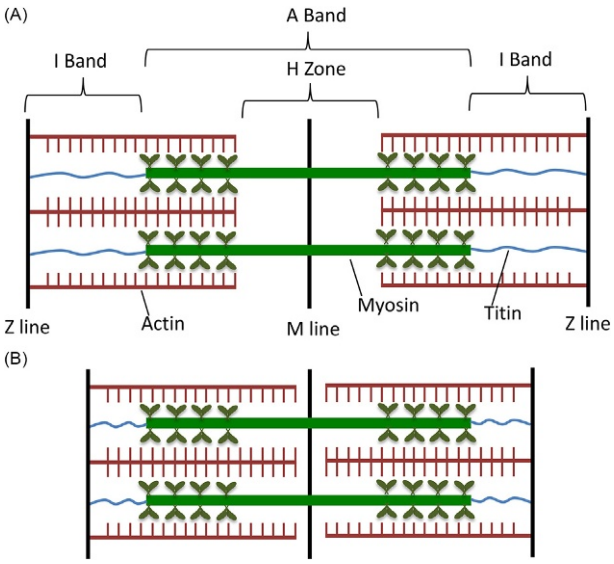

Fig. 3 Schematic of sarcomere in relaxed and contracted state. (A) The sarcomere is relaxed when calcium is not bound to Troponin C in the thin filament. (B) When calcium is bound to Troponin C, the tropomyosin reconfigures along the F-actin allowing for the myosin head in the thick filament to attach to the thin filament and form a cross-bridge. The myosin performs a power stroke and, in the aggregate, creates a contraction within the sarcomere.

The thin filaments in the heart are comprised of F-actin, nebulette, tropomyosin, and the troponin complex. Tropomyosin is positioned in a groove along and between the two actin strands (Solaro, 2011). The troponin complex consists of troponin C, troponin I, and troponin T and is clustered at approximately 400 Å intervals to each other, connecting the ends of the tropomyosin.

The thick filament is an aggregate of individual myosin molecules. Myosin is composed of two heavy chains and two light chains and discussed in terms of a tail and pair of heads. The tail is a coiled coil where the two myosin heavy chains are wound around each other. The heads consist of the globular region of one myosin heavy chain along with the two myosin light chains. The binding affinity between the troponin complex, tropomyosin, and actin is dependent on whether calcium is bound to troponin C. A contraction can proceed when calcium binds to troponin C.

For calcium to bind to troponin C, the depolarization of the cell membrane occurs, triggering an action potential with a less negative value inside the cell membrane compared to the outside. The change in action potential along the cellular membrane is typically modeled with five phases (Carmeliet and Vereecke, 2002). The voltage-gated calcium channels are typically triggered by an influx of sodium, with a potassium equilibrium potential precursory to the sodium influx. The calcium that enters the cardiomyocyte induces calcium release from stored calcium within the SR. Ultimately, calcium then binds to troponin C triggering a contraction.

For a contraction to occur, energy is also necessary. The mitochondria are primarily responsible for providing the energy for cells to function, governed through the synthesis of ATP via the Krebs cycle. Muscle cells, cardiac in particular, require a very large amount and constant supply of ATP. Therefore, cardiomyocytes require a high content of mitochondria, which has been measured as 30% of normal adult cardiac cell volume (Eisenberg, 1983; Piquereau et al., 2013). The constant supply of energy allows for sarcomeres within the cardiomyocyte to contract.

The force generated during the contraction within the cell is transmitted externally via the mechanical linkages from cell-to-cell ends at the intercalated discs. Additionally, force is transmitted laterally by the costameres at the Z-disc located at the peripheral cell membrane (Samarel, 2005). The focal adhesion complex plays an important role in attaching a cell to the extracellular matrix (ECM) (LaNasa and Bryant, 2009; Samarel, 2005). The complex additionally connects myofibrils to the membrane and serves as a mediating site for relaying external and internal forces and intracellular signaling (Kaprielian et al., 2000). The focal adhesion complex is built upon signaling from a focal adhesion kinase (FAK) where phosphorylation initiates a cascade of protein signaling (Samarel, 2005); it is also through FAK that cellular motility is controlled (Mitra et al., 2005). When the complex is mature, the focal adhesion serves as a strong anchorage site and hub for force absorption and transmission within the cardiomyocyte.

To understand cellular mechanics, structural engineering is applicable. Basic structural mechanics relies on three primary aspects to describe a body: the static equilibrium, the stiffness, and the compatibility conditions (Heyman, 2008). The static equilibrium is based on a body's internal structure equilibrium, from forces and moments, with respect to the external load acting on the system. The stiffness is based on the body's elastic deformation properties, such as internal tension and compression, defined by Young's Modulus (stress over strain). This leads to the compatibility conditions, which are the balance between a body's static equilibrium with an external load and its internal stiffness properties, described by surface stress and surface tension. These structural engineering principles apply to all cells but are demonstrated here in cardiomyocytes.

Using advanced imaging and analysis techniques, the cellular mechanics are subdomain regulated (Curtis et al., 2013; Broughton and Russell, 2015), and cardiomyocyte contractility has variation down to the individual sarcomere within the myofibril (Jacot et al., 2008; Chapin et al., 2014). The myofibrils are anchored to the substrate through the combination of focal adhesions, costameres, and ECM ligands (Samarel, 2005; Petit and Thiery, 2000). The substrate represents a static load, and therefore external force, acting against a deformable elastic body, the myocyte. The myocyte will then internally respond to counterbalance this load and stabilize through its own deformation properties within the mechanotransduction proteins, such as the focal adhesion protein paxillin. To explain this concept mathematically, one should first turn to Maxwell's reciprocal theorem that states:

$$\nabla_{ij} = \nabla_{ji}$$

This theorem provides that when an elastic structure is subjected to a force applied in a specific direction at location i, an elastic deflection occurs a distance ji. Similarly, an elastic deflection ij results from a force applied at location j (Yang, 2005; Heyman, 2008). This essentially provides that force symmetry occurs at the matrix of elastic flexibility coefficients. However, as evidenced from line scan and traction force analyses, the contractility properties are unique at each individual point within the cell to balance the internal forces to the external static load. This principle, therefore, leads to the recognition that the myocyte and its contractility are mechanically anisotropic.

Anisotropic elasticity is based on the basic concept that properties are not similar throughout an entire object (Patnaik et al., 1999). In the theory of linear elasticity, stress is a measurement of the intensity of force and measured in force per unit area. Cardiomyocyte function is found to be directionally dependent, and therefore, plane stress is defined in tensor notation, such that:

$$\sigma_{ij} = \begin{bmatrix} \sigma_{xx} & \tau_{yx} & \tau_{zx} \\ \tau_{xy} & \sigma_{yy} & \tau_{zy} \\ \tau_{xz} & \tau_{yz} & \sigma_{zz} \end{bmatrix}$$

The static equilibrium described using tensor notation to define normal and shear stress is evident within cardiomyocytes configured in 2D, with a flat substrate, or 3D, with a microtopography substrate, surface; the static equilibrium is further regulated

by the stiffness of the substrate, which influences the external load. This leads to the discussion of cellular stiffness based on elastic deformation.

The stiffness of the cell is described in terms of the elastic deformation properties and may be described in terms of tension. The active tension in the cell is generated by the interaction between actin and myosin, which is a dynamic system that continually changes during each individual contraction (Granzier and Irving, 1995). Active tension behaves under an oscillating frequency with each cellular contraction and undergoes both a tensile force at the focal adhesion sites and a compressive force in the myofibril centroid during shortening in each contraction. If adjacent myofibrils do not synchronously contract, these asynchronized myofibrils will also experience a shear force during the cycle. This active tension cycle is based on a strain since it is a length change along the myofibril. To mathematically explain this principle, strain is also defined in tensor notation, such that:

$$\varepsilon_{ij} = \begin{bmatrix} \varepsilon_{xx} & \gamma_{yx} & \gamma_{zx} \\ \gamma_{xy} & \varepsilon_{yy} & \gamma_{zy} \\ \gamma_{xz} & \gamma_{yz} & \varepsilon_{zz} \end{bmatrix}$$

Each strain displacement of any point within the system is related by the differential subparts of the normal and shear components of the plane, denoted by:

$$\varepsilon_x = \frac{\partial u}{\partial x},\ \varepsilon_y = \frac{\partial v}{\partial y},\ \varepsilon_z = \frac{\partial w}{\partial z}$$

$$\gamma_{xy} = \frac{\partial u}{\partial y} + \frac{\partial v}{\partial x},\ \gamma_{xz} = \frac{\partial u}{\partial z} + \frac{\partial w}{\partial x},\ \gamma_{yz} = \frac{\partial v}{\partial z} + \frac{\partial w}{\partial y}$$

Whereas u, v, and w are the displacement components of the body, or cell, as described in this model. The use of tensor notation to describe the strain within the cell is also applicable in summation with the passive or resting tension to describe cellular stiffness as total tension (Granzier and Irving, 1995).

Passive or resting tension is a steady-state system that may increase overtime to counterbalance the load acting upon the cell. This passive tension is related by the shortening velocity and the stress development within the cardiomyocyte (Katz, 1939; Granzier and Irving, 1995). This static load in combination with the dynamic load caused from contractility leads to the total tension and force generation with the cell. It is from the balance of forces generated within the cell to respond to the accumulation of internal and external loads that the conditions of compatibility must be satisfied.

In structural mechanics, the balance of external and internal forces of a body is related by three equilibrium equations and three compatibility conditions (Yang, 2005). This balancing of external and internal forces is evident in all cell types including, but not limited to, neurons, adipose, bone, and muscle cells, as cells respond to the forces caused by the stiffness of the surrounding microenvironment (Discher et al., 2005; Hazeltine et al., 2012; Hersch et al., 2013). This balancing and internal response to external cues is so critical that stem cell differentiation can be directed down certain lineages just by modification to the stiffness of the external microenvironment (Engler et al., 2006). The compatibility conditions to describe the balance of forces within a body are exceptionally complex; and Saint-Venant mathematically determined how to solve for the compatibility conditions by relating such conditions to tractions along the boundary of the body (Yang, 2005; Patnaik et al., 1999).

Saint-Venant's principle essentially states that when a localized stress distribution occurs in response to the immediate region of the load, an essentially equivalent stress will occur at a distance from this localized stress to counterbalance the forces (Yang, 2005; Heyman, 2008). It was from the manipulation of complex equations representing conditions of compatibility into easier, solvable terms using tractions along the boundary of a body that this third element of structural mechanics is solved for real-world problems. These tractions define boundary conditions of a three-dimensional body consist of surface stress and surface tension that stabilize the body (Patnaik et al., 1999). Applying boundary conditions to an elastic body, such as a cell, the results are experimentally found using traction force microscopy techniques. Although some labs have referenced a bead displacement as a readout of sarcomere shortening within the cell (Hazeltine et al., 2012; Broughton and Russell, 2015), the line scan is a direct measurement of sarcomere shortening. The bead displacement is better applied to solve for boundary conditions of the cell and defines such balancing of subdomain forces between the cell and the substrate surface.

Surface stress of a solid is defined as the amount of reversible work per unit area to elastically deform a surface (Gurtin and Ian Murdoch, 1978). It is from this balance of force described in terms of surface stress why previous research, correctly, indicated cells demonstrate a stress response to increased substrate stiffness (Discher et al., 2005; Patnaik et al., 1999; Hazeltine et al., 2012; Hersch et al., 2013). To complete the discussion of boundary conditions, the surface tension is complementary to surface stress.

Surface tension of solids is not well understood or scientifically described in comparison to the surface tension of fluids (Style et al., 2013). Surface tension is a measurement of force per unit length or energy per unit area. Historically, surface tensions of solids were not studied in depth because the force generated by surface tension of a solid was considered negligible. However, recent research is demonstrating the importance of surface tension for soft elastomer solids (Style et al., 2013). It is from this combined analysis of the surface stress, forces per unit area, and surface tension, force per distance, that demonstrate the overall cellular compatibility conditions demonstrating a balance of the extrinsic to intrinsic loads imposed upon and within the local microenvironment of the cell.

The intricate details of each protein structure and its function within the cardiomyocyte are critical to the proper functioning of individual contractions within each sarcomere along the myofibrils of each cardiomyocyte and, in the aggregate, the heart. It is when

a primary mechanism—mechanical, chemical, or genetic—strays from the normal state in which a cellular dysfunction occurs, resulting in a cardiac myopathy.

Cardiac Dysfunction

Cardiomyopathies and Heart Failure

Environmental stress, disease, genetics, and age that lead to pathophysiology of heart failure are common in human populations. Some common cardiovascular conditions such as myocardial infarction (MI), valvular heart disease, and systemic hypertension may lead to secondary changes, collectively known as "remodeling," in response to abnormal mechanical forces working against otherwise normal cardiomyocytes. In the aggregate, cardiomyocyte remodeling may lead to alterations in the interstitial matrix, chamber enlargement, abnormalities of systolic and diastolic pump function and myocyte concentric or eccentric hypertrophy, arrhythmia and necrosis (Pfeffer et al., 1991).

Congestive heart failure is most likely caused by ischemia, with earlier reports stating 20% of all failures (Manolio et al., 1992) to more recent reports of 60% (He et al., 2001). Additionally, systemic diseases, such as metabolic derangements, toxin exposures, and genetic disorders, may additionally affect cardiac function resulting in a cardiomyopathy (Felker et al., 2000). These causes leading up to heart failure, particularly in the left ventricle, have myocyte hypertrophy, fibrosis deposition, and LV chamber enlargement. Abnormalities to the systole, diastole, or both functions contribute to the dysfunction leading to such heart failure. These failures can be based on single point mutations within the myofilament proteins (Tartiff, 2005).

When the heart responds to a MI, chamber remodeling occurs. The left ventricle is particularly prone to experience the brunt of remodeling and can undergo immediate contour changes as a result of thinning and elongation of the region affected by the regional ischemia inducing myocardial necrosis. During the critical initial minutes of a MI, when acute ischemia progresses to necrosis, regional systolic dysfunction sets in. Once cell death occurs, particularly if the MI and cell death involved the ventricular apex region, there is likelihood for infarct expansion (Colan et al., 1992). As a whole, infarct expansion causes regional stretching and thinning of the ventricle wall until the connective tissue within the infarcted region provides sufficient resistance to oppose further deformation (Colan et al., 1992). At the individual cardiomyocyte level, the cardiomyocyte will remodel with an increase of sarcomeres in series along the myofibrils to create elongated myocytes due to the stretching of the ventricle wall (Pangonytė et al., 2008).

During ventricle remodeling and expansion as a result of infarction or lack of sarcomeric contractractility, the apex radius increases causing increased wall stress and wall tension on the remaining myocardium, leading to the likelihood of further cavity enlargement and shape distortion (Jugdutt and Michorowski, 1987). This phenomenon, known as dilated cardiomyopathy, can be mathematically described using the Law of Laplace. Using this theory, the wall stress and wall tension increases proportionately to the increased apex radius. The changes in wall thickness of the heart that lead to a myopathy may be hypertrophic, evident by an increase in absolute wall thickness, or dilated, evident by an increase in radius with no change or thinning in wall thickness (**Fig. 4**). As a result there is an increased volume of blood pumped into the left ventricle, causing an increased pressure/volume relationship.

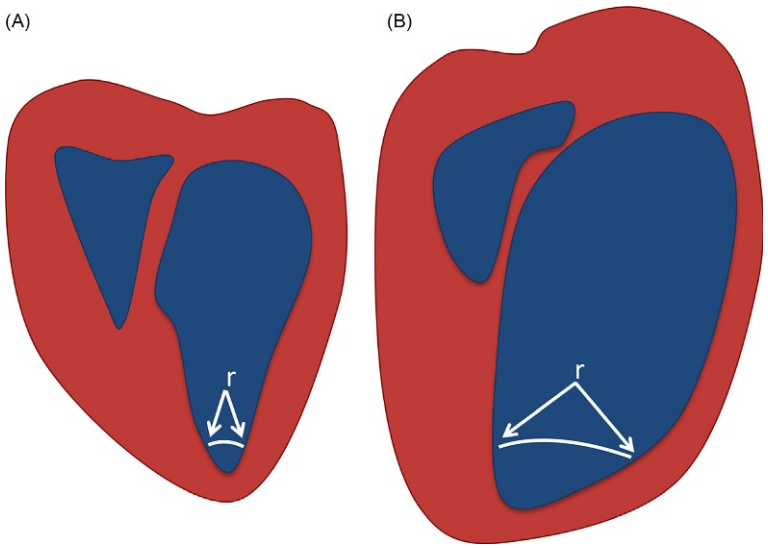

Fig. 4 Interpretive drawing of normal and dilated left ventricle. (A) A normal heart with smaller apex. (B) Infarct expansion due to remodeling affecting the apex region of the left ventricle, resulting in increased apex radius, increased wall stress and wall tension on remaining myocardium and likelihood of further cavity enlargement and shape distortion. Mathematically, using the Law of Laplace, the wall stress and wall tension increases proportionately to this increased radius. Interpretation based on Pfeffer et al. (1991).

It is in the weeks to months after the infarction that the extent of damage caused by the MI can be assessed regarding regional remodeling and months to years to assess the damage of the infarct expansion and the global heart enlargement (Pfeffer et al., 1991; Gaudron et al., 1993).

When a cardiomyopathy is present, the blood supply does not meet the oxygen demand. This failure to eject blood against the load acting upon it relates to the ventricular wall stress (σ) and aortic pressure, or afterload, and therefore cardiomyocyte shortening. The Law of LaPlace essentially states that wall tension (surface tension) is proportionate to the pressure multiplied by radius for thin-walled spheres or cylinders. Transcribing this law in application of the heart, this relationship is known as:

$$T \propto \frac{P \cdot r}{2}$$

where T = ventricular wall tension, P = ventricular pressure, r = ventricular radius. Wall stress is wall tension divided by wall thickness and the geometric shape of the ventricle affects the wall stress, thus wall stress is defined as:

$$\sigma \propto \frac{P \cdot r}{2h} = \frac{T}{h}$$

where σ = ventricular wall tension and h = ventricular wall thickness. Under the assumption of constant pressure, in cases of a thickened wall, or hypertrophied ventricle, the wall stress and afterload are reduced, whereas in the cases of an increased ventricular radius, or dilated ventricle, the wall stress and afterload increase. The increase of aortic pressure, and therefore increase in afterload, causes an ESV increase that leads to a decrease in SV and eventual heart failure and can again be described in terms of pressure volume loops (**Fig. 5**). This tension relationship describes both the hypertrophic and dilated cardiomyopathy heart condition, and cardiac function can be predicted at the cellular level (Davis et al., 2016). Using human-induced pluripotent stem cells derived into cardiomyocytes (hIPS-CMs), researchers can predict if experimental pharmacological treatment serves as a potential therapeutic intervention to treat patients with hypertrophic and dilated cardiomyopathy caused by protein mutations (Matsa et al., 2011; Zhang et al., 2014; Broughton et al., 2016). The mechanics of cardiac function and dysfunction at the organ, cellular, and molecular levels are extremely complex and continue to hold exceptional importance in one of the most relevant human diseases in the 21st century.

Heart Failure Clinical Relevance

In the United States, about 5.1 million people suffer from congestive heart failure, and such failure is a contributing cause of death for one in nine people (Go et al., 2014). The cost of congestive heart failure is an estimated $32 billion every year, which includes the cost of health care services, medications to treat heart failure, and missed days of work (Heidenreich et al., 2011). However, the direct and indirect costs of heart disease per year in the United States total $207.3 billion dollars in 2011–12 and remain the leading cause of death (Mozaffarian et al., 2015). Globally, heart disease is the number one cause of deaths per year, and the trend is expected to continue (Mozaffarian et al., 2015). There is, therefore, a strong need to better understand, prevent, and treat heart disease and heart failure.

The survival after clinical diagnosis of congestive heart failure in the Framingham Heart Study was found to be 3.2 years for men and 5.4 years for women (Ho et al., 1993). For clinicians to classify the severity of the congestive heart failure and better treat the patient, the degree of functional impairment conferred by the abnormality may be utilized as defined by the New York Heart Association (NYHA) Functional Classification system (Fleg et al., 2000). Using the NYHA Functional Classification System, studies have shown that individuals with class IV congestive heart failure, compared to those without any congestive heart failure diagnosis,

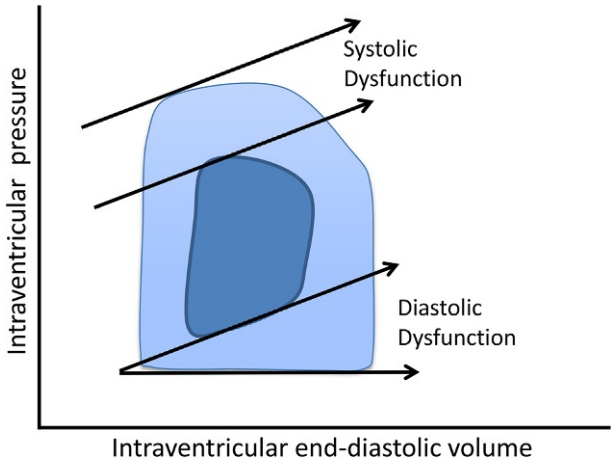

Fig. 5 Pressure–volume relationship of the failing heart. The pressure–volume relationship of the heart is compromised after dilated cardiomyopathy. Inotropy decreases as the pressure volume shifts to the right. Systolic dysfunction occurs when the left ventricle cannot properly contract to eject blood. Diastolic dysfunction occurs when the ventricles do not properly relax, eventually causing stiffness and incapability to fill with blood.

have a 27% survival rate within 3 years of class IV diagnosis (Califf et al., 1982). These example statistics demonstrate the overwhelming burden and need for researchers to study mechanisms leading to systolic and diastolic dysfunction of the heart. It is in further understanding the molecular and cellular form–function relationships that a better appreciation of cardiac mechanics is achieved. With recognizing these small but powerful genetic and environmental influences on the function of the heart, more impactful therapeutic interventions to prevent, diagnose, and treat heart disease will integrate into the healthcare of people.

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Cardiac Fibroblast

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Glossary

Collagen volume fraction Quantification of the area occupied by collagen as percent of total area, commonly determined in tissue sections by staining for fibrillar collagen.
Endomysium Layer of connective tissue surrounding each muscle fiber.
Epicardial-derived cells Cells arising from the embryonic epicardium that include cardiac fibroblasts, vascular smooth muscle cells, and pericytes.
Epicardium Mesothelial covering of the heart that during development contains cardiac fibroblast and coronary vascular smooth muscle cell progenitors.
Fibrocyte Cell that expresses both leukocyte and mesenchymal cell markers.
Fibrosis Excess deposition of extracellular matrix resulting in scarring and thickening of tissue.
Hypertrophy Increase in either heart size or cardiomyocyte size in the absence of proliferation.
Matricellular proteins Secreted proteins that interact with ECM to regulate cell function without directly contributing to the organization of ECM.
MicroRNA A small and highly conserved noncoding RNA that regulates gene expression.
Myofibroblast A fibroblast that expresses contractile actin filaments most commonly identified by smooth muscle actin alpha (αSMA) expression.
Perimysium Layer of connective tissue surrounding each muscle fascicle.
Pressure overload Experimentally, constriction of the transverse aorta to impose hypertrophy and fibrosis in the left ventricle and pathological condition where heart must contract in the presence of excessive afterload.
Reactive fibrosis Deposition of extracellular matrix without significant cardiomyocyte loss.
Remodeling Modification, deposition, and degradation of ECM.
Replacement fibrosis Deposition of extracellular matrix to compensate for the loss of cardiomyocytes most commonly after myocardial infarction.

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Introduction

Fibroblasts are mesenchymal cells that secrete extracellular matrix (ECM) macromolecules. Within the heart ventricles, atria, and septum, the cardiac fibroblast is the cell responsible for ECM production. Under normal physiological conditions, cardiac fibroblasts are thought to regulate ECM homeostasis by producing, degrading, and organizing collagen, processes that are essential for proper heart function. In addition to controlling ECM, fibroblasts secrete cytokines, growth factors, and proteases. Under pathological conditions and during aging, cardiac fibroblasts proliferate, become activated, and produce components of the ECM including collagens and proteoglycans. Collectively, these events are termed cardiac fibrosis. When ECM-producing fibroblasts persist, fibrosis expands and leads to cardiac dysfunction. Cardiac fibrosis occurs in most forms of heart disease and is an important determinant for progression to heart failure (Querejeta et al., 2004; Berk et al., 2007; Hutchinson et al., 2010). Fibrosis remains a major concern of heart disease, and the importance of the fibroblast in cardiac pathology is apparent as we learn more about the functions of this cell. In this article, we summarize current concepts regarding the cardiac fibroblast including their origins and roles in normal heart function and fibrosis. We will also discuss potential therapeutic strategies for reducing fibrosis that target cellular pathways associated with fibroblast activation and ECM remodeling.

Characteristics of the Cardiac Fibroblast

The heart is composed of many different cell types including cardiomyocytes, fibroblasts, smooth muscle cells, pericytes, and endothelial cells (Nag, 1980; Camelliti et al., 2005a). Although much of our current knowledge of the cardiac fibroblast is from in vitro systems and animal models of heart stress and injury, in this section, we focus on the identification and function of the fibroblast in the uninjured heart.

Cardiac Fibroblast Identity

Resident fibroblasts

At the center of many of the uncertainties regarding cardiac fibroblasts is the relative difficulty in clearly distinguishing them from other cell types, such as pericytes and macrophages. Traditionally, cardiac fibroblasts have been defined as cells that are derived from embryonic mesoderm and embedded within the fibrillar collagen matrix of the heart (Kanekar et al., 1998; Chang et al., 2002; Goldsmith et al., 2004). Fibroblasts have a flat spindle-shaped morphology, a highly developed rough endoplasmic reticulum, the abundant cytoplasmic granules, and a heterochromatic, oval nucleus (Nag, 1980; Camelliti et al., 2005a). At the ultra structural level, the main distinction between a fibroblast and a pericyte is that the pericyte resides next to a capillary (Nag, 1980). While characterizing a fibroblast by electron microscopy can be informative, more practical approaches have been adopted for routine identification.

A variety of markers has been used to identify the cardiac fibroblast by immunohistochemistry, in situ hybridization, and flow cytometry, although few of these markers are specific to the cardiac fibroblast (**Table 1**). Identification of the fibroblast can be accomplished by detection of proteins that are enriched in fibroblasts combined with specific markers that permit the exclusion of other cell types. As one of the defining features of a fibroblast, production of collagen types I and III can be used to distinguish fibroblasts from other cells (Weber, 1989; Eghbali et al., 1989a). But because these are secreted proteins, detection is limited to

Table 1 Markers of cardiac fibroblasts

Cardiac fibroblast markers	*Human gene(s)*	*Function*	*Location*
Collagen type I	*COL1A1*, *COL1A2*	Matrix protein	Extracellular
Collagen type III	*COL3A1*	Matrix protein	Extracellular
Discoidin domain receptor tyrosine kinase 2	*DDR2*	Receptor tyrosine kinase for collagen	Surface (low)
Lysyl oxidase, lysyl oxidase homolog 2	*LOX*, *LOXL2*	Collagen cross-linking enzyme	Extracellular
Vimentin	*VIM*	Intermediate filament protein	Intracellular
Platelet-derived growth factor receptor alpha	*PDGFRA*	Receptor tyrosine kinase for PDGF	Surface
Prolyl 4-hydroxylase	*P4HA1*, *P4HB*	Collagen-modifying enzyme	Intracellular
Tcf21	*TCF21*	Transcription factor	Intracellular
Activated cardiac fibroblast/myofibroblast markers	*Human gene*	*Function*	*Location*
Fibronectin ED-A	*FN1*	Matrix protein, alternative splice variant of fibronectin 1	Extracellular
Smooth muscle alpha-actin	*ACTA2*	Actin molecule in contractile cells	Intracellular
Periostin	*POSTN*	Matrix protein	Extracellular

Listed are proteins used to identify fibroblasts. Many are expressed by other cells, such as lymphocytes, smooth muscle cells, and endothelial cells. Detection and specificity may vary from species to species.

either in situ hybridization (Bing et al., 1997; Smith et al., 2011), staining for intracellular collagen pro-proteins (Zhou et al., 2010), or single-cell RNA expression analysis (Kanisicak et al., 2016). Expression of other proteins involved in collagen production and assembly, such as prolyl 4-hydroxylase or lysyl oxidase, not only is used less often but also may identify fibroblast populations (Gonzalez-Santamaria et al., 2016; Yang et al., 2016).

There are several other commonly used fibroblast markers. Vimentin is an intermediate filament protein that can be used for cardiac fibroblast identification (Camelliti et al., 2004b, 2005a,b; Kanisicak et al., 2016). Platelet-derived growth factor receptor α (PDGFRα) is a receptor tyrosine kinase that is expressed by cardiac fibroblasts during development and in the adult heart (Smith et al., 2011; Moore-Morris et al., 2016; Kanisicak et al., 2016; Ieronimakis et al., 2013). Discoidin domain receptor 2 (DDR2), a transmembrane receptor involved in collagen maturation, has also been used to detect cardiac fibroblasts (Goldsmith et al., 2004; Camelliti et al., 2005a; Vogel et al., 1997) but is also expressed by leukocytes (Goldsmith et al., 2004). Other markers that have been used to identify cardiac fibroblasts include surface antigens recognized by MEFSK4 (Pinto et al., 2016), Thy1 (CD90) (Ali et al., 2014), Sca1 (Ieronimakis et al., 2013), and Fsp1 (Zeisberg et al., 2007). These markers not only may identify a subset of cardiac fibroblasts but also are expressed by immune and/or endothelial cells (Pinto et al., 2016; Holmes and Stanford, 2007; Raff, 1971; Jurisic et al., 2010; Kong et al., 2013).

Activated fibroblasts and myofibroblasts

Smooth muscle alpha-actin (αSMA) is the definitive marker for the activated cardiac fibroblast population known as the myofibroblast (Frangogiannis et al., 2000), but this actin isoform is also abundant in vascular smooth muscle cells (Skalli et al., 1989). Upon activation, fibroblasts switch fibronectin isoform usage to the extra domain A isoform, and there are antibodies that can bind specifically to this isoform (Santiago et al., 2010; Serini et al., 1998). More recently, periostin expression has been demonstrated to be uniquely upregulated in fibroblasts responding to heart injury (Kanisicak et al., 2016; Kaur et al., 2016).

In the mouse, genetic tools have been developed to follow fibroblasts (reviewed in Swonger et al., 2016). Genetically engineered mice using cis-regulatory elements for collagen 1a1 (Yata et al., 2003), 1a2 (Zheng et al., 2002), PDGFRα (Hamilton et al., 2003), Tcf21 (Acharya et al., 2012), and periostin (Kanisicak et al., 2016; Kaur et al., 2016; Takeda et al., 2010) provide means for identifying and tracing the cardiac fibroblast lineage in vivo (see **Fig. 1**), but use of these mouse lines requires additional breeding and can be time-consuming.

As characterization of the cardiac fibroblast improves, identification of additional fibroblast-specific genes is likely to occur. Until then, the use of multiple markers is advised to distinguish fibroblasts from other cellular populations in the heart.

Cardiac Fibroblast Number

Early studies using transmission and scanning electron microscopy determined that nonmyocytes constitute 70% of the cells in adult rat heart (Nag, 1980; Zak, 1974). These studies used location and morphology to classify the cell types and did not report the relative number of fibroblasts. Later studies using flow cytometry and histology comparing fibroblast content in mouse and rat hearts found that the relative numbers of cardiomyocytes and nonmyocytes differ depending on the species, age, gender, and fibroblast markers used (Banerjee et al., 2007; Moore-Morris et al., 2016; Camelliti et al., 2004a). Recently, studies in the mouse and

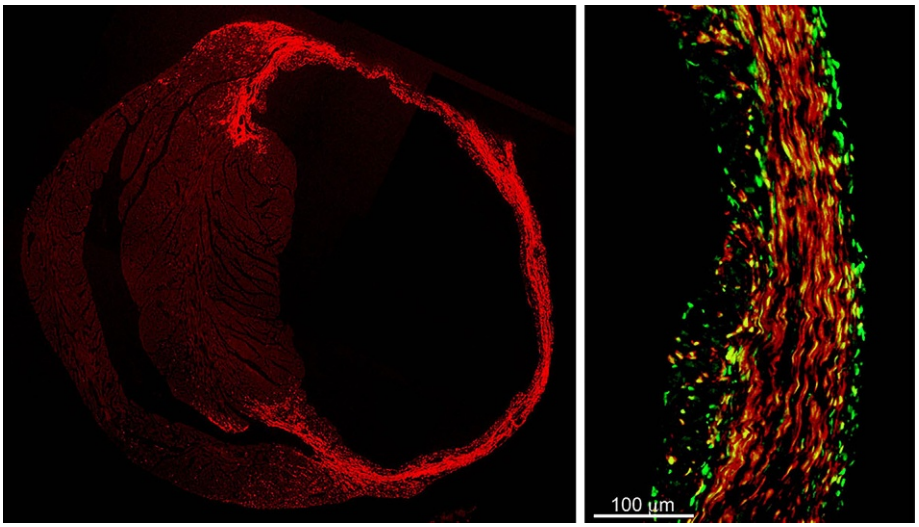

Fig. 1 Fibroblast accumulation after myocardial infarction. *Left panel*, transverse section through infarct region of a murine heart after permanent ligation of the left coronary artery. *Bright-red staining* identifies activated fibroblasts responding to the injury. *Right panel*, higher magnification view of *infarct region green* identifies the nuclei of PDGFRα-positive fibroblasts, and *red* identifies activated fibroblasts that express periostin.

human have refined our understanding of the relative numbers of fibroblasts in the heart. Using a combination of flow cytometry, histology, and reporter mouse lines to identify resident fibroblast populations, the number of fibroblasts within the mouse heart was found to be 10%–12% (Pinto et al., 2016), a number that is substantially lower than previous estimates (Banerjee et al., 2007). Similar analyses in the human heart, using immunohistochemistry and flow cytometry to identify cardiomyocytes and endothelial cells, estimated that the percent of mesenchymal cells ranges from 20% to 58% depending on the identification method (Pinto et al., 2016; Bergmann et al., 2015).

Cardiac Fibroblast Distribution

Despite the proposed role for fibroblasts in maintaining the ECM of the heart, details are lacking regarding the location and dynamics of the fibroblast population. Most studies have investigated regional fibroblast density after heart injury, but few studies have examined fibroblast location in the healthy heart, possibly due to inability to clearly distinguish fibroblasts from other cell populations. In the heart, fibroblast density varies depending on the region. The highest concentrations of fibroblasts can be found in the valves, annulus fibrosus (Opthof et al., 1987), and adventitia of the coronary arteries. Typically, the fibroblast population within the valves is referred to as valve interstitial cells or valvular fibroblasts. Due to their anatomical location, these cells are unlikely to play a role in ventricular fibrosis. However, some heart dissociation protocols for isolation and culture of cardiac fibroblasts may include contamination by this cell population. The densely organized fibroblasts found around the sinoatrial node (Shiraishi et al., 1992; Keith and Flack, 1907) and in the annulus fibrosus are essential for insulating the electric impulses of the conduction system (Kolditz et al., 2008). In coronary vessels, fibroblasts comprise the majority of the cells in the collagen-rich adventitia, but several other cell populations also reside in this space (reviewed in ATVB Majesky, 2015).

Little is known about the relative density and distribution of interstitial cardiac fibroblasts. Located between muscle fibers, these solitary cells are spindle shaped with long filopodia (Goldsmith et al., 2004) that contain no stress fibers (Tomasek et al., 2002). Connexin-40 positive gap junctions can be observed between cardiac fibroblasts (Camelliti et al., 2004b). One report showed fibroblasts in close apposition to the heart capillary network (Bowers et al., 2010), but a distinction between fibroblast and pericyte was not made. A population of fibroblasts has also been described that is tightly associated with cardiomyocyte fibers (Nag, 1980), and occasionally, connexin-45 positive gap junctions have been observed between fibroblasts and cardiomyocytes (Camelliti et al., 2004b).

Cardiac Fibroblast Developmental Origins

Resident cardiac fibroblasts arise from two developmental sources, the epicardium and the endocardial cushions. Classic studies in the avian system used fate mapping to show that many fibroblasts originate from the proepicardial organ. These progenitor cells migrate over the heart tube forming the embryonic epicardium. Between embryonic days 12 and 14, a subset of these cells invades the atrial and ventricular myocardium after undergoing an epithelial-to-mesenchymal transition (EMT). Epicardial-derived cells differentiate into vascular smooth muscle cells and fibroblasts that are interspersed throughout the myocardium and also surround the coronary vessels (Perez-Pomares et al., 2002; Snider et al., 2009; Gittenberger-de Groot et al., 1998; Mikawa and Gourdie, 1996; Dettman et al., 1998). More recently, several groups have identified a population of fibroblasts arising from the endocardial cushions. These endocardial-derived fibroblasts constitute a portion of fibroblasts that reside in the ventricular septum and right ventricle (Moore-Morris et al., 2014; Ali et al., 2014). Despite distinct developmental origins, these two fibroblast populations have similar gene expression patterns and proliferation rates after pressure overload (Moore-Morris et al., 2014; Ali et al., 2014).

While many genes play crucial roles in epicardial formation and EMT (Krainock et al., 2016; von Gise and Pu, 2012), only two genes have been identified that are required specifically for ventricular fibroblast formation. When PDGFRα expression is disrupted in epicardial cells during embryonic development, ventricular fibroblasts fail to develop (Smith et al., 2011; Rudat et al., 2013; Acharya et al., 2012). Similarly, hearts lacking the bHLH transcription factor, Tcf21, have a deficiency in cardiac fibroblasts (Acharya et al., 2012). The importance of these two genes was observed in the epicardial-derived cardiac fibroblasts, but it remains to be determined if the endocardial-derived fibroblast population is also disrupted in the absence of these genes.

Additional Putative Sources of Fibroblasts

In addition to the two resident fibroblast populations, several other cell types have been identified that contribute to the fibrotic process after injury. These sources include pericytes (Kramann et al., 2015; Henderson et al., 2013), epicardial cells (Zhou and Pu, 2011; Duan et al., 2012; van Wijk et al., 2012), endothelial cells (Zeisberg et al., 2007; Widyantoro et al., 2010), and fibrocytes or circulating hematopoietic cells (van Amerongen et al., 2008a; Haudek et al., 2006, 2010; Bucala et al., 1994; Sopel et al., 2012; Lei et al., 2013; Mollmann et al., 2006). The estimated participation of each of these cell populations varies from 3% to 10% depending on the fibroblast source, injury type, and method of quantification (Zeisberg et al., 2007; Haudek et al., 2006; Crawford et al., 2012). Although it is definite that cells other than resident fibroblasts play important roles in the fibrotic process, it is less clear if all of these sources of fibroblasts have a significant role in ECM production after injury. Several recent studies using parabiosis, bone marrow chimeras, lineage tracing, and single-cell RNA sequencing have suggested that resident cardiac fibroblasts account for the majority of ECM production in the heart after injury (Ali et al., 2014; Moore-Morris et al., 2014; Ruiz-Villalba et al., 2015; Banerjee et al., 2007; Guimaraes-Camboa et al., 2017; Kanisicak et al., 2016).

Cardiac Fibroblast Function

Function During Homeostasis

The heart, unlike other organs, is in a constant state of relaxation and contraction, and the ECM provides structural integrity and scaffolding for cardiomyocytes, ensuring appropriate distribution of mechanical forces throughout the cardiac tissue. As cardiac fibroblasts are the major source of ECM components, it has been postulated that they monitor and regulate ECM by balancing ECM synthesis and degradation (Butt et al., 1995; Atance et al., 2004; Kandalam et al., 2011; Spinale, 2007; Petrov et al., 2002). Collagens (I, III, IV, V, and VI) constitute the majority of proteins in the space between the myofibers, with types I and III comprising 90% of the ECM content (Bashey et al., 1992b; Medugorac and Jacob, 1983; Weber, 1989). In the healthy heart, fibroblasts maintain a relatively slow turnover of fibrillar collagen (Bonnin et al., 1981; Laurent et al., 1978). Collagen fibers form by cross-linking facilitated by lysyl oxidases (LOXs) (Reiser et al., 1992; Robins, 2007). In addition to collagens, fibroblasts make a great number of other proteins required for ECM organization and cardiac signaling including fibronectin, elastin, laminins, fibrillins, glycoproteins, and proteoglycans (Speiser et al., 1992).

Fibroblasts reportedly control other essential processes such as angiogenesis, vascular homeostasis, and cardiomyocyte proliferation (Ieda et al., 2009; Zhao and Eghbali-Webb, 2001; Ubil et al., 2014). One way that fibroblasts communicate with cardiomyocytes, endothelial cells, and immune cells is by secretion of growth factors and cytokines. Fibroblast secretion of the proangiogenic factors fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) stimulates blood vessel formation upon injury (Murakami and Simons, 2008; Lavine et al., 2006). Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) produced by fibroblasts also modulate neutrophil–endothelial cell adhesion and angiogenesis (Fernandez-Patron et al., 2001; Qi et al., 2003; Nikolova et al., 2012). Fibroblast-derived transforming growth factor-beta (TGF-β) and interleukin-6 (IL-6) signaling have been implicated in the regulation of myocardial function in both healthy and diseased hearts (Dobaczewski et al., 2011; Fredj et al., 2005). During development, fibroblasts secrete matrix molecules that activate beta-1 integrin on cardiomyocytes and regulate their proliferation (Ieda et al., 2009). As identification and classification of fibroblasts become more precise, so will our understanding of the basal functions of the resident cardiac fibroblast.

Function After Injury

While relatively few studies focus on the homeostatic role of the cardiac fibroblast, there is a significant amount of information regarding cardiac fibroblast behavior after injury. In general, fibrosis is divided into two categories, replacement or reparative fibrosis and reactive fibrosis. The former describes the process where ECM is deposited by cardiac fibroblasts to compensate for cardiomyocyte loss. Reactive fibrosis, on the other hand, is located in both the interstitium and adventitia and is generally not associated with immediate cardiomyocyte death. Fibroblasts undergo a phenotypic transition from a resting state to an activated state, predominantly characterized by increased ECM production. This activated fibroblast is often referred to as a myofibroblast, but recent data have suggested that identification of the myofibroblast using αSMA may underestimate the number of fibroblasts participating in the remodeling process (Moore-Morris et al., 2014). Other studies also suggest that cardiac fibroblasts can acquire additional distinct phenotypes described as proinflammatory and matrix-degrading phenotypes during the early inflammatory phase. In vitro studies suggest that interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and oncostatin M may regulate the acquisition of distinct fibroblast phenotypes (Turner et al., 2009; Lafontant et al., 2006; Siwik et al., 2000).

Fibrotic remodeling permits the heart to adapt to mechanical, metabolic, and inflammatory stress, and the activated cardiac fibroblast has three main tasks: proliferation, secretion of growth factors and cytokines, and ECM deposition and remodeling. The location and duration of these activities depend on the underlying cause of fibrosis. Growth factors and cytokines (e.g., TNF-α, IL1-β, TGF-β, and platelet-derived growth factor (PDGF)), as well as mechanical stretch, have a demonstrated capacity to activate cardiac fibroblasts (Awad et al., 2010; Tyagi et al., 1998; Atance et al., 2004; Dobaczewski et al., 2011; Smith et al., 2011; Zhao et al., 2011; Ponten et al., 2005). Secreted factors, including profibrotic factors (TGF-β, angiotensin II, and endothelin-1 (ET-1)) (Leask, 2010; Yamamoto et al., 2000; Kulasekaran et al., 2009), mast cell-specific proteases (tryptase and chymase) (Zhao et al., 2008; Lu et al., 2012; Somasundaram et al., 2005), and PDGFs (Zymek et al., 2006), are also associated with regulation of fibroblast activation.

Fibroblast proliferation

Signaling pathways that direct fibroblast proliferation have been identified using both in vitro and in vivo methods. Some of the best understood cardiac fibroblast mitogenic signals occur after activation of G protein-coupled receptors, including β-adrenergic and angiotensin type I (AT1) receptors (McEwan et al., 1998; Turner et al., 2003; Schorb et al., 1993; Samuel et al., 2004). Receptor tyrosine kinase activation by PDGFs, insulin-like growth factor, and FGFs also regulates fibroblast proliferation (Ponten et al., 2005; Zhao et al., 2013; Yi et al., 2014; Samuel et al., 2004; Virag et al., 2007; Gallini et al., 2016; Olson and Soriano, 2009). Inflammatory cytokines have variable effects on fibroblast proliferation. For example, the proinflammatory cytokine IL-1β reduces cardiac fibroblast proliferation in vitro (Palmer et al., 1995). The role of IL-6 on fibroblast proliferation is less clear. Perinatal fibroblasts in IL-6 null hearts exhibit increased proliferation (Banerjee et al., 2009), while inhibition of IL-6 in vitro leads to decreased cardiac fibroblast numbers (Fredj et al., 2005). Interestingly, fibroblast proliferation is regulated not only by secreted factors but also by altered hormone production, such as relaxin and aldosterone (Samuel et al., 2004; Rombouts et al., 2001).

Inflammation

A delicate interplay exists between cardiac fibroblasts and immune/inflammatory cells. Regardless of the event initiating fibrosis, there are some common attributes of the fibroblast's role in inflammation (reviewed by Frieler and Mortensen, 2015; Epelman et al., 2015; Van Linthout et al., 2014). While it is assumed that fibroblasts secrete some proinflammatory cytokines after injury, often, the cellular source of cytokine production is not clearly delineated. The paucity of information regarding fibroblast mediation of inflammation is due to the lack of reliable means for identifying the fibroblast from other cell types in vivo. Nonetheless, there is some information regarding the role of the fibroblast during the inflammatory stage of heart injury. Studies using ischemia/reperfusion and myocardial infarction (MI) models of injury have shown that inflammasome activation in fibroblasts is essential for the initial inflammatory response (Kawaguchi et al., 2011; Sandanger et al., 2013). Cardiac fibroblasts activated in vitro express neutrophil-recruiting chemokines (CXCL1, CXCL2, and CXCL5) and other proinflammatory cytokines (IL1β, IL6, and TNFα) (Lafontant et al., 2006; Turner et al., 2009). In addition to having direct effects on inflammatory cells, some fibroblast activities occur indirectly by their influence on other cell populations (Fernandez-Patron et al., 2001). The field is still in its infancy regarding our understanding of cardiac fibroblast communication with inflammatory cells, and future studies are likely to reveal important insights into this topic.

Matrix remodeling

ECM components play a pivotal role in the remodeling process. One of the hallmarks of an activated fibroblast is the abundant secretion of collagens, resulting in scar formation or fibrosis. In the case of acute MI, the collagen network is essential for replacing lost cardiomyocytes and preventing ventricular rupture (Davis et al., 2015; van Amerongen et al., 2007; Schellings et al., 2009). During cardiac remodeling, resting or quiescent fibroblasts become activated by multiple factors produced in response to cardiomyocyte injury and inflammation (Siwik et al., 2000; Awad et al., 2010; Kulasekaran et al., 2009; Kanisicak et al., 2016). Not only cardiac fibroblasts produce the majority of collagen following injury (Petrov et al., 2002; Bashey et al., 1992a; Brilla et al., 1994), but also they modify ECM architecture by catalyzing collagen cross-linking through the action of LOX and lysyl oxidase-like 2 (LOXL-2) proteins (Yang et al., 2016). Fibroblast-produced MMPs and TIMPs also balance degradation of ECM permitting movement of cardiac fibroblasts and immune cells into the site of injury (Turner et al., 2010; Chiao et al., 2012; Spinale, 2007; Cleutjens et al., 1995; Kandalam et al., 2010). It is important to note that while cardiac fibroblasts do upregulate expression of MMPs after activation, many other cell types also produce these proteases (Saxena et al., 2013).

Matricellular proteins are components of the ECM that act as biological mediators of cell function. One matricellular protein that regulates cardiac fibrosis is periostin. Periostin expression in fibroblasts is increased in the infarct area after MI (Liu et al., 2012), and periostin-deficient mice showed reduced fibrosis after acute MI due to reduction of the recruitment of activated fibroblasts (Shimazaki et al., 2008; Oka et al., 2007). It has been suggested that one action of periostin is to promote cardiac repair via integrin-mediated induction of cardiomyocyte proliferation (Shimazaki et al., 2008). These results suggest that periostin contributes to cardiac repair, but the role of periostin during chronic remodeling is less clear.

TGF-β is one of the key mediators of the fibrogenic response. TGF-β leads to cardiac fibroblast activation potentially mediated by integrins αvβ5 and αvβ3 or PDGF-D as shown in vitro (Zhao et al., 2013; Sarrazy et al., 2014). In vivo work also suggests a pivotal role for TGF-β in regulation of fibrosis. TGF-β1 secretion is increased quickly in response to heart injury. TGF-β1-overexpressing mice exhibited significant cardiac hypertrophy and interstitial fibrosis (Rosenkranz et al., 2002). Moreover, the loss of Smad3, a downstream effector of TGF-β, resulted in reduced interstitial fibrosis in the remote myocardium in hearts but had no defect in resolution of inflammation in healing infarcts compared with their wild-type counterparts (Bujak et al., 2007).

Causes of Fibrosis

In the heart, there are multiple pathological processes that result in fibrosis. In most cases, fibrosis is not the primary disease, but a secondary response to tissue stress and inflammation. Later, we describe the heart pathologies that can culminate in fibrosis and focus on the actions of the fibroblast in fibrotic progression.

Myocardial infarction

MI is a frequent cause of cardiac injury. Following MI, a large number of myocardial cells die due to ischemia. Because there is negligible regenerative capacity in the adult heart, the loss of cardiomyocytes triggers a reparative response in which the dying cells are replaced with a fibrotic scar (Frangogiannis, 2006; van den Borne et al., 2010). This scar formation is driven by deposition of fibrin, which acts as a provisional matrix, providing a foundation for invading inflammatory cells and cardiac fibroblasts that are involved in repair of damaged tissue (Greiling and Clark, 1997). These fibrin scaffolds are eventually degraded and ultimately a scar is formed that covers up to 80% of the infarct area (reviewed by Turner and Porter, 2013; Dobaczewski et al., 2006; Sun and Weber, 2000).

Following MI, a number of secreted factors influence cardiac fibroblast and inflammatory cell responses (Briaud et al., 2001; Frangogiannis, 2012; Arslan et al., 2011; Timmers et al., 2012). Proinflammatory cytokines (TNF-α, TGF-β, and IL-6) and vasoactive peptides (ET-1 and natriuretic peptides) are increased in the myocardium (Ma et al., 2012). The recruited immune cells clear damaged and dying cells, while cardiac fibroblasts produce MMPs that degrade ECM and permit migration of cells into the infarct area (Dobaczewski et al., 2006; Li et al., 2002).

Increased production of TNF-α, IL-6, and VEGF after MI also promotes fibroblast activation and collagen synthesis (Saini et al., 2005; Taimeh et al., 2013; Wang et al., 2016a). The injury area expands, and over time, the infarct zone begins to thin. This replacement fibrosis prevents cardiac rupture after the ischemic insult but may become maladaptive. Sustained collagen deposition combined with increased cross-linking disturbs tissue architecture leading to ventricular wall stiffening, hypertrophy of the remaining cardiomyocytes, and potentially resulting in heart failure.

Experimentally, molecular mechanisms of cardiac remodeling after MI have been studied in mouse and rat injury models focused on the left ventricle through cryoablation (van den Bos et al., 2005; van Amerongen et al., 2008b), ligation of the left anterior descending artery (Eberli et al., 1998; Johns and Olson, 1954), and ischemia–reperfusion (Nossuli et al., 2000).

Pressure overload/hypertension

Pressure overload results in hypertrophy of the left ventricular wall with little or no change in chamber size (Norton et al., 2002; Weber et al., 1988). It can be caused by hypertension, aortic stenosis, or coarctation of the aorta. Chronic pressure overload causes excessive collagen synthesis and myocardial thickening (Thiedemann et al., 1983; Jalil et al., 1988) through the activation of profibrotic growth factors (TGF-β, angiotensin II (Ang II), ET-1, and aldosterone) (Hutchinson et al., 2010; Creemers and Pinto, 2011; Koitabashi et al., 2011; Senbonmatsu et al., 2000; Yorikane et al., 1993).

Hypertensive heart disease represents two key pathological processes, cardiomyocyte hypertrophy and accumulation of fibrotic tissue within the cardiac interstitium (Fagard, 1992; Berk et al., 2007; Querejeta et al., 2004). Hypertensive patients have increased collagen volume fraction of the myocardium compared with their normotensive counterparts (Querejeta et al., 2004; Kasner et al., 2011). The severity of fibrosis in hypertension disease may vary. With mild hypertension, increased collagen deposition in the endomysium and perimysium and cardiomyocyte hypertrophy are observed, although ECM structure is preserved. However, severe hypertension leads to cardiomyocyte cell death and replacement fibrosis resulting in expanded focal areas of disorganized ECM (Olivetti et al., 1994; Rossi, 1998).

Experimentally, thoracic banding achieves pressure overload. Infusion of Ang II/phenylephrine, isoproterenol, or aldosterone can also initiate hypertension-mediated cardiac fibrosis in rodent models (Hasenfuss, 1998). Both hemodynamic (pressure overload) and nonhemodynamic factors (TGF-β, Ang II, ET-1, and aldosterone) induce fibrosis in hypertensive disease (Berk et al., 2007; Kahan, 1998; Ganau et al., 1990). Experimental models have shown that recruitment and activation of macrophages through T-cell responses act as critical modulators for hypertension and reparative fibrosis (Ma et al., 2014; Laroumanie et al., 2014).

Aging

In the absence of MI and pressure overload, the aged heart exhibits alterations in collagen and ECM deposition (Eghbali et al., 1989b; Capasso et al., 1990). Aging is associated with left ventricular hypertrophy and fibrosis, ultimately leading to heart failure. The state of normal systolic function combined with diastolic dysfunction has been termed heart failure with preserved ejection fraction (HFpEF), and aging is considered one factor in the HFpEF epidemic (Aronow et al., 1998; Bryg et al., 1987; Loffredo et al., 2014). Common features of aging-associated heart failure are the loss of cardiomyocytes and increased fibroblast-mediated ECM remodeling (Olivetti et al., 1991; Horn et al., 2012) resulting in reduced pump function and ventricular wall stiffening (Melissari et al., 1991; Horn and Trafford, 2016). One mechanism to explain this fibrosis is that cardiomyocytes become more susceptible to mitochondrial permeability transition pore (mPTP)-triggered cell death leading to replacement fibrosis (Di Lisa et al., 2001; Morita et al., 2009). Also, increased oxidative stress from dysfunctional mitochondria leads to reactive oxygen species and fibrosis (Dai et al., 2011). Analyses of human hearts have suggested that reactive fibrosis is common in the aged heart (Jellis et al., 2010; Liu et al., 2013). Studies have suggested that in the aging heart, ECM disorganization and matricellular protein changes contribute more to the disease state rather than overall increases in collagen levels (Annoni et al., 1998; Thomas et al., 2000; McCurdy et al., 2010; Bradshaw et al., 2010; de Castro Bras et al., 2014; Horn, 2015; Chiao et al., 2012).

Diabetes mellitus

Cardiac fibrosis has been documented in both type I and II diabetes mellitus (Turkbey et al., 2011; Shimizu et al., 1993; Sutherland et al., 1989). Histological analysis has demonstrated that cardiac fibrosis in diabetic patients occurs independent of coronary atherosclerosis or hypertension and is accompanied by cardiomyocyte hypertrophy and microvascular abnormalities (Rubler et al., 1972; Gherasim et al., 1985). Fibrosis in diabetes is characterized by excessive accumulation of collagen types I and III in both left and right ventricles (Shimizu et al., 1993).

Type I diabetes models have shown interstitial myocardial fibrosis with cardiomyocyte hypertrophy, induction of profibrotic factors, and microvascular rarefaction (Ares-Carrasco et al., 2009; Black et al., 2010; Shiomi et al., 2003). Also, type II diabetes models have shown fibrosis with cardiomyocyte hypertrophy and diastolic dysfunction (Daniels et al., 2012; Huynh et al., 2012). Hyperglycemia leads to accumulation of advanced glycation end products that cross-link ECM proteins in both the intracellular and extracellular space (Cooper, 2004; Liu et al., 2003; Brownlee et al., 1986). In addition, hyperglycemia stimulates cardiac fibroblast proliferation and activation in vitro (Shamhart et al., 2014; Aguilar et al., 2014; Fowlkes et al., 2013), but the roles of hyperglycemia in fibrotic remodeling in vivo remain elusive. Angiotensin signaling is associated with diabetes-mediated fibrosis, which is accompanied by increased TGF-β-Smad signaling, accentuated TGF-β responses and generation of ROS to induce fibroblast activation (Brown et al., 1997; Zaman et al., 2001; Vazquez-Medina et al., 2013). Also, increased systemic levels of proinflammatory cytokines and chemokines, such as TNF-α and IL-1β, contribute to accelerate fibrosis development in diabetes (Suzuki et al., 2015; Westermann et al., 2007).

Therapeutic Targets

Because there are few therapies directed specifically at fibroblasts and their activities, there is a broad opportunity for the development of new clinical interventions targeting the fibroblast. Later, we describe a few examples of potential treatments that may reduce fibrosis.

Growth Factors

TGF-β

The role of TGF-β signaling in fibroblast proliferation and matrix production is an intensely studied topic. Many studies indicate a crucial role of TGF-β in the pathogenesis of cardiac fibrosis and hypertrophic remodeling (Dobaczewski et al., 2011; Bujak and Frangogiannis, 2007; Koitabashi et al., 2011). In vitro studies have demonstrated that TGF-β stimulation induces fibroblast conversion to myofibroblasts and enhances ECM protein synthesis and deposition (Petrov et al., 2002; Desmouliere et al., 1993). Treatment of soluble TGF-β receptor in vivo has either protective or detrimental effects in MI depending on the time point of TGF-β signaling blockage after MI (Okada et al., 2005; Ikeuchi et al., 2004). Moreover, a small molecule inhibitor of ALK5 (a TGF-β type I receptor) attenuated cardiac fibrosis after pressure overload and MI (Tan et al., 2010; Engebretsen et al., 2014).

Angiotensin II

Studies have demonstrated that Ang II promotes cardiomyocyte hypertrophy (Gray et al., 1998; Schultz Jel et al., 2002) and enhances ECM protein synthesis (Lijnen et al., 2000; Brilla et al., 1994, 1997). Ang II upregulates TGF-β expression through the AT1 receptor in cardiomyocytes and fibroblasts, indicating that TGF-β acts downstream of Ang II (Gao et al., 2009). Administration of angiotensin receptor blockers, such as losartan, results in reduced fibrosis both in cardiac experimental injury models and in patients (Shibasaki et al., 2005; Diez et al., 2002; Lim et al., 2001). Although manipulation of renin–angiotensin signaling may have direct influences on fibroblast behavior, this pathway functions in many cell type; and identification of more specific means for targeting this pathway in fibroblasts would be a significant advance.

MicroRNAs

It has become clear that cardiac microRNAs (miR) actively participate in different aspects of cardiac remodeling, such as cell proliferation, apoptosis, and fibrosis, and miR has become attractive targets for therapeutic interventions (van Rooij and Olson, 2012). Evidence is accumulating that multiple miRs function in cardiac fibrosis, and studies have demonstrated that manipulation of these miRs can impact fibroblast behavior during cardiac fibrosis. Due to their ability to regulate several proteins and pathways at once, manipulation of miR activity by injecting either stable miR analogs or miR inhibitors called antagomirs or antimiRs has become an active area of drug discovery.

Connective tissue growth factor (CTGF) has emerged as an important modulator of fibrotic responses in tissues and acts as a downstream mediator that enhances the fibrogenic action of TGF-β in fibroblasts (Ahmed et al., 2004; Chen et al., 2000; Chujo et al., 2005). It has been reported that cardiac miR, miR-30, (cardiomyocytes and cardiac fibroblasts), and miR-133 (cardiomyocytes) regulate CTGF, and expression level of these miR is negatively correlated with CTGF levels, collagen production, and fibrosis. These results suggest that these two miRs may act as negative regulators of fibrosis and could therefore be useful in broadly altering fibrotic responses (Duisters et al., 2009). miR-21 is upregulated in cardiac fibroblasts after injury (van Rooij et al., 2008), and in vivo silencing of miR-21 in a pressure overload model attenuates fibrosis and heart failure via suppression of ERK–MAPK signaling (Thum et al., 2008). Recent findings also show that overexpression of miR-9 attenuates cardiac fibrosis via PDGFR-β-mediated cardiac fibroblast proliferation and collagen synthesis (Wang et al., 2016b; Zhang et al., 2011). miR-101a can modulate TGF-β signaling, suggesting another potential avenue for fibrosis regulation (Zhao et al., 2015; Pan et al., 2012). miR-24 expression is correlated with fibrosis in hypertrophic hearts, and intramyocardial injection of miR-24 improves heart function and attenuates fibrosis after MI (Wang et al., 2012). miR-1 attenuates pathological remodeling and cardiac hypertrophy in pressure overload model (Karakikes et al., 2013). Taken together, these findings point to the manipulation of miRNAs as novel therapeutic targets during cardiac fibrosis.

Lysyl oxidase

Tissue stiffness is dependent not only on the abundance of collagen but also on the amount of collagen cross-linking (Herrmann et al., 2003; Capasso et al., 1989; Iimoto et al., 1988; Lopez et al., 2010). Thus, strategies for modulation of matrix stiffness are being considered as a therapeutic target for cardiac fibrosis. LOX activity is associated with fibrosis in multiple organs, and increased levels of LOX have been reported in patients with hypertensive and chronic heart disease. Higher LOX expression is observed in areas of interstitial and perivascular fibrosis (Maki, 2009; Lopez et al., 2010). It has been shown that treatment with a LOX inhibitor, β-aminopropionitrile, reduced collagen cross-linking and myocardial stiffness in healthy pig hearts (Kato et al., 1995), age-related myocardial fibrosis (Rosin et al., 2015), and MI (Gonzalez-Santamaria et al., 2016). A recent study has shown that LOXL2 secretion is increased in the serum of patients with heart failure, and LOXL2 correlates with collagen cross-linking and diastolic abnormalities. Specifically, inhibition of LOXL2 activity using a neutralizing antibody after transverse aortic constriction reduces interstitial fibrosis and improves heart function (Yang et al., 2016), suggesting that it could also be used as a biomarker. Therefore, regulation of LOX activity has become an attractive therapeutic target for cardiac fibrosis.

Summary

Cardiac fibroblasts are essential for regulating ECM homeostasis and remodeling in heart, but an accurate understanding of the multifaceted role of the fibroblast has been constrained due to limitations in studying these cells. As our knowledge of fibroblast composition and behavior in normal and pathological heart conditions improves, so will our ability to modulate these cells during disease processes.

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Cardiac Hypertrophy: Signaling and Cellular Crosstalk

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Introduction

Heart failure is a clinical manifestation of cardiovascular disease, a leading cause of death worldwide. In the United States, nearly 800,000 patients die of cardiovascular disease each year (Mozaffarian et al., 2016). Almost all etiologies of heart failure involve pathological cardiac hypertrophy and remodeling.

Hypertrophic growth is the primary mechanism by which the heart responds to stimuli that increase intraventricular wall stress and demand enhanced cardiac performance. In accordance with Laplace's law, which states that ventricular wall stress is proportional to both ventricular pressure and cavity radius and inversely proportional to ventricular wall thickness, cardiac hypertrophy is an adaptive response to reduce wall stress and hence is beneficial for heart function (Burch et al., 1952). Cardiac hypertrophy is classified as physiological when associated with physiological stimuli or as pathological when associated with hemodynamic stress. Physiological hypertrophy occurs during normal postnatal growth from birth to adulthood, pregnancy, or in response to sustained exercise training. In this case, the wall thickness increases proportionally to the chamber size and normal cardiac structure and function are maintained. In contrast, pathological hypertrophy develops in response to chronic increase in the hemodynamic load caused by hypertension, aortic stenosis, aortic coarctation, mitral regurgitation, or myocardial infarction, among others. In these conditions, hemodynamic stress increases intraventricular pressure or volume and promotes an adaptive compensatory hypertrophic response, which progresses into maladaptive remodeling with prolongation increase in wall stress. Pathological hypertrophy is often associated with impaired myocardial vascularization, fibrosis, cell death, inflammation, and chamber dilation, which ultimately lead to cardiac dysfunction and heart failure (Shimizu and Minamino, 2016; Diwan and Dorn, 2007; Dorn, 2007).

Heart failure is broadly classified into two types: heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF). HFrEF is mainly due to cardiomyocyte death or reduced cardiac function, fibrosis, and adverse remodeling caused by myocardial infarction, hypertension, and cardiomyopathy. The common pathophysiology responsible for HFpEF is associated with concentric myocardial hypertrophy and diastolic left ventricular (LV) dysfunction caused by a variety of risk factors including hypertension, metabolic syndrome, and aging. The molecular mechanisms underlying HFpEF involve myocyte hypertrophy, endothelial dysfunction, inflammation, fibrosis, and oxidative stress (Heinzel et al., 2015; Jumean and Konstam, 2015).

The human heart contains an estimated 2–3 billion cardiac muscle cells, accounting for only a third of the total number of cells. Other non-myocyte cells include fibroblasts, vascular cells (smooth muscle and endothelial cells of the coronary vasculature and endocardium), and immune cells. Unlike other cell types, cardiomyocytes become terminally differentiated shortly after birth and lose the ability to proliferate, although a very low renewal rate occurs throughout life (Bergmann et al., 2009; Porrello et al., 2011). Thus, "the sine qua non" of myocardial hypertrophy is the increase in size of individual cardiomyocytes through new sarcomere addition and organization, rather than cell proliferation. Postnatal growth, from birth to adulthood, is an example of cardiomyocyte hypertrophy increasing heart mass to keep pace with increase in body weight (Hew and Keller, 2003). Physiological and pathological stimuli promote a dynamic range of growth response. Pregnancy causes a reversible "physiological" LV hypertrophy characterized by an increase of up to 34% of LV mass which returns to the original mass after delivery (Schannwell et al., 2002). In athletes, depending on athletic conditioning, cardiac mass has been reported between 9% and 90% greater, as compared with

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nonathletes (Pluim et al., 2000). By contrast, surgical constriction of the aorta induces an increase of cardiac mass by 40% within 1 week (Rockman et al., 1991).

In addition to changes in myocyte morphology (size and shape), electrophysiology, and metabolism, intercellular signaling and communication between different cell types are critical in the pathophysiology of ventricular hypertrophy and transition to heart failure (Tirziu et al., 2010). This article highlights the molecular basis of hypertrophic signaling and the contribution of cellular communication to myocardial hypertrophy and heart failure.

Cardiomyocyte Hypertrophy

Phenotypic Features

Consistent efforts have been made to uncover phenotypic differences in cardiomyocyte hypertrophy under physiological versus pathological conditions. A hallmark feature of pathological hypertrophy is the induction of the "hypertrophic program of fetal genes," absent in physiological hypertrophy. The well-known markers of pathological hypertrophy include: atrial natriuretic peptide, B-type natriuretic peptide, and the fetal isoform of contractile proteins including skeletal α-actin and the β-isoform of myosin heavy chain (β-MHC) (Dorn et al., 2003). However, it should be noted that in adult human heart the reduction in α-MHC expression is associated with pathological hypertrophy and heart failure (Dorn et al., 2003). In addition, altered expression of the calcium cycling protein, sarco(endo)plasmic reticulum Ca^{2+}ATPase 2a (SERCA2a), instrumental in calcium handling and cardiomyocyte contractility, is often associated with contractile dysfunction and progression to heart failure (Shareef et al., 2014).

One long-lasting theory was that the duration of hemodynamic stress, intermittent versus constant, determines whether the hypertrophy is physiological or pathological. Physiological overloads such as exercise are considered intermittent, whereas pathological overloads such as hypertension are constant. However, intermittent pathological pressure overload in mouse hearts provides convincing evidence that the nature of pathological stress is the molecular trigger of cardiac dysfunction, rather than its duration (intermittent vs. constant) or hypertrophic growth per se (Perrino et al., 2006).

At the cellular level, cardiomyocytes enlarge by enhancing protein synthesis and increasing the number of the sarcomere units. The alignment of additional sarcomeres either in parallel or series determines whether the myocytes are thicker or elongated (Lorell and Carabello, 2000). Based on these alignments, two different hypertrophic phenotypes could be identified: concentric and eccentric (Grossman et al., 1975). Concentric hypertrophy, induced by LV pressure overload in aortic stenosis and hypertension, is associated with thicker cardiomyocytes, increased LV wall thickness, and no changes in LV chamber size. In contrast, eccentric hypertrophy induced by volume overload in aortic and mitral insufficiency is associated with elongated cardiomyocytes and increases of both wall thickness and LV chamber size. In both cases, hypertrophy helps to normalize systolic stress, but not diastolic wall stress (Grossman et al., 1975). A proportional increase in myocyte length and width is typically observed in exercise-induced physiological hypertrophy. In heart failure, cardiomyocytes are disproportionately elongated relative to their width.

Morphological changes in size and shape ultimately affect cardiomyocyte to cardiomyocyte communication. This is particularly important as adverse remodeling impairs myocyte electrical coupling. The electrical impulse is propagated among adjacent cardiomyocytes through gap junctions. In the heart, gap junctions are composed of three major connexins, connexin (Cx)43 (α_1 connexin), Cx45 (α_6 connexin), and Cx40 (α_5 connexin) and each exhibits different channel properties and regional expression pattern (Lo, 2000; Teunissen et al., 2004). Gap junction remodeling, a set of molecular and functional changes that occur during pathological progression, alters the expression and phosphorylation of connexins and induces the lateralization of the gap junction from the intercalated disk (Severs et al., 2008; Kieken et al., 2009). Gap junction remodeling ultimately increases the risk for fatal ventricular arrhythmias. It has been suggested that Cx43, the major connexin expressed in working ventricular myocardium, is upregulated during the compensatory phase of hypertrophy and downregulated and heterogeneously redistributed to the lateral sides of cardiomyocytes with the progression to heart failure (Fontes et al., 2012). However, it is worth mentioning that electrical uncoupling and arrhythmogenicity in pathological hypertrophy and heart failure are most likely a synergistic effect between reduced Cx43 expression and increased fibrosis and collagen deposition (de Jong et al., 2011).

Research over the past few decades has identified numerous factors and signaling pathways involved in cardiomyocyte hypertrophy. In physiological hypertrophic response to growth factors, the activation of the phosphatidylinositol 3-kinase (PI3K) α/serine–threonine protein kinase v-akt murine thymoma viral oncogene homolog (Akt) 1 pathway is a typical mechanism. In response to hemodynamic stress and neurohumoral factors, the activation of calcium–calmodulin–calcineurin/nuclear factor of activated T-cells (NFAT) pathway through the G-protein-coupled receptors (GPCRs) is a potent inducer of hypertrophy. However, the propagation of hypertrophic stimulation extends to a more complex network of signaling events and pathways concurrently integrated within cellular crosstalk that ultimately may determine the outcome: either physiological or compensatory or pathological hypertrophy.

Signaling Mechanisms of Physiological Hypertrophic Response

Growth factors and signaling

The growth factors involved in physiological hypertrophic response are well-characterized and include thyroid hormone (TH), insulin, growth hormone, and insulin-like growth factor 1 (IGF-1) (Maillet et al., 2013). The role of TH in postnatal growth in children is well-recognized and its beneficial effects in preventing pathological heart remodeling have started to emerge (Pantos and

Mourouzis, 2015). A mounting body of evidence demonstrates the benefit of TH replacement therapy in animal models of heart failure, often associated with reduced TH levels (Gerdes and Iervasi, 2010). For instance, TH optimized LV chamber geometry and attenuated adverse remodeling after myocardial infarct (Pantos et al., 2007, 2008). Likewise, in a rat model of LV pressure overload, treatment with TH restored cardiomyocyte calcium sensitivity and contractility by upregulating SERCA2a and α-MHC expression (Chang et al., 1997). Also, atrial fibrillation post-myocardial infarction was attenuated by TH treatment (Zhang et al., 2014). TH induces physiological hypertrophy most likely through activation of p38 mitogen-activated protein kinase (MAPK) and PI3K/Akt/mammalian target of rapamycin complex 1 (mTORC1) signaling (Kenessey and Ojamaa, 2006; Kinugawa et al., 2005). TH effects are not limited to cardiomyocytes. TH activates the Akt pathway and platelet-derived growth factor (PDGF) signaling in endothelial cells and promotes angiogenesis and increased capillary density, which has the potential to increase the delivery of blood supply and nutrients in hypertrophied myocardium (Chen et al., 2012; Makino et al., 2009). However, although therapeutic activity in animal models is promising, large clinical trials are still awaited to test the efficacy of TH treatment in patients with heart failure (Pantos and Mourouzis, 2015).

In the heart, insulin signaling regulates glucose and lipid metabolism, cell growth and differentiation, protein synthesis, and fatty acid oxidation (Saltiel and Kahn, 2001). Insulin binding to the insulin receptor (IR) leads to recruitment and phosphorylation of insulin receptor substrate (IRS)-1 and -2, which activate the PI3K-Akt signaling pathway. Insulin signaling is critical for normal heart development. Gene deletion of IRS-1, IRS-2, and IR resulted in smaller hearts and worsened response to hypertrophic stimuli (Hu et al., 2003; Boudina et al., 2009; Belke et al., 2002; Araki et al., 1994).

IGF-1 signaling is the best known contributor to physiological hypertrophy in response to endurance exercise (**Fig. 1**). IGF-1 regulates cell proliferation, differentiation, metabolism, and survival and is critical for normal development and postnatal growth. In athletes, increase in cardiac IGF-1 has been associated with eccentric hypertrophy (Neri Serneri et al., 2001). IGF-1 binds to its tyrosine kinase receptor IGF1-R, which activates the PI3K/Akt pathway through IRS-1 and IRS-2 (Troncoso et al., 2014). The role of Akt1 as downstream effector of IGF-1 signaling was demonstrated in Akt1-deficient mice, where physiological hypertrophy in

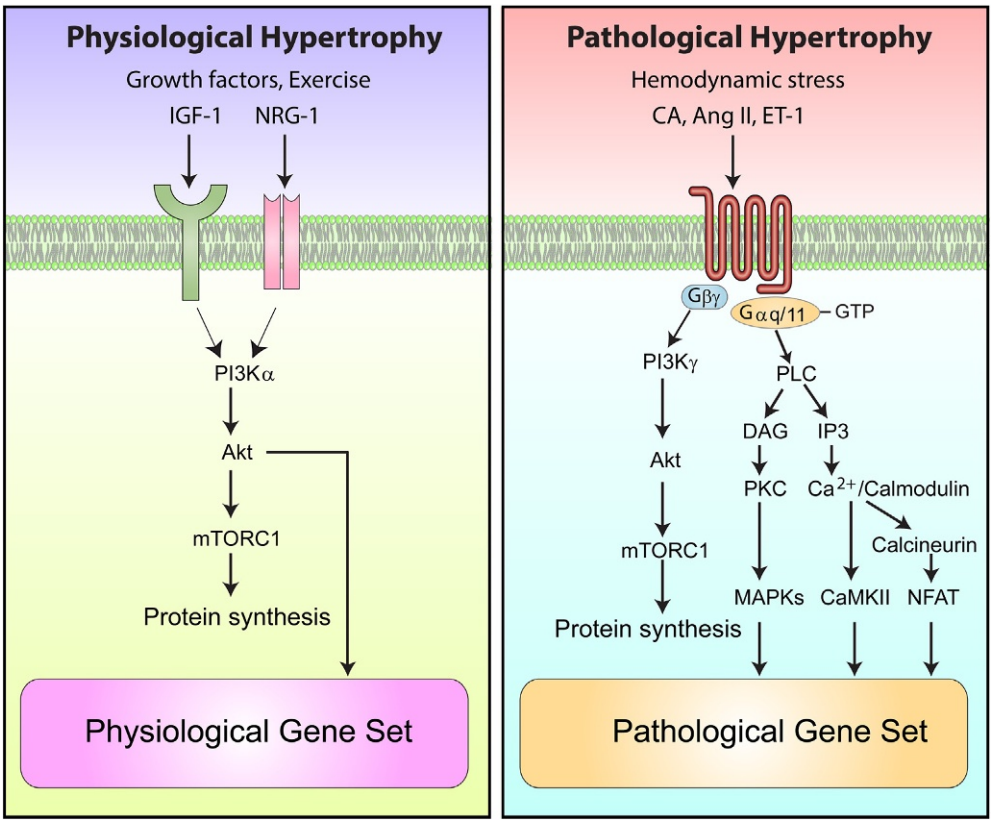

Fig. 1 Key signaling pathways involved in physiological and pathological cardiac hypertrophy. In physiological hypertrophic response to growth factors or exercise, the activation of the PI3Kα/Akt pathway is a typical mechanism to induce protein synthesis and the hypertrophic program of gene expression. In response to hemodynamic stress and neurohumoral factors, the activation of the Gαq/11-PLC pathway through G-protein-coupled receptors is a major mechanism of cardiomyocyte hypertrophy. Downstream, the pathological hypertrophic gene set is induced through several pathways including calcineurin/NFAT, PKC, MAPKs, and CaMKII. In addition, Gβγ-induced activation of the PI3Kγ-dependent Akt/mTOC1 pathway contributes to protein synthesis. *IGF-1*, insulin-like growth factor 1; *NRG-1*, neuregulin-1; *PI3K*, phosphoinositide kinase 3; *Akt*, v-akt murine thymoma viral oncogene homolog 1; *mTORC1*, mammalian target of rapamycin complex 1; *CA*, catecholamine; *Ang II*, angiotensin II; *ET-1*, endothelin 1; *PLC*, phospholipase C; *DAG*, diacylglycerol; *IP3*, inositol trisphosphate; *PKC*, protein kinase C; *NFAT*, nuclear factor of activated T-cells; *CaMKII*, Ca^{2+}/calmodulin-dependent protein kinase II; *MAPKs*, mitogen-activated protein kinases.

response to swimming was completely abolished (DeBosch et al., 2006). Akt1 activation in response to exercise training and transgenic expression of PI3K, IGF-1, and IGF1-R varies from two- to sixfold increase (McMullen et al., 2003, 2004; Shioi et al., 2000). Cardiomyocyte-specific overexpression of IGF1-R promotes a specific physiologic hypertrophy response in a PI3K(p110α)-dependent manner with no pathological features (McMullen et al., 2004). The expression of a dominant active PI3K(p110α) mutant promotes physiological hypertrophy and preserves contractile function, while a dominant negative PI3K(p110α) mutant prevents exercise-induced hypertrophy and exacerbates cardiac dysfunction under hemodynamic stress (McMullen et al., 2003, 2007; Lin et al., 2010). Akt1 promotes myocardial hypertrophy in response to exercise by downregulating the transcription factor CCAAT/enhancer-binding protein β (C/EBPβ). C/EBPβ interacts with serum response factor and contributes to the regulation of the so-called "physiological gene set" involved in cardiomyocyte hypertrophy and differentiation: GATA-binding protein 4 (GATA4), T-box 5 (Tbx5), and NK2 homeobox 5 (Nkx2.5) (Bostrom et al., 2010; Lerchenmüller and Rosenzweig, 2014). Mice with reduced cardiac expression of C/EBPβ have a physiological hypertrophic phenotype and are protected against adverse remodeling (Bostrom et al., 2010). Reduction in C/EBPβ is linked to increased expression of Creb-binding protein/p300-interacting transactivator with ED-rich carboxy-terminal domain-4 (CITED4). Cardiac-specific transgenic CITED4 expression in adult mice was sufficient to promote cardiomyocyte hypertrophy and normal cardiac function, comparable with that induced by exercise training (Bezzerides et al., 2016). CITED4 activates mTORC1, most likely accounting for protein synthesis and cardiomyocyte growth (Bezzerides et al., 2016; Kemi et al., 2008; Ma and Blenis, 2009).

Traditionally, endurance exercise has been regarded as a well-balanced physiological growth, where cardiomyocyte hypertrophy is accompanied by appropriate increase in coronary microvasculature and blood flow (Lerchenmüller and Rosenzweig, 2014). The molecular mechanism most likely involves the vascular endothelial growth factor A (VEGFA) signaling and endothelial nitric oxide synthase (eNOS) activation through the Akt pathway (Iemitsu et al., 2006). In this context, it is worth mentioning that IGF-1 was found to promote angiogenesis in vitro and ex vivo experiments (Su et al., 2003).

miRNAs and physiological hypertrophic response

Several miRNAs are involved in the regulation of physiological hypertrophic response (Fernandes et al., 2015). Cardiac-specific miR-1 and miR-133, downregulated in pathological hypertrophy, are also reduced in physiological hypertrophy induced by cardiac-specific Akt1 transgenic overexpression and exercise training, suggesting their contribution to a wide-spectrum of hypertrophic responses (Care et al., 2007; Ellison et al., 2012). MiR-1 negatively regulates cardiac mass by modulating the IGF pathway and calcium signaling (Ikeda et al., 2009; Elia et al., 2009), while miR-133 is a negative modulator of calcineurin/NFAT signaling (Care et al., 2007; Li et al., 2010).

Cardiac miR-222 is an important modulator of exercise-induced cardiac growth and is protective against adverse remodeling and cardiac dysfunction (Liu et al., 2015). Interestingly, exercise training increased miR-222 expression in both healthy volunteers and heart failure patients (Baggish et al., 2011; Liu et al., 2015). Both gain- and loss-of-function studies showed that miR-222 targets cyclin-dependent kinase inhibitor 1B (p27), homeobox containing 1 (HMBOX1), and homeodomain-interacting protein kinase 1/2 (HIPK1/2) to regulate myocardial hypertrophy (Liu et al., 2015). Most likely, the reduction of p27 and HIPK1/2 has a proliferative effect, while the reduction of HMBOX1 stimulates cardiomyocyte hypertrophy.

An endothelial-specific miRNA, miR-126, is also upregulated by exercise. MiR-126 promotes angiogenesis and increases capillary density by repressing two negative regulators of the VEGF pathway: sprouty-related protein 1 (Spred-1), a suppressor of the Ras/MAPK pathway, and PI3K regulatory subunit 2, a negative regulator of the PI3K/Akt/eNOS pathway (Silva et al., 2012). Exercise training in pathological conditions, such as hypertension, could prevent microvascular rarefaction by inducing miR-126 expression (Fernandes et al., 2012).

Another interesting miRNA induced by exercise is miR-29c, a negative modulator of extracellular matrix (ECM) components collagen I and III. High performance training in rats is well-tolerated and ventricular compliance improved due to an inhibitory effect of miR-29c on ECM deposition and cardiac fibrosis (Soci et al., 2011). In exercise-trained myocardial-infarcted rats, the increase of miR-29 prevents excessive fibrosis and is therefore beneficial for heart function (Melo et al., 2014).

Hyperplasia and physiological hypertrophic response

Although controversial, recent studies support the idea that physiological adaptation to endurance exercise is a combination of cardiac hypertrophy and hyperplasia, involving cardiomyocytes and endothelial cells (Bostrom et al., 2010; Waring et al., 2014). Cardiomyogenesis has been recently reported in a rat model of endurance exercise where a significant number of newly myocytes arose from endogenous c-kit$^+$ cardiac stem cells (CSCs) (Waring et al., 2014). Consistent with this idea, growth factors such as IGF-1 and neuregulin 1 (NRG-1) have been previously reported to induce cardiomyocyte proliferation in vitro and in vivo (Bersell et al., 2009; Bostrom et al., 2010). Furthermore, in exercise-trained rats with myocardia infarct, the increased production of NRG-1 promoted cardiac repair and the recruitment of c-kit$^+$ CSCs by binding to receptor tyrosine kinase ErbB2/ErbB4 followed by downstream activation of the PI3K/Akt signaling (Cai et al., 2016). The recognition of the exercise-induced regenerative capacity in the adult heart may lead to future therapeutic avenues for patients with heart failure.

Signaling Mechanisms of Pathological Hypertrophic Response

Major triggers of pathological hypertrophy include biomechanical stress and neurohumoral factors (Hill and Olson, 2008). The mechanisms by which biomechanical stress is transduced within cardiomyocytes are uncertain, but suggest the involvement of

stretch-activated cation channels, ECM, integrins, and cellular cytoskeleton among others (Sadoshima and Izumo, 1997; Sharif-Naeini et al., 2010; Lyon et al., 2015). The adrenergic and renin-angiotensin systems have been extensively studied as neurohumoral mechanisms of pathological hypertrophy in chronic heart failure. Beta-adrenergic receptor blockers (β-blockers), angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARB), and a recent combination of an ARB with an inhibitor of neprilysin (ARNI) are first-line therapies for the heart failure (Yancy et al., 2016). The key signaling pathways involved in the pathological hypertrophic response are briefly summarized.

Signal transduction through myocardial GPCRs, including adrenergic, angiotensin, and endothelin receptors, is critically involved in the cardiac response to hemodynamic stress and the development of cardiac hypertrophy, fibrosis, heart failure, and arrhythmia. GPCRs transduce agonist-induced signals to intracellular effectors through the activation of three principal classes of heterotrimeric G protein complexes composed by one GTP-binding Gα subunit (Gαs, Gαq/$G\alpha_{11}$, or Gαs) tightly associated in the GDP-bound transition state with an obligate Gβγ subunit.

Adrenergic receptors (ARs) bind catecholamines, such as noradrenaline and adrenaline, and mediate chronotropic and inotropic effects, as part of the acute fight-or-flight response, in both physiological and pathological conditions. In patients with heart failure, elevated levels of catecholamines and chronic activation of the adrenergic system result in a poor clinical outcome and shortened life expectancy (Cohn, 1995). There are two main ARs in the heart: β-ARs, roughly 90%, and α_1-ARs, likely 10% of the total ARs. Increased β-adrenergic activity causes the heart to beat fast, while increased α-adrenergic activity constricts the arterioles and increases the blood pressure.

β-ARs signaling and cardiomyocyte hypertrophy

Cardiomyocytes mainly expresses β1 and β2 ARs. The β1-AR subtype is the most predominant (80%–90% of total β-ARs) and is mainly responsible for the positive chronotropic and inotropic effects of catecholamines (Brodde et al., 2006; Fu et al., 2012; Lohse et al., 2003). Both, β1- and β2-ARs couple to the heterotrimeric Gs protein complex and activate adenylyl cyclase (AC) to generate cAMP. Then, cAMP activates protein kinase A (PKA), which regulates key Ca^{2+}-handling proteins involved in cardiac contractility: L-type channels, phospholamban (PLN), troponin I, ryanodine receptors (RyR), myosin-binding protein-C, and protein phosphatase inhibitor-1 (Lohse et al., 2003). Although acute activation of β1-AR is initially adaptive, chronic activation leads to cardiac dysfunction and heart failure associated with β1-AR downregulation and desensitization (Bristow et al., 1982; Ungerer et al., 1994; Dorn, 2009).

Despite strong indication of cardiomyocyte hypertrophy in mice with cardiac β1-ARs transgenic overexpression or upon stimulation with isoproterenol (ISO, a non-selective β-AR agonist), or dobutamine (a β1-AR selective agonist) the mechanism of the hypertrophic response is not fully understood (Osadchii, 2007; Engelhardt et al., 1999). One scenario plays out the possibility that the cAMP/PKA pathway increases the transcriptional activity of cAMP response element (CRE) on CRE-responsive genes in a manner dependent on cAMP-response element modulator (CREM). The loss- and gain-of-function studies showed that CREM is required for β1-AR-induced hypertrophy (Lewin et al., 2009). Likewise, inhibition of CRE-mediated transcription by the over-expression of inducible cAMP early repressor was also shown to prevent ISO-induced hypertrophic response (Tomita et al., 2003). Another scenario supports the idea that the activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), whether dependent or independent of PKA activity, modulates the β-AR-induced hypertrophic response (Grimm and Brown, 2010). This is also supported by the cardiac-specific overexpression of a peptide AC3-I, a CaMKII inhibitor, which significantly prevented myocardial hypertrophy and pathological remodeling in response to β-agonist stimulation (Zhang et al., 2005). Although the precise mechanism by which the β-AR/CaMKII axis contributes to the hypertrophic response is still elusive, recent studies have suggested that CaMKII is rather involved in mitochondrial dysfunction, cardiac fibrosis, and adverse cardiac remodeling (Grimm et al., 2015; Xu et al., 2016).

Beta-AR desensitization, probably the most detrimental effect induced by chronic receptor stimulation, is regulated by the GPCR kinases GRK2 and GRK5. Intriguingly, the inhibition of β-AR desensitization substantially prevented the progression of pathological hypertrophy and restored cardiac function in several animal models of heart failure (Hullmann et al., 2016). The inhibition of GRK2 with a C-terminal peptide known as βARKct showed that preventing GRK2 binding to Gβγ improved cardiac contractility (Koch et al., 1995; Shah et al., 2001; White et al., 2000). Furthermore, the recent interest in non-canonical signaling induced by GRK2 and GRK5 has led to discovery of unexpected roles of GRK2 in cardiac metabolism and survival and GRK5 in pathological hypertrophy (Woodall et al., 2014; Traynham et al., 2015, 2016).

There is also support for the idea that sustained β-AR activation promotes cardiomyocyte hypertrophy via neurohumoral factors such as angiotensin II (Ang II), interleukin 6 (IL-6), and endothelin 1 (ET-1) (Ferreira et al., 2008; Yin et al., 2003; Morimoto et al., 2001).

α-ARs, Ang II and ET-1 receptor signaling, and cardiomyocyte hypertrophy

Cardiac α1-ARs, angiotensin II type 1 receptor (AT1), and endothelin receptor (ET_A) couple to the Gq/11 (Gαq)-phospholipase C pathway, which is considered a major mechanism involved in the cardiomyocyte hypertrophic response (**Fig. 1**). The canonical effectors of activated GTP-bound family Gαq subunits are β-isoforms of phospholipase C (PLC-β). Activation of PLC-β cleaves phosphatidylinositol, increasing inositol trisphosphate (IP3) and diacylglycerol (DAG). IP3 binds to the IP3-receptor to release calcium and DAG activates protein kinase C (PKC). Downstream, hypertrophic signaling is propagated through several pathways: calcineurin/NFAT, MAPKs, and CAMKII, among others (Hubbard and Hepler, 2006; Sugden and Clerk, 1997; van Berlo et al., 2013). In addition, Gβγ subunit activates the PI3K p110γ-dependent Akt/mTORC1 pathway of cardiomyocyte hypertrophy (Dupre

et al., 2009; Kerchner et al., 2004; Naga Prasad et al., 2000; Ma and Blenis, 2009). The key role of Gαq was demonstrated in mouse models, where cardiac-specific Gαq transgenic overexpression exacerbated pathological hypertrophy and failure, whereas Gαq conditional deletion prevented the hypertrophic response to chronic pressure overload (D'Angelo et al., 1997; Mende et al., 1998; Wettschureck et al., 2001).

Calcineurin, a Ca^{2+}/calmodulin-activated serine/threonine phosphatase, dephosphorylates and induces the translocation of cytoplasmic NFAT to the nucleus. Subsequently, NFAT forms complexes with the cofactors GATA4 and myocyte enhancer factor-2 (MEF-2) to activate transcription of the hypertrophic program of gene expression (Heineke and Molkentin, 2006). Although the role of the calcineurin /NFAT pathway in pathological hypertrophy is well-recognized, smaller hearts in calcineurin-deficient mice suggest potential contribution of the calcineurin/NFAT signaling to normal heart development (Wilkins et al., 2004).

CaMKII is also regulated by the Gαq-induced Ca^{2+}/calmodulin activity and, like PKA and PKC, modulates acute cellular Ca handling (Maier and Bers, 2002). It has been shown that myocardial transgenic overexpression of the predominant cardiac CaMKIIδc isoform, like overexpression of Gαq, was sufficient to induce cardiac hypertrophy and transition to heart failure (Zhang et al., 2003). Conversely, CaMKIIδ gene deletion attenuated cardiac dysfunction and pathological remodeling in response to chronic pressure overload (Backs et al., 2009; Ling et al., 2009). However, a recent study employing a compound mouse model with cardiac-specific Gαq transgenic overexpression and CaMKIIδ deletion concluded that CaMKIIδ induces mitochondrial gene reprogramming responsible for the transition to heart failure and has no effect on hypertrophy (Westenbrink et al., 2015). Interestingly, similar changes in mitochondrial gene expression have also been observed in pressure overload-induced pathological hypertrophy (Westenbrink et al., 2015). It is worth noting that CaMKII activation in response to β-agonist led to mitochondrial dysfunction as well.

MAPK signaling cascades consist in successively acting protein kinases that ultimately result in the activation of three main pathways: extracellular signal-regulated kinases (ERKs), c-Jun amino-terminal kinase (JNK), and p38. The role of ERK1/2 in cardiomyocyte hypertrophy is very complex, often complicated by their potential contribution to both physiological and pathological growth mechanisms. Cardiac-restricted expression of activated MEK1 (upstream of ERK1/2) in mice induced compensated hypertrophy with no cardiac dysfunction or fibrosis (Bueno et al., 2000). Genetic inhibition of cardiac ERK1/2 had no effect on hypertrophy, but exacerbated heart failure in response to chronic pressure overload (Purcell et al., 2007). Recent evidence has further suggested that autophosphorylation of ERK1/2 at Thr188 via Gβγ is critical for ERK1/2-induced hypertrophy associated with pathological stimuli (Lorenz et al., 2009). Subsequently, employing an ERK2(T188A) mutant that interferes with ERK1/2^{Thr188} phosphorylation, the pathological hypertrophic response to pressure overload was significantly attenuated, while physiological hypertrophy in response to exercise was unchanged (Ruppert et al., 2013). JNK and p38 are stress-activated kinases and their roles in cardiomyocyte hypertrophy are still controversial (Shimizu and Minamino, 2016). On the other hand, it has been asserted that JNK and p38 promote cardiomyocyte apoptosis, adverse remodeling, and heart failure (Baines and Molkentin, 2005).

miRNAs and pathological hypertrophic response

During the past decade, several miRNAs have been associated with myocardial hypertrophy (Sayed et al., 2007; van Rooij et al., 2008). The first discovered miRNA, miR-208, has been shown to play a key role in the upregulation of β-MHC (a fetal slow contracting ATP isoform of MHC) and cardiac dysfunction in response to hemodynamic stress (van Rooij et al., 2007). Other early reports found the expression of miR-1 and miR-133, which belong to the same transcriptional unit, inversely correlated with cardiac hypertrophy in response to pressure overload, Akt1 transgenic expression, and exercise (Care et al., 2007). Interestingly, knocking down miR-133 in mice with an infusion of antisense RNA oligonucleotide appeared to be sufficient to induce cardiac hypertrophy and activation of the fetal program of gene expression (Care et al., 2007). Consistent with the role in pathological hypertrophy, gain- and loss-of-function studies showed that miR-133 negatively regulates NFATc4 and components of the β1-AR/cAMP/PKA pathway (Castaldi et al., 2014; Li et al., 2010). MiR-1, although not as potent as miR-133, can prevent cardiomyocyte hypertrophy by targeting calmodulin, GATA4, and MEF-2 (Ikeda et al., 2009).

Other miRNAs are consistently upregulated in cardiomyocytes in the settings of pathological pressure overload in vivo and upon treatment with neurohumoral factors in vitro. Interesting examples are calcineurin/NFAT-responsive miRNAs: miR-199b and miR-23a. MiR-199b enhances calcineurin/NFAT-mediated pathological cardiac hypertrophy by targeting the nuclear NFAT kinase dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1a (Dyrk1a) in an apparent pathogenic feed forward mechanism (da Costa Martins et al., 2010). MiR-23a conveys the hypertrophic signal by suppressing two anti-hypertrophic effectors: muscle ring finger protein-1 (MuRF1) and forkhead box O3 (FOXO3) (Lin et al., 2009; Wang et al., 2012). FOXO3, a regulator of the atrophy-related gene program and suppressor of calcinurin/NFAT signaling, is also targeted by miR-212/132 (Ucar et al., 2012). Both miR-212 and miR-132 are upregulated by cardiac stress and are necessary and sufficient to induce pathological hypertrophy in vitro and in vivo. In addition, pharmacological inhibition of miR-132 with antagomir prevented cardiac hypertrophy and heart failure induced by chronic LV pressure overload (Ucar et al., 2012).

Cardiomyocyte Hypertrophy and Endothelial Cells/Cardiac Angiogenesis

Cardiomyocytes secrete a large variety of factors that affect the coronary vasculature during development and stress response. Among these are VEGFA, fibroblast growth factor 2 (FGF2), PDGF, transforming growth factor β (TGF-β), ET-1, urocortin, adenosine, angiopoietins, and myokines. For instance, cardiomyocyte-specific deletion of VEGFA in mice during development

resulted in hearts with fewer coronary microvessels, thinned ventricular walls, and abnormal response to β-adrenergic stimulation. This demonstrates a critical role for cardiomyocyte-derived VEGFA for cardiac angiogenesis and normal myocardium development (Giordano et al., 2001).

A balanced hypertrophic growth implies that myocardial requirements for oxygen and nutrients are met by a commensurate growth of the coronary microvasculature and increase in blood flow. Failure of the vasculature to match cardiomyocyte growth can lead to cardiac dysfunction and ultimately failure. Compelling evidence for the importance of myocyte-vascular balance was provided in mice with cardiac hypertrophy induced by overexpression of Akt1. Akt1 modulates postnatal physiological hypertrophy in response to insulin signaling and nutritional status (Shiojima et al., 2002). However, transgenic overexpression of activated Akt1 in the mouse heart led to a large variety of phenotypes from cardiac hypertrophy with preserved systolic function, to cardiac dilatation and failure (Matsui et al., 2002). Initial activation of the Akt pathway promoted hypertrophy while preserving cardiac function (Condorelli et al., 2002; Kim et al., 2003; Shioi et al., 2002), whereas long-term activation led to pathological hypertrophy and cardiac dysfunction (Shiojima et al., 2005). The progression to pathological hypertrophy was thought to be caused by inadequate expansion of coronary angiogenesis to support the increase in cardiac mass with matching blood supply and nutrients. To directly demonstrate this, a tetracycline-inducible system of the cardiac Akt1 transgene was used. Short-term (2 weeks) Akt1 induction in cardiomyocytes promoted a physiological hypertrophic response accompanied by an increase in capillary density. Angiogenesis was induced at least in part by increased secretion of cardiomyocyte-derived VEGFA and angiopoientin-2 (Shiojima et al., 2005). However, activation of Akt1 for longer than 2 weeks resulted in a disproportional increase in cardiac mass relative to the extent of angiogenesis. This demonstrates the importance of coordination of cardiac muscle and the coronary vasculature in order to maintain cardiac function and suggests that angiogenic adaptation might be limited and imbalanced relative to the extent of hypertrophy (Walsh and Shiojima, 2007). Whether this limitation is due to a diminution in myocyte production of angiogenic growth factors, or increased production of antiangiogenic factors, or perhaps incapacitated endothelial response to angiogenic factors remains uncertain.

The importance of vascular balance during the hypertrophic response is further emphasized in peripartum cardiomyopathy (PPCM), a life-threatening condition characterized by sudden onset of heart failure in previously healthy women. Though PPCM etiology remains unknown, the current paradigm suggests that late-gestational secretion of antiangiogenic factors superimposed with reduced production of angiogenic factors in the heart causes vascular rarefaction that fails to provide sufficient amount of blood supply and nutrients to support the heart adaptation to increased hemodynamic and metabolic challenges (Bello and Arany, 2015; Patten et al., 2012). Ultimately, this mismatch leads to the development of systolic heart failure. Although approximately half of the affected women recover cardiac function postpartum, a large number develops chronic heart failure (Fett and Markham, 2015). There are two potential antiangiogenic factors contributing to this vascular imbalance (Bello and Arany, 2015). The first factor is a soluble version of the VEGF receptor 1 or soluble Fms-like tyrosine kinase 1 (sFLT1) secreted by the placenta in late gestation. sFLT1 binds and traps circulating VEGFA. The second factor is a 16 kDa fragment produced by proteolytic cleavage of the nursing hormone prolactin (PRL), secreted by the pituitary gland. The 16 kDa PRL fragment induces endothelial cell apoptosis and release of endothelial miR-146a into exosomes. Taken up by cardiomyocytes, miR-146a impairs metabolic activity and cardiac function by suppressing ErbB4, a NRG-1 receptor and an important modulator of physiological hypertrophic response during pregnancy (Halkein et al., 2013; Hilfiker-Kleiner et al., 2007). Additionally, superimposed reduction in cardiomyocyte-derived VEGFA exacerbates the angiogenic imbalance in the maternal heart (Patten et al., 2012). No specific therapy is available for PPCM other than standard treatment for heart failure. An ongoing randomized, controlled clinical trial is being performed in Germany to evaluate the efficacy and safety of bromocriptine (an inhibitor of PRL production) for improvement of LV function in PPCM patients. Although bromocriptine can lower PRL, it does not address the causes leading to PPCM (Hilfiker-Kleiner et al., 2012). Overall, there is a scientific consensus for a better understating of the PPCM mechanism of disease and more efficient therapeutic options.

Myocardial infarction is another clinical setting that provides evidence of the importance of cardiac myocyte-derived factors influencing the vasculature. In fact, there is strong evidence that VEGFA is substantially increased in the ischemic myocardium (Li et al., 1996) and the vasculature in ischemic heart exhibits greater sensitivity to VEGF- and other growth factor-induced vasodilation (Sellke et al., 1996). Transcriptional regulation of the ischemic response is broadly mediated by hypoxia-inducible factor 1 (HIF-1). HIF-1 levels increase in response to hypoxia and alter the expression of factors involved in angiogenesis and vasomotor reactivity including all the glycolytic enzymes, the Glut1 glucose transporter, VEGFA, PDGF-B, hepatic growth factor, TGF-β1, iNOS, ET-1, heme oxygenase, connective tissue growth factor (CTGF), and many others (Giordano, 2005; Semenza, 2001). Although HIF potentially could play an important role in vascular adaptation during myocardial hypertrophy, accumulation of p53 inhibits HIF-1 activity and angiogenesis in response to chronic LV pressure overload (Sano et al., 2007). Conversely, inducing angiogenesis with angiogenic factors or inhibiting p53 accumulation enhanced hypertrophic response and restored cardiac function under chronic pressure overload. Interestingly, the recently discovered angiogenic factor Canopy 2 (CNPY2), a HIF-1α regulated gene, prevents the antiangiogenic effect of p53 and vascular rarefaction in chronic pressure overload. Moreover, cardiac-specific CNPY2 transgenic overexpression attenuated the transition from compensatory hypertrophic response to ventricular dysfunction (Guo et al., 2015).

Transcription factors can also alter the expression of paracrine factors produced by cardiomyocytes to adjust angiogenic response. An interesting example is GATA4, which modulates cardiomyocyte differentiation and adaptive hypertrophic response in the adult heart. Cardiomyocyte-specific transgenic expression of GATA4 promoted myocardial angiogenesis, increased coronary flow, and perfusion-dependent cardiac contractility (Heineke et al., 2007). GATA4-induced angiogenesis in this model was

independent of the hypertrophic response and was mediated through direct regulation of VEGFA promoter activity (Heineke et al., 2007).

Recently, PDGF receptor β (PDGFR-β) has been shown to be required for cardiomyocyte-induced angiogenesis. The role of PDGFR-β in heart was initially noticed in cancer patients who developed heart failure following treatment with PDGFR-β inhibitors. Mice with cardiomyocyte-specific PDGFR-β gene deletion develop heart failure in response to pressure overload associated with impairment in cardiac angiogenesis (Chintalgattu et al., 2010). However, the mechanism by which PDGFR-β controls the paracrine angiogenic potential of cardiomyocytes is not clear. Also, the identity of the paracrine factors responsible for PDGFR-β-dependent angiogenic capacity of cardiomyocytes remains to be determined (Chintalgattu et al., 2010).

Myokines, cardioprotective factors secreted by cardiomyocytes, can act in a paracrine manner to induce angiogenesis. For instance, follistatin-like 1 (Fstl1), which is highly expressed by cardiomyocytes in response to pressure overload and myocardial infarction, activates the Akt pathway to promote survival in cardiomyocytes and angiogenesis in endothelial cells (Oshima et al., 2008; Ouchi et al., 2008). Different than other members of the follistatin family that function as extracellular-binding partners of the TGF-β superfamily, Fstll binds disco-interacting protein 2 homolog A, a cell-surface receptor, present on both cardiomyocytes and endothelial cells (Ouchi et al., 2010).

Endothelial Cells/Cardiac Angiogenesis and Cardiomyocyte Hypertrophy

The vasomotor control of coronary arteries plays a critical role in maintaining sufficient supply of oxygen and nutrients to the myocardium in response to exercise training, hemodynamic stress, and neurohumoral factors. A recent report has proposed that endothelial cells account for more than 60% of non-myocyte cell population and most likely their role in cardiac physiopathology is greater than previously anticipated (Pinto et al., 2016). Endothelium contributes to cardiovascular homeostasis by regulating vascular permeability, vascular resistance, and myocardial stiffness. Endothelium releases vasodilator and vasoconstrictor factors (nitric oxide (NO), ET-1), prostaglandins, pro- and anticoagulant factors, and growth factors (FGF, VEGFA, PDGF). In addition, the luminal surface of the endothelium harbors a number of enzymes, including ACE, capable of affecting myocardial and vascular function under hemodynamic stress.

Myocardial angiogenesis and increase in capillary density are critical for the development of cardiac hypertrophy. For instance, trapping VEGFA with a soluble VEGF receptor 2 decoy during pressure overload accelerates the progression of heart failure due to inhibition of the compensatory growth response, or possibly due to a reduction in size of the existing vasculature (Izumiya et al., 2006). Furthermore, myocardial hibernation and cardiac function appear to be regulated by the endothelial cell mass. A reduction in cardiac VEGFA levels induced by a cardiac-specific expression of a sFLT1 decoy reduced vascular density and promoted hypoperfusion and myocardial hibernation (May et al., 2008). The effect was reversible, since normal coronary vessel density and recovered heart function occurred once expression of the VEGF trap was discontinued.

The endothelium secretes factors, such as NRG-1 and apelin, which support the compensatory hypertrophic response to hemodynamic stress. In the heart, NRG-1 expression is restricted to the endocardial and microvascular endothelium, whereas its tyrosine kinase receptors ErbB2 and ErbB4 are expressed on cardiomyocytes (Lemmens et al., 2006). Disruption of NRG-1/ErB2 with trastuzumab (Herseptin), an inhibitory antibody against ErbB2, used in the treatment of mammary carcinomas, was discovered to induce cardiomyopathy in treated patients (Lemmens et al., 2007). NRG/ErbB signaling is indispensable during cardiac and neuronal development (Meyer and Birchmeier, 1995; Gassmann et al., 1995) and regulates the cardiac subtype specification nodal vs. ventricular myocytes in differentiating human embryonic stem cells (Zhu et al., 2010). Furthermore, increased NRG-1/ ErbB4 signaling by NRG-1 injection or ErbB4 overexpression promoted cardiomyocyte proliferation and myocardial regeneration after myocardial infarction (Bersell et al., 2009). In response to pressure overload, NRG-1/ErbB signaling increases during the compensatory phase of concentric growth and then decreases as the heart begins to fail (Lemmens et al., 2006, 2007). Interestingly, activation of NRG-1 signaling improves cardiac function in pressure overload-induced heart failure by inhibiting the abnormal sympathetic hyperactivity (Matsukawa et al., 2014). Mechanistically, the activation of NRG-1/ErbB signaling promotes cardiomyocyte hypertrophy via ERK1/2 and PI3K/Akt pathways (Baliga et al., 1999).

Apelin acts via its GPCR receptor APJ and likely plays an important role in cardiovascular pathophysiology by opposing actions to the rennin-angiotensin system (Ashley et al., 2006). While Ang II increases vascular tone and raises blood pressure, apelin promotes vasodilation via a NO-dependent mechanism. In the heart, apelin is primarily expressed by the endothelium, while APJ is expressed on cardiomyocytes, smooth muscle cells, and endothelial cells, suggesting a paracrine mode of action (Chen et al., 2003; Kleinz and Davenport, 2004; Kleinz et al., 2005; Rosenkranz, 2004). Apelin expression is induced in response to hypoxia in the ischemic heart (Chun et al., 2008; Sheikh et al., 2008) and promotes cardiac neovascularization after myocardial infarction by recruiting stem cells (Tempel et al., 2012). Several reports associate apelin deficiency with myocardial decompensation and human heart failure (Chen et al., 2003; Foldes et al., 2003; Chong et al., 2006; Charo et al., 2009). In hypertensive rats, the levels of apelin and APJ are maintained during the compensatory LV hypertrophic response and decline as heart failure sets in (Iwanaga et al., 2006). This may be due to an inhibitory effect of Ang II, because an AT1 receptor blocker, telmisartan, restores apelin/APJ expression. Apelin also improves cardiac contractility through a mechanism dependent on the activation of the PKC epsilon and ERK1/2 signaling (Perjes et al., 2014). However, whether apelin is beneficial for cardiac hypertrophy is still controversial, considering its effect to promote myocardial hypertrophy and cardiac dysfunction in the absence of pathological stimulation (Lu et al., 2016).

It has become evident that the endothelium has a role in the regulation of the heart size. The endothelium controls the size of adipose tissue, the prostate gland, and the liver (Tirziu and Simons, 2009). In the heart, early studies provide evidence of an angiogenesis-induced myocardial hypertrophy mechanism under stimulation with TH in rats. Although TH initially induced an increase in capillary density, this event was followed by the development of physiological hypertrophy and enhancement of ventricular function (Tomanek and Busch, 1998). The TH-induced angiogenic response required in part an early upregulation of FGF2 expression (Tomanek et al., 1998).

A more direct approach in analyzing the role of endothelium in determining heart size was addressed in conditional mouse models by cardiac-specific overexpression of angiogenic factor PR39 or placental growth factor (PlGF) (Tirziu et al., 2007; Jaba et al., 2013). PR39 or PlGF transgene expression for short-term (3 weeks) in the adult mouse heart led to a significant increase in endothelial cell mass and capillary/myocyte ratio with no changes in heart size. However, longer induction (6 weeks) promoted cardiomyocyte hypertrophy and increase in heart size. In both models, the hypertrophy was physiological, preserved contractile function, and was not accompanied by fibrosis or induction of markers associated with pathological hypertrophy. Mechanistically, higher baseline NO production due to increased vascular density promotes cardiomyocyte hypertrophy by targeting regulator of G protein signaling type 4 (RGS4) and relieving the repression of Gβγ-dependent PI3Kγ activation of the Akt/mTORC1 pathway (Jaba et al., 2013). In this sequence of events, NO is important for its role in proteosomal degradation of RGS4 through the Arg/N-end rule pathway of protein degradation (Hu et al., 2005). Consequently, a conditional RGS4-gain-of-function mouse, eNOS inhibitors, and angiogenic stimulation in eNOS knockout mice prevented myocardial hypertrophy. Furthermore, miR-182 was shown to be targeted by the endothelium to cardiomyocyte communication (Li et al., 2016). Although the mechanism by which endothelial NO induces miR182 in cardiomyocytes is not clear, miR-182 contributes to the cardiomyocyte hypertrophic response. MiR-182 fine-tuned the activation of the Akt/mTORC1 pathway in cardiomyocytes by reducing the expression of branched chain aminotransferase 2, responsible for branched chain amino acids (BCAAs) catabolism and FOXO3 (Li et al., 2016). Although cardiomyocyte-derived angiogenic growth factors cannot be excluded, the paracrine action of endothelium-derived NO appears to be an important hypertrophic mechanism in the context of superfluous supply of nutrients, in the absence of external stimuli. In another report, cardiomyocyte transgenic PlGF expression did not induce angiogenesis at the baseline. However, in this model PlGF induced greater hypertrophy in response to pressure overload through a paracrine signaling generated from the endothelium-fibroblast crosstalk (Accornero et al., 2011).

Another member of the VEGF family, VEGFB, promoted cardiac hypertrophy (Karpanen et al., 2008; Bry et al., 2010; Kivela et al., 2014). However, opposed to the PlGF model (Jaba et al., 2013), transgenic overexpression of VEGFB in the heart induces an increase in vessel diameter rather than an increase in vessel density and hypertrophy is independent of NO (Kivela et al., 2014). This apparent mechanistic difference between PlGF and VEGFB, both binding to VEGFR1, may reflect the capability of PlGF to induce angiogenesis and activate endothelial eNOS through VEGFR1 phosphorylation at Tyr794 (Ahmad et al., 2006; Roncal et al., 2008). In contrast, the VEGFB gain- and loss-of-function models provide evidence of a VEGFB-induced metabolic reprogramming mechanism in cardiomyocytes which favors glucose oxidation and protein synthesis, without increasing fatty acid uptake (Kivela et al., 2014). In the light of recent evidence that a BCAA metabolite released by cardiomyocytes instructs endothelium to facilitate fatty acid import into muscle (Jang et al., 2016), it is highly probable that VEGFB-induced metabolic changes mediate cardiomyocyte-endothelial cell crosstalk.

Cardiomyocyte Hypertrophy and Fibroblasts/Cardiac Fibrosis

Cardiac fibrosis, or excessive deposition of the ECM proteins, is a hallmark of pathological hypertrophy and heart failure. In response to increased wall stress, cardiac fibroblasts proliferate and produce the majority of ECM proteins (fibronectin, laminin, proteoglycan, collagen I and III) deposited in the interstitium (around cardiomyocytes) and perivascular region (in the adventitia of coronary arteries) (Piek et al., 2016). Fibroblasts sense and respond to biomechanical stress (MacKenna et al., 2000) and humoral factors including Ang II, ET-1, TGF-β, FGF2, and IGF-1 (Manabe et al., 2002; Bujak and Frangogiannis, 2007; Bouzegrhane and Thibault, 2002). Once activated, fibroblasts transdifferentiate into myofibroblasts, cells that express smooth muscle type contractile proteins and exhibit increased proliferative and secretory properties (Tomasek et al., 2002). While fibrosis may initially preserve cardiac function, a sustained fibrotic response promotes myocardial stiffness and atrial fibrillation and deprives the stressed myocardium of vascular supply. On the other hand, in myocardial infarction, α-smooth muscle actin (α-SMA) expressing myofibroblasts prevent wall rupture and adverse remodeling by facilitating scar formation and contraction (Shinde et al., 2017). In addition, fibroblasts can interact directly with cardiomyocytes via hetero-cellular gap junction coupling, which ultimately alters impulse generation and conduction (Tanaka et al., 2017).

Fibroblasts previously were thought to be an abundant non-myocyte cell population in the heart. However, advanced tracing techniques have recently estimated that the resident fibroblast makes up less than 20% of all non-myocyte cells (Pinto et al., 2016). Under pathological conditions, fibroblasts exhibit significant heterogeneity and plasticity, but the lack of reliable and specific markers has hampered efforts to understand their origin and biology. Not long ago, it was reported that up to 70% of cardiac fibroblasts in chronic pressure overload was derived from pathologically induced endothelial-to-mesenchymal

transition (EndoMT) (Zeisberg et al., 2007). This process was mediated by TGF-β1 and inhibited by bone morphogenic protein 7, a TGF-β superfamily member known to antagonize TGF-β signaling (Potenta et al., 2008; Zeisberg et al., 2007). Many other cell types have been suggested as a source of activated fibroblasts. For instance, pericytes were reported to be a major source of myofibroblasts containing α-SMA (Kramann et al., 2015). Cells of hematopoietic origin and infiltrating immune cells have been described to transdifferentiate into cardiac fibroblasts in the diseased heart (Crawford et al., 2012; van Amerongen et al., 2008). However, recent studies employing genetic lineage tracing revealed that fibroblast heterogeneity observed in pressure overload results from activated resident fibroblasts, derived from developmentally lineages of epicardial and endocardial/endothelial origin (Ali et al., 2014; Moore-Morris et al., 2014, 2016). Besides, endothelial-lineage tracing (Moore-Morris et al., 2014) and bone marrow transplant (Ali et al., 2014) argue against contribution of EndoMT and infiltrating hematopoietically derived fibroblasts to the pool of activated fibroblasts and cardiac fibrosis under hemodynamic stress. Similarly, another study employing periostin-lineage tracing provided evidence of myofibroblasts arising from the tissue-resident fibroblasts (Kanisicak et al., 2016).

Several signaling pathways have been implicated in fibroblast activation (Travers et al., 2016). In addition, bidirectional fibroblast-cardiomyocyte crosstalk plays an important role in myocardial hypertrophy and fibrosis. For instance, TGF-β, a potent mediator of fibroblast activation and cardiac fibrosis, is induced in fibroblasts and cardiomyocytes by Ang II via AT1 receptor (Campbell and Katwa, 1997; Schultz Jel et al., 2002). In fibroblasts, TGF-β activates the expression of profibrotic genes by binding to the type I TGF-β receptor and phosphorylation of Smad2/3, which subsequently bind Smad 4 and translocate to the nucleus. In cardiomyocytes, TGF-β binds to the type II TGF-β receptor and promotes cardiac hypertrophy by the activation of TGF-β-activated kinase-1 (TAK1) and induction of hypertrophic responsiveness to β-adrenergic stimulation (Rosenkranz, 2004). In addition, chronic stimulation of β2-ARs can induce fibroblast proliferation and ECM deposition (Turner et al., 2003). Interestingly, ET-1 is thought to interact with Ang II signaling. Deletion of ET-1 in endothelium prevented the development of cardiac fibrosis and hypertrophic response to Ang II stimulation (Adiarto et al., 2012).

It has become evident that miRNAs are involved in the fibroblast-cardiomyocyte crosstalk. For instance, CTGF, a TGF-β-activated factor, is directly regulated by miR-133 and miR-30 (Duisters et al., 2009). While miR-133 is specifically expressed in cardiomyocytes, miR-30 is expressed in both cardiac fibroblasts and myocytes. The decrease of these two miRNAs in pathological hypertrophy allows CTGF levels to increase. This suggests that cardiomyocyte-released CTGF contributes in a paracrine manner to augment cardiac fibrosis and collagen deposition (Duisters et al., 2009; Care et al., 2007; van Rooij and Olson, 2009). Furthermore, miRNA-enriched exosomes secreted by cardiac fibroblasts are taken up by cardiomyocytes and promote hypertrophy (Bang et al., 2014). MiR-21*, one of passenger strand miRNAs which normally undergo intracellular degradation, was identified as a paracrine signaling mediator of cardiomyocyte hypertrophy.

Recently, cardiac fibroblasts have been appreciated for their cardioprotective potential. For example, IGF-1 produced and secreted by fibroblasts is required for the adaptive response to pressure overload and cardiomyocyte hypertrophy. The transcription factor Krüppel-like factor 5 (Klf5) regulates IGF-1 expression and its haploinsufficiency in fibroblasts attenuates cardiomyocyte hypertrophy and fibrosis, whereas cardiomyocyte-specific Klf5 deletion has no effect on cardiomyocyte hypertrophy (Takeda et al., 2010). Interestingly, the conversion of fibroblasts to functional cardiomyocytes could be enhanced by Akt1. The addition of Akt1 to a pre-established reprograming cocktail boosted the generation of cardiomyocytes and cardiomyocyte hypertrophy potentially through the IGF-1/PI3K/Akt/mTORC1 pathway (Zhou et al., 2015). Furthermore, cardiac repair could be enhanced by cardiac fibroblasts undergoing mesenchymal-endothelial transition to generate de novo endothelial cells. This switch in fibroblast fate appears to be regulated by the transcription factor p53 (Ubil et al., 2014).

It is worth noting that cardiac fibroblasts in the adult heart promote myocyte hypertrophy, whereas during embryonic development they promote myocyte proliferation. The latter is mediated in part by secretion of fibronectin, collagen, and heparin-binding EGF-like growth factors that collaboratively interact and promote cardiomyocyte proliferation via β1-integrin signaling (Ieda et al., 2009). Therefore, a shift in the fibroblast genetic program changes the way fibroblasts communicate with cardiomyocytes in postnatal life versus embryonic development.

Cardiomyocyte Hypertrophy and Immune Cells/Cardiac Inflammation

There is increasing evidence that inflammatory signaling and resident and recruited immune cells contribute to the regulation of cardiomyocyte hypertrophy (Frieler and Mortensen, 2015; Kamo et al., 2015). Cardiomyocytes and non-cardiomyocyte cells in the heart secrete and respond to numerous pro-inflammatory cytokines, but responses are complex, depending on the cell type and pathophysiological circumstances: hypertrophic remodeling, cardiac injury, or heart failure. For instance, in patients with heart failure, the circulating levels of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β correlated with the severity of disease (Testa et al., 1996). Cardiac TNF-α is induced by chronic pressure overload and appears to be involved in cardiac hypertrophy, myocyte apoptosis, inflammatory response, and cardiac dysfunction (Sun et al., 2007). Mice with cardiac-specific TNF-α transgenic overexpression recapitulate some of the features of pathological hypertrophy including cardiac hypertrophy and fibrosis, but they die suddenly of congestive heart failure (Kubota et al., 1997). On the other hand, clinical trials with TNF-α inhibitors, entanercept

(a recombinant human TNF receptor that binds to circulating TNF-α) and infliximab (a chimeric monoclonal antibody against TNF-α), failed to provide any benefit in patients with chronic heart failure (Chung et al., 2003; Mann et al., 2004). In the case of IL-6, various pleiotropic effects confound the understanding of the signaling mechanism (Fontes et al., 2015). For instance, IL-6 infusion alone, in the absence of hypertension, resulted in cardiomyocyte hypertrophy, increased fibrosis, and diastolic dysfunction, whereas IL-6 genetic deletion was protective in Ang II-induced cardiac fibrosis, by interfering with macrophage-stimulated fibroblast production of TGFβ-1 (Ma et al., 2012; Melendez et al., 2010). Furthermore, loss of gp130, a component of the IL-6 receptor complex, impaired the compensatory hypertrophic response and accelerated the onset of dilated cardiomyopathy and cardiomyocyte death during pressure overload (Hirota et al., 1999). IL-1β signaling has been associated with myocardial hypertrophy, cardiomyocyte apoptosis, and cardiac dysfunction and is the most promising therapeutic target to reduce the adverse remodeling in patients with heart failure (Bujak and Frangogiannis, 2009). Interestingly, low levels of IL-1β generated in response to pressure overload caused fibroblast-derived IGF-1 release to activate the Akt pathway and maintain the hypertrophic response in cardiomyocytes during early adaptation to hemodynamic stress (Honsho et al., 2009).

Macrophages also play an important role in the hypertrophic response. In the adult mouse heart, there are 4 distinct macrophage subsets that have different functions and origins (Epelman et al., 2014). During Ang II infusion, expansion of macrophage populations occurs through both local proliferation and monocyte recruitment as early as 2 days, before hypertrophy and fibrosis set in (Epelman et al., 2014). Importantly, Ang II infusion induced a CCR2+, $CD11c^{hi}$ macrophage subset, predominantly derived from circulating monocytes, enriched in genes regulating the inflammasome activity and IL-1β production. In response to pressure overload, $Ly6c^{lo}$ macrophages were specifically increased, while the number of $Ly6c^{hi}$ macrophages remained apparently unchanged (Weisheit et al., 2014). In addition, the depletion of macrophages by intravenous administration of liposomal clodronate in hypertensive Ren-2 rats had no effect on cardiac hypertrophy, but exacerbated cardiac dysfunction, indicating a potential role of macrophages in preserving the cardiac function (Zandbergen et al., 2009). Furthermore, recent evidence shows the contribution of macrophage miR-155 to cardiomyocyte hypertrophy. The gain- and loss-of-function studies showed that macrophage miR-155, not cardiomyocyte miR-155, promotes cardiomyocyte hypertrophy, cardiac inflammation, and dysfunction in response to pressure overload (Heymans et al., 2013). MiR-155-induced downregulation of suppressor of cytokine signaling 1 (Socs1) in macrophages was responsible for the paracrine-induced growth response in cardiomyocytes (Heymans et al., 2013). However, the precise identity of the paracrine signaling remains to be determined.

Recent studies have implicated mast cells in myocardial hypertrophy and cardiac fibrosis. Mast cells reside in the heart and their number is increased in response to pressure overload (Levick et al., 2011). Interestingly, pressure overload increased mast cell infiltration and fibrosis in the atrium, causing atrial fibrillation (Liao et al., 2010). This apparent communication between mast cells and fibroblasts led to the upregulation of PDGFA expression, responsible for fibroblast proliferation and collagen deposition. In contrast, treatment with the mast cell stabilizer cromolyn or bone reconstitution from mast cell-deficient mice prevented the upregulation of atrial PDGFA expression and atrial fibrosis (Liao et al., 2010) Furthermore, mast cell-deficient mice showed significant reduction in chronic pressure overload-induced cardiac hypertrophy and fibrosis and protection against adverse cardiac remodeling and failure (Hara et al., 2002). The effect of mast cells on fibroblast proliferation and ECM synthesis is at least in part mediated by the secretion of chymase and tryptase. Chymase increases TGF-β1 expression and signaling, while tryptase activates protease-activated receptor 2 and MAPK signaling in fibroblasts (McLarty et al., 2011; Zhao et al., 2008).

Conclusions

While cardiac hypertrophy is thought to be an adaptive response to hemodynamic stress, the complexity of molecular signaling congruently and inadvertently may irreversibly cause cardiac dysfunction with chronic exposure to stress. Once it has been said: "no cell is an island entire of itself" and "The constitutional endowment and the environment of a cell are both vital to its performance. An organism cannot be broken down into its component parts and rebuilt step by step as a house may be" (Smithers, 1969). There are no better words to describe the complex paradigm of cardiac hypertrophy. Although physiological and pathological hypertrophies have molecular and functional differences, an important question is whether mechanisms responsible for physiological response can be exploited to the benefit of patients with pathological hypertrophy and heart failure. Exercise training reduces cardiac risk factors and improves the quality of life in a variety of cardiovascular diseases (Johnson et al., 2015). However, it remains to be determined whether exercise can leverage the protective features of physiological response against pathological remodeling in patients with heart failure.

There are intricate conversations among multiple cell types within the cardiac microenvironment. The implications of these interactions are various and affect critical processes of the hypertrophic response: cardiomyocyte hypertrophy, angiogenesis, and cardiac fibrosis (**Fig. 2**). Elucidation of the molecular mechanisms mediating this cellular communication may lead to the discovery of novel therapeutic targets or biomarkers. It is also possible that paracrine factors mediating cellular crosstalk will show the benefit of inducing protective pathways in several cell types and generate a balanced growth response with preserved cardiac function. Despite the fact that there have been major breakthroughs, it is clear that a deeper understanding of cardiac hypertrophy will be critical to achieve exciting advancements in the treatment of cardiac hypertrophy and heart failure.

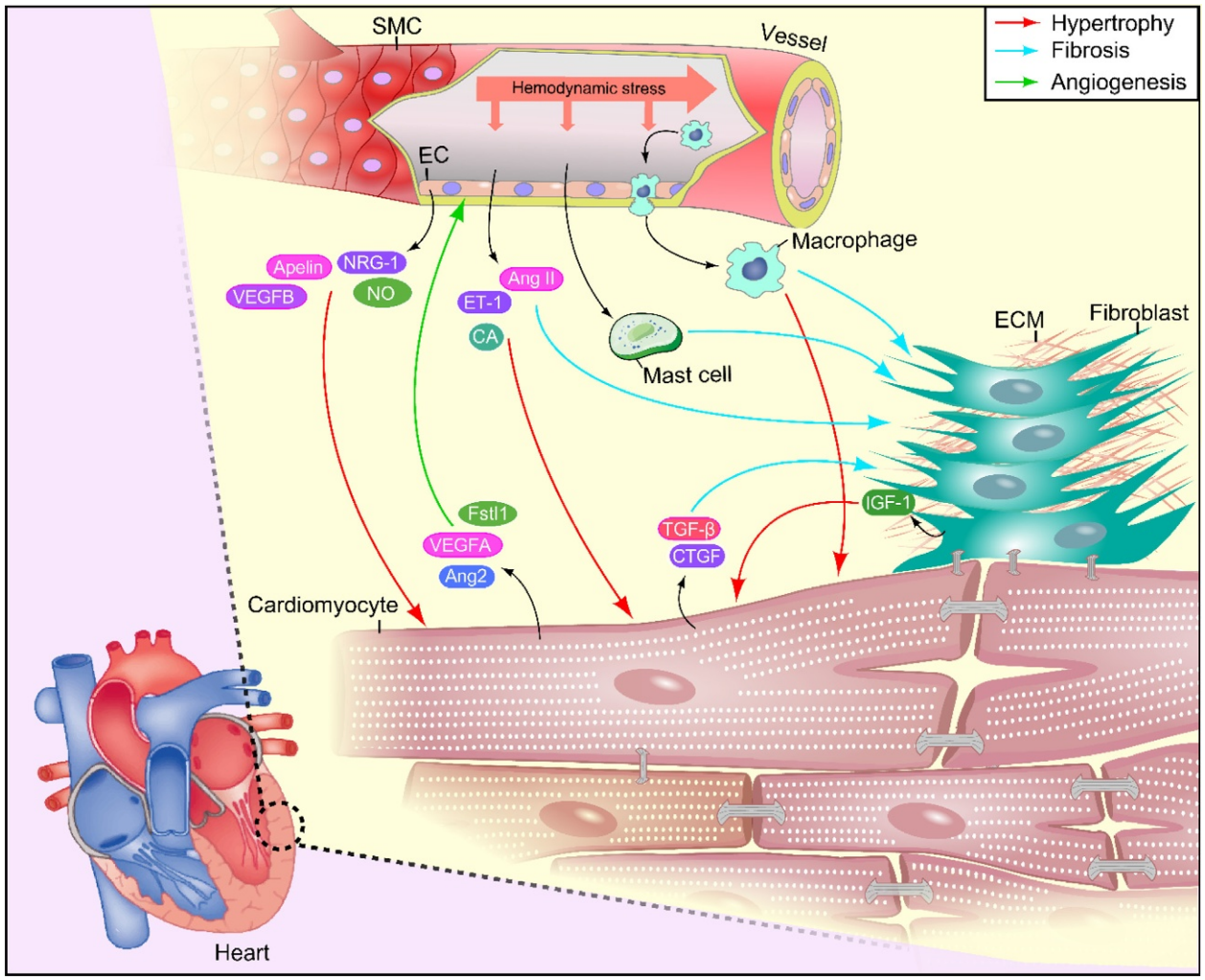

Fig. 2 Cardiac hypertrophy cellular crosstalk paradigm. The propagation of the hypertrophic response to hemodynamic stress extends beyond cardiomyocytes, the major players, to a complex network of signaling events concurrently integrated within cellular crosstalk. Several paracrine factors, known and unknown, drive cellular communications: cardiomyocytes to endothelium, endothelium to cardiomyocytes, cardiomyocytes to fibroblasts, fibroblasts to cardiomyocytes, immune cells to fibroblasts, immune cells to cardiomyocytes. In addition, direct cell–cell contacts via gap junctions may occur between cardiomyocytes and fibroblasts. Overall, this cellular crosstalk contributes to the key processes of cardiomyocyte hypertrophy, angiogenesis, and fibrosis. In particular, angiogenesis is important in meeting demands in blood supplies, oxygen and nutrients of the growing heart, as an imbalance in the extent of hypertrophic and angiogenic responses leads to a progressive deterioration of heart function. Hypertrophic signaling from endothelium and even fibroblasts may provide support for the compensatory hypertrophic response to cardiac stress. However, perturbations in the balance of this network during sustained hemodynamic stress can lead to a variety of pathological events including heart failure as a result of inadequate vascular compensation for myocyte hypertrophy, cardiac fibrosis, and adverse remodeling. *SMC*, smooth muscle cell; *EC*, endothelial cell; *ECM*, extracellular matrix.

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Cardiac Magnetic Resonance Imaging in Heart Failure

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Introduction

Cardiomyopathy, and the heart failure associated with it, is a leading cause of cardiovascular and overall morbidity and mortality in the United States and throughout the world (McMurray et al., 1998; Mozaffarian et al., 2016). Determining the etiology of heart failure is a crucial step in choosing the appropriate management strategy to achieve the best patient outcomes. Advances in cardiac magnetic resonance (CMR) have led to a detailed and comprehensive evaluation of the cardiovascular system. CMR is a valuable tool in diagnosis and management of cardiomyopathy and allows for patient-centered and personalized cardiovascular care.

The comprehensive nature of CMR not only provides detailed evaluation of structure and function but more recently tissue characterization. CMR is the gold standard for quantification of cardiac volumes and function. Valvular heart disease can be evaluated in detail with phase contrast imaging yielding quantification of flow and velocity. CMR is the optimal imaging modality in the assessment and management of congenital heart disease. Angiographic imaging, with its recent advances in flow dynamics, can provide an all-inclusive evaluation of aortopathies and vasculopathies. However, the most striking and revolutionary capability of CMR has been tissue characterization. Late gadolinium enhancement identifies focal ischemic and nonischemic scar. T1 mapping allows for quantification of diffuse myocardial fibrosis by measuring the extent of interstitial expansion via extracellular volume fraction. T2 mapping is able to identify areas of edema and inflammation, while T2 star mapping quantifies myocardial iron content. Cutting edge sequences are now evaluating myocardial fibrosis without the use of gadolinium contrast. Together, these CMR techniques provide highly diagnostic and prognostic information, especially in the evaluation of cardiomyopathy. This information is acquired without the use of ionizing radiation, and while some limitations exist in the use of CMR in patients with implanted metallic devices and contrast in those with renal dysfunction, CMR remains a rapidly evolving and cutting edge tool for clinicians in the evaluation of heart failure. This chapter highlights the utility of CMR in diagnosis and management of patients with commonly encountered cardiomyopathies.

Ischemic Cardiomyopathy

Ischemic heart disease is one of the most common causes of heart disease (Mozaffarian et al., 2016). Multiple imaging techniques exist to evaluate ischemic cardiomyopathy; however, CMR is one of the more comprehensive diagnostic strategies. CMR has the capability to identify ischemic myocardium with stress testing, myocardium at risk in the acute setting, localize infarct scar to provide information about viability, as well as identify the presence of thrombus—all in one exam.

The hallmark of ischemic cardiomyopathy is a typical pattern of subendocardial to transmural scar as noted with late gadolinium enhancement imaging (LGE) (Choi et al., 2001). During an acute myocardial infarction, damaged myocytes allow gadolinium to diffuse into the intracellular space, resulting in increased tissue concentration of gadolinium compared with normal myocardium. While in chronic infarction, as necrotic tissue is replaced by collagenous scar, the interstitial space expands, which leads to increased gadolinium tissue concentration. Higher tissue concentrations of gadolinium shorten the T1 relaxation time and thus appear bright, or "hyperenhanced," while viable regions appear black, or "nulled"(Rehwald et al., 2002). The transmural extent of infarction is a key predictor of improvement in contractile function, with 51%–75% transmural extent associated with limited viability and 76%–100% associated with virtually no viability (Choi et al., 2001; Kim et al., 2000). CMR is the only modality that can accurately determine the transmural extent of infarct scar given its high spatial resolution. This is an important distinction as it has been repeatedly shown that revascularization of patients with viable myocardium leads to significant improvement in clinical outcome (Kim et al., 2000). **Fig. 1** demonstrates a transmural myocardial infarction in the mid inferolateral wall consistent with nonviable

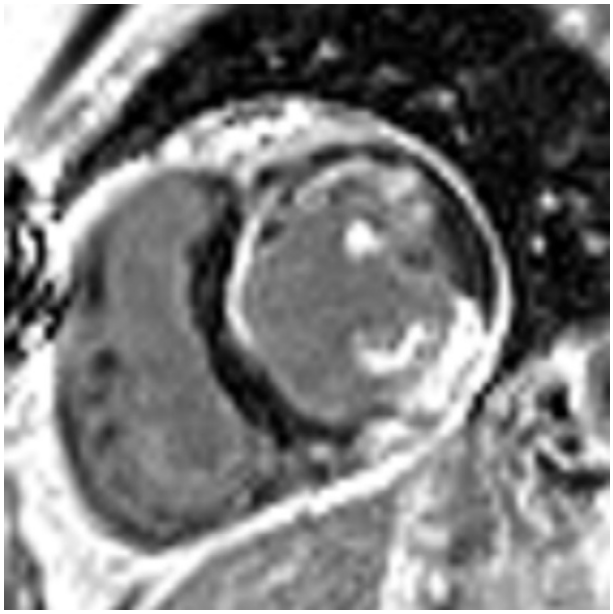

Fig. 1 Subendocardial and transmural myocardial infarction on late gadolinium enhancement short-axis image.

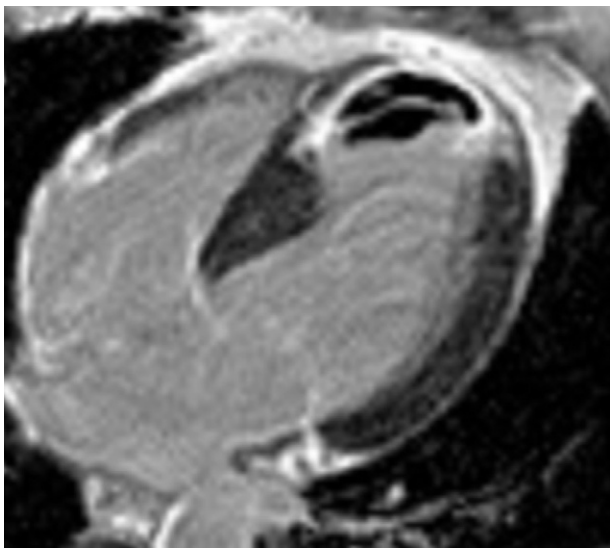

Fig. 2 Horizontal long-axis late gadolinium enhancement image demonstrates myocardial infarction (*white*) and a large apical thrombus (*black*).

myocardium, while the anterior, anteroseptal, and anterolateral walls exhibit a subendocardial infarction involving 50% of myocardial wall thickness suggesting partial viability.

Additional evaluation of the ischemic myocardium can be accomplished with stress CMR (adenosine, dobutamine, or treadmill stress). Multiple studies have shown the superior diagnostic performance of stress CMR as compared with standard imaging (Schwitter et al., 2008; Hamon et al., 2010; Flotats et al., 2012). Stress CMR can demonstrate ischemia with stress-induced wall motion and perfusion abnormalities, which together with LGE viability data paint a thorough picture of the myocardium. CMR also plays a role in the acute setting of a myocardial infarction by identifying the myocardium at risk with T2-based edema imaging. The additional information of the salvageable myocardium can impact the need and timing of revascularization (Klocke, 2010).

An important component of the CMR exam in patients with ischemic cardiomyopathy is the identification of ventricular thrombi. The presence of a ventricular thrombus is associated with larger infarct size, lower ejection fraction, and ultimately major adverse cardiac events (Pöss et al., 2015). LGE imaging has high sensitivity in identifying even small thrombi which appear as a low signal intensity mass surrounded by high intensity structures including the ventricular cavity and surrounding myocardium. Fig. 2 demonstrates significant infarct scar with a large apical thrombus.

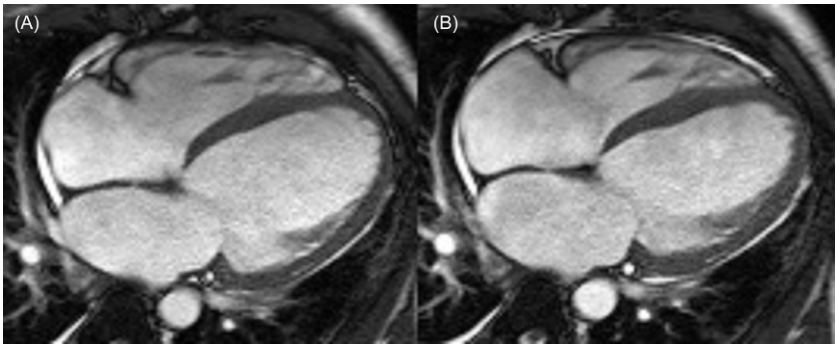

Fig. 3 Horizontal long-axis cine images at end-diastole and end-systole in a patient with dilated cardiomyopathy.

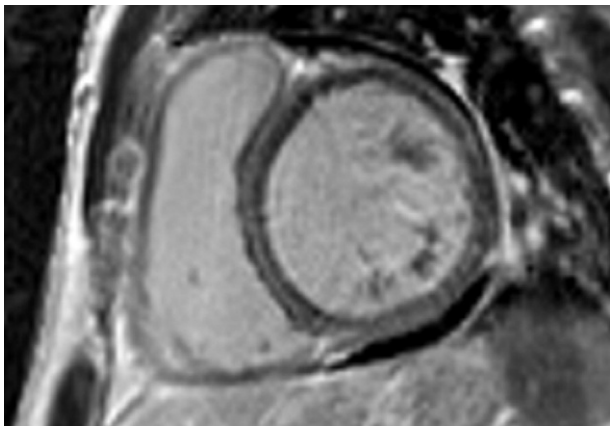

Fig. 4 Short-axis late gadolinium enhancement images with linear midmyocardial septal fibrosis in a patient with dilated cardiomyopathy.

Comprehensive CMR imaging in ischemic cardiomyopathy permits clinicians to confidently make key decisions about the need and timing of revascularization, use of systemic anticoagulation, and tailoring medical and device-based therapy.

Dilated Cardiomyopathy

Dilated cardiomyopathy comprises a large number of etiologies and a broad genetic basis (Haas et al., 2015). The evaluation of nonischemic dilated cardiomyopathy often involves CMR which not only identifies the structure, degree of ventricular dysfunction, but provides important prognostic information about myocardial tissue. The diagnosis is established with a typical LGE pattern of linear midwall fibrosis, particularly present in the septum (Mahrholdt et al., 2005; Almehmadi et al., 2014). **Fig. 3** demonstrates end-diastolic and end-systolic cine frames of a patient with severe biventricular dilation and dysfunction. LGE imaging reveals prominent midwall fibrosis in the septum in the same patient (**Fig. 4**). The presence of midwall fibrosis has high prognostic value and is strongly associated with ventricular arrhythmias, heart failure, and sudden cardiac death (Gulati et al., 2013; Di Marco et al., 2017). These findings are independent of ejection fraction thus raising important questions about appropriate patient selection for implantable cardioverter-defibrillator (ICD) placement (currently only based on ejection fraction). LGE presence could guide selection of ideal candidates for ICD implantation irrespective of the severity of systolic dysfunction.

Additional diagnostic and prognostic information can be obtained from evaluation of diffuse myocardial fibrosis. While LGE can accurately identify focal myocardial replacement fibrosis, it cannot assess diffuse interstitial fibrosis as image contrast relies on the different signal intensities between fibrotic and "normal" myocardium (Mewton et al., 2011). In diffuse nonischemic processes, these differences may not exist. However, T1 mapping can quantify diffuse fibrosis by measurement of the extracellular volume fraction (ECV). An elevated ECV has been shown to be associated with worse outcomes in multiple disease states, including the nonischemic cardiomyopathy population (Zareba et al., 2013).

Hypertrophic Cardiomyopathy

CMR is a powerful diagnostic and prognostic tool in hypertrophic cardiomyopathy (HCM). Assessment of the location and extent of myocardial wall thickness, the presence of myocardial crypts, and visualization of abnormal mitral apparatus can all be

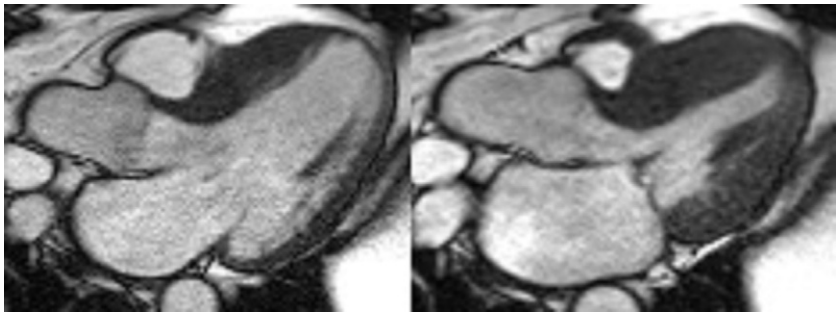

Fig. 5 Three-chamber cine images at end-diastole and end-systole in a patient with hypertrophic cardiomyopathy.

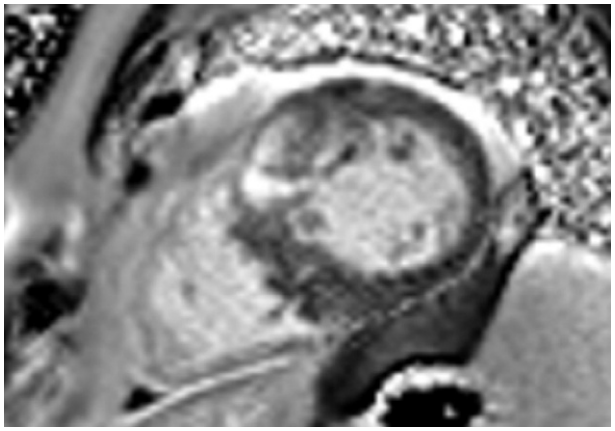

Fig. 6 Short-axis late gadolinium enhancement images demonstrating prominent patchy midmyocardial fibrosis in a patient with hypertrophic cardiomyopathy.

accomplished with cine imaging. Phase contrast imaging can identify the degree of left ventricular outflow tract obstruction secondary to the hypertrophic process. The presence and amount of myocardial fibrosis can be measured by LGE. **Fig. 5** shows prominent asymmetric septal hypertrophy with a maximum wall thickness of 2.5 cm in a patient with genetically confirmed HCM. Short-axis LGE images demonstrate extensive midmyocardial septal fibrosis—a poor prognostic indicator (**Fig. 6**).

The presence of LGE is an independent predictor of adverse outcomes in HCM patients. Studies have demonstrated a significant relationship between LGE and cardiac as well as all-cause mortality in this patient population (Green et al., 2012; Chan et al., 2014). Quantification of LGE can also aid in risk stratifications—an important study demonstrated that LGE $\geq$ 15% of LV mass is linked to a twofold increase in the risk of sudden cardiac death in otherwise lower risk patients. CMR allows for earlier diagnosis and identification of high-risk HCM patients. HCM thought leaders advocate for including CMR fibrosis assessment in the overall risk stratification of HCM patients when deciding on primary prevention ICD placement (Maron and Maron, 2016).

Cardiac Sarcoidosis

The evaluation of cardiac sarcoidosis with CMR has added significant value to the diagnosis and management of a disease process whose patchy cardiac involvement can be difficult to confirm with myocardial biopsy. Cardiac sarcoidosis can lead to conduction abnormalities, arrhythmias, heart failure, and sudden cardiac death. Left ventricular ejection fraction has traditionally been the major predictor of mortality in patient with cardiac sarcoid (Hulten et al., 2016).

CMR can evaluate both the reversible inflamed and irreversible scarred myocardium in patients with cardiac sarcoidosis. T2 mapping allows identification of active inflammation which has been shown to correspond to electrocardiographic and arrhythmic abnormalities (Crouser et al., 2014). **Fig. 7** demonstrates abnormal signal in the inferior wall on T2 mapping, indicating inflammation in a patient with cardiac sarcoid; while **Fig. 8** reveals normalization of T2 signal after immunosuppressive therapy with prednisone and methotrexate in the same patient.

The typical LGE pattern in cardiac sarcoidosis involves patchy midmyocardial fibrosis, particularly in the inferolateral wall and septum; however, other patterns can be seen (Hulten et al., 2016). The presence of LGE has been shown to be associated with ventricular arrhythmias and mortality in patients with cardiac sarcoidosis despite a normal ejection fraction (Murtagh et al., 2016). Recent consensus statements advocate for including myocardial inflammation and fibrosis data in appropriate ICD selection in sarcoidosis patients with cardiac involvement (Birnie et al., 2014).

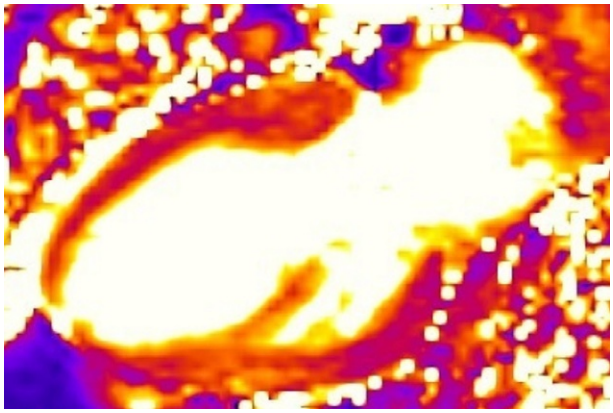

Fig. 7 Vertical long-axis T2 maps demonstrating abnormal T2 signal in the inferior wall consistent with myocardial inflammation in a patient with active cardiac sarcoidosis.

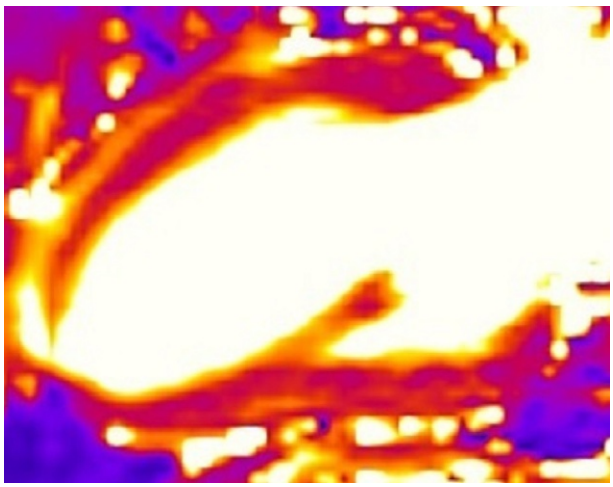

Fig. 8 Vertical long-axis T2 maps demonstrating normalization of T2 signal after immunosuppressive therapy in a patient with cardiac sarcoid.

Chemotherapy-Induced Cardiomyopathy

Chemotherapy-induced cardiomyopathy is a well-described form of left ventricular dysfunction that occurs as a result of myocardial toxicity related to chemotherapy. Various cardiotoxic chemotherapeutic agents have been implicated; however, anthracycline-induced cardiotoxicity is the most well studied. CMR can accurately and reliably assess left ventricular function with cine imaging without the need for gadolinium-based contrast (Armstrong et al., 2012). MR is also ideally suited for the serial nature of cardiotoxicity assessment given the lack of radiation needed to image.

Late gadolinium enhancement in chemotherapy-induced cardiomyopathy can demonstrate midwall fibrosis of nonischemic etiology. However, earlier detection of cardiotoxic myocardial tissue changes provides an opportunity for prompt therapeutic intervention. Studies have shown that patients treated with anthracyclines have abnormal T1 mapping, particularly higher native T1 times and elevated ECVs, in the absence of left ventricular dysfunction (Jordan et al., 2014; Neilan et al., 2013). Furthermore, these elevations were shown to be independent of underlying cancer or cardiovascular comorbidities, but rather related to the cardiotoxic cancer treatment regimen (Jordan et al., 2016). As CMR continues to become part of routine clinical practice, further studies will determine how myocardial tissue changes can be modified.

Cardiac Amyloidosis

Cardiac infiltration in amyloidosis results in a restrictive cardiomyopathy leading to heart failure, ventricular arrhythmias, and death. While the genetics of amyloidosis are fairly well-understood (Gertz et al., 2015), the diagnosis of cardiac involvement had in the past relied on clinical suspicion and biopsy. The prognosis varies based on the type of cardiac amyloidosis with life expectancy ranges of 5 months to 5 years (Kyle et al., 1996; Pinney et al., 2013). Current therapeutics, including targeted chemotherapy along

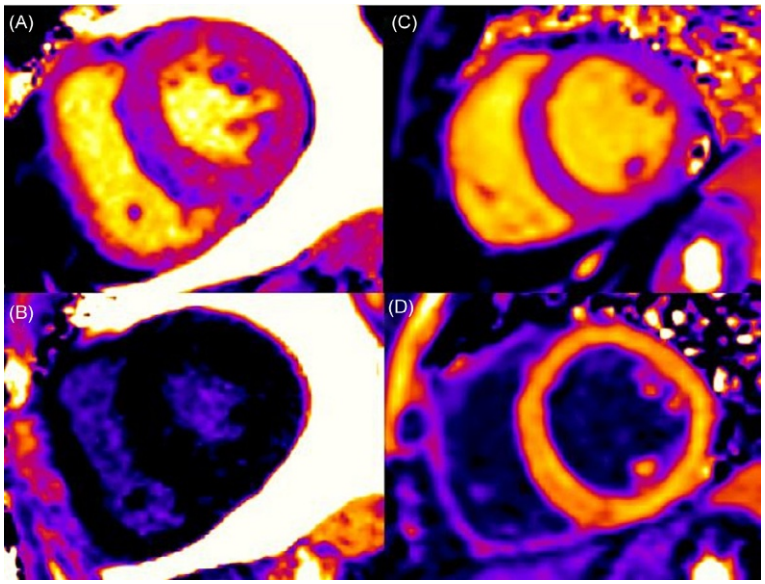

Fig. 9 Pre- and postcontrast T1 maps (MOLLI sequence) in a patient with cardiac amyloidosis (panels A and B) and a healthy individual (panels C and D).

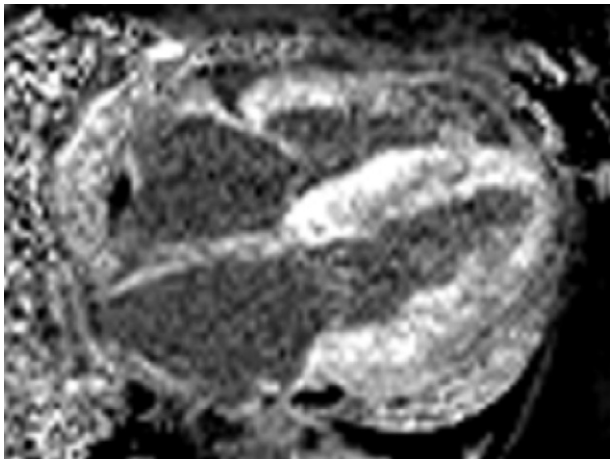

Fig. 10 Diffuse myocardial hyperenhancement and characteristic dark blood pool in a horizontal long-axis late gadolinium enhancement image classic for cardiac amyloidosis.

with heart failure medications, provide variable response rates; however, earlier detection is thought to improve outcomes. The amyloid protein deposits in the myocardial interstitium, thereby expanding the extracellular matrix. CMR is uniquely suited for the diagnosis of cardiac amyloid given the ability to quantify the extracellular space via the measurement of extracellular volume fraction with T1 mapping (Moon et al., 2013). ECV is significantly elevated in cardiac amyloid and also highly prognostic (Banypersad et al., 2013, 2015). **Fig. 9** demonstrates the striking difference between pre- and postcontrast T1 maps in a patient with cardiac amyloid (panels A and B) and a healthy individual (panels C and D).

A distinct LGE pattern is seen with cardiac amyloid with prominent and often diffuse myocardial hyperenhancement which can be circumferential, transmural, or subendocardial (Syed et al., 2010). Different subtypes of amyloid can also present with different LGE patterns (Dungu et al., 2014). **Fig. 10** demonstrates diffuse hyperenhancement (biventricular and biatrial) in a patient with cardiac amyloidosis.

Arrhythmogenic Right Ventricular Cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is caused by an autosomal dominant genetic mutation in desmosomal proteins, resulting in fibrofatty replacement of the myocardium leading to wall thinning and aneurysm formation (Basso et al., 2009). This abnormal myocardial structure leads to ventricular dysfunction and potentially fatal arrhythmias. While ARVC was historically felt to be isolated to the right ventricle, more evidence suggests possible left ventricular involvement. CMR is the imaging

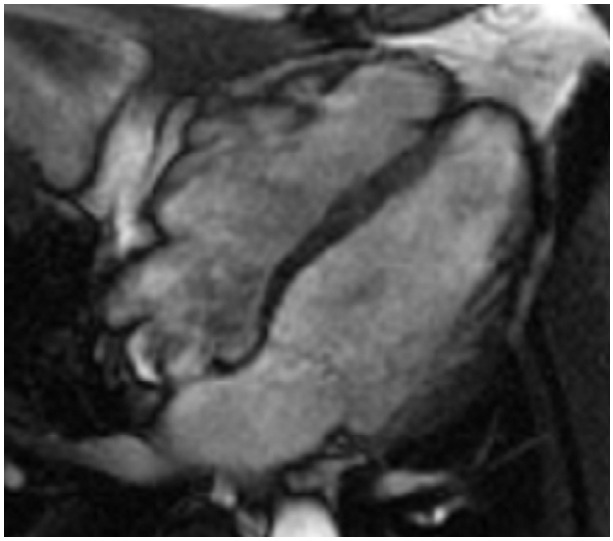

Fig. 11 Horizontal long-axis cine image at end-diastole in a patient with ARVC showing RV dilatation and multiple irregularities and aneurysms of the RV free wall.

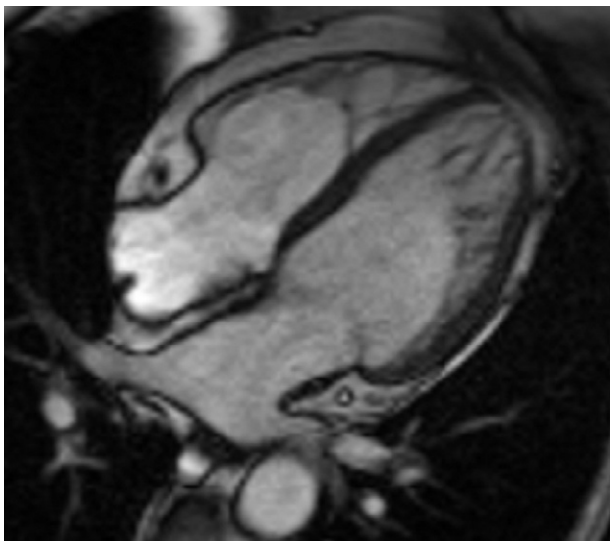

Fig. 12 Horizontal long-axis cine image at end-diastole showing prominent biventricular trabeculae in a patient with noncompaction cardiomyopathy.

modality of choice in ARVC and has been incorporated into the revised Task Force Criteria (Marcus et al., 2010). Cine imaging allows for assessment of right ventricular wall motion, presence of aneurysms, myocardial fat deposition, and precise quantification of right ventricular function (Tandri et al., 2008). **Fig. 11** is an end-diastolic cine frame of a patient with ARVC demonstrating RV dilation and aneurysm formation in the RV free wall.

Left Ventricular Noncompaction

Left ventricular noncompaction cardiomyopathy is characterized by abnormal trabeculae in the left ventricular apex and lateral wall. The disease results from cessation of the normal compaction process of the myocardium during cardiac development, and can result in a variety of patterns of cardiomyopathy, including arrhythmogenic, hypertrophic, dilated, and restrictive cardiomyopathy (Towbin et al., 2015). Patients affected by left ventricular noncompaction cardiomyopathy are at risk for heart failure, ventricular arrhythmias, and sudden death. There is also elevated risk of thrombus formation within the trabeculae due to decreased flow, whether from systolic dysfunction or the morphology of the LV cavity between trabeculae.

CMR provides high-resolution images of noncompacted myocardium and studies have shown that a noncompacted to compacted layer ratio greater than 2.3 has high diagnostic accuracy for distinguishing pathologic noncompaction (Petersen et al., 2005). Numerous LGE patterns have been described including trabecular, midwall, subendocardial, and transmural, which correlate with disease severity (Nucifora et al., 2011). LGE imaging can also identify ventricular thrombi in patients with noncompaction cardiomyopathy. **Fig. 12** is an end-diastolic cine frame demonstrating prominent biventricular trabeculae.

Myocarditis

Myocarditis is an inflammatory disease of the myocardium with an often heterogeneous clinical presentation and etiology. Although infectious etiologies (direct viral infections or postviral immune mediate response) account for the majority of cases, myocardial inflammation can be triggered by any immunologic reaction (Friedrich et al., 2009). Patients often present with a viral syndrome, chest pain, ST segment abnormalities on EKG, and troponin elevation.

CMR plays an important role in diagnosing myocarditis as it can demonstrate the extent of inflammation along with differentiate between ischemic and nonischemic injury. T2 mapping allows for quantitative assessment of myocardial T2 signal which reflects water content. Elevated T2 signal identifies myocardial tissue inflammation and/or edema and has been shown to reliably detect myocardial involvement in patients with myocarditis (Giri et al., 2009; Thavendiranathan et al., 2012). The typical LGE pattern in myocarditis involves midmyocardial to epicardial enhancement. LGE also has significant prognostic value in myocarditis (Grun et al., 2012), with patients exhibiting extensive LGE having poorer outcomes than those with no or minimal LGE. CMR images provide additional insight in myocarditis, including ventricular function, presence of pericardial effusion, and evidence of pericardial thickening and enhancement if concomitant pericarditis is present. **Fig. 13** is a short-axis T2 map demonstrating elevated T2 values in the anterior and lateral walls. LGE images reveal striking enhancement predominantly in the lateral wall (**Fig. 14**).

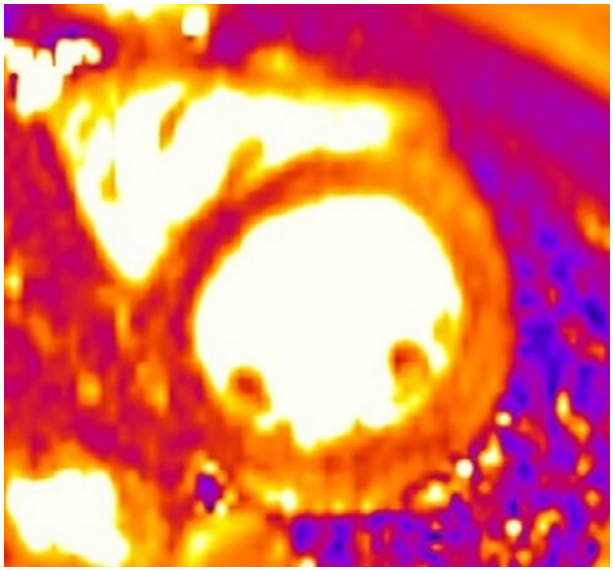

Fig. 13 Short-axis T2 map demonstrating elevated T2 signal in the anterior and lateral walls in a patient with myocarditis.

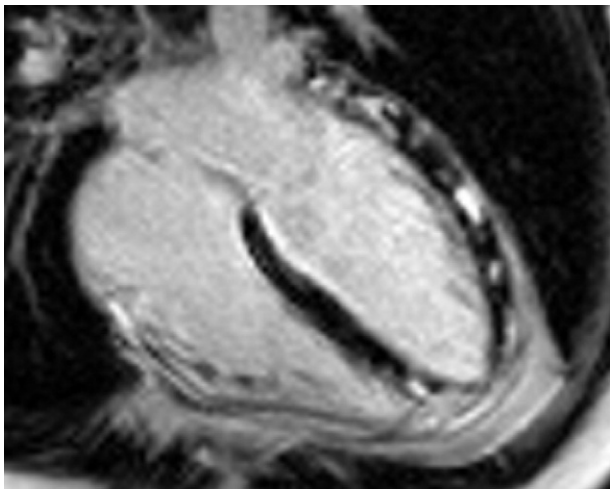

Fig. 14 Horizontal long-axis late gadolinium enhancement imaging demonstrating striking lateral enhancement in a patient with myocarditis.

Takotsubo Cardiomyopathy

Takotsubo, or stress-induced cardiomyopathy, typically presents with signs and symptoms concerning for acute coronary syndrome despite lack of obstructive coronary artery disease. It often follows emotional or physical stress and is possibly thought to be related to an excess of catecholamines leading to myocardial stunning as well as microcirculatory dysfunction (Kohan et al., 2014).

CMR is particularly useful in Takotsubo cardiomyopathy due its tissue characterization capabilities. Cine images demonstrate wall motion abnormalities typically in the mid an apical segments with basal sparing, often in a noncoronary distribution. **Fig. 15** demonstrates this wall motion pattern in end-diastolic and end-systolic images. T2 imaging reveals myocardial edema/inflammation in the affected segments, often without significant LGE burden (Thavendiranathan et al., 2012; Eitel et al., 2011). **Fig. 16** reveals elevated T2 signal in the mid and apical segments. Diagnosis of Takotsubo cardiomyopathy usually portends favorable survival and prognosis; however, the presence of LGE has been associated with more profound heart failure and delayed recovery in this patient population (Naruse et al., 2011).

Cardiac Iron Overload

Chronic iron overload states seen in hemochromatosis or chronic transfusion syndrome can result in iron deposition into the myocardium leading to overt cardiomyopathy. Early disease is characterized by diastolic dysfunction, leading to ventricular dilation with eventual systolic dysfunction, atrial and ventricular arrhythmias (Gujja et al., 2010; Kirk et al., 2009). Myocardial iron uptake

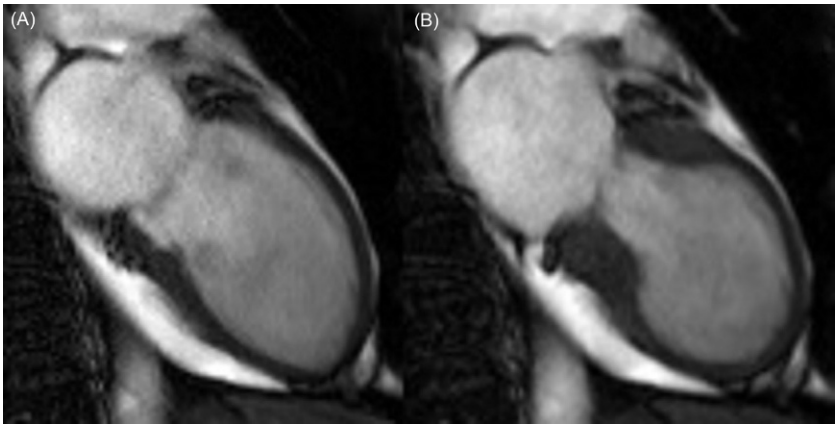

Fig. 15 Vertical long-axis cine images at end-diastole and end-systole in a patient with takotsubo cardiomyopathy.

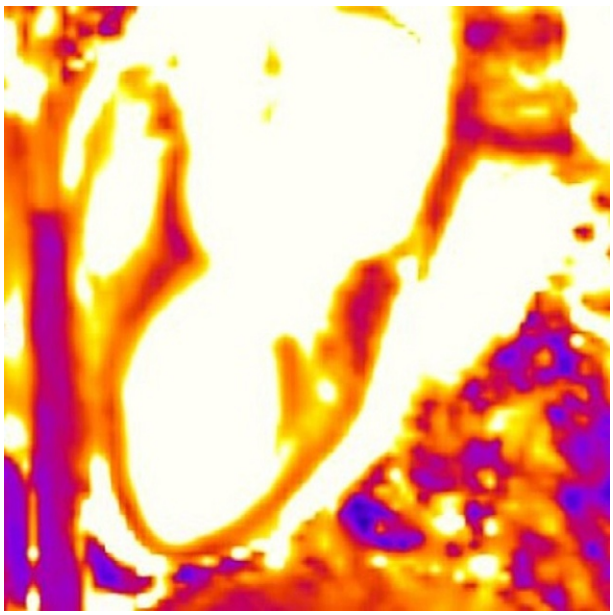

Fig. 16 T2 map in a patient with takotsubo cardiomyopathy demonstrating diffusely abnormal T2 signal in mid and apical segments.

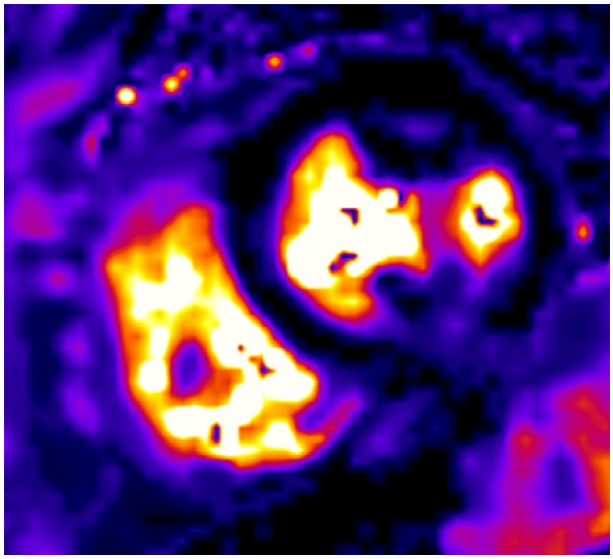

Fig. 17 Short-axis T2* map in a patient with hereditary hemochromatosis demonstrating severe myocardial iron overload.

often lags behind the iron deposition rate in other organs, particularly the liver (Kremastinos and Farmakis, 2011); however, once heart failure ensues prognosis is significantly worsened. Strategies aimed at early diagnosis to institute prompt therapy have had a significant impact on survival.

CMR is especially well suited for early detection and quantification of cardiac and hepatic iron overload as well as for monitoring response to therapy. CMR T2* relaxometry imaging is more reliable than serum ferritin or liver iron measurements in determining the degree of iron overload in the myocardial tissue (Kirk et al., 2009). T2* values <20 ms inversely correlate with ejection fraction, and severe iron overload with T2* values of <10 ms is associated with the development of heart failure and arrhythmias (Gujja et al., 2010; Kirk et al., 2009). **Fig. 17** is a short-axis T2* map demonstrating severe myocardial iron overload with a T2* value of 1.8 ms.

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Cardiac Pacing and Monitoring: Past, Present, and Future

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Introduction

Cardiac pacing has evolved dramatically over the past half century from simple externalized devices that treated life threatening bradycardias to sophisticated, highly programmable platforms to treat cardiomyopathies and heart failure. Electrical stimulation to create cardiac activity was first pioneered in the early 1930s by a prominent cardiologist Albert Hyman who developed the "Hyman Otor" a device with a manual crank generator that created an electrical current conducted through a long needle placed intercostally to stimulate myocardial tissue (Beck et al., 2010). Large externalized pacing devices with myocardial wires were later developed and perfected by C. Walton Lillehei, a prominent cardiac surgeon at the University of Minnesota, and used for postoperative cardiac pacing. It was not until a power outage in 1957 that Lillehei along with an equipment engineer, Earl Bakken, developed the first portable transistorized battery powered pacemaker. On October 8th, 1960, the first self-contained battery powered internalized pacemaker was inserted into Anne Larsson, a patient with complete AV block and Stokes Adams attack (Aquilina, 2006; Beck et al., 2010).

For the past 60 years, despite remarkable breakthroughs in pacing technologies, device size, battery longevity, remote monitoring and lead performance, the overall configuration of the cardiac pacemaker has not changed; one or more leads placed transvenously in contact with myocardial tissue and attached to a pulse generator placed subcutaneously. Nonetheless, innovations in catheter based techniques, energy sources, and miniaturized electronic platforms has brought in a new era in cardiac pacing; the leadless cardiac pacemakers (LCP) comprising of either a multicomponent leadless pacemaker system or a self-contained electrode and generator in a single device (El-Chami et al., 2017). The remarkable evolution of cardiac pacing has been paralleled with an unprecedented development in cardiac rhythm diagnostic tools that went from portable Holter monitors to miniaturized fully implantable loop recorders. In this review we will summarize the basics in cardiac pacing and pacing physiology and moreover highlight advances in pacing modalities (cardiac resynchronization therapy, multipoint pacing, His bundle pacing) and novel pacing technologies (leadless pacing and battery-less devices) in addition to a brief overview on advances in cardiac rhythm monitoring.

Basics of Cardiac Pacing

Pulse Generator and Leads

The pacemaker system comprises two main components; the leads that are placed transvenously in contact with myocardial tissue and the pulse generator or "can" that is connected to the leads and placed subcutaneously typically in the pectoral region. A pacemaker generates an electrical pulse capable of depolarizing the underlying myocardial tissue in addition to sensing any intrinsic cardiac electrical activity. The leads consist of conductor cables and electrodes separated by insulation material (silicone or polyurethane). A side-by-side relationship of the conductor cables denotes a coradial design; whereas, a conductor coil within a conductor coil implies a coaxial design. The electrodes are typically located at the lead tip and more proximally along the lead (ring electrode). The lead tips are attached to the myocardium by a screw in penetrating helix (active fixation mechanism) or via tines projecting from the lead tip that attach to trabeculae (passive fixation mechanism). Newer generation leads consist of steroid eluting

collars, which minimize inflammation and fibrosis at the lead tips that lead to elevated capture thresholds and failure to pace. Whereas pacemakers generate a stable voltage, the leads are typically designed to feature higher resistances (400–1200 ohms) to minimize current drain and battery depletion. The pulse generator in turn is comprised of the electrical circuitry and lithium iodide batteries that became the battery of choice for modern pacemakers owing to the smaller size, production cost, and highly predictable voltage decay and battery life (Mulpuru et al., 2017).

Pacemaker Function

As eluded to earlier, pacemakers not only generate an electrical current to depolarize the underlying myocardium but also sense myocardial electrical activity. The minimum amount of energy needed to depolarize the myocardium is termed capture threshold. A pacemaker stimulus is characterized by its amplitude (volts) and pulse width (milliseconds), the duration over which the stimulus is delivered. Optimizing both these parameters is integral in maintaining battery longevity while minimizing capture of nearby structures. The electrical current delivered is either bipolar or unipolar. With bipolar pacing, the current delivered is between the tip electrode (cathode) and the ring electrode (anode). With unipolar pacing, energy delivered is between the lead tip and the can (anode). Bipolar pacing is generally more desirable because of minimal extracardiac stimulation making bipolar leads the standard in endocardial pacing (Mulpuru et al., 2017; Beck et al., 2010). Sensing, on the other hand is a feature by which the pacemaker depicts myocardial depolarization in proximity to the lead tip. For optimal function a pacemaker must sense 'near-field' (contact) signals and reject 'far-field' (noncontact signals) and repolarization signals. The advent of bipolar sensing, between closely spaced tip and ring electrodes has minimized myopotential oversensing, far-field signals, and electromagnetic interference (EMI), which were present with unipolar sensing (tip to can). In conclusion, the safety and reliability of pacemaker leads exhibit 97%–99% 5-year survival.

Pacemaker Modes

The pacing mode is reported as a 4-letter code, in which the first position identifies the chamber paced (A for atrium, V for ventricle, D for both chambers), the second position denotes the sensed chamber, the third position denotes the device's response to any sensed events; whereby the device will inhibit pacing (I) trigger pacing output (T) or both (D). The fourth letter code indicates whether rate response is on. The rate responsive feature provides chronotropic response. Devices use different sensors to determine rate responsiveness to physiological demands.

The most commonly utilized pacing modes are DDD, DDI, VVI, and AAI. DDD mode, the standard dual-chamber pacing mode, is used when the sinus mode is intact, but AV conduction impaired. Sinus activity is sensed and will trigger ventricular pacing following a programmed AV delay. Rate response (DDDR) is sometimes used when sinus function is impaired. Ventricular-only pacing, VVI(R), is used in patients with chronic atrial fibrillation, or infrequent pauses or bradycardias. The potential for tracking fast atrial rhythms is excluded. Rate response provides chronotropic support when needed. AAI(R) or atrial only pacing, on the other hand, is reserved for isolated sinus node dysfunction with intact AV nodal conduction. DDI mode is utilized in individuals with paroxysmal atrial tachyarrhythmias to prevent tracking of fast atrial rhythms. Asynchronous modes (DOO/VOO) are programmed to avoid recognition of electrical activity, most commonly electrocautery, MRI signals, or other EMI. These modes prevent sensing of extrinsic electrical activity that can otherwise inhibit pacing.

Basic Pacemaker Programming

Right ventricular pacing activates the interventricular septum before the left ventricular lateral wall, which is manifested as a left bundle branch block pattern on the surface electrocardiogram (ECG). This results in LV dyssynchrony, with deleterious effects on left ventricular function and adverse clinical outcomes, including heart failure and increase in mortality (Wilkoff et al., 2002). Pacing-induced cardiomyopathy is a well-recognized consequence of chronic right ventricular pacing, even among patients with preserved baseline ejection fraction (EF) and occurs in as much as 20% of individuals with chronic right ventricular pacing (Khurshid et al., 2014). It is reported that a pacing burden of 20% is associated with pacing-induced cardiomyopathy (Khurshid et al., 2014). Basic programming features of pacemakers are centered on minimizing ventricular pacing. Different features were developed in dual chamber devices that utilize atrial-only pacing with automatic switching to dual chamber pacing with failed AV conduction or algorithms that automatically prolong AV intervals with shortening of these intervals with no intrinsic AV conduction. These various programming algorithms have decreased significantly the rates of right ventricular pacing in patients without complete AV block (Stockburger et al., 2015; Sweeney et al., 2007).

Rate response or rate-adaptive pacing is a feature in modern pacemakers to increase the pacing rate in response to physical or emotional exertion. Rate-adaptive pacing improves exercise capacity in patients with chronotropic incompetence. Accelerometers are the most commonly used in rate adaptive pacing and increase pacing heart rate in response to upper extremity movements. Other physiological sensors utilized in some devices rely on minute ventilation, cardiac contractility, and blood temperature to increasing pacing rate.

Cardiac Resynchronization Therapy

Overview

Cardiac pacing has evolved over the years from treating life-threatening bradyarrhythmias to standard of care in the management of cardiomyopathies and heart failure. Cardiac resynchronization therapy was introduced as a concept to treat heart failure by improving dyssynchrony, where the lateral segment of the left ventricle is both electrically and mechanically delayed than the earlier activated interventricular septum. Dyssynchrony which is manifest with a left bundle branch block on ECG is either interventricular (between the right and left ventricle) or intraventricular (between the septum and lateral LV wall).

Early animal studies in the late 1980s demonstrated the presence of ventricular dyssynchrony with widening of the QRS complex (Burkhoff et al., 1986). Later studies suggested that simultaneously pacing the LV along with the RV, resulted in a more synchronous ventricular activation manifested with a reduction in QRS complex (Foster et al., 1995). Over the years, numerous randomized studies have shown benefit for CRT by improvement of stroke volume, LV contractility, EF, cardiac output, improvement in heart failure symptoms (Bristow et al., 2004; Cleland et al., 2005; Moss et al., 2009; Tang et al., 2010; Abraham et al., 2002; Cazeau et al., 2001), and overall reduction in mortality (Bristow et al., 2004; Cleland et al., 2005; Moss et al., 2009; Tang et al., 2010).

CRT unfortunately has not been shown to be effective in as many as one third of patients, who are nonresponders; therefore, careful patient selection, optimal lead placement, and device programming are integral in managing patients referred for resynchronization therapy (Jaffe and Morin, 2014; Madhavan et al., 2017). Electrocardiographic criteria and severity of heart failure play a central role in patient selection. In many studies, the presence of LBBB was the most powerful predictor for CRT response (Bristow et al., 2004; Tang et al., 2010; Moss et al., 2009; Cleland et al., 2005; Cazeau et al., 2001; Abraham et al., 2002). In addition a wide QRS (>150 ms) predicted the likelihood of response where the wider the QRS was, the greater the likelihood of response. This is reflected in current guidelines for CRT placement, where the presence of a LBBB with a QRS duration of >150 ms is a class I recommendation for resynchronization therapy in patients with clinical heart failure.

Left ventricular lead placement, which is placed transvenously within a coronary sinus tributary, is an important factor for achieving optimal resynchronization. In general, the farther the right and left ventricular electrodes are, the more favorable the CRT response, where posterolateral and lateral positions are preferred to a more apically placed lead that has been associated at times with adverse outcomes. As for device programming, CRT devices are programmed in a fashion to maximize biventricular (BiV) pacing. Heart failure outcomes and mortality correlate with the percentage of BiV pacing where >98.4% BiV pacing has been associated with favorable outcomes (Bleeker et al., 2006). In modern CRT devices, right ventricular sensed events result in triggered pacing of the left ventricle with less favorable hemodynamic effects than "true" BiV pacing. PVCs and rapidly conducted atrial fibrillation frequently result in trigger pacing, and treatment of PVCs and rate control of atrial fibrillation (Ganesan et al., 2012) are two critical strategies to achieve optimal resynchronization therapy.

Multisite and Multipoint Pacing in CRT

Multisite pacing using two LV leads positioned in various branches of the coronary sinus was developed as a means to treat dyssynchrony with early promising results involving improvement of EF, LV dimensions, and symptoms in small nonrandomized studies. This approach was soon abandoned due to the complexity of the procedure, the need for multiple adapters, rapid battery depletion, and the advent of the quadripolar lead. The quadripolar lead is comprised of widely spaced electrodes along the LV lead offering different bi-pole options for pacing from different points along the left ventricle with best capture threshold and optimal delays. The quadripolar lead also laid the foundation for multipoint pacing, which is near-simultaneous pacing from widely spaced electrodes along the multipolar LV lead in a fashion to capture a large area of myocardium. Multipoint pacing has been shown to improve hemodynamic parameters like stroke volume, stroke work, EF, and various diastolic parameters when compared with conventional BiV pacing (Pappone et al., 2014).

Endocardial LV Pacing

Endocardial LV pacing has been proposed as a means for resynchronization therapy to overcome obstacles encountered with traditional epicardial pacing from a coronary sinus branch, including difficult anatomy, lead dislodgement, and phrenic nerve capture. Moreover, LV endocardial pacing allows a choice for optimal lead placement in nonscarred myocardium and a favorable endocardial to epicardial pacing activation resulting in a narrower QRS and improved hemodynamics (Garrigue et al., 2001). Some small studies have demonstrated the feasibility of LV endocardial pacing through leads placed transapically, or through the interatrial or interventricular septum (Betts et al., 2014; Garrigue et al., 2001). This approach to CRT has nonetheless resulted in an increased number of thromboembolic events and mitral regurgitation.

His Bundle Pacing

The nonphysiological effects RV pacing are well recognized notably with an increase in heart failure and atrial fibrillation (Sweeney et al., 2003; Wilkoff et al., 2002). Cardiac resynchronization therapy has limited benefits in a number of patients including those with non-LBBB and mild heart failure. His bundle pacing has evolved as an attractive pacing modality, as the most physiological means for

pacing. Capture of the His bundle at high pacing outputs recruits the right and left ventricular conduction system resulting in a narrow paced QRS complex. His bundle pacing is typically performed with a small caliber active fixation lead through a specialized delivery sheath delivered to the membranous septum proximal to the tricuspid valve. His bundle pacing has been successfully performed with or without concomitant mapping of the His bundle location or placing a backup RV pacing lead (Sharma et al., 2015). Procedural success has been as high as 80%–90% with the use of newer tools (Sharma et al., 2017) and the lead dislodgement rate has been comparable with conventional pacing leads (Zanon et al., 2011). His bundle pacing has also been proposed as an alternative therapy for cardiac resynchronization therapy in individuals with cardiomyopathy and LBBB (Lustgarten et al., 2015).

Leadless Cardiac Pacing

Despite breakthroughs in pacemaker and lead designs along with increasing complexity of pacing algorithms, the overall paradigm of the permanent pacemaker remains to be transvenous lead(s) attached to a generator implanted typically in the pectoral region. Lead related complications including lead failure, venous occlusion and thrombosis, valvular trauma, myocardial perforation, and infection are well recognized. In theory, the advent of a complete intracardiac device would mitigate many of these lead related complications something that was recognized over 20 years ago. To date, there are two distinct strategies to leadless pacing; the single-component platform and the multicomponent platform.

The single component pacemaker system comprises of the pulse generator, electrodes, and sensors in one compact capsule delivered via a catheter system through a transfemoral venous approach into the right ventricle. The only FDA approved device is the Micra (Medtronic Inc., Minneapolis MN) Transcatheter Pacing System (TPS) that has been widely studied. The TPS, which weighs approximately 2 g with a volume of 0.8 cc, is functionally similar to a conventional pacemaker with a rate adaptive accelerometer and battery longevity of 12 years. The device is delivered on a catheter system through a 23F femoral venous introducer and navigated into the right ventricle with a steerable mechanism. The TPS is then fixed into the right ventricle using four electrically inert nitinol tines. The tether is thereafter cut and the delivery system is removed. The device has been compared to a cohort of conventional transvenous pacemakers with favorable short and intermediate complication rates with an overall efficacy rate of low and stable capture thresholds of >90% (Reynolds et al., 2016). Another single component leadless system that has also been studied is the Nanostim (St. Jude medical, St. Paul MN) LCP with early promising results (Reddy et al., 2015), though this system awaits FDA approval.

The experience with the multicomponent pacemaker system is largely from the WiSE CRT platform (Auricchio et al., 2014). It consists of a subcutaneous pulse generator coupled with a receiver electrode implanted in the LV endocardium. Candidates for the WiSE CRT system have an existing conventional RV pacing device. The pulse generator detects a right ventricular pacing pulse from the existing device (pacemaker or defibrillator) and delivers ultrasonic energy to the LV electrode, which converts ultrasound energy emitted from the pulse generator into pacing pulses. The WiSE CRT trial was terminated early due to safety concerns (Auricchio et al., 2014) yet design modifications to the device and the delivery system have addressed the procedural complication rate; nonetheless, the overall efficacy of the device is yet to be determined (Miller et al., 2015). Another multicomponent system utilizing electromagnetic energy (induction energy) from a transmitter located subcutaneously to a receiver electrode has been studied in animal models, yet no human data is available to date (Wieneke et al., 2009).

Cardiac Rhythm Monitoring

Cardiac rhythm monitoring has long played a central role in diagnosis of cardiac related syncope, monitoring of certain arrhythmias and symptomatic events correlating with cardiac rhythm disturbances. Lately, the role of cardiac monitoring has expanded to monitoring suspected atrial fibrillation in individuals with cryptogenic stroke.

Ambulatory Holter monitors (24 or 48 h) provide continuous ECG monitoring through three leads attached to a patient's chest. The two main advantages to Holter monitors are continuous ECG monitoring and quantification of arrhythmia burden. The brief duration of monitoring and the lack of real time transmissions are among the main drawbacks to Holter monitoring (Zimetbaum and Goldman, 2010). The ZioPatch monitor is a water resistant adhesive patch that provides extended continuous ambulatory electrocardiographic monitoring for 14 days. Similar to Holter monitoring, the ZioPatch lacks real time transmission although symptoms could be accurately logged by recording on the device.

Loop recorders, with “loop memory”, on the other hand continuously analyze the rhythm and store data a few minutes before arrhythmia onset or once activated by the patient. They can reliably correlate symptoms with arrhythmia as well as exclude an arrhythmia as the cause of symptoms. External loop recorders (ELR) are attached to a patient's chest and monitor the rhythm for a maximum of 30 days. Internal loop recorders (ILR) are small devices that are implanted subcutaneously in the left parasternal region by a minimally invasive procedure (Giada et al., 2012). Newer ILRs have been miniaturized to the size of a paperclip with a battery life of 3 years, yet allow registration along one ECG lead making arrhythmia interpretation difficult in some cases. Data is transmitted remotely through a monitor that wirelessly connects with the ILR. ILRs are commonly used in clinical practice in the management of patients with syncope and early detection of atrial fibrillation in patients with cryptogenic stroke. The ISSUE-2 trial showed that therapy guided by ILR findings led to a significant reduction in the number of syncopal events per year (92% relative risk reduction) (Brignole et al., 2006). Likewise, the CRTSTAL AF trial showed that ILRs were superior to conventional 24-h monitoring in detection of atrial fibrillation in patients with cryptogenic stroke (Sanna et al., 2014).

Mobile Cardiac Outpatient Telemetry (MCOT) is an external ambulatory monitor that monitors patients for 30 days providing continuous analysis of the ECG and real-time transmission of every event. Despite the fact that recent ELRs have auto-triggered and symptom-triggered features, episodes are stored in devices until transmitted or downloaded. ELRs also do not have the capability to record and store all 30 days of ECG information making real-time MCOTs advantageous to other forms of outpatient cardiac monitoring (Rothman et al., 2007).

In conclusion, cardiac pacing has come a long way since the early days of treating life threatening bradycardias and has evolved over the years as a treatment strategy in individuals with heart failure. Leadless pacing has catapulted the field into a new era. The future of cardiac pacing lies with miniaturizing the technology and the lead that once stood as the cornerstone for pacing will soon become obsolete.

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Cardiac Regeneration and Stem Cells as Therapy for Heart Disease

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Introduction to Heart Regeneration and Stem Cells

Promoting heart repair and regeneration after injury is a long sought-after target among clinical and basic scientists; some consider this the "holy grail" for heart failure research. Researchers in the field of regenerative medicine have utilized unique model systems in lower vertebrates such as zebrafish and amphibians to study heart regeneration. These have been helpful in investigating the regenerative capabilities of the heart but the difficulty arises when similar research is attempted in mammals. In mammalian species, heart regeneration after injury has been found to occur primarily in early stages of life. After the adult heart has completed development, the heart appears to be incapable of regenerating large areas of damage such as that caused by a heart attack. This has led to considerable effort and attention on the hypothesis that adult progenitor or stem cells can be manipulated to achieve the ultimate goals of restoring damaged myocardium and improving cardiac function in patients with heart injury and heart failure. There has been considerable success using stem cells as therapy in other clinical applications, which has helped to accelerate this line of investigation.

Stem cells by definition have two major roles, the capability of self-renewal and their capacity to differentiate into a variety of cell lineages. Many types of stem cells have been utilized as therapies to treat a variety of diseases and disorders. The most widely known use of stem cells clinically is bone marrow or stem cell transplantation for hematological disorders and cancers such as aplastic anemia and leukemia, respectively. The first successful stem cell transplant in humans was performed in the 1950s by Dr. E. Donnall Thomas to treat leukemia in a patient who had a perfect donor match from his/her identical twin. In the 1950s this was considered radical, but decades later stem cell therapy has become quite common in certain clinical situations. The field of cardiovascular medicine has the opportunity to learn from the work performed in these other disciplines.

Stem cell therapy introduces either allogeneic or autologous stem cells to the body to repair the damaged tissue, regenerating and rejuvenating the organ in need of repair. Allogeneic stem cell transplantation uses stem cells from either a matched relative or unrelated donor while autologous transplantation utilizes stem cells harvested from the patient receiving the stem cell therapy. Currently, clinical trials of stem cell therapy for heart regeneration more commonly use autologous stem cell transplantations over allogeneic transplants. Both sources of stem cells have their own combination of advantages and disadvantages. Autologous stem cell transplants can limit side effects and complications that come with allogeneic transplantations such as graft versus host disease, a disease where the donor stem cells (graft) target the patient's (host's) normal tissues as foreign to the body. A limitation of autologous stem cell transplantation is patient age and wellness. For example, clinical comorbidities such as diabetes as well as age can negatively impact the likelihood that harvested cells will engraft. Thus allogeneic cell transplantation brings forth the benefit of receiving cells donated by a young and healthy donor. This observation has obvious implications for use of autologous stem cell for treatment of cardiovascular diseases. Here we will discuss multiple facets of stem cell therapy including the types of stem cells used, how the stem cells work in heart rejuvenation, and the direction the field is going for clinical trials.

How Stem Cell Therapy Works

Types of Stem Cells Studied in Heart Disease

A variety of stem cells have been studied in preclinical and clinical platforms as cardiovascular disease therapy. Stem cells in heart regeneration studies include bone marrow-derived mesenchymal stem cells, hematopoietic stem cells, endothelial progenitor cells,

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and cardiac progenitor cells. Stem cells by definition have the capability to self-renew, as well as the ability to differentiate into mature cardiac cell types such as cardiac myocytes, fibroblasts, endothelial cells, and smooth muscle cells. Introducing stem cells to a weakened heart muscle might allow these cells to differentiate into mature cardiac myocytes that improves muscle strength. Differentiation to endothelial cells can help promote neovascularization of the damaged myocardium and improve cardiac function. A more detailed assessment of these different stem cell types will be discussed below (Table 1).

Bone marrow-derived mesenchymal stem cells

Bone marrow-derived mesenchymal stem cells are adult nonhematopoietic stromal stem cells discovered in the 1970s by A.J. Friedenstein. They are one of the most studied types of cells for therapy of cardiovascular diseases and are thought to be one of the most promising types of cells for treatment. Their multipotent ability to differentiate into multiple mature cardiac cell lineages such as cardiac myocytes, endothelial cells, smooth muscle cells, and cardiac fibroblasts makes them an attractive stem cell for therapy. Extensive studies on bone marrow-derived mesenchymal stem cells support this impression. In addition to these cells having the potential to differentiate into mature cardiac lineages, many studies have focused on their immune modulating functions such as the secreted factors (secretome) these cells produce that work in both paracrine and autocrine mechanisms to regulate cardiac response to injury. The secretome of stem cells will be discussed in more detail below. The immune modulating functions of these specific stem cells include suppression of neutrophil and T cell proliferation, in part by suppressing expression of proinflammatory cytokines as well as increasing production of anti-inflammatory cytokines. A clear benefit of bone marrow-derived mesenchymal stem cells is the ability to harvest these cells from a bone marrow biopsy. This makes these cells fairly easy to obtain. Since these cells are collected through a bone marrow biopsy, if a donor match was available, allogeneic stem cell transplantation could be utilized or the patient could undergo a biopsy and receive an autologous transplant pending his/her wellness status.

Hematopoietic stem cells

Hematopoietic stem cells are the cells which maintain the populations of blood and immune cells. Hematopoietic stem cells can be isolated from blood, bone marrow, and umbilical cord blood. Though these cells naturally reside in the body to repopulate the mature hematopoietic cells of lymphoid and myeloid lineage, studies have investigated their efficacy to repair damaged myocardium. These cells were mostly tested due to the ease of obtaining them. As with bone marrow-derived mesenchymal stem cells, hematopoietic stem cells can be utilized for both autologous and allogeneic cell transplantation due to the relatively noninvasive nature of collecting these cells. Whether hematopoietic stem cells can directly contribute to heart regeneration is controversial. Some studies report the ability of these stem cells to transdifferentiate into cardiomyocytes, but this has not been reproduced by all investigators working in this field. Nevertheless, these cells continue to be investigated and have even moved into clinical trials.

Endothelial progenitor cells

Endothelial progenitor cells are described as a class of circulating cells that can be identified by cell markers similar to those of endothelial cell and hematopoietic stem cell markers. While these cells are very similar to hematopoietic stem cells, they are distinguished by their ability to respond to endothelial cell stimuli such as vascular endothelial growth factor (VEGF), differentiate to mature endothelial cells, and incorporate into as well as form vascular structures. In vivo, these cells have been reported to home to sites of ischemia and injury, where they promote vascular repair through new vessel formation. It is due to this and given that they can be found in the circulation that endothelial progenitor cells are an attractive choice as therapy.

Cardiac progenitor cells

Cardiac progenitor cells (also referred to as cardiac-derived mesenchymal stem cells) are mesenchymal-like multipotent cells isolated from atrial or ventricular myocardial tissue. These cells are highly proliferative, and are capable of differentiating into multiple mesenchymal-derived cell lineages including mature cardiac cells. A major advantage of these cells is that they are derived and isolated from the myocardium; thus they are presumably adapted to the cardiac microenvironment. A major problem with cell

Table 1 Major stem cell types used in stem cell therapy for heart disease

Stem cell type	*Cell surface markers*
Bone marrow-derived mesenchymal stem cell	CD105+CD73+CD90+CD44+CD34-CD45-
Hematopoietic stem cell	CD34+CD90+cKit+Lin-CD38-CD31-
Endothelial progenitor cell[a]	CD34+CD31+CD133+VEGFR2+
Cardiac progenitor cell[b]	CD105+CD31-CD45-

Stem cells are characterized by specific combinations of cell surface markers. Endothelial progenitor cells and cardiac progenitor cells are classified with additional characterization as noted in the footnotes.

[a]It should be noted that in addition to these markers these cells are characterized by their ability to grow ex vivo, especially in response to endothelial stimuli such as VEGF.

[b]These cells also require further analysis such as identification of transcription factors GATA4 and Nkx2.5. The markers listed are the three major markers; others reported in the literature require further analysis and confirmation.

therapies that use cells from noncardiac tissue is that those cells are taken from their stem cell niche and introduced into damaged and stressed cardiac tissue where the signals necessary to stimulate the desired cell responses may or may not exist. Given that cardiac progenitor cells reside locally, harvesting these cells to expand the population ex vivo prior to returning to their host environment theoretically makes these an attractive stem cell choice for therapy. The challenge with these cells is to safely obtain them from a patient, which requires an invasive biopsy from an endovascular or open surgical approach. A subpopulation of these cells is highly proliferative; therefore, cells can be expanded ex vivo prior to reimplantation via another invasive procedure. Thus use of cardiac progenitor cells as therapy is essentially more invasive than using cells that can be harvested from the blood or even bone marrow.

Mixed stem cell therapies

Another area of interest for heart regeneration with stem cell therapy is the use of multiple types of stem cells as a stem cell hybrid. The idea behind this approach is to combine complimentary stem cell types to improve their efficacy and functionality. One such combination involves cotreatment with bone marrow-derived mesenchymal stem cells and cardiac progenitor cells as a way to combine their unique properties. The benefit of the cardiac progenitor cells, as discussed previously, is their familiarity with the microenvironment, whereas the benefit of the bone marrow-derived mesenchymal stem cells is perhaps the secreted factors these cells provide to the local myocardial microenvironment. Another mixed stem cell population have been termed CardioChimeras and CardioClusters (Quijada et al., 2015). These cells are a mix of cardiac progenitor cells and mesenchymal stem cells engineered ex vivo. Though this method uses the same cells others have investigated in combination stem cell therapies, this method has the benefit of allowing for direct cell–cell interactions prior to introduction to the heart. The CardioClusters are a 3D mixture of mesenchymal stem cells, cardiac progenitor cells, endothelial progenitor cells, and fibroblasts that are meant to create their own microenvironment that might improve survival of the implanted cells. Whole bone marrow or bone marrow mononuclear cells are another mixed cell population that has been tested. Whole bone marrow includes a variety of cell types; at least two of the cell types discussed previously (hematopoietic stem cells and bone marrow-derived mesenchymal stem cells) would contribute to the cell combination.

Delivery

One of the challenges of stem cell therapy for heart disease is development of methods to deliver these cells in a way that allows them to incorporate into and survive at the site of injury. In stem cell therapy for blood disorders and cancers, the hematopoietic stem cells are delivered intravenously as the cells are inherently able to move from the blood stream to the bone marrow where they engraft. The two major strategies for delivery of stem cells to the heart are intravenous infusion versus local delivery of stem cells by intracoronary delivery and direct intramyocardial injection of cells. Local delivery by intramyocardial injection can occur via an endocardial catheter or injection into the epicardium with direct visualization of the heart.

Systemic delivery of stem cells with intravenous infusion is much like the methodology of delivering current stem cell therapies to support treatment of malignancies. This delivery method is minimally invasive making it an attractive approach to treat patients. The challenge of this method is to ensure the cells are capable of engrafting in the area of therapeutic interest. Since these cells are delivered intravenously, these cells will passage through the heart, though the first capillary bed they encounter will be pulmonary. In preclinical trials using systemic intravenous delivery, the majority of cells do in fact accumulate in the lungs. There are signals created by damaged tissues that might improve homing. For example stromal-derived factor 1 (SDF1) is induced by tissue injury and appears to activate stem cells within the vicinity to migrate to and repair the damaged tissue. Understanding and manipulating stem cells to respond to local cardiac signals may allow for intravenous delivery of stem cells to succeed.

Local delivery of stem cells to the heart can be performed by two different methods. Studies with intramyocardial injection take the approach of delivering the cells directly to the site of damage. This requires visualization of the myocardium, as in direct access during open heart surgery with direct epicardial injection of cells at or near the site of damage. This method is obviously more invasive and adds a level of risk. Nevertheless, injection of stem cells directly to the cardiac muscle is attractive because of the certainty that one has placed the cells at the site of injury. A similar, though somewhat less invasive, method is to use a catheter-based approach for endocardial injections. This method requires the placement of an intravascular catheter that reaches the left ventricular endocardial space through the aortic valve.

Additionally, through bioengineering methodologies, the use of a hydrogel via patch, sheet, or injectable is another methodology for local delivery of the stem cells (Sepantafar et al., 2016). This method requires seeding the cells within the hydrogel to be placed onto the myocardium during an open heart surgery. The hydrogel can also be engineered to release essential nutrients or factors such as basic fibroblast growth factor (bFGF) which aid in the self-renewal and antiapoptotic capabilities of the stem cells. The hydrogel provides an environment where the cells are able to build a network with the adjoining tissue due to the less toxic environment it has in the damaged myocardium. This method is primarily tested in preclinical trials but is progressing to some Phase I/II clinical trials (Fig. 1).

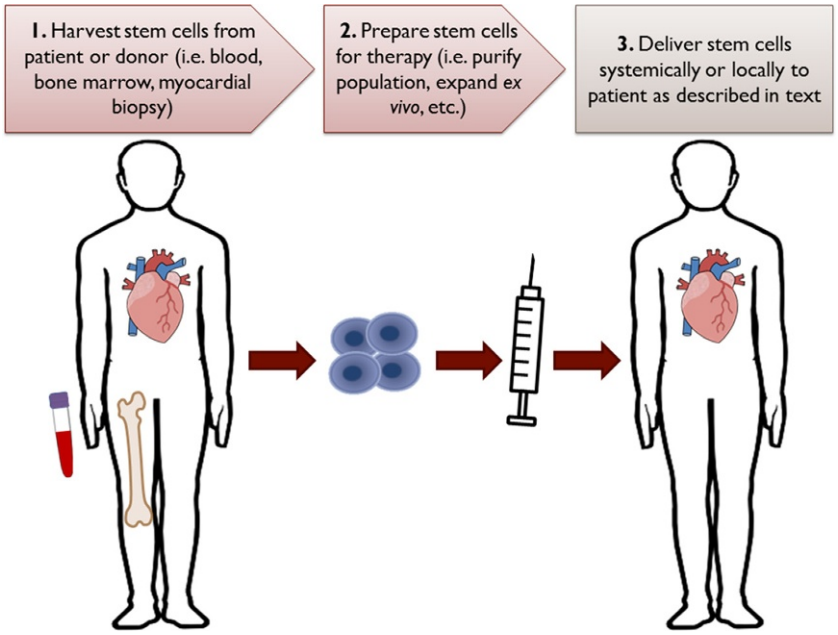

Fig. 1 Stem cell therapy for heart regeneration. Different types of stem cells isolated from bone marrow, blood, and cardiac tissue are currently being tested in clinical trials as treatments for heart disease and heart failure. Stem cells can be harvested from the patient directly or from a matched donor to be injected locally or systemically to the patient who requires stem cell therapy intervention.

Stem Cell Functions: Homing, Engraftment, Survival, Self-Renewal, Proliferation, and Differentiation

The success of stem cells as therapy depends upon the cell's ability to home to the appropriate site for engraftment as well as survive in the new environment (Chavakis et al., 2008). With the exception of cardiac progenitor cells, the stem cells are taken from noncardiac tissues. There are many steps involved in cellular homing including migration and adhesion. Stem cells such as bone marrow-derived mesenchymal stem cells are known to have high expression of integrins on their cells' surface. One of the major functions of the family of integrin proteins is their ability to promote both migration and adhesion. Integrins are commonly activated by the extracellular matrix and couple the matrix to intracellular signaling. The extracellular matrix composition can act via integrins to determine what function the cells take. For example, fibronectin is known to activate integrin alpha 5 beta 1 in mesenchymal stem cells to promote migration via the phosphorylation of platelet-derived growth factor receptor (PDGFR)-β (Veevers-Lowe et al., 2011).

The goal of stem cell therapy is for the stem cells to engraft into the myocardium and contribute to tissue repair. Ideally cells maintain their stemness and proliferative properties and are able to provide the cells necessary for tissue repair over a long time course. It appears that stem cells have autocrine and paracrine mechanisms that are important determinants of their proliferation as well as differentiation. This is regulated in part by the microenvironment these cells reside in. Under normal conditions, a healthy tissue appears to maintain a microenvironment that is optimal for local stem cells involved in tissue maintenance. A diseased heart requiring consideration for stem cell therapy is most likely a toxic microenvironment for stem cells. Thus the success of stem cell therapies requires considerable understanding of and strategies to help stem cells overcome the microenvironmental conditions. Garnering a better understanding of stem cell's needs as well as the challenges cells face within the damaged myocardium is needed for cell therapies to reach their potential (Fig. 2).

Secretome

It is clear that some benefits of stem cell therapy arise from the stem cell's secreted factors which are known to regulate tissue morphogenesis and repair. Stem cells are known to have autocrine and paracrine factors that make up their secretome. The autocrine effects of the secretome promote proliferation of these stem cells driving their self-renewal capabilities. These factors appear essential for the maintenance of stem cell stemness, and regulate their self-renewal such that cells are available for tissue renewal. One such factor is secreted frizzled related protein (SFRP-2) which is reported to promote self-renewal and survival of mesenchymal stem cells but also aid in myocardial repair (Gallina et al., 2015). The paracrine effects of the secretome regulate the other cells within the environment of the stem cells. The secretome of the stem cells regulates the generation of chemokines, cytokines,

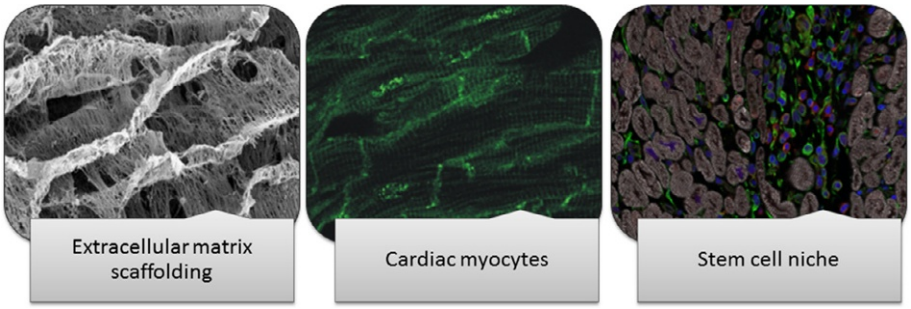

Fig. 2 Stem cell niche in the heart. The heart has many contributors to the structure and microenvironment for stem cells within the tissue. The extracellular matrix (image on *left*) provides the scaffolding between cells to maintain tissue structure while also lending cellular signals. Cardiac myocytes (*middle* image) aid in the contractility of the cardiac tissue while the stem cell niche (image on *right*) in the heart can be a combination of cardiac myocytes with other cell types such as endothelial cells and fibroblasts. The niche for stem cells with the heart can be made of a combination of extracellular matrix, cardiac myocytes, endothelial cells, fibroblasts, and other cells within the cardiac tissue. Disruption of this environment can alter the stem cell function which is common in heart disease.

exosomes and other secreted factors that will promote myocardial regeneration and repair (Gnecchi et al., 2008; Ranganath et al., 2012; Gallina et al., 2015). It should be noted that the stem cells themselves are not the only cells to produce a secretome. The cells within the damaged and surrounding healthy myocardium also produce their own secretome. These factors in combination with the factors produced from the stem cells play a part in the success of this therapeutic approach.

One of the most studied factors within the secretome is stromal-derived factor 1 (SDF1, also known as CXCL12). SDF1 is a chemokine that is important for angiogenesis and cell trafficking. After injury, SDF1 is secreted from cells located within the damaged tissue. SDF1 has been demonstrated to promote migration and homing of stem cells to the injured tissue (Chavakis et al., 2008). SDF1 promotes the recruitment and homing capabilities of stem cells through its receptor CXCR4 which is commonly expressed on stem cells (Zaruba and Franz, 2010). This has led to the concept that SDF1 might be useful as an adjunct to stem cell therapies. SDF1 is one of many factors that have been shown to have beneficial roles in aiding the repair of the damaged tissue. There are many other growth and survival factors in the secretome, such as insulin growth factor for activating nearby cardiac stem cells and having antiapoptotic roles, VEGF contributes to cardiomyocyte and endothelial cell survival, and cysteine-rich angiogenic inducer (Cyr61) promotes angiogenesis (Gallina et al., 2015).

Proinflammatory and anti-inflammatory factors also contribute to the secretome and influence how these cells function. The innate immune system is activated in damaged myocardium, leading to proinflammatory cytokine expression that can alter the function of the stem cells. In response, stem cells themselves are reported to counteract with production of anti-inflammatory cytokines. This is perhaps a mechanism to promote a microenvironment that is required for stem cell survival (Bernardo and Fibbe, 2013). A related article on this topic, *Immune Mechanisms in Cardiac Physiology*, covers more broadly the inflammatory response and which pro- and anti-inflammatory cytokines contribute to the condition of the myocardium following injury.

Overview of Recent Stem Cell Clinical Trials for Heart Disease

Stem cell therapy for heart disease has yet to reach regulatory approval, and is still in development. The first clinical trial to use stem cell therapy for heart failure was reported in 2002 (Vrtovec et al., 2013). Preclinical models and the current stem cell therapies used in the clinic for other noncardiac-related diseases have set the groundwork for stem cell therapy as a method to regenerate the damaged heart. As described earlier, there are many factors that need to be determined in order to find the most successful approach in this therapeutic area. The combination of the type of stem cells, source of the stem cells (allogeneic vs. autologous), mode of delivery, and the clinical problem which needs to be treated all need to be weighed carefully. In **Table 2** a few of the ongoing and recently completed clinical trials are listed, and in **Table 3** select clinical trials are organized to demonstrate the variety of clinical conditions being treated with stem cell therapy as well as the different stem cell therapy approaches in delivery, cell type, and source of cells.

Conclusions

The work before us in both developmental biology research and stem cell transplantation in other areas of clinical medicine are the key to developing successful stem cell therapies for cardiovascular disease and heart failure. Developmental biology researchers have spent decades uncovering the mechanisms to how a functional heart is created through an organized program starting with pluripotent stem cells. Clinical medicine such as the development of bone marrow transplantation techniques for treatment of hematological malignancies lends insight into the importance of proper isolation, HLA typing (if allogeneic transplantation), and

Table 2 Background on select clinical trials utilizing stem cell therapy to treat heart disease

Trial name	*Clinicaltrials.gov ID*[d]	*Trial status*	*Trial phase*	*Type of cells used*	*Allogeneic or autologous*	*Study results*
Prospective randomized study of mesenchymal stem cell therapy in patients Undergoing cardiac surgery (PROMETHEUS)	NCT00587990	Completed	Phase I/II	Mesenchymal stem cells	Autologous	Intramyocardial injection of the stem cells improved overall left ventricular function. Findings should be confirmed with the required placebo group[a]
Allogeneic stem cell therapy in heart failure (CSCC_ASCII)	NCT03092284	Recruiting	Phase II	Adipose-derived stromal stem cells	Allogeneic	Study ongoing
CSCC_ASC therapy in patients with severe heart failure	NCT02387723	Completed	Phase I	Adipose-derived stromal stem cells	Allogeneic	Results not yet reported but has moved on to Phase II (CSCC_ASCII)
Stem cells therapy in advanced heart failure	NCT02871466	Completed	Phase IV	Stem cells, type undisclosed	Autologous	Results not yet reported
Stem cell therapy in patients with severe heart failure and undergoing left ventricular assist device placement (ASSURANCE)	NCT00869024	Completed	Phase I/II	Bone marrow-derived mononuclear cells	Autologous	Results not yet reported
Repetitive Intramyocardial CD34+ cell therapy in dilated cardiomyopathy (REMEDIUM)	NCT02248532	Recruiting	Phase II/III	CD34+ stem cells isolated from peripheral blood (hematopoietic stem cells)	Autologous	Study ongoing. estimated completion January 2018
AutoLogous human CArdiac-derived stem cell to treat ischemic cArdiomyopathy (ALCADIA)	NCT00981006	Completed	Phase I	Cardiac-derived stem cells	Autologous	Early results presented at conference confirm safety and efficacy of this method[b]
The Transendocardial Autologous Cells (hMSC or hBMC) in Ischemic Heart Failure Trial (TAC-HFT)	NCT00768066	Completed	Phase I/II	Mesenchymal stem cells or bone marrow cells	Autologous	Results indicated the transendocardial delivery of both cell types was safe; no definitive results on efficacy but enough evidence to proceed to a larger trial[c]

[a]Heldman et al. (2014).
[b]Takehara et al. (2012).
[c]Karantalis et al. (2014).
[d]Information was taken from www.clinicaltrials.gov; reference Clinical Trial ID for more information on the specific clinical trial.

Table 3 Clinical trial parameters by stem cell type to treat heart disease

Cell type	*Clinical problem*	*Source*	*Delivery*	*Clinicial Trial ID*[a]
Mesenchymal stem cells	Acute myocardial infarction	Autologous	Intracoronary	NCT01392105
	Chronic ischemic left ventricular dysfunction secondary to myocardial infarction	Autologous	Intramyocardial	NCT00587990
	Nonischemic heart failure	Allogeneic	Intravenous	NCT02467387
	Nonischemic dilated cardiomyopathy	Both—one arm of study is autologous the other is allogeneic	Transendocardial	NCT01392625
Hematopoietic stem cells	Chronic heart failure/dilated cardiomyopathy	Autologous	Intramyocardial	NCT00694642
Endothelial progenitor cells	Coronary artery disease/refractory angina	Autologous	Transendocardial	NCT00694642
Cardiac progenitor cells	Ischemic cardiomyopathy	Autologous	Hydrogel with cells and bFGF	NCT00981006
	Ischemic cardiomyopathy	Autologous	Intracoronary	NCT00474461
Mixed stem cell therapy	Ischemic cardiomyopathy	Autologous (mesenchymal stem cells and cardiac progenitor cells alone or in combination)	Transendocardial	NCT02501811

[a]Information was taken from www.clinicaltrials.gov; reference Clinical Trial ID for more information on the specific clinical trial.

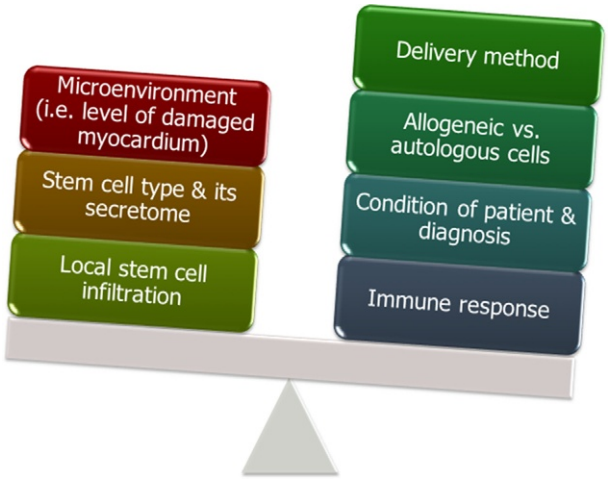

Fig. 3 Balancing barriers to stem cell therapy success. Stem cell therapy success relies on a balance of many contributing factors. The combination such as type of stem cell, delivery method, and clinical diagnosis of the patient all factor into the success of the stem cell therapy. Finding the appropriate combination to balance the pros and the cons is still being determined in basic and clinical research.

delivery of cells to ensure a successful therapeutic. The investigations into utilizing various stem cell types as a therapy for cardiovascular medicine are still in their infancy stages but the value in this therapeutic approach is clear. Cardiovascular medicine needs to identify the proper combination of stem cell type, source of stem cells, and delivery method required for the most successful stem cell therapy to treat the patient's specific clinical diagnosis. Further collaboration between basic and clinical researchers in this field is required to expand our knowledge and fine-tune what is needed for a successful transition from the bench to the bedside (Fig. 3).

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Cardiac Resynchronization Therapy

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Introduction

The prevalence of heart failure is approximately 2% in the population of the western world and despite pharmacological and nonpharmacological therapy it is still associated with severe morbidity and high mortality rates (Ponikowski et al., 2016). Widening of the QRS complex on the surface ECG is found in around 30% of patients with heart failure with reduced ejection fraction (HFrEF) and may indicate the presence of a dyssynchronous activation and contraction pattern (Baldasseroni et al., 2002). It became clear in the 1990s that ventricular dyssynchrony could be reversed by cardiac resynchronization therapy (CRT) and during the past 15 years several randomized controlled trials showed that CRT significantly decreases morbidity and mortality in patients with HFrEF and dyssynchrony. QRS prolongation in HFrEF is mainly caused by left bundle branch block (LBBB), nonspecific QRS prolongation, right bundle branch block (RBBB), and right ventricular pacing (RVP). In the case of LBBB, the interventricular septum is initially activated followed by a further rapid activation of the right bundle branch and finally a delayed activation of the left ventricle (LV) posterolateral wall (**Fig. 1**). During implantation of a CRT device, an additional ventricular pacing lead is positioned in a side branch of the coronary sinus to stimulate LV regions simultaneously with the right ventricle resulting in a homogeneous LV activation and contraction pattern (**Video 1–6**).

Randomized Controlled Trials Establishing the Role of CRT in HFrEF

The first randomized controlled clinical trials investigated CRT efficacy in patients with HFrEF (EF $\leq$ 35%), moderate-to-severe symptoms, evident as New York Heart Association (NYHA) functional class III-IV and electrical dyssynchrony (QRS duration $\geq$ 120 ms). The MUSTIC trial included 67 patients (Cazeau et al., 2001) and significant improvement of some functional endpoints was observed after CRT implantation (**Table 1**.). Two larger randomized controlled trials also showed beneficial CRT effects on morbidity and mortality in similar patients. In the COMPANION trial, 1520 patients were randomized in three arms: optimal medical therapy, CRT with a defibrillator (CRT-D), or CRT without a defibrillator (CRT-P). After a 12-month follow-up CRT-D or CRT-P significantly reduced combined death or hospitalization for any cause (Bristow et al., 2004). The CARE-HF study (Cleland et al., 2005) randomized 813 patients and showed for the first time that during a 30-month follow-up CRT-P was associated with a significant reduction in all-cause mortality as compared to optimal medical therapy. In addition, these large clinical trials reported improvement of LV size and function, peak oxygen consumption, 6-minute walking distance, quality of life scores, and mitral regurgitation severity. Long-term follow-up of the MUSTIC and CARE-HF studies showed lasting beneficial effects of CRT on reverse remodeling (Linde et al., 2002) and further trials investigated whether CRT was able to reduce clinical events in patients with less severe HFrEF (i.e., NYHA class I-II symptoms) and QRS prolongation. In the REVERSE study (Linde et al., 2008), CRT-P or CRT-D was implanted in 610 patients and CRT was turned on in two thirds of them. In the CRT ON group, CRT was associated with reverse remodeling and significantly increased time to first hospitalization but it did not significantly improve symptoms or exercise capacity in these patients with little functional impairment at baseline. The MADIT-CRT trial (Moss et al., 2009) compared CRT-D with ICD alone in 1820 patients with NYHA class I and II symptoms, LVEF $\leq$ 30%, and QRS interval $\geq$ 130 ms. CRT-D implantation resulted in a significant reduction of combined all-cause death or heart failure events during a follow-up of 2.4 years as compared to ICD alone (17.2% vs. 25.3%, $p = 0.001$). This was mainly driven by a reduction in heart

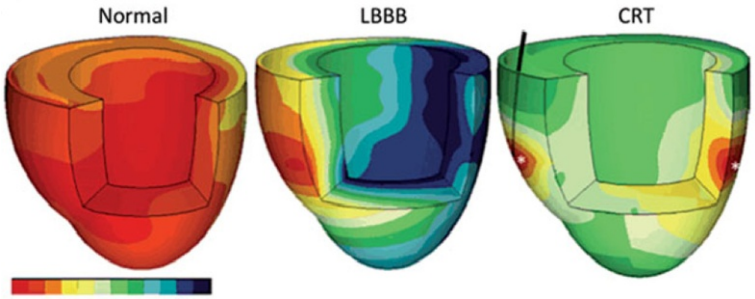

Fig. 1 Homogenous electrical activation pattern in the heart with normal intrinsic conduction (left panel). During LBBB activation (middle panel), septum is activated early followed by a late activation of the LV lateral wall. However, these regional differences in electrical activation are ameliorated by performing biventricular pacing after CRT implantation (right panel) (Gorcsan and Prinzen, 2012). Reprinted with permission from Elsevier.

Table 1 Clinical studies of CRT in patients with HFrEF

Study (reference)	*Inclusion criteria*	*Patient number*	*Study design*	*Follow-up (M)*	*Results*
MUSTIC SR (Cazeau et al., 2001)	EF ≤ 35%, QRS ≥ 150 ms	67	1:1 CRT ON/OFF	3/12	Improved 6MWT, pVO_2, reduced HF hospitalization
MUSTIC AF (Linde et al., 2002)	EF ≤ 35%, QRS ≥ 150 ms	37	1:1 CRT ON/OFF	3/12	Improved 6MWT, pVO_2, reduced HF hospitalization
MIRACLE (Abraham et al., 2002)	EF ≤ 35%, QRS ≥ 130 ms	453	1:1 CRT ON/OFF	6	Improved 6MWT, pVO_2
MIRACLE-ICD (Young et al., 2003)	EF ≤ 35%, QRS ≥ 130 ms	555	1:1 CRT ON/OFF	6	Improved NYHA, VE/CO_2, LV volumes
PATH-CHF (Auricchio et al., 2003)	EF ≤ 35%, QRS ≥ 120 ms	41	CRT/RV pacing/ epicardial LV pacing	12	Improved 6MWT and pVO_2
CONTAK CD (Higgins et al., 2003)	EF ≤ 35%, QRS ≥ 120 ms	290	1:1 CRT ON/OFF	6	Improved NYHA, VE/CO_2, LV volumes
COMPANION (Bristow et al., 2004)	EF ≤ 35%, QRS ≥ 120 ms	1520	1:2:2 OMT/CRTP/CRTD	15	Reduced mortality or hospitalization
CARE-HF (Cleland et al., 2005)	EF ≤ 35%, QRS ≥ 120 ms	813	1:1 OMT/CRTP	30	Reduced mortality and hospitalization
REVERSE (Linde et al., 2008)	EF ≤ 40%, QRS ≥ 120 ms	610	2:1 CRT ON/OFF	12–24	Reverse remodeling and longer time to first hospitalization
MADIT-CRT (Moss et al., 2009)	EF ≤ 30%, QRS ≥ 130 ms	1820	3:2 CRT-D/ICD	28.8	Reduced combined mortality and hospitalization
RAFT (Tang et al., 2010)	EF ≤ 30%, QRS ≥ 120 ms	1798	1:1 CRT-D/ICD	40	Reduced mortality and hospitalization
MADIT-CRT extended (Goldenberg et al., 2014)	EF ≤ 30%, QRS ≥ 130 ms	854	3:2 CRT-D/ICD	84	Reduced mortality

EF, ejection fraction; *M*, months; *ms*, milliseconds; *NYHA*, New York Heart Association Functional Class; *OMT*, optimal medical therapy; *pVO_2*, peak oxygen consumption; *6MWT*, distance covered during a 6-minute walk test.

failure events and all-cause mortality was not reduced. A 7-year follow-up of the study showed, however, that CRT was associated with a reduction in all-cause mortality in patients with LBBB (38% relative risk reduction, $p = 0.003$) (Goldenberg et al., 2014). The RAFT trial (Tang et al., 2010) also showed a mortality benefit from CRT in patients with less symptomatic heart failure. After a follow-up of 40 months, in 1798 patients, the primary outcome—death or hospitalizations for heart failure—was significantly reduced among recipients of CRT-D compared with those with an ICD alone (33.2% vs. 40.3%; $p < 0.001$), as were the secondary outcomes of death from any cause and hospitalizations for heart failure. The RAFT trial included also patients with atrial fibrillation (AF) and patients with HFrEF and RVP for bradycardia indication.

Improving CRT Therapy

Patient Selection

QRS width and morphology

RBBB and nonspecific QRS prolongation

These large randomized trials established the pivotal role of CRT in the management of patients with HFrEF and prolonged QRS. Nevertheless, around 30% of patients do not show echocardiographic signs of reverse remodeling and/or improved symptoms after CRT implantation and they are considered nonresponders. 2/3 of the patients randomized in the clinical trials had widened QRS with LBBB morphology, which means that the major evidence is within this subgroup. The effects of CRT in patients with HFrEF and RBBB are, however, much less known as only 5%–10% of the patients had RBBB in the randomized and controlled studies (Linde et al., 2008). Posttrial subgroup analyses of the large trials and studies on medical registries have highlighted that CRT is more

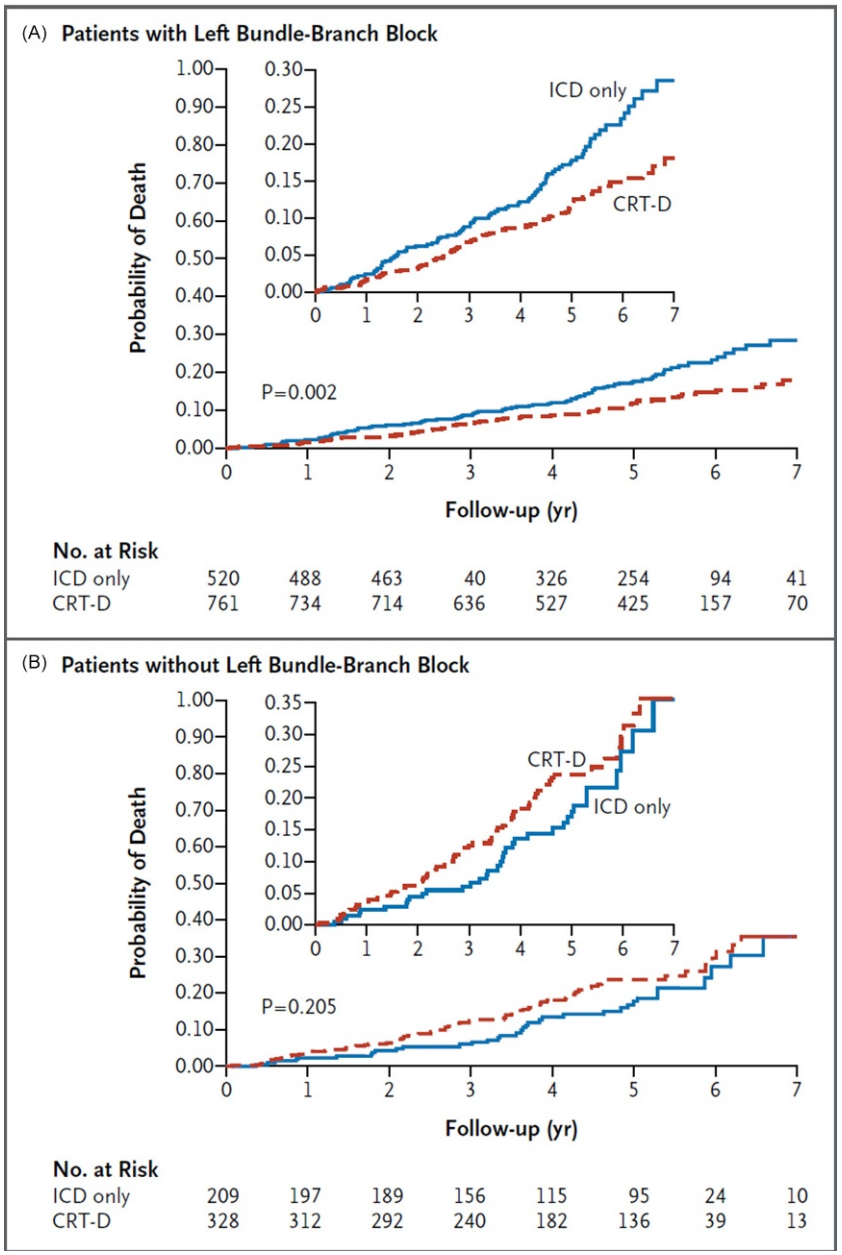

Fig. 2 Long-term analysis of the MADIT-CRT study shows lower cumulative probability of death from any cause in patients with LBBB (A) but not in patients without LBBB (B) (Goldenberg et al., 2014). Reprinted with permission from the Massachusetts Medical Society.

effective in patients with LBBB compared to RBBB (Bilchick et al., 2010; Tang et al., 2010; Peterson et al., 2013; Goldenberg et al., 2014, **Fig. 2**). In contrast, another large case-based meta-analysis (Cleland et al., 2013) found only prolonged QRS duration to be strongly predictive of the response to CRT even in patients with RBBB or nonspecific QRS prolongation. It may be speculated that some patients with RBBB may potentially benefit from biventricular pacing. Especially in ischemic cardiomyopathy, the electrical activation wave fronts in RBBB are heterogeneous and a proportion of patients with RBBB and concomitant LV intraventricular conduction defect have significant LV delay (Fantoni et al., 2005). In one of the early studies CRT had beneficial effects in a proportion of RBBB subjects showing echocardiographic signs of concomitant LV intraventricular dyssynchrony (Garrigue et al., 2001a). Similarly, the presence of left anterior hemiblock among patients with RBBB was associated with greater response to CRT in another study (Chandra et al., 2010). Left ventricular (LV) activation delay was found to correlate well with QRS duration in both LBBB and RBBB (Varma, 2009) and, furthermore, patients with non-LBBB and longer LV activation delay had a lower heart failure hospitalization rate after CRT implantation (Kandala et al., 2013, **Fig. 3**). In a secondary analysis of the MADIT-CRT trial, patients with non-LBBB and prolonged PR interval had a significantly better outcome as compared to non-LBBB patients with normal PR (Kutyifa et al., 2014). Similarly, in a post-hoc analysis of the COMPANION trial prolonged PR interval resulted in a better outcome

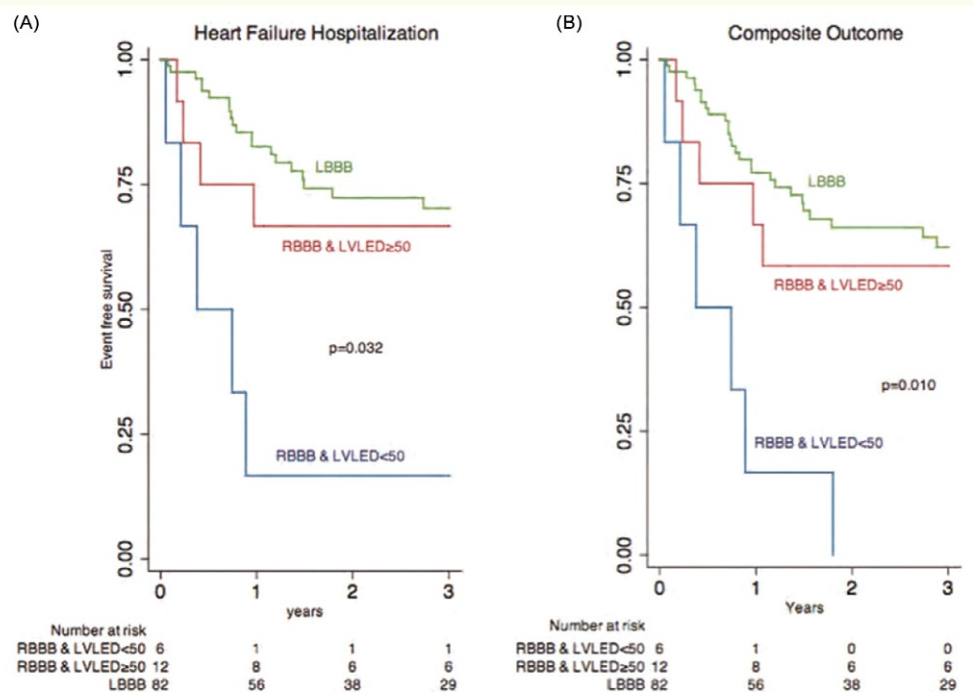

Fig. 3 Event-free survival for heart failure hospitalization (A) and the composite outcome at 3 years (B) comparing LV lead electrical delay <50% versus LV lead electrical delay ≥50% within LBBB and non-LBBB (Kandala et al., 2013). Reprinted with permission from the European Society of Cardiology.

after CRT implantation in both LBBB and non-LBBB patients (Lin et al., 2017). Identification of patients with RBBB or intraventricular conduction defect who may benefit from CRT is being evaluated in a prospective study with individualized targeting of the LV lead in patients with a non-LBBB morphology (ENHANCE CRT; ClinicalTrials.gov NCT01983293).

Definition of LBBB

As LBBB was recognized as the primary target for CRT, there was growing interest how to exactly characterize LBBB morphology. The conventional and widely used criteria (Linde et al., 2008; Moss et al., 2009) of complete LBBB are a QRS duration ≥120 ms, with a QS complex in the V1 lead, and the absence of Q waves in the lateral leads. Some studies have, however, suggested that 1/3 of patients diagnosed with LBBB by conventional electrocardiographic criteria may likely have a combination of LV hypertrophy and left anterior fascicular block (Grant and Dodge, 1956; Vassallo et al., 1984; Auricchio et al., 2004) and not true LBBB. On the basis of computer simulations, stricter criteria for complete LBBB were suggested that include a QRS duration ≥140 ms for men and ≥130 ms for women, along with mid-QRS notching or slurring in two or more contiguous leads of V1, V2, V5, V6, I, and aVL (Strauss et al., 2011, **Fig. 4**). The presence of mid-QRS notching was shown to distinguish true complete LBBB from LV hypertrophy, LV dilatation, and incomplete LBBB in a simulation study (Galeotti et al., 2013). Additionally, a mechanical LBBB pattern in 2D longitudinal strain echocardiography is frequently associated with the presence of notching on electrocardiography (Risum et al., 2013). Refinement of the definition of LBBB morphology with notching and slurring significantly improved prediction of CRT response and clinical outcome compared to the conventional definition (Mascioli et al., 2012; Tian et al., 2013). The inclusion of QRS notching or slurring in the lateral leads might improve patient selection but further studies are necessary to evaluate which electrocardiographic criterion for LBBB correlates best with CRT response.

Narrow QRS

Conversely, later studies consequently showed that CRT is not indicated in patients with narrow QRS. In the ECHO-CRT trial, patients with HFrEF, QRS < 130 ms, and echocardiographic signs of mechanic dyssynchrony by tissue Doppler imaging or speckle tracking were randomized (Ruschitzka et al., 2013). The study was stopped for futility as there was a significantly increased mortality in the CRT ON arm. Patients with a QRS duration of <130 ms may thus even be harmed by CRT regardless of LV dyssynchrony on echocardiography. This applies not only to those with a QRS duration of <120 ms but also to those with QRS 120–130 ms (Steffel et al., 2015).

Ischemic heart disease

Ischemic heart disease (IHD) and hypertension are common underlying causes of HFrEF and approximately 50%–60% of CRT recipients have underlying IHD with or without a previous myocardial infarction. In these patients, the overall outcome is worse and the LV reverse remodeling is less prominent than in nonischemic patients (Linde et al., 2010). Nevertheless, subgroup analyses



Fig. 4 Two ECGs with a complete left bundle branch block according to the classical criteria (A, Linde et al., 2008) and the stricter criteria from Strauss (B, Strauss et al., 2011).

of large randomized clinical trials indicate that patients with underlying IHD have similar outcome benefits as compared to those with nonischemic etiology. In the CARE-HF trial, patients with ischemic etiology had a significant reduction in total mortality after CRT implantation and it was similar as for those with nonischemic cardiomyopathy (Wikstrom et al., 2009). Similar observations were made in the REVERSE trial of patients with milder HFrEF (Linde et al., 2010, **Fig. 5**).

Atrial fibrillation

HFrEF is associated with AF in a substantial proportion of patients ranging from 10% in NYHA I to 50% in NYHA IV (Maisel and Stevenson, 2003). The large randomized clinical trials, however, have almost exclusively included patients with sinus rhythm at baseline. The only exception was an early small, randomized trial (Linde et al., 2002) and a small population of the RAFT trial (Tang et al., 2010). Therefore, the efficacy of CRT is less known in patients with symptomatic HFrEF, wide QRS, and AF. CRT may be less effective in AF as compared to sinus rhythm because it is not able to improve ventricular filling by optimizing atrioventricular coupling. Suboptimal ventricular filling due to delayed atrioventricular timing is frequently present in HFrEF and sinus rhythm. In addition, it may be more difficult to achieve sufficiently high percentage of biventricular pacing in patients with AF due to short RR intervals and fusion beats. Even a small decrease (<5%–10%) in the percentage of biventricular pacing is associated with worse outcome in CRT recipients (Koplan et al., 2009). Some meta-analyses compared the effect of CRT in patients with AF and sinus rhythm. The results from these meta-analyses are not consistent since some indicate that CRT is less effective in AF (Wilton et al., 2011) while others show no difference between CRT efficacy in sinus rhythm versus AF (Upadhyay et al., 2008). Patients with HFrEF and AF can be suitable CRT candidates in two clinical situations. First, patients may have QRS prolongation and heart failure symptoms similarly to patients in sinus rhythm. Second, patients may have AF where pharmacological rate control is difficult and atrioventricular junction ablation is considered. In a smaller clinical trial, CRT implantation after atrioventricular junction ablation was associated with improved LV function and reduction of adverse clinical events as compared to RVP (Brignole et al., 2011). Large-scale clinical studies are necessary in the future to further investigate the evidence of CRT efficacy in patients with AF.

Continuous RVP

Conventional ventricular pacing for bradycardia is delivered by RVP. In a proportion of patients RVP is similar to LBBB in terms of electrical activation and contraction pattern and may compromise systolic function (Prinzen and Peschar, 2002). Most patients without structural heart disease tolerate chronic RVP well. In patients with EF ≤ 40%, however, frequent RVP (>40%) was associated

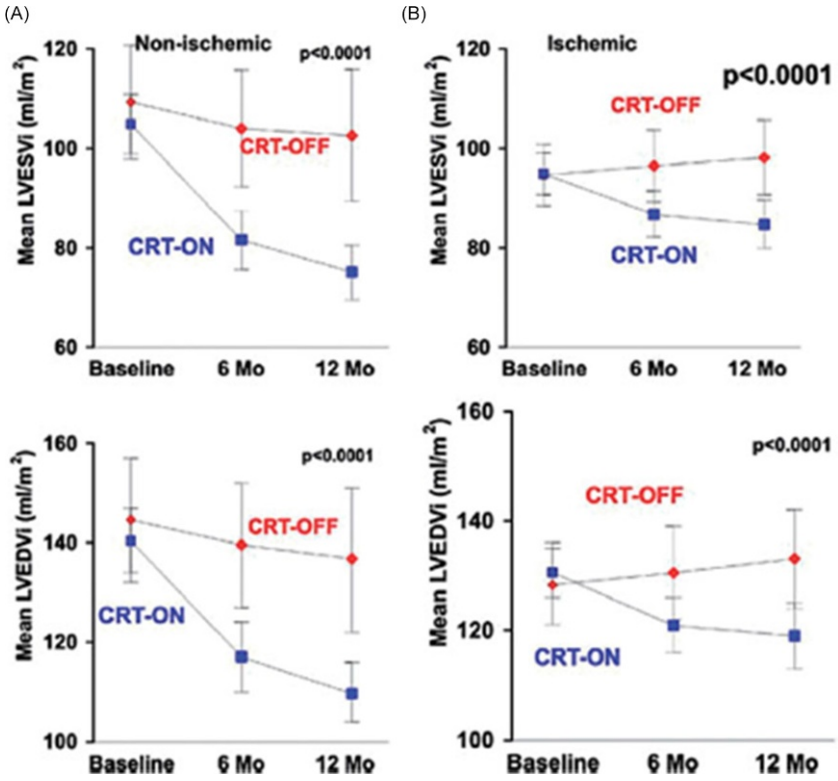

Fig. 5 CRT has a smaller but significant effect on reverse remodeling in ischemic (B) versus nonischemic cardiomyopathy (A) (Linde et al., 2010). Reprinted with permission from Elsevier.

with further deterioration of cardiac function and increased clinical events (Wilkoff et al., 2002). In a clinical context, there are two scenarios where this may be important. First, patients may develop LV dysfunction during antibradycardia pacing using RVP and may need an additional LV lead. There are some studies suggesting that upgrading a conventional RVP device to a CRT in the case of HFrEF and high percentage RVP may be beneficial. The RAFT trial was the only large randomized clinical CRT trial that included a small subgroup of these patients (Tang et al., 2010) but the subgroup was too small to draw firm conclusions on. However, several smaller clinical studies have investigated the effect of CRT versus RVP in patients with EF $\leq$ 40% and symptoms of heart failure. These studies have generally shown that CRT results in an echocardiographic reverse remodeling and amelioration of heart failure symptoms (Hoijer et al., 2006). In addition, data from both observational studies and registries support the upgrade of RVP devices to CRT in patients with HFrEF. In the second scenario, patients with preexisting LV dysfunction may develop an indication for chronic antibradycardia pacing. De novo implantation of CRT was compared to a conventional RVP device in patients with a bradycardia indication for RVP (mostly high degree AV block) and moderate-to-serve systolic dysfunction in several randomized controlled clinical trials. The HOBIPACE (Kindermann et al., 2006), COMBAT (Martinelli Filho et al., 2010), and BLOCK HF (Curtis et al., 2013) trials included approximately 880 patients and consistently showed improved outcome of de novo CRT compared to RVP device implantation in terms of improved cardiac structure and function, improved quality of life, and reduction in clinical events. The PACE trial (Yu et al., 2009) randomized patients with preserved EF and a bradycardia indication for ventricular pacing to receive either CRT or RVP. This study showed that CRT could prevent the deterioration of cardiac function and progression of remodeling. The efficacy of CRT on clinical endpoints in the case of preserved EF remains largely unknown as no sufficiently powered clinical trials have been conducted.

Lead Positioning

The LV region, which is activated latest during LBBB, RVP, or other conduction abnormalities, should be theoretically the optimal site for lead placement and subsequent stimulation in order to obtain maximal resynchronization. During the conventional approach, the LV lead is placed transvenously in a lateral or posterolateral second- or third-order branch of the coronary sinus vein. This strategy is based on the expected pattern of delayed electrical and mechanical activation of the lateral and posterolateral wall in patients with LBBB. Placement of the LV lead can be challenging due to heterogeneity of coronary sinus venous anatomy (Blendea et al., 2007). The response to CRT is often variable even when the LV lead is placed in this optimal anatomical position (Derval et al., 2010) reflecting the heterogeneity of ventricular activation even within similar QRS duration or bundle branch morphology (Rodriguez et al., 2003; Fung et al., 2004) as was also found in canine models of LBBB (Ploux et al., 2014).

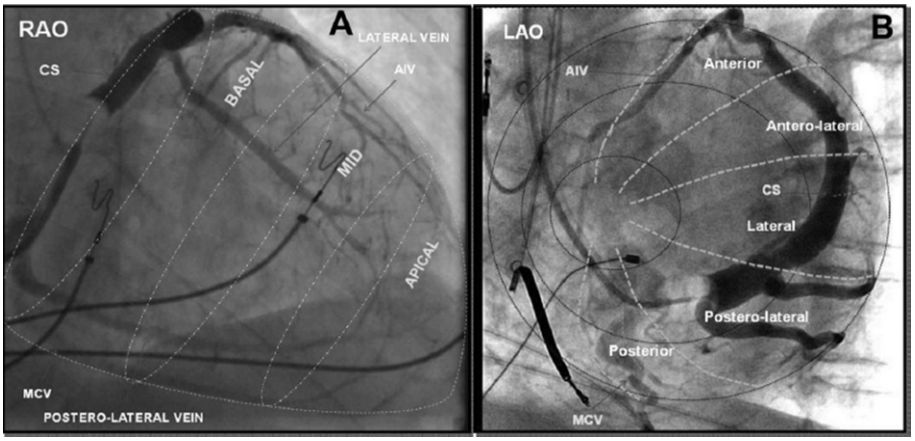

Fig. 6 Angiographic classification of LV lead position (Singh et al., 2011). Right anterior oblique (RAO) view (A) is used to divide the LV wall along the long axis of the heart into basal, midventricular (MID), and apical segments. The segmentation of the LV wall along the short axis of the heart is performed using the left anterior oblique (LAO) view (B). LV wall is divided then into anterior, anterolateral, lateral, posterolateral, and posterior segments. AIV indicates anterior interventricular vein; *CS*, coronary sinus; *MCV*, middle cardiac vein. Reprinted with permission from the American Heart Association.

Anatomically, placement of the LV lead can be organized along the long and short axes (Singh et al., 2011, **Fig. 6**). Subgroup analyses of the large, randomized CRT trials are not consistent regarding the superiority of positioning the LV lead in a specific anatomical region during implantation. In the COMPANION trial, no difference was observed in the CRT effect between anterior, lateral, or posterior LV lead positions. On the other hand, posttrial analyses of the REVERSE and MADIT-CRT studies indicated that a lateral lead position should be preferred and that a basal or mid ventricular LV region is more optimal as compared to an apical position (Singh et al., 2011). However, there is no clear evidence that a supreme anatomical area should routinely be targeted during CRT implantation. Considering the heterogeneous activation of the LV it is tempting to establish more individualized approaches to find an optimal LV lead position. Theoretically, it may be achieved by targeting regions of maximal electrical delay (Singh et al., 2006; Gold et al., 2013), maximal mechanical delay (Bilchick et al., 2008; Saba et al., 2013), or lead positions that maximize hemodynamic improvement (Duckett et al., 2011). Since CRT is a form of electrical therapy for patients with abnormalities of electrical conduction, an optimal CRT response may be achieved by targeting the site of latest electrical activation. It can most practically be identified by measuring the time between the beginning of the surface QRS and the sensed intracardiac signal of the LV lead (QLV) (Ellenbogen et al., 2010). The QLV delay can subsequently be corrected for baseline QRS duration to determine the LV lead electrical delay (Singh et al., 2006). Implantation of LV lead at sites of longer QLV was associated with a significantly greater LV reverse remodeling and improvement in patient symptoms. The site of latest electrical activation can also be detected by electroanatomical mapping catheters (Ryu et al., 2010). Although mechanical dyssynchrony has failed to predict CRT response or identify patients with narrow QRS who may benefit from CRT, newer methods to assess mechanical dyssynchrony (e.g., speckle tracking and cardiac MRI) (Bilchick et al., 2008; Khan et al., 2012; Saba et al., 2013) were studied. In the largest randomized trials it was shown that speckle tracking guided lead placement to the areas with the latest activation but without scar resulted in significantly reduced adverse clinical events (Saba et al., 2013, **Fig. 7**) and higher response rates (Khan et al., 2012). Importantly, exact concordance was only achieved in 30% of patients demonstrating the limitations of coronary venous anatomy (Saba et al., 2013). In a recent study, LV lead placement guided by the combined use of MRI and longitudinal myocardial strain was associated with a significantly better echocardiographic response after CRT implantation (Bertini et al., 2016). Pacing in myocardial scar might lead to inadequate resynchronization. LV lead implantation at sites of scar (even in optimal anatomical location) has been associated with poor response to CRT in large single-center studies (Adelstein and Saba, 2007; Leyva et al., 2011; Khan et al., 2012).

Multisite Pacing

A beneficial clinical response to CRT is based on an optimized LV electrical stimulation resulting in synchronous myocardial contraction. The LV is conventionally stimulated by one pacing vector but multisite pacing (MSP) also emerged as a strategy to enhance clinical response to CRT. More pacing locations in the LV may increase the chance to capture an LV region ideal for resynchronization. MSP can be achieved by introducing two LV electrodes in different side branches of the coronary sinus (**Fig. 8**) or by multipolar LV leads (**Video 1–6**).

Multiple LV leads

It was demonstrated in animal models that addition of further LV pacing electrodes resulted in an acute hemodynamic improvement as compared to single-lead LV pacing (Ploux et al., 2014). Human studies also showed significantly better CRT responses by implanting multiple LV leads (Rogers et al., 2012). It was hypothesized that stimulation by more LV leads may have induced a rapid and potentially more physiological LV activation (Ramanathan et al., 2004). The beneficial effects of MSP may be different in

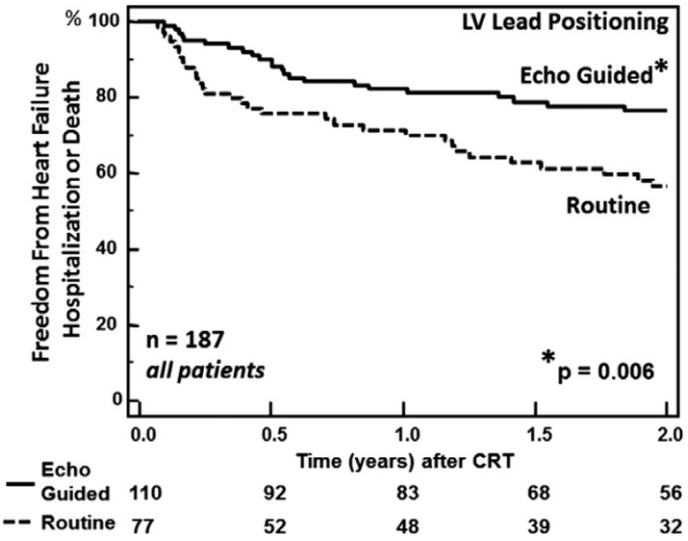

Fig. 7 Kaplan–Meier plots of the results of freedom from heart failure hospitalization or death after CRT. Patients randomized to echocardiography-guided LV lead positioning strategy had a significantly more favorable clinical outcome in comparison to routinely treated patients (Saba et al., 2013). Reprinted with permission from the American Heart Association.

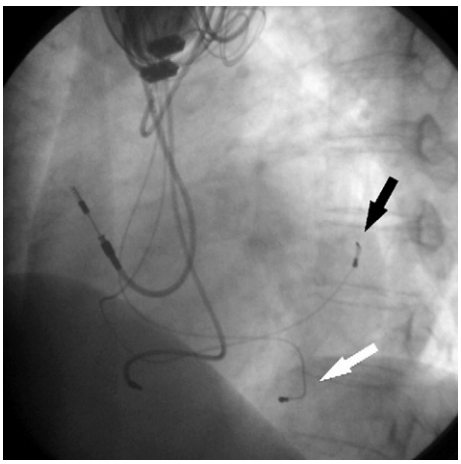

Fig. 8 Multisite pacing achieved by two electrodes in different side branches of the coronary sinus (Lenarczyk et al., 2007). The electrode in an anterolateral branch is marked with a black arrow and the lead in a posterolateral branch is marked with a white arrow. Reprinted with permission from Elsevier.

patients with HFrEF of ischemic or nonischemic etiology. Patients with nonischemic cardiomyopathy responded adequately to conventional CRT while MSP resulted in a significantly higher acute hemodynamic response in patients with myocardial scarring and ischemic HFrEF may be a more appropriate target group for MSP (Ginks et al., 2012a,b). Additional studies have also shown an improvement in the acute LV hemodynamic response (LVdP/dtmax) (Pappone et al., 2000), LV ejection fraction (Lenarczyk et al., 2007), and LV end-systolic volume (Leclercq et al., 2008). As regards clinical endpoints, the results are not consistent as some studies reported improvement in the NYHA functional status (Lenarczyk et al., 2009) and exercise tolerance (Rogers et al., 2012) while others have not shown such clinical benefit (Leclercq et al., 2008).

Multipolar LV leads

Multipolar LV leads can induce electrical activation usually at four stimulation sites, and therefore, MSP can be achieved by a single quadripolar LV lead. High implant success rates, satisfactory pacing parameters, and stable positioning were reported (Tomassoni et al., 2013). Multipolar LV leads offer a considerably large number of available pacing configurations and, therefore, problems such as high capture thresholds and diaphragmatic stimulation can easily be solved. A few preliminary studies were performed to investigate the effects of MSP delivered via multipolar LV leads. Some studies showed increase of the LV acute systolic function (Thibault et al., 2013; Pappone et al., 2014), which was consistently observed regardless of QRS duration or LV lead position (Zanon et al., 2015). Interestingly, acute hemodynamic response to MSP via multipolar LV leads was not significantly different as compared to MSP via multiple LV leads (Shetty et al., 2014). Other studies reported improvement of radial strain (Rinaldi et al.,

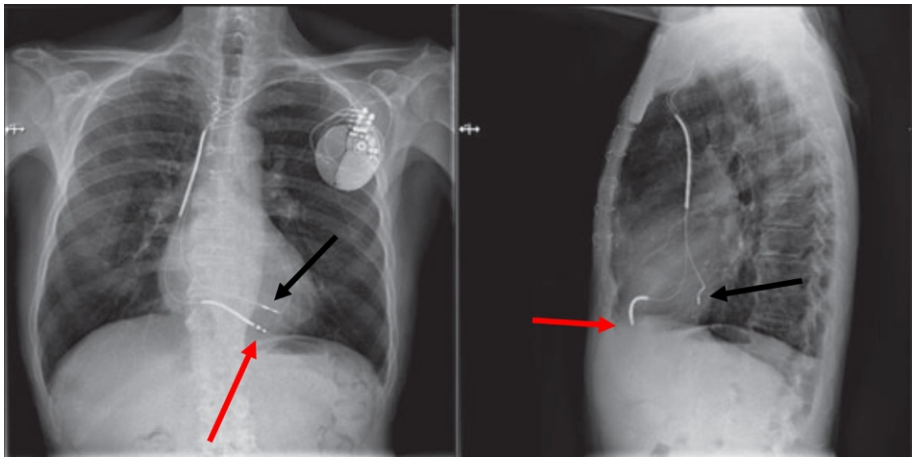

Fig. 9 Position of an endocardial LV lead after a transseptal implantation. Anteroposterior and lateral X-ray images show the final position of an endocardial LV lead (*black arrow*) and a RV lead (*red arrow*) (Calvo et al., 2014). Reprinted with permission from the European Society of Cardiology.

2014) and mechanical dyssynchrony (Rinaldi et al., 2013; Osca et al., 2016) during multipolar pacing as compared to conventional CRT. Subsequently, a small clinical study was conducted to investigate the clinical effects of multipolar leads. After a 12-month follow-up improved echocardiographic and clinical response was observed in the group with multipolar pacing (Pappone et al., 2015). Similarly to MSP by two LV leads, bioengineering simulations have suggested that MSP by multipolar LV leads may be more beneficial in subjects with ischemic cardiomyopathy and heterogeneous LV activation (Niederer et al., 2012). Randomized, prospective, multicenter studies investigating the safety and efficacy of MSP via a single multipolar LV lead are ongoing. The MOre REsponse on Cardiac Resynchronization Therapy With MultiPoint Pacing (MORE-CRT MPP) aims to recruit patients with class I or IIa indications for CRT therapy. After a 6-month period of conventional LV pacing nonresponders (<15% reduction in LV end-systolic volume) are randomized to standard pacing or MSP via a quadripolar lead. The primary endpoint is >15% reduction in LV end-systolic volume after further 6 months.

Endocardial LV Pacing

Endocardial LV pacing (Calvo et al., 2014, **Fig. 9**) has a couple of theoretical benefits as compared to epicardial pacing: positioning of the lead is not limited by the anatomy of the coronary sinus branches; it likely requires lower pacing energy outputs, and there is no phrenic nerve stimulation; furthermore, it is probably more physiological and possibly less pro-arrhythmic by reduced dispersion of ventricular repolarization. The importance of a good lead position was observed in animal studies, in which endocardial pacing resulted in larger acute hemodynamic effects and better resynchronization of ventricular activation than with epicardial pacing (Bordachar et al., 2012; Strik et al., 2012). The possible reason of the more efficient electrical resynchronization is a faster impulse conduction and a shorter activation path length in the endocardial than in the epicardial layers. The results of some small observational clinical studies (Derval et al., 2010; Spragg et al., 2010; Ginks et al., 2012a) of endocardial CRT are consistent with the findings of the animal experiments. In these studies, acute hemodynamic responses at the best endocardial LV pacing site were significantly greater than those achieved via epicardial LV pacing (Spragg et al., 2010; Ginks et al., 2012a) and a small clinical study showed even long-term benefits (Garrigue et al., 2001b). However, endocardial LV lead placement might increase the risk of systemic thromboembolism, mitral insufficiency, and endocarditis (Rademakers et al., 2014). The recently published prospective, multicenter Alsync trial (Morgan et al., 2016) evaluated the feasibility and safety of LV endocardial pacing. The success rate of the implantation was 89% but 23% of the patients had periprocedural or mid-term complications during a 6-month follow-up. Clearly, better and easier techniques, such as wireless pacing, are required for endocardial CRT, after which randomized studies can be performed to investigate the benefits compared to epicardial CRT. Recently, a system for leadless RVP has been developed (Reddy et al., 2015, **Fig. 10**). This technique does not require a pacemaker pocket or lead and during the implantation the pacemaker, the size of which is a small coin, is introduced transvenously and screwed directly in the endocardium of the heart. It may be speculated that leadless LV endocardial pacing may be potentially beneficial for resynchronization without the major complications caused by endocardial LV leads. However, leadless pacing has not yet been tested as an alternative to conventional LV pacing and its place in a CRT context is therefore unknown.

Patient Care After CRT Implantation

In addition to routine follow-up of a patient with heart failure, care of the patient following implantation of CRT includes control of proper functioning of the device and the leads, optimization of device programming, and early recognition of patients at risk for nonresponse. A standard 12-lead ECG is very useful to detect LV noncapture, fusion beats (**Video 7–9**), and

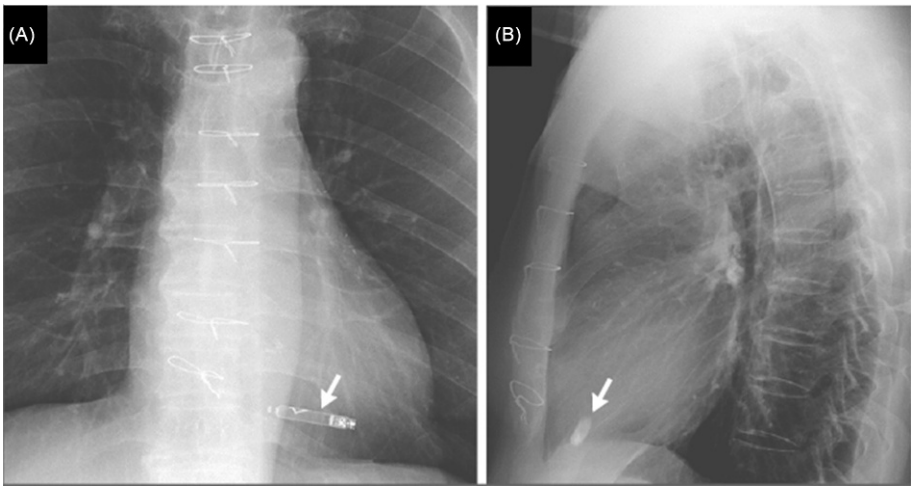

Fig. 10 Leadless cardiac pacemaker. Frontal (A) and lateral (B) chest radiographs of a patient after implantation of a Nanostim leadless cardiac pacemaker. The arrows indicate the location of the device in the region of the distal right ventricular septum and right ventricular apex (Reddy et al., 2015). Reprinted with permission from the Massachusetts Medical Society.

tachyarrhythmias. A chest X-ray can be used to determine LV lead position and in case of nonresponse repositioning of the LV lead may be considered (**Video 10–12**). As regards optimization of device programming, the delay between atrial sensing or stimulation and ventricular stimulation (AV-delay) and the timing interval between stimulation of each ventricle (VV-delay) are programmable parameters and their optimization may affect resynchronization and thereby improve clinical outcome. Optimizing the AV delay in patients with CRT resulted in an optimal atrioventricular coupling, improved hemodynamics, and cardiac function (Auricchio et al., 1999; Gras et al., 2009). Optimization can be performed by device algorithms based on intrinsic AV intervals, interventricular timing, and LV lead location (Ellenbogen et al., 2010; Kamdar et al., 2010; Singh et al., 2013) or by echocardiography guided assessment of mitral inflow patterns (Barold et al., 2008). AV optimization was shown to be beneficial to patients demonstrating nonresponse after CRT implantation (Mullens et al., 2009). In most studies, however, intrinsic device based on routine echocardiographic optimization of AV intervals did not significantly improve clinical outcome (Ellenbogen et al., 2010; Kamdar et al., 2010; Singh et al., 2013). In addition to AV optimization, optimization of VV delay was also demonstrated to improve hemodynamic parameters (Leon et al., 2005; Boriani et al., 2009). Similar to AV optimization, VV optimization can be performed by using intrinsic device algorithms or guided by echocardiography. Routine optimization of VV delay, however, did not result in a significantly better clinical outcome (Gras et al., 2009; Singh et al., 2013; Boriani et al., 2009). VV optimization may be of more importance to patients with ischemic cardiomyopathy and consequently heterogeneous activation patterns as compared to nonischemic cardiomyopathy (Marsan et al., 2009). Recently, a dynamic LV only pacing algorithm has been developed (AdaptivCRT; Medtronic, Minneapolis, MN) (Martin et al., 2012), which creates an effective fusion of LV pacing and native RV conduction. It was hypothesized that the physiologic activation of the RV and avoidance of RVP-induced dyssynchrony may be beneficial during resynchronization. Patients randomized to the AdaptivCRT algorithm experienced a 44% reduction in RVP compared to those undergoing standard echocardiography-guided optimization (Martin et al., 2012). Increased percentage of LV only pacing in all patients, as well as the use of the AdaptivCRT algorithm in patients with normal PR interval, were associated with a clinical benefit (Singh et al., 2013; Birnie et al., 2013). There may be a particular benefit of LV only pacing algorithms in patients demonstrating nonresponse to CRT as recently suggested (Thibault et al., 2011). Another optimization algorithm uses the Peak Endocardial Acceleration (PEA) signal system implanted in the RV lead (SonR sensor; Sorin Group, Milan, Italy) (Ritter et al., 2012; Delnoy et al., 2013). The CLEAR (Clinical Evaluation on Advanced Resynchronization) study aimed to assess the effects of CRT with automatically optimized AV and VV delays, based on this accelerometer-based technology (Ritter et al., 2012). After a short-term follow-up, the accelerometer-based algorithm was associated with an improved NYHA class as compared to standard echocardiographic optimization (Ritter et al., 2012) and a post hoc analysis suggested a possible mortality benefit in patients undergoing frequent, systematic optimization compared with standard care (Delnoy et al., 2013). The recently published RESPOND CRT study, a randomized controlled clinical trial, showed noninferiority but not superiority of the contractility sensor-guided optimization as compared to standard care in terms of clinical and echocardiographic CRT response (Brugada et al., 2017). Contemporary CRT devices offer a large number of monitoring parameters that are relevant for the longitudinal care of patients with heart failure (Singh et al., 2009). Information regarding heart rate, atrial and ventricular ectopic burden autonomic activity, transthoracic impedance, and patient activity are available in current CRT devices and may help to recognize worsening heart failure status earlier and, thus, prevent poor clinical outcome and heart failure hospitalizations (Auricchio et al., 2014; Vegh et al., 2014). Biventricular pacing percentage has been reported as an important determinant of clinical outcomes following CRT. A recent report from the LATITUDE investigators (Boston Scientific, Marlborough, MA) of nearly 37,000 patients suggested biventricular pacing percentages greater than 98% were associated with improved survival as compared to lower pacing percentages (Hayes et al., 2011). Device data including

biventricular pacing percentage, atrial, or ventricular ectopic burden or tachyarrhythmias may facilitate early intervention and improve clinical outcomes (Lakkireddy et al., 2012).

Conclusions

CRT, with or without ICD, has been well established in multiple large trials to improve reverse remodeling, symptoms, hospitalizations, and mortality in well-selected patients with heart failure when used in addition to optimal medical therapy. Consensus guidelines have identified the patients most likely to benefit from CRT as those with symptomatic (NYHA II-III) heart failure, LVEF $\leq$ 35%, and wide QRS duration. Subsequent analysis showed that patients with wide QRS and LBBB pattern benefit most from CRT whereas the positive effects of CRT in RBBB or nonspecific QRS prolongation are less clear and require further investigation by randomized controlled trials. CRT was subsequently shown to have beneficial effects in patients with HFrEF and wide QRS duration in case of ischemic cardiomyopathy, AF or continuous RVP. The proportion of nonresponders may be reduced by better patient selection and more optimal lead positioning individually targeting areas with the latest activation but without scar. MSP by multiple and multipolar LV leads may result in a more synchronous myocardial contraction in selected patients. Randomized controlled trials are ongoing to evaluate the clinical efficacy of MSP in larger patient populations. Endocardial LV pacing is an alternative therapeutic option but its widespread clinical use has been questioned by safety considerations. Leadless LV endocardial pacing may be a future technique potentially beneficial for resynchronization and without the major complications caused by endocardial LV leads.

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Cardiac Transplantation

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Introduction

Advanced therapy with mechanical support and cardiac transplantation for refractory chronic heart failure have emerged as standard treatment for those considered end-stage despite advances in both pharmacologic and device treatment in the past decades. The survival benefit of defibrillators, cardiac resynchronization devices, and medical therapy has been well established, but morbidity and mortality associated with advanced heart failure remains disproportionate, reaching nearly 50% for 5-year mortality and greater than 50% 1-year mortality for end-stage patients. Cardiac transplantation has a median survival range from 10 to 13 years based upon a patient surviving the first year; 1 year survival has improved in the most recent era with an unadjusted rate of 86% in 2009–13 compared with 84% in the 2002–08 era (Lund et al., 2015). Although

replacement of the heart is standard treatment for select patients with refractory symptoms, mechanical support has emerged as an acceptable alternative for those who are either not candidates or require more urgent definitive therapy. In fact, around 42% patients were transplanted from left ventricular assist devices (LVAD) in 2013. Although the first successful human heart transplant was performed in 1967 in South Africa and followed by the first United States case the following year, cardiac transplantation faced many challenges that limited survival ranging from medical management to surgical techniques. Improvement in immunosuppression was a key factor with the development of calcineurin inhibitors (i.e., cyclosporine) in the 1980s (Reitz et al., 1981) and continues to improve with refinements in surgical technique, recipient and donor selection, donor management, immunosuppression, and treatment of comorbidities. Estimated annual transplant rates are >4000 cases with the limiting factor being shortage of available donors (**Fig. 1**).

Evaluating the Transplant Candidate

Indications

Cardiac transplantation is reserved for advanced heart failure, refractory angina, and intractable ventricular arrhythmias (Macdonald, 2008) (**Table 1**). Candidates for cardiac transplantation have New York Heart Association (NYHA) Class III–IV symptoms despite optimal medical and device therapy, including resynchronization therapy and often surgical treatment of ischemic and/or valvular heart disease. Etiology of heart failure most commonly involves coronary artery disease or nonischemic cardiomyopathy and less often restrictive cardiomyopathy, valvular heart disease, and congenital heart disease (**Fig. 2**). Trends in the most recent era of 2009 to June 2014 include an increase in dilated cardiomyopathy from 52.1% in the previous era (2004–08) to 55.6% currently and a decline in coronary artery disease from 38.8% to 35.1% in the current era (Lund et al., 2015). Other less commonly transplanted indications involve (1) repeated ventricular arrhythmias despite pharmacologic and ablative treatment due to the risk of sudden death and hemodynamic intolerance with the arrhythmias, and (2) chronic angina unresponsive to medical therapy which is not amenable to further revascularization. These indications are the result of end-stage disease failing maximal therapy and reducing quality of life and survival.

Cardiopulmonary Exercise Testing as a Guide to Candidacy

Cardiopulmonary exercise testing assesses peak oxygen consumption (VO_2) and provides an objective measure in addition to NYHA class, ejection fraction, and clinical status (blood pressure, heart rate, readmissions, tolerance of medical therapy). A VO_2 result between 10 and 14 mL/kg/min is associated with a reduced survival; evaluation for transplantation is recommended.

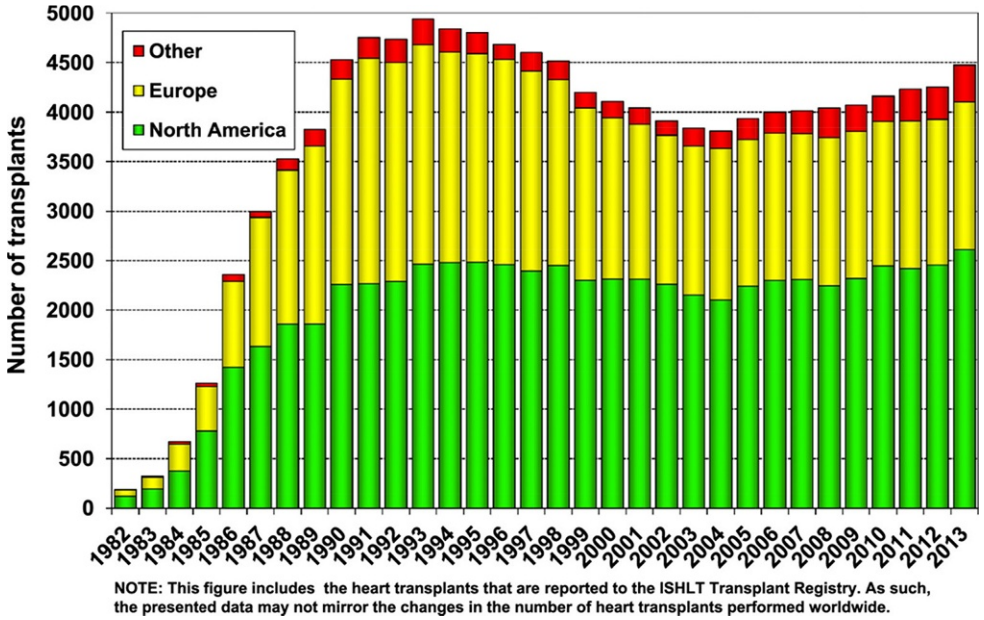

Fig. 1 Heart transplant volume as reported to the ISHLT Transplant Registry. Reprinted from Lund, L. H., Edwards, L. B., Kucheryavaya, A. Y. et al. (2015). The registry of the International Society for Heart and Lung Transplantation: Thirty-second official adult heart transplantation report—2015; focus theme: Early graft failure. *The Journal of Heart and Lung Transplantation* **34** (10), 1244–1254. Copyright 2015, with permission from Elsevier.

Table 1 Indications for heart transplantation

Advanced systolic heart failure
- NYHA class III–IV symptoms
- Low cardiac output
- Cardiopulmonary exercise testing with peak VO_2 max <12–14 mL/kg/min or <50% predicted in select patients

Acute heart failure not expected to recover
- Acute myocardial infarction
- Acute myocarditis

Refractory angina in ischemic disease
- Not amenable to percutaneous or surgical revascularization
- Unresponsive to maximal medical therapy

Intractable ventricular arrhythmias
- Maximal antiarrhythmic therapy and ablative therapy

Advanced diastolic heart failure symptoms in restrictive or hypertrophic cardiomyopathy
Congenital heart disease with refractory symptoms

NYHA, New York Heart Association; *Vo_2*, maximal oxygen uptake.

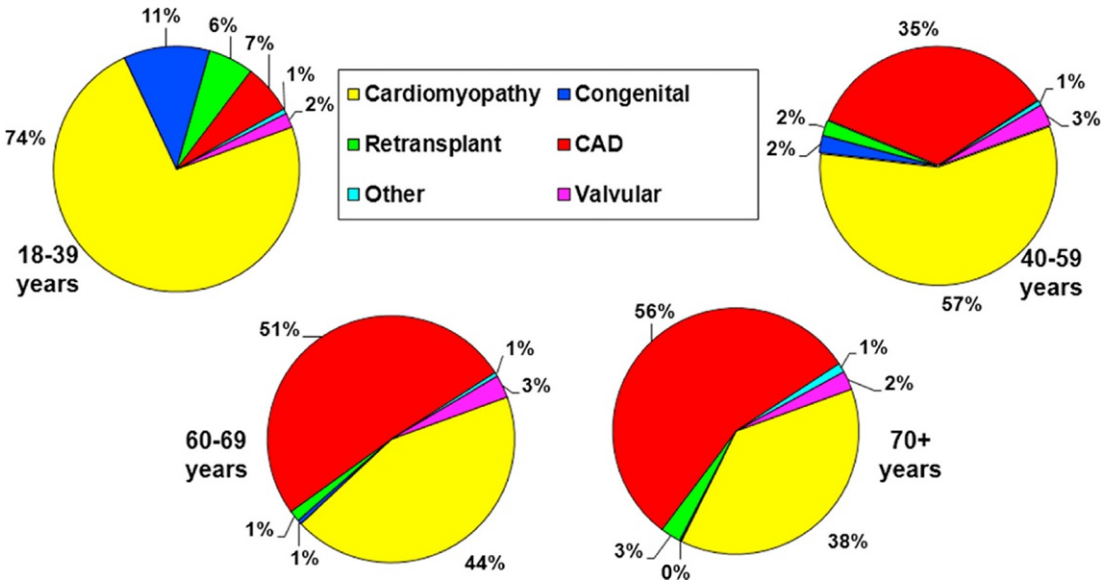

Fig. 2 Etiology of adult heart transplantation indication from January 2009 to June 2014. Reprinted from Lund, L. H., Edwards, L. B., Kucheryavaya, A. Y. et al. (2015). The registry of the International Society for Heart and Lung Transplantation: Thirty-second official adult heart transplantation report—2015; focus theme: Early graft failure. *The Journal of Heart and Lung Transplantation* **34** (10), 1244–1254. Copyright 2015, with permission from Elsevier.

Although listing criteria detail peak $VO_2 \leq 12$ mL/kg/min in those taking a beta-blocker and ≤ 14 mL/kg/min if intolerant of a beta-blocker, it is acceptable to evaluate alternate parameters from peak exercise in younger patients <50 years of age and women (Mehra et al., 2006, 2016). In these patients due to variations in peak VO_2 based on gender and age, a peak $VO_2 < 50\%$ predicted can also guide candidacy.

Patients should reach anaerobic threshold, which is defined as a respiratory exchange ratio (RER) >1.05 and represents carbon dioxide production exceeding oxygen consumption during exercise. If patients have a submaximal test, perhaps due to deconditioning, parameters such as the ventilatory response to exercise, or the ventilation to carbon dioxide slope (VE/VCO_2), can be measured throughout the duration of exercise. A higher slope, $VE/VCO_2 > 35$, signifies worse prognosis and is noted in patients with low cardiac output, pulmonary hypertension, and a higher dead space volume.

Current guidelines recommend using heart failure prognosis scores adjunctively to cardiopulmonary exercise testing, but not using the prognostic scores independently of this data or other objective data as some data suggests risk is underestimated with the prognostic scores alone (Mehra et al., 2016; Alba et al., 2013). If the estimated 1-year survival in the Seattle Heart Failure Model (SHFM) is <80% or Heart Failure Survival Score (HFSS) is medium/high risk, it is reasonable to use this score to assist VO_2 data when determining listing candidates for transplantation (Table 2).

Table 2 Heart failure survival score: variables

Ischemic etiology
Interventricular Conduction delay
Left ventricular ejection fraction
Heart rate
Serum sodium
Mean arterial pressure
Peak VO_2 or maximal oxygen uptake

Contraindications

The majority of contraindications to transplant are relative contraindications as opposed to absolute and are weighed according to the patient's comorbidities and clinical status. These contraindications may vary among transplant centers as to absolute or relative in terms of patient candidacy (**Table 3**).

Age

Cardiac transplant recipient age has increased over the last several decades until 2010 and has remained stable around age 54 over the past few years (Lund et al., 2015). Historically, candidates who were 65 years or older were considered higher risk with lower expected survival, but current guidelines recommend considering patients ≤70 years of age based on improved survival in older patients and select patients >70 years of age (Mehra et al., 2006, 2016). Long-term follow-up of patients >65 years after cardiac transplant revealed similar 10-year survival and causes of death when compared with younger patients (groups included recipients <60, 60–64, and >65 years); there was no difference in freedom from severe infection, cancer, or severe graft vasculopathy (Zuckermann et al., 2003). Moreover, older patients displayed immunosenescence, or a gradual decline in the immune system with age, leading to fewer rejection episodes and a larger percentage of patients who were not on maintenance steroid therapy. Review of the UNOS database ($n = 50{,}432$) for transplanted patients from 1987 to 2014 confirmed similar outcomes for recipients in their 70s compared with recipients in their 60s despite being more likely to receive organs from donors with a history of tobacco, alcohol, or cocaine use or donors considered high risk for transmission of disease (Cooper et al., 2016). Contrary evidence has endorsed reduced survival in older recipients (Tjang et al., 2008; Marielli et al., 2008), therefore leading to current guideline recommendations to carefully select older patients based on comorbidities, such as renal function, with specific donor and recipient criteria in place at the transplanting center (Mehra et al., 2016). Upper age limit remains center-specific criteria with each individual center determining if they will have an absolute cutoff for candidacy based upon chronologic age.

Obesity

Obesity in cardiac surgery is associated with complications of infections, poor wound healing, pulmonary status, and thromboembolic events. Risk associated with transplantation involves higher risk of rejection, vasculopathy, and mortality (Lietz et al., 2001). Weight gain is a likelihood posttransplant due to cardiac cachexia with end-stage heart failure, and is often related to age and gender (Williams et al., 2006); progression of preexisting comorbidities is a reality for this reason in addition to immunosuppression regimens that can affect weight gain and blood sugar. Therefore, recipient weight is an essential parameter for candidacy, measured as body mass index (BMI, weight in kilograms per height in meters squared) or percent ideal body weight (PIBW) for a given height and gender. Both measurements take into account height as opposed to considering weight only. The International Society for Heart and Lung Transplantation (ISHLT) guidelines recommend a pretransplant BMI <35 kg/m^2 or PIBW <140%, and state it is "reasonable" to recommend weight loss in candidates who do not meet this criteria due to the association with poor outcomes (Mehra et al., 2006). This criteria has been amended from previous recommendation of 30 kg/m^2 in the most recent guidelines based upon data suggesting that BMI <35 kg/m^2 is not clearly associated with higher mortality although patients above this cutoff have longer wait times and lower likelihood of suitable donors (Russo et al., 2010a; Weiss et al., 2009a). Given the prevalence of obesity, many centers accept a higher BMI or PIBW in recipients and the upper limit is usually center specific.

Pulmonary hypertension assessment

Due to the risk of right heart failure posttransplantation, irreversible pulmonary hypertension is an absolute contraindication to candidacy and right heart catheterization is required in all candidates (Mehra et al., 2006). In advanced heart failure, secondary or venous pulmonary hypertension develops as a result of elevated filling pressures. Although it is usually considered reversible with a low pulmonary vascular resistance (PVR), an irreversible or fixed component may develop due to persistent elevation of filling pressures as a result of chronic intimal changes and medial hypertrophy in the pulmonary vasculature. A donor heart cannot withstand the high afterload of an elevated PVR, which results in failure of the right ventricle and adverse outcomes including acute cardiogenic shock and mortality (Butler et al., 2005). Therefore, routine evaluation of candidates includes invasive assessment of pulmonary pressures, filling pressures, cardiac output, and PVR. When the PVR >3 Woods units, pulmonary artery systolic pressure (PASP) >50 mmHg, PVR index >6, or a transpulmonary gradient >15 mmHg (difference of the mean PA and wedge pressure), transplant candidacy is usually not considered unless provocative testing with a vasodilator reduces the pressures and PVR to an acceptable level while maintaining a systemic blood pressure >85 mmHg

Table 3 Contraindications in cardiac transplantation (Mehra et al., 2016)

Advanced age (select patients >70 years)
Obesity
● BMI >35 kg/m^2 or PIBW >140%
Fixed pulmonary hypertensiont
● PVR >5 Woods units
● TPG >15 mmHg
● PA systolic pressure >50–60 mmHg or >50% systemic pressure in conjunction with the above parameters
● Inability to reduce PVR <2.5 Woods units with vasodilator or inotrope with systemic BP >85 mmHg
Diabetes mellitus complications
● Persistent poorly controlled (Hgb A_{1c} >7.5 mg/dL)
● End-organ complications other than nonproliferative retinopathy
Irreversible renal, hepatic, or pulmonary disease unless undergoing dual organ transplantation
● Irreversible renal dysfunction with eGFR <30 mL/min/1.73 m^2 is a relative contraindication to heart transplant only
Active infection or malignancy
● Recent malignancy
● Risk of recurrence
Clinically severe peripheral or cerebral vascular disease not amenable to revascularization or that limits rehabilitation
Psychosocial factors hindering compliance and/or comprehension of management
● Active or recent substance abuse (alcohol, illicit drugs, tobacco)
● Poor compliance with medical regimen or follow-up
● Insufficient social support system
● Psychiatric illness that is not well controlled on therapy

BMI, body mass index; *PIBW*, percent ideal body weight; *PVR*, pulmonary vascular resistance; *TPG*, transpulmonary gradient; *PA*, pulmonary artery; *BP*, blood pressure; *Hgb*, hemoglobin; *eGFR*, estimated glomerular filtration rate; *Hgb A1c*, glycosylated hemoglobin.

(Mehra et al., 2006). If the PVR is reduced to <2.5 Woods units in the setting of a BP <85 mmHg, the patient remains high risk for right heart failure and mortality after transplantation. If patients fail acute vasodilator testing, further hemodynamic monitoring with more aggressive intervention, for example, inotropes and diuretics, is usually undertaken to determine reversibility of pulmonary hypertension. Mechanical devices such as intraaortic balloon pump or left ventricular assist devices (LVAD) are reasonable therapies to effectively unload these patients; reevaluation of hemodynamics is usually performed 3–6 months after LVAD to allow time for reversal and successful bridge to transplantation (Kutty et al., 2013). Patients who remain irreversible with fixed, elevated PVR may also have underlying lung disease, concurrent obstructive sleep apnea, or chronic thromboembolic pulmonary hypertension, which should be excluded.

Diabetes mellitus

Diabetes with end-organ damage (proliferative retinopathy, nephropathy, and neuropathy) or poorly controlled blood sugars (glycosylated hemoglobin, HbA_{1c}, >7.5 mg/dL) is considered a relative contraindication. In a review of >20,000 recipients in the United Network for Organ Sharing (UNOS) database, Russo et al. (2006) found that uncomplicated diabetic patients have improved survival when compared to those with complicated diabetes, defined as the collective number of diabetic-related complications such as severe obesity, peripheral vascular disease, cerebrovascular accident, and history of renal failure; 10-year survival was negatively impacted by the presence of diabetes in another review of predictive factors (Kilic et al., 2012a). Further evidence is lacking to further delineate what degree of end-organ involvement is acceptable, but guidelines recommend considering only those with nonproliferative retinopathy (Mehra et al., 2006). Dual organ candidacy with a combined heart–kidney transplantation should be evaluated in select cases of nephropathy. Consultation with an endocrinologist should be part of the evaluation process to reach HbA_{1c} <7.5 mg/dL.

Renal dysfunction

Estimated glomerular filtration rate (eGFR) or creatinine clearance should be used to assess renal function, and evaluation includes workup of intrinsic renal disease, which would be considered irreversible or unlikely to correct after improving cardiac function. This assessment of chronic kidney disease (CKD) includes imaging, quantifying proteinuria, and evaluating for renal arterial disease. Transplant programs are faced with the effect of renal dysfunction on outcomes. In a review of 1732 patients from 1996 to 2007 in the United Kingdom, patients with CKD stage IV, V, or on dialysis had an increased risk of mortality with the highest risk associated with CKD stage V not receiving dialysis. Initiating dialysis reduced this risk but not to that of the CKD stage IV patients. Those with impaired renal function at the time of listing were more likely to develop CKD with function often deteriorating within the first year after transplantation, progressing from 50% with CKD stage III or higher immediately before transplantation to 77% during the first

year (Thomas et al., 2012). Risk factors for developing CKD included recipient age, female gender, diabetes, and temporary dialysis or hemofiltration perioperatively (Thomas et al., 2012; Hamour et al., 2009). ISHLT listing criteria state that it is reasonable not to consider irreversible renal dysfunction with eGFR <30 mL/min/1.73 m^2 for heart transplantation alone; dual organ candidacy should be considered in appropriate cases (Mehra et al., 2006).

Active infection and malignancy

Both active infection and malignancy, with the exception of nonmelanoma skin cancers, are absolute contraindications to transplantation due to the risk of progression with immunosuppression. The duration to wait after remission for malignancy is arbitrary and dependent on the type of cancer, response to treatment, and risk of recurrence (Mehra et al., 2006). Malignancy that is in remission and has a low risk of recurrence will usually be considered after oncology consultation, especially when considering the urgency of the patient clinical presentation. For example, a patient with end-stage heart failure and prostate cancer within the past few years would be a reasonable candidate based on the success rate of treatment and slow-growing nature of this cancer as opposed to a patient who presents with chemotherapy-induced cardiomyopathy closer to treatment for breast cancer. Pretransplant evaluation includes routine screening for malignancy.

Active infection must be resolved before considering transplantation with documentation of negative cultures and resolving signs of infection. Chronic infections such as hepatitis B or C and human immunodeficiency virus (HIV) present a more complex circumstance for listing; in the past, such patients would not have been considered. With growing experience, patients with these chronic infections have been successfully transplanted if they do not have extensive organ involvement with the disease; however, experience is limited and many centers still consider such infections a contraindication. Hepatitis C positive heart transplant recipients have a worse survival in two database reviews: (a) UNOS from 1991 to 2014, higher mortality at 5 and 10 years with late renal and liver dysfunction occurring more frequently despite similar baseline pretransplant liver function when compared with hepatitis C negative recipients and (b) Scientific Registry of Transplant Recipients from 1995 to 2013, HCV+ was independently associated with a higher mortality posttransplant (Kumar et al., 2016; Stepanova et al., 2016). However, the success of current antiviral agents may significantly impact survival in these patients, leading to consideration of listing based upon HCV genotype and liver biopsy (Mehra et al., 2006).

Select HIV-positive patients without active or opportunistic infections who are stable on combined antiretroviral therapy with an undetectable viral load and CD4 count >200 μL have been successfully transplanted (Uriel et al., 2009; Agüero et al., 2016. Current ISHLT guidelines support consideration in the appropriate patient with chronic infections, but close collaboration with transplant infectious disease specialists should be undertaken in these situations given the challenges of managing antiretroviral therapy and immunosuppression.

Peripheral vascular disease

Symptomatic cerebral or peripheral vascular disease is considered a contraindication to candidacy if clinically severe or not amenable to revascularization when rehabilitation posttransplant would be limited. Asymptomatic disease will likely be considered, but it is an individual center decision. Retrospective data suggested progression of peripheral vascular disease posttransplantation; another registry found patients with symptomatic cerebrovascular disease pretransplant were at a higher risk of stroke and functional decline posttransplantation but not a higher mortality rate (Patlolla et al., 2011). Cardiac rehabilitation capability is critical in any candidate with vascular disease.

Psychosocial factors

A detailed psychosocial assessment of all transplant candidates is a fundamental step in the evaluation. This includes compliance with medical therapy and clinical follow-up, psychiatric history, substance abuse history, and social support network. Initial assessment is usually performed by dedicated social workers in transplant programs in which caregivers and patients usually meet with the social worker. If the patients have concerning psychiatric history or active illness, many are often referred to a psychiatrist or psychologist for further evaluation or neurocognitive testing based on concerns with cognitive function. Substance abuse (alcohol, illicit drugs, or tobacco) are all contraindications to listing and programs require 6–12 months of abstinence documented with negative toxicology screens; current alcohol abuse or illicit drug use are absolute contraindications in transplant programs. Various interventions are recommended including rehabilitation or substance abuse programs, counseling, and sponsorship. Active tobacco use is a relative contraindication due to the association with poor outcomes. Medical marijuana poses a new controversial issue without a clear answer regarding candidacy; it should likely be treated similar to alcohol, which is legal, but abuse, inability to quit, or cognitive impairment are barriers to transplant candidacy. Many patients are denied candidacy based on their psychosocial situation as this has been critical to long-term success (Rivard et al., 2005). Patients who have demonstrated poor medication compliance are not considered candidates, and severe cognitive-behavioral disabilities and dementia are considered contraindications since a benefit has not been established and there is potential for harm (Mehra et al., 2006). Comprehensive screening tools are now incorporated into the psychosocial assessment in many transplant programs for standardization and predicting psychosocial outcome (Maldonado et al., 2012). Success of heart transplantation is dependent upon the recipient's compliance with therapy and follow-up in addition to coping with the challenges of receiving such a scarce resource which includes an emotional and financial burden to patients and caregivers.

Ventricular assist devices as bridge to transplantation

Patients with comorbidities that limit current candidacy have been bridged to transplantation (BTT) with ventricular assist devices (VAD). This has been successful when pulmonary hypertension is present as reversal has been documented over time with mechanical support (Etz et al., 2010). Other treatable comorbidities, such as obesity, renal failure, tobacco use, and cancer, are also reasonable as BTT indications for VAD support. Renal failure should be evaluated for the degree of intrinsic renal disease related to other comorbidities, such as diabetes, that would likely not improve with improved cardiac function. Weight loss surgeries have been performed successfully in morbidly obese VAD patients to improve BMI to an acceptable range for transplant candidacy (Chaudhry et al., 2015). Patients with restrictive cardiomyopathy represent a complicated patient population that is not typically bridged with VAD support due to the restrictive physiology of the heart that does not respond similarly to the dilated ventricle with systolic dysfunction.

Amyloidosis

Treatment of amyloidosis with heart transplantation depends on the subtype and extent of cardiac and/or systemic involvement. Due to the higher mortality and progression of disease, only select patients with primary or AL amyloidosis may be considered for heart transplant and autologous stem cell transplant in centers with collaborative heart transplant and hematology programs, but is considered a contraindication for heart transplant alone (Mehra et al., 2006). Patients with transthyretin-related (TTR) amyloidosis may be considered for combined heart–liver transplantation since the protein is synthesized in the liver, and liver transplantation is the definitive treatment in familial amyloidosis. In a single-center review, combined transplantation in this situation has a survival similar to heart only patients (Raichlin et al., 2009). Older patients with TTR amyloid and only cardiac manisfestation may be considered for heart transplant alone, but younger patients would be combined heart–liver candidates to prevent systemic progression including cardiac involvement posttransplantation.

Waitlist Management

Listing candidates

Patients are waitlisted for heart transplant after decision by a multidisciplinary selection committee consisting of the various team members involved in the evaluation process: cardiac surgeons, heart failure cardiologists, coordinators, pharmacists, social workers, and subspecialty consultants such as pulmonology, nephrology, and infectious disease. Current listing status in 2016 comprises three levels, 1A, 1B, and 2 from the most urgent status to the least urgent. See **Table 4** for details on the current listing status (OPTN Policy, 2017). Patients accrue time on the waitlist regardless of status and are matched to available organs based on size, blood type,

Table 4 United network for organ sharing waitlist status criteria (as of 2016) (OPTN Policy, 2017)

Status 1A: recertified every 14 days from initial listing as status 1A

- Mechanical circulatory support
  - LVAD and/or RVAD if clinically stable can be listed as 1A at any point after implantation for 30 days without a complication
  - LVAD or RVAD with significant device-related complication: thromboembolism, infection, device failure, ventricular arrhythmias
  - IABP, TAH, or ECMO
- Mechanical ventilation
- Inotrope support
  - Continuous hemodynamic monitoring plus multiple inotropes or high dose of single inotrope

Status 1AE (by exception): recertified every 14 days from initial listing as status 1AE

- Urgent clinical status but patient does not meet above criteria (i.e., ventricular tachyarrhythmias preventing inotrope use or hemodynamic monitoring)
- Approved by appropriate Regional Review Board

Status 1B

- LVAD and/or RVAD beyond 30 days without a complication
- Continuous infusion of single inotrope without hemodynamic monitoring

Status 1B (by exception): Does not meet status 1B criteria, similar requirements as status 1AE

Status 2

- Candidates not meeting status 1A or 1B criteria

Status 7

- Temporarily unsuitable for transplantation

LVAD, left ventricular assist device; *RVAD*, right ventricular assist device; *IABP*, intraaortic balloon pump; *TAH*, total artificial heart; *ECMO*, extracorporeal membrane oxygenation.

and medical urgency or waitlist status. The goal of organ allocation is to transplant those who are the most critical and have the shortest survival. Average wait times on the list will vary by region. Patients who are large and blood type O will typically wait longer than those who are nonblood type O and smaller. For this reason, VADs are implanted in those who are less likely to survive while waiting for an available organ and are termed "bridge to transplantation." Some transplant programs have an alternate list for higher risk recipients (age >70 years, renal dysfunction, retransplantation) and donors (high risk behavior, older donors, or presence of coronary artery disease). The United Network for Organ Sharing currently is in the process of updating thoracic organ allocation, which will risk stratify recipients into more categories based on the extent of support to reflect our utilization of mechanical circulatory support devices and address geographic disparities in donor access. Patients who are grouped into status 1A in our current scheme will be further categorized based on the type of support, such as the highest status given to temporary or nondischargeable mechanical support, and certain VAD complications while the stable VAD patient is considered similar to the chronic inotrope patient without hemodynamic monitoring.

Right heart catheterization

Routine right heart catheterization (RHC) is performed as part of the transplant evaluation and periodically as part of waitlist management, which is center specific based upon the individual patient and usually varies in the 3- to 6-month range (Mehra et al., 2006). A vasodilator challenge is indicated when PASP >50 mmHg and TPG >15 mmHg or PVR >3 Woods units while maintaining systolic blood pressure >85 mmHg; commonly utilized agents are nitroprusside or nitroglycerin as an acute vasodilator and milrinone or dobutamine for inotrope support. If the patient becomes hypotensive with SBP <85 mmHg despite an appropriate decline in PVR with a vasodilator challenge, poor outcomes have been associated due to a high risk of right heart failure and mortality in this scenario (Mehra et al., 2006). If this challenge is unsuccessful, unloading of the LV will be attempted using diuretics for decongestion, continuous inotrope infusion, or in cases refractory to medical therapy, some type of mechanical support with temporary intraaortic balloon pump (IABP) or long-term with VAD. VAD in secondary PH has successfully reduced severe pulmonary hypertension to normal with PVR declining from 5 WU pre-VAD to 2.1 WU ($P < 0.05$) after VAD with similar 1-year posttransplant survival when compared with those receiving primary heart transplant (Kutty et al., 2013); other studies have found similar reversal in this population (John et al., 2010; Krishnamurthy et al., 2016). Reevaluation of hemodynamics after VAD is usually performed after 3–6 months of support to allow time for PH reversal. Patients with a fixed component of PH or elevated PVR should be evaluated for intrinsic pulmonary disease, chronic thromboembolic disease, and sleep apnea. Right heart catheterizations are regularly performed on waitlisted patients; our center's protocol requires RHC every 3 months if a vasodilator study was required to reverse PH or the patient is on a chronic inotrope infusion and every 6 months for the rest of the patients. Accurate assessment of hemodynamics and reversibility of PH is crucial for the success of heart transplantation due to the mortality associated with right heart failure in the setting of PH. Fixed PH or pulmonary arterial hypertension are considered contraindications to transplantation due to elevated and nonreversible PVR, but even reversible PH carries risk with a significantly higher risk of mortality posttransplant (Butler et al., 2005) and a nonsignificant trend to increased mortality in another study (Mogollón et al., 2008) emphasizing careful monitoring of these patients pre- and posttransplant.

Perioperative Transplant Management and Surgical Considerations

Donor Evaluation and Selection

Assessing the donor organ focuses on avoiding use of an inferior organ in critically ill patients waiting for transplantation. Organ procurement organizations (OPO) take on the responsibility of increasing the number of registered donors and coordinating the donation process. Procurement coordinators with a local OPO will evaluate potential donors; this process involves a comprehensive medical and social history, laboratory studies including blood type, infectious serologies (human immunodeficiency virus, hepatitis B and C, and cytomegalovirus), size including height and weight, and evaluation of cardiac function with an echocardiogram. Further cardiac studies, that is, coronary catheterization or right heart catheterization, are recommended based upon donor cause of death or risk factors, especially age. Substantial left ventricular hypertrophy (LVH), obstructive coronary disease, excessive inotrope use, ventricular arrhythmias, or reduced ejection fraction <40% are cardiac abnormalities that in general are not accepted for use in transplantation (Costanzo et al., 2010). Donors with chest trauma should be carefully assessed for any damage to the heart. Donor comorbidites may influence decisions on organ utilization. Data is limited regarding donor risk factors on transplant outcomes since these hearts are typically not transplanted.

Management of the donor by the OPO optimizes organ function through support of blood pressure, volume resuscitation, oxygenation, correction of electrolyte, and metabolic abnormalities including hormonal imbalances noted in brain death (insulin, corticosteroids, arginine vasopressin, tri-iodothyronine). The ischemic time may be referred to as the "cold" or "warm" ischemic time, but the total ischemic time is the total time from organ procurement in the donor to reperfusion in the recipient, also termed the "cold" ischemic time. "Warm" ischemic time refers to the duration of implantation or removal of the organ from ice until reperfusion. When evaluating a potential donor, the total ischemic time is considered, and guidelines recommend an ischemic time between 4 and 6 h with preference ≤4 h for older donor organs (Costanzo et al., 2010). ABO incompatibility is a well-established contraindication in adults due to hyperacute rejection. Size matching is usually within 20%–30% of recipient body size, although an average size male donor of 70 kg should typically be an appropriate size match for any recipient. Gender mismatching holds a mortality risk and poor outcomes due to the smaller female heart that is less likely to match the demands of a larger male recipient

or higher PVR (Kittleson et al., 2011; Weiss et al., 2009b). With an endpoint of 10-year survival, Kittleson et al. found a reduced survival in both types of gender mismatch when compared with gender-matched groups; in this single-center study of 857 patients from 1994 to 2008, findings suggest that an immunologic effect could also have a role in outcomes for mismatched recipients (Kittleson et al., 2011). Conversely, in a large UNOS database review of 18,240 transplants between 1999 and 2007, 5-year survival was the highest for matched male patients (male donor to male recipient) at 74.5%, while female donor to male recipients had a 15% increase in the risk of adjusted cumulative mortality (hazard ratio, 1.15; [1.02–1.30], $P=0.02$) (Weiss et al., 2009b). Female recipients of gender-matched or -mismatched organs showed no survival difference.

Utilization of donor hearts has decreased in the past decade and is around 40% in recent data (Khush et al., 2013). Predictors of donor nonuse were reviewed by Khush et al. within the California Transplant Donor Network from 2001 to 2008 with turndown of 1872 donors. Potential donors that were not accepted had the following: >50 years, female sex, cerebrovascular accident (CVA) as the cause of death, hypertension, diabetes, positive troponin, LV dysfunction with regional wall motion abnormalities, and LVH (Khush et al., 2013). Only CVA as the donor cause of death minimally predicted prolonged recipient posttransplant hospitalization (odds ratio, 1.41 [1.00–2.00]), while diabetes mellitus was the only donor predictor of reduced recipient 1-year survival (odds ratio 3.58, [1.18–10.84]). Experts have suggested that our current criteria for donor selection have resulted in discarding potentially useful organs leading to our present-day shortage of donors, and it seems likely that donor characteristics have a small contribution to posttransplant adverse events based on available data (Wittwer and Wahlers, 2008). Efforts to expand donor selection by liberalizing acceptance criteria and developing innovative organ perfusion techniques are ongoing as heart transplantation continues to remain limited by donor availability.

Organ Allocation

Organs are allocated according to the region, status on the waitlist, and matching by size and blood type. The allocation policy changed on June 12, 2006, to concentric 500 mile zones with the goal of prioritizing higher risk recipients, status 1A and 1B, and increasing the availability of a donor; prior to this updated policy, organs were offered to all status patients within a UNOS region before they were available to bordering regions (**Fig. 3**). Since this last revision, there has been an overall decline in the national waitlist mortality rate up to 180 days from 13.3% to 7.9% ($P<0.001$) and in the 2-year mortality rate from 17.3% to 14.6% ($P<0.001$). However, waitlist time has increased across the nation for status 1A and 1B candidates—maybe partly related to the rise in LVAD bridging, which was variable based upon the region with a range of <20%–40% (Schulze et al., 2014). Currently, LVAD patients have 30 days of 1A time regardless of a complication after which they are listed as 1B, but discussion is ongoing about the low waitlist mortality of continuous flow LVAD patients, which is similar to status 2 patients but lower than status 1A and 1B in a review of the UNOS database between 1999 and 2011 (Wever-Pinzon et al., 2013). However, patients with biventricular or temporary support or an LVAD complication were found to have a higher mortality. For this reason, the current allocation policy is under revision to create further tiers for the most urgent status based on type of support (temporary, biventricular support, temporary support) versus the stable LVAD patient or those with complications. Additional goals of the revision are to reduce the number of exception requests and increase organ sharing for the more urgent status candidates rather than the current scheme, which favors less urgent candidates in the local donor service area over more urgent candidates outside of the local donor area (https://optn.transplant.hrsa.gov/governance/public-comment/adult-heart-allocation-changes-2016/).

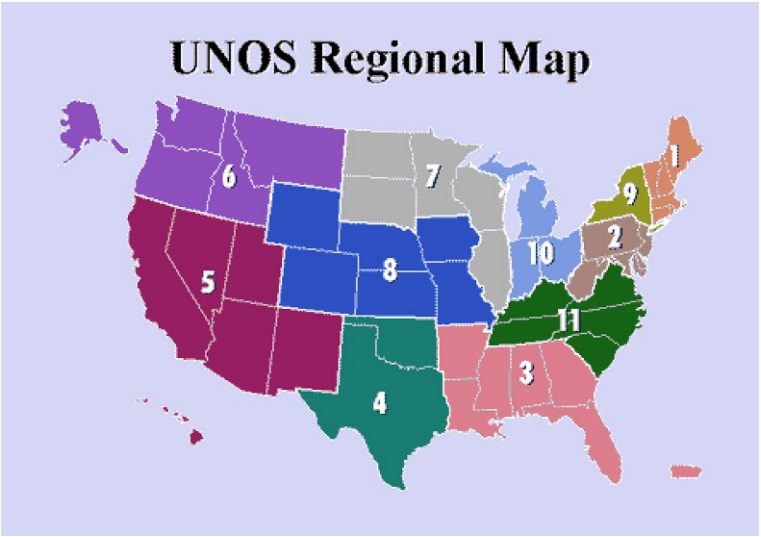

Fig. 3 The United Network for Organ Sharing (UNOS) manages organ allocation in the United States based upon these 10 geographic regions.

The Transplant Surgery

Donor procurement focuses on organ preservation and minimizing organ injury; both procuring and transplanting teams are involved in this process in order to limit the ischemic time. The organ is placed in a cold cardioplegia solution and transported to the transplanting center. Fig. 4 shows a comparison of the difference in size between the donor heart and recipient's dilated heart from heart failure. Experimental animal models have shown promise in using novel techniques to help reduce ischemia and reperfusion injury: targeted complement inhibition in recipients (Atkinson et al., 2013), cardioplegia solution supplemented with erythropoietin (Watson et al., 2013), a hypothermic hydrogen-rich water bath (Noda et al., 2013), and ex vivo heart perfusion after cardiocirculatory death (White et al., 2013; Tolboom et al., 2016). These methods require further investigation before incorporating into standard procedure.

Orthotopic heart transplantation was founded by Norman Shumway, Richard Lower, Adrian Kantrowitz, and Christiaan Barnard with Lower and Shumway first describing biatrial anastomosis in 1960 (Lower and Shumway, 1960). Biatrial anastomosis involves midatrial transection of both donor and recipient hearts along with transection of the great vessels just above the semilunar valves; the atrial septum is first reanastomosed followed by the great vessels. This method keeps the pulmonary venous connection to the left atrium intact in the recipient and includes the donor sinoatrial node in the right atrium. This method has evolved into the bicaval technique, first reported in 1991 by Dreyfus et al., in which the atria are not resected and anastomosis occurs at the inferior and superior vena cavae and pulmonary veins keeping the donor atria intact (Dreyfus et al., 1991). One benefit with the biatrial method is fewer connections with only two in the atria versus six atrial connections in the bicaval manner (two to the vena cavae and four to the pulmonary veins), possibly reducing ischemic time (Davies et al., 2010). The drawbacks to the biatrial technique are the large geometry of the atria and its possible effect on hemodynamics and arrhythmia risk along with the chance of damaging the sinoatrial node. Revising the implantation scheme was meant to reduce atrial size, atrial arrhythmias, tricuspid valve regurgitation, and bradycardia requiring a pacemaker which were considered to occur with more frequency in the biatrial method. Davies et al. reviewed almost 21,000 transplants from 1997 to 2007 with 59.3% biatrial and 38.1% bicaval anastomoses performed; they found bicaval anastomosis less often required postoperative pacemaker implantation and had a significant yet small survival advantage at 30 days and 1, 5, and 10 years (Davies et al., 2010). Despite the increasing use of the bicaval anastomosis, tricuspid regurgitation persists after transplantation in part due to repeated endomyocardial biopsies.

Physiology of the Transplanted Heart

When transplanted, the new heart is surgically "denervated" without afferent and efferent nerve supply (Pham et al., 2011). The recipient will often have a higher resting heart rate up to 100 beats per minute due to the loss of the efferent nerve supply from the central nervous system to the heart; due to this loss of vagal tone, they also will not respond to drugs dependent on the autonomic nervous system, such as atropine. The afferent supply, or that from the heart to the central nervous system, is responsible for angina and is not generally present in transplant patients due to denervation. Without angina, patients who are ischemic tend to present with heart failure symptoms or arrhythmias instead. Exercise intolerance or a blunted response to exercise is a trademark feature after transplantation related to efferent denervation since the recipient is now reliant upon circulating catecholamines for chronotropic effect. The heart is slow to increase the heart rate in response to exercise and also slow to recover postexercise as these levels decline. The baroreceptor reflex is not intact in these patients either due to sympathetic and parasympathetic denervation, which leads to a more sensitive orthostatic response and absent effect of carotid sinus massage. Reinnervation is a phenomenon that tends to arise with time as is evident by a lower resting heart rate and the presence of angina in some patients, but it is not a predictable event as to who, when, or how much (Pham et al., 2011).

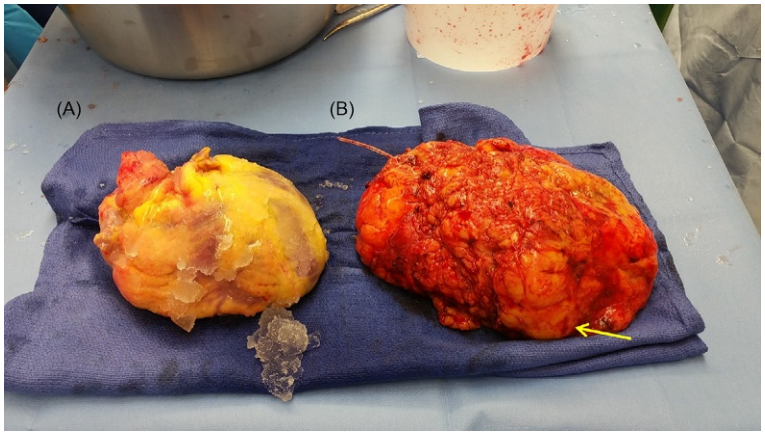

Fig. 4 Donor heart (A) compared to an enlarged recipient heart (B) in a patient with dilated cardiomyopathy. Courtesy of Ohio State University Wexner Medical Center Transplant Program.

Post-transplant Management and Challenges

Immunosuppression

The immunosuppression regimen consists of multiple drugs with many pharmacologic interactions and side effects, but this regimen lays the groundwork for successful outcomes and prevention of rejection in transplant recipients. The early regimen tends to have the highest intensity since rejection risk is the greatest in the early posttransplant period, and maintenance therapy tends to require lower doses as this risk declines with time in the majority of patients. An appropriate regimen will vary for the individual patient based upon the crossmatch, extent of sensitization (or pre- and posttransplant antibodies), and comorbidities in order to balance preventing rejection with avoiding complications associated with chronic overimmunosuppression such as infection and malignancy.

Induction

Approximately half of transplant centers use induction therapy as an adjuvant treatment in the postoperative management of a transplant recipient due to the risk of early rejection (Lund et al., 2015); immunologic mechanisms in this early stage include the concern that donor antigen expression is triggered by donor brain death, ischemia and reperfusion, and the trauma of surgery (Lindenfeld et al., 2004a). Induction allows delay of calcineurin antagonists in the setting of acute or worsening chronic kidney disease during the early postoperative period. Induction therapy includes T cell cytolytic agents such as polyclonal antithymocyte antibodies derived from rabbits (thymoglobulin) or horses (ATGAM) and the monoclonal antibody OKT3 derived from mice; these agents result in marked depletion of T lymphocytes. Interleukin-2 (IL-2) antagonists, such as basiliximab and daclizumab, inhibit proliferation and differentiation of T cells activated through the IL-2 pathway. The decision to induce any patient must take into consideration the infectious/malignancy risk versus rejection risk of these agents leading many centers to treat patients based upon the individual risk profile rather than inducing all transplant patients. Studies have shown varying results in terms of infectious risk with the choice of induction agent but similar efficacy in preventing rejection that requires treatment in small comparison studies of ATG and daclizumab (Carlsen et al., 2005; Mullen et al., 2014).

Maintenance regimen

Maintenance regimens usually consist of triple therapy in the early transplantation period (calcineurin inhibitor, antimetabolite, and corticosteroids), and usually reduce to two drugs if possible as corticosteroids are gradually withdrawn. The transplant physician must be familiar with the numerous drug interactions and toxicities of an immunosuppressive regimen. The multiple targets of the various agents in a combination drug regimen are detailed in **Fig. 5**.

Corticosteroids

Corticosteroids inhibit cytokine production and lymphocyte proliferation (T and B cells, monocytes, granulocytes, and macrophages), and are part of the maintenance immunosuppression regimen during the first year of transplantation when rejection risk tends to be the highest. Steroids are also used for the treatment of acute and/or chronic cellular and humoral rejection (Lindenfeld et al., 2004b). Doses tend to be high initially and are tapered during the early posttransplant period with discontinuation in approximately 50% of patients during the first year based on their rejection history. Chronic use is avoided as much as possible due to the considerable side effect profile of corticosteroids, which includes poor wound healing, tremors, hypertension, depression/anxiety, skin fragility, weight gain, osteoporosis, cataracts, peptic ulcer disease, infectious risk, and myopathy. Adverse metabolic effects include hyperglycemia, fluid retention, salt retention, osteopenia, and hyperlipidemia. Adrenal insufficiency will develop in some patients on chronic steroid treatment.

Calcineurin inhibitors

Calcineurin inhibitors (CNI), cyclosporine and tacrolimus, inhibit calcineurin activation by binding this protein, and therefore hinder transcription of IL-2 and several cytokines. These drugs represent the foundation of immunosuppressive therapy since the beginning of cyclosporine use in the 1980s due to a significant increase in survival. Comparison studies of both drugs have provided some evidence that although survival is similar with both CNI, less rejection is associated with tacrolimus, which has led to its greater utilization in maintenance regimens (Kobashigawa et al., 2006a; Grimm et al., 2006; Ye et al., 2009), Kobashigawa et al. found a significantly lower occurrence of grade 3A or higher rejection in both groups treated with tacrolimus and an improved side effect profile with lower median serum triglycerides and creatinine in the tacrolimus/mycophenolate mofetil group (groups were tacrolimus plus mycophenolate or sirolimus and cyclosporine plus mycophenolate) (Kobashigawa et al., 2006a). Likewise, in a meta-analysis of seven randomized controlled trials ($n = 885$), similar survival and less rejection for tacrolimus versus cyclosporine microemulsion was confirmed along with a different side effect profile; a lesser extent of hypertension and dyslipidemia was found with tacrolimus but a higher rate of new onset diabetes (Ye et al., 2009). Nephrotoxicity is associated with both drugs.

Antimetabolite agents

Mycophenolate mofetil (MMF) has replaced azathioprine as the favored antimetabolite agent in the maintenance of immunosuppression regimens. Both drugs inhibit T and B lymphocyte proliferation by inhibiting DNA replication. The primary difference is that MMF is more selective in its action on de novo purine synthesis and, consequently, does not have the degree of bone marrow suppression noted with azathioprine. The latter acts upon both de novo and salvage pathways targeting all cell lines as opposed to

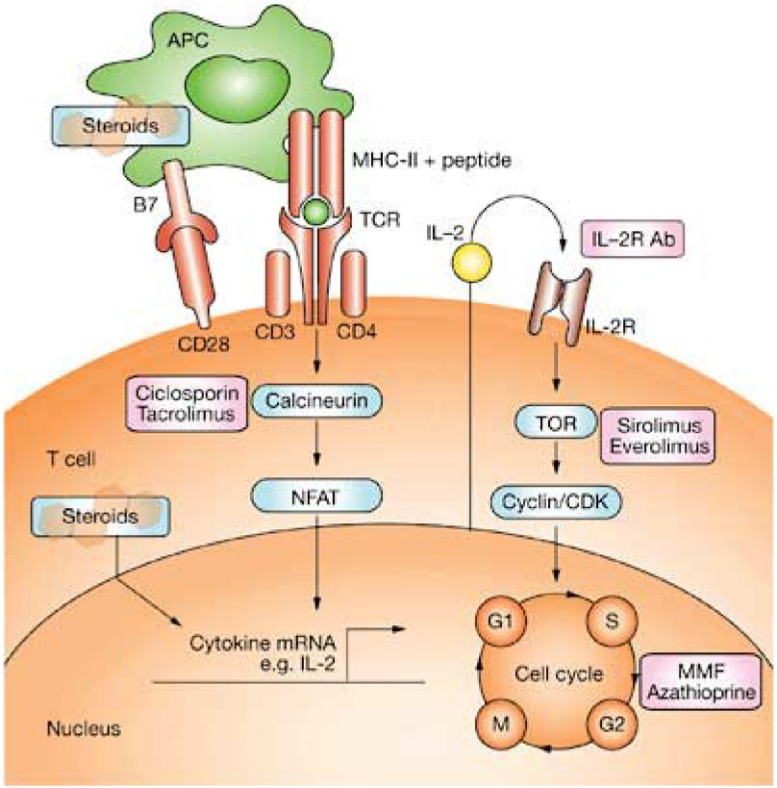

Fig. 5 Sites of action of immunosuppressive drugs. Presentation of donor antigen to antigen-presenting cells results in immunologic response, T-cell proliferation. This mechanism results in activation of calcineurin, which leads to production of interleukin-2 and subsequent cell proliferation. Immunosuppressive drugs *(in pink boxes)* exert their effects at a number of different targets to prevent T-cell proliferation. G1 (first growth phase), S (synthesis of DNA), G2 (second growth phase), and M (cell division) represent the phases of the cell cycle (Adapted with permission from reference Grimm, M., Rinaldi, M., Yonan, N. A. et al. (2006). Superior prevention of acute rejection by tacrolimus vs. cyclosporine in heart transplant recipients—A large European trial. *American Journal of Transplantation* **6**, 1387–1397. © (2001) American Society of Transplantation). *APC*, antigen-presenting cell; *CDK*, cyclin-dependent kinase; *IL-2*, interleukin-2; *IL-2R*, interleukin-2 receptor; *IL-2R Ab*, interleukin-2-receptor antibody; *MHC*, major histocompatibility complex; *MMF*, mycophenolate mofetil; *mRNA*, messenger RNA; *NFAT*, nuclear factor of activated T cells; *TCR*, T-cell receptor; *TOR*, target of rapamycin protein (Reprinted from Kobashigawa, J. A., Patel, J. K. (2006). Immunosuppression for heart transplantation: Where are we now? *Nature Clinical Practice. Cardiovascular Medicine* **3**, 203–212. Copyright 2006, with permission from Nature Publishing Group.

lymphocytes only (Lindenfeld et al., 2004b). Comparison of MMF to azathioprine in cardiac transplant patients revealed an improved late survival using an MMF regimen in a randomized trial and ISHLT/UNOS registry data (Eisen et al., 2005; Hosenpud and Bennett, 2001). Adverse effects of MMF are primarily gastrointestinal with nausea, vomiting, diarrhea, and tend to be dose responsive; mycophenolate sodium (MPS) is an enteric-coated, delayed release formulation with less gastrointestinal upset and therefore fewer dose reductions along with a similar efficacy in maintenance regimens (Lehmkuhl et al., 2008; Kobashigawa et al., 2006b. Fewer dose reductions with MPS were associated with a higher average daily dose of MPS (88.4% MPS vs. 79.0% MMF of the recommended dose, $P = 0.016$) and a significantly lower rate of biopsy proven and treated rejection grade $\geq$3A (23.4% MPS vs. 44.0% MMF, $P = 0.032$) (Segovia et al., 2008).

Proliferation signal inhibitors

The mammalian target of rapamycin (mTOR) inhibitors, sirolimus and everolimus, has been gaining in use after cardiac transplantation and several clinical trials have demonstrated their efficacy. These drugs prevent T and B lymphocyte proliferation in addition to smooth muscle and endothelial cell proliferation in response to growth factors (Lindenfeld et al., 2004b). The latter clarifies advantages of these drugs in potentially preventing transplant coronary allograft vasculopathy (CAV) and possibly tumor regression. Both drugs have been associated with a lower incidence of CAV and rejection when compared with azathioprine (Keogh et al., 2004; Eisen et al., 2003). Combination therapy with CNI can aggravate CNI-related nephrotoxicity, but evidence suggests that reduced CNI levels plus earlier initiation of mTOR inhibitors preserve renal function without the loss of immunosuppressive protection (Gonzalez-Vilchez et al., 2014). Major side effects include hypertriglyceridemia, bone marrow suppression, diarrhea, capillary leak, poor wound healing, pneumonitis, and aphthous ulcers; the side effect profile of these agents often limit tolerance and result in withdrawal of treatment.

Rejection

Management of immunosuppression has shifted through the years with initial introduction of calcineurin inhibitors followed by mycophenolate mofetil replacing azathioprine and now tacrolimus as the leading CNI. Acute rejection is more common early after transplant with the risk subsequently declining over time. Hyperacute rejection occurs within hours of transplantation, but is unusual since it is related to ABO incompatibility. Cellular- and antibody-mediated rejection presents as acute or chronic occurring alone or in a mixed scenario. Acute cellular rejection (ACR) is T cell mediated and the most common form of rejection occurring within the first year posttransplant. Diffuse inflammation, myocyte injury, hemorrhage, and/or necrosis are graded according to the ISHLT grading scale (**Table 5**) (Stewart et al., 2005). Acute antibody-mediated rejection (AMR) is B cell related with immunopathologic findings of antibody deposition in the vasculature (arteriolar vasculitis) and complement activation, histopathologic findings of endothelial swelling and interstitial edema, and clinical features of hemodynamic compromise and allograft dysfunction (**Table 6**).

Symptoms

Symptoms of rejection are nonspecific or even clinically silent in earlier stages, often not apparent until rejection has progressed to an advanced stage. Patients present with heart failure signs, symptoms, or hypotension; exam findings could include volume overload or S_3 gallop. They may have vague symptoms of fatigue, cough, or a low-grade fever. Atrial arrhythmias or new pericardial effusions have also been a presenting sign.

Diagnosis

Given the absence of symptoms in early rejection, surveillance endomyocardial biopsies and right heart catheterization are performed in the routine management of heart transplant recipients in order to diagnose rejection before it has reached more advanced stages. **Tables 5** and **6** review the grading system for diagnosis of ACR and AMR, and **Figs. 6** and **7** are examples of biopsies with moderate to severe rejection. Endomyocardial biopsies (EMB) are performed preferentially through the right internal jugular vein followed by the femoral vein via inserting a bioptome into the right ventricle to obtain a few samples of endomyocardium. Centers perform EMB with echocardiography or fluoroscopy to identify the appropriate location and avoid complications of sampling the tricuspid valve apparatus or the free wall. A less invasive test using a blood sample is now in use, Allomap, which detects cellular rejection based on gene expression in peripheral blood mononuclear cells in the stable patient at low risk of rejection (Pham et al., 2010). Benefits included significantly fewer biopsies reducing the risk of biopsy-related complications such as tricuspid regurgitation, arrhythmias, and perforation. More recent data suggests that score variability may be used to predict probability that an event will occur (Crespo-Leiro et al., 2015). Routine surveillance with EMB in stable patients without a clear indication of heart failure symptoms or allograft dysfunction has been shown to have a low yield beyond 6 months posttransplant with the majority of episodes being mild and not requiring treatment (Orrego et al., 2012); therefore, noninvasive testing represents an advancement in this discipline. A novel 13-point risk score was developed and validated from the UNOS database between 1998 and 2008 ($n = 14{,}265$). The primary outcome was drug-treated rejection within 1 year of transplantation; four variables were associated with a strong correlation between observed and predicted rejection rates in the validation cohort ($r^2 = 0.96$, $P < 0.001$): younger age, female sex, race (Asian < white, black, Hispanic, and other), and degree of human leukocyte antigen (HLA) mismatching (4–6 antigen mismatches) (Kilic et al., 2012b).

Table 5 International Society for Heart and Lung Transplantation grading system for acute cellular rejection

2004 revised		*1990*	
Grade 0R	No rejection	Grade 0	No rejection
Grade 1R, mild	Interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage	Grade 1, mild	Focal perivascular and/or interstitial infiltrate without myocyte damage
		A—focal	
		B—diffuse	Diffuse infiltrate without myocyte damage
Grade 2R, moderate	2 or more foci of infiltrate with associated myocyte damage	Grade 2, moderate (Focal)	One focus of infiltrate with associated myocyte damage
		Grade 3, moderate	
		A—focal	Multifocal infiltrate with myocyte damage
Grade 3R, severe	Diffuse infiltrate with multifocal myocyte damage ± edema, ±hemorrhage ± vasculitis	B—diffuse	Diffuse infiltrate with myocyte damage
		Grade 4, severe	Diffuse, polymorphous infiltrate with extensive myocyte damage ± edema, ±hemorrhage + vasculitis

Adapted from Stewart, S., Winters, G. L., Fishbein, M. C. et al. (2005). Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *The Journal of Heart and Lung Transplantation* **24**, 1710–1720. Copyright 2004, with permission from Elsevier.

Table 6 International Society for Heart and Lung Transplantation Grading System for Pathologic Diagnosis of Acute Antibody Mediated Rejection (AMR).

- pAMR 0—negative for pathologic AMR: histopathologic and immunopathologic studies are both negative.
- pAMR 1 (H+)—histopathologic AMR alone: histopathologic findings present and immunopathologic findings negative.
- pAMR 1 (I+)—immunopathologic AMR alone: histopathologic findings negative and immunopathologic findings positive (CD68+ and/or C4d+ for immunohistochemical staining and C4d+ with or without C3d+ for immunofluorescence staining).
- pAMR 2—pathologic AMR: histopathologic and immunopathologic findings are both present.
- pAMR 3—severe pathologic AMR: interstitial hemorrhage, capillary fragmentation, mixed inflammatory infiltrates, findings of dying cells (pyknosis, karyorrhexis), marked edema, and immunopathologic findings are present.

Adapted from Berry, G. J., Burke, M. M., Anderson, C. et al. (2013). The 2013 International Society for Heart and Lung Transplantation working formulation for the standardization of nomenclature in the pathologic diagnosis of antibody-mediated rejection in heart transplantation. *The Journal of Heart and Lung Transplantation* **32**, 1147–1162. Copyright 2013, with permission from Elsevier.

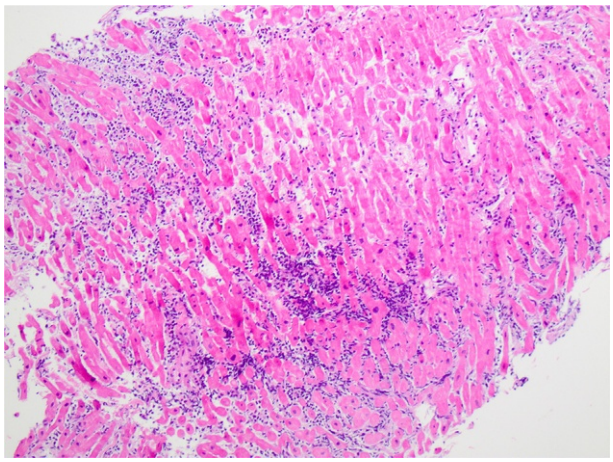

Fig. 6 Diffuse patchy interstitial inflammatory cell infiltrate with associated myocyte injury suggestive of at least moderate acute rejection Grade 3R cellular rejection. This patient had risk factors of sensitization, race, and noncompliance with immunosuppression regimen. Courtesy of Ohio State University Wexner Medical Center.

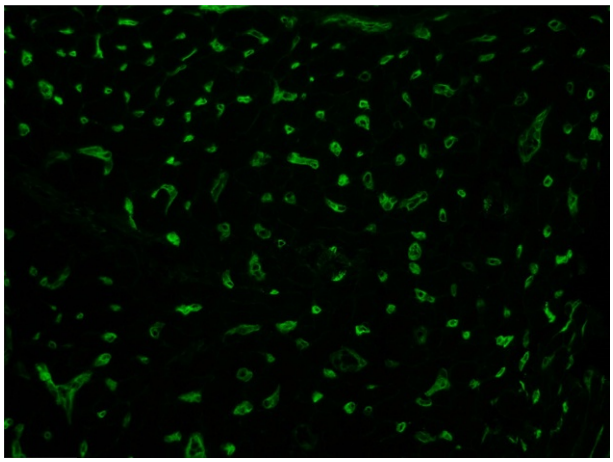

Fig. 7 Antibody-mediated rejection in a heart transplant recipient. Risk factors were younger age, female gender, and noncompliance with medication regimen. The patient was treated multiple times for rejection with subsequent graft dysfunction and chronic AMR despite aggressive therapy. Diffusely positive C4d staining in the capillaries appears as a "donut" appearance on this immunofluorescence sample. Courtesy of Ohio State University Wexner Medical Center.

Treatment

Therapy is based on clinical presentation, hemodynamic compromise on RHC and/or imaging, and histologic grade of the biopsy. Low-grade rejection in an asymptomatic patient is typically not treated. In scenarios of graft dysfunction or hemodynamic deterioration, treatment of ACR includes pulse and oral steroids, augmentation of oral immunosuppression (CNI, MMF, mTOR inhibitor), changing cyclosporine to tacrolimus, and the addition of antithymocyte antibody or cytolytic antibody.

AMR has historically been more challenging to treat than ACR and can lead to allograft dysfunction and cardiac vasculopathy (Nair et al., 2011). For this reason, treatment protocols are not standardized and will vary for transplant centers. Therapy focuses on the following four targets: suppression of T lymphocytes (ATG, CNI, MMF), inhibition of circulating antibodies (intravenous immunoglobulin or IVIG), elimination of circulating antibodies (apheresis), and suppression of B lymphocytes (rituximab) (Nair et al., 2011). Promising treatment includes proteasome inhibitors to deplete plasma cells, but limited data is available in cardiac transplantation. Although more difficult to treat than ACR and escalating immunosuppression does not always result in resolution, a combined approach seems best along with routine follow-up of anti-HLA antibodies. Chronic or refractory rejection, both cellular and humoral, can be further treated with total lymphoid radiation, or extracorporeal photopheresis, which requires central access. Other considerations include infection, such as cytomegalovirus, that can lead to rejection, and obtaining a detailed history to define the etiology of rejection since psychosocial factors, noncompliance, and changes in the patient's drug regimen resulting in lower drug levels could also play a role.

Complications

Primary graft dysfunction

A consensus statement from the ISHLT released in 2014 defined primary graft dysfunction within 24 h of completion of transplant surgery and involving the left and/or right ventricular function (Kobashigawa et al., 2014). It is not related to hyperacute rejection, pulmonary hypertension, or surgical complications, which would lead to secondary graft dysfunction; outcomes are usually death or retransplant associated with graft failure within the first 30 days after transplant. Recipient and donor risk factors contribute to primary graft dysfunction. Donor risk factors include cause of brain death, donor age, and female gender, while recipient risk factors include age, pulmonary hypertension (even within transplant acceptable criteria), and the more critically ill recipient (mechanical support, ventilation, inotrope) (Lund et al., 2015; Kobashigawa et al., 2014; Russo et al., 2010b). Surgical considerations are ischemic time, donor–recipient weight mismatch, and center volume with lower center volume associated with a higher rate of primary graft dysfunction. If graft function does not improve with supportive measures, retransplantation is an option although less likely due to multiorgan failure that may develop. In a UNOS database review from 1999 to 2007, Russo et al. found that 414 heart transplants were complicated by primary graft failure; of these, 85.5% died and 14.5% were retransplanted (Russo et al., 2010b).

Right ventricular dysfunction

Right ventricular (RV) dysfunction is common after cardiac transplantation, in as much as half of recipients, but acute, severe RV failure is high risk for early mortality (Bozbas et al., 2013). Pretransplant pulmonary hypertension increases the risk of peri- and postoperative RV dysfunction, explaining why high or fixed PVR is a contraindication to transplantation. When present, RV dysfunction is treated with inhaled vasodilators, such as nitric oxide or epoprostenol, and pulmonary afterload reduction with milrinone. Inotrope support of the RV with dobutamine or milrinone and volume control (diuretics, dialysis, ultrafiltration) are key management steps in this setting. Maintaining a higher heart rate also supports the RV. If RV dysfunction does not improve, worsening renal failure ensues contributing to a higher volume load on the transplanted heart.

Bradycardia

Posttransplantation, patients have a higher resting heart rate, usually around 110 bpm, which is related to denervation of the transplanted heart. A lower heart rate is considered “relative” bradycardia, which may be related to sinus node dysfunction, pretransplant negative chronotropic agents such as amiodarone, and possibly the type of anastomosis (Davies et al., 2010). Patients are dependent upon chronotropic response to maintain exercise tolerance, and in cases of chronotropic incompetence (or heart rates of 70 bpm), beta-agonists such as terbutaline could increase heart rate or, when persistent and symptomatic, a permanent pacemaker would be indicated.

Infection

Infection risk is greatest during the first year after transplantation due to the higher intensity immunosuppression regimen (Fishman, 2009, 2014). Risk assessment for a higher infectious risk involves critical illness entering transplantation, induction therapy, high dose corticosteroids, prior colonization with antimicrobial-resistant infections, graft dysfunction, early graft rejection, and technical complications such as prolonged intubation, surgical or urinary catheters, and wound infection. Early posttransplant infections tend to involve donor or recipient derived infection or are associated with surgical complications; therefore, they generally occur within the first month. These include nosocomial bacterial infections related to indwelling lines/catheters, pneumonia and wound infection with organisms such as *Staphylococcus*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Escherichia coli*, and *Clostridium difficile* colitis (**Fig. 8**). Opportunistic infections usually occur later in the 1–6-month period after transplant arising from various sources: viral (cytomegalovirus, CMV), fungal (aspergillus, candida), and parasites (toxoplasmosis, pneumocystis). After 6 months, community-acquired infections are more common since immunosuppression tends to be less intense than initial regimens. Antiviral (CMV, herpes simplex virus) and pneumocystis carini prophylaxis are standard protocol in transplant centers; antifungal prophylaxis is not standardized and varies per center.

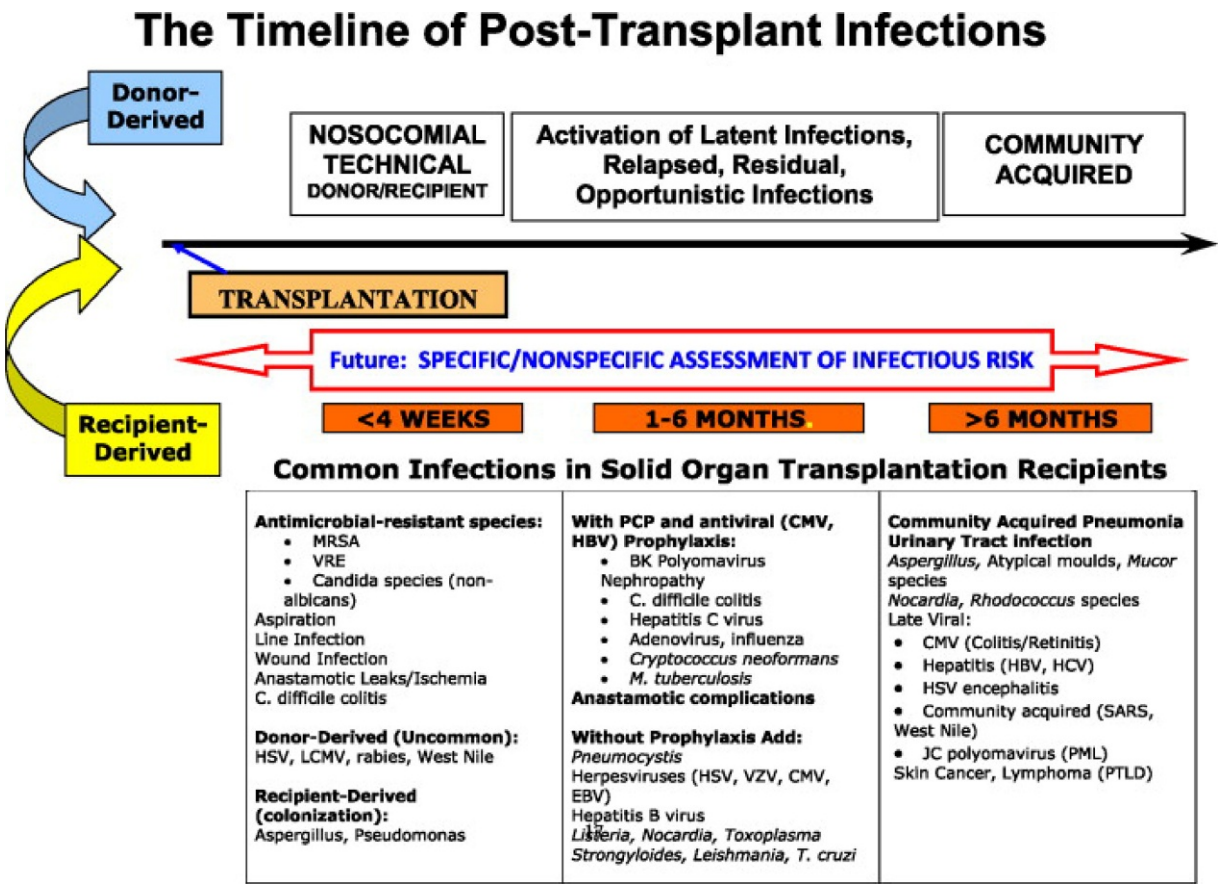

Fig. 8 Common infections in solid organ transplant recipients. Reprinted from Fishman, J. A. (2014). From the classic concepts to modern practice. *Clinical Microbiology and Infection***20**, 4–9. Copyright 2014, with permission from John Wiley and Sons

Malignancy

Malignancy is a common cause of late death ranging from 20% to 24% for patients beyond 3–10 years posttransplantation (**Fig. 9**) (Lund et al., 2015). Risk is related to the intensity and duration of chronic immunosuppression leading to decreased immune surveillance for cancer and activation of oncogenic viruses such as Epstein-Barr virus (EBV in posttransplant lymphoproliferative disorder, PTLD), human herpes virus 8 (HHV-8 in Kaposi's sarcoma), and human papilloma virus (HPV in anogenital cancer). The most common de novo posttransplant malignancy is skin cancer followed by lymphoproliferative disorders, PTLD. Squamous and basal cell cancers occur at a much higher rate in the solid organ transplant population and in younger patients, at many sites, and are also more aggressive than in the general population (Ajithkumar et al., 2007). Melanoma risk is greater but not as excessive as other skin cancers; the risk of Kaposi's sarcoma is increased tremendously. PTLD is the most common noncutaneous malignancy and most cases are related to EBV reactivation or primary infection. Breast, lung, prostate, bladder, and colon cancers also occur in transplant recipients, although evidence varies in terms of additional incidence in these patients, but may present more aggressively due to immunosuppression.

Multiple factors contribute to the development of malignancy in these patients including disruption of DNA repair mechanisms related to the immunosuppressive regimen and upregulation of angiogenic growth factors (**Fig. 10**) (Nair et al., 2014). Nair et al. suggest an algorithm that involves risk factor modification, cancer surveillance, modification of immunosuppression doses after diagnosis of malignancy, and nonimmune therapies (Nair et al., 2014). Prevention is key which follows current recommendations for cancer screening with mammograms, papanicolaou testing and gynecologic examinations, colonoscopy, dermatology examination, and prostate-specific antigen. Emphasis is placed on minimizing and protecting against sun exposure, smoking cessation, and antiviral prophylaxis in the early posttransplant period. If malignancy develops, immunosuppression is reduced, especially in the case of PTLD, with the goal of maintaining a balance of adequate immunosupression; MMF or CNI might be replaced with sirolimus which may have antiproliferative properties. The pleiotropic benefits of statins could play a role in reducing cancer-free survival posttransplant, but needs further clinical trial evaluation (Fro¨hlich et al., 2012). Additionally, future research could focus on malignancy prevention strategies such as biomarkers for tolerance prediction, memory B cell reduction, and naive B cell repopulation.

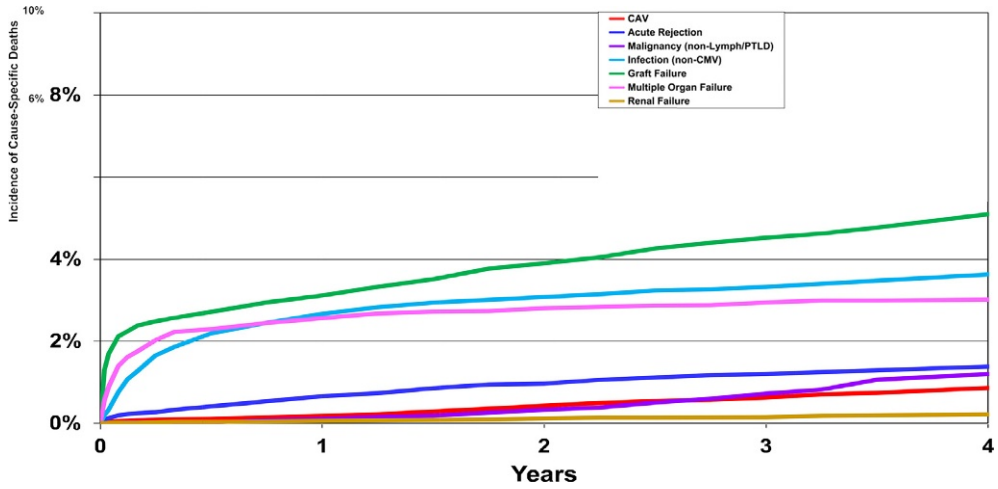

Fig. 9 Leading causes of mortality in posttransplant course (Jan 2009 to June 2013). *CAV*, cardiac allograft vasculopathy. Reprinted from Lund, L. H., Edwards, L. B., Kucheryavaya, A. Y. et al. (2015). The registry of the International Society for Heart and Lung Transplantation: Thirty-second official adult heart transplantation report—2015; focus theme: Early graft failure. *The Journal of Heart and Lung Transplantation* **34** (10), 1244–1254. Copyright 2015, with permission from Elsevier.

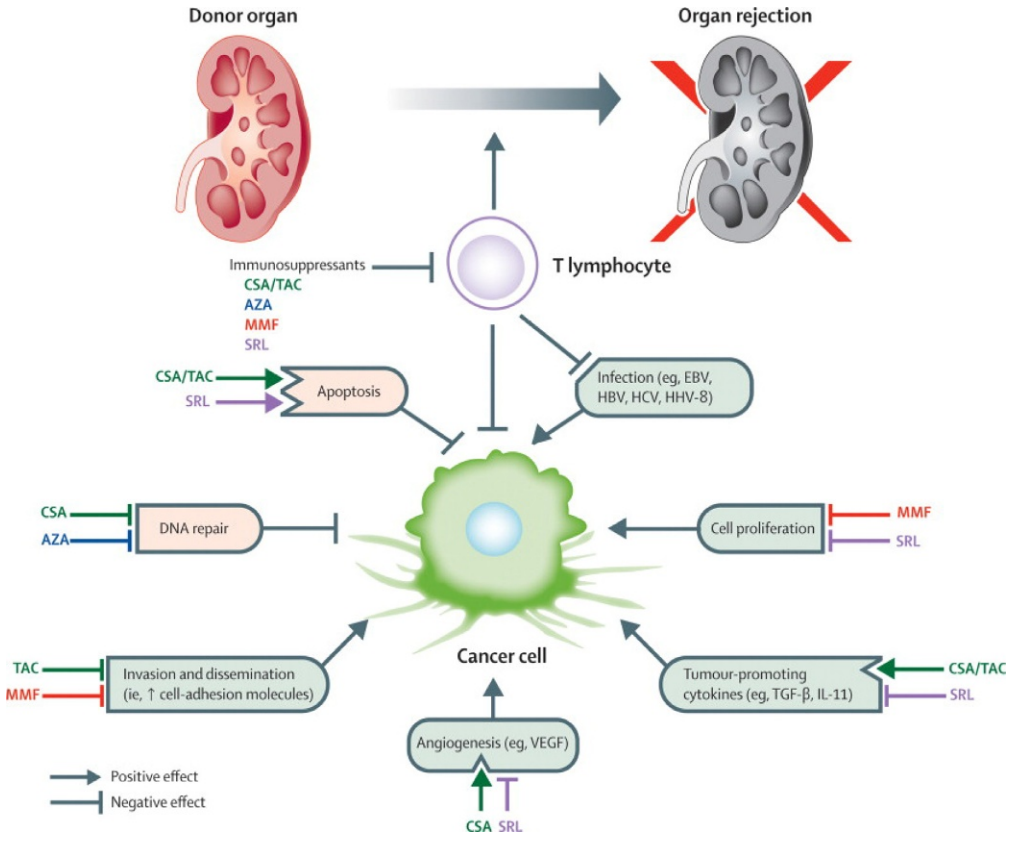

Fig. 10 Mechanisms of transplant-associated malignancies including possible effects related to immunosuppressive agents. Cyclosporin impairs repair of radiation-induced DNA damage and both cyclosporin and tacrolimus induce expression of tumor promoting cytokines. *CSA*, cyclosporin; *TAC*, tacrolimus; *AZA*, azathioprine; *MMF*, mycophenolate mofetil; *SRL*, sirolimus; *HBV*, hepatitis B virus; *HCV*, hepatitis C virus; *VEGF*, vascular endothelial growth factor; *TGF*, transforming growth factor; *IL*, interleukin; ↑, increased quantities. From Ajithkumar, T. A., Parkinson, C. A., Butler, A. et al. (2007). Management of solid tumours in organ-transplant recipients. *Lancet Oncology* **8**, 921–932. Copyright 2007, reprinted with permission from Elsevier.

Comorbidities

Hypertension

Hypertension is prevalent in transplant patients and for the most part related to CNI therapy, with tacrolimus resulting in less hypertension than cyclosporine in a meta-analysis by Yu et al. of seven randomized controlled trials (Ye et al., 2009) Yu. The majority of patients will be hypertensive requiring multiple drugs in an antihypertensive regimen for control. Transplant patients have higher ambulatory BP including clinic, mean 24-h, mean daytime, and mean nighttime systolic/diastolic BP along with increased 24-h heart rate and decreased heart rate variability (Kotsis et al., 2005). There is also a reduced decline in nocturnal BP noted posttransplantation. Hypertension is a risk factor for other posttransplant complications such as renal failure and coronary vasculopathy. Beta-blockers are usually avoided in the early posttransplant course due to the recipient's denervation and dependence on circulating catecholamines for chronotropic response; symptoms of fatigue may be more pronounced if taking beta-blockers. Commonly used drug regimens for treatment include angiotensin-converting enzyme inhibitors or angiotensin receptor blockers based on renal dysfunction or hyperkalemia, calcium channel blockers such as amlodipine or diltiazem (which will increase CNI levels), thiazide diuretics, hydralazine, and nitrates.

Hyperlipidemia

Dyslipidemia is largely related to drug-induced side effects after transplantation, particularly from corticosteroids, CNI, and proliferation signal inhibitors. Development of hyperlipidemia contributes to CAV, peripheral vascular disease, and cerebrovascular disease. Statins, or HMG-CoA reductase inhibitors, are effective in reducing the lipid profile and have pleiotropic effects that extend the benefit beyond cholesterol lowering. These include promotion of apoptosis, inhibition of angiogenesis, downregulation of circulating cytokines, plaque stabilization, inhibition of smooth muscle cell proliferation, and improved endothelial function (Luo et al., 2014; Vallakati et al., 2016). Rhabdomyolysis is a risk of use in patients due to a drug interaction with CNI therapy, although it is likely lower with pravastatin since it is not metabolized by cytochrome enzymes like the other statins. Risk of rhabdomyolysis increases with higher statin doses or addition of other agents such as fibrates or niacin. There is also a potential immunomodulator role of statins due to an association with lower rejection in heart transplant recipients, and have been associated with a better long-term survival compared with patients who were not on statins (Luo et al., 2014; Vallakati et al., 2016; Som et al., 2014). A review of seven randomized controlled trials of statins in cardiac transplants ($n = 353$) confirmed a modest survival benefit of statins, low incidence of adverse effects but no difference in rejection episodes (Marchetti, 2004). In a larger meta-analysis of 2295 patients, pooled data also suggested improved survival with statin use but also decreased fatal rejection episodes, terminal cancer, and the incidence of coronary vasculopathy (Vallakati et al., 2016).

Diabetes mellitus

Diabetes is very common posttransplantation with approximately one-third of patients affected by 5 years (Marchetti, 2004). Many factors contribute to the development of diabetes: age, obesity, pretransplant diabetes, ethnicity, family history, and drug-induced side effects from corticosteroids and CNI drugs (tacrolimus more than cyclosporine). Diabetes has a negative impact on survival, renal function, and CAV risk, been associated with reduced long-term survival, and is associated with comorbidities such as CAV and renal dysfunction. A guideline consensus statement was developed for renal, liver, and heart transplantation to reduce individuals' risk of developing new onset diabetes and improve long-term associated complications from diabetes; guidelines include recommendations on screening, managing risk factors, and adjusting contributing immunosuppressive drugs (Wilkinson et al., 2005).

Renal failure

Renal dysfunction is a common posttransplantation complication and is associated with poor outcomes (Thomas et al., 2012). Risk factors for development include older recipient age, female gender, diabetes, temporary dialysis or hemofiltration perioperatively, and impaired renal function pretransplant or early posttransplant. The risk of death is higher for recipients with CKD stage 4, CKD stage 5, or those on dialysis when compared with CKD stage 3 or lower. In a series of renal biopsies performed in renal failure postcardiac transplantation, a diverse range of histopathologic findings were noted with multiple patient-specific factors contributing to CKD: acute kidney injury from hypotension, vasoconstrictor use and cardiopulmonary bypass, hypertensive and diabetic nephropathy, ischemic disease, and nephrotoxic medications, including CNI with vasoconstrictor effects (Pinney et al., 2012). In addition, CNI-induce vasoconstriction appears to worsen renal function when these underlying factors or intrinsic renal disease are present, rather than CNI being the sole etiology. Prevention involves managing comorbidities such as hypertension and diabetes along with nephrology involvement early. Renal sparing immunosuppression regimens are also initiated in these patients and include reducing CNI dose or converting from CNI to sirolimus/everolimus maintenance therapy alone or in combination with lower dose CNI (Arora et al., 2012; Zuckerman et al., 2012; Potena et al., 2012). Success of this conversion to proliferation signal inhibitors is earlier utilization since the presence of proteinuria seems to limit any benefit from this change in maintenance regimens (Pinney et al., 2012; Potena et al., 2012). Sirolimus is associated with a higher discontinuation rate due to the side effect profile and more episodes of acute rejection (Zuckerman et al., 2012). Other preventive measures include close surveillance of renal function and risk factors posttransplantation and minimizing nephrotoxic insults as much as possible.

Coronary vasculopathy

Coronary allograft vasculopathy is present in one-third of transplant recipients at 5 years posttransplantation and remains a major comorbidity limiting long-term survival after transplantation (**Fig. 9**) (Lund et al., 2015). Similar to surveillance biopsies, surveillance coronary angiography is performed routinely to detect development since patients do not present with typical angina symptoms due to denervation status. Symptoms might develop in some individuals over time if reinnervation is present, but most will have acute heart failure symptoms, arrhythmias, myocardial infarction, or sudden death (Pham et al., 2011).

Vasculopathy does not resemble native coronary artery disease (CAD) and has unique features that contribute to a difficult diagnosis with angiography. Involved areas are diffuse rather than focal and have less lipid and calcium deposition in the plaque when compared with CAD in nontransplant patients. CAV is a proliferative process consisting of diffuse intimal hyperplasia of the entire of length of the vessel, which can include the epicardial vessels and their branches and the microvasculature with intramyocardial arteries, arterioles, and capillaries (Vecchiati et al., 2014). "Distal pruning" is noted in the distal, smaller caliber epicardial vessels but intervention is not an option due to the size (**Fig. 11**). The concentric nature of involvement along the entire vessel often leads to underestimation of luminal narrowing and disease burden.

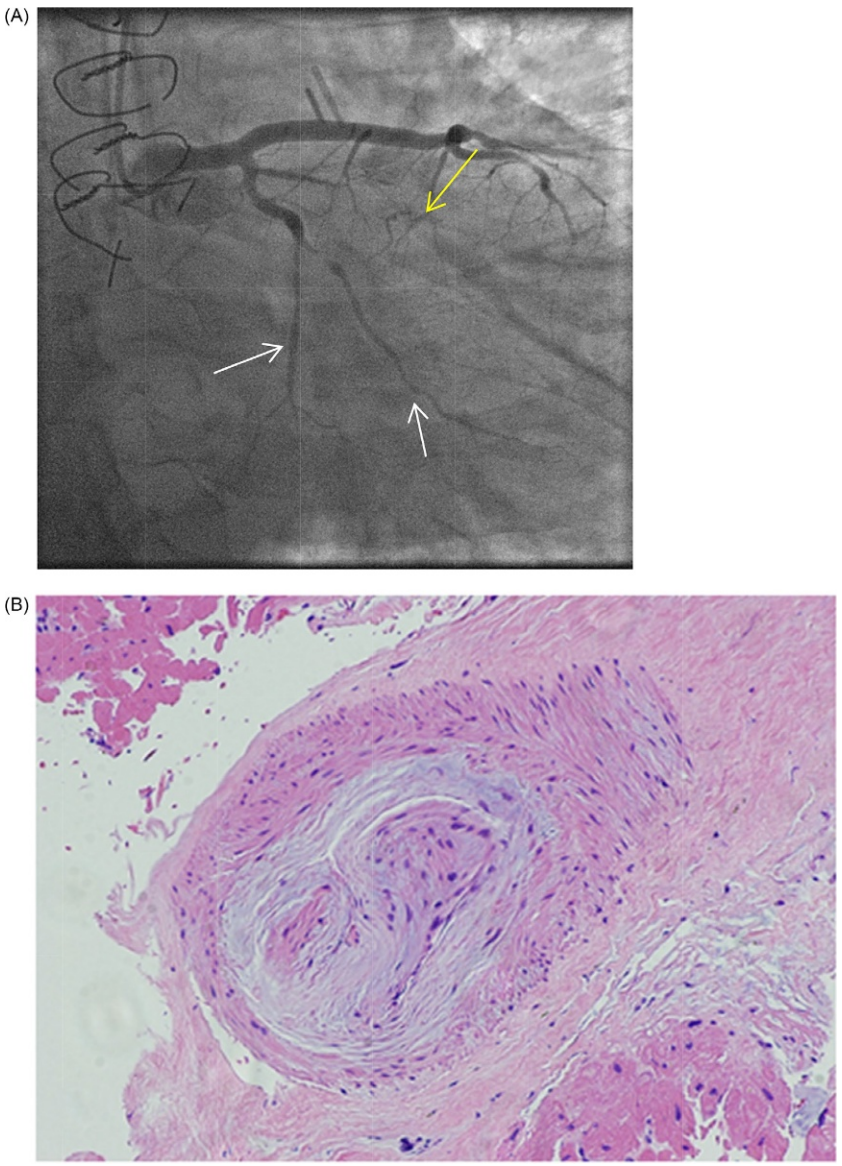

Fig. 11 Extensive coronary allograft vasculopathy (A) in large and distal vessels in a patient with recurrent cellular rejection and history of CMV. Arrows (*yellow*): total obliteration of the mid and distal left anterior descending artery. Arrows (*white*): severe transplant vasculopathy in the left circumflex and obtuse marginal branches. Pathology sample of coronary artery vasculopathy (B) demonstrating diffuse concentric intimal thickening. Courtesy of Ohio State University Wexner Medical Center.

Endothelial dysfunction is an early marker of CAV; evidence suggests both immunologic and nonimmunologic mechanisms contribute to endothelial damage and the subsequent inflammatory response (Vecchiati et al., 2014; Lim et al., 2016). The following immunologic mechanisms lead to a chronic immune response: (a) alloimmune injury from the degree of HLA antigen compatibility, (b) non-HLA allo- and auto-antibodies (or alloreactive T and humoral cells), and (c) dysregulation of the nitric oxide pathway leading to endothelial vasodilator dysfunction. The ensuing inflammatory response activates proliferation of smooth muscle cells and mononuclear infiltrate. Nonimmunologic factors are older donor age, longer ischemia–reperfusion time, CMV infection, and traditional risk factors such as hyperlipidemia and diabetes. In a single-center study, postoperative renal replacement therapy was also associated with CAV (Lim et al., 2016). Arteriosclerosis in CAV can result in de novo lesions but also remodeling of preexisting lesions in the donor heart. An important feature of CAV is intraplaque hemorrhages as a result of endothelial damage that may cause rapid increase of stenosis and generate a substrate for plaque destabilization (Angelini et al., 2014). Histopathology of CAV divides lesions into different phenotypes (inflammatory, smooth muscle, and fibrotic) that could be related to time after transplantation, age at transplantation, infection history, and the amount of atherosclerotic disease (Hulbers et al., 2014).

Routine angiography is an important screening tool for the detection of CAV, but other imaging modalities have added detailed assessment due to the challenging nature of CAV identification as mentioned earlier. Modalities include invasive, intracoronary imaging such as intravascular ultrasound (IVUS) and optical coherence tomography (OCT), and noninvasive imaging, such as dobutamine stress echocardiogram and coronary-computed tomography (Kobashigawa et al., 2005; Khandar et al., 2013; Sade et al., 2008; Wever-Pinzon et al., 2014). IVUS has been the gold standard and measures coronary intimal thickening, which has been associated with poor outcomes. Patients with intimal thickening on IVUS within the first year after transplantation had a higher incidence of death, graft loss, nonfatal major cardiac events, and angiographic CAV after 5 years (**Fig. 12**) (Kobashigawa et al., 2005). IVUS is limited due to low spatial resolution and cannot detect more subtle changes in intimal thickness. A newer intravascular imaging technology with higher spatial resolution has emerged with OCT, which allows for a more detailed evaluation of intimal thickness and characterization of atherosclerotic plaque having been shown to be very accurate when compared with histology (Khandar et al., 2013). Long-term outcome data correlating findings with cardiac events is limited since it is a newer technique. Noninvasive alternatives to coronary angiography utilize dobutamine stress echocardiography with a moderate sensitivity and specificity and a favorable meta-analysis of coronary-computed tomography angiography (Sade et al., 2008; Wever-Pinzon et al., 2014).

Prevention of CAV is key; management should focus on surgical factors such as reducing ischemic time and recipient factors to prevent recurrent rejection and infection, and modify traditional risk factors (hypertension, diabetes, smoking, and hyperlipidemia). Pharmacologic management utilizes traditional treatment with aspirin prophylaxis and statins, but also focuses on the immunosuppression regimen and inflammatory response. Early conversion to a PSI regimen may be more effective in preventing CAV progression as noted in three trials: a sirolimus-based regimen within the first 2 years and everolimus within the first year posttransplant (Matsuo et al., 2013; Masetti et al., 2013; Kobashigawa et al., 2013). Conversely, a recent prospective trial compared long-term outcomes in 126 patients receiving sirolimus + low-dose tacrolimus versus full-dose tacrolimus + MMF and found no significant difference between the two strategies in terms of survival, renal function, and CAV over the 8-year follow-up (Guethoff et al., 2015). In addition to lipid lowering, the pleiotropic benefits of high dose statins (antiinflammatory properties, inhibition of

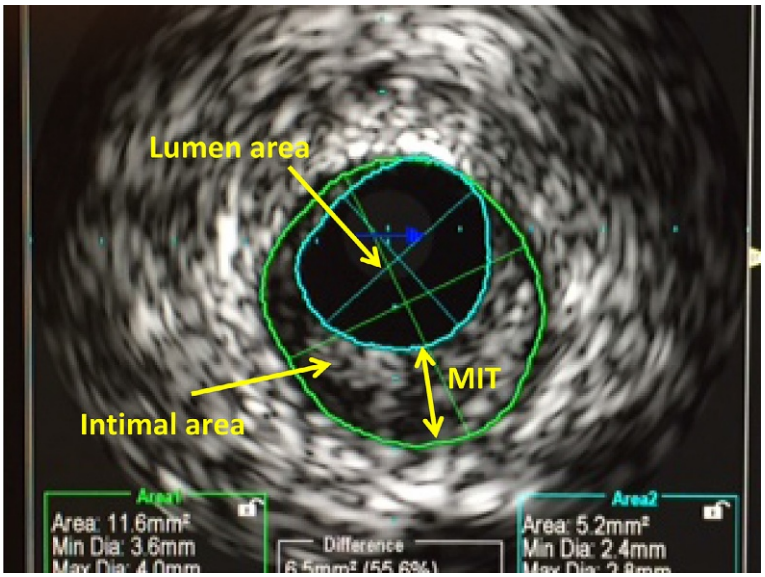

Fig. 12 Example of IVUS of mid LAD posttransplantation in a patient who present with anterior ST elevation. Example of intimal thickening on intravascular imaging with MIT. The vessel area = lumen area + intimal thickness. The percent area stenosis = (intimal area/vessel area) × 100%. *MIT*, maximal intimal thickness. Courtesy of Ohio State University Wexner Medical Center.

smooth muscle cell proliferation, and improved endothelial function) have been associated with reduced early and late CAV progression (Masetti et al., 2013).

The ISHLT established the following classification system for defining the extent of CAV: (a) CAV_0, no detectable angiographic lesion, (b) CAV_1, mild, (c) CAV_2, moderate, (d) and CAV_3, severe, which are based on the degree of stenosis, number of vessels, and which vessels are involved (left main, primary vessel or branch vessel) (Mehra et al., 2010). This grading system was shown to have prognostic significance in a retrospective study assessing CAV at 1-year posttransplant and predicted major adverse cardiac events (death, acute coronary syndrome, coronary revascularization, heart failure admission unrelated to an acute rejection episode, and cardiac retransplantation) (Prada-Delgado et al., 2012).

Revascularization poses many challenges starting with lack of symptoms for many patients and whether to attempt revascularization. Limited outcome data exists and an appropriate choice of treatment is still questionable. Coronary artery bypass grafting in CAV has been associated with poor outcomes, which is not unexpected due to the nature of CAV with less proximal and mid-vessel stenosis and suboptimal distal anastomotic targets (Halle et al., 1995). In-stent restenosis with percutaneous coronary intervention is higher when compared with nontransplant patients (Bader et al., 2006). Database review of drug-eluting stents in six studies of CAV found a reduction in in-stent restenosis at 12 months when compared with bare metal stents, but there was no significant difference in mortality or major adverse cardiac events (Dasari et al., 2011). Further clinical trial data is needed to assess an outcome benefit of drug-eluting stents beyond the reduction in in-stent restenosis. Therefore, with the limited evidence on revascularization in this patient population, retransplantation is the definitive answer for severe CAV; candidacy is often restricted by comorbidities.

Osteoporosis

Osteoporosis and fragility fractures are a serious complication usually occurring in the first year after solid organ transplantation. Exposure to high doses of corticosteroids and CNI may contribute to rapid bone loss (Kulak et al., 2014). Other contributing factors to bone loss are immobilization and pretransplant risk factors including chronic disease. Bone density assessment is part of the pretransplant evaluation. Prevention should include combination therapy with calcium supplementation, vitamin D, and bisphosphonates in addition to weight-baring exercises.

Psychosocial factors

Psychosocial and behavioral factors have been shown to predict long-term survival after heart transplantation and are an important criteria for patient selection. In a quality of life assessment of more than 500 patients, Farmer et al. found that higher levels of social and economic satisfaction and education level predicted improved survival while poor compliance and higher NYHA class were associated with a worse survival (Farmer et al., 2013). Education level and socioeconomic status have a role in posttransplantation outcomes. In a large modern cohort of heart transplant recipients, patients with Medicare and Medicaid had a reduced long-term survival when compared to those with private insurance or self-pay; the survival difference was evident at 1-year and increased with time (Allen et al., 2012). In multivariable analysis, Medicare and Medicaid increased mortality risk by 18% and 33%, respectively ($P \leq 0.001$). Similar results were noted for college education with an 11% reduction in mortality risk and an absolute increase of 7% for 10-year survival. Older patients (≥ 60 years) have also reported more satisfaction with quality of life, social support, psychosocial adjustment, and adherence when compared with younger (<45 years) and middle-aged patients (between 45 and 59 years), suggesting they are better adjusted after cardiac transplantation (Shamaskin et al., 2012). Psychosocial factors have a major impact on transplant survival due to the required frequent follow-up by specialized health care professionals and management of a complicated medication regimen and comorbidities.

Survival

In the 32nd ISHLT adult and pediatric heart transplant report, 1- and 5-year survival are 82% and 69%, respectively, with a median survival of 11 years for all and 13 years for those surviving the first year (Lund et al., 2015). Survival has improved with successive eras, but the most recent cohort of 2009–13 shows improvement in the 1-year unadjusted survival at 86% in this most recent era as compared with 84% in the 2002–08 era ($p < 0.05$) (**Fig. 13**). Leading causes of death are related to graft failure, infection, and multiorgan failure. Cause of death varies with the duration after transplant. Earlier death tends to result from graft failure and infection while long-term mortality is more commonly from malignancy, cardiac allograft vasculopathy, and renal failure. The most common posttransplant morbidities are diabetes, hypertension, hyperlipidemia, and renal dysfunction. Functional status returns to normal activity levels in 90% but the percentage of patients returning work remains less than 50% at 1- and 3-year follow-up. In multivariable analysis, the most important continuous risk factors for 1-year mortality are donor age, recipient age, and recipient pretransplant creatinine. The number of older recipients >60 years is increasing, and age is a risk factor for mortality at all follow-up points according to the most recent ISHLT report with a focus theme on age (Lund et al., 2015).

Factors associated with long-term survival are age younger than 55 years, annual center volume of 9 or more transplants, younger donor age, and shorter ischemic time; each 1 h reduction in ischemic time improved the odds of 10-year survival by 11% ($p < 0.001$) (Kilic et al., 2012c). Similar improvement in survival was associated with each decade decrease in donor age. Mechanical ventilation and diabetes decreased the probability of long-term survival. A risk stratification tool, IMPACT (Index for Mortality Prediction After Cardiac Transplantation) using UNOS data, has been introduced and validated as a predictor of short-term and long-term mortality, and has potential as a prognostic aid in appropriate patient selection for transplant (Kilic et al., 2013). The score assigns points to

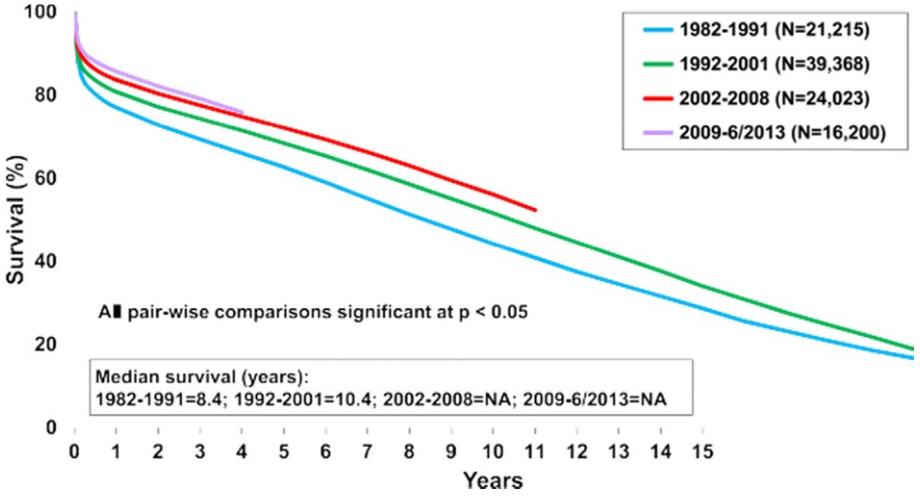

Fig. 13 Improvement in long-term survival in adult heart transplant recipients as documented by era. Reprinted from Lund, L. H., Edwards, L. B., Kucheryavaya, A. Y. et al. (2015). The registry of the International Society for Heart and Lung Transplantation: Thirty-second official adult heart transplantation report—2015; focus theme: Early graft failure. *The Journal of Heart and Lung Transplantation* **34** (10), 1244–1254. Copyright 2015, with permission from Elsevier.

various pretransplant clinical features, such as age >60 years, bilirubin, creatinine clearance, dialysis before transplant, recent infection, female gender, type of ventricular assist device, and race.

Despite the overall improvement in posttransplant survival in the current area, racial outcome differences still persist. Racial differences in several studies have shown a persistently elevated mortality among black recipients and attribute the documented higher rate of graft loss in this patient population to a higher risk profile, immune-related mechanisms, differential drug absorption, and systemic complications of therapy (Liu et al., 2011; Singh et al., 2011). Racial differences also exist among waitlist outcomes in the United States; both black and Hispanic patients were more likely to be listed as an urgent status (1A/1B) when compared with white patients (Singh et al., 2012). Listed Hispanic patients are at a higher risk of dying on the waitlist or becoming too ill for a transplant in comparison with white patients while black patients have a similar waitlist mortality but a higher early posttransplant mortality. Further research is necessary to identify mechanisms for outcome differences among minorities and improve these disparities.

Retransplantation

Survival after retransplantation is overall very poor; the best results are associated with CAV as the indication. Other indications are associated with poor survival with acute rejection associated with the lowest survival (32% and 8% at 1 and 5 years, respectively) followed by early graft failure (50% and 39% at 1 and 5 years, respectively) (Radovancevic et al., 2003). Nevertheless, retransplantation is limited by patient comorbidities and graft failure.

Risk factors associated with a high risk of 1-year graft failure rates in patients who underwent retransplantation are older recipient age, increasing serum creatinine, and mechanical ventilation. The effect of multiple risk factors is cumulative as patients with all three factors had a 32% higher risk of graft failure within 5 years as compared with patients with no risk factors. Each decade increase in recipient age was associated with a 20% increase in 1-year graft failure and each 1-mg/dL increase in serum creatinine increased the risk of graft failure by 58% (Kilic et al., 2012d).

Conclusion

In summary, cardiac transplantation is the preferred treatment for end-stage heart failure in select patients with improved survival and quality of life in the current era due to multiple advances in the field. Bridging patients with mechanical support continues to increase and has improved outcomes, including reduced waitlist mortality if patients do not have a VAD-related complication. Enhancement of immunosuppression regimens has reduced acute allograft rejection episodes, and donor procurement and surgical techniques have contributed to improved donor function. Posttransplant care continues to utilize complex medical regimens with multiple drug interactions. Pre- and posttransplant comorbidities affect quality of life and survival with medical survival of 10 and 13 years for those surviving the first year. Early complications of graft failure and infection affect survival while later development of malignancy, renal failure, vasculopathy, and rejection reduce long-term survival. Investigative focus has been in the direction of risk-stratifying patient selection, expanding the donor pool, and further optimizing immunosuppression to prevent posttransplant complications. Continued advances in cardiac transplantation will lead to enhancement of patient management, long-term outcomes, and racial disparities.

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Cardiohepatic Interactions

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Introduction

The liver is the largest gland in the body constituting 2% of total body weight. It is a highly vascular organ that receives 25% of the cardiac output with a dual blood flow system via the hepatic artery (~20%) and hepatic portal vein (~80%) (Bacon, 2012). It is responsible for multiple critical functions, including blood filtration, metabolism of carbohydrates, proteins, fats, hormones and foreign chemicals, biliary formation, storage of vitamins and iron, protein synthesis, and formation of coagulation factors.

The Centers for Disease Control and Prevention list chronic liver disease as the twelfth leading cause of mortality in 2014 in the United States with a mortality rate that has increased from 9.4% to 12% over the past decade (Kowalski and Abelmann, 1953). Chronic liver failure can result in cirrhosis, which is characterized by late-stage progressive hepatic fibrosis, architectural distortion, and formation of regenerative nodules (Bacon, 2012). The most common causes of cirrhosis are alcohol-related liver disease, chronic hepatitis C virus, chronic hepatitis B virus, and nonalcoholic fatty liver disease. Due to its diversity in function, it is not surprising that the complications of cirrhosis can adversely multiple organ systems, including neurological (e.g., hepatic encephalopathy), gastrointestinal (e.g., portal hypertension and varices), hematologic abnormalities (e.g., portal vein thrombosis), and cardiovascular (e.g., cirrhotic cardiomyopathy).

The physiologies of the heart and liver are closely intertwined. Liver dysfunction can result in cardiovascular pathology. Cardiac dysfunction can lead to hepatopathy. And there are diseases that simultaneously affect both heart and liver. The goal of this review is to help the reader better understand the challenges of diagnosing and managing this diverse group of patients.

Clinical Effects of Liver Dysfunction on the Cardiovascular System

Numerous studies over the last half century have documented the detrimental effect of liver dysfunction on the cardiovascular system due to a hyperdynamic circulatory state (Kowalski and Abelmann, 1953). "Cirrhotic cardiomyopathy" was a term coined back in 1980s to describe chronic cardiac dysfunction with normal to increased cardiac output and contractility at rest but a suboptimal response to pharmacological or physiological stress (Baik et al., 2007; Moller and Henriksen, 2010; Zardi et al., 2010). Cardiovascular imaging usually demonstrates normal or hyperdynamic left ventricular (LV) ejection fraction at rest, LV dilatation, reduced peak systolic velocity, and diastolic dysfunction (Kowalsky and Abelmann, 2003). Diastolic dysfunction affects approximately 50% of the patients (Alexopoulou et al., 2012). Echocardiography demonstrates impaired ventricular relaxation (reduction in early (E) and late (A) phases of trans-mitral ventricular filling) (Bernardi et al., 1998). Underlying mechanisms include myocardial hypertrophy, interstitial fibrosis, and subendothelial edema through activation of the renin–angiotensin–aldosterone system (Patel and Mehta, 2012). It has been hypothesized that other mechanical factors, such as poor intrathoracic compliance and the physical pressure of hepatomegaly, can cause diaphragmatic displacement and worsen underlying diastolic dysfunction.

Contributing to the phenotype of cirrhotic cardiomyopathy are a multitude of electrophysiologic abnormalities including chronotropic incompetence and prolonged QT interval. Chronotropic incompetence, defined as the lack of heart rate response to stress, is seen in 50%–80% of patients with cirrhosis and limits exercise capacity. The inability to reach target heart rate during physiologic or pharmacologic stress makes it difficult to assess for ischemia. Severity of cirrhosis and degree of portal hypertension are proportionally associated with chronotropic incompetence (Bernardi et al., 2012). Underlying mechanisms will be discussed in the next section of this review.

QT prolongation is seen on the electrocardiograms of more than 50% of patients with cirrhosis (Bernardi et al., 2012; Lazzeri et al., 1997). The exact mechanism behind QT prolongation in liver cirrhosis is not well understood; however, studies have implicated increased sympathetic tone along with cardiotoxins such as endotoxins and cytokines to be major contributing factors (Mohamed et al., 1996; Carey and Douglas, 2005). Trevisaniet al. demonstrated increased QT prolongation after transjugular intrahepatic portosystemic shunt procedure in cirrhotic patients suggesting increased venous return after portosystemic shunt as a putative mechanical mechanism (Chaudhry et al., 1999). The clinical significance of QT prolongation in cirrhotic patients remains indeterminate and controversial (Baik et al., 2007; Day et al., 1993; Bernardi et al., 1998). Studies of cirrhotic patients using an adjusted Child–Turcotte–Pugh score demonstrated that while the QTc interval prolongation was common in cirrhotic patients, its presence had no independent effect on mortality or risk of SCD. However other studies showed lower survival rate in cirrhotic patients with QT prolongation that was not attributable to sudden cardiac death or torsade de pointes which occur rarely in this population (Kim et al., 2017). Several studies show that prolonged QTc significantly improves in most patients after liver transplantation (Adigun et al., 2005; Mohamed et al., 1996; Carey and Douglas, 2005) and that improvement in the QT interval may be associated with improved survival after transplant (Mohamed et al., 1996).

Autonomic dysfunction appears to be present in approximately 50% of cirrhotic patients regardless of etiology (Chaudhry et al., 1999). Although it is usually asymptomatic, its presence correlates with increased mortality and morbidity before, during, and after liver transplantation due to hemodynamic instability in the setting of any stressful event. These patients have depressed heart rate variation with predominantly parasympathetic dysfunction and seemingly intact sympathetic function (Chaudhry et al., 1999; Milovanovic et al., 2009). Prevalence and severity of autonomic dysfunction are related to severity of liver dysfunction (Chaudhry et al., 1999; Milovanovic et al., 2009). One study has shown that autonomic dysfunction manifesting as parasympathetic impairment and sympathetic predominance is associated with risk for sudden cardiac death and ventricular arrhythmias (Milovanovic et al., 2009). Autonomic dysfunction regresses after liver transplantation in 63%–70% of patients. The improvement seen after liver transplantation suggests that the neuropathy is metabolically, neurohormonally, and/or hemodynamically mediated as opposed to structural damage of the parasympathetic system (Carey et al., 2008).

Liver dysfunction has direct effects on cardiovascular hemodynamics which in turn affect multiple organ systems making cirrhotic cardiomyopathy a challenging syndrome to treat and manage. Guidelines for the management of cirrhotic cardiomyopathy are similar to noncirrhotic patients with heart failure and include normalizing the preload and restoring the effective circulating volume using sodium restriction and diuretics, use of supplemental oxygen, afterload reduction, and noncardio-selective beta-blockers (Starr and Raines, 2011; Runyon and Committee, 2009). Due to the significant regenerative capacity of the liver, efforts to treat the underlying cause of liver dysfunction, for example alcohol abstinence, result in improvement in liver function (Pares et al., 1986; Veldt et al., 2002). Further highlighting the causality and reversibility of these disease states are reports of cirrhotic cardiomyopathy fully resolving after orthotopic liver transplantation (Milani et al., 2007; Cosardereliogluet al., 2016; Huffman et al., 2010).

Cardiovascular Molecular Mechanisms Affected by Chronic Liver Disease

The dysregulation of several molecular mechanisms, including adrenergic receptors, channelopathies, nitric oxide, carbon monoxide, endocannabinoids, and retinoids, has been described in cirrhotic cardiomyopathy. Both detrimental and beneficial effects on cardiac function through these mechanisms have been described in the literature.

The dynamic interaction between stimulatory β-adrenergic receptors and inhibitory muscarinic receptors plays an important role in the normal maintenance of heart rate and cardiac contractility. Cirrhotic patients have a blunted chronotropic and inotropic response in part due to decreased β-adrenergic receptor density (Battarbee et al., 1990; Ma et al., 1994; Gerbes et al., 1986; Lee et al., 1990), and in part attributed to receptor dysfunction. Impaired biliary clearance is known to be directly involved in decreased plasma membrane fluidity. The cardiomyocyte membranes of bile duct ligated rats with liver cirrhosis had increased cholesterol-to-phospholipid ratio causing decreased membrane fluidity, reduced cyclic adenosine monophosphate (cAMP) production, and suboptimal response to β-adrenergic stimulation (**Fig. 1**) (Ma et al., 1994; Gazawi et al., 2000). The role of various muscarinic acetylcholine receptor subtypes in cirrhotic cardiomyopathy has yet to be clearly defined. Of the five known muscarinic acetylcholine receptors, M2 and M3 are expressed in cardiac myocytes with greater density in the atria compared to ventricles (**Fig. 1**). Rodent models of cirrhosis have demonstrated blunted muscarinic responsiveness via a postreceptor effect (Jaue et al., 1997).

Nitric oxide is a vasodilator synthesized by vascular endothelium and cardiac myocytes. There are three different isoforms of nitric oxide namely, neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), and inducible nitric oxide synthase (iNOS). iNOS has a cardiotoxic effect via negative chronotropic effect and induction of apoptosis, while the former two are cardioprotective (Zardi et al., 2010; Kim et al., 1999). Animal models have shown elevated iNOS mRNA expression in cirrhotic patients likely due to transient bacteremia and ongoing inflammatory process (Liu et al., 2000). Blocking nonspecific nitric oxide

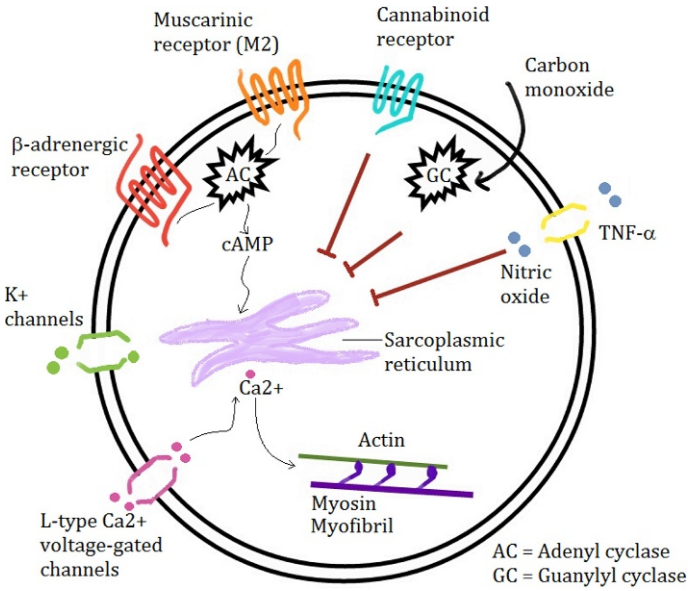

Fig. 1 Diagram of the various molecular mechanisms known to be involved in the pathophysiology of cardiovascular disease due to liver disease.

synthase improves cardiac contractility further supporting roles for nitric oxide synthases in cirrhotic cardiomyopathy (Herring et al., 2002; Han et al., 1995).

Carbon monoxide is produced via enzymatic action of heme oxygenase and primarily acts as a physiological vasodilator in splanchnic circulation. Bile duct ligated cirrhotic rats have increased carbon monoxide activity secondary to increased transcriptional activity. Carbon monoxide may result in negative inotropic effect via activation of cyclic guanosine monophosphate (Liu et al., 2001).

Animal models have implicated increased endocannabinoids activity in cirrhotic rats as a cause of decreased ventricular contractility. Studies have demonstrated that endocannabinoids, including anandamide and 2-arachidonoylglycerol, may result in decreased myocyte contractility via G-protein-coupled receptor pathway involving cannabinoid receptor type 1. Activation of this pathway causes inhibition of adenylate cyclase activity and reduction in intracellular calcium concentration (**Fig. 1**). Endocannabinoids also induce splanchnic vasodilatation. Cardiac contractility in the cirrhotic rats is restored with introduction of endocannabinoid/CB1 receptor blockade (Batkai et al., 2007).

Retinoids play an important role in cellular physiologic processes including cellular proliferation, differentiation, and apoptosis. Studies have shown that approximately 66%–75% of dietary retinoid is stored as retinyl esters in the liver (Li et al., 2014; Harrison, 2012). Liver retinoid levels vary among individuals in alcoholic liver disease (Bell et al., 1989). Murine models have demonstrated that cirrhotic mice with depleted hepatic retinoid stores while maintaining effective circulating Vitamin A levels are protected from cardiac injury and have increased ventricular gene expression and progenitor cells. The authors speculated that subclinical liver disease with depleted hepatic retinoid stores might have a cardioprotective benefit (Asson-Batres et al., 2016).

The numerous functions of the liver that are absolutely necessary for daily life make its study challenging. Treatment for the cardiovascular complications of cirrhosis remains limited by our incomplete knowledge about the underlying mechanisms. That disease reversal or regression is seen after liver transplantation suggests the possibility that a better understanding of these mechanisms may result in the development treatment options other than liver transplantation.

Clinical Effects of the Cardiovascular System on the Liver

Acute Cardiogenic Liver Injury

Acute cardiogenic liver injury, also known as hypoxic or ischemic hepatitis, is a condition where diminished cardiac output fails to meet the metabolic demands of hepatic cells in patients with underlying diagnosis of heart failure. The clinical features of ischemic hepatitis include right-upper quadrant abdominal pain, hepatomegaly, elevated serum bilirubin, significant transaminitis (ranging up to 10 × the upper limit of normal), and elevated lactate dehydrogenase levels (up to 10–20 × the upper limit of normal) (Seeto et al., 2000; Waseem and Chen, 2016). Early and rapid increase in LDH with ratio of alanine aminotransferase (ALT) to LDH < 1.5 is characteristic of cardiogenic cause of liver injury (Samsky et al., 2013).

The liver is a highly vascular organ, which can compensate for decrease in cardiac output by releasing adenosine which causes dilation of the hepatic vasculature and increased blood flow. The hepatic sinusoids are highly permeable permitting increased oxygen diffusion to the hepatocytes with oxygen extraction levels reaching close to 90%. However, this process is impaired in patients with venous congestion from heart failure. When these patients experience acute hemodynamic instability, it results in

impaired perfusion with hepatic insult (Waseem and Chen, 2016; Birrer et al., 2007; Henrion et al., 1994). Studies have demonstrated that profound hypotension by itself is not enough to precipitate ischemic hepatitis and suggest that the presence of hepatic congestion is a major contributing factor (Seeto et al., 2000).

Recommended treatments are directed toward optimizing circulation with the use of inotropes. Some studies have demonstrated benefit with the use of dopamine in patients with normotensive cardiogenic shock and acute kidney injury, and use of dobutamine in patients with reduced cardiac index (Kram et al., 1988; Naschitz and Yeshurun, 1987). Decongestion and maintenance of euvolemia may be preventative, though that is purely conjectural and not yet studied. The prognosis of ischemic hepatitis remains poor with in-hospital mortality rate exceeding greater than 50% (Waseem and Chen, 2016).

Congestive hepatopathy

Congestive hepatopathy is a chronic form of ischemia characterized by passive hepatic congestion due to congestive heart failure. Patients with congestive hepatopathy are often asymptomatic. Physical exam findings early in the course of hepatic congestion include peripheral edema, hepatomegaly with hepatojugular reflux, and pulsatile liver. Late in the course of congestive hepatopathy, physical exam findings are essentially those of end-stage liver disease such as ascites and jaundice. Chronic passive hepatic congestion can cause hepatic sinusoidal dilation, sinusoidal hypertension, and eventually lead to progressive hepatocyte atrophy and centrilobular fibrosis with development of portal hypertension and cirrhosis. Laboratory findings are notable for cholestatic pattern of injury with mild to moderate increase in serum γ-glutamyl transferase (GGT), alkaline phosphatase, and bilirubin levels. Elevations in GGT and alkaline phosphatase independently portend a poor prognostic outcome (Poelzl et al., 2012). The degree of injury is proportional to the severity of heart failure and valvular dysfunction such as tricuspid regurgitation.

As mentioned above, congestive hepatopathy can lead to cirrhosis if left untreated. Prevention and management of congestive hepatopathy is geared toward both treatment of the underlying cause of the heart failure and normalization of intracardiac filling pressures with diuresis. It has been observed that successful decongestion can result in significant liver recovery, which also supports the possibility that maintaining euvolemia may prevent the development of congestive hepatopathy. Patients with congestive hepatopathy (but not cirrhosis) who are status post cardiac transplantation demonstrate sustained improvement in biochemical liver function tests and 10-year long-term survival as shown by standard model for end-stage liver disease (MELD) scores (Dichtl et al., 2005; Chokshi et al., 2012).

Fontan liver disease

The Fontan operation is a palliative procedure for congenital heart disease patients who are born with either functional or anatomic single ventricle physiology. The procedure involves connecting central venous return (superior vena cava and inferior vena cava) directly to the pulmonary arteries and creating a functionally single pump system. Postoperative 15–20-year survival rate after Fontan procedure ranges from 60% to 85% with patients frequently requiring revision surgery or consideration of heart transplantation. The physiologic changes in the circulatory system post-Fontan include chronically reduced cardiac output (i.e. heart failure) and elevated hepatic venous pressure in the setting of high central venous pressure (Rychik and Gui-Yang, 2002; Kiesewetter et al., 2007). Hence, post-Fontan patients are a classic example of chronic congestive hepatopathy with liver cirrhosis, developing in the majority of patients by about eleven to fifteen years post-Fontan (Baek et al., 2010).

Patients post-Fontan are usually clinically asymptomatic during the first five years but demonstrate abnormalities during laboratory screening or radiological study (Baek et al., 2010). Consistent with the natural history of congestive hepatopathy, studies demonstrate a recurring pattern of hepatic injury with chronic congestion and tissue hypoxia. CT scan is notable for zonal or reticular enhancement, i.e., peripheral diffuse patchy enhancement. Reticular enhancement signifies extensive hepatic fibrosis. Liver biopsies are notable for sinusoidal dilation with variable and nonuniform fibrosis (Ford et al., 2015).

A growing number of patient status post Fontan are reaching adulthood and developing both failing Fontan and liver cirrhosis. These patients are difficult to assess because use of morbidity and mortality predictor scores such as the MELD score and Child–Turcotte–Pugh is confounded by cardiac ascites, use of diuretics, presence of cardiorenal syndrome, unreliable INR interpretation in the setting of vitamin K antagonist use, and impaired albumin assessment due to malnutrition and protein losing enteropathy (Carey and Douglas, 2005). Often the only treatment option is combined heart and liver transplantation, which is both high risk and limited as an option due to availability of organs (Ford et al., 2015).

Diseases that Affect Both the Liver and the Heart

Infections

Hepatitis C

While the most common manifestation of hepatitis C Virus (HCV) infection is in the form of a chronic hepatitis, HCV infection also has direct and indirect effects on the cardiovascular system. PCR studies have demonstrated that HCV can infect cardiomyocytes resulting in an acute myocarditis which can then progress to a chronic cardiomyopathy (Matsumori et al., 1998; Frustaci et al., 2002; Okabe et al., 1997). Both dilated and hypertrophic cardiomyopathies have been described in patients with HCV infection (Matsumori et al., 1995; Teragaki et al., 2003). There is strong evidence for genetic susceptibility to development of cardiomyopathy

in the presence of HCV infection. Specific HLA alleles are more prevalent in HCV-infected patients who develop dilated cardiomyopathy while other HLA alleles are more prevalent in those who develop hypertrophic cardiomyopathy (Matsumori, 2005; Naruse and Inoko, 2000). Myocyte damage is believed to be caused by several mechanisms. HCV infection is known to induce the expression of the cytokine TNF-α which is well described in the literature as a key mediator in the development and progression of myocarditis by causing myocyte depression, impaired β-adrenergic response (Matsumori, 2005; Sanchez and Bergasa, 2008). The HCV core protein can directly damage the myocardium (Omura et al., 2005). Transgenic mice for the HCV-core gene develop cardiomyopathies with significant histopathological findings (Omura et al., 2005). Another hypothesized mechanism is the activation of the immune response by anti-HCV antibodies causing myocyte injury. Supporting this hypothesis is a report that treatment with immunosuppressives of patients found to have HCV myocarditis with B cell infiltrates and anti-HCV antibodies resulted in normalization of cardiac chamber size and function (Frustaci et al., 2002).

Various studies have demonstrated that HCV infection is associated with a higher prevalence of insulin resistance and diabetes (Younossi et al., 2016; Macaluso et al., 2013; Petta et al., 2013; Chang, 2016; Lonardo et al., 2014). Studies show a causal effect—that the virus is able to alter lipid metabolism and insulin signaling and eradication of the virus reverses these metabolic abnormalities (Younossi et al., 2016; Macaluso et al., 2013; Pavone et al., 2016). Multiple studies also show a correlation between active HCV infection and the presence and severity of atherosclerosis in the carotids and coronary arteries (Ishizaka et al., 2003; Targher et al., 2007; Alyan et al., 2008; Yelken et al., 2009; Butt et al., 2009). Although there are other reports that found no association, meta-analyses performed for each of these conditions suggest that the presence of active HCV infection conveys significantly higher risk for cerebrovascular events and myocardial infarction even after adjusting for risk factors such as smoking and diabetes (Petta et al., 2016). How much of an effect the metabolic abnormalities invoked by HCV infection (visceral obesity, insulin resistance, diabetes mellitus) has on promoting atherosclerosis versus direct effects of the virus itself is unknown as there is evidence for both possibilities (Ishizaka et al., 2003).

Whether treatment of HCV infection is beneficial in reversing or halting its associated cardiovascular disease states remains unknown, but there is accumulating evidence that viral eradication may be beneficial. Case reports suggest that treatment resulting in viral clearance may result in myocardial recovery in patients with HCV-related myocarditis or cardiomyopathy (Matsumori et al., 1998; Okabe et al., 1997). One small study demonstrated improvement of myocardial perfusion defects in HCV-infected patients with myocarditis after treatment with interferon therapy (Matsumori et al., 1995). A recent retrospective study of interferon-free-based anti-HCV treatment regimen provides evidence that treatment may reverse HCV-related cardiomyopathy (Yelken et al., 2009). A community-based study of HCV subjects reported increased mortality due to cardiovascular disease in subjects with active HCV infection compared with uninfected subjects, but no increased risk in those subjects who were HCV-cleared (Lee et al., 2012). An observational study reported risk of lethal cerebrovascular events to be directly proportional to viral load (Lee et al., 2010). Cohort studies looking at treatment with interferon demonstrated significant reduction in acute coronary syndrome, cerebrovascular events, and end-stage renal disease (Hsu et al., 2013, 2014). Historically treatment of HCV has been limited in patients with cardiovascular disease as the physiological stress reaction induced by interferon treatment was reported to result in cardiomyopathy (Zhao et al., 2014; Martin et al., 2014). In recent years the development of interferon-free, anti-HCV viral regimens with minimal adverse events that are highly effective in viral eradication may offer new treatment opportunities for this group of patients.

Human immunodeficiency virus

Human immunodeficiency virus (HIV) is a type of retroviral infection that affects and compromises the immune system of the affected patient. Chronic HIV infection can result in acquired immunodeficiency syndrome (AIDS) which is defined as having a CD4 cell count less than 200 cells/uL. HIV/AIDS affects and effects both hepatic and cardiac diseases.

That HIV/AIDS patients are at substantially higher risk (1.5–2.0-fold) of developing coronary artery disease has been known for many years. HIV/AIDS patients can also develop both myocarditis and a nonischemic cardiomyopathy. The reported prevalence of cardiomyopathy ranges from 15% to 38% in HIV/AIDS patients (De Castro et al., 1992; Longo-Mbenza et al., 1998; Sliwa et al., 2012). Other cardiac diseases for which HIV/AIDS patients are at higher risk of developing include aortitis from occlusion of the vasa vasorum, inflammation-related wall weakness and autoimmune mechanisms, pericardial effusion with or without pericarditis, pulmonary arterial hypertension, major conduction defects, prolongation of the QT interval, and atrial fibrillation (Manga et al., 2017).

The mechanisms by which HIV/AIDS affect the heart are controversial. The HIV population is known to have at baseline a higher prevalence of traditional coronary artery disease risk factors such as smoking, diabetes, dyslipidemia, central obesity, and hypertension (Vachiat et al., 2017). The immune dysfunction of HIV is associated with ongoing inflammation, which is another known risk factor for the development of coronary artery disease. Treatment with combination antiretrovirals may not be effective because protease inhibitors are known to exacerbate dyslipidemia, endothelial dysfunction, and hypertension. It is thought that in HIV/AIDS-related myocarditis/cardiomyopathy direct HIV infection of the cardiomyocyte is the mechanism of disease. This is supported by studies demonstrating the presence of HIV virus intracellularly (Grody et al., 1990). This hypothesis is challenged by the lack of CD4 receptors in cardiomyocytes leading to the question of how the HIV virus could enter a cardiomyocyte. Other studies suggest that the immunocompromised state by HIV/AIDS results in a susceptibility and nonclearance of other viruses that are cardiotoxic (Shaboodien et al., 2013). The converse has also been suggested that HIV/AIDS infection renders cardiomyocytes more susceptible to the entry of cardiotropic viruses such as Epstein-Barr virus (Goldblum et al., 1990). Patients with HIV infection have increased levels of TNF-α and endothelin cytokines levels, resulting in indirect cardiotoxic effect. As already described above with HCV infection, elevated TNF-α levels cause myocyte depression, impaired β-adrenergic response. In HIV infection elevated TNF-alpha levels in conjunction with high endothelin levels result in enhanced catecholamine response, and LV hypertrophy

(Bloomfield and Leung, 2017; Lumsden and Bloomfield, 2016; Barbaro, 2005). The prevalence of myocarditis, nonischemic cardiomyopathy, and pericardial disease has decreased since the introduction of combination antiretroviral therapy (Bloomfield et al., 2015; Remick et al., 2014; Lind et al., 2011), suggesting viral and presence and load may play important mechanistic roles.

The liver can be both directly and indirectly affected by HIV/AIDS. Patients are often concomitantly infected with hepatitis B and hepatitis C and combined infection has been shown to increase their risk for developing cirrhosis and some of the cardiovascular diseases described above (Osibogun et al., 2017; Foca et al., 2016). Before the development of combination antiretroviral therapy, the most common cause of liver pathology was infection (Sonderup and Wainwright, 2017). Studies reported significant findings by biopsies of opportunistic infections such as mycobacterium avium complex which can precipitate hepatobiliary disease, tuberculosis, cytomegalovirus, schistosomiasis, histoplasmosis, and cryptococcosis (Sonderup and Wainwright, 2017).

Combined antiretroviral therapy has led to a decrease in opportunistic infections and, as a consequence, marked improvement in life expectancy for HIV infected patients. However as discussed above the treatments can exacerbate cardiac disease. They also significantly affect the liver with as many as 42% of patients showing evidence of drug-induced liver injury in one study (Sonderup and Wainwright, 2017). Antiretroviral agents can cause drug-induced liver injury via several mechanisms, including direct drug toxicity, hypersensitivity, mitochondrial toxicity, and immune related (Sonderup and Wainwright, 2017). Antiretroviral agents can also potentiate the hepatotoxic potential of other drugs, especially cardiac drugs. One common example of this are statins, which are the mainstay treatment regimen for hyperlipidemia treatment and frequently prescribed in this patient population due to the increased risk for coronary artery disease. Most of the statin medications are metabolized by cytochrome P450 3A4 (CYP 3A4). Antiretroviral medication class such as protease inhibitors downregulate the activity of CYP3A4 and can cause statin toxicity which can cause liver dysfunction and rhabdomyolysis (Dube et al., 2003).

Tropical diseases

Malaria, Q-Fever, and dengue are some of the tropical diseases known to cause cardiac and liver dysfunction via cytokine-mediated pathway (Herr et al., 2011; Malavige et al., 2004; Elgouhari and Huntington, 2016). Detailed discussion about these disease processes is beyond the scope of this chapter.

Infiltrative Diseases

Amyloidosis

Amyloidosis is a general term to describe a group of disorders with multiorgan system involvement because of extracellular tissue deposition of insoluble fibrillary proteins also called amyloid fibrils (Bejar et al., 2015). There are three different types of amyloidosis—AL, ATTR, and wild-type TTR. We will briefly review AL amyloidosis and discuss ATTR amyloidosis. Wild-type TTR amyloidosis will not be discussed as it is not known to significantly affect the liver.

AL amyloidosis is an aggressive form of amyloidosis caused by deposition of light-chain immunoglobulins in various tissues including kidney, soft tissue, liver, and heart due to an underlying plasma cell dyscrasia (Bejar et al., 2015). Clinical features of AL amyloidosis are variable and can include fatigue, weight loss, peripheral neuropathy, restrictive cardiomyopathy, hepatomegaly with elevated liver enzymes, macroglossia, purpura, and bleeding diathesis. Cardiac involvement is common in patients with AL amyloidosis and portends a poor prognosis. Amyloid deposition in the myocardium and microvasculature results in restrictive cardiomyopathy, cardiac arrhythmias, and heart failure (Falk et al., 2016). There are successful chemotherapeutic options for treatment of plasma cell dyscrasia, including melphalan with autologous hematopoietic stem cell transplantation or bortezomib-based chemotherapy (Zumbo et al., 2017). However, there are patients in whom cardiac involvement is so significant that they require heart transplantation prior to treatment for the plasma cell dyscrasia. In select patients at select centers this can be done with acceptable outcomes (Grogan et al., 2016).

ATTR amyloidosis is an autosomal dominant condition that is caused by the genetic mutation in the transthyretin (TTR) gene resulting in unstable TTR protein. Clinical manifestations of TTR amyloidosis depend on the protein mutation. For example, Val122Ile mutation is characterized by late amyloid cardiomyopathy characterized by progressive severe heart failure, Val30Met mutation results in lower extremity sensorimotor peripheral neuropathy. These patients typically present in 4th–6th decades of their life (Bejar et al., 2015; Galant et al., 2017). Orthotopic liver transplant can halt the disease in ATTR amyloidosis patients and can be performed with minimal risk and improve survival (Popescu and Dima, 2012). Unique to this group of patients is the domino liver transplant, a strategy where the explanted ATTR liver can be used as a living donor liver for other patients on the liver transplant waiting list, consequently not affecting the number of livers available as they are able to donate their own livers at the time of transplant (Bejar et al., 2015). Combined heart and liver transplantation is considered in select patients with severe heart disease. While additional data and registry information are essential to predict long-term success rate, an Italian study demonstrated promising results with 5-year survival rate of 82% among 14 patients who underwent combined orthotopic heart and liver transplantation (Careddu et al., 2015).

Hemochromatosis

Hemochromatosis is a syndrome characterized by excess of iron deposition leading to multiorgan disease including liver cirrhosis, diabetes, and cardiomyopathy. Primary or hereditary hemochromatosis is an autosomal recessive disorder with variable penetrance associated with *HFE* gene mutations on chromosome 6 encoding for human hemochromatosis protein. Mouse models have

demonstrated that human hemochromatosis protein is involved in iron homeostasis. Mutations in *HFE* gene result in increased gastrointestinal iron absorption, elevated plasma iron and transferrin saturation level, and iron overload in hepatocytes. Secondary hemochromatosis is a complication of another condition that results in chronic exposure to high levels of iron, such as chronic blood transfusions, long-term hemodialysis, or chronic liver disease. Cardiac iron deposition causes infiltrative cardiomyopathy, biventricular heart failure, and conduction abnormalities. Patients with hemochromatosis have a 14-fold increase in mortality due to heart disease (Bejar et al., 2015; Ripoll et al., 2011; Moller and Bernardi, 2013).

Wilson's Disease

Wilson's disease is an autosomal recessive disorder of copper metabolism characterized by excessive copper deposition in the liver, brain, heart, and other tissues. Cardiac manifestation of Wilson's disease includes arrhythmias, hypertrophic cardiomyopathy, and autonomic dysfunction (Factor et al., 1982; Kuan, 1987).

Autoimmune Diseases

Systemic Lupus Erythematosus

Systemic lupus erythematous (SLE) is an inflammatory disease with multiple immunologic abnormalities affecting multiple organs. Significant liver disease in patients with SLE is rare but reported hepatic manifestations include liver enzyme elevations and hepatomegaly. Hepatic complications as a result of antiphospholipid antibody syndrome include Budd–Chiari syndrome or veno-occlusive disease. Cardiac findings in patients with SLE include nonseptic pericarditis with or without effusion, verrucous (Libman–Sacks) endocarditis, coronary artery disease, and rare cases of myocarditis (Miner and Kim, 2014; Grover et al., 2014).

Sarcoidosis

Sarcoidosis is a multisystem granulomatous disorder due to unclear etiology that is histopathologically characterized by noncaseating granulomas in the involved organs. While sarcoidosis typically affects young adults and pulmonary system, it can also involve other organ systems including liver (~15%) and heart (~2%–5%) (Baughman et al., 2001; Yigla et al., 2002). Most patients with hepatic sarcoidosis are asymptomatic with minimal laboratory abnormalities including elevated alkaline phosphatase and gamma glutamyltranspeptidase levels. However, there are case reports of sarcoidosis patients suffering from cholestatic liver disease, biliary strictures, obstructive jaundice, hepatic vein thrombosis and developing cirrhosis (Devaney et al., 1993; Efe et al., 2012). Clinical manifestations of cardiac sarcoidosis range from heart block to valvular dysfunction to life-threatening arrhythmias and cardiomyopathy.

Conclusion

The hepatic and cardiovascular organ systems are intimately linked. Liver disease can cause profoundly abnormal cardiovascular physiology resulting in cirrhotic cardiomyopathy, HPS, and hepatorenal syndrome. Chronic congestion from heart failure can result in hepatopathy, cirrhosis, and lower the threshold for the development of acute ischemic liver disease. That liver transplantation and decongestion reverse the aforementioned syndromes, respectively, warrants further investigation to better understand the pathophysiology behind cardiohepatic disease. Improved understanding of the underlying molecular mechanisms may help with the development of targeted therapies to improve clinical outcomes for patients with heart and liver dysfunction.

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Cardiopulmonary Exercise Testing

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Introduction

Heart failure (HF) is a major and growing public health problem in the United States. Approximately 5 million patients in this country have HF and over 550,000 patients are diagnosed with HF for the first time each year (Mancini et al., 2009; Ansari and Massie, 2003). The number of HF deaths has increased steadily despite advances in treatment, in part, because of increasing numbers of patients with HF and better treatment of acute myocardial infarctions. Patients with HF commonly present with exercise intolerance, clinically manifested as shortness of breath and fatigue. The ability to perform individual tasks, particularly activities of daily living, is the leading determinant of prognosis. The New York Heart Association (NYHA) functional class is a subjective estimate of a patient's exercise ability and includes a subjective assessment, such as: "How many flights of stairs can you climb or how many blocks can you walk?" However, patients vary in their reporting and may inaccurately estimate their true functional capacity.

Cardiopulmonary exercise testing is a noninvasive test that grades the severity of exercise intolerance (American Thoracic Society and American College of Chest Physicians, 2003; Balady et al., 2010; Malhotra et al., 2016; Arena et al., 2007a). Studies have demonstrated that familiarization and fluctuations in motivation do not influence the results of the test. During CPX, oxygen consumption (VO_2), carbon dioxide elimination (VCO_2), and minute ventilation (Ve) are measured directly using a tight-fitting mask or nose clip, mouthpiece, and a metabolic cart. Performance of CPX follows the same absolute and relative contraindications to exercise testing established by the American College of Cardiology and American Heart Association (Fletcher et al., 2013). Unstable angina and ventricular arrhythmias and severe aortic stenosis are unequivocal reasons why an exercise test should not be performed until the patient is clinically stable. Generally, patients should abstain from ingesting caffeine for at least 12 h and/or eating for 3 h prior to CPX and should be dressed suited for exercise with comfortable clothes.

Peak VO_2

A peak VO_2 (maximal oxygen consumption) over 20 mL/kg/min is considered in the normal range in the average adult and suggests there is no serious cardiac, pulmonary, or neuromuscular pathology. There is a great deal of variability in test results depending on age and conditioning, and a trained athlete can reach a peak VO_2 as high as 80 mL/kg/min.

Peak VO_2 has proven to be a powerful prognosticator of survival in patients with HF (Mancini et al., 1991; Lund et al., 2005; Sue and Hansen, 1984), which is generally thought to represent a surrogate for maximal cardiac output and is used to identify high risk patients who would benefit from high priority listing and early transplantation. The American Heart Association/American College of Cardiology (AHA/ACC) guidelines on exercise testing recommend the CPX to distinguish between cardiac and pulmonary causes of unexplained exercise intolerance (Class I), evaluation of exercise capacity when a standard stress test is unreliable (IIa) and determination of intensity level for cardiac rehabilitation (IIb) (Sue and Hansen, 1984). The AHA/ACC (Mancini et al., 2009; Fletcher et al., 2013), European Society of Cardiology (ESC) (Swedberg et al., 2005), and the International Society for Heart and Lung Transplantation (ISHLT) guidelines (Mehra et al., 2006) recommend the use of peak VO_2 for heart transplant selection.

While there is no single parameter that qualifies a patient for transplant, those patients with peak VO_2 >14 mL/kg/min had a 1-year survival of 94% on optimal HF therapy, which was comparable to survival after transplant (Mancini et al., 1991). For such patients, listing for transplant is not indicated. Patients who were found to have a peak VO_2 of <14 mL/kg/min, however, had significantly lower survival rates, making listing for transplant appropriate. Based on these data, clinical use of CPX with a cutoff value for peak VO_2 of 14 mL/kg/min emerged as a key component of the transplant selection algorithm. Notably hemodynamic responses to exercise are greatly affected by gender, and a threshold value of 14 mL/kg/min may be quite inappropriate for women. In women, it is reasonable to consider using alternate standards in conjunction with peak VO_2 to guide listing, including percent of predicted (≤50%) peak VO_2 (Sue and Hansen, 1984; Swedberg et al., 2005; Mehra et al., 2006). Furthermore, most of the studies evaluating the relationship between peak VO_2 and HF prognosis were performed before the beta-blocker era, and recent studies have reassessed the value of peak VO_2 since this a major shift in therapy (Lund et al., 2003). The ISHLT (Mehra et al., 2006) currently

recommends a peak VO_2 <12 mL/kg/min as the value below which transplant should be considered for those treated with beta-blockers.

Peak VO_2 is the goal standard measurement of a patient's functional capacity as well as long-term prognosis; however, the interpretation of the test should take into consideration other parameters as well to obtain appropriate prognostic information.

Ventilatory Threshold

This physiologic event marks the exercise level at which the Ve increases exponentially (no longer in a linear fashion) relative to the increase in VO_2, where anaerobic metabolism increases in exercising muscles to sustain the work, as aerobic metabolic capacity can no longer meet the physiologic demands, and the body shifts to anaerobic metabolism as a source of energy (Balady et al., 2010; Forman et al., 2010). The key utility of ventilatory threshold (VT) is considered an index of submaximal exercise and independent of motivation. Often, it is a more practical performance index for those patients who are too anxious or debilitated to exercise to maximal exercise.

Anaerobic, ventilatory, and lactate threshold are used interchangeably; however, they are different but related events. VT usually occurs at 45%–65% of measured peak VO_2 in healthy untrained subjects. It occurs at a higher percentage of maximal effort in endurance-trained subjects. Exercise training has been shown to increase VO_2 at the VT, typically 10%–25% for previously sedentary individuals.

Several methods have been proposed for determination of VT; however, no universal agreement exists regarding which is best. The most common definitions of VT are the following: (1) The departure of VO_2 from a line of identity drawn through a plot of VCO_2 versus VO_2, often called the V-slope method; (2) the point at which a systematic increase in the ventilatory equivalent for oxygen (VE/VO_2) occurs without an increase in the ventilatory equivalent for carbon dioxide (VE/VCO_2); and (3) the point at which a systematic rise in end-tidal oxygen pressure ($PETO_2$) occurs without a decrease in the end-tidal carbon dioxide pressure ($PETCO_2$) (Balady et al., 2010). The assessment of all three of the aforementioned VT detection techniques in combination may result in an improved approximation of anaerobic threshold by blood lactate. The determination of the appropriate VT is fundamental for the interpretation of CPX given that important parameters like ventilatory efficiency (VE/VCO_2 slope) are determinates at VT.

Respiratory Exchange Ratio

The respiratory exchange ratio (RER) is the ratio between VCO_2 and VO_2 and an indicator of validity of the patient's effort during CPX (Balady et al., 2010; Forman et al., 2010). Achievement of at least 85% of age-predicted maximal heart rate is a well-recognized indicator of good effort by a patient during stress testing. However, the widespread use of beta-blocker in the HF population limits the use of this maximal heart rate to evaluate the quality of effort provided by a patient and determine if results are applicable. Peak RER is the most accurate measurement of a subject's effort. A peak RER $\geq$ 1.1 is considered an indication of excellent effort, however, not an indication to stop the test (similar to peak heart rate of 85% of predicted). A peak RER <1.0 in a CPX that is terminated by subject request, absent any electrocardiographic or hemodynamic abnormalities, generally reflects submaximal cardiovascular effort and the peak VO_2 should be interpreted with caution (Balady et al., 2010; Mezzani et al., 2003).

Assessment of peak RER is also important in clinical trials to demonstrate comparable participant effort among serial exercise tests (Balady et al., 2010; Brawner et al., 2008; Arena et al., 2007a). Reproducibility among tests could be difficult particularly if testing is performed by different personnel or in multi-institutional laboratories. If not adequately controlled, such variables can be expected to affect peak VO_2 which is an endpoint in most clinical trials in HF. Defining an acceptable magnitude of difference in functional capacity between serial tests, particularly at baseline, is valuable. Although there are no universal criteria for test reproducibility, peak/maximal VO_2 is generally considered reproducible if values vary by <10% on separate days. The ability of determine peak RER is an important reason that the incorporation of CPX should be strongly considered for interventional trials with a functional endpoint. Further, VT can occasionally be detected in subjects who achieve a RER <1.0, given that VT is detected somewhere in the RER range of 0.8–0.99 (Balady et al., 2010).

Ventilatory Efficiency

The most widely studied index of ventilatory efficiency is the Ve/VCO_2 slope (Balady et al., 2010; Forman et al., 2010). The relationship between Ve and VCO_2 during exercise is tightly coupled, because the former is modulated by the metabolic and anaerobic production of the latter. A Ve/VCO_2 slope <30 is considered normal without modification for age and sex (Balady et al., 2010; Sun et al., 2002). Abnormalities in ventilatory efficiency during exercise (VE/VCO_2 slope $\geq$ 34) portend a worse prognosis in patients with systolic HF and should lead to intensification of therapy in HF patients (Robbins et al., 1999; Arena et al., 2007b,c; Tsurungaya et al., 2006; Guazzi et al., 2008; Mancini and LeJemtel, 2007). Further, in patients with relatively preserved functional capacity (peak VO_2), a Ve/VCO_2 slope >34 still demonstrated a significantly higher cardiac event rate than other patients (35.0% vs. 13.3%, $P < 0.01$) (Tsurungaya et al., 2006), and some have proposed that it predicts cardiovascular risk irrespective of aerobic capacity (Arena et al., 2007c; Guazzi et al., 2008; Mancini and LeJemtel, 2007).

In patients with cardiopulmonary problems, there can be significant ventilation-perfusion mismatching and up-regulated ergo- and chemoreceptors leading to an elevated VE/VCO_2 slope. Further, there is an important relationship between ventilatory efficiency and right ventricular performance during exercise. A significant correlation was demonstrated between VE/VCO_2 slope and pulmonary vascular tone as assessed by exercise pulmonary vascular resistance and a negative correlation was shown between VE/VCO_2 slope and exercise right ventricular ejection fraction (Lewis et al., 2008). In fact, a patient with pulmonary hypertension can far exceed the normal threshold, some having Ve/VCO_2 slopes >60 if they have severe disease (Holverda et al., 2008).

CPX Advantages

Cardiopulmonary exercise testing is a well-established method for evaluation of cardiopulmonary diseases, provides an objective assessment of maximal aerobic capacity, estimates prognosis, and allows the physician to discriminate among many subtle and often overlapping etiologies. In general, when the anaerobic threshold is <40% of the predicted peak VO_2, circulatory insufficiency is suggested. A breathing reserve <20% would indicate ventilatory impairment, especially when accompanied by oxygen desaturation with exercise. If the patient has a reduction in both anaerobic threshold and breathing reserve, the more dominant finding may indicate the primary cause of the patient's functional limitation (Balady et al., 2010; Malhotra et al., 2016; Palange et al., 2007).

Further, the test allows for other cardiovascular diagnostic evaluations such as heart rate reserve, blood pressure response to exercise and coronary ischemia as well as the detection of oxygen desaturation during exercise. Some cardiopulmonary diseases have a distinct pattern during exercise. Patients with HF have a reduced peak VO_2 and anaerobic threshold and a shallower profile (or even flattening) of the oxygen-pulse increase with increasing VO_2. A steep increase in Ve/VCO_2 slope is observed and occasional periodic breathing at rest and during the early phase of exercise has been noted (Balady et al., 2010; Malhotra et al., 2016; Palange et al., 2007).

CPX Limitations

The test requires sophisticated expensive equipment, specially trained personnel and is labor- and cost-intensive, therefore usually limited to specialized centers. Interpretation of the CPX also demands specialized expertise, which is of vital importance. Quality assurance protocols have been described to help ensure the gas exchange data is physiologically reasonable and reproducible (Brawner et al., 2008). Even centers conducting a high volume of CPXs, clinically or for research, do not always pass quality assurance protocols, the most common reasons being: use of treadmill handrails by the patient for support, talking during the test, air leak around the mask, mouthpiece, or nose clip, inaccurate calibration procedures, errant pneumotach or flow sensor, error of gas analyzer or software that was not identified through calibration. Holding onto the treadmill handrails usually decreases the metabolic cost and should be discouraged (Balady et al., 2010; Forman et al., 2010). A valid test interpretation is crucially dependent on the calibration of airflow and gases (O_2 and CO_2) that are performed immediately prior to testing.

An area of current discussion is the optimal exercise modality (cycle ergometer vs. treadmill) has not been established (Balady et al., 2010; Forman et al., 2010; Maeder et al., 2008). Treadmill exercise testing has several advantages over cycle ergometer (**Table 1**) and for most subjects, treadmill walking is a more familiar activity than cycling; however, it tends to be criticized as patients tend to rely on handrails, which confounds assessments of work capacity. Walking on the treadmill involves a larger muscle mass and more work against gravity. Consequently, treadmill peak VO_2 is on average 5%–10% higher compared to exercise on a cycle ergometer. A cycle ergometer is less prone to noise artifact and allows for better quantification of the metabolic cost. Generally, it is less expensive and requires less space than a treadmill and the preferred method for patients with gait, balance instability, or orthopedic problems.

An adequate set of normal reference values has not been established (American Thoracic Society and American College of Chest Physicians, 2003; Forman et al., 2010; Miles et al., 1980; Wasserman et al., 2005). The selection of peak VO_2 predicted values (both

Table 1 Comparison of cycle ergometer and treadmill CPET

Variable	*Cycle*	*Treadmill*
Peak VO_2	Lower	Higher
Work rate measurement	Yes	No
Blood gas collection	Easier	More difficult
Noise and artifacts	Less	More
Safety (fewer musculoskeletal injuries)	Safer	Less safe (?)
Weight bearing in obese subjects	Less	More
Degree of leg muscle training	Less	More
Usefulness in supine position	Yes	No
Familiarity of exercise	Less	More
More appropriate for	Patients	Active normal subjects

mean and at the 95% confidence level) is a challenging problem, especially because body sizes and activity levels of a specific clinical population may differ from those of reference populations. Peak VO_2 during exercise varies with age, gender, body size, lean body mass, level of ordinary activity, and type of exercise. Peak VO_2 is generally expressed as mL/kg/min; however, the selection of an appropriate predicted peak VO_2 in overweight patients is difficult. Overweight individuals are more likely to have predominantly an increase in adipose tissue rather than an increase in potential exercising muscle mass. Thus, a direct correlation between increasing peak VO_2 and body weight cannot be implied. The current recommendations are to increase the predicted peak VO_2 by 6 mL/min for each kilogram of weight above normal (Wasserman et al., 2005) or to normalize the peak VO_2 by ideal body weight (instead of actual weight) estimated from the patient's height (Cicoira et al., 2004). Normalizing peak VO_2 for gender and age (using the percent predicted VO_2) in terms of listing criteria for heart transplantation has been recommended by the ISHLT guidelines (Mehra et al., 2006), particularly for young patients and women, for whom a threshold of 14 mL/kg/min may be inappropriate.

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Cardiovascular Complications and Management in Sarcoidosis: A Review

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Introduction

Sarcoidosis is a disease known for invading many organ systems in a diverse group of patients, both by age and by ethnicity (Valeyre et al., 2014; Culver and Valeyre, 2016; Birnie et al., 2016a). Inflammatory involvement of these organs by noncaseating granulomas is a key diagnostic criterion. While pulmonary disease is the most common cause of death in this population, cardiac causes including arrhythmia and decompensated heart failure are the second most common, despite only 5% clinically diagnosed cardiac disease involvement (ATS, ERS, WASOG, 1999). This issue has been helped somewhat by advanced imaging, which has informed us that subclinical cardiac disease may be present in 25% of patients, and has led to earlier treatment with steroids and other immunomodulators, as well as antiarrhythmic procedures (Bussinguer et al., 2012). Prognosis is worse with cardiac involvement and depends on cardiac function at the time of treatment (Sadek et al., 2013).

Background

Sarcoidosis is a systemic inflammatory disorder characterized histologically by noncaseating granulomatous formation with potential for multisystem tissue involvement. The disease process disrupts physiologic architecture and function, often complicated by resultant scar formation. The cause of sarcoidosis remains poorly understood and the case-to-case diagnosis is often difficult, especially as it manifests in quite diverse and often nebulous ways (Valeyre et al., 2014; Culver and Valeyre, 2016; Yee, 2016). Modern diagnostic modalities have detected involvement in essentially every organ system, including pulmonary, cardiac, neural, hepatic, splenic, glandular, ocular, dermal, and bone tissues (Yee, 2016; Saidha et al., 2012; Birnie et al., 2015). The most recent joint consensus statements by the American Thoracic Society, the European Respiratory Society, and the World Association for Sarcoidosis and Other Granulomatous Disorders (WASOG) requires evidence of disease involvement of two organ systems for the diagnosis of sarcoidosis, although organ diagnosis itself is a subject of varying and evolving practice (Sperry et al., 2016).

Pathogenesis and Organ Involvement of Sarcoidosis

While the pathogenesis of sarcoidosis is incompletely known, the contemporary understanding is that there is an antigen-specific immune response to a likely infectious trigger, with interplay between both innate and adaptive immunity, ultimately contributing to noncaseating granulomatous inflammation (Culver and Valeyre, 2016). There is no clear evidence that sarcoidosis is a primary infectious disease. Yet, it is hypothesized that dead or partly degraded mycobacteria and propionibacteria, along with potentially other substances, may linger in macrophages due to their acid-fast nature, and thus chronically supply antigens that could be responsible for an immune response (Ishige et al., 1999; Chen and Moller, 2008). Granulomas that form then are thought to trap remnants of these agents that cannot be degraded further and continue to influence inflammation (Zissel et al., 2010).

The pathologic result varies widely by organ involvement, with poorly understood regional geographic prevalence (Cozier, 2016). Organ systems commonly involved include the skin, peripheral lymph nodes, eyes, liver, spleen, heart, and nervous system. Less commonly involved systems include the kidneys, parotid glands, larynx, bones, skeletal muscles, and gastrointestinal tract.

Epidemiology

Sarcoidosis has a worldwide prevalence estimated between 5 and 64 in 100,000, with the highest worldwide incidence reported in northern Europeans and in African Americans, particularly in women (Birnie et al., 2016a; ATS, ERS, WASOG, 1999). In Europe, Scandinavians seem to have the highest annual incidence, with an estimated 50–60 per 100,000 (Te et al., 2016). Meanwhile, in the United States, the annual incidence is estimated at 10.9 per 100,000 in Caucasians versus a markedly higher incidence of 35.5 per 100,000 in African Americans (Te et al., 2016). Worldwide, there is an age distribution pattern showing that 70% of cases occur between the ages 25 and 45, and only sparse incident cases exist for the pediatric population and for people older than age 70 (Birnie et al., 2016a). In Europe and Japan, there appears to be a bimodal distribution, with a later peak occurring in women older than age 50 (Te et al., 2016). For cardiac sarcoidosis, there is an unclear gender bias, with Japanese studies suggesting a higher prevalence in woman, and American studies suggesting a higher prevalence in men (ATS, ERS, WASOG, 1999; Sperry et al., 2016; Martusewicz-Boros et al., 2016; Yoshida et al., 1997; Sekiguchi et al., 1985).

Cardiac Sarcoidosis: Epidemiology, Prognosis, Morbidity, and Mortality

While sarcoidosis involves the lungs in more than 90% of patients, an estimated 5% of cases have clinically apparent cardiac involvement (Birnie et al., 2016a). In addition to this 5%, though, an estimated 20%–30% of patients with extracardiac sarcoidosis and no diagnosis of cardiac involvement are thought to have subclinical or simply undiagnosed cardiac sarcoidosis, as based on autopsy findings (Birnie et al., 2016a; Bussinguer et al., 2012; Birnie et al., 2016b). Imaging studies have shown similar percentages of asymptomatic cardiac involvement via the use of cardiac MRI and FDG-PET (Mehta et al., 2008; Nagai et al., 2014; Patel et al., 2009; Pizarro et al., 2016). Among all organ involvement, cardiac involvement is the second leading cause of death, behind pulmonary involvement (ATS, ERS, WASOG, 1999; Yee, 2016; Kandolin et al., 2015). In clinically diagnosed cases of cardiac sarcoidosis, the five-year mortality rate ranges from 10% to 85%, a number that varies geographically (Kandolin et al., 2011; Akashi et al., 2012). While this mortality rate is closer to 10%–40% in the United States, the rate attributed to cardiac involvement is estimated to be as much as 85% in Japan, where cardiac sarcoidosis seems to be the most common (Te et al., 2016; Yoshida et al., 1997; Sekiguchi et al., 1985).

Mortality due to cardiac sarcoidosis occurs from either sudden cardiac death (ventricular arrhythmia) or refractory decompensated heart failure (Birnie et al., 2016a). The degree of left ventricular systolic dysfunction appears to be the most important predictor of survival among patients with cardiac sarcoidosis (Birnie et al., 2016a; Chiu et al., 2005). The ten-year survival of patients with left ventricular ejection fraction (LVEF) of less than 30% has been found to be as low as 19%, compared to 100% ten-year survival in patients with preserved LVEF, according to a Japanese single-center retrospective study in 2005 (Chiu et al., 2005). While survivorship among those with cardiac sarcoidosis and LVEF less than 30% is likely somewhat higher with the now wider use of prophylactic implantable cardiac defibrillators (ICDs), low systolic function in the setting of cardiac sarcoidosis remains a high risk for sudden cardiac death due to the unpredictable substrate resulting from granuloma and scar formation. The data from clinical studies remain insufficient, though, to adequately risk-stratify for sudden cardiac death specifically due to sarcoidosis (Epstein et al., 2013). The data is also quite divergent about the prognosis of clinically silent cardiac sarcoidosis, with studies showing either quite good survival or a significant decline in survival (Mehta et al., 2008; Nagai et al., 2014; Patel et al., 2009; Greulich et al., 2013; Murtagh et al., 2016; Smedema et al., 2005a).

Manifestations of Sarcoidosis in the Heart

Granulomatous inflammation of the myocardium and the conduction system produces a broad range of sequelae, from sudden cardiac death to the insidious onset of heart failure (Yee, 2016). As cardiac sarcoidosis often is not diagnosed until the patient presents with arrhythmia, the Heart Rhythm Society recommends that workup for cardiac sarcoidosis be done in any patient with known extracardiac sarcoidosis who has an abnormal electrocardiogram (ECG), abnormal echocardiogram, or symptoms to suggest cardiomyopathy or arrhythmia (Birnie et al., 2014). The types of cardiac involvement can be most simply divided into three main categories: conduction abnormalities, ventricular arrhythmias, and heart failure (Birnie et al., 2016a; Birnie et al., 2016b; Kusano and Satomi, 2016; Cohen Aubart et al., 2016).

Sarcoid-induced conduction abnormalities include primarily atrioventricular block (up to 62% of cases) and bundle branch block (up to 61% of cases) (Birnie et al., 2016c; Nery et al., 2012). Interestingly, up to 33% of patients younger than age 60 presenting with clinically apparent high-grade conduction system disease are found in retrospect to have cardiac sarcoidosis as the primary etiology (Birnie et al., 2016c). High-grade atrioventricular conduction block occurs due to granulomatous involvement of the basal septum, thus directly damaging the conduction system. In association with this basal involvement, mitral or tricuspid insufficiency is often seen due to granulomatous encroachment producing direct valvular damage. Notably, inflammation at the same basal septal territory which results in conduction abnormalities can produce septal edema that can mimic the structural appearance of hypertrophic cardiomyopathy, but sarcoid involvement can be distinguished by new bundle branch block (Uchida et al., 2012).

Cardiac sarcoidosis also manifests in arrhythmias frequently. Up to 42% of cases present with ventricular tachyarrhythmias, while an additional 15% of cases present with supraventricular arrhythmias (Houston et al., 2016). Ventricular tachyarrhythmias, usually due to macro-reentrant arrhythmias around areas of scar, are especially worrisome, as they are presumably associated with sudden cardiac death. Consensus statements suggest that data from randomized defibrillator trials is applicable in this patient population (Birnie et al., 2014). Unfortunately, sarcoidosis of the heart presents clinically as sudden cardiac death in an estimated 60% of cases (Te et al., 2016; Houston et al., 2016). Predicting risk for sudden cardiac death is particularly problematic because distribution of the pathologic substrate is often variable and dynamic, much more so than other cardiomyopathies (Zipse and Tzou, 2016).

Heart failure in cardiac sarcoidosis occurs from two different processes, both which can lead to nonischemic systolic and/or diastolic dysfunction. The first is replacement of normal myocardium with fibroblastic scar tissue in response to granulomatous inflammation, thereby producing stiff and dysfunctional myocardium (Sperry et al., 2016; Epstein et al., 2013; Yufu et al., 2017). The other process is through insidious myocardial remodeling from chronic arrhythmia due to the underlying granulomatous involvement of the conduction system.

Outside of the above typical presentations of conduction disease, ventricular arrhythmias, and heart failure, cardiac sarcoidosis has also been demonstrated to produce coronary artery disease via granulomatous arterial infiltration (Smedema et al., 2005b). This process seems to create vasculitis and coronary spasm, manifesting as angina and what is essentially simulated myocardial infarction without significant epicardial coronary involvement (Roberts et al., 2014). In addition, there is an indeterminate prevalence of clinically silent cardiac sarcoidosis, with at least 25% of cases being either asymptomatic or undiagnosed due to diagnostic challenges (Birnie et al., 2016c). Postmortem diagnosis of cardiac sarcoidosis accounts for an estimated 20%–30% of cases in otherwise assumed extracardiac sarcoidosis, compared to the approximately 5% of cases diagnosed with clinically apparent cardiac involvement (Bussinguer et al., 2012; Birnie et al., 2016b).

Diagnostics

While it may appear that the incidence of cardiac sarcoidosis is increasing, this is likely more a reflection of improving diagnostic techniques to detect occult cases, rather than truly increased pathologic incidence (Birnie et al., 2016a). There is currently no formal consensus on screening for cardiac sarcoidosis as an etiology for nonischemic cardiomyopathy in patients without a known diagnosis of extracardiac sarcoidosis, but its patterns of presentation should influence suspicion and prompt diagnostic workup (Kandolin et al., 2011). In general, screening for cardiac sarcoidosis should be considered for patients younger than 55 years with unexplained high-grade atrioventricular block, patients younger than 55 years with new onset ECG abnormalities in the absence of known obstructive coronary artery disease, patients with sustained monomorphic ventricular tachycardia, and patients with a histologic diagnosis of extracardiac sarcoidosis, regardless of cardiac symptoms (Bussinguer et al., 2012; Blankstein and Waller, 2016; Natarajan et al., 2016).

Both echocardiogram and ECG are recommended for initial screening in suspected or known cardiac sarcoidosis (Birnie et al., 2014). Suspicious echocardiographic findings are LV dilatation (EDD $>$ 55 mm in women, $>$60 mm in men), septal thinning, local wall thinning or thickening, and impaired left ventricular (LV) longitudinal strain (Kusano and Satomi, 2016; Blankstein and Waller, 2016). Granulomatous involvement and scar tissue will tend to appear hyperechogenic on echocardiogram. Common ECG findings include new right or left bundle branch block, ventricular tachycardia, frequent PVCs, and abnormal Q waves (Nery et al., 2012; Sobue et al., 2015; Tanaka et al., 2016). Despite the ECG findings being quite nonspecific, rarely does a patient with cardiac sarcoidosis present with a normal ECG. In fact, a single center retrospective study in the United States demonstrated abnormal ECGs in all patients with cardiac sarcoidosis (Sperry et al., 2016). If there remains suspicion for cardiac sarcoidosis in a patient with a normal ECG, 24-h ambulatory Holter monitoring looking for these typical arrhythmias is recommended (Kashiwabara et al., 1999; Suzuki et al., 1994).

Advanced imaging modalities have become routine in the workup for cardiac sarcoidosis, increasing the diagnostic sensitivity and specificity (Blankstein and Waller, 2016; Youssef et al., 2011; Perez et al., 2016). Either magnetic resonance imaging (MRI) or ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) is recommended following abnormal echocardiogram or ECG findings, to confirm diagnosis and to determine myocardial involvement.

MRI is the current imaging modality of choice in cardiac sarcoidosis workup, due mostly to its more common accessibility over PET and high diagnostic sensitivity (Natarajan et al., 2016; Greulich et al., 2016; Bateman, 2016). Acute inflammation of the myocardium is best seen on T2-weighted imaging and with early gadolinium enhancement. Meanwhile, more chronic changes, such as wall thinning, hypertrophy, and fibrosis, are best demonstrated on T1-weighted imaging and with late gadolinium enhancement. The most common patterns of MRI enhancement in cardiac sarcoidosis are mid-myocardial and sub-epicardial, usually not in a vascular distribution (**Fig. 1**). In addition to its diagnostic utility, MRI is of particular use in predicting mortality from cardiac involvement, with late gadolinium enhancement findings being the most telling (Greulich et al., 2013; Greulich et al., 2016).

While FDG-PET may not be as widely utilized as MRI, it appears to be even more sensitive in detecting active disease. It also notably can attain higher image resolution (Bateman, 2016; Lee et al., 2016; Ishida et al., 2014). The cellular uptake of 18-Fluorodeoxyglucose (^{18}FDG), a glucose analog used in this study, in cardiac sarcoidosis is related to high macrophage activity

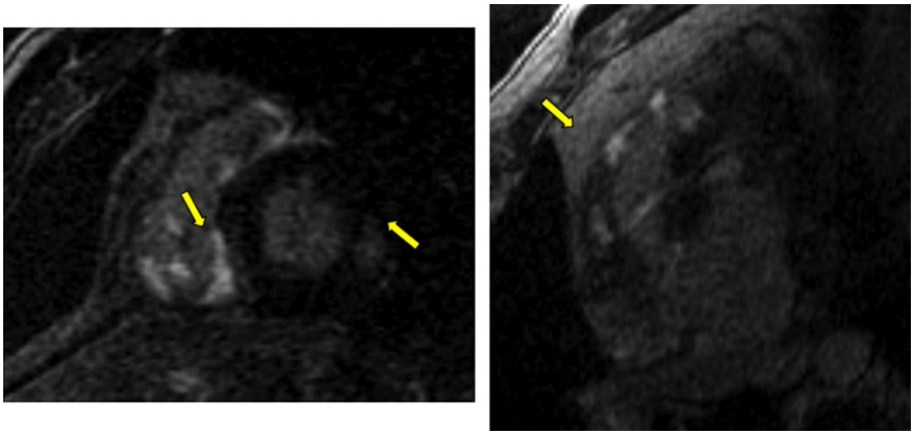

Fig. 1 Cardiac MRI imaging of cardiac sarcoidosis. The image shows patchy late gadolinium enhancement of the right ventricular outflow tract and mid-inferolateral wall (A, arrows) and the right ventricular free wall (B, arrow).



Fig. 2 FDG-PET imaging of cardiac sarcoidosis. The image included shows increased FDG uptake involving the septum and apex (arrow).

in the inflammatory milieu of active disease (Ishida et al., 2014). Accordingly, FDG PET is especially useful in detecting the inflammatory distribution in known disease and in gauging response to antiinflammatory treatment (**Fig. 2**). Generally, patterns of involvement can be seen just in focal areas or on a background of diffuse uptake. One challenge to this study is that imaging is entirely dependent on suppression of glucose utilization in normal, nonaffected myocardium, which requires significant dietary and behavioral rules for 24 h prior to the procedure (Bhambhvani, 2017). Another challenge is that ^{18}FDG activity is not specific to cardiac sarcoid. It can also be seen in hibernating myocardium, myocarditis, or even systemic rheumatologic conditions. Of note, fusion imaging, a composite of both FDG PET and MRI, is beginning to gain momentum in its ability to detect both high-resolution acute inflammation and more of the chronic changes that are seen better on MRI late gadolinium enhancement (Ferreira and Piechnik, 2016).

Endomyocardial biopsy, while diagnostic, is unfortunately quite limited by its low sensitivity (Kandolin et al., 2011; Kusano and Satomi, 2016). This shortcoming is attributed to the patchy myocardial distribution, which limits the likelihood of obtaining a successful diagnostic biopsy (Yee, 2016; Sperry et al., 2016; Kandolin et al., 2011; Kusano and Satomi, 2016). A diagnostic biopsy contains the histology that defines the disease: noncaseating granulomas and multinucleated giant cells, characteristically without myocardial necrosis (**Fig. 3**). In practice, though, biopsies are diagnostic of cardiac sarcoidosis in less than a quarter of patients who are later proven to indeed have cardiac sarcoidosis (Birnie et al., 2016c).

The most recent worldwide joint consensus statement on diagnosis of cardiac sarcoidosis, as prepared by the Heart Rhythm Society, European Heart Rhythm Association, World Association for Sarcoidosis and other Granulomatous Disorders (WASOG), American College of Chest Physicians, American Heart Association the American College of Cardiology and the Asia Pacific Heart Rhythm Society, aims to assist in diagnosis especially when faced with patients with inconclusive clinical data (Birnie et al., 2016c). As this consensus is a class C statement, it is intended as guidance rather than as criteria for diagnosis (**Table 1**).

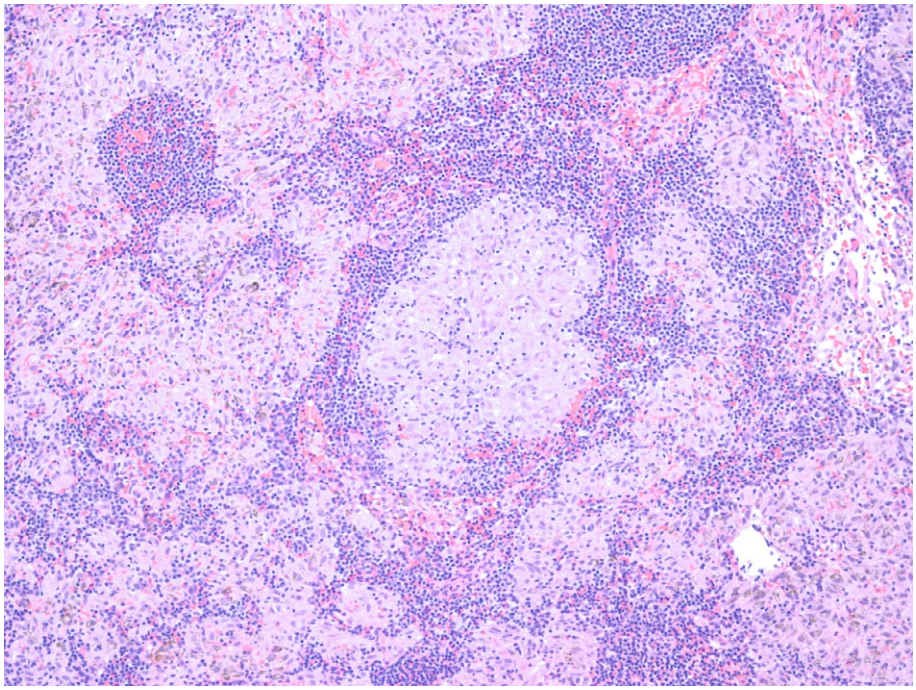

Fig. 3 Microscopic view of a right ventricular endomyocardial biopsy showing noncaseating granulomata as well as myocardial damage. Hematoxylin-eosin. Credit to Robert Christman, MD, Maine Medical Center Department of Pathology.

Table 1 The first international expert consensus on the diagnosis and management of cardiac sarcoidosis

Options for diagnosis of cardiac sarcoidosis
1. Histological diagnosis from endomyocardial biopsy
– Noncaseating granuloma of myocardial tissue with no other causeidentified, including infectious staining
2. Clinical diagnosis
Diagnosis is probable (adequate for diagnosis) if the following are present
(A) Histological proof of extracardiac sarcoid and
(B) At least one of the following
(i) Steroid (with or without immunosuppressant) responsive heart block or cardiomyopathy
(ii) Ejection fraction $<$40% without other explanation
(iii) Sustained ventricular tachycardia
(iv) High-grade heart block
(v) FDG-PET (cardiac) shows sarcoid-consistent patchy tracer uptake
(vi) Cardiac MRI shows late Gadolinium enhancement in a sarcoid-consistent pattern
(vii) Positive gallium uptake in a sarcoid-consistent pattern and
(C) Other causes of these findings have been excluded

Modified from Ref. (Birnie et al., 2016c).

Prior to the aforementioned worldwide consensus statement, the Japanese Ministry of Health and Welfare has created diagnostic guidelines, first in 1993 and revised in 2006 (Soejima and Yada, 2009) (**Table 2**). These guidelines have often been applied to aid in diagnosis of cardiac sarcoidosis outside of Japan, despite this not being the primary intention. The flaw in these criteria, arguably, is their basis on the Japanese population, which does not necessarily represent other patient populations.

Management

Management of cardiac sarcoidosis is twofold: treatment aimed at limiting the inflammation, and interventions aimed at preventing arrhythmia from the disease.

Despite limited observational data, glucocorticoids are the standard antiinflammatory pharmacotherapy for limiting the inflammation and progression to fibrosis that occurs in cardiac sarcoidosis (Armstrong et al., 2015). Response to glucocorticoids

Table 2 Revised guidelines for diagnosing cardiac sarcoidosis 2006 (Japan Society of Sarcoidosis and other Granulomatous Disorders) (Soejima and Yada, 2009)

1. Histologic diagnosis group:
   Cardiac sarcoidosis is confirmed when myocardial biopsy specimens demonstrate noncaseating epithelioid cell granuloma with histological or clinical diagnosis of extracardiac sarcoidosis.
2. Clinical diagnosis group:
   Although myocardial biopsy specimens do not demonstrate noncaseating epithelioid cell granuloma, extracardiac sarcoidosis is diagnosed histologically or clinically and satisfies the following conditions and more than one in six basic diagnostic criteria.
   (1) More than 2 of 4 major criteria are satisfied.
   (2) One in 4 major criteria and more than 2 in 5 minor criteria are satisfied.

   Major criteria
   - Advanced AV block.
   - Basal thinning of the interventricular septum.
   - Positive cardiac 67Ga uptake.
   - Depressed ejection fraction of the left ventricle (LVEF $< 50\%$).

   Minor criteria
   - Abnormal ECG findings: Ventricular arrhythmias (VT, multifocal, or frequent PVCs), CRBBB, axis deviation, or abnormal Q-wave.
   - Abnormal echocardiography: Regional abnormal wall motion or morphological abnormality (ventricular aneurysm, wall thickening).
   - Nuclear medicine: Perfusion defect detected by 201Tl myocardial scintigraphy or 99Tc myocardial scintigraphy.
   - Gd-enhanced MRI: Delayed enhancement of myocardium.
   - Endomyocardial biopsy: Interstitial fibrosis or monocyte infiltration over moderate grade.

depends heavily on whether the cardiac dysfunction is attributed more to active inflammation or to fibrosis that is already present (Zipse and Tzou, 2016). In theory, the immunosuppressive effect of glucocorticoids will be of much less benefit in cases where there is already extensive fibrosis. It follows that early initiation of steroids is recommended to prevent fibrotic progression of disease.

There is no proven science to the steroid dosing, but 0.5 mg/kg of daily prednisone is typically the starting dose (Kusano and Satomi, 2016; Houston et al., 2016). We generally recommend repeating a PET scan 3 months after treatment initiation, which, if negative, can allow a 3-month steroid taper to 0.2 mg/kg daily, which would continue for another 9 months. We would then repeat a PET scan 3 months post treatment end, and if negative, would follow a patient with echocardiogram and EKG every 6-12 months. If at any point a PET scan is positive for relapse or continued inflammation, combination therapy with Methotrexate should be considered. The efficacy of other immunosuppressive therapy as an alternative to glucocorticoids is less known, but can be considered in cases of contraindication or intolerance to glucocorticoids.

Similar to the role in primary and secondary prevention of arrhythmia in other types of cardiomyopathies, options in cardiac sarcoidosis include ablation, permanent pacemaker, and ICD. Radiofrequency catheter ablation is an option for limiting tachyarrhythmia burden in the majority of cardiac sarcoidosis patients, although results are mixed (Muser et al., 2016; Birnie et al., 2016c; Nery et al., 2012). There is variable efficacy, likely due to the complex and dynamic substrate changes in sarcoidosis. Permanent pacemaker implantation is recommended for high grade or complete atrioventricular conduction block in the setting of cardiac sarcoidosis, even if the block appears to transiently resolve, as there is a very high probability for disease progression and risk for conduction block recurrence (Epstein et al., 2013).

As in patients with cardiomyopathy in general, ICD implantation is a class I indication for primary prevention in those with NYHA class II to III symptoms and an LVEF of 35% or less, as well as for secondary prevention in those with structural heart disease with sustained ventricular tachyarrhythmias (Epstein et al., 2013). Cardiac sarcoidosis is included in potentially both categories, and the Heart Rhythm Society has provided consensus recommendations for the use of devices (Epstein et al., 2013; Birnie et al., 2016c; Nery et al., 2012). ICDs have a IIa recommendation in patients with an indication for pacemaker and/or syncope or near-syncope felt to be arrhythmic. They also have a IIa recommendation for ventricular arrhythmias including over 30 s of ventricular tachycardia, or any relevant episodes of polymorphic ventricular tachycardia or fibrillation. There is a IIb recommendation in patients with LVEF 36%–49% or right ventricular EF $<40\%$ despite optimal medical therapy and immunosuppression for a period of time, with potential help with risk stratification from MRI or FDG-PET. Finally, if none of these categories are achieved, the patient may undergo an MRI, and if there is significant scar, an electrophysiology study can be performed, which, if positive, would give a IIa indication for ICD implantation (Epstein et al., 2013).

Notably, cardiac resynchronization therapy (CRT) guidelines fail to encompass a specific role in cardiac sarcoidosis, as there is currently a paucity of outcome data for this population (Zipse and Tzou, 2016). One pertinent question that could guide future research is whether CRT might be beneficial earlier in the course of cardiac sarcoidosis in the setting of LBBB or AV block, potentially limiting the progression to severe heart failure.

In cases of advanced heart failure attributed to cardiac sarcoidosis, left ventricular assist device (LVAD) implantation or heart transplant can be considered. The criteria for heart transplant include New York Heart Association class III-IV symptoms despite appropriate therapy and cardiogenic shock, which may occur in cardiac sarcoidosis. Importantly these criteria also include refractory ventricular arrhythmias despite appropriate defibrillation and ablation therapies.(Colvin et al., 2017) Criteria for LVAD implantation include poor cardiac output in the setting of heart failure, but also notably include systolic dysfunction with intractable

arrhythmias.(Kirklin et al., 2015) Therefore for patients with significant physical compromise or life-threatening arrhythmias, advanced therapies are an option. Notably, there is controversy regarding potential recurrence in the transplanted heart. In 14 patients with cardiac sarcoidosis as primary reason for heart transplant out of 825 patients undergoing heart transplant at a single center, 2 out of the 14 patients had confirmed sarcoidosis recurrence in the transplanted heart, and there was a trend toward higher 1 and 5 year mortality in all cardiac sarcoid patients who received transplants (Akashi et al., 2012).

Future Directions

Sarcoidosis has proven difficult to understand, with difficulty even accurately characterizing disease frequency within various populations and regions (Cozier, 2016). Multiple trials are seeking to better understand the genetic and regional markers for sarcoidosis activity. Medication trials, such as using atorvastatin or pentoxifylline for treatment, are awaiting results after recruitment, and therapies as advanced as hematopoietic stem cell transplant have haltingly been tried in recent studies (Clinicaltrials.gov). Much of the other thrust of current or recent research is in better understanding the utility of imaging for diagnostic and therapeutic purposes. It is our hope that existing treatment dosing strategies can be compared for efficacy in a systematic way, in order to allow for growth of our understanding of how best to help our patient survive and thrive with cardiac sarcoidosis.

Conclusion

Sarcoidosis is a diverse disease that in a minority of patients has severe effects on the heart. The diagnosis is difficult to make, but once made, treatment strategies including immunomodulatory therapies and electrophysiology procedures are often quite successful. It is our hope that this project helps to spread the knowledge of this uncommon presentation and facilitates the treatment of patients in the future.

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Cardiovascular Disease and Obesity

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Guidelines of World Health Organization (WHO) entitled "Prevention of Cardiovascular Disease" (World Health Organization, 2007) aims to prevent cardiovascular disease (CDV) in a number of WHO member states and contains abundant data in each region. For example, predicted 10-year risk of CDV for each sex in each region is summarized in risk prediction charts. With these risk prediction charts, it is possible to easily predict the absolute risk of CDV and establish a therapeutic target.

Risk prediction charts have also been prepared for regions where cholesterol cannot be measured, and are applicable in a wide range of countries including those of low- and high-income levels. From the viewpoint of CVD prevention, it particularly emphasizes the importance of life habits, including dietary habits, and control of risk factors in the therapeutic aspect.

The total risk concerning onset of CVD is judged by the combined effects of CVD risk factors, which coexist in many cases and act in a synergistic manner. Thus, a person who has several low-level risk factors is possible to have a higher total risk of CVD than a person having only one risk factor of a similar level. The risk prediction charts of WHO proposed that this time is also applicable to persons having metabolic syndrome.

Total risk thresholds vary depending on the financial resources level. The 10-year CVD risk thresholds that require intensive intervention are as follows:

High financial resources level: 20%
Intermediate financial resources level: 30%
Low financial resources level: 40%

Low thresholds for intervention increase the number of beneficiaries but also increase the total costs for chemotherapy and the number of adverse events.

Characteristics of the Risk Prediction Charts

The majority of risk prediction methods for predicting total CVD risk employ prediction formula obtained from diverse information sources. The most widely used information source is Framingham Heart Study (Wilson et al., 1998; British Heart Foundation, n.d.; McCormack et al., 1997; Jackson, 2000; Wood et al., 1998; D'Agostino et al., 2001; Marrugat et al., 2003). In risk charts and tables prepared, diverse age categories, risk evaluation periods, and risk factor profiles are deployed (**Figs. 1** and **2**) (World Health Organization, 2007). The current New Zealand CVD risk charts (Jackson, 2000) and the charts of Joint British Societies (Williams et al., 2004; British Heart Foundation, n.d.) have similar conception.

Risk scores prepared by using the Framingham equation have been widely examined in populations of European Americans and Europeans (Ferrario et al., 2005; D'Agostino et al., 2001; Marrugat et al., 2003; Brindle et al., 2003) as well as in Chinese population (Liu et al., 2004). The European guidelines on CVD prevention uses a new model for estimating the total risk based on the Systematic Coronary Risk Evaluation (SCORE) system (Conroy et al., 2003). Risk is estimated based on sex, age, smoking status, systolic blood pressure, and total cholesterol (TC) or the ratio of TC to high-density lipoprotein cholesterol (HDL-C). Unlike risk scoring based on the Framingham equation, SCORE predicts only the possibility of a fatal CVD event. Risk of fatal CVD of 5% or higher is defined as the threshold for high risk, instead of composite endpoints (including fatal and nonfatal coronary artery disease) of 20%.

Risk factors included in the current SCORE system are the same as those used in the Framingham score, but inclusion of new risk factors is being discussed including C-reactive protein, fibrinogen, and waist–hip ratio (Asia Pacific Cohort Studies Collaboration, 2006). It is likely necessary to continue investigating indices to include so as to respond to changing times.

Position for Measuring Waist Circumference

There is no international standard about the position for measuring waist circumference. We thus investigated the most effective position for measuring waist circumference to be used for diagnosis of metabolic syndrome.

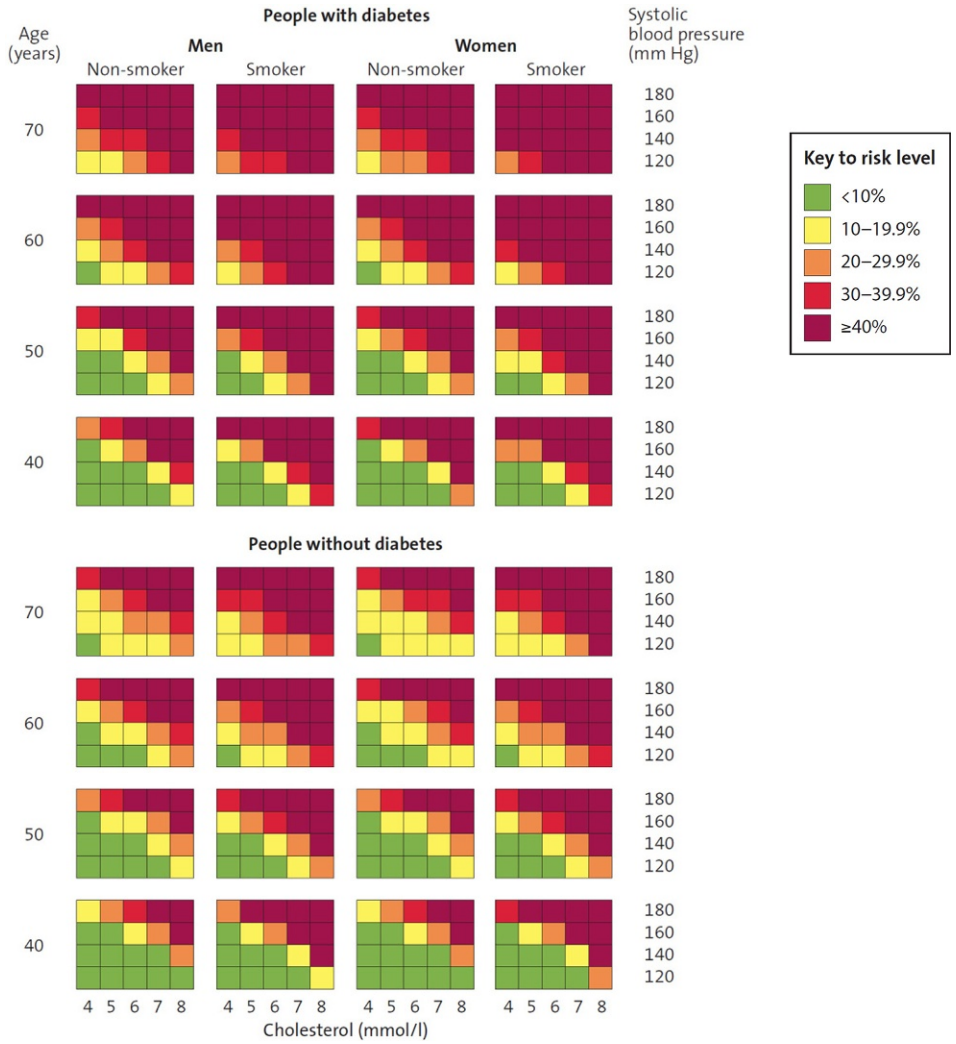

Fig. 1 Sample WHO/ISH risk prediction chart, for use where measurement of cholesterol level is possible (World Health Organization, 2007). The chart below indicates total 10-year risk of a fatal or non-fatal cardiovascular event (myocardial infarction or stroke), according to age, sex, blood pressure, presence or absence of diabetes, smoking status, and cholesterol level, for the WHO Region of South-East Asia.

Waist circumference of 1140 persons (including males and females) was measured at four typical body positions used to measure waist circumference in the world, that is, (1) the narrowest part, (2) the middle point between the lower edge of the costal arch and the upper line of the crista iliaca, (3) umbilical position, and (4) the upper line of the crista iliaca. The optimum position for predicting multiple risk factors of metabolic syndrome was investigated by drawing receiver operating characteristic curves. The mean difference between the largest and smallest waist circumference values was 3.9 cm in males and 12.6 cm in females, showing a large difference by measuring position in females. It was also found that there is no significant difference in the capability of predicting metabolic syndrome between positions measured (Fig. 3). This study revealed that waist circumference measured at any position was useful for diagnosis of metabolic syndrome in both males and females. It was suggested important to correctly measure waist circumference at the position specified by each diagnosis standard. However, it is desirable to internationally standardize the position for measuring waist circumference in order to facilitate comparing prevalence of metabolic syndrome among regions of the world. Conversion expressions among the measuring positions are also stated (Matsushita et al., 2010a).

Effects of Visceral Fat Area on Development of Multiple Risk Factors of Metabolic Syndrome

The effects of visceral fat area on development of multiple risk factors of metabolic syndrome were investigated by comparing with other physique indices.

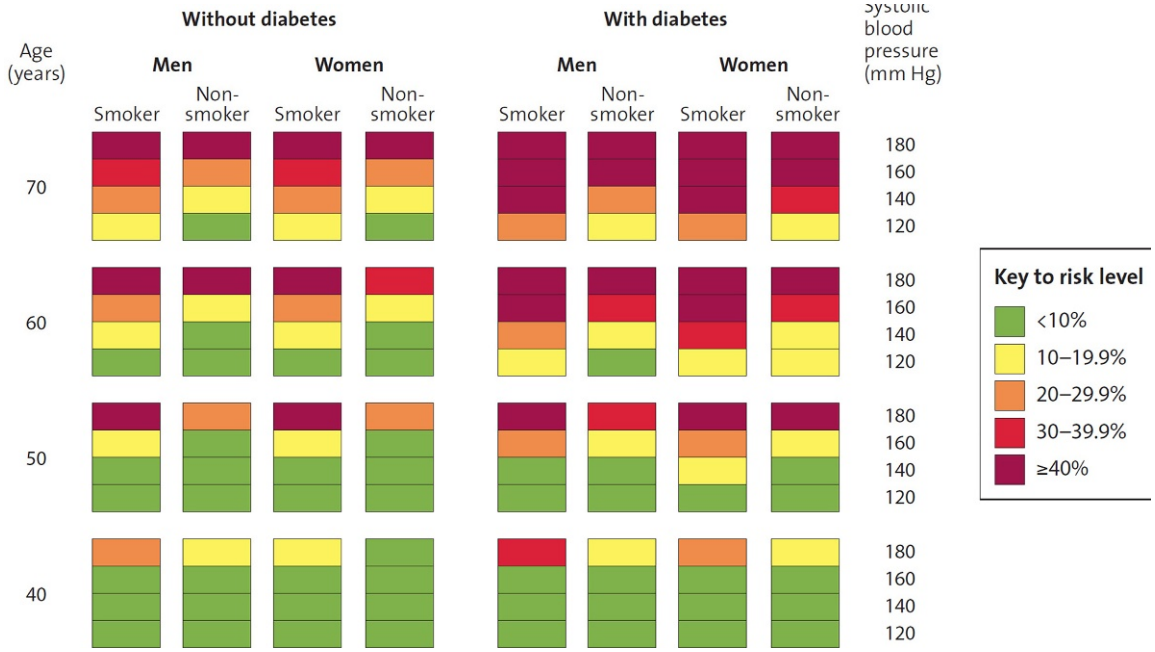

Fig. 2 Sample WHO/ISH risk prediction chart for use where measurement of cholesterol level is not possible (World Health Organization, 2007). The chart below indicates total 10-year risk of a fatal or non-fatal cardiovascular event (myocardial infarction or stroke), according to age, sex, blood pressure, presence or absence of diabetes, and smoking status, for the WHO Eastern Mediterranean Region.

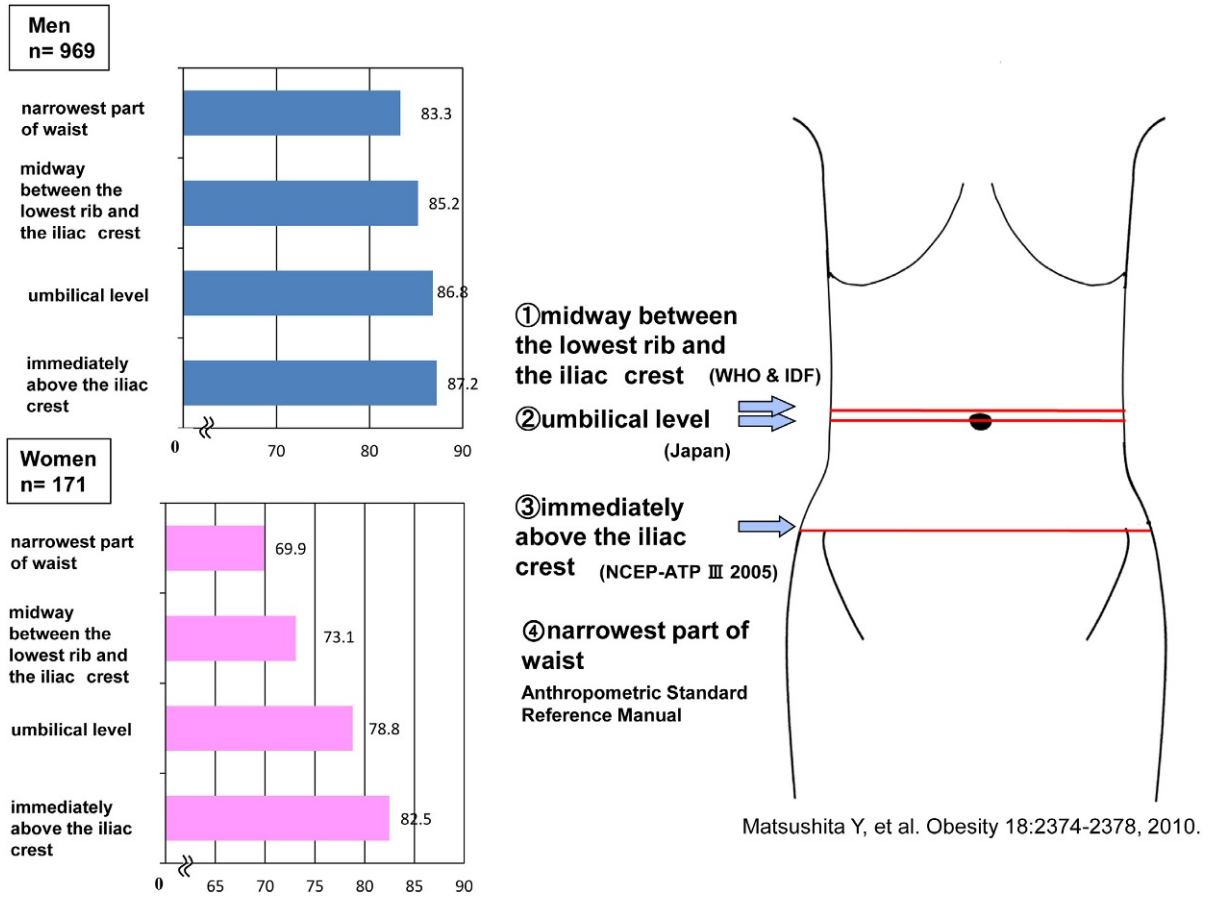

Fig. 3 Comparisons among waist circumference measurements at different sites.

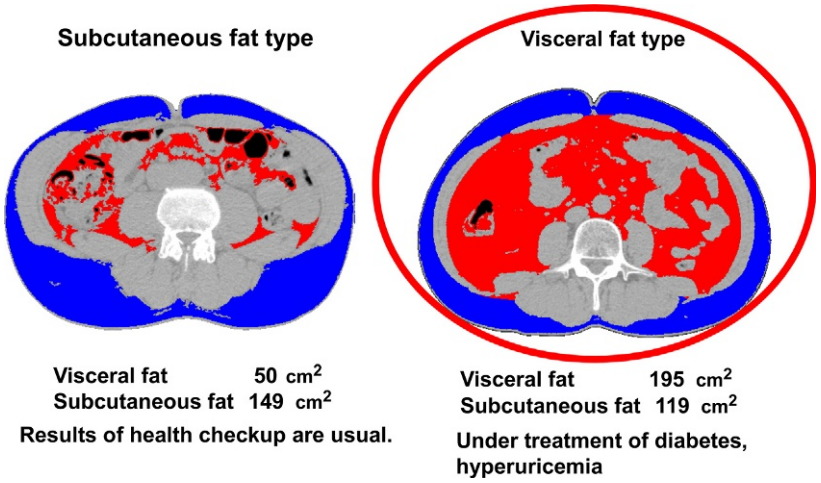

Fig. 4 Although the waist circumferences of these persons are both 86 cm, CT imaging disclosed such a difference.

These are CT photographs of abdomen (Fig. 4). Visceral fat is shown in red. The blue part is subcutaneous fat.

Although the waist circumferences of these persons are both 86 cm, CT imaging disclosed such a difference. We cannot know the inside just by measuring the circumference with a tape. The left is a subcutaneous fat type, and the right is a visceral fat type. The person of a subcutaneous fat type does not show abnormal data in medical checkup, but the person of a visceral fat type is receiving treatment for diabetes mellitus and hyperuricemia. Their pathology is different.

Transverse analysis was conducted on 6292 workers. Based on their CT data, we investigated how visceral fat area, subcutaneous fat area, waist circumference, and BMI are related to each factor of metabolic syndrome (Matsushita et al., 2010b).

For each physique index, the subjects were divided into quintiles, separately for males and females. The odds ratio of persons having multiple risk factors of metabolic syndrome was determined by using the smallest quintile as the standard. Increases in any of the physique indices (visceral fat area, subcutaneous fat area, waist circumference, and BMI) were found to increase the risk of having multiple risk factors of metabolic syndrome. Visceral fat area, which is shown in blue curve, was found to particularly increase the risk. Compared to visceral fat area, waist circumference, which is used as a criterion for metabolic syndrome today, can detect the risk of metabolic syndrome in only 70% in males and 50% in females. There are sex differences in waist circumference, which is shown in red, and BMI, shown in black. It was suggested better to use waist circumference for males and BMI for females (Fig. 5).

As a simple index of metabolic syndrome, waist circumference is used today. However, waist circumference was found to have a limit in detecting the risk.

We also performed a longitudinal study. The relationship between changes in visceral fat area in 3 years and factors of metabolic syndrome and new development of multiple risk factors is shown.

Subjects were 1106 male persons who received abdominal CT scan in 2004 and 2007 and were not receiving treatment for hypertension, hyperlipidemia, or diabetes mellitus during the period (Matsushita et al., 2013).

The subjects were divided into seven groups based on the changes in visceral fat area. The group that had undergone changes of $\pm 10\ cm^2$ or smaller was used as the standard. The odds ratios of developing (1) high triglyceride level, (2) low HDL cholesterol level, (3) high blood pressure, (4) disorder of carbohydrate metabolism, and (5) multiple risk factors of metabolic syndrome in the 3 years were determined. The odds ratio for developing multiple risk factors of metabolic syndrome was significantly high in the group of visceral fat area increase of 50 cm^2 or more. The odds ratio for developing high triglyceride level was significantly low in the group of visceral fat area decrease of 50 cm^2 or more and was significantly high in groups of increases of 30 cm^2 or more (Fig. 6).

The study suggested that suppressing increases in visceral fat would prevent metabolic syndrome.

Risk of CDV

On about 7000 persons in their 20s–70s (including males and females), we investigated the relationship between the amount of visceral fat and the percentage of people having at least two risk factors of lifestyle disease, that is, hypertension, hyperglycemia, and dyslipidemia (or the percentage of people having multiple risk factors of metabolic syndrome) (Matsushita et al., 2014a).

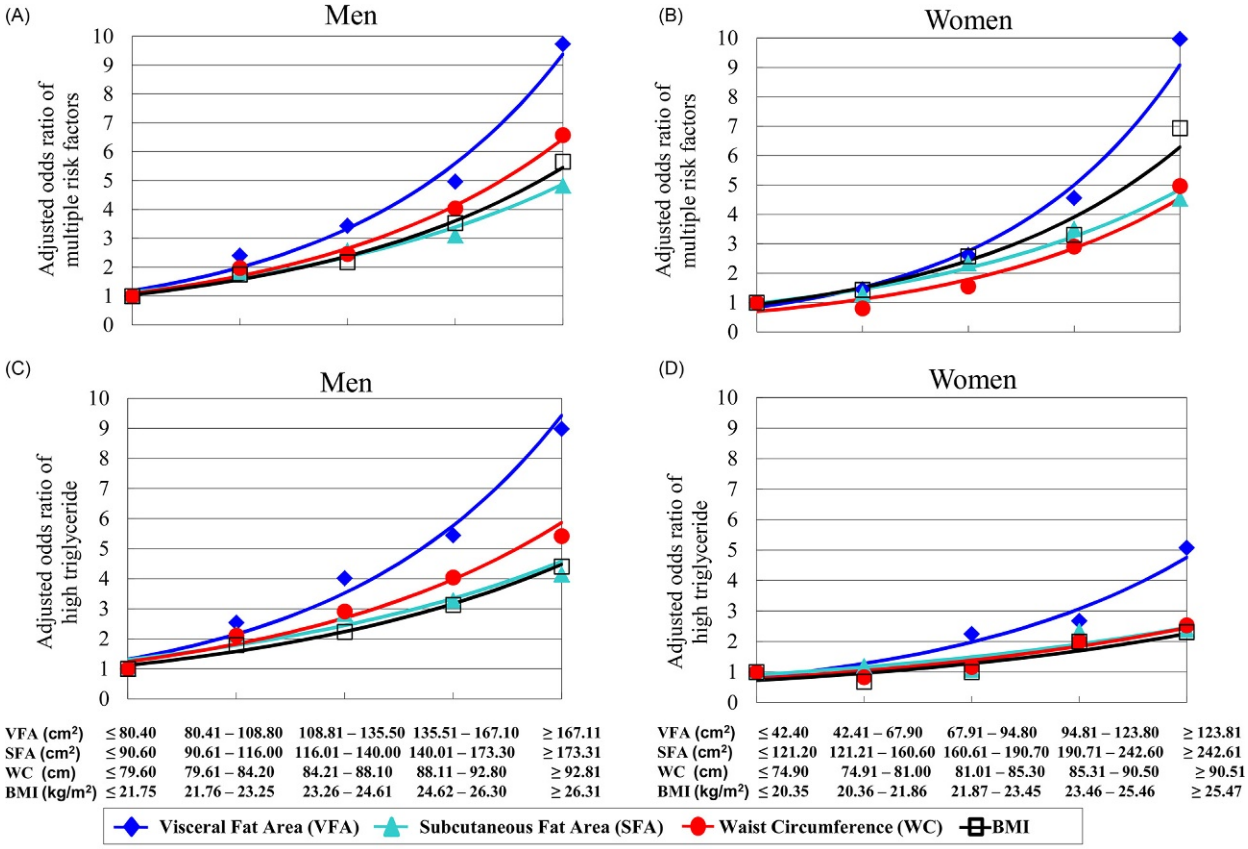

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Fig. 5 Odds ratios for high triglyceride and the clustering of metabolic risk factors according to the quintiles (Q1–Q5) of each anthropometric value.

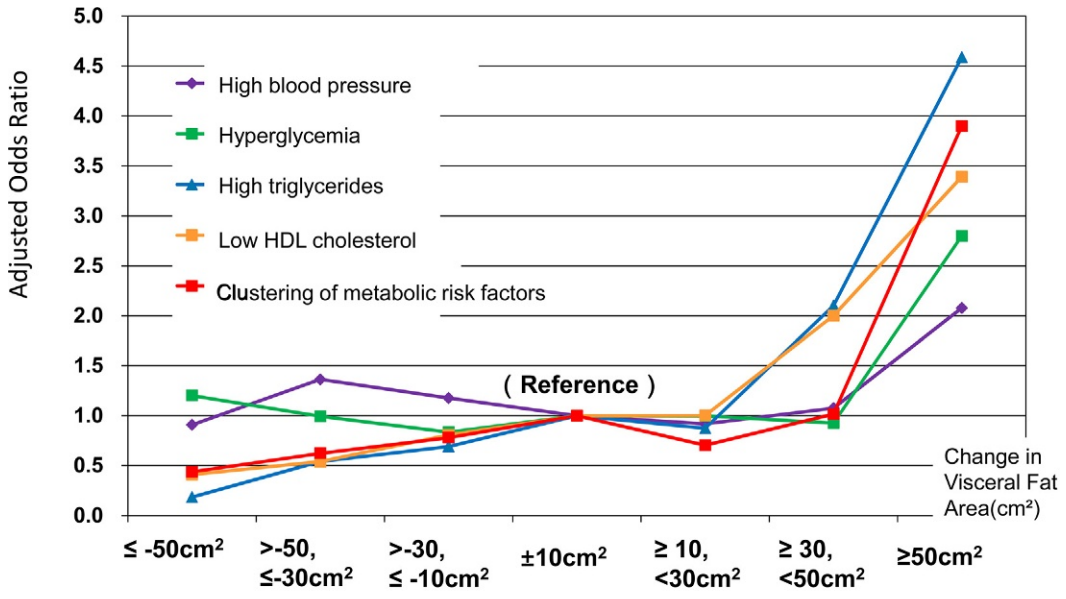

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Fig. 6 Odds ratio of metabolic risk factors (defined by NCEP-ATPIII) at the 3-year follow-up survey.

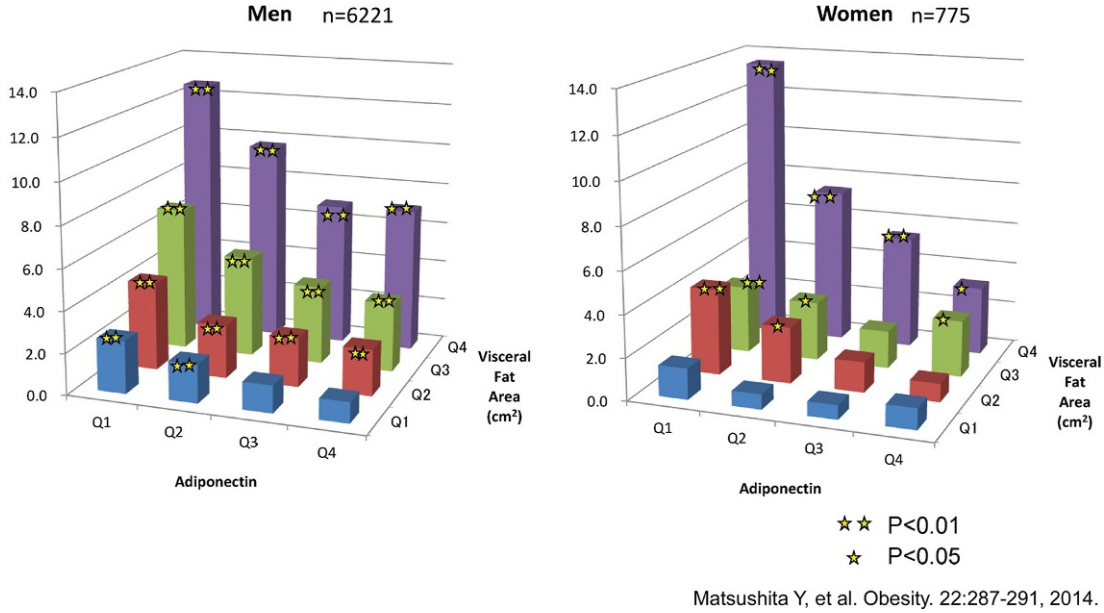

Fig. 7 Odds ratios for multiple risk factors of metabolic syndrome according to combined groups of VFA and adiponectin.

As a result, it was discovered that the risk of developing multiple risk factors of metabolic syndrome of a male who has much visceral fat and low level of a hormone called "adiponectin" in the blood was two times higher compared to a person having high adiponectin level (12.65:7.01 in VFA Q4 (=1.8)) (Fig. 7).

Even in persons having small amount of visceral fat, the risk of developing multiple risk factors of metabolic syndrome was 2.5 times higher in those who have low "adiponectin" level compared to persons having high adiponectin level (2.54:1.00 in VFA Q4).

"Adiponectin" is a hormone secreted by adipocytes and has been shown in animal experiments to prevent arteriosclerosis and stimulate combustion of fatty acids. We discovered that the risk of lifestyle disease in human differed by the level of adiponectin even when the amount of visceral fat is the same.

How Can Waist Circumference Predict the Body Composition?

Each component of the WC may differ between the sexes and generations, even if they have the same WC. Between 2004 and 2009, employees and their spouses working at a Japanese company underwent a health examination after more than 12 h of fasting. We analyzed the data of 11,570 subjects (9874 men and 1696 women), aged from 20 to 76 years, who underwent a computed tomography (CT) examination. VFA, SFA, WC, muscle, intramuscular fat, viscera, and bone were measured using a CT scanner. We conducted stratified analyses by generational age, and calculated the Pearson's correlation coefficients between the VFA and WC, BMI, and VFA plus SFA. To establish the equations for converting the WC to the corresponding VFA and VFA plus SFA, linear regression analyses were used to obtain the regression coefficients and intercepts.

As the generations increased in age, the VFA tended to increase. However, the differences in the WC values of each generation did not coincide with the VFA values in men ($r=-0.275$ and 0.979 for men and women, $n=5$ generations), but did correlate with the difference in the sum of the VFA plus SFA for both sexes ($r=0.915$ and 0.996 for men and women, $n=5$ generations). Older generations had lower WC values when they had the same VFA values as the younger generations (Fig. 8) (Matsushita et al., 2014b).

The WC value corresponding to a certain VFA value differed significantly by generational age. Thus, revised optimal cutoff values for the WC may be needed for each generation.

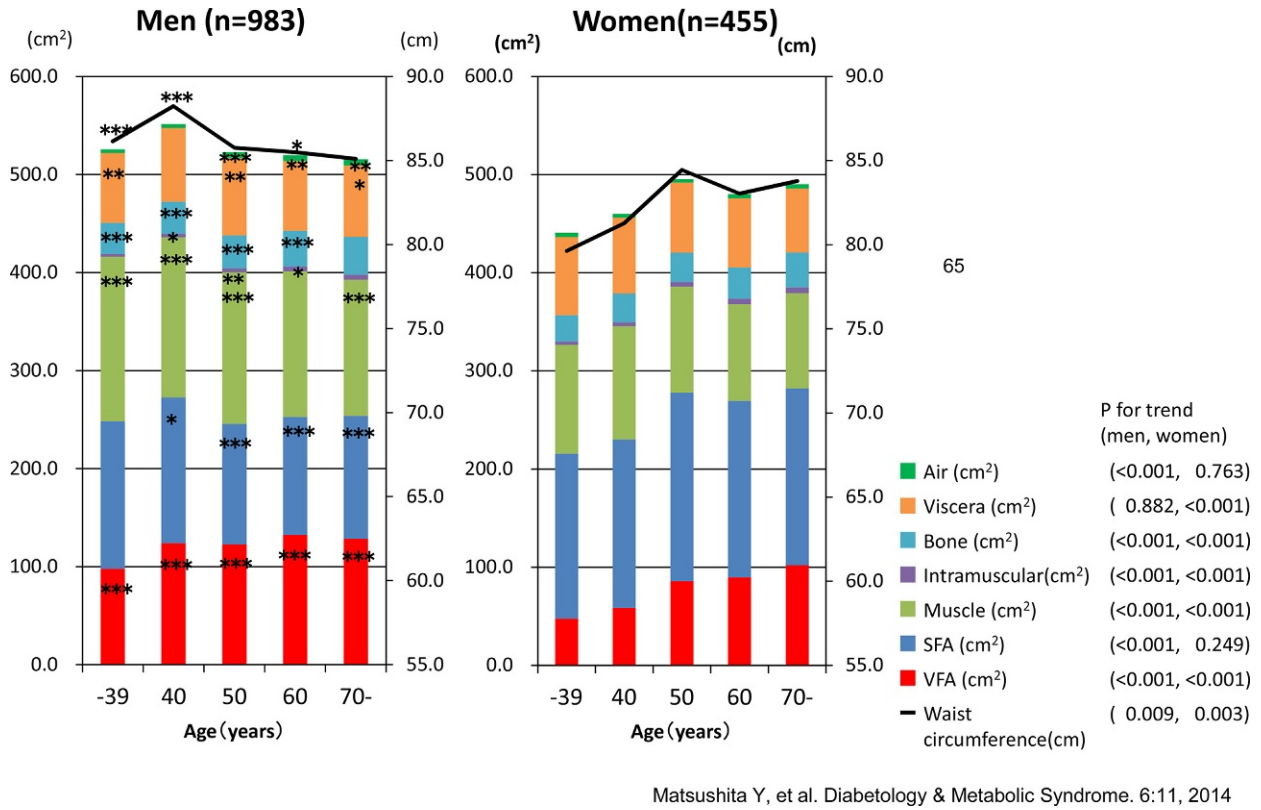

Fig. 8 Mean body components according to the age group measured by CT. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ for differences in mean values between men and women in each age group by *t*-test.

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Catecholaminergic Polymorphic Ventricular Tachycardia

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Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a cardiac ion channelopathy with a high risk of sudden death (SD). It is a relatively newly recognized disease, which can easily escape detection and requires a high index of suspicion for timely diagnosis. Advances in our understanding of the genetic etiology and molecular mechanisms have elicited new and exciting therapeutic approaches. In this manuscript the history of the disease, from the earliest descriptive case reports to the most recent large multicenter studies will be reviewed, along with a discussion of the genetic and molecular mechanisms, as well as current and future approaches to therapy.

Diagnosis

The classic diagnostic feature of CPVT is polymorphic or bidirectional ventricular tachycardia (VT) during exercise or stress. There is a typical progression (**Fig. 1**), from a normal resting electrocardiogram (ECG), to the development of single premature ventricular contractions (PVC's), to bigeminy (alternating sinus beats with PVC's), to ventricular couplets, and nonsustained VT (NSVT). Bidirectional VT can often be confused for bigeminy, and is generally relatively slow and well-tolerated. It should be emphasized that the bidirectional/polymorphic VT seen in CPVT is not life-threatening. Indeed, it is usually asymptomatic or minimally symptomatic. Sustained polymorphic VT can degenerate into ventricular fibrillation (VF), which results in syncope or sudden death.

As stated previously, the resting ECG is normal in CPVT, except perhaps for a relative sinus bradycardia seen in some patients, even prior to therapy (Sumitomo et al., 2003). Occasionally patients will not display arrhythmias on exercise testing, but develop polymorphic VT with epinephrine or isoproterenol infusion (Sy et al., 2011). The heart is also structurally normal, and imaging with echocardiography or cardiac MRI is useful to rule out other potential diagnoses in the differential, such as arrhythmogenic cardiomyopathy. Atrial arrhythmias, including atrial ectopic beats, atrial tachycardia, and fibrillation, are also common features of CPVT, and can be adrenergically driven as well. The most common symptom related to CPVT is syncope, followed by cardiac arrest. Symptom onset is typically during childhood, and a delay from first symptom to diagnosis is typically several months to years. Cardiac events that occur during diving or swimming are almost exclusively due to Long QT syndrome type 1 or CPVT (Choi et al., 2004). The true prevalence of CPVT is difficult to determine, as the resting ECG and imaging studies are unremarkable, and diagnosis requires rhythm assessment during exercise, but it has been estimated at roughly 1 in 10,000 (Priori et al., 2013).

History

The earliest cases of what would now be identified as CPVT were reported in the 1960s. Berg described three sisters with frequent ventricular ectopy, two with repeated syncope with exertion, and one who died suddenly at 19 years of age while biking (Berg, 1960). **Fig. 2** shows a recording of bidirectional VT in this subject at 12 years of age, obtained in 1944. This first report made several notable observations, including the presumed cause of syncope due to VT or VF, the familial occurrence, and the absence of "organic heart disease." In 1962 Horan and Venables reported a case of a 5-year-old girl with recurrent syncope while frightened, in whom they recorded various arrhythmias, including bidirectional VT (Horan and Venables, 1962). In hospital they were able to reproduce an episode of VF by "deliberately induced emotional upset, caused by the threat of making her walk downstairs, to which she had a

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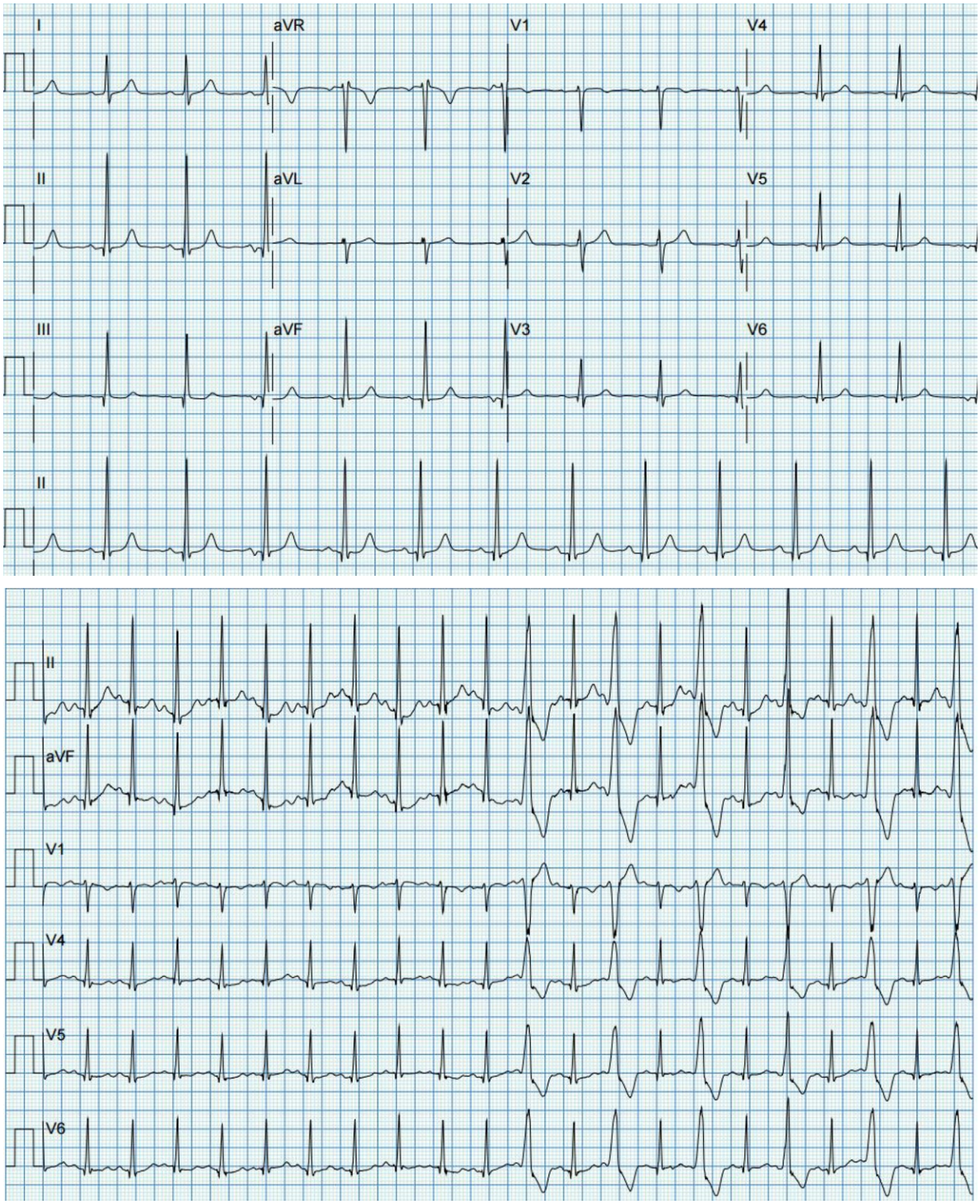
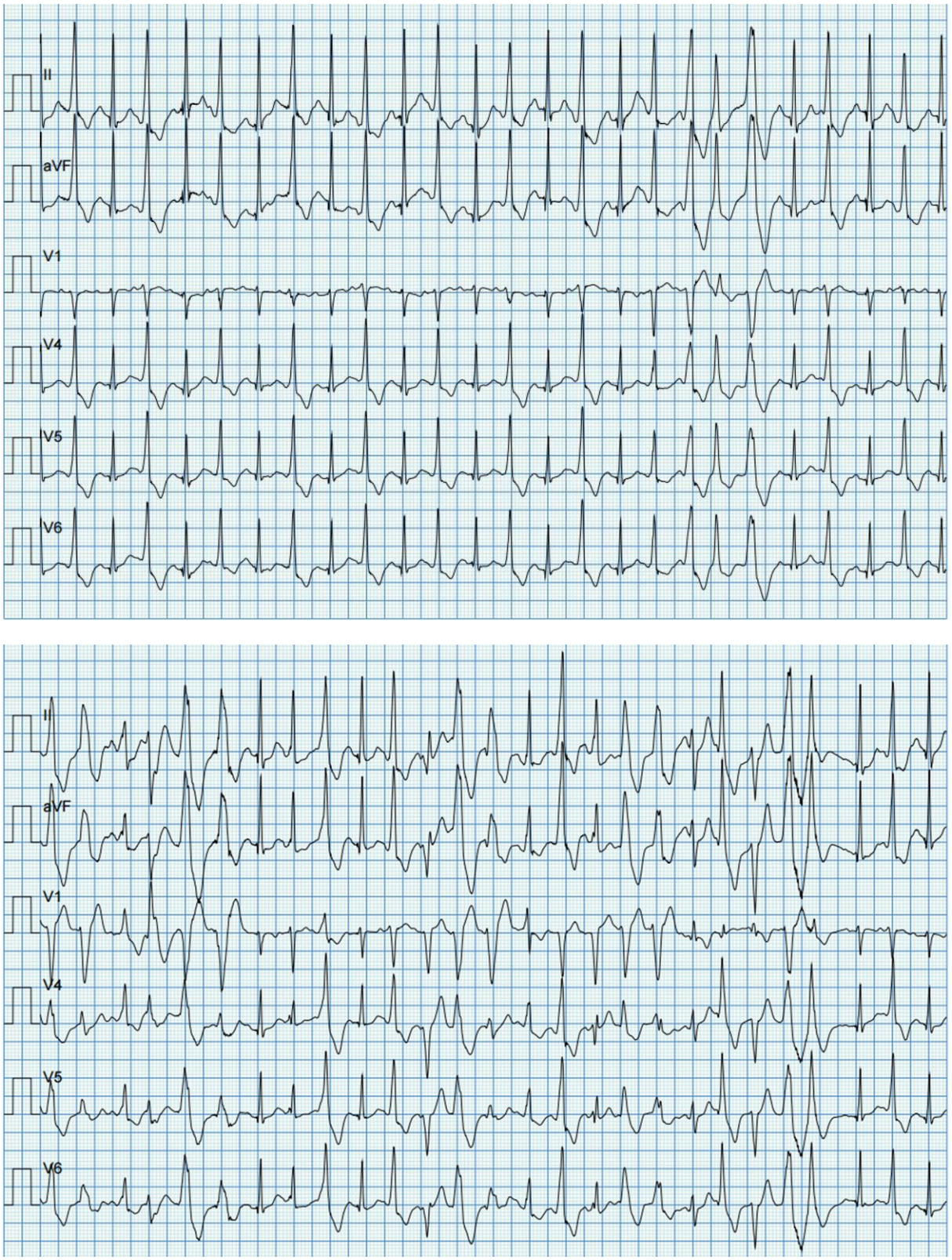


Fig. 1 Classic progression of ventricular arrhythmias in CPVT. The resting ECG is normal. As exercise progresses, single premature ventricular beats begin to emerge in a bigeminal pattern. These can be monomorphic as seen here, or polymorphic. With continued exercise, ventricular ectopy increases and nonsustained or sustained polymorphic or bidirectional VT develops.



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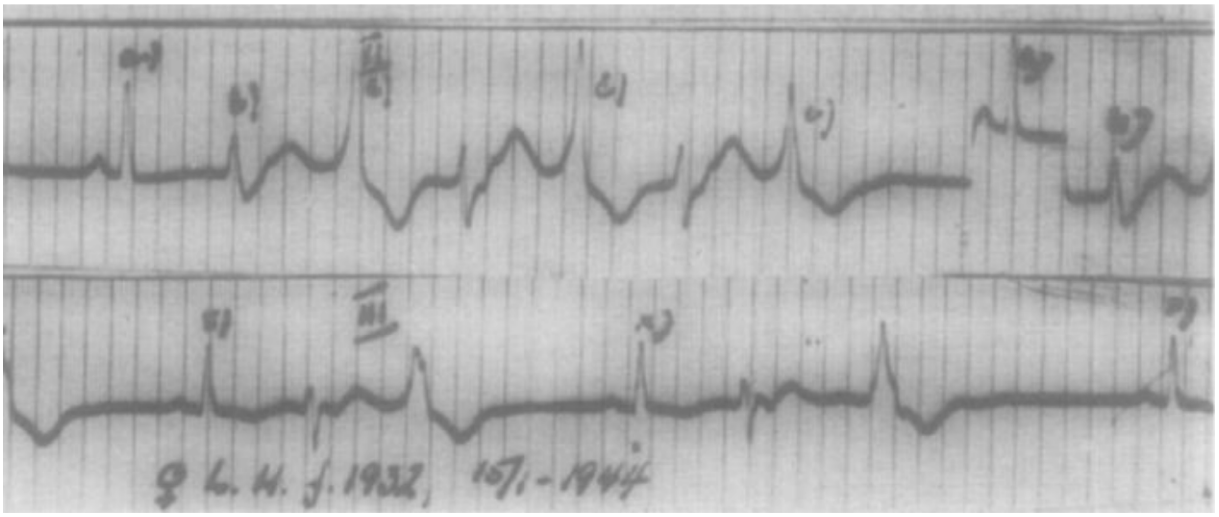

Fig. 2 Recording of nonsustained bidirectional VT from 1944. This ECG was obtained in a 12 year-old girl with a history of syncope who died suddenly while biking at 19 years of age. In retrospect, this was probably the first reported case of CPVT. From Berg K.J. (1960). Multifocal ventricular extrasystoles with Adams-Stokes syndrome in siblings. *American Heart Journal* 60, 965–970.

particular aversion." They too speculated that the etiology involved "excessive liberation of adrenalin combined with a particularly sensitive myocardium." In 1975, His-bundle recording was used in a 6-year old with effort/stress induced bidirectional tachycardia, proving the mechanism was indeed ventricular (Reid et al., 1975). Coumel published the first series in 1978, but the comprehensive series of 21 children published by Leenhardt in 1995 unified the term "CPVT" (Coumel et al., 1978; Leenhardt et al., 1995).

Genetics and Molecular Mechanisms

Under normal conditions, after myocyte depolarization, voltage-gated L-type calcium channels open and a small amount of calcium enters the cytoplasm. This results in calcium-induced calcium release (CICR) from the sarcoplasmic reticulum (SR), where the bulk of intracellular calcium is stored, bound to calsequestrin (**Fig. 3**). Massive amounts of calcium then enter the cytoplasm from the SR through calcium release channels, called ryanodine receptors (see below). Calcium activates the contractile apparatus, and then is taken back into the SR via the sarco/endoplasmic reticulum calcium-ATPase (SERCA2), and a small amount gets extruded via the sodium-calcium exchanger (NCX). The primary arrhythmogenic mechanism underlying CPVT is excessive calcium leak from the SR during periods of adrenergic stress, resulting in delayed after depolarizations (DADs) and triggered activity (Liu et al., 2006).

The Cardiac Ryanodine Receptor

The most common form of CPVT is familial and inherited in an autosomal dominant fashion, resulting from mutations in the gene encoding the cardiac ryanodine receptor (*RYR2*). Ryanodine receptor is an unusual name for an ion channel, and has an interesting etymology (Yuchi and Van Petegem, 2016). In 1796, Danish botanist Martin Vahl described a new plant that he named *Ryania speciosa*, in honor of John Ryan, a physician who had sent him many plant samples. This plant has insecticidal properties, and when the active molecule ($C_{25}H_{35}NO_9$) was isolated in 1948, it was named "ryanodine." This poison binds with high affinity to the major calcium release channels of the sarcoplasmic reticulum, hence the term "ryanodine receptors." The identification of mutations in *RYR2* underlying CPVT was found nearly simultaneously by two independent groups in 2001 (Priori et al., 2001; Laitinen et al., 2001). Current literature suggests that over half of clinically affected CPVT patients carry a putative pathogenic *RYR2* mutation (Hayashi et al., 2009).

The underlying proarrhythmic molecular mechanism in CPVT is excessive calcium release from the SR, leading to DADs and triggered activity. There are multiple mechanisms by which mutations in *RYR2* can lead to excessive calcium release (Watanabe and Knollmann, 2011). One mechanism involves spontaneous calcium release when SR calcium content reaches a critical level [store-overload-induced calcium release (SOICR)]. Certain mutant RyR2 channels have an increased sensitivity to activation by luminal calcium, and thus a lower threshold for SOICR (Jiang et al., 2004). Another mechanism involves a protein called FKBP12.6 (calstabin 2), which normally binds to RyR2 and stabilizes the channel in the closed state. During exercise, calstabin 2 partially dissociates from the channel, resulting in increased calcium release. Certain CPVT-linked mutations in *RYR2* reduce the affinity of calstabin 2 for RyR2 channels and increase single-channel activity (Wehrens et al., 2003). Another mechanism involves defective interdomain interactions of the RyR2 protein. In this hypothesis, the N-terminal domain and the central domain, common sites for disease-linked mutations, interact with each other, effectively closing the channel in the resting state. A mutation in either domain

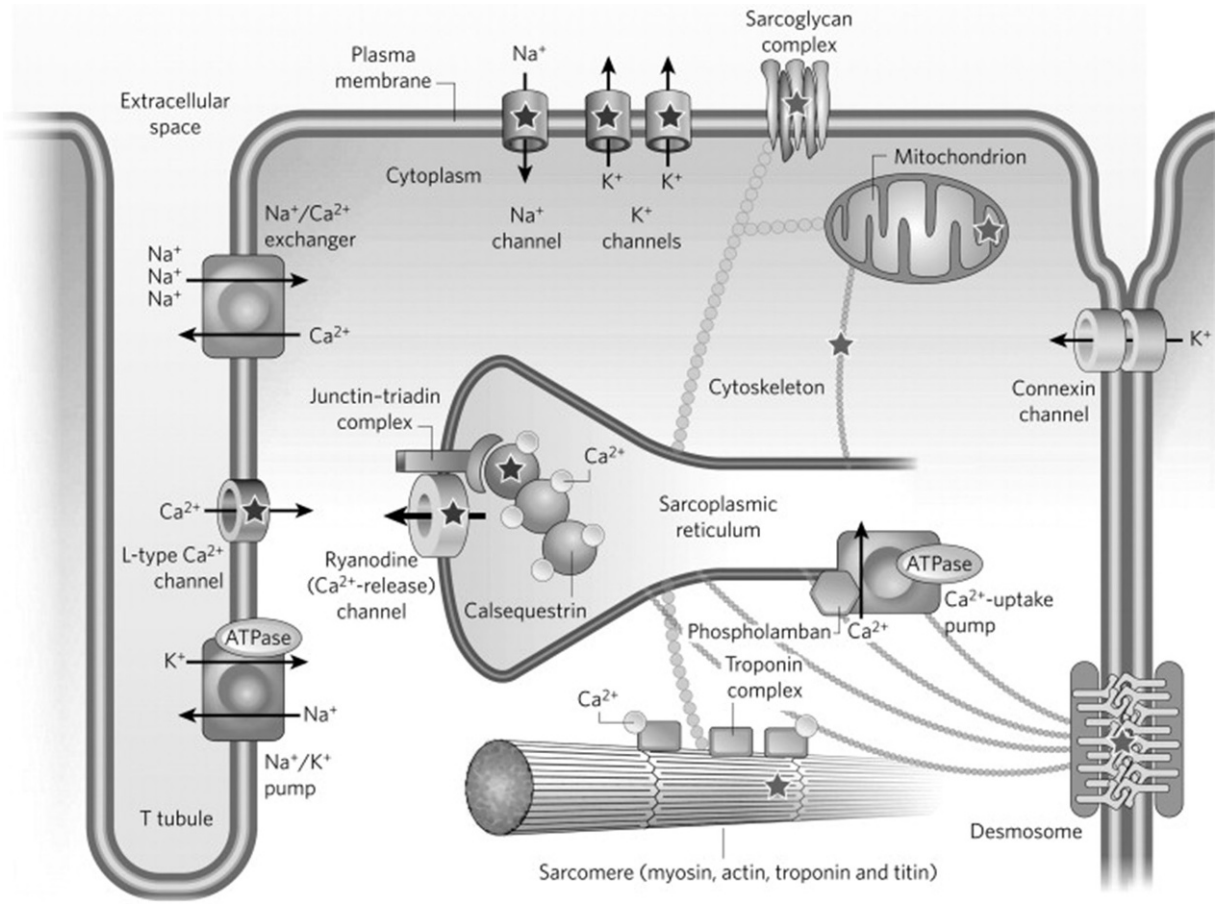

Fig. 3 Protein complexes, cardiomyocyte architecture, and intracellular organelles involved in excitation–contraction coupling. Entry of calcium ions into the cell triggers the release of calcium from the sarcoplasmic reticulum through the RyR2 channel. Calcium then binds to the troponin complex and activates the contractile apparatus (the sarcomere, bottom). Relaxation occurs on removal of calcium from the cytosol via calcium uptake into the sarcoplasmic reticulum where calcium binds to calsequestrin and via calcium transport out the cell by the Na/Ca exchanger. From Watanabe H., Knollmann B.C. (2011). Mechanism underlying catecholaminergic polymorphic ventricular tachycardia and approaches to therapy. *Journal of Electrocardiology* 44, 650–655.

can weaken the interdomain interaction, resulting in domain "unzipping" in the resting state, resulting in activation of the channel and diastolic calcium leak (Suetomi et al., 2011).

Cardiac Calsequestrin

Mutations in the cardiac calsequestrin gene (*CASQ2*) were identified to underlie CPVT in 2001 (Lahat et al., 2001). Cardiac calsequestrin is the major calcium binding protein in the sarcoplasmic reticulum. Classically, *CASQ2*-mediated CPVT is inherited in an autosomal recessive fashion, with disease manifestation in homozygous (or compound heterozygous) individuals, while heterozygous family members appear unaffected (de la Fuente et al., 2008). In some cases, heterozygous family members have been described with a mild phenotype (exercise or catecholamine-induced arrhythmias), and recent work has demonstrated fully penetrant autosomal dominant CPVT from heterozygous *CASQ2* mutations (Postma et al., 2002; Gray et al., 2016). CPVT due to *CASQ2* is reported in several consanguineous families described from one particular geographic region, but it is a relatively uncommon (≈5%) cause of CPVT worldwide. Nevertheless, lack of cardiac calsequestrin in model systems has informed much of the molecular mechanisms underlying CPVT, including some emerging new therapies, as outlined below.

Cardiac Triadin

After a decade with only two identified CPVT genes (*RYR2* and *CASQ2*) mutations in triadin (*TRDN*) were associated with autosomal recessive CPVT in 2012 (Roux-Buisson et al., 2012). The *TRDN* gene undergoes alternative splicing to encode a family of multiple triadin proteins which are expressed in skeletal muscle (TRIadin SKeletal, or Trisk), with one isoform expressed in both cardiac and skeletal muscle (Cardiac Triadin, or CT1/Trisk32). In the initial report, mutations in triadin were identified in 2/97

CPVT probands without mutations in *RYR2* or *CASQ2*, using a candidate gene strategy investigating both triadin and junctin. After the initial report, two other families with autosomal recessive CPVT due to *TRDN* mutations have been reported (Rooryck et al., 2015; Walsh et al., 2016). Most of the reported mutations result in a premature stop or an unstable protein that is degraded, thus it is expected that affected patients have a complete lack of triadin in both cardiac and skeletal muscle. Interestingly, mild skeletal muscle weakness or motor delay has been described in some *TRDN* CPVT patients.

Calmodulin

Mutations in calmodulin have also been associated with autosomal dominant CPVT (Nyegaard et al., 2012). Calmodulin is encoded by three distinct genes (*CALM1* on chromosome 14, *CALM2* on chromosome 2, and *CALM 3* on chromosome 19), but all encode a protein product with the identical amino acid sequence, each of which is expressed in human heart tissue (Crotti et al., 2013). Calmodulin is an essential calcium binding protein that transduces calcium signals in a wide range of biological processes including muscle contraction, by binding to larger proteins and functioning as a local calcium sensor. Initially, a mutation in *CALM1* was identified by linkage analysis in a large CPVT family, and a second CALM1 mutation was identified in an unrelated CPVT proband. Subsequently, mutations in *CALM2* and *CALM3* have been identified in CPVT patients (Makita et al., 2014; Gomez-Hurtado et al., 2016). Mutations in these three genes have also been identified in cases of long QT syndrome (Crotti et al., 2013; Makita et al., 2014; Reed et al., 2015). Calmodulin mutations that cause CPVT promote arrhythmogenic calcium disturbances (Hwang et al., 2014), while those that cause LQTS prevent inactivation of L-type calcium currents (Limpitikul et al., 2014).

TECRL

Recently, homozygous mutations in the trans-2,3enoyl-CoA reductase-like (*TECRL*) gene have been implicated in an arrhythmia syndrome with features overlapping CPVT and LQTS (Devalla et al., 2016). These patients had normal to mild QT prolongation at rest, some with paradoxical QT prolongation during catecholamine infusion, but all had exercise-induced ventricular arrhythmias, more characteristic of CPVT. *TECRL* encodes an endoplasmic reticulum protein expressed predominantly in human heart and skeletal muscle. Investigation of disease-associated mutant *TECRL* in human induced pluripotent stem cell cardiomyocytes (hiPSC-CMs) showed downregulation of RYR2 and CASQ2, and increased propensity for DADs under adrenergic stimulation, as well as mildly prolonged action potential duration. Although it appears to be an uncommon cause of CPVT, *TECRL* could be considered in autosomal recessive CPVT.

Therapy

As patients with CPVT are at risk for syncope, cardiac arrest, and sudden death, therapy is warranted, even in asymptomatic patients. It is recommended that patients with CPVT should limit or avoid competitive sports and strenuous exercise (Priori et al., 2013). Furthermore, beta-blockers (see below) are recommended for all patients with a clinical diagnosis of CPVT. Exercise testing has been shown to provide prognostic information, with persistent ventricular couplets or NSVT portending higher risk of subsequent cardiac events (Hayashi et al., 2009). Additional pharmacologic and/or nonpharmacologic therapies should be considered for patients who continue to have couplets or NSVT on exercise testing despite maximally tolerated nonselective beta-blocker therapy.

Beta-blockers

The benefits of beta-blocker therapy in CPVT have been recognized since the earliest reports (Berg, 1960), and it has been shown that events after diagnosis are significantly reduced by beta-blocker therapy (Hayashi et al., 2009). Currently, nearly all CPVT patients are treated with beta-blockers (Roston et al., 2015). Nadolol, a long-acting nonselective beta-blocker, has been the preferred drug in CPVT, typically at doses of at least 1–2 mg/kg/day. Large cohort studies have suggested a lower event rate with nadolol compared to other beta-blockers (Hayashi et al., 2009). In a study examining the incidence and severity of ventricular arrhythmias during serial exercise tests in 34 CPVT patients, nadolol was superior to metoprolol at equipotent doses (Leren et al., 2016). Of note, exercise-induced arrhythmias during treatment with metoprolol were similar to those observed on no therapy (**Fig. 4**). Current evidence supports using nadolol for CPVT. Where nadolol is unavailable, propranolol has been suggested as the best alternative. While beta-blockers are the mainstay of CPVT pharmacologic therapy, event rates despite beta-blockers remain unacceptably high warranting additional therapy in the subset with persistent ventricular couplets or NSVT on exercise testing (van der Werf et al., 2012).

Calcium Channel Blockers

Excessive calcium release through RyR2 channels, the molecular defect in CPVT, is initiated by calcium influx through the L-type calcium channels. Thus, calcium channel blockers would seem to have a credible role in CPVT therapy. Indeed, prior to 2009, many experts used verapamil in addition to beta-blockers for severely affected CPVT patients. Intravenous verapamil was associated with a decrease in ventricular arrhythmias during exercise testing in six *RYR2* mutation-positive CPVT patients (Swan et al., 2005). Oral

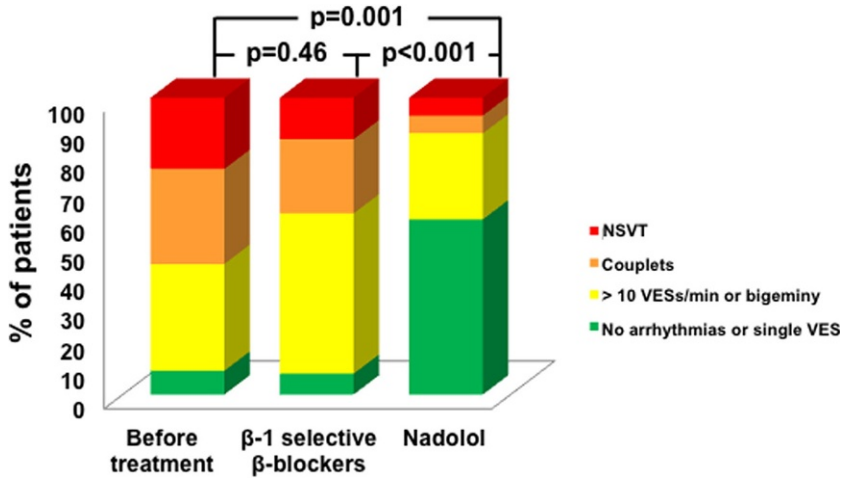

Fig. 4 Incidence and severity of arrhythmias during exercise stress tests in 34 patients with CPVT before the initiation of β-blocker treatment *(left)*, during treatment with β1-selective β-blockers *(middle)*, and during treatment with nadolol *(right)*. Arrhythmias were less frequent and less severe during nadolol treatment than both before the initiation of β-blocker treatment and during β1-selective β-blocker treatment, whereas there was no difference between β1-selective β-blocker treatment and before the initiation of β-blocker treatment. *CPVT*, catecholaminergic polymorphic ventricular tachycardia; *NSVT*, nonsustained ventricular tachycardia; *VES*, ventricular extrasystoles. From Leren I.S., Saberniak J., Majid E., Haland TF2, Edvardsen T., Haugaa K.H. (2016). Nadolol decreases the incidence and severity of ventricular arrhythmias during exercise stress testing compared with β1-selective β-blockers in patients with catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm* 13, 433–40.

verapamil, when added to beta-blocker, was associated with a decrease in exercise-induced arrhythmias in five CPVT patients, compared to beta-blockers alone (Rosso et al., 2007). Unfortunately, in medium-term follow-up, these patients continued to have arrhythmic events (Rosso et al., 2010), an experience mirrored by other groups (van der Werf et al., 2012). Most practitioners now use flecainide (see below) rather than calcium channel blockers as an additional pharmacologic agent in CPVT.

Flecainide

Flecainide is known primarily as a class IC sodium channel blocker, and was initially FDA-approved for the suppression of VT in 1984. In 2009, it was reported that flecainide directly targets the molecular defect in CPVT by blocking RyR2 calcium release (Watanabe et al., 2009). In a calsequestrin knockout mouse model of CPVT, flecainide was highly effective in preventing exercise-induced VT, and flecainide showed striking efficacy in two severely affected human CPVT patients. The precise mechanism underlying flecainide's efficacy in CPVT is controversial; some groups have implicated sodium channel block and/or the sodium/calcium exchanger, rather than direct RyR2 block by flecainide (Smith and MacQuaide, 2015). Nevertheless, the clinical data supporting a protective effect in human CPVT patients is fairly robust. In three retrospective cohort studies, flecainide added to beta-blockers significantly reduced exercise-induced ventricular arrhythmias compared to beta-blockers alone in 29 genotype-positive (primarily *RYR2*) patients, 12 genotype-negative patients, and 10 *CASQ2* homozygous mutation-positive patients (van der Werf et al., 2011; Watanabe et al., 2013; Khoury et al., 2013). In a prospective, placebo-controlled, randomized crossover clinical trial, flecainide + maximally tolerated beta-blockers (primarily nadolol) resulted in significantly reduced ventricular arrhythmias during exercise testing compared to placebo + beta-blockers (Kannankeril et al., 2017). Flecainide doses in the trial consisted of 100–200 mg twice daily (200–400 mg total daily dose), with target trough serum levels >0.5 mcg/mL. In children aged 1–12 years, doses of 100–200 mg/m^2/day and given at 8 h intervals (three times per day) may be required to achieve effective levels (Perry and Garson, 1992). Flecainide is now recommended for CPVT patients who have incomplete arrhythmia suppression on beta-blockers (Priori et al., 2013). Although it is recommended that all CPVT patients take beta-blockers, flecainide as monotherapy may be an option for patients who are truly beta-blocker intolerant (Padfield et al., 2016).

Propafenone

After the discovery that flecainide blocks RyR2 channels, all class I drugs marketed in the United States were studied with respect to RyR2 channel inhibition (Hwang et al., 2011). Only flecainide and propafenone inhibited RyR2 channels, while quinidine, procainamide, disopyramide, lidocaine, and mexiletine had no effect. Propafenone, also known primarily as a Class IC antiarrhythmic drug, is a racemic mixture of S- and R-enantiomers. Each enantiomer has similar potency for blocking sodium channels, while the S-enantiomer has moderate beta-blocking activity (Stoschitzky et al., 1990). R-propafenone was nearly as potent as flecainide in reducing RyR2 open probability, while S-propafenone was less potent. Reduction in exercise-induced VT in a CPVT mouse model was directly related to potency of RyR2 block, rather than sodium channel block (Hwang et al., 2011). Propafenone use was associated with a significant reduction in implantable cardioverter-defibrillator (ICD) discharges and exercise-induced

ventricular arrhythmias in a single patient (in Turkey, where flecainide is not available for clinical use). Data are limited, but propafenone would appear to be a reasonable alternative to flecainide for CPVT in regions of the world where flecainide is unavailable.

Implantable Cardioverter-Defibrillators

The ICD has been used in the management of CPVT, particularly after aborted cardiac arrest or recurrent VT/syncope despite optimal medical therapy. However, ICD use in CPVT is confounded by several factors. First, the target population is young compared to the general ICD population and younger age is associated with a higher rate of complications. Second, the relatively high rate of supraventricular arrhythmias may lead to inappropriate shocks. Third, the catecholamine surge after an ICD shock (appropriate or inappropriate) may trigger VT storm. Indeed, instances of SD despite an ICD have been reported (Sy et al., 2011). Finally, data from two independent cohorts demonstrate that ICD shocks are highly ineffective for treating polymorphic/bidirectional VT in CPVT (Fig. 5) (Miyake et al., 2013; Roses-Noguer et al., 2014). Combined, these studies analyzed 37 CPVT patients with ICD's who received 236 shocks, 138 of which were appropriate (delivered for VT or VF). ICD shocks in CPVT patients were effective (resulting in primary termination) in 62/66 (94%) VF episodes, but only effective in 1/72 (1.4%) VT episodes. Inappropriate shocks were observed in 43% of the combined cohorts. Presently, many experts avoid ICD implantation in CPVT, favoring beta-blockers in combination with flecainide or left cardiac sympathetic denervation (LCSD) as an alternative. When implanted, ICD programming should be tailored with longer detection times and higher detection rates, ideally to treat VF but not VT. This can be difficult if not impossible in some cases.

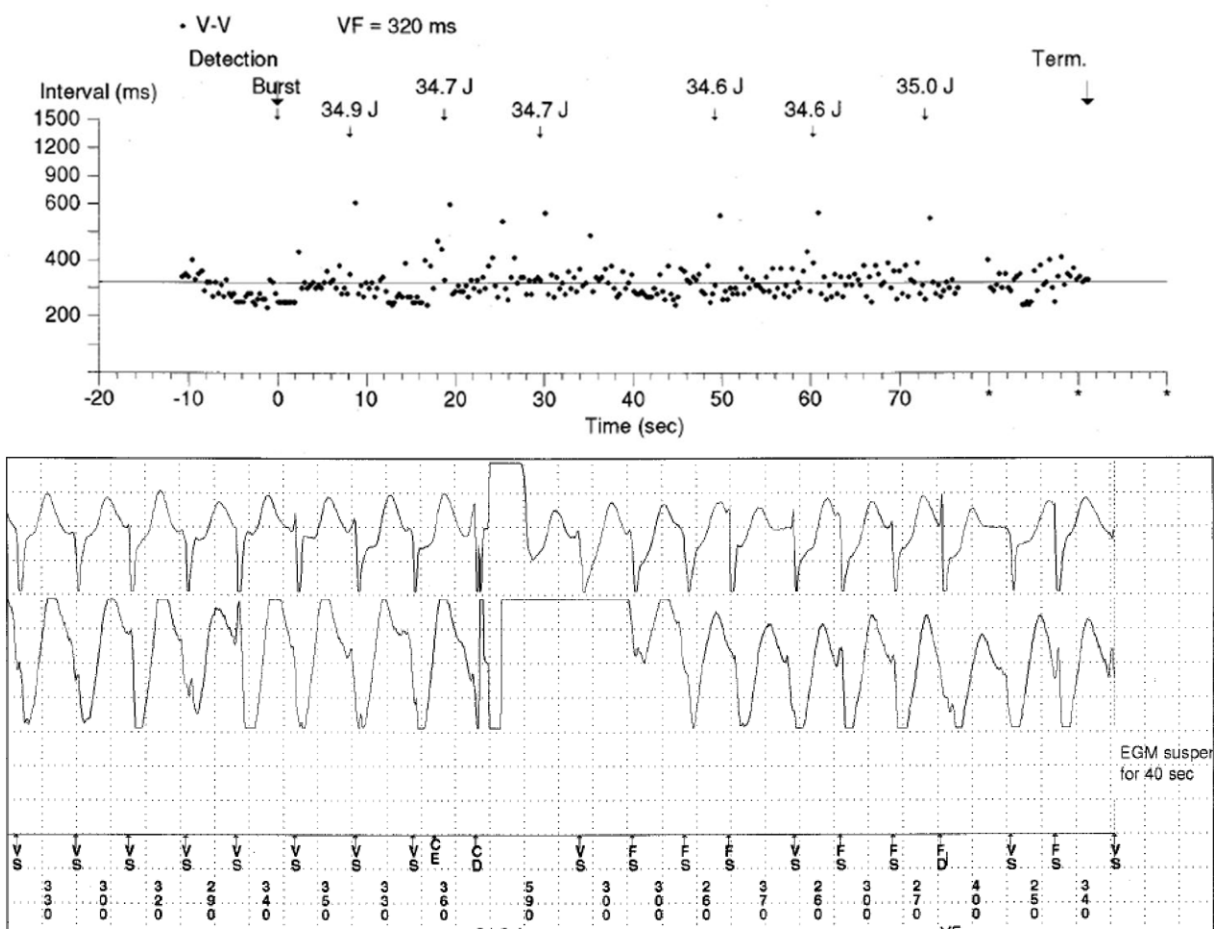

Fig. 5 ICD tracing from a CPVT patient showing ineffective shocks for polymorphic VT. Top panel displays RR interval (ms) on the *Y*-axis and time (s) on the *X*-axis. The RR interval is irregular, and after the programmed 24/32 beats fall below the threshold for tachycardia detection by the ICD, an ineffective burst of antitachycardia pacing (Burst) is delivered, followed by a series of six ineffective shocks (34.9 J, 34.7 J, 34.7 J, 34.6 J, 34.6 J, and 35.0 J delivered). *Bottom* panel displays the near-field *(top)* and far-field *(middle)* electrograms and marker channel *(bottom)* showing polymorphic ventricular tachycardia which is unaffected by the ICD shock. After six shocks, the ICD is not programmed to deliver more therapy, and eventually the VT spontaneously terminates.

Left Cardiac Sympathetic Denervation

LCSD is a surgical procedure which prevents norepinephrine release in the heart and raises the threshold for ventricular fibrillation (Schwartz, 2014). The procedure has evolved from one that required a thoracotomy or supraclavicular incision, to the minimally invasive video-assisted thoracoscopic surgical (VATS) approach. The high thoracic left sympathectomy technique, in which the lower half of the stellate ganglion and the first four or five left thoracic ganglia are removed results in a very low incidence of Horner's syndrome and provides the antifibrillatory effect (Cho, 2016). LCSD in CPVT was first reported in a series of three patients in 2008 (Wilde et al., 2008), followed by other small case series, and one relatively large (63 patients) international report (De Ferrari et al., 2015). The data show a clear reduction, but not complete elimination, of cardiac events post-LCSD in CPVT patients who comprise a relatively high-risk group. LCSD should be considered for CPVT patients with cardiac events despite optimal medical therapy.

Gene Therapy

Preliminary work toward gene therapy for *CASQ2*-mediated CPVT is currently being performed. In 2012, Silvia Priori's group reported successful viral gene transfer of *CASQ2* using an adeno-associated viral vector (AAV9) into neonatal *casq2* knockout mice (Denegri et al., 2012). After 20 weeks, these mice showed normal levels and subcellular localization of calsequestrin and associated proteins, as well as a lack of the typical epinephrine-induced arrhythmias so readily apparent in the *casq2* knockout mouse. Subsequent work from the same group used the AAV9-*CASQ2* construct to deliver normal *casq2* into mice homozygous for the R33Q *casq2* mutation—a model more representative of human CPVT patients (Denegri et al., 2014). A single injection in newborn mice resulted in normalization of calsequestrin protein levels and lack of an arrhythmia phenotype even 1 year after injection. Similar findings were observed when injected into adult mice as well. The lack of an arrhythmia phenotype was observed despite only an AAV9-*casq2* infection rate of only about 40% of myocytes. While there are still several hurdles to overcome, these exciting results open the prospects for gene therapy in *CASQ2*-mediated CPVT.

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Catheter Ablation for Atrial Fibrillation (Methods)

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Outline

Atrial fibrillation (AF) is the most common sustained arrhythmia encountered in clinical practice. The contemporary management of AF is centered on symptomatic improvement, as well as reduction in the AF-associated morbidity and mortality. For many symptomatic patients catheter ablation offers an efficacious alternative to maintaining sinus rhythm when antiarrhythmic drugs have been ineffective, are contra-indicated, or cannot be tolerated. Over the past 15 years, catheter ablation has moved from an "experimental therapy" to the standard of care for the maintenance of sinus rhythm. Unfortunately, while the results of ablation are unequivocally superior to medical therapy, recognized limitations of the contemporary AF ablation procedures have spurred several developments designed to improve the efficacy of the index ablation procedure, while limiting adverse events. The purpose of this review is to discuss the procedural refinements, and technological innovations proposed to improve the outcome of patients undergoing a percutaneous catheter ablation procedure for AF. This article focuses on a high-level review of the methods of a first

procedure for catheter ablation of AF, as it is impossible to provide a detailed discussion of the nuances of each approach to AF ablation in a single review. Moreover, we have not included a discussion on the approach to a repeat AF ablation, or the mapping and ablation of atypical flutter.

Evolution of Current Ablation Strategies and Techniques

Initial attempts at catheter ablation for atrial fibrillation attempted to recreate the surgical maze procedure, which followed the "multiple wavelet hypothesis" of AF (Leitch et al., 1991; Cox et al., 1991). This theory postulated that AF results from the continuous annihilation and regeneration of multiple independent coexisting wavelets that occur simultaneously and propagate randomly throughout the left and right atria. This hypothesis suggested that if the atrium had a sufficient area (or electrical mass) with adequately short refractory periods AF could be initiated and then indefinitely perpetuated. As such, the early attempts at interventional AF treatment aimed to decrease arrhythmia perpetuation by compartmentalizing the atrium into smaller regions incapable of sustaining the critical number of circulating wavelets (i.e., reducing the excitable mass of atrial tissue).

Unfortunately these percutaneous approaches were only marginally successful and carried relatively high complication rates. The discovery in the late 1990s by Haïssaguerre and colleagues that AF was a triggered arrhythmia initiated by rapidly repetitive discharges originating predominantly from the pulmonary veins (PVs) represented a monumental shift in thinking around AF (Haissaguerre et al., 1998). As a result percutaneous procedures designed to isolate these PV triggers were developed. While isolation of the pulmonary veins remains the cornerstone of the invasive management of paroxysmal AF the ablation strategy itself has undergone significant evolution over the past 15–20 years. Initially, the procedure targeted elimination of the focal triggers within the PV however this approach was associated with modest clinical efficacy (frequent PV reconnection), and an elevated risk of complication (PV stenosis). Thereafter the procedure evolved to target the more proximal extensions of the PV myocardial sleeves, whereby the earliest PV potentials were targeted at the tubular ostium of each vein (usually guided by a multipolar, circular catheter). Although this "segmental" or "ostial" isolation resulted in improved outcomes, the recognition that sites of AF initiation and/or maintenance were frequently located within the PV antrum resulted in a shift in ablation strategies to target the more proximal perivenous LA tissue. These early circumferential PV ablation strategies aimed to "compartmentalize" a significant portion of the LA via the creation of wide encircling lines of ablation outside and around the PV antra within the LA body (LACA or WACA). As this approach did not necessarily document electrical isolation of the PVs it was limited by the induction of a high rate of iatrogenic macro reentrant arrhythmias due to residual LA–PV connections. The contemporary AF ablation procedure has evolved to a hybrid of the above approaches, whereby circumferential ablative lesions are placed within the LA myocardium outside of the tubular veins with a goal of electrical pulmonary vein isolation (i.e., bidirectional conduction block documented on a circular mapping catheter placed at the PV ostia). This contemporary approach, as recommended by major heart rhythm societies, therefore targets not only the initiating triggers of AF (the PVs) but also the mass of electrically active LA tissue capable of sustaining the AF perpetuating fibrillatory wavelets (Calkins et al., 2012).

Given the certainty of this endpoint more recent studies have focused on procedural refinements aiming to increase the effectiveness of the lesion set to obtain more durable PVI in paroxysmal AF patients. Specifically, postisolation adenosine testing (to unmask dormant PV conduction or reconnection) and the use of contact force sensing catheters have demonstrated improved clinical outcomes. Likewise, novel technological innovations such as cryoballoon ablation, laser ablation, and multielectrode ablation designed specifically for PVI will continue to evolve in the hopes that alternative energies or delivery mechanisms will lead to improved outcomes.

While the isolation of the PV antra has become the "cornerstone" of all contemporary AF ablation procedures, it may not be sufficient for all patients. In the case of persistent AF, electrical and structural remodeling leads to a preponderance of non-PV triggers as well as a shift in the sites required for AF perpetuation to regions outside the PV–LA junction. Proposed adjunctive ablation strategies include: (1) Linear ablation, which is derived from the surgical Cox MAZE procedure and follows the aforementioned "multiple wavelet hypothesis" for AF; (2) Complex fractionated atrial electrograms (CFAE) ablation, which are local signals during AF that are either at a very short cycle length or fractionated with two or more components. It is postulated that they represent areas of slow conduction, conduction block, or "pivot" points for a local AF perpetuating reentry; (3) "Rotor ablation," which is based on the "localized source hypothesis" that proposes that AF is perpetuated by discrete, organized, and rapid micro reentrant circuits (rotors) or focal impulses that disorganize into fibrillatory waves at their periphery. Several techniques have been proposed to identify these potential pathophysiologic sites including: (a) Shannon entropy analysis of invasive bipolar signal amplitude distribution to differentiate the pivot from surrounding peripheral regions (Ganesan et al., 2013) and (b) simultaneous multielectrode activation analysis using a proprietary 64-electrode catheter and a software algorithm (Focal Impulse and Rotor Modulation, FIRM, RhythmView, Topera, Inc.). With respect to the latter, a spatiotemporal activation analysis of simultaneously recorded electrograms using this panoramic mapping catheter is performed. Using this proprietary technology a digital electroanatomic map is created revealing focal impulses (defined by centrifugal activation isochrones from a point origin) or electrical rotors (defined as sequential isochrones around a center of rotation with waves emanating outwardly) (Ganesan et al., 2013; Cuculich et al., 2010). While the incremental benefit of each of these approaches requires extensive study, it is most likely that the major innovations in the interventional management of more advanced AF will be centered on refining the overall ablation strategy, as well as tailoring the specific approach to the individual patient.

Preablation Management

Patient Selection

In current practice, AF ablation is recommended for patients with symptomatic atrial fibrillation intolerant of, or refractory to ≥ 1 antiarrhythmic drugs. In selective circumstances AF ablation may be considered as a first line therapy in patients who have not trialed antiarrhythmic drug therapy.

Preablation Antiarrhythmic Drug Management

Drug treatment for nonarrhythmic indications is generally continued prior to an AF ablation. In terms of antiarrhythmic drug therapy, there is no consensus with regard to continuation or discontinuation. If the latter approach is chosen then they should be discontinued at least four half-lives prior to the procedure in order to avoid confounding the results of the ablation procedure.

Preablation Anticoagulation Management

An effective anticoagulation therapy is most often required prior to an ablation procedure. The majority of patients undergoing an AF ablation procedure will require preprocedure oral anticoagulation, with the exception of a paroxysmal AF patient with a CHADS Score of 0. All other patients have an indication for continuous anticoagulation owing to elevated stroke risk (CHADS Score ≥ 1) or the need for cardioversion at the time of ablation (persistent AF >48 h prior to ablation). If warfarin is the chosen agent for preprocedure anticoagulation, then current practice would suggest continuation of OAC therapy throughout the procedure (perform the procedure on a therapeutic INR). For patients receiving NOACs there is debate regarding the safety of performing an ablation without interruption. Until clear evidence of safety, most procedures would be performed after NOAC interruption (discontinuation the day prior to the procedure for rivaroxaban, apixaban, and dabigatran, except in the case of dabigatran with an CrCl <50 mL/min in which case the medication would be discontinued 2 days prior).

Preablation Imaging

Transthoracic echocardiogram is usually performed as part of the routine AF management and can be utilized to assess cardiac function, chamber dimension, and to exclude significant valvular pathology. Cardiac CT or MRI imaging can be performed as part of the preprocedure planning in order to define variant pulmonary anatomy, assess PV dimensions and angulation, as well as define atrial scar burden (MR imaging). These cardiac CT or MR images may be integrated with the electroanatomic mapping system. Preablation transesophageal echocardiographic (TEE) imaging may be used to exclude LA thrombus, which is especially useful in those who did not receive adequate preprocedure anticoagulation. If performed in the EP laboratory the TEE (or intracardiac echocardiography) can be utilized to guide transseptal puncture.

Intraprocedural Management

Left Atrial Access

A discussion regarding left atrial access technique is beyond the scope of the current article. In general an AF ablation procedure necessitates 1 or 2 transseptal punctures to facilitate left atrial access for the ablation catheter and the circular mapping catheter. These punctures can be performed with fluoroscopic guidance, intracardiac echocardiographic guidance, or TEE-guidance using standard techniques.

Intraprocedural Anticoagulation

In order to minimize the risk of intraprocedural thromboembolic complication anticoagulation should be administered as soon as left atrial access is obtained. In our practice we administer a bolus of IV unfractionated heparin of 100–130 IU/kg (100 IU/kg for warfarin anticoagulated patients, 120 IU/kg for dabigatran anticoagulated patients, and 130 IU/kg for rivaroxaban and apixaban anticoagulated patients), followed by additional boluses q15–30 min to maintain an activated clotting time (ACT) target of 300–350 s. Alternatively, an infusion of 15–18 IU/kg/h may be employed. The rationale underlying this ACT target is the observation that intravascular sheath thrombus markedly decreased with a target ACT >300 s.

Sheath Management

In our practice the left atrial sheaths are continuously irrigated with heparinized saline at >60 mL/h.

Atrial Fibrillation Ablation—Strategies

At present, multiple approaches have been developed for catheter ablation of patients with paroxysmal and persistent AF. The current techniques of catheter ablation target the elimination of AF initiating triggers and as well as AF maintaining substrate, while

Table 1 Ablation Strategies for Atrial Fibrillation.

Approach	*Techniques*	*Endpoint*
Wide-area or left atrial circumferential ablation (WACA/LACA)	Circumferential point-by-point ablation Multielectrode ablation	Abatement of bipolar voltage by 90% (or <0.05 mV within the encircled area)
PV Isolation Alone	Circumferential point-by-point antral PVI (preferred to ostial or segmental PVI) Multielectrode circumferential PVI (e.g., PVAC, nMARQ) Cryoballoon-based PVI	PV Isolation (bidirectional conduction block)
PVI + defragmentation	Point-by-Point PVI + Complex Fractionated Atrial Electrogram (CFAE) ablation Multielectrode ablation (PVAC + MAAC/MASC)	PVI plus elimination of high-grade CFAE (secondary—AF interruption, AF noninducibility)
PVI + LA Compartmentalization	PVI/LACA + linear left atrial ablation (roof, mitral isthmus) ± cavotricuspid isthmus ● Consider CFAE ± CS isolation if immediate relapse or AF refractory to cardioversion Multielectrode ablation (PVAC + TVAC)	PVI plus bidirectional linear conduction block
PVI + defragmentation + LA Compartmentalization	PVI + CS and SVC isolation followed by CFAE ablation followed by linear ablation (roof, mitral isthmus ± CTI)	PVI plus bidirectional linear conduction block plus sinus rhythm restoration
Scar-based Ablation	Identification and sequestration or elimination of abnormal LA fibrosis	Elimination or isolation of low voltage areas
Rotor Ablation	Ablation at the center of rotation or focal impulse origin	AF termination or ablation time at that source reached 10 min
Non-PV Triggers	Targeted point-by-point ablation of AF initiating triggers	
Autonomic ganglionated plexi ablation	Targeted point-by-point ablation of left atrial gangionated plexi sites	Abolition of vagal reflexes induced by high frequency stimulation

Legend: AF - atrial fibrillation, CFAE - complex fractionated atrial electrograms, CS - coronary sinus, LA - left atrial, MAAC - multi-array ablation catheter, MASC - multi-array septal catheter, PV - pulmonary vein, PVI - pulmonary vein isolation, PVAC - Pulmonary Vein Ablation Catheter, TVAC - tip versatile ablation catheter.

employing the least amount of ablation necessary. The primary endpoint of AF ablation is the completion of the predetermined lesion set, with determination of bidirectional linear block (in order to limit the development of proarrhythmia and late procedural failure). Secondary procedural endpoints include AF termination and/or noninducibility. A summary of the different approaches proposed for catheter ablation of AF, and their procedural endpoints are presented in **Table 1**.

Pulmonary Vein Isolation

Since assuming the dominant ablation strategy for AF, pulmonary vein-based ablation has undergone significant modification over the past 15–20 years. Initially, the procedure targeted elimination of the focal triggers within the PV musculature; however this "segmental" approach was associated with modest clinical efficacy (frequent PV reconnection), and an elevated risk of complication (PV stenosis). Thereafter the procedure evolved to target the more proximal extensions of the PV myocardial sleeves, whereby the earliest PV potentials were targeted at the tubular ostium of each vein. These "ostial" PVI procedures are usually guided by a multipolar, circular mapping catheter (15, 20, or 25 mm fixed diameter, or 15–25 mm variable diamete) positioned at the atrial side of each pulmonary vein ostia. A series of segmental lesions are then created until isolation of the vein can be demonstrated by disappearance of the venous potentials on the circular mapping catheter. Although these "segmental" and "ostial" isolation resulted in improved arrhythmia-free outcomes, the recognition that sites of AF initiation and/or maintenance were frequently located within the PV antrum resulted in a shift in ablation strategies to target the more proximal perivenous LA tissue.

These early circumferential PV ablation strategies aimed to "compartmentalize" a significant portion of the LA via the creation of wide encircling lines of ablation 0.5–2 cm outside and around the PV antra within the LA body. The procedural endpoint for these 3D EAM-guided LACA (left atrial circumferential ablation) or WACA (wide circumferential ablation) procedures was a 90% reduction in local bipolar voltage (or to less than 0.05 mV). As this approach did not necessarily document electrical isolation of the PVs it was limited by the induction of a high rate of iatrogenic macro reentrant arrhythmias due to residual LA–PV connections.

The contemporary AF ablation procedure has evolved to a hybrid of the above approaches, whereby circumferential ablative lesions are placed within the LA myocardium outside of the tubular veins (0.5–2 cm outside the PV ostia), but with a goal of electrical pulmonary vein isolation [bidirectional conduction block consisting of entrance block—the stable absence of conduction from the LA into the PV, and exit block—stable absence of conduction from the PV into the LA]. This contemporary approach, as recommended by major heart rhythm societies, therefore targets not only the initiating triggers of AF (the PVs) but also the mass of electrically active LA tissue capable of sustaining the AF perpetuating fibrillatory wavelets (Calkins et al., 2012).

Waiting Phase

An observation period after completion of PVI may be used to assess and reablate spontaneous recovery of PV-atrial conduction. In recent studies using a waiting period post-PVI, acute PV reconnection was observed in 27% of patients with a waiting period of 20 min (Macle et al., 2015), 33%–48% of patients with a waiting period of 30 min (Rajappan et al., 2008; Wang et al., 2007; Ninomiya et al., 2009), and in 42%–93% of patients with a waiting period of 60 min (Wang et al., 2007; Cheema et al., 2007; Bansch et al., 2013; Kobori et al., 2015). The prevalence of time-induced PV reconnection is most frequent at 30 min, with a significant proportion of patients having further reconnection at 60 min and very few between 60 and 90 min (Yamane et al., 2011). In a retrospective study including patients undergoing a repeat ablation procedure for recurrent AF, ROC curve analysis revealed a strong negative correlation between the observation time after complete PVI during the initial procedure and chronic PV reconnection (Nakamura et al., 2013). This study suggested an optimal cut-off value was 35 min, although the diagnostic accuracy did not seem to be very high (sensitivity 66.9%, specificity 60.6%) (Nakamura et al., 2013). Unfortunately, randomized studies evaluating the utility of a waiting period reported conflicting results. In one study, the success rate of ablation was 60.7% in patients randomized to no observation period, 84.3% in patients assigned to a 30-min, and 86.7% in those assigned to a 60-min observation time post-PV isolation ($p=0.04$) (Wang et al., 2007). Another trial randomly assigned patients to no observation time versus 60 min waiting time post-PV isolation and reported no difference in arrhythmia-free survival between the two groups (Bansch et al., 2013). Based on the available data, monitoring for at least 20 min following initial PVI should be considered. In practice, the main limiting factor for a longer observation period is the impact on the total procedure duration and workflow in the electrophysiology laboratory.

Intraprocedural Assessment of Durable Isolation

Unfortunately, while electrical PVI may be achieved acutely the transient injury induced at the time of index ablation may recover as the acute effects of RF-induced tissue injury resolve, revealing gaps in the initial line of ablation and allowing PV triggers to excite the adjacent LA and induce AF (Kowalski et al., 2012; Ouyang et al., 2005; Verma et al., 2005). As a result several techniques have been proposed to identify regions of incomplete ablation and/or residual gaps within the index ablation line. One such technique is the use of intravenous adenosine (or adenosine triphosphate, ATP) to transiently restore cellular excitability and thus differentiate permanent PV-atrial conduction block from dormant conduction (i.e., viable but latently nonconducting tissue). This is typically achieved by the administration of 12–18 mg adenosine as an IV push to attain one blocked P wave or a sinus pause ≥ 3 s. Sites of dormant conduction, as defined by the re-appearance of PV activity for ≥ 1 beat, are then targeted for ablation until all sites of acute, adenosine-provoked reconnection have been eliminated on sequential adenosine testing (Miyazaki et al., 2012; Matsuo et al., 2007; Kumagai et al., 2007; Macle et al., 2012; Matsuo et al., 2011). This approach was evaluated in the randomized Adenosine Following Pulmonary Vein Isolation to Target Dormant Conduction Elimination (ADVICE) trial, which demonstrated a prevalence of dormant conduction in 53% of patients post-PVI. While the presence of dormant conduction was associated with increased risk of of atrial tachy-arrhythmia recurrence, the elimination of dormant conduction resulted in a 56% relative risk reduction (27% absolute risk reduction, $P<0.0001$) in recurrence of atrial tachy-arrhythmias when compared to those in whom DC was not targeted for ablation.

An alternative strategy is the "pace-capture-guided" approach whereby, after completion of PVI, the antral ablation line encircling the ipsilateral PVs is mapped while pacing at high output from the ablation catheters distal electrode pair (Eitel et al., 2010; Steven et al., 2010). Additional ablation can be performed in areas where local LA capture is identified, with the goal of closure of these residual gaps. While there is a reasonable rationale underlying this latter approach, the evidence base support such a strategy is limited (Eitel et al., 2010; Steven et al., 2010). Specifically a recent randomized study demonstrated that this technique significantly improved arrhythmia-free survival compared to conventional PVI; however, comparable long-term success rates have been observed between an adenosine-guided PVI approach and a pace-capture-guided ablation strategy (Andrade et al., 2013; Steven et al., 2013).

Novel Tools for PVI

While focal point-by-point radiofrequency catheter ablation has shown considerable success, the procedure is time-consuming and highly dependent on operator competency (Calkins et al., 2009). As such, considerable effort has been directed toward developing technologies to achieve safer and more effective PV isolation that is less reliant on operator dexterity.

Contact Force Sensing Catheters

Ablation electrode–tissue contact is important determinant of lesion size and durability. Unfortunately the widely used surrogates of catheter–tissue contact (fluoroscopic imaging of the catheter tip motion, tactile feedback, and local electrogram attenuation, as well as impedance reductions during energy delivery) have poor accuracy when compared to direct assessment. In contrast, contact force sensing technology provides the operator with an accurate real-time quantitative estimation of the contact force between the tip of the catheter and the target myocardium. It is hoped that assessment of contact force will improve efficacy by minimizing the delivery of ineffective lesions with inadequate contact force, as well as decrease the risk of complications. Recent data suggest that

incorporating contact force data into the ablation strategy results in a reduction in procedure time, ablation time, and total energy delivery, with a comparable safety profile to that observed with standard irrigated RF (Reddy et al., 2012; Neuzil et al., 2013). However, the two largest multicenter trials evaluating this technology demonstrated a 1-year success of 68% (TactiCath, TOCCASTAR (Reddy et al., 2015)) and 74% (SmartTouch, SMART-AF(Natale et al., 2014)). In the case of the former the success was no different from that observed with standard noncontact force RF ablation. Interestingly, posthoc analyses of these studies have suggested that the outcomes were improved when the procedure was performed with adequate CF parameters (84% 1-year freedom from AF in the 47% of patients in whom ablation was in the target range $\geq$80% of the time in SMART-AF, and 76% 1-year freedom from AF in the 57% of patients in whom $\geq$90% of the lesions were >10 g in TOCCASTAR). To date, no differences in the incidence of complications have been reported between patients undergoing ablation with the contact force vs. noncontact force sensing RF ablation catheters in randomized (TOCCASTAR) or nonrandomized studies (Reddy et al., 2015; Andrade et al., 2014; Marijon et al., 2014).

Cryoballoon Ablation

The cryoballoon system (Arctic Front; Medtronic, Minneapolis, MN) consists of a steerable 10.5-Fr catheter with distally mounted polyurethane and polyester balloons specifically designed to facilitate PVI. Cryothermal ablation has the advantages of freeze-mediated catheter adhesion ansures catheter stability, which is beneficial when ablating unstable regions such as the ridge between the left atrial appendage and left-sided PVs. Moreover, the lesions resulting from cryothermal ablation have: (1) a well-demarcated and homogeneous border, which is thought to be less arrhythmogenic and may decrease the incidence of post-PVI atrial tachycardia; (2) minimal endocardial surface disruption and are less thrombogenic the lesions produced with RF energy, which may reduce the risk of thromboembolic complications; and (3) a relatively preserved ultrastructural tissue integrity, which should result in a lower acute perforation risk and a lower longer-term incidence of PV (Herrera Siklody et al., 2011; Gaita et al., 2011). Moreover, the use of a large cryoballoon catheter results in a large antral circumferential lesion resulting in both electrical PVI and substantial left atrial substrate modification (mean extent of LA ablation 40.2 $\pm$ 3.9%).

Recently the multicenter, randomized FIRE and ICE trial reported was designed to determine whether cryoballoon ablation was noninferior to RF ablation in symptomatic patients with drug-refractory paroxysmal AF (Kuck et al., 2016). Patients were randomized to cryoballoon ablation (374 patients) vs. irrigated RF ablation (376 patients). The primary efficacy endpoint (documented recurrence of AF > 30s/AT/AFL, AAD prescription, or reablation) occurred in 138 patients in the cryoballoon group and in 143 in the RF group (1-year Kaplan–Meier event rate estimates, 34.6% and 35.9%, respectively; hazard ratio, 0.96; 95% confidence interval [CI], 0.76 to 1.22; $P < 0.001$ for noninferiority). However, the study was not an exclusive comparison of advanced technologies, with the majority of patients receiving noncontact force irrigated RF ablation (284/376 in the RF group) and 90 of 374 patients receiving first generation cryoballoon ablation. Taken together the cumulative experience with this established technology suggests that cryoballoon ablation is efficacious and comparable to RFCA for maintaining sinus rhythm at 1 year in patients with paroxysmal AF undergoing a PV isolation procedure.

Multielectrode Radiofrequency Ablation

Multielectrode catheters are another purpose-built AF ablation toolset that was developed to address the technical difficulties associated with created continuous radiofrequency lesions. The pulmonary vein ablation catheter (PVAC, Medtronic Ablation Frontiers, Carlsbad, CA) is a 9-F deflectable circular multielectrode catheter that enables mapping and circumferential PV ablation (en electrodes with 3-mm interelectrode spacing mounted on a nitinol frame). For persistent AF, two additional catheters, i.e., the multiarray septal catheter (MASC) and the multiarray ablation catheter (MAAC), were developed to facilitate LA mapping and substrate modification for patients with more persistent forms of AF. The accompanying GENius multichannel, duty-cycled RF generator enables the delivery of energy in a unipolar (between electrode and indifferent electrode on the body) or bipolar (between any two adjacent electrodes) configuration to all electrodes simultaneously or individually. Together, the catheters potentially allow the performance of PVI and LA substrate ablation with less effort than standard catheter ablation. Recently the irrigated multi-electrode nMARQ ablation system (Biosense Webster) has been developed. Similar to the PVAC the nMARQ allows multielectrode ablation with any or all of the 10 catheter electrodes. The key difference between the nMARQ system is its integration into the CARTO3 platform allowing full visualization of the catheter loop and electrodes, as well as the fact that the catheter electrode is circumferentially irrigated.

Non-PV Trigger Ablation

Although the PVs are the major site of ectopic AF initiating triggers, recent evidence has demonstrated the importance of nonpulmonary vein triggers. Depending on the population being studied non-PV triggers have been documented in approximately 10%–20% of patients undergoing AF ablation and have been described (in descending order of frequency) in the superior vena cava, the left atrial free wall or left atrial appendage, the coronary sinus ostium, the ligament of Marshall, the crista terminalis or right atrial free wall, the interatrial septum, and rarely a persistent left SVC (Chang et al., 2012; Lin et al., 2012; Chen and Tai, 2005).

Non-PV triggers are found more frequently in female patients, patients with nonparoxysmal AF, patients with more advanced atrial substrate (i.e., dilated or electrically abnormal atrial tissue), and those with more comorbidities (Chang et al., 2012). As the presence of non-PV triggers is independently associated with a worse outcome after catheter ablation (HR 2, 95% CI 1.4–2.85), the elimination of non-PV triggers has been suggested to improve long-term outcomes for patients with persistent AF (Chang et al., 2012; Lin et al., 2012; Dixit et al., 2012). It is important to note only 30% of patients with identified non-PV triggers are able to have them succesfully eliminated due to difficulties with localization; however those in whom the non-PV triggers were eliminated had an improved arrhythmia-free outcome compared to those in whom triggers were idenitifed but could not be eliminated (86% vs. 37%; $P=0.09$).

Non-PV triggers may be noted spontaneously or after the infusion of isoproterenol (1–20 mcg/min). To facilitate the identification of non-PV triggers catheters are typically placed in the left atrial appendage (CMC), the superior vena cava (CMC), the coronary sinus (decapolar catheter), and the inferior right atrium (decapolar). Following the induction of an AF triggering PAC the mapping catheter is displaced toward the trigger origin site, the patient cardioverted and the procedure repeated. Once identified, the procedure for ablation of these non-PV trigger sites is variable depending on the source. Isolation of the superior vena cava or persistent left superior vena cava can be employed in a method similar to PVI, while using great care to avoid inadvertent collateral damage to the phrenic nerve. Ablation of a CS musculature course is performed in a manner similar to other focal sources and does not require CS isolation. Ablation of the ligament of Marshall can often be targeted from the LA endocardium and only rarely requires direct ablation from the CS.

Linear Left Atrial Ablation

Conceptually the rationale underlying linear LA lesions originates from the surgical Cox maze procedure and follows the aforementioned "multiple wavelet hypothesis" (Haissaguerre et al., 1994). Linear lesions are generally deployed across the left atrial roof and/or at the mitral isthmus in an effort to compartmentalize the LA. In doing so they may prevent macro reentrant tachycardias that can occur post-AF ablation, as well as the modify the structures that harbor key "rotor" sites for AF maintenance (e.g., LA posterior wall, roof, mitral annulus, and septum). Unfortunately, the efficacy of linear ablation is dependent on the achievement of complete contiguous and transmural lesions that result in complete conduction block, which can be very difficult to achieve. Thus, whereas complete lines may result in conversion of AF directly to sinus rhythm and prevent recurrent arrhythmias, if incomplete they may be proarrhythmic. There does not appear to be any benefit to the addition of linear lesions in combination with PVI in paroxysmal AF patients (77% 1 year success for PVI + linear ablation versus 73% with PVI alone in one study), although the results are conflicting (Verma, 2011; Hocini et al., 2005). While two small, randomized trials have suggested a slight advantage may be present in persistent AF patients (aggregate arrhythmia-free survival of 54% 1 year success for PVI + linear ablation versus 23% with PVI alone) the large randomized multicenter STAR-AF 2 trial did not demonstrate any clinical benefit (59% freedom from recurrent atrial fibrillation after 18 months with PVI, vs. 46% of patients assigned to pulmonary-vein isolation plus linear ablation; $P=0.15$) (Verma, 2011; Gaita et al., 2008; Verma et al., 2015).

Linear ablation of the left atrial roof is performed for the treatment of a roof dependent perivenous flutter. The line of ablation is extended from the superior margins of the encircling PV lesions using an irrigated RF catheter employing one of three approaches: (1) *Direct extension* from the LSPV to RSPV using a superiorly oriented catheter (perpendicular). The line is extended inferiorly and septally via clockwise rotation and catheter flexion; (2) *Large loop* (parallel tip)—Catheter is looped around the lateral, inferior, septal, and then cranial walls before entering the LSPV ostia. Withdrawal of the catheter drags a line back from the LSPV to the RSPV; (3) *Small loop* (parallel tip)—Catheter is maximally deflected near the left superior PV so that the tip is facing the right PV. Releasing the curve positions the catheter tip adjacent to the right superior PV ostia and allows dragging as the sheath/catheter assembly is pushed toward the LSPV.

Linear ablation of the mitral isthmus is performed for the treatment of perimitral flutter—clockwise or counterclockwise. The line of ablation is extended from the lateral mitral annulus and the left inferior PV ostium. The irrigated RF ablation catheter is introduced through a long sheath and positioned on the lateral mitral annulus (usually at around 4 o'clock in the LAO projection) at an angle between 90 degrees (perpendicular; poor contact) and 180 degrees (parallel; good contact) to the plane of the CS. The lesion is usually commenced at the ventricular aspect of the lateral mitral annulus (targeting an AV ratio of 1:1 or 2:1 on the ablation catheter). The sheath and catheter are rotated clockwise to extend the lesion posteriorly to the ostium of the LIPV (approximately 2 o'clock in LAO). Unfortunately it can be difficult to achieve a transmural lesion across the mitral isthmus due to the myocardial thickness as well as cooling effect of the CS. In approximately 50% of cases ablation will need to be performed within the coronary sinus at a site opposite the endocardial ablation lesions. The catheter should be deflected toward the endocardium to maximize contact with the atrial side of the CS and to reduce the risk of left circumflex artery injury.

Linear ablation of the anterior left atrium is performed for the treatment small anterior reentrant circuits that can occur in persistent or permanent AF. Beginning at the anterior mitral annulus the ablation catheter is progressively withdrawn while the sheath is rotated counterclockwise to increase contact with the anterior and anteroseptal wall of the LA. At the level of the transseptal puncture, clockwise rotation is applied to the sheath, and the ablation catheter is progressively extended to the right PVs or to the roof line. Note that this lesion set may substantially delay activation of the lateral LA and the LA appendage resulting in a significant reduction in the contribution of LA appendage emptying.

Linear Ablation Endpoint

The electrophysiological endpoint of linear lesions is the demonstration of bidirectional line of block, i.e., widely separated local double potentials along the length of the ablation line, with demonstration of activation moving toward the line when pacing from both sides of the line. For the *roof line* the activation pattern should be caudocranial on the posterior wall during left appendage pacing, and the conduction time to the LAA should shorten as the pacing site is moved inferiorly from the ablation line along the posterior wall. For the *mitral isthmus line* the activation sequence of the coronary sinus should invert from distal–proximal to proximal–distal during pacing anterior to the line in the LA close to the mitral annulus. Pacing on the posterior aspect of the line via the CS results in late activation on the opposite side, with progressively shorter activation in the anterior LA (e.g., LAA) as the pacing site is moved toward the septum from distal to proximal CS. For the *anterior left atrium line* pacing at the anterior LA immediately lateral to the ablation line results in activation propagating laterally and posteriorly around the mitral annulus to reach the septal aspect of the LA and the opposite side of the line. As the anterior pacing site is moved further from the ablation line (i.e., toward the lateral LA) the conduction time to the opposite side should correspondingly shorten.

Complex Fractionated Atrial Electrogram Ablation

Complex fractionated atrial electrograms (CFAEs) are local low-voltage (usually <0.25 mV) multipotential signals during AF that are either at a very short cycle length (<120 ms), or are fractionated with two or more components, and/or a continuous perturbation of the baseline. It is postulated that they represent areas of slow conduction, conduction block, or "pivot" points for a local AF perpetuating reentry. The primary goal of the CFAE-guided ablation is complete elimination of these substrate areas, with subsequent conversion to sinus rhythm (or alternatively organization of AF into an atrial tachycardia). Unfortunately, the underlying mechanisms causing CFAEs remain controversial. While fractionation may be recorded close to the core of a rotor, it may also be recorded at the periphery of rotors as well as at sites not actively participating in the AF process (i.e., bystander sites of passive wavelet collision). It is hoped that automated algorithms to facilitate the objective determination of CFAE sites, and/or the coadministration of intravenous ibutilide might facilitate the identification of CFAE sites critical to AF maintenance by eliminating areas of passive atrial activation.

Similar to linear lesion, studies have demonstrated that CFAE ablation as a lone ablation strategy is inadequate for both paroxysmal and persistent AF (Verma et al., 2010; Estner et al., 2008). Likewise, in the paroxysmal AF population there appears to be limited benefit for adjunctive CFAE ablation (three small studies all individually failed to demonstrate significant benefit, however the pooled success was greater in the PVI + CFAE group [83% vs. 72% for PVI alone]) (Verma, 2011; Verma et al., 2010; Deisenhofer et al., 2009; Di Biase et al., 2009). Similar to the results of linear ablation observational and randomized studies have suggested that ablation of CFAE areas in addition to PVI improves the procedural outcome (two positive randomized trials, and one negative randomized trial; pooled single procedure success 51% in the PVI + CFAE group vs. 38% in the PVI alone group); however the large randomized multicenter STAR-AF 2 trial did not demonstrate any clinical benefit (59% freedom from recurrent atrial fibrillation after 18 months with PVI, vs. 49% of patients assigned to pulmonary-vein isolation plus complex electrogram ablation; $P = 0.15$) (Verma, 2011; Verma et al., 2015; Verma et al., 2010; Elayi et al., 2008; Oral et al., 2009).

CFAE Identification

CFAE are typically located in the interatrial septum (57%), PV ostia (40%), LA roof (near appendage; 32%), proximal CS (32%), mitral annulus (20%), and LA posterior wall, with less common sites including the crista terminalis, cavotricuspid isthmus, left atrial appendage. CFAE can be identified using visual inspection, or the aid of an electroanatomic mapping system with automated detection algorithms (**Table 2**). The CARTO (Biosense-Webster) EAM has two automated detection algorithms; (1) The *Interval Confidence Level (ICL)* examines the number of intervals identified between consecutive complexes over a specified duration (i.e., 2.5 s). The more repetitions observed (i.e., higher ICL), the more confident the categorization of CFAE (**Table 3**). (2) *Average Complex Interval (ACI)* examines the average interval between consecutive CFAE complexes (in msec) observed over a specified duration (i.e., 2.5 s). The shorter the interval, the more confident the categorization of CFAE. The NAVx EAM (St. Jude Medical) utilizes a *Fractionation Index*, which examines the averaged time between multiple, discrete deflections ($-dV/dT$; inter-deflection time intervals). The shorter the mean CL of the local EGM during AF the more rapid and fractionated the local EGM.

Table 2 CFAE Severity Gradation by detection algorithm

Fractionation Index	*CARTO—ICL*	*CARTO—ACI*
High grade	High grade ≥17	High grade 50–70
Intermediate	Intermediate 10–17	Intermediate 70–90
Low grade −120 ms	Low grade 3–9	Low grade 90–120

Table 3 CFAE Settings

Parameter	*Rationale*	*NAVx*	*CARTO*
Minimum detection threshold (peak-to-peak sensitivity)	Avoids noise	0.03–0.05 mV	0.05–0.15 mV
Interval Duration			50–120
Internal/external Projection	Avoids collection of EGMs from electrodes that are not in good contact with map shell	4–6 mm	4-6 mm
EGM refractory period	Avoids double-counting of multiphasic EGMs	35–45 ms	
EGM width	Avoids detection of broader, far-field EGMs	15–20 ms	
EGM segment length	Total recording duration at each point Obtains a mean CL for that point	5 s	
Interpolation	Maximum distance between points which will be used to assign average values for a vertex	4–6 mm	

CFAE Ablation and Endpoints

Ablation of CFAE is typically commenced at regions of highest grade CFAE (i.e., shortest intervals). Local ablation is continued until significant attenuation ($\geq$80% decrease) elimination of local EGMs occurs (local voltage $<$0.1 mV). Ablation then shifts to regions with longer grade CFAE until: (1) complete elimination of all areas with CFAE in both atria or (2) conversion of AF to normal sinus rhythm with subsequent noninducibility.

Left Atrial Scar Modification

Experimental models have shown that AF results in progressive structural changes due to electrical and structural remodeling of the atria. These interstitial fibrotic changes promote atrial arrhythmogenesis and increase the likelihood of AF initiation and perpetuation, possibly by acting as anchor sites for micro reentrant circuits or rotors. As such, procedures have evolved to target these regions in an effort to improve ablation outcomes for patients with a predominantly substrate-based arrhythmia (i.e., persistent AF).

To date six studies have evaluated the impact of scar-based ablation for persistent AF (Cutler et al., 2016; Jadidi et al., 2016; Kottkamp et al., 2016; Rolf et al., 2014; Yamaguchi et al., 2016; Yang et al., 2016). These studies are limited by small numbers of patients (31–85 patients) and significant heterogeneity (definition of scar ranging from $<$0.5 to $<$1.5 mV, variable use of multipolar catheters, rhythm during voltage mapping [sinus vs. AF], timing of mapping [before or after PVI], and duration of follow-up). However, given these limitations the success of scar-based ablation was observed to be superior in each of these low-moderate quality nonrandomized studies (27%–57% freedom from recurrent AF in the control arm vs. 69%–80% success in the scar-based ablation arm).

Scar Identification

A multipolar mapping catheter is the preferred modality to create a detailed left atrial endocardial voltage map and identify areas of scarring within the atria. This map should be created in sinus rhythm and should include the acquisition of a minimum of 500 intracardiac points. Normal myocardium is defined as a bipolar endocardial amplitude $>$0.5 mV; dense fibrosis is defined as a voltage $<$0.25 mV; and nontransmural fibrosis is defined as a voltage between 0.25 and 0.5 mV.

Scar Ablation and Endpoints

Ablation should be delivered to sites that demonstrate fibrosis (voltage $\leq$0.5 mV). For regions with an area $<$4 cm^2 the lesion set should be delivered to cover the affected area. For regions with an area $>$4 cm^2 the lesion set should be delivered to encircle the affected area.

Focal Impulse and Rotor Modulation

While the "multiple wavelet hypothesis" proposes that AF perpetuation is maintained by continuous annihilation and regeneration of randomly meandering electrical waves it fails to adequately explain the observed spatial nonuniformities in AF. An alternative "localized source hypothesis" proposes that AF may be perpetuated by organized and fast discrete micro reentrant circuits (rotors) or focal impulses that disorganize into fibrillatory waves at their periphery (i.e., central highly organized activation with surrounding variable propagation and fractionation).

Several techniques have been proposed to identify putative pathophysiologic rotor sites. These include: (1) The use of noninvasive Electrocardiographic Mapping (ECM), which allows global chamber mapping in a single beat through the use of a 252-lead surface ECG combined with computed tomography-scan-based biatrial anatomy (CardioInsight Inc., Cleveland, USA) (Shah et al., 2013); (2) Shannon entropy analysis of invasive bipolar signal amplitude distribution to differentiate the pivot from surrounding peripheral regions (Ganesan et al., 2013); (3) simultaneous multielectrode activation analysis using a novel software mapping system (FIRM, RhythmView, Topera, Inc.). With respect to this latter approach, using a 64-electrode panoramic mapping catheter the fibrillatory wave activity of widely sampled atrial sites is analyzed in the context of rate-dependent atrial repolarization and conduction. Thereafter a spatiotemporal activation analysis of simultaneously recorded electrograms and fibrillatory wave activity is performed, and a subsequent digital electroanatomic FIRM map is created revealing focal impulses (defined by centrifugal activation isochrones from a point origin), or electrical rotors (defined as sequential isochrones around a center of rotation with waves emanating outwardly). Using this approach it has been demonstrated that stable rotors and focal impulses may be the drivers of both paroxysmal and persistent AF in significant number of patients. In the CONFIRM study 2.1 ± 1.0 localized rotors (70%) or focal impulses (30%) were observed in 98 of 101 cases of sustained AF. These were found widespread across locations in the left (76%—including sites outside the pulmonary veins, posterior, inferior, roof, and anterior regions), and in the right atrium (24%—inferolateral, posterior, and septal regions). Ablation achieved AF termination in 20/36 cases (56%) with 4.3 ± 6.3 min of FIRM ablation at the primary source. In the 11/36 cases in whom AF did not terminate, AF slowed by 33 ± 12 ms. By comparison, in the FIRM-blinded group, the acute endpoint was achieved in only 13 of 65 cases with sustained AF (20%) after 43.4 ± 28.0 min of ablation ($p < 0.001$). At a median follow-up of 273 days the FIRM-guided group achieved a significantly greater long-term freedom from AF (82.4% vs. 44.9%). Interestingly, and in contrast to the studies of CFAE ablation, FIRM ablation without PVI has been shown to result in an excellent long-term freedom from recurrent AF suggesting that paroxysmal AF, like persistent AF, may be predominantly a substrate-based disease (PRECISE study).

While the targeting of focal impulses and rotors may represent a new paradigm in the invasive management of AF this approach is not without limitations. While the results of CONFIRM and PRECISE are optimistic, these results have not been widely replicated. Moreover, while FIRM has identified "rotors" in the majority of patients, alternative series have not been as successful (Ganesan et al., 2013; Cuculich et al., 2010). In addition, the temporal stability of proposed rotor sites is variable suggesting that, like CFAE, a proportion of the proposed pathogenic regions represent passive bystanders (Ganesan et al., 2013; Cuculich et al., 2010).

Ablation of Autonomic Ganglionated Plexi

Major left atrial ganglionated have been identified within the perivenous epicardial fat pads. They are located in the Anterior descending LA (anterior to the LAA), Posterolateral LA (along the anterior aspect of the left PVs), Superior LA (posterior wall behind the LSPV and the superior LIPV), Posteromedial LA (posterior wall inferior to the right PVs), Superior RA (anterior RA at the RA–SVC junction), and Posterior RA (posterior to the RA but anterior to the right PVs). They can be identified through high-frequency stimulation (HFS—cycle length 50 ms, 12 V, 1–10 ms pulse width) from the left atrial mapping catheter. This stimulation increases vagal tone, followed by either AF induction or transient bradycardia/AV block. Ablation may then be performed at the sites of positive vagal response to high-frequency stimulation until the response to high-frequency stimulation is eliminated. At this point it is uncertain whether these sites should be independently targeted for ablation, as they often reside within the circumferential pulmonary venous lesions set, or within areas of CFAE. Outcomes have thus been variable.

Postprocedure Management

The immediate postprocedural management consists of supportive treatment, achieving and maintaining hemostasis at the puncture sites, reinitiation and maintenance of anticoagulation, and rhythm monitoring.

Supportive Treatment

Vagal episodes may occur in the early postprocedure period and are typically remedied by fluid infusion and/or atropine. In the event of significant hypotension a hemodynamically significant pericardial tamponade must be excluded. Pericardial chest discomfort may occur during the first few days post ablation. Rarely this is accompanied by a mild self-limited febrile syndrome. If present it should be treated with oral analgesics, and/or anti-inflammatories (ASA, or NSAIDs) with/or without colchicine.

Hemostasis

Hemostasis is usually achieved through manual compression. In some cases percutaneous closure devices have been utilized for large caliber sheaths.

Re-initiation and Maintenance of Anticoagulation

Following the procedure therapeutic anticoagulation should be re-established as soon as possible. For those undergoing procedures without interruption, then OAC therapy should be continued. In those undergoing OAC interruption it is our practice to reinitiate

NOAC therapy 4 h postablation. In those undergoing warfarin interruption then we start LMWH 4 h post procedure and continue this until the INR is therapeutic. For NOACs there is no need for bridging as the anticoagulation effect is reestablished within several hours of the first dose.

Antiarrhythmic Drug Therapy

The use of antiarrhythmic drugs postablation has not been standardized. In some cases the AADs are discontinued prior to ablation and only restarted in the case of symptomatic or sustained recurrence. Others take the approach of prophylactically readministering AAD therapy for the first 8 weeks postablation in order to promote the sustained maintenance of sinus rhythm and facilitate reverse remodeling. As early AF recurrence usually peaks within the first few weeks following PV isolation, the temporary routine administration of antiarrhythmic drugs (AAD) in the immediate postablation period has been proposed as a potential preventive strategy (Oral et al., 2002; Roux et al., 2009). While the true efficacy of this approach is unknown, studies have suggested that transient AAD use does not prevent late arrhythmia relapses (Oral et al., 2002; Leong-Sit et al., 2011). The 5A study randomized 110 consecutive patients with paroxysmal AF undergoing ablation to empirical AAD therapy versus no AAD therapy for the first 6 weeks after RF catheter ablation (Roux et al., 2009). While the authors noted a significantly lower incidence of clinically significant atrial arrhythmias (AF >24 h or associated with severe symptoms), cardioversions, and arrhythmia-related hospitalization during the 6-week treatment period (13% vs. 28% in the AAD vs. non-AAD group; $P<0.05$), there was no difference in the 6-month freedom from recurrent AF (72% vs. 68%; $P=0.84$) (Roux et al., 2009; Leong-Sit et al., 2011). These results were paralleled in the observational study by *Oral et al.* where no difference in the occurrence of ERAT was noted between those discharged on temporary AAD therapy and those without AADs (41% recurrence on AAD [16/39 patients] vs. 32% recurrence off AAD [23/71 patients]) (Oral et al., 2002). As such, a uniform approach of empirical AAD therapy can be expected to reduce morbidity and cost of care early in the post ablation period but does not appear to have an effect on later recurrences. If administered postablation it is our practice to discontinue them 8 week postablation in order to assess for arrhythmia recurrence at the conclusion of the 3-month blanking period.

Conclusion

The invasive approach to AF management is an ever-evolving field, which encompasses several techniques designed to target the triggers and perpetuators of atrial fibrillation.

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Catheter Ablation for Ventricular Tachycardia

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Introduction

Sustained ventricular arrhythmias are typically a manifestation of significant structural heart disease and often associated with a high risk of sudden cardiac death. Although implantable cardioverter defibrillators (ICD) remain the mainstay of therapy for the prevention of sudden cardiac death associated with these arrhythmias, recurrent ventricular arrhythmias can lead to multiple ICD shocks that are painful, decrease quality of life, and cause posttraumatic stress disorder (Zipes et al., 2006; Sweeney, 2010). Spontaneous ventricular tachycardia (VT) and ICD shocks are also associated with an increased risk of death, and progressive heart failure(Sweeney, 2010). Antiarrhythmic drugs, amiodarone in particular, are useful in reducing arrhythmic events, but have limited efficacy for long-term management, particularly for scar-related reentrant arrhythmias. In addition, antiarrhythmic drugs have the potential for significant adverse effects that include negative inotropy, proarrhythmia, and noncardiac organ toxicities that limit their long-term use. Catheter ablation offers an alternative therapy for preventing arrhythmias, and can be life saving when frequent episodes (VT storm) threaten survival.

Less commonly, VT or repetitive premature ventricular contractions (PVC) occur in the absence of structural heart disease. These arrhythmias are often termed "idiopathic" and generally carry a benign prognosis, but this is a diagnosis of exclusion and structural heart disease should be ruled out. Importantly, ventricular arrhythmias can be the initial presentation of early myocardial disease that is not detectable by echocardiography. Imaging with cardiac MRI or ^{18}F-FDG PET (for detection of sarcoid) might be warranted, especially when underlying disease is suggested by an abnormal resting ECG, family history of arrhythmias or sudden death, or unusual VT QRS morphologies. In addition to symptoms, frequent PVCs or VT, even if idiopathic, can lead to worsening of left ventricular contractile function (Lee et al., 2012).

Mapping and Ablation Technologies and Procedural Considerations

Radiofrequency (RF) current remains the most commonly used energy source. Lesion size is limited by coagulum formation on the electrode when electrode temperature exceeds 70°C. Hence, the use of cooled tip catheters that prevent excessive heating at the electrode/tissue interface has gained popularity as they allow greater delivery of RF energy to achieve deeper lesions. Cooling is achieved by fluid circulated internally or by open saline irrigation of the electrode tip (Stevenson et al., 2008). Open irrigation catheters carry the risk of intravascular fluid overload during ablation but may have a smaller risk for thrombus formation. Newer catheters incorporate feedback on contact force and have the potential for improving catheter contact with myocardium, thereby

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potentially facilitating the creation of deeper and more consistent lesions (Ikeda et al., 2014; Hoffmayer and Gerstenfeld, 2015). Despite irrigation, some VTs originate from intramural sources that are difficult to access from the endocardial or epicardial surface. RF energy delivered through extendable, retractable needle electrodes, and via warm saline infusions has been tested in preliminary studies with promising results(Sapp et al., 2013; John et al., 2014). Simultaneous dual site delivery of unipolar RF or bipolar RF delivery across the septum or between the epicardium and endocardium has also been reported to have success in reaching deep circuits in a small number of patients (Iyer et al., 2014).

Cryoenergy delivered via catheters has the advantage of ice formation at the catheter tip with adherence to structures making it useful in situations where catheter stability is difficult to achieve as in ablation of VTs originating from the papillary muscle (Rivera et al., 2015). Experience of this technology in VT ablation is limited.

Electroanatomic mapping systems to create a three-dimensional shell of the chamber of interest improve the appreciation of the arrhythmia substrate to the anatomy and allow for catheter manipulation with limited fluoroscopy. Point by point charting of electrograms can be used to create visual maps of voltage (voltage map) or impulse propagation (activation map). Concomitant use of intracardiac echocardiography aids in the creation of the anatomic shell visualization of valve structures including papillary muscles. It also helps monitor for related complications, such as deteriorating ventricular function and pericardial effusion during the procedure (Rivera et al., 2015).

For endocardial ablations, access to the left ventricle can be obtained either retrogradely via the aortic valve or via a transeptal approach. The latter is the preferred approach for patient with severe peripheral artery disease and is mandatory for left ventricular access in patients with a mechanical aortic valve prosthesis. In a significant number of patients especially those with nonischemic cardiomyopathies and arrhythmogenic right ventricular cardiomyopathy, ventricular arrhythmia may arise from the subepicardium. In such cases, the epicardium can be approached via percutaneous access to the pericardial space. In the absence of prior cardiac surgery or pericarditis, the pericardial space can be accessed via the subxiphoid approach by introducing a needle under fluoroscopy and injection of small amounts of contrast to identify the pericardial space. Once the pericardial space is entered, a guide wire is advanced followed by a sheath for the mapping and ablation catheter. In the absence of pericardial adhesions, catheters can be moved freely on the epicardial surface for mapping (Sosa and Scanavacca, 2005). Prior to any ablation, proximity to the coronary arteries is usually assessed by coronary angiography. Ablation close to a coronary artery (within 5 mm) poses a risk of acute coronary occlusion and should be avoided (Roberts-Thomson et al., 2009). Left phrenic nerve injury is another concern and high output pacing is performed prior to ablation along the anatomical course of the nerve to assess proximity of the nerve. Phrenic nerve protection with an inflatable balloon or instillation of saline into the pericardial space may be needed in some patients. In some cases, a subxiphoid pericardial window created surgically can allow epicardial ablation in patients who have had prior cardiac surgery and have pericardial adhesions (Soejima et al., 2004). If epicardal mapping and ablation is anticipated, it is our preference to obtain access to the pericardial space prior to endocardial access and systemic anticoagulation. If, however, the need for epicardial mapping and ablation becomes apparent after endocardial access, anticoagulation can be reversed prior to pericardial puncture, and reimplemented if there are no bleeding concerns after pericardial access.

The 12-lead ECG tracing of the clinical arrhythmia is helpful in determining potential targets for ablation that can also be assessed during pacemapping (see what follows). While the ECG is often available for patients with idiopathic VT, most patients with structural heart disease will have had their arrhythmia terminated by an ICD. In cases where the ECG of VT is not documented, we often perform noninvasive programmed stimulation using the patient's ICD before induction of general anesthesia (GA) that may suppress inducibility of the arrhythmia. If spontaneous arrhythmia is absent, programmed stimulation and burst pacing with or without isoproterenol infusion or IV epinephrine boluses is utilized to provoke the arrhythmia.

Both conscious sedation and GA can potentially suppress spontaneous or inducible arrhythmia by reducing sympathetic tone. GA, however, has several advantages. Ablation procedures can be complex and can last for several hours, during which the patients are required to remain still. The frequency and depth of breathing plays an important role in determining good catheter contact and stability. Epicardial access is often easier when respiratory motion can be controlled as under GA. In general, we have adopted the use of GA for mapping and ablation of most scar-related VTs and start with conscious sedation for idiopathic PVC/VT.

Approximately two-thirds of patients with structural heart disease who are referred to us for ablation have hemodynamically unstable VT precluding extensive mapping and pacing during induced VT. In addition, patients with severe LV dysfunction can experience hemodynamic deterioration during prolonged procedures under GA. To secure hemodynamic stability during VT ablation, the availability of hemodynamic support is important. Inotropic infusions are used most commonly. An intraaortic balloon pump is the most commonly used assist device. It can improve stability after recovery from induced VT and under anesthesia, but has limited efficacy for maintaining hemodynamic stability during VT. Percutaneous LV assist devices have the advantage of maintaining end-organ perfusion during tachycardia enabling longer periods of mapping(Reddy et al., 2014). However, vascular complications are a significant concern and the overall outcome does not appear to be significantly better. Extracorporeal membrane oxygenation offers biventricular support (4–6 L/min) and can be useful during VT storms associated with hemodynamic instability. Right ventricular function can be critical in the outcomes of VT ablation; patients with severe right ventricular cardiomyopathies can deteriorate hemodynamically due to myocardial stunning even if blood pressure is maintained during induced VT (Ujeyl et al., 2013).

VT in Patients With Structural Heart Disease

The mechanism of ventricular arrhythmia is a major consideration in selection of patients for ablation. Scar-related VT is the most common mechanism of sustained monomorphic VT associated with heart disease. Reentry involving channels formed by surviving muscle bundles within or adjacent to areas of fibrosis is often the cause. Scar that supports reentrant arrhythmias can be due to prior myocardial infarction, cardiomyopathies, inflammation, infiltrative disorders, familial cardiomyopathies, or prior cardiac surgery. A single scar can support multiple reentry circuits giving rise to multiple VT morphologies. Progressive ventricular remodeling can alter the substrate for ventricular arrhythmias over time, such that the frequency and nature of arrhythmias can change. Hence, ablation of VT/VF in structural heart disease is primarily aimed at reducing episodes and ICD shocks and is usually not stand-alone therapy, without an ICD. A small number of patients with structural heart disease will have VT that involves the Purkinje system, most commonly reentry within the bundle branches that can be easily abolished by ablation.

Catheter ablation should be an early consideration for patients with recurrent monomorphic VT that requires therapy to prevent episodes (**Table 1**). For recurrent polymorphic VT, ablation is largely reserved for patients who have an identifiable triggering focus, often recognized from spontaneous PVCs that can be targeted for ablation (Alliot et al., 2009). The exception is the ventricular arrhythmias associated with Brugada syndrome. Low voltage areas are often identified in the anterior free wall and outflow tracts of the right ventricular epicardium and ablation of these areas can abolish the characteristic repolarization changes of the Brugada syndrome and prevent VT and ventricular fibrillation (VF) inducibility (Brugada et al., 2015).

The recurrence rate for sustained monomorphic VT after ablation is high with more than 40% of patients experiencing at least one recurrence over the next 2 years, but the frequency of VT is reduced for most patients (Kuck et al., 2010). Initial studies in patients with drug refractory, recurrent VT showed that catheter ablation reduced VT recurrences in over two-thirds of patients(-Stevenson et al., 2008). The VANISH trial randomized patients with prior MI and recurrent sustained monomorphic VT despite antiarrhythmic drug therapy to ablation or escalation of antiarrhythmic drug therapy. Ablation was superior to escalation of drug therapy in the combined endpoint of death, ICD shocks, or VT storm (Sapp et al., 2016).

In view of the safety and efficacy, patients are no longer required to exhaust antiarrhythmic drug therapy or present with a VT storm (commonly defined as 3 or more VT episodes within 24 h) in order to be considered for ablation. The recognition that VT is associated with adverse outcomes despite the presence of an ICD raises the question as to whether ablation should be performed early, after initial development of an arrhythmia, in patients who are receiving an ICD. The substrate mapping and ablation in sinus rhythm to halt ventricular tachycardia (SMASH-VT) study included patients presenting for the first time with VF, hemodynamically unstable VT, or syncope with inducible VT during invasive electrophysiological testing (Reddy et al., 2007). Ablation to modify the arrhythmia substrate significantly reduced the incidence of appropriate ICD therapy. The ventricular tachycardia ablation in coronary heart disease (VTACH) study included patients with ischemic heart disease, and spontaneous VT who were receiving an ICD (Kuck et al., 2010). Patients randomized to VT ablation prior to ICD implant had a 40% relative reduction in VT recurrences and fewer hospitalizations during follow-up, although approximately half of the patients treated with ablation still had at least one episode of VT. Although these studies were too small to address the effect of VT ablation on mortality, they provide support for a strategy of early intervention in centers with substantial experience in VT ablations.

Table 1 Indications for catheter ablation for ventricular tachycardia

Patients with structural heart disease
1. Symptomatic sustained monomorphic VT that recurs despite antiarrhythmic drug therapy or when drugs are not tolerated or desired
2. Incessant VT or VT storm that is not due to a reversible cause
3. Frequent PVCs, nonsustained VT or VT that is presumed to cause ventricular dysfunction
4. Bundle branch rentry or interfascicular reentrant VTs
5. Recurrent polymorphic VT or VF refractory to antiarrhythmic drugs when there is a suspected trigger that can be targeted for ablation

Patients without structural heart disease
1. Symptomatic monomorphic VT with severe symptoms
2. Monomorphic VT when antiarrhythmic drugs are not effective, not tolerated, or not desired
3. Sustained polymorphic VT or VF that is refractory to antiarrhythmic drugs when there is a suspected trigger that can be targeted for ablation

Catheter ablation for VT is contraindicated
1. Mobile ventricular thrombus in the chamber from which VT originates (epicardial ablation or alcohol ablation can be considered)
2. Asymptomatic PVCs or nonsustained VT that are not causing or contributing to ventricular dysfunction
3. VT due to transient reversible causes, such as torsade de Pointes VT related to prolonged QT

Adapted from reference Alliot, E. M., Stevenson, W. G., Almendral-Garrote, J. M., et al. (2009). EHRA/HRS Expert consensus on catheter ablation of ventricular arrhythmias. Europace, 11, 771–817.

Mapping and Ablation Techniques for Scar-Related VT

A 12-lead ECG offers multiple clues to the VT mechanisms and potential sites of origin. A left bundle branch block configuration in Lead V1 (dominant S wave) suggests an exit site in the right ventricle or the interventricular septum. A dominant R wave or right bundle branch block pattern in V1 indicates a left ventricular exit site. The QRS axis defines VT origins in the vertical plane; an inferiorly directed QRS axis suggests a superior or anterior wall exit, whereas a superiorly directed axis indicated an inferior wall exit. The precordial leads are more indicative of directionality in the horizontal plane. Deep S waves in the apical leads (V3 to V6) indicate earliest activation in the LV apex, whereas prominent R waves in these leads point to a basal origin of activation. Areas of scar, conduction block, and abnormal ventricular anatomy reduce the validity of these general rules. In patients with nonischemic heart disease, subepicardial origin of VT is suggested by wider QRS complexes and delayed initial upstrokes in the precordial leads (Valles et al., 2010). Areas of scar containing the reentrant circuits can extend over several centimeters. With current technology, it is often impossible to use mapping during VT to identify all potential sources of VT within a scar. Hence, other techniques have been developed to identify potential reentrant circuit sites.

Mapping and Ablation During VT

Scar-related VTs usually utilize a central isthmus bordered by anatomical or functional conduction barriers (Stevenson et al., 1993). When VT is hemodynamically stable, mapping during VT can often identify the isthmus where a focal ablation can terminate VT and usually render it noninducible. The location of the exit site from an isthmus is usually along the scar border and determines the QRS morphology of the VT. In the exit recordings during VT demonstrate electrograms that precede the onset of the surface QRS complexes. Early, diastolic electrograms during VT are consistent with sites proximal to the exit in the central or proximal isthmus of the circuit (**Fig. 1**). However, electrogram timing alone is not entirely reliable as a guide to successful ablation sites due to the frequent presence of multiple conduction channels, some of which may be bystanders or blind loops and other abnormal areas. Hence, additional information is sought with pacing maneuvers. Capture during pacing from the tip electrode in contact with the tissue of interest indicates electrically excitable tissue.

During VT, pacing at a rate slightly faster than the VT rate will result in continuous resetting (entrainment) of a reentrant VT. If the pacing site is within the circuit, the interval from the last paced complex to the first return cycle (postpacing interval (PPI) will approximate the tachycardia cycle length (**Fig. 1**)). Ablation is more likely to terminate tachycardia if the PPI is within 30 ms of tachycardia cycle length, but short PPIs can occur at outer loops in a broad reentry circuit where ablation is often not effective. The degree of fusion between paced and VT complexes is helpful in further refining assessment of the pacing site location relative to an isthmus involved in the VT. QRS fusion occurs if the stimulated wavefront alters activation over a large area to change the ECG. If pacing is performed from the isthmus, the stimulated wavefront emerges from the circuit replicating ventricular activation during VT, a process termed entrainment with concealed fusion (ECF) because the fusion between stimulated antidromic and orthodromic

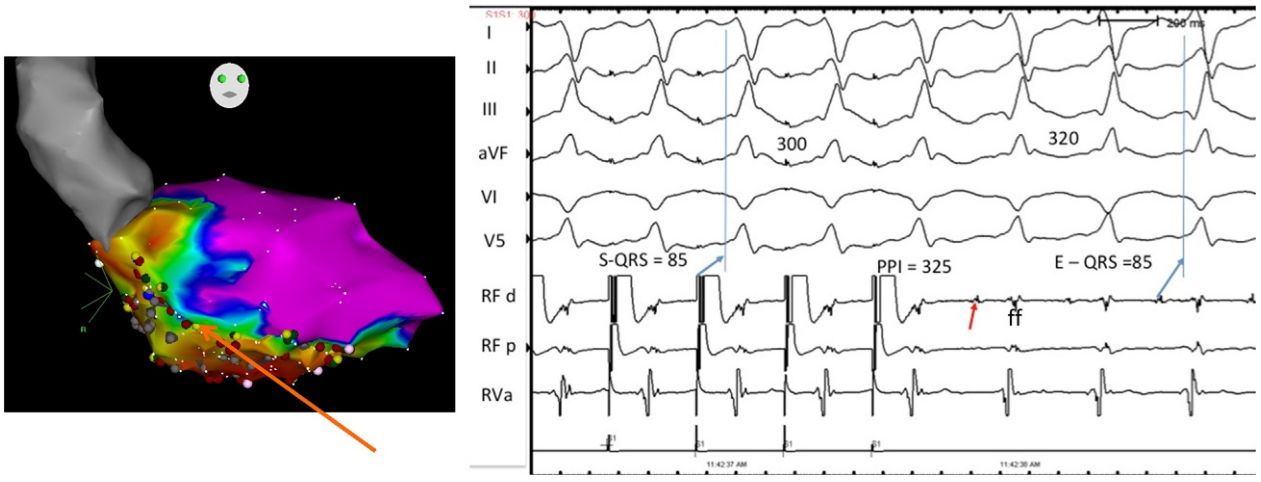

Fig. 1 Entrainment of ventricular tachycardia in a critical isthmus. Left panel—An electroanatomic map of the left ventricle in a 50 year-old male with a prior inferior myocardial infarction and sustained monomorphic VT is shown. Voltage is represented by colors overlaid on an anatomical shell of the chamber in right anterior oblique view. Normal voltage is represented by *purple* and progressively lower voltage is represented by *blue*, *green*, *yellow*, and *orange* with *red* representing scar area. Nonexcitable areas are marked with *gray dots*. Right panel: Pacing is performed at a cycle length of 300 ms during induced VT (CL 320 ms) from the septal border of a large inferior wall scar (*orange arrow* in left panel). The tachycardia is accelerated to the paced cycle length with minimal change in QRS morphology indicating concealed fusion and the site of pacing as a critical isthmus for the VT. On cessation of pacing, the tachycardia continues and the postpacing interval (PPI) approximates the tachycardia cycle length (see text for details). The *red arrow* indicates a mid-diastolic potential that is captured by the pacing; the subsequent adjacent electrogram is a far field signal (ff). The stimulus to QRS duration (S-QRS) equates the mid-diastolic potential to the QRS (E-QRS) duration of 85 ms. Ablation at this site was successful in terminating tachycardia.

wavefronts are confined in or near the circuit and is thereby concealed in the surface ECG (Fig. 1). During ECF, the S-QRS indicates the conduction time between the pacing site and the reentry circuit exit and matches the local electrogram to QRS during VT (Almendral et al., 2013). The stimulus to QRS interval (S-QRS) interval is indicative of the conduction time from the pacing site to the exit. A short S-QRS suggests a stimulus closer to the exit, whereas a long S-QRS is consistent with a more proximal pacing site location, as near the entrance to the isthmus.

Substrate Mapping During Sinus or Paced Rhythm

In patients with hemodynamically unstable VTs or in whom VT is not reliably inducible, the substrate for the arrhythmia can be targeted for ablation during sinus rhythm or a paced rhythm. For substrate mapping, a 3-D electroanatomic mapping system is used to define areas of scar by charting electrogram amplitude in a voltage map. In the ventricular endocardium, peak-to-peak bipolar electrogram voltage less than 1.5 mV is generally used as representative of areas of scarred myocardium (Fig. 1). Areas of slow conduction and late potentials within these areas of low voltage have been correlated with critical isthmus sites for reentry. Pacing from within the scar is useful to identify areas of slow conduction indicated by long stimulus to QRS time (usually >40 ms). Pacemapping in the exit site region will usually replicate the QRS morphology of the VT. A stimulated wavefront that emerges from the exit with a long S-QRS and complexes that are similar to the morphology of the VT is strongly suggestive of an isthmus involved in the reentrant VT (Bogun et al., 2006).

Several strategies for modifying the VT substrate have been described. These include targeting of sites with late potentials or fractionated electrograms that are likely to represent channels that are poorly coupled to the surrounding myocardium, circumferential ablation of the scar area, circumferential core isolation around critical VT circuit elements, or "homogenization" of the scar with extensive overlapping ablation lesions (Kuhne et al., 2010; Jais et al., 2012; de Chillou et al., 2014; Vergara et al., 2012; Tilz et al., 2014; Tzou et al., 2015; Di Biase et al., 2012; Berruezo et al., 2015). However, the optimal strategy resulting in effective ablation while minimizing unnecessary RF application and procedure time is unclear. Anatomically ablating unexcitable scar might not be necessary and abnormal electrograms may arise from tissue remote from the ablation catheter (Tung et al., 2013; Baldinger et al., 2015).

At our center, substrate modification of the scar is performed targeting regions of slow conduction evident as late or fractionated potentials. We aim for sites where pacemapping approximates the morphology of an induced clinical VT and has a stimulus to QRS delay >40 ms (Fig. 1). Ablation is usually performed with a contact-sensing catheter aiming for 10–20 g of contact force and impedance drop of 10–15 Ω during ablation. As an acute endpoint we aim to achieve noninducibility of all sustained monomorphic VT by rendering the whole scar or scar areas felt to be involved in VT circuits unexcitable to pacing (10 mA at 2 ms pulse width) or to disconnect them from healthy myocardium.

Epicardial Approach to VT Ablation

In approximately 15% of patients with VT who are referred for ablation, circuits are found to be intramural or subepicardial and not accessible with an endocardial catheter (Sacher et al., 2010). Epicardial ablation has become an essential component of ablation strategies especially for VT due to nonischemic cardiomyopathies. In ischemic heart disease, epicardial ablation is usually performed after failed endocardial ablation. An initial endo-epicardial approach can be beneficial in selected patients.

The presence of endocardial or epicardial scar can sometimes be suspected based on analysis of voltage maps created from endocardial unipolar electrograms. Unipolar signal amplitude of less than <8.3 mV may identify potential intramural or epicardial substrate (Sacher et al., 2010; Hutchinson et al., 2011). Once access to the pericardial space is obtained as described (see above), the approach is generally similar to endocardial mapping techniques. Epicardial fat can insulate the myocardium and overall, voltages tend to be lower, especially in close vicinity to large epicardial vessels and valve structures. Bipolar electrogram voltages for defining scar on the epicardium are similar to the endocardial EGM although an abnormal epicardial bipolar voltage of <1.0 mV may be more specific than 1.5 mV (Cano et al., 2009).

Transcoronary Ethanol Ablation

Deep intramural VT substrates can be difficult to interrupt permanently with endocardial, epicardial, or even simultaneous dual site ablations. Transcoronary ethanol ablation (TCEA) can be helpful in such situations (Yoshida et al., 2011). Success of TCEA depends on the presence of a patent vessel supplying the area of interest. Once a suitable branch is identified, its relationship to a critical portion of the VT circuit has to be established by observance of termination of VT with injection of iced saline or by occlusion of the branch by balloon inflation. Then, 1 cm^3 of absolute alcohol is injected distally into the branch after balloon occlusion to prevent reflux into other branches. In a series from our center, acute clinical success was obtained in 56% of patients; all VT recurrences were prevented in 36% and improved arrhythmia control was achieved in an additional 27% (Tokuda et al., 2011a). Complications include heart block and extension of infarction with further deterioration of LV function. However, in patients with intractable VT unresponsive to traditional ablation techniques, this approach can be life saving.

Other VTs

Purkinje System-Mediated Ventricular Arrhythmias

The Purkinje system can be involved in VT in approximately 10% of patients with structural heart disease and recurrent arrhythmias (Tokuda et al., 2011a). VT may be due to catecholamine sensitive automaticity or reentry involving the Purkinje system. Monomorphic PVCs, usually from the Purkinje system, have been shown to be a trigger for initiation of VF in rare patients with idiopathic VF and no recognized structural heart disease as well as in patients with various forms of cardiomyopathy including amyloid cardiomyopathy (Lopera et al., 2004; Haissaguerre et al., 2002; Bansch et al., 2003). There are also reports of successful catheter ablation of inciting PVCs in electrical storms associated with the long QT syndrome and the Brugada syndrome (Bansch et al., 2003). Suppression of recurrent VF can be achieved with catheter ablation of the local Purkinje network origin of the triggering PVCs.

Bundle Branch Reentry

Sustained monomorphic VT may be due to reentry between bundle branches (Tan et al., 2012). Usually, the circulating wavefront propagates up the left bundle and antegrade via the right bundle resulting in VT with typical LBBB pattern. This VT can be entrained from the RV apex with postpacing intervals approximating the tachycardia cycle length. A constant relationship of the His deflection preceding the QRS is seen, usually with any oscillation in the H–H interval preceding V–V cycle length alteration. Less commonly, the circuit revolves up the right bundle and down the left producing a typical RBBB type VT. Ablation of either of the bundle branches will lead to termination of the VT; ablation of the right bundle is easier and preferable to avoid induction of ventricular dysynchrony from LBBB. Most patients have evidence for conduction system disease and a third of patients will develop heart block requiring pacing support. Many patients also have other scar-related VTs requiring additional therapy.

Idiopathic Ventricular Tachycardia

Idiopathic VT, a diagnosis made after exclusion of significant structural heart disease, is usually due to focal automaticity, or reentry between fascicular bundles. Focal automaticity often results in isolated or repetitive monomorphic PVCs or VT. Catheter ablation, when successful, is often curative in these patients.

Outflow Tract VT

Approximately 80% of outflow tract VTs arise from the RV outflow tract, the majority from an area adjacent to pulmonary valve or within the proximal pulmonary artery musculature (Alliot et al., 2009; Blanck et al., 1993). VT from the RV outflow tract has a left bundle branch block pattern with transition in the precordial leads at V3 or V4. A free wall (rightward) focus is suggested by wider QRS complexes (>140 ms) and notching in the inferior leads. A more leftward focus produces deeper S waves in leads 1 and aVL, whereas a lower focus in the RV closer to the His bundle is indicated by positive QRS in aVL (Alliot et al., 2009). Precise mapping for ablation requires inducible VT or PVCs to allow activation mapping. Earliest activation at successful ablation sites precedes the surface QRS by 15–45 ms. Bipolar electrograms often show sharp rapid deflections and unipolar recordings demonstrate a QS pattern. Pacemapping to identify the site where pacing reproduces the VT QRS can be effective in guiding ablation when the arrhythmia is not present for activation mapping; but it should be recognized that exact pacemaps can sometimes be generated from sites within 4–5 mm of earliest activation (Liu et al., 2014). RF application often induces acceleration of VT before complete suppression and noninducibility of the arrhythmia.

Less frequently, outflow tract VTs originate from the LV outflow tract. These include sites in the aortic sinuses in close relation to the right and left coronary cusps or the commissure between these cusps. The noncoronary aortic sinus being in proximity to the atrial musculature is rarely the origin of these arrhythmias. Other LV outflow sites of origin are the LV summit, the region of aortomitral continuity or LV epicardium often clustered around the course of the great cardiac vein or anterior interventricular branch of the coronary sinus. QRS complexes demonstrate an inferior axis but show prominent R waves in V1 or V2. Proximity to coronary arteries often limits application of RF energy in these regions (Azegami et al., 2005; Hachiya et al., 2002).

Aortic cusp VTs emanate from extensions of the ventricular myocardium above the aortic annulus. These foci are approached from the left or right sinus of Valsalva and defined by early activation rather than pacemapping (Hachiya et al., 2002). Typically, a double component electrogram is seen in sinus rhythm due to late activation of this muscle bundle. During PVCs or VT, the second component becomes early preceding the QRS by 30–40 ms. Ablation of these targets requires that the origin of the coronary arteries be clearly defined and a safe distance between the ablation site and the arteries (>4 mm) established. Intracardiac echocardiography is useful to define the origins of the coronary arteries and in conjunction with an electroanatomic mapping system, to define the relative locations for safe ablation. Coronary angiography may be necessary for precise definition.

It is important to recognize that the electrocardiographic morphology of PVCs or VT may not be accurate in predicting the location of the initiating focus. A systemic approach is essential. In our laboratory, we begin with systematic mapping starting with the RVOT, and progress sequentially to the great cardiac vein, aortic root, and LV outflow tract including the aortomitral continuity,

and in selected cases, the epicardium based on clues from the ECG and initial mapping. In markedly symptomatic patients or those with tachycardia-induced cardiomyopathy, we have, in rare cases, utilized a surgical approach using cryoablation after mobilizing coronary vessels away from the area of interest (Lerman, 2015).

VT Arising From the Tricuspid Annulus and RV Body

In a recent report of 278 patients undergoing ablation for idiopathic PVCs or VT, 29 (10%) had VT from the lower RV body with half of them arising from regions within 2 cm of the tricuspid valve annulus (Choi et al., 2015). All had LBBB morphology. Ablation was effective in preventing long-term recurrence of PVCs or VT in 80% of patients. It is particularly important to exclude arrhythmogenic RV cardiomyopathy in such patients; voltage maps of the right ventricle can be helpful in excluding endocardial areas of RV scar, but epicardial areas of scar can escape detection during endocardial mapping.

Mitral Annulus VT

Focal VTs from the region of the mitral annulus accounted for 5% of idiopathic VTs in one series(Van Herendael et al., 2011). Areas of the valve annulus included the anteroseptal region in 58%, and posterior or posteroseptal in the remaining. Similar to aortic cusp VT, a delayed potential is observed during sinus rhythm at the valve annulus. During VT, this potential preceded the QRS by as early as 70 ms. The possibility of a remnant of the atrioventricular ring of conduction tissue has been suggested as the potential mechanism for these annular VTs. Endocardial ablation is usually successful in suppressing VT; occasionally, an approach via the coronary sinus may be necessary.

Interfascicular VT

Belhassen and coworkers demonstrated that a RBBB, left axis VT occurring in patients with structurally normal hearts could be terminated with intravenous verapamil (Tada et al., 2005). This VT typically involves the posterior fascicle in the left ventricular septum and comprises the majority of idiopathic LV VTs. Reentry involving, in part, the fascicle of the left bundle branch can be demonstrated by multielectrode mapping that often identifies the circuit along the inferoseptal aspect of the LV. Two other forms of verapamil sensitive VTs have been described; a left anterior fascicular VT with RBBB right-axis deviation and an upper septal fascicular VT with a narrow QRS configuration and a normal or right-axis deviation(Belhassen et al., 1981). For the typical posterior fascicular VT, RF ablation is usually directed at the anterograde Purkinje potentials more apically rather than proximally to avoid injury to the left bundle. Barotrauma from catheter manipulation can terminate tachycardia and render them noninducible. Empiric lines of ablation along the region may be successful in preventing recurrence.

Papillary Muscle VT

Idiopathic VT arising from an LV papillary muscle is a distinct subgroup but can demonstrate QRS morphology similar to fascicular VT(Yamada et al., 2010). The arrhythmias can be exercise induced and catecholamine sensitive, but most commonly present as PVCs rather than sustained VT. They appear to have a focal automatic mechanism. Differentiation from fascicular VT is based on absence of early Purkinje potentials, spontaneous variations in QRS morphology, and early activation located in the region of the papillary muscle identified by echocardiography. Variations in QRS morphology can be subtle and may be due to preferential conduction to different exit sites or multiple regions of origin(Yamada et al., 2010). Ablation is technically challenging due to unstable catheter contact with the contracting papillary muscle; the use of intracardiac echocardiography is helpful for visualization of catheter positioning. The use of a cryo-catheter for better catheter contact with the papillary muscle has been reported to have a higher success rate (see above) (Rivera et al., 2015).

Outcomes of Ablation

Outcomes vary depending on the presence or absence of structural heart disease. For VT associated with structural heart disease, ablation typically aims at palliation to reduce recurrent arrhythmias and prevent defibrillator shocks. Hence, interpretation of results of ablation is often clouded by the presence of inducible VTs that have not been seen to occur spontaneously and that are not targeted by ablation. The target VT is rendered acutely noninducible in 70%–90% of patients (Alliot et al., 2009; Yamada et al., 2010). The success rate of abolishing all inducible VTs is significantly lower (approximately 50%) (Yamada et al., 2010). A meta-analysis of four randomized and one observational study of VT ablation for structural heart disease demonstrated a significant 38% reduction in VT recurrence compared with medical therapy (Tanner et al., 2010). In addition, adjunctive catheter ablation showed a trend toward reduction in electrical storms provoking multiple ICD shocks. Ablation did not influence mortality in this patient population; over a mean follow up of approximately 2 years, 12% in the ablation group and 14% in medical therapy group died.

Even after acutely successful VT ablation, 30%–50% of the patients may subsequently have a recurrence, although the frequency of VT episodes is substantially reduced for the majority (Stevenson et al., 2008; Yamada et al., 2010). Patients should be aware of the need for repeat ablations and/or the continued use of antiarrhythmic drugs and to temper expectations. In patients with VT storm,

catheter ablation can be life saving (Mallidi et al., 2011). Patients who have recurrent VT after ablation are at greater risk for death than those whose VT is controlled.

Success rate for ablation of idiopathic VT is higher although, when sites other than the right ventricular outflow tract or aortic sinuses are involved, these arrhythmias can be difficult to localize for successful ablation. When success from ablation is defined as at least an 80% reduction in ectopic activity, rates are lower than previously published. In a recent multicenter retrospective analysis of 1185 patients undergoing ablations at 8 specialized centers, acute overall procedural success was 84%. However, when these patients were followed with postprocedure Holter monitoring, success rate without antiarrhythmic drugs was 71% (Bansch et al., 2003). Predictors of success were RVOT origin (82% success), a nonepicardial focus, and absence of multiple morphologies. Repeat procedures were required in 22%, most commonly for arrhythmias of epicardial origin that required a mean of 1.6 procedures. Another common reason for failure is the inability to induce the arrhythmia for adequate mapping.

Risks and Complications in VT Ablation

Catheter ablation of VT can be challenging but is generally safe in large volume centers. Complications occur in approximately 6% of patients, most related to vascular access. Procedure mortality is less than 1% (Latchamsetty et al., 2015; Tung et al., 2015). Cardiac tamponade occurs in about 1% of patients (Bohnen et al., 2011). The majority of ablation-related mortality is due to uncontrolled ventricular arrhythmia and associated heart failure.

Planning for VT ablation is crucial in preventing complications. Patients with severe LV dysfunction can have major hemodynamic consequences following prolonged procedures. The risk of hemodynamic deterioration can be reduced by optimization of heart failure therapy prior to the procedure, intraprocedural monitoring of filling pressures, hemodynamic support if necessary, attention to sedation and anesthesia management, and careful attention to fluid management. Preprocedure echocardiography should be routine to exclude intracardiac thrombus in patients with structural heart disease who are undergoing LV mapping and attention to anticoagulation is mandatory to preventing thromboembolic complications. The HRS/ EHRA VT consensus report recommend a target ACT level of ≥ 250 s during procedures involving the LV (Alliot et al., 2009). Anticoagulation with warfarin for 6 weeks is often employed in cases with extensive ablation, but there are no data evaluating warfarin or aspirin. Limiting RF power with attention to reducing power if marked impedance falls are observed during ablation in areas vulnerable to rupture such as RV free wall can potentially avoid "steam pops" that are a cause of cardiac tamponade(Bohnen et al., 2011; Tokuda et al., 2011b). Prompt cardiac surgery can be lifesaving in event of cardiac perforation and onsite surgical backup is an important safeguard (Bohnen et al., 2011). In cases of epicardial access, a safe distance from epicardial coronary arteries and the phrenic nerve has to be established by angiography and high output pacing, respectively, prior to ablation.

Summary

Catheter ablation is an effective therapy for reducing VT recurrence in patients with structural heart disease and often curative in idiopathic VT. However, the procedure can be complex especially when patients are hemodynamically unstable or in VT storm. It is essential to plan in advance with the team participating in the procedure. Careful planning and knowledge of the various techniques, approaches, and management of complications are the key components for successful VT ablation.

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Cellular Sinoatrial Node and Atrioventricular Node Activity in the Heart

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Introduction

In order for blood to be delivered properly to the tissues of the body, the heart has to contract in an organized and coordinated fashion. The mammalian heart consists of both contractile (i.e., "working") cardiomyocytes (located in the atria and ventricles) as well as specialized pacemaker and conduction system cells that demonstrate automaticity (present at specific locations within the heart). The precise activity of the specialized cell types demonstrating automaticity in the heart results in the coordinated excitation of the contractile cardiomyocytes in the atria and ventricles, thereby ensuring proper pump function of the heart.

The components of the heart that demonstrate automaticity include the sinoatrial node (SAN), the atrioventricular node (AVN), and the His-Purkinje network (**Fig. 1**). Myocytes in each of these locations are capable of generating spontaneous action potentials (APs). Under normal conditions, the SAN demonstrates the highest rate of intrinsic spontaneous activity (and hence is normally the primary pacemaker of the heart). The AVN has a lower rate of intrinsic spontaneous activity and normally functions to transmit electrical signals (i.e., APs) from the atria to the ventricles. The His-Purkinje network, located in the ventricles, demonstrates the lowest rate of intrinsic spontaneous activity and is responsible for the rapid delivery of electrical signals to the working ventricular myocardium. Abnormal activity in any of these specialized components of the heart can result in cardiac arrhythmia.

Anatomy of the SAN and AVN

The SAN is a small, condensed area of tissue located subendocardially, adjacent to the cristae terminalis, and within the intercaval region of the right atrium (Keith and Flack, 1907; Boyett et al., 2003; Dobrzynski et al., 2007; Mangoni and Nargeot, 2008; Liu et al., 2007) (**Fig. 1**). Developmentally, the SAN and AVN are formed from specific populations of progenitor cells and are regulated by specific transcriptional pathways that result in the specification and differentiation of the SAN and AVN. The reader is referred to excellent reviews on the topic of patterning of the SAN and cardiac conduction system for additional information on these topics (Christoffels et al., 2010; van Weerd and Christoffels, 2016; Vedantham, 2015).

SAN myocytes are smaller than atrial myocytes and exist in a number of morphologies including spindle shaped, elongated spindle shaped, or spider cells (Anderson et al., 2009; Boyett et al., 2000; Mangoni and Nargeot, 2001). The SAN is a heterogeneous structure that can be divided into the SAN center, which most frequently contains the leading pacemaker site, and SAN periphery

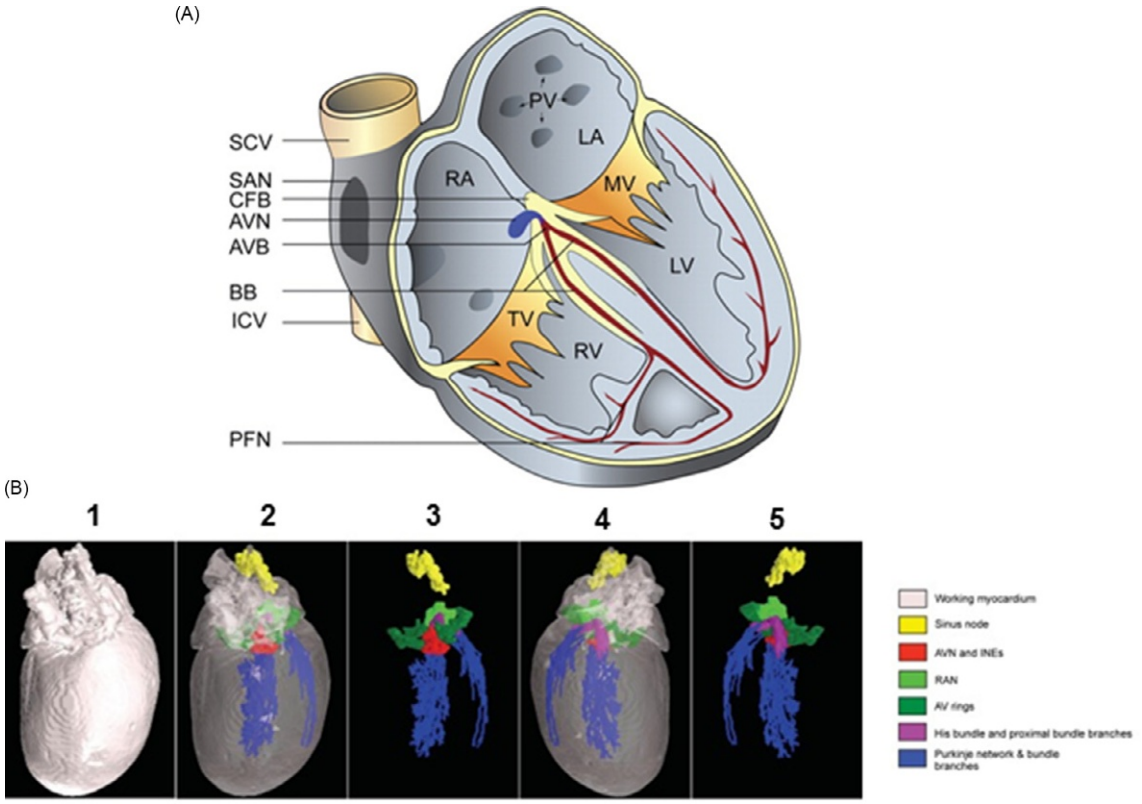

Fig. 1 (A) Schematic illustration of the heart showing the location of the sinoatrial node (SAN) and the components of the cardiac conduction system including the atrioventricular node (AVN), the atrioventricular bundle (AVB), the bundle branches (BB), and the Purkinje fiber network (PFN). LA, left atrium; RA, right atrium; SCV, superior vena cava; IVC, inferior vena cava; CFB, central fibrous body; PV, pulmonary vein; TV, tricuspid valve; MV, mitral valve; RV, right ventricle; LV, left ventricle. (B) The three-dimensional model of the SAN and cardiac conduction system including (1) a model of the heart from the dorsal epicardial surface, (2) a transparent model of the heart with a view of the sinoatrial node and cardiac conduction system from the dorsal surface, (3) a model of the SAN and cardiac conduction system in isolation as viewed from the dorsal surface, (4) a transparent model of the heart with a view of the sinoatrial node and cardiac conduction system from the ventral surface, and (5) a model of the SAN and cardiac conduction system in isolation as viewed from the ventral surface. (A) Reproduced with permission (Mangoni, M. E. and Nargeot, J. (2008). Genesis and regulation of the heart automaticity. Physiological Reviews 88, 919–982). (B) Modified and reproduced with permission (Atkinson, A.J., Logantha, S.J., Hao, G., Yanni, J., Fedorenko, O., Sinha, A., Gilbert, S.H., Benson, A.P., Buckley, D.L., Anderson, R.H., Boyett, M.R. and Dobrzynski, H. (2013). Functional, anatomical, and molecular investigation of the cardiac conduction system and arrhythmogenic atrioventricular ring tissue in the rat heart. Journal of the American Heart Association 2, e000246).

(Dobrzynski et al., 2007). SAN myocytes in the center are smaller compared with those in the periphery, and have distinct molecular and electrophysiological properties that will be discussed further throughout this chapter.

There is considerable variability in the structure of the SAN between different species. In the human and canine heart, the SAN is commonly "cigar shaped" with the head located lateral to the crista terminalis near the opening of the superior vena cava and a tail that extends toward the opening of the inferior vena cava to varying extents. In the rabbit heart, the SAN spans the region between the superior and inferior vena cava (Inada et al., 2014). In contrast, the mouse SAN is "comma shaped," with a dense region at the top of the comma near the superior vena cava and a tail that runs adjacent to the cristae terminalis (Liu et al., 2007). SAN myocytes are interspersed within a network of connective tissue that serves as an insulator (Opthof, 1988; Dobrzynski et al., 2007; Boyett et al., 2003; Chandler et al., 2009). This connective tissue, along with the SAN arteries and adipose tissue, insulate the SAN from the surrounding atrial myocardium (Fedorov et al., 2009, 2012). The mouse SAN contains relatively low levels of connective tissue and has finger-like projections that are one to three cells long and one to two cells wide, which extend into the surrounding atrial myocardium to a length of $\sim$200 μm (Liu et al., 2007).

The anatomy of the AVN was first detailed in 1906, where Sunao Tawara meticulously detailed the cardiac conduction system in various mammals, including humans (Tawara, 2000). The AVN is often separated into two regions including the compact node and the lower nodal bundle (**Fig. 2**). The compact node is a half-oval shaped, small, dense region of interweaving spindle-shaped cells situated at the apex of the triangle of Koch adjacent to the central fibrous body (Hucker et al., 2008; Dobrzynski et al., 2013; Meijler and Janse, 1988; Inoue and Becker, 1998; Anderson and Ho, 1998). Morphologically, cells within the compact node are small and spindle or ovoid shaped (Munk et al., 1996). The anterior portion of the AVN is occupied by the lower nodal bundle and includes the rightward inferior nodal extension (INE). INEs are regions of specialized tissue that extend from the coronary sinus ostium and are continuous with the compact node, which have been identified in the dog and human heart (Hucker et al., 2008; Inoue and

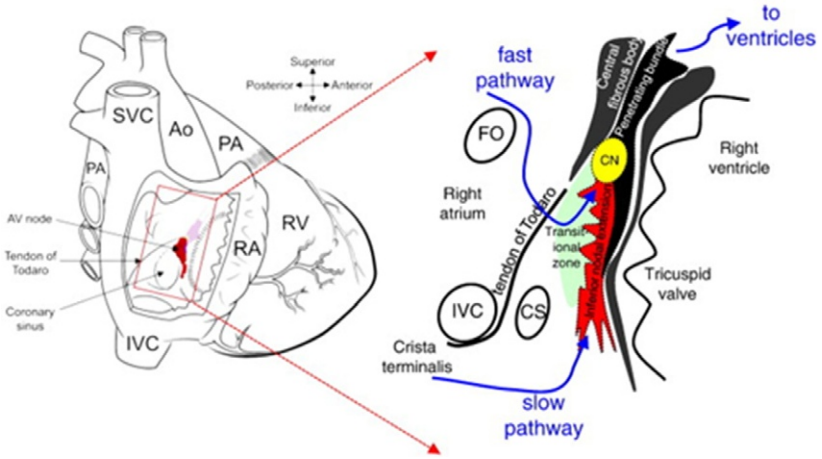

Fig. 2 Location of the atrioventricular node within the heart (left) and schematic illustration of the atrioventricular node junction including the fast and slow pathways (right). Ao, aorta; CN, compact node; CS, coronary sinus; FO, fossa ovalis; IVC, inferior vena cava; SVC, superior vena cava; RA, right atrium; RV, right ventricle. Reproduced with permission from Dobrzynski, H., Anderson, R.H., Atkinson, A., Borbas, Z., D'Souza, A., Fraser, J.F., Inada, S., Logantha, S. J., Monfredi, O., Morris, G.M., Moorman, A.F., Nikolaidou, T., Schneider, H., Szuts, V., Temple, I. P., Yanni, J. and Boyett, M.R. (2013). Structure, function and clinical relevance of the cardiac conduction system, including the atrioventricular ring and outflow tract tissues. Pharmacology and Therapeutics 139, 260–288.

Becker, 1998; Anderson and Ho, 1998). Cells located in this region are smaller than atrial myocytes, yet larger than those in the compact node. They are tightly packed and organized in a parallel configuration. Surrounding the compact node and INE are transitional cells. These cells have a unique cellular phenotype as they exhibit morphological and electrophysiological characteristics of cells within the compact node as well as the surrounding myocardium in which they are located (Anderson and Latham, 1971; Anderson et al., 1974; Temple et al., 2013; Efimov et al., 2004). Electrophysiology studies have identified three cell types within the AVN including the atrio-nodal, the nodal, and the nodo-his cells (Nikolaidou et al., 2012).

SAN and AVN Conduction

In the intact SAN, the leading pacemaker site is the location of initial breakthrough of electrical activity and is most commonly located within the intercaval region (Dobrzynski et al., 2007; Mangoni and Nargeot, 2008). It is estimated that the leading pacemaker site accounts for roughly 1% (approximately 5000 cells) of the SAN in the rabbit heart (Bleeker et al., 1980; Kodama and Boyett, 1985). In response to physiological and pathophysiological conditions, the location of the leading pacemaker site can change. For example, a superior shift occurs in response to sympathetic nervous system activation with β-adrenergic receptor (β-AR) agonists (Fedorov et al., 2012; Azer et al., 2014). Similarly, activation of the parasympathetic nervous system or application of muscarinic (M_2) agonists produces an inferior shift in leading pacemaker site (Fedorov et al., 2012; Vinogradova et al., 1998; Krishnaswamy et al., 2015). Circulating hormones, such as natriuretic peptides (NP), can also modulate SAN function in association with shifts in leading pacemaker site (Azer et al., 2014). Furthermore, in the aged mouse heart it has been shown that the location of the leading pacemaker site can alternate between a superior and inferior position in subsequent beats and change as a function of frailty (Moghtadaei et al., 2016a). It has been proposed that these shifts in leading pacemaker site alter heart rate because of different properties of SAN myocytes at the different locations within the SAN.

Conduction across the SAN spreads from the leading pacemaker site toward the periphery of the SAN at the cristae terminalis where it excites the surrounding atrial myocardium. Interestingly, there is a band of nonexcitable tissue, commonly referred to as a "block zone," at the periphery of the SAN, which slows or prevents conduction toward the left atrium (Boyett et al., 2000, 2003; Bleeker et al., 1980; Nikolaidou et al., 2012). Myocytes at the center of the SAN exhibit poor electrical coupling, which has been attributed to cell orientation, increased levels of connective tissue, and gap junction protein expression patterns (Boyett et al., 2000, 2003; Dobrzynski et al., 2007; Chandler et al., 2009).

Connexins (Cxs) are critical proteins in the SAN (and AVN) that facilitate the spread of electrical signals between adjacent cells via gap junctions and are expressed heterogeneously in the SAN (and atria). The center of the SAN expresses the low conductance Cx45, but lacks expression of Cx40 and Cx43 (Boyett et al., 2000; Coppen et al., 1999; Verheijck et al., 2001). In the SAN periphery, both Cx43 and Cx45 are expressed, resulting in both an increase in electrical coupling between myocytes and increased conduction velocity (Coppen et al., 1999; Verheijck et al., 2001). Heterogeneity in Cx expression results in a slower conduction velocity within the SAN center compared with the periphery as well as the surrounding atrial myocardium and contributes to protecting the SAN from the hyperpolarized atrial myocardium (Joyner and van Capelle, 1986; Boyett et al., 2000; Mangoni and Nargeot, 2008; Bartos et al., 2015). Cxs are also critical for the activation of the atria by the SAN whereby the coupling of Cx45 expressing regions of the SAN communicate with Cx40 and Cx43 positive regions of the atria (Verheijck et al., 2001). It has been proposed that the SAN

communicates with the surrounding atrial myocardium via distinct exit pathways in the SAN (Fedorov et al., 2012; Csepe et al., 2016).

The AVN serves as a gateway to the ventricles as APs generated in the atria must pass through the AVN to excite the ventricular myocardium. Conduction throughout the AVN is much slower than the atria, which has been attributed to numerous factors including cell orientation, intracellular coupling, and ion channel expression. In larger animals, including humans, two distinct pathways of conduction through the AVN have been identified based on electrophysiological characteristics and molecular properties. These are referred to as the fast and slow pathways because of the ease with which conduction spreads through each, which is correlated with Cx43 expression (Hucker et al., 2008; Kurian et al., 2010; Temple et al., 2013). The fast pathway demonstrates relatively rapid conduction velocity, a long refractory period, and has very little to no Cx43 expression. This pathway includes the transitional cells, leftward INE, and compact node (Temple et al., 2013; Hucker et al., 2008; Greener et al., 2011; Efimov et al., 2004; Mani and Pavri, 2014). In contrast, Cx43 is expressed throughout the slow pathway (Cx43 positive region), which includes the rightward INE and the lower nodal bundle, and is continuous with the His bundle (Hucker et al., 2008). As the name suggests, conduction velocities are slow in this pathway (Mani and Pavri, 2014).

Sinoatrial Node Action Potentials

The quintessential feature of the SAN is its ability to generate spontaneous APs characterized by the presence of a slow diastolic depolarization (DD) between successive APs (**Fig. 3A**). The DD occurs during diastole and is characterized by a gradual increase in resting membrane potential, starting from the maximum diastolic potential (MDP) until the threshold for the next AP is reached (DiFrancesco, 1993; Irisawa et al., 1993). In SAN myocytes the AP upstroke velocity (V_{max}) is slower, the overshoot (OS) is smaller, and AP duration (APD) is longer compared to the surrounding contractile atrial myocytes (Monfredi et al., 2010; Dobrzynski et al., 2007). AP morphology is also heterogeneous within the SAN from center to periphery (**Fig. 3B**). Using small ball preparations of consecutive regions within the SAN, intrinsic differences in AP morphology have been characterized. Specifically, from the periphery to the center of the SAN, there is a gradual decrease in AP V_{max}, OS, and MDP (Kodama and Boyett, 1985; Kodama et al., 1997; Boyett et al., 1999). Furthermore, there is an increase in APD from the periphery toward the center of the SAN (Kodama et al., 1997; Yamamoto et al., 1998; Boyett et al., 1999).

Morphologically, APs recorded from the periphery of the SAN have some resemblance to atrial APs while still retaining properties of spontaneously active SAN myocytes (Yamamoto et al., 1998) (**Fig. 3B**). For example, peripheral SAN myocytes have a prominent DD and display automaticity; however, V_{max} is faster and APD is shorter in peripheral versus central SAN myocytes (Boyett et al., 1999; Dobrzynski et al., 2007). Collectively, these AP differences across the SAN result in the leading pacemaker site depolarizing first, but repolarizing last (Yamamoto et al., 1998; Boyett et al., 2000). This is of functional importance because it prevents re-entrant activity.

Atrioventricular Node Action Potentials

APs within the AVN are unique compared with other regions in the heart. Specifically, AVN myocytes generate spontaneous APs that are characterized by much lower V_{max} values compared to the working myocardium. Furthermore, APs in AVN myocytes are shorter in duration compared with ventricular myocytes, but not atrial myocytes (Munk et al., 1996). While AVN myocytes are

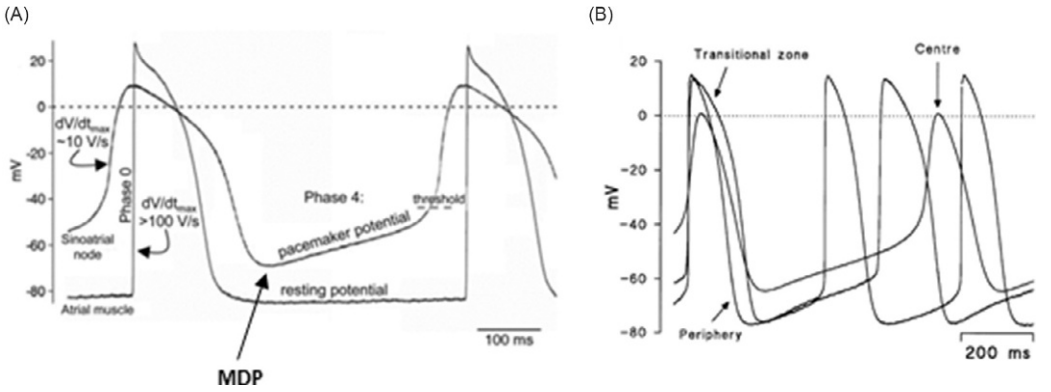

Fig. 3 Action potential morphologies in the sinoatrial node and atrial myocardium. (A) Overlay of representative action potentials from the working atrial myocardium and the sinoatrial node. In comparison to atrial muscle, sinoatrial node action potentials are characterized by a depolarized MDP, a diastolic depolarization (DD; also referred to as a "pacemaker potential"), a reduced upstroke velocity (V_{max}, dV/dt_{max}), lower action potential amplitude and longer action potential duration. (B) Heterogeneity in spontaneous action potential morphology in the center, transitional zone, and periphery of the sinoatrial node. (A) Modified and reproduced with permission from Boyett, M. R. (2009). "And the beat goes on." The cardiac conduction system: The wiring system of the heart. Experimental Physiology 94, 1035–1049. (B) Reproduced with permission from Boyett, M.R., Honjo, H., Yamamoto, M., Nikmaram, M.R., Niwa, R. and Kodama, I. (1999). Downward gradient in action potential duration along conduction path in and around the sinoatrial node. The American Journal of Physiology 276, H686–H98.

spontaneously active, their APs are characterized by slower spontaneous beating rates, reduced DD slopes, more negative MDPs, and reduced AP thresholds compared with SAN myocytes (Marger et al., 2011b).

Regional differences in AP morphology have been characterized in atrio-nodal cells (rod-shaped cells) and compact node or nodo-his cells (ovoid cells) isolated from the rabbit heart. Compact AVN cells are characterized by the presence of a rounded AP OS (Munk et al., 1996). Furthermore, these compact AVN cells have the lowest V_{max}, OS, APD, and MDP compared with the other regions of the AVN (Munk et al., 1996). In contrast, AP morphology of atrio-nodal cells demonstrates characteristics of both AVN and atrial myocytes (Munk et al., 1996; Yuill and Hancox, 2002).

Ionic Basis for SAN APs

The unique morphology of the SAN AP comes about due to the activities of a number of underlying ionic currents in SAN myocytes. These include the hyperpolarization-activated current (I_f) carried by hyperpolarization-activated cyclic-nucleotide gated (HCN) channels, T- and L-type Ca^{2+} currents, a number of voltage-dependent K^+ currents, a sustained inward current (I_{st}), and a Na^+–Ca^{2+} exchange (NCX) current (I_{NCX}) driven by the release of Ca^{2+} from the sarcoplasmic reticulum (SR) (Mangoni and Nargeot, 2008; Lakatta et al., 2010). Other currents and ion channels that have been shown to affect SAN AP firing include sodium channels, two pore K^+ channels, inward rectifier K^+ channels, Ca^{2+} activated K^+ channels, transient receptor potential (TRP) channels, and stretch-activated ion channels. A terminology that has seen widespread adoption is that ionic currents across the plasma membrane that affect SAN AP potential firing may be collectively referred to as a "membrane clock," while the SR Ca^{2+} release/I_{NCX} mechanism may be referred to as the "Ca^{2+} clock" (Lakatta et al., 2010). There has been considerable debate regarding whether any one ionic mechanism is essential for automaticity (i.e., generation of the DD) and regarding the relative importance of each of the ionic mechanisms that underlie spontaneous activity in the SAN (Lakatta and DiFrancesco, 2009). There is currently no consensus on these issues; however, when considered collectively, it appears that there is some redundancy in the ionic mechanisms that generate the DD and automaticity in the SAN.

HCN channels

The hyperpolarization-activated current, also known as the "funny" current (I_f), is encoded by HCN channels and contributes to the DD phase of the AP. Multiple HCN isoforms are expressed in the heart, including HCN1–4. HCN4 is thought to be the predominant isoform in the SAN with contributions from HCN1 and HCN2 as well. Functionally, HCN isoforms assemble into heteromultimeric complexes and the resulting I_f current has biophysical properties consistent with this structural arrangement (Monfredi et al., 2010; Accili et al., 2002; Bucchi et al., 2012). In many species, including mouse, rabbit, and dog, HCN4 expression accounts for upward of 80% of the total HCN expression in the SAN (Shi et al., 1999; Zicha et al., 2005; Liu et al., 2007). However, in the human SAN, HCN1 is highly expressed in addition to HCN2 and HCN4 (Chandler et al., 2009; Li et al., 2015).

As their name suggests, HCN channels are activated by hyperpolarization of the membrane potential and play a role in the early phase of DD (Baruscotti et al., 2010; DiFrancesco and Borer, 2007). I_f channels conduct both Na^+ and K^+, are blocked by Cs^+ in a voltage-dependent fashion, have a reversal potential between −10 and −20 mV, and exhibit slow activation and deactivation kinetics (Baruscotti et al., 2010; DiFrancesco and Borer, 2007; Accili et al., 2002). Inside-out patch-clamp experiments have shown direct activation of I_f channels by cAMP (i.e., independently of protein kinase A (PKA) phosphorylation); therefore, these channels are activated by both membrane voltage and cAMP (DiFrancesco and Tortora, 1991; Wainger et al., 2001). Physiologically, these effects allow for cAMP-dependent regulation of I_f channels, which functions to shift the voltage dependence of activation of HCN channels, increasing I_f currents and DD slope in SAN myocytes. In isolated rabbit SAN myocytes, 2 mM Cs^+ almost completely blocks I_f (Denyer and Brown, 1990). Interestingly, the same concentration of Cs^+ slows AP firing frequency by ∼30% rather than causing a complete cessation of AP firing. Similarly, in isolated rabbit SAN myocytes, ivabradine, a selective I_f blocker, reduced the spontaneous beating rate by 16%, which was attributed to a 32% reduction in early DD slope (Bucchi et al., 2007). Similar effects of Cs^+ on I_f and AP firing have been reported in the human heart (Verkerk et al., 2007).

Strong evidence of a role for I_f in the SAN, as well as the involvement of specific ion channel isoforms in its generation, comes from studies using genetically modified mice. For example, HCN1 knockout mice display a significant reduction (∼30%) in I_f density and spontaneous AP firing frequency in isolated SAN myocytes (Fenske et al., 2013). Furthermore, HCN1 knockout mice are characterized by bradycardia, impaired SAN conduction, and sinus pauses in vivo. Collectively, these findings confirm that HCN1 is present in the SAN and contributes functionally to I_f current generation.

Genetic deletion of HCN2 channels also results in a reduction in I_f density (∼30%) in isolated SAN myocytes as well as a slowing of I_f activation, indicating that HCN2 contributes to I_f in the mouse SAN (Ludwig et al., 2003). HCN2 knockout mice do not show a change in heart rate, but they are characterized by enhanced SAN-mediated arrhythmias, suggesting that HCN2 is important for normal SAN function.

Consistent with the hypothesis that HCN4 is the major isoform in the SAN, global deletion of HCN4 is embryonic lethal; embryos lacking HCN4 die by day 12 post coitum (Stieber et al., 2003). Prior to day 12 HCN4 knockout embryos show near complete absence of I_f and slow heart rates (Stieber et al., 2003). The impacts of inducible and tissue-specific HCN4 ablation have also been investigated. In one study, Cre-lox methods were used to delete HCN4 from the adult mouse SAN, which reduced I_f by ∼75% (Herrmann et al., 2007). Isolated SAN cells from these mice displayed irregular patterns of AP firing, but this could be restored to normal upon application of isoproterenol (ISO), indicating that the cells were capable of beating spontaneously despite the loss of I_f. The authors concluded that HCN4 was necessary for stable basal automaticity, especially at low heart rates

(Herrmann et al., 2007). A second study that investigated inducible, cardiac-specific deletion of HCN4 in mice reported a more severe phenotype in which mice were characterized by severe bradycardia, as well as AV node block, ultimately resulting in death within 5 days of deletion (Baruscotti et al., 2011). Isolated SAN myocytes from these mice exhibited reductions in I_f of ~70%, while spontaneous AP firing was reduced by 60%. Taken together, the available data clearly demonstrate that I_f is importantly involved in generating automaticity in the SAN, although there is still continued debate on whether it is essential for this function.

T-type and L-type calcium channels

Voltage-gated calcium channels are expressed in the SAN and contribute to the DD in SAN myocytes (Mangoni et al., 2006a; Marionneau et al., 2005). In the heart, L-type calcium current ($I_{Ca,L}$) is encoded by two subunits, $Ca_V1.2$ and $Ca_V1.3$, while the T-type calcium current ($I_{Ca,T}$) is encoded by the subunits $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$ (Marionneau et al., 2005). These α subunits show distinct patterns of expression in different parts of the heart, as noted later.

With regard to $I_{Ca,L}$ channel expression, studies have revealed that $Ca_V1.2$ is expressed through the myocardium including in the SAN, while $Ca_V1.3$ has a more restricted expression pattern, being present in the SAN, AVN, and atrial myocardium, but absent from the ventricular myocardium (Mangoni et al., 2003). Functionally, $Ca_V1.3$-mediated $I_{Ca,L}$ is activated at more negative membrane potentials (compared with $Ca_V1.2$-mediated $I_{Ca,L}$), which correspond to membrane voltages during the DD (Mangoni et al., 2003; Mesirca et al., 2016b). $Ca_V1.2$, on the other hand, is primarily active during the AP upstroke. Bradycardia is observed in $Ca_V1.3$-deficient mice, which is attributed to a significant increase in the cycle length between successive APs in association with a reduction in the slope of the DD (Mangoni et al., 2003; Torrente et al., 2016). $Ca_V1.3$-deficient mice display a 60%–70% reduction in $I_{Ca,L}$ density and a positive shift in $I_{Ca,L}$ activation kinetics in SAN myocytes consistent with the remaining $I_{Ca,L}$ being generated by $Ca_V1.2$ (Mangoni et al., 2003). Combined, these data indicate $Ca_V1.3$-mediated $I_{Ca,L}$ contributes importantly to SAN pacemaker activity during the late phase of the DD and that this is the predominant L-type Ca^{2+} channel contributing to automaticity. $Ca_V1.2$-mediated $I_{Ca,L}$, while contributing importantly to the generation of the AP upstroke, does not appear to be a major participant in the generation of the DD.

For $I_{Ca,T}$, both $Ca_V3.1$ and $Ca_V3.2$ mRNAs have been found in the SAN; however, $Ca_V3.1$-mediated $I_{Ca,T}$ appears to play a more prominent functional role (Mesirca et al., 2014; Mangoni and Nargeot, 2008). $I_{Ca,T}$ demonstrates slower activation kinetics and accelerated inactivation kinetics compared with $I_{Ca,L}$ (Mesirca et al., 2014; Mangoni and Nargeot, 2008). Furthermore, $I_{Ca,T}$ activates at more negative membrane potentials than $Ca_V1.2$- and $Ca_V1.3$-mediated $I_{Ca,L}$ and the peak $I_{Ca,T}$ density is lower than that of $I_{Ca,L}$ in SAN myocytes. Nevertheless, $Ca_V3.1$-mediated $I_{Ca,T}$ has been shown to contribute to pacemaker activity in the SAN. Specifically, mice lacking $Ca_V3.1$ completely lack detectable $I_{Ca,T}$ in SAN myocytes, which resulted in a 37% reduction in spontaneous AP firing frequency (Mangoni et al., 2006b). Functionally, while basal heart rate was unchanged in $Ca_V3.1$ knockout mice, intrinsic heart rate (measured in the presence of autonomic nervous system blockade) was reduced by 10%. The precise mechanisms by which $I_{Ca,T}$ affects the DD are still unclear, but may involve the generation of a window current or the coupling of $I_{Ca,T}$ to intracellular Ca^{2+} handling.

Sarcoplasmic reticulum Ca^{2+} release and Na^+–Ca^{2+} exchange

Evidence of a role for sarcoplasmic reticulum (SR) Ca^{2+} release in cardiac automaticity was first shown in latent pacemaker cells in the atria and in the SAN, whereby application of ryanodine was found to slow spontaneous pacemaker activity (Rubenstein and Lipsius, 1989; Rigg and Terrar, 1996; Li et al., 1997). Since then, this phenomenon has been explored in great detail in the mammalian SAN (Lakatta et al., 2010). The central tenet of this hypothesis is that localized subsarcolemmal Ca^{2+} release (LCR) events from the SR occur during the late DD. These LCRs occur rhythmically and lead to the activation of NCX, which extrudes this Ca^{2+} from the SAN myocytes, thereby generating an inward current (I_{NCX}) during the DD. This SR Ca^{2+} release/I_{NCX} mechanism appears to depend on a high level of basal PKA activity in the SAN and is controlled by phosphodiesterases, which are responsible for hydrolyzing cAMP (Vinogradova et al., 2006, 2008). It has been proposed that the LCR events present during the DD occur spontaneously and do not depend on membrane depolarization (Lakatta et al., 2010). On the other hand, more recent evidence has demonstrated that $Ca_V1.3$ Ca^{2+} channels are colocalized with ryanodine receptors in the SAN (Christel et al., 2012) and it has been suggested that Ca^{2+} influx via $Ca_V1.3$ channels during the DD may lead to the opening of ryanodine receptors by Ca^{2+}-induced Ca^{2+} release (Torrente et al., 2016). There has been considerable debate on the relative importance of this Ca^{2+} clock mechanism in SAN automaticity with some studies ascribing it a dominant or even essential role and others concluding that the Ca^{2+} clock is an important contributor, but that spontaneous activity in the SAN can continue without it, albeit with reduced rates and/or stability (Lakatta and DiFrancesco, 2009; Honjo et al., 2003; Lakatta et al., 2010). Nevertheless, the evidence is overwhelming that SR Ca^{2+} release occurs during the late DD and this leads to the activation of I_{NCX}, which increases the rate of depolarization during diastole and thus enhances spontaneous AP firing in the SAN.

Further evidence of a role for SR Ca^{2+} release in automaticity comes from studies of IP_3 receptors (IP_3Rs) in the SAN. All three IP_3R subtypes (IP_3R1, IP_3R2, and IP_3R3) are expressed in the heart and IP_3R1 and IP_3R2 have both been detected in the SAN (Ju et al., 2011, 2012). Activation of IP_3Rs with either membrane-permeable IP_3 or indirectly via application of endothelin-1 (which increases endogenous IP_3 levels via the phospholipase C signaling pathway) caused a significant increase in Ca^{2+} spark frequency near the cell membrane, indicating SR Ca^{2+} release. Furthermore, these IP_3R agonists also increased spontaneous AP generation in the SAN (Ju et al., 2011). Consistent with these findings, IP_3R2 knockout mice as well as pharmacological inhibition of IP_3Rs result in a reduction in spontaneous Ca^{2+} spark frequency and SAN AP firing rate (Ju et al., 2011; Kapoor et al., 2015). Combined, these data indicate IP_3R2-mediated SR Ca^{2+} release can contribute to pacemaker activity in the SAN.

Sustained inward current

The sustained inward current, I_{st}, is a current carried by Na^+ that activates at ~−70 mV and peaks at ~−50 mV. I_{st} is enhanced by β-AR agonists, is insensitive to TTX, and is blocked by dihydropyridine receptor antagonists as well as divalent cations (Mg^{2+}, Ni^{2+}) (Mitsuiye et al., 2000; Cho et al., 2003; Shinagawa et al., 2000; Toyoda et al., 2005). I_{st} has been detected in SAN myocytes and, interestingly, appears to be present only in spontaneously beating, but not quiescent, SAN cells (Mitsuiye et al., 2000; Zhang et al., 2002). Based on its biophysical and kinetic properties, I_{st} has been predicted to contribute to the generation of the DD, particularly in the central regions of the SAN were I_{Na} is small or absent (Shinagawa et al., 2000). The molecular identity of the channels responsible for I_{st} has not yet been identified and until this is accomplished it will remain difficult to determine the precise role of I_{st} in the SAN.

Sodium channels

The heart expresses multiple sodium channel isoforms that can be categorized based on their sensitivity to tetrodotoxin (TTX). The dominant cardiac sodium current (I_{Na}) is carried by the TTX-resistant $Na_V1.5$ channels. $Na_V1.5$ is absent from the center of the SAN (Lei et al., 2004, 2005, 2007), which is consistent with the low V_{max} characteristic of central SAN myocytes. In contrast, $Na_V1.5$, and the resulting I_{Na}, is detected in the larger cells of the peripheral SAN.

TTX-sensitive sodium channels (sometimes referred to as neuronal sodium channels) are also expressed in the heart, including in the SAN. Specifically, $Na_V1.1$ and $Na_V1.3$ subunits have been detected in the mouse SAN (Maier et al., 2003) and $Na_V1.1$, $Na_V1.3$, and $Na_V1.6$ have been identified in the rat SAN (Huang et al., 2015). There is now evidence to suggest that TTX-sensitive Na^+ channels play an important role in pacemaker activity in the SAN. For example, in Langendorff perfused mouse or rat hearts, low concentrations of TTX result in a significant reduction in heart rate (Maier et al., 2003; Huang et al., 2015). Furthermore, in the isolated rabbit SAN, application of nanomolar concentrations of TTX results in a reduction in spontaneous AP firing frequency (Lei et al., 2004).

Detailed investigations have identified differential roles for TTX-sensitive and resistant sodium channels in cardiac pacemaking. In optical mapping studies, nanomolar concentrations of TTX (which would only affect neuronal Na^+ channels) elicit an increase in SAN cycle length, which is consistent with a slower heart rate, but no change in SAN conduction velocity. In contrast, application of micromolar concentrations of TTX (which would block $Na_V1.5$ as well as neuronal Na^+ channels) resulted in a greater increase in cycle length as well as a reduction in conduction velocity across the SAN (Maier et al., 2003). Consistent with these observations, $Na_V1.5$-deficient mice have impaired SAN function resulting in a reduced heart rate attributed to slowed SAN conduction at the peripheral regions of the SAN (Lei et al., 2005). Central SAN myocytes from $Na_V1.5$ knockout mice showed no changes in AP morphology or automaticity while larger peripheral SAN myocytes from $Na_V1.5$ knockout mice had reduced spontaneous AP firing and smaller AP peaks. Collectively, based on these findings, it has been suggested that TTX-sensitive I_{Na} contributes to pacemaker function whereas, TTX-resistant I_{Na} does not contribute to automaticity in the central SAN, but can affect heart rate by modulating conduction through the peripheral SAN and into the atrium (Lei et al., 2004, 2007).

Inward rectifier potassium channels

The inward rectifier K^+ current (I_{K1}) is carried by K^+ channels made up of $K_{ir}2.1$ and K_{ir} 2.2 subunits. I_{K1} is importantly involved in maintaining the resting membrane potential in working contractile myocytes; however, it is largely absent in SAN myocytes. The lack of I_{K1} partly explains the more depolarized MDP in SAN myocytes relative to the hyperpolarized resting membrane potential in contractile myocytes of the surrounding atrium (Mangoni and Nargeot, 2008). It should be noted that while not present in central SAN myocytes, I_{K1} can be measured in peripheral SAN myocytes although at much lower densities than in the working myocardium (Cho et al., 2003; Mangoni and Nargeot, 2001).

Delayed rectifier potassium channels

The ether-a-go-go family of proteins are responsible for the rapidly activating delayed rectifier potassium current (I_{Kr}), which is blocked by compounds such as E-4031 and dofetilide. The slow delayed rectifier K^+ current (I_{Ks}) is encoded by KCNQ1 in the heart and is sensitive to chromonol 293B. Both of these delayed rectifier currents have been described in the SAN, with some species differences as noted later.

In mouse SAN myocytes, three splice variants for mERG1 (1a, 1a′, and 1b) are expressed (Clark et al., 2004). E-4031 application results in depolarization of the MDP, a reduced AP amplitude and can completely prevent spontaneous AP firing in SAN myocytes (Ono and Ito, 1995; Verheijck et al., 1995). Experiments performed in rabbit heart indicate that a gradient of I_{Kr} is present across the SAN such that the effect of E-4031 is greater in the center of the SAN compared with the periphery (Kodama et al., 1999).

Functionally, I_{Kr} contributes to repolarization of the AP and determines the MDP in SAN myocytes (Clark et al., 2004; Verheijck et al., 1995). Furthermore, kinetic analysis of I_{Kr} indicates that these channels have an accelerated rate of deactivation, but I_{Kr} may not deactivate fully during the cardiac cycle so that a sustained current may be present throughout the DD. Inhibition of I_{Kr} shifts the MDP and prolongs AP repolarization resulting in a slowing of spontaneous AP firing. This slowing effect is largely due to less activation of I_f and Ca^{2+} channels.

While I_{Kr} appears to the dominant delayed rectifier K^+ current in rabbit and rodent SAN, in the pig SAN I_{Ks} has been found to be responsible for repolarization of the SAN AP (Ono et al., 2000). In the pig, I_{Ks} appears to function in the same way as I_{Kr}. Consistent with this, block of I_{Ks} in pig SAN with chromanol 293B halts spontaneous AP firing in a similar fashion to E-4031 in rabbit SAN.

Transient outward potassium channels

The transient outward potassium current (I_{to}) plays a critical role in the working myocardium, but is less well understood in the SAN. I_{to} is characterized by fast activation and inactivation kinetics and can be subdivided into fast and slow components based on differences in inactivation kinetics. The fast component of I_{to} ($I_{to,f}$) is encoded by $K_V4.2$ and $K_V4.3$, whereas the slow component ($I_{to,s}$) is encoded by $K_V1.4$ (Nerbonne and Kass, 2005).

I_{to} density is significantly lower in rabbit SAN myocytes as compared to atrial myocytes (Uese et al., 1999). Within the SAN, sensitivity to the I_{to} channel blocker 4-aminopyradine (4-AP) was correlated with cell size such that larger SAN myocytes (presumably from the peripheral SAN) exhibited larger responses to 4-AP compared with the smaller cells from the center of the SAN (Lei et al., 2000). Consistent with these observations, 4-AP also results in AP prolongation in isolated SAN, but the effect is more pronounced in larger SAN myocytes. Collectively, these findings demonstrate that I_{to} is functional in the SAN, perhaps more prominently in the SAN periphery, but more studies are needed to determine the precise role of I_{to} in regulating SAN function.

Two-pore potassium channels

Two-pore domain potassium channels (K_{2P}) are a family of K^+ channels with emerging roles in the heart. Functionally, they facilitate stabilization of the resting membrane potential and AP repolarization in cardiomyocytes (Wiedmann et al., 2016). K_{2P} channels are not voltage dependent; rather they continually conduct currents throughout the various phases of the AP and are hence referred to as background K^+ currents. Regulation of these channels is diverse, and includes factors such as temperature, pH, lipids, and membrane stretch. Six subfamilies of K_{2P} channels exist with the TWIK (two-pore domain weak rectifying potassium channel), TREK (TWIK-related potassium channel), and TASK (TWIK-related acid-sensitive potassium channel) subfamilies being expressed in the heart (Wiedmann et al., 2016; Gaborit et al., 2007).

Emerging evidence is suggestive of a role for K_{2P} channels in SAN electrophysiology. For example, the stretch-sensitive TREK channel is expressed in the mouse SAN and TREK-1 knockout mice have a reduced heart rate (Unudurthi et al., 2016). SAN myocytes isolated from TREK-1-deficient animals exhibit an increased rate of DD and spontaneous AP firing rate as well as an increase in APD at 50% repolarization. Furthermore, background K^+ current was significantly reduced in isolated SAN myocytes from TREK-1 knockout mice. Thus, TREK-1 contributes to SAN automaticity by altering the rate of DD. Additional studies are needed to further investigate the role of K_{2P} channels in the SAN.

Calcium-activated potassium channels

Three types of calcium-activated K^+ channels have been categorized based on their single-channel conductance, including the big conductance (BK), intermediate conductance (IK), and small conductance (SK) channels. These channels are coupled to L-type Ca^{2+} channels and, as their name suggests, are activated by an increase in intracellular calcium levels (Schmitt et al., 2014). Relatively little is known about the role of these channels in the SAN, however emerging evidence is suggestive of a role for BK channels in SAN excitability. Both pharmacological inhibition and genetic ablation of BK channels in isolated SAN myocytes leads to a significant reduction in spontaneous AP firing with a corresponding increase in DD duration (Lai et al., 2014). A role for SK channels has also been proposed in the SAN (Weisbrod et al., 2016).

TRP channels

TRP channels are a large and diverse family of ion channels divided into a number of subfamilies, including the short canonical (TRPC) channels, the vanilloid receptor (TRPV) channels, the melastatin (TRPM) channels, the Ankyrin-repeat (TRPA) channels, the polycystin (TRPP) channels, and the mucolipin (TRPML) channels (Clapham et al., 2001, 2003). Within each of these subfamilies a number of channel isoforms exist. Several TRP channels have been implicated in SAN function including TRPM7, TRMP4, and TRPC channels.

TRPM7 channels are permeable to divalent cations and generate an outwardly rectifying current. Furthermore, TRPM7 is expressed in the SAN and TRPM7 currents are readily detectable in isolated SAN myocytes (Sah et al., 2013). Targeted global knockout of TRPM7 as well as inducible SAN restricted deletion of TRPM7 in mice results in the generation of sinus pauses and AVN block (Sah et al., 2013). Consistent with these observations, TRPM7 knockout mice display a reduction in spontaneous AP frequency in association with a reduction in the DD slope in SAN myocytes. Interestingly the effects of TRPM7 ablation on DD slope appear not to be related directly to the loss of TRPM7 current. Rather, TRPM7 knockout mice display a substantial reduction in HCN channel expression and I_f current density, which appears to account for the reduction in DD slope. Consistent with this hypothesis, a TRPM7 blocker, FTY720, did not affect spontaneous AP frequency in the SAN (Sah et al., 2013).

TRPM4 is a calcium-activated nonselective cation channel that is permeable to Na^+ and K^+, but not Ca^{2+} ions (Little and Mohler, 2013). TRMP4 is activated by intracellular calcium and phosphatidylinositol 4,5-bisphosphate, but inhibited by increases in intracellular ATP. TRPM4 is expressed in the mouse SAN and a nonselective cation current with the properties of TRPM4 currents has been measured in mouse SAN myocytes (Demion et al., 2007). Furthermore, pharmacological inhibition of TRPM4 with 9-phenanthrol or genetic deletion of TRMP4 results in a significant reduction in beating rate within the right atria (Hof et al., 2013). These effects have been attributed to a decrease in the rate of DD as assessed by microelectrode recordings in multicellular atrial preparations. Interestingly, this effect was greater at lower beating rates, thereby suggesting TRPM4 functions in preventing bradycardia. Importantly, the effects of TRPM4 deletion have yet to be studied in isolated SAN myocytes.

The mouse SAN has also been shown to express a number of TRPC channel transcripts, including TRPC1, 2, 3, 4, 6, and 7 and it has been suggested that one or more of these TRPC channels may be involved in mediating Ca^{2+} influx into SAN myocytes in a store operated fashion (Ju et al., 2007). Consistent with this hypothesis, Gd^{3+} and SKF-96355, two compounds known to antagonize several TRPC channels, were shown to block Ca^{2+} entry into SAN myocytes. Furthermore, SKF-96365 reduced pacemaker activity (Ju et al., 2007). While these findings are very interesting, it must be noted that many of the pharmacological agents used in these studies are not specific to TRPC channels and more work is needed to determine the precise role of TRPC channels in the SAN.

AVN Ion Channels

Like SAN myocytes, myocytes isolated from the AVN are characterized by their ability to generate spontaneous APs with DDs. Spontaneous AP frequency in AVN myocytes is lower than in SAN myocytes and less is known about ion channel function in the AVN. At the molecular level, mRNA transcripts for HCN1, HCN4, $Na_V1.1$, $Ca_V1.3$, $Ca_V3.1$, $K_{ir}3.4$, and Cx45 have been detected in the AVN and these transcripts are expressed at higher levels compared with the surrounding atrial myocardium (Greener et al., 2011; Marionneau et al., 2005). On the other hand, $Na_V1.5$, $K_{ir}2.1$, and Cx43 expression was lower in the AVN compared with the atrium.

Consistent with their ability to intrinsically generate spontaneous APs, AVN cells display a prominent I_f, although I_f density in the AVN is ~50% smaller than in the SAN (Marger et al., 2011a,b; Mesirca et al., 2014). While I_f is detected throughout the AVN, regional differences exist such that I_f in nodal or nodo-his cells is significantly higher compared with atrio-nodal cells (Munk et al., 1996; Choisy et al., 2015).

Similar to the SAN, sodium channel expression is variable within the AVN. The compact AVN lacks I_{Na}, which is consistent with the low V_{max} characteristic of the AVN (Efimov et al., 2004; Greener et al., 2011). On the other hand, I_{Na} is detected in all atrio-nodal cells and approximately one-third of nodo-his cells in rabbit and Guinea-pig hearts (Munk et al., 1996; Yuill and Hancox, 2002). Furthermore, both TTX-resistant and TTX-sensitive I_{Na} have been recorded from isolated rabbit AVN myocytes, and blockade of these channels using high levels of TTX can result in cessation of spontaneous AP firing (Marger et al., 2011b).

Multiple K^+ currents have been identified in AVN myocytes. I_{to} density is variable within the AVN such that it is more commonly detected in atrio-nodal cells compared with nodal cells (Marger et al., 2011b; Munk et al., 1996). In mouse AVN, the delayed rectifier I_{Kr} has been identified and found to be larger than in SAN myocytes (Marger et al., 2011b; Efimov et al., 2004). I_{Ks}, on the other hand, was not detected in mouse AVN myocytes. I_{K1} has been recorded in AVN myocytes from mouse and rabbit AVN myocytes (Choisy et al., 2015).

In AVN myocytes, both $I_{Ca,T}$ and $I_{Ca,L}$ contribute to automaticity and AVN function and transcripts for several Ca^{2+} channels can be detected including $Ca_V1.2$, $Ca_V1.3$, and $Ca_V3.1$ (Marger et al., 2011a,b; Marionneau et al., 2005). $I_{Ca,L}$ in the AVN is approximately half the magnitude of that in the SAN and application of L-type Ca^{2+} channel blockers can completely suppress spontaneous APs (Marger et al., 2011a; Greener et al., 2011). $I_{Ca,L}$ density is approximately twice as large in rabbit AVN as compared with mouse AVN (Choisy et al., 2015).

Consistent with an important role for Ca^{2+} channels in AVN function, $Ca_V3.1$ knockout mice are characterized by prolongation of the P–R and P–Q intervals (indicators of conduction through the AVN) and a significant increase in AVN effective refractory period (Mangoni et al., 2006b; Marger et al., 2011a). Furthermore, $Ca_V1.3$ knockout mice show a greater prolongation of the PR interval than $Ca_V3.1$ knockout mice. This prolongation of the P-R interval is exacerbated in double knockouts lacking both Cav1.3 and Cav3.1. In isolated AVN myocytes, the generation of spontaneous APs was reduced by 70% in $Ca_V3.1$ knockout mice and absent in Cav1.3 knockout animals.

SK2 knockout mice exhibit impaired SAN and AVN function, as indicated by a reduction in heart rate and prolongation of the P–R interval (Zhang et al., 2008). AP firing frequency, the rate of DD, and V_{max} were reduced, whereas APDs were prolonged in the AVN of SK2 knockout mice. In contrast, overexpression of SK2 results in the opposite effects, including an increase in spontaneous AP firing rate (Zhang et al., 2008). These effects were attributed to alterations in I_{KCa} densities in AVN myocytes. Combined, these data indicate SK2 channels contribute to AVN function.

SR calcium release also affects AVN function. In isolated rabbit AVN cells, calcium transients correlate with AP generation (Hancox et al., 1994). These Ca^{2+} transients result in an increase in intracellular calcium at the periphery of the myocyte that subsequently spreads throughout the cell. Pharmacological inhibition of SERCA pumps leads to a significant reduction in Ca^{2+} transients and slowed the frequency of AP generation. Furthermore, interfering with SR calcium release by application of ryanodine significantly decreases Ca^{2+} transient amplitude in the AVN. This in turn slows the rate of spontaneous APs in AVN myocytes (Ridley et al., 2008; Choisy et al., 2015). There is also data to suggest SR Ca^{2+} release is coupled to I_{NCX} because inhibition of NCX prevents spontaneous Ca^{2+} transients in AVN (Ridley et al., 2008). Combined, these data indicate both SR calcium release and I_{NCX} facilitate the generation of spontaneous APs in the AVN, although compared to the SAN this is relatively poorly understood.

Regulation by the Autonomic Nervous System

The SAN and AVN are both heavily innervated, allowing heart rate and conduction through the AVN to be altered by the release of neurotransmitters (Pauza et al., 2013, 2014). The autonomic nervous system can be divided into two branches including the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). Stimulation of the β-ARs by SNS agonists results in an increase in heart rate, whereas stimulation of muscarinic (M_2) receptors by the PNS results in a decrease in heart rate. The

interplay between sympathovagal activity, combined with the intrinsic activity of the SAN, determines the rate of spontaneous AP firing in SAN myocytes and hence the heart rate (Mangoni and Nargeot, 2008).

β-AR activation by catecholamines results in the activation of stimulatory G proteins (G_s) that cause an increase in cAMP production by adenylyl cyclases and an increase in protein kinase A (PKA) activity. Increasing cAMP levels and PKA activity causes a positive chronotropic effect and enhances conduction through the AVN following the activation of a number of targets including ion channels and SR Ca^{2+} handling.

In isolated SAN myocytes, β-AR activation leads to a rapid increase in the slope of the DD and a subsequent increase in spontaneous AP firing frequency without affecting APD (Accili et al., 2002; Irisawa et al., 1993) (**Fig. 4**). I_f is modulated by cAMP following the direct binding of cAMP to HCN channels (DiFrancesco and Tortora, 1991), which causes a positive shift in the I_f activation curve and increases I_f during the DD. These effects of cAMP on I_f have been largely thought to occur independently of PKA, although recent evidence suggests that HCN channels can also be phosphorylated by PKA (Liao et al., 2010).

L-type Ca^{2+} currents contribute to the increase in spontaneous AP firing in the SAN following β-AR activation. Consistent with this hypothesis, ISO can dose-dependently increase $I_{Ca,L}$ in SAN myocytes with similar efficacy to I_f (Zaza et al., 1996). Furthermore, SAN myocytes isolated from $Ca_V1.3$ knockout mice show a blunted response to ISO, including impaired effects on DD slope and AP firing frequency (Torrente et al., 2016). β-AR agonists are thought to modulate $I_{Ca,L}$ ($Ca_V1.2$ and $Ca_V1.3$) in a PKA-dependent fashion (Mangoni and Nargeot, 2008).

SR Ca^{2+} release is also modulated by β-AR agonists and it has been suggested that this makes a major contribution to the effects of the SNS on AP firing in the SAN (Lakatta et al., 2010). Enhancement of intracellular cAMP, PKA, and CaMKII activity following β-AR activation leads to an increase in SR Ca^{2+} spark amplitude, frequency, and SR filling rate (Vinogradova et al., 2006; Gao et al., 2011). These effects have been attributed to increases in phosphorylation of ryanodine receptors and phospholamban. In SAN and AVN myocytes, these effects result in increased SR calcium release, which enhances I_{NCX} during the DD and increases AP firing frequency (Vinogradova et al., 2002; Gao et al., 2011; Hancox et al., 1994). The relative importance of each of these mechanisms (I_f, $I_{Ca,L}$ SR Ca^{2+} release) in mediating the positive chronotropic effects of SNS activation has not been resolved.

PNS (i.e., vagus nerve) stimulation causes a negative chronotropic effect and slowing of conduction in the SAN and AVN following the release of acetylcholine (ACh), which activates M_2 receptors and inhibitory G proteins ($G_{i/o}$) (Irisawa et al., 1993). The α_i subunit of these G proteins inhibits AC activity and cAMP signaling. Reduced cAMP levels decrease the DD slope in SAN myocytes and these effects can involve reductions in I_f, $I_{Ca,L}$, and SR Ca^{2+} release (Mangoni and Nargeot, 2008; Lakatta et al., 2010) (**Fig. 4**). In addition to these effects, the βγ subunits of the $G_{i/o}$ proteins can directly activate GIRK ($K_{ir3.x}$) channels that mediate I_{KACh} currents (Mangoni and Nargeot, 2008). Activation of I_{KACh} hyperpolarizes the SAN myocyte, which also slows spontaneous AP firing. A significant amount of investigation has gone into trying to determine which ionic mechanism(s) are particularly important for the negative chronotropic effects of PNS agonists. For example, $K_{ir3.4}$ or $G_{\beta\gamma}$ knockout mice have significant reductions in PNS responsiveness, indicating that I_{KACh} makes a major contribution to the response (Wickman et al., 1998; Gehrmann et al., 2002; Mesirca et al., 2016a). On the other hand, biophysical studies have suggested that I_f is more sensitive to acetylcholine than I_{KACh} or $I_{Ca,L}$ (DiFrancesco et al., 1989; Zaza et al., 1996). From these studies it was concluded that I_f contributes more importantly to changing pacemaker activity in the SAN. Still other studies have shown that PNS agonists suppress local Ca^{2+} releases from the SR during the DD and this is a major determinant of the slowing in pacemaker activity, including in conditions where I_{KACh} would not be activated (Lyashkov et al., 2009; van Borren et al., 2010). Additional studies will be required to resolve these issues.

Hormonal Regulation of the SAN by Natriuretic Peptides

Natriuretic peptides (NPs) are a family of hormones with a number of potent effects in the cardiovascular system (Potter et al., 2006; Kuhn, 2004). Several members of the NP family have been identified including atrial NP (ANP), B-type NP (BNP), and C-type NP (CNP). Each of these peptides is expressed in the heart and is present in both cardiomyocytes and cardiac fibroblasts (Moghtadaei et al., 2016b).

Three NP receptors, called NPR-A, NPR-B, and NPR-C, mediate the effects of NPs (Potter et al., 2006). NPR-A (which binds ANP and BNP) and NPR-C (which is selectively activated by CNP) are guanylyl cyclase (GC) linked receptors that enhance cGMP

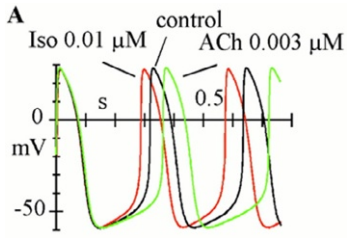

Fig. 4 Effects of the β-adrenergic receptor agonist ISO and the muscarinic agonist acetylcholine (ACh) on spontaneous action potential cycle length and diastolic depolarization slope in the sinoatrial node. Note that these autonomic nervous system agonists modulate action potential cycle length in association with changes in the slope of the diastolic depolarization. Reproduced with permission from Accili, E.A., Proenza, C., Baruscotti, M. and Difrancesco, D. (2002). From funny current to HCN channels: 20 years of excitation. News in Physiological Sciences 17, 32–37.

signaling (Lucas et al., 2000) while NPR-C (which binds all NPs with similar affinity) is coupled to inhibitory G proteins that inhibit AC signaling (Rose and Giles, 2008; Anand-Srivastava, 2005).

NPs can potently regulate heart rate through effects on pacemaker myocytes in the SAN (Springer et al., 2012; Azer et al., 2012; Rose et al., 2007). In isolated SAN myocytes, BNP and CNP each increase spontaneous AP firing frequency in association with increases in the DD slope and APD, but without differences in MDP. These effects on SAN AP firing occurred in association with increases in HR in intact hearts. Voltage clamp studies demonstrate that these changes in AP firing properties were the result of increases in I_f and total $I_{Ca,L}$ in the presence of BNP or CNP along with shifts in the voltage dependence of channel activation ($V_{1/2\,(act)}$). To determine the mechanism for these electrophysiological effects, NPR-C knockout (NPR-C$^{-/-}$) mice and pharmacological approaches have been used. The stimulatory effects of BNP and CNP on spontaneous AP firing, I_f and $I_{Ca,L}$ in NPR-C$^{-/-}$ mice are indistinguishable from wild-type mice in basal conditions. In contrast, the effects of BNP and CNP were occluded by the PDE3 inhibitor milrinone and the effects of BNP were antagonized following NPR-A blockade. Thus, these experiments illustrate that BNP and CNP can potently increase HR and spontaneous AP firing in SAN myocytes by activating the GC-linked NPR-A and NPR-B receptors and inhibiting PDE3 activity (Springer et al., 2012). Consistent with these findings, ANP has also been shown to elicit a cGMP-dependent increase in I_f in human atrial myocytes (Lonardo et al., 2004).

Although NPR-C does not affect SAN function in basal conditions, it does contribute importantly in the presence of β-adrenergic receptor (β-AR) activation (Azer et al., 2012; Springer et al., 2012). Specifically, in the presence of ISO, cANF dose-dependently decreases HR and slows spontaneous AP firing by decreasing the DD slope in SAN myocytes (Azer et al., 2012). These effects of cANF are completely absent in NPR-C$^{-/-}$ mice confirming they are mediated by the NPR-C receptor. To explore the contributions of different NPRs to these responses, we compared the effects of BNP and CNP (which can activate NPR-A/B as well as NPR-C) to cANF (which only activates NPR-C). These measurements show that BNP and CNP increase HR and AP firing in submaximal (10 nM) doses of ISO, but these effects are smaller than those observed in basal conditions because, in the presence of ISO, BNP, and CNP activate NPR-A/B (which mediate an increase in HR and AP firing) as well as NPR-C (which mediates a decrease in HR and AP firing). Interestingly, in the presence of maximum doses of ISO (1 μM) BNP and CNP elicit reductions in HR and cause slowing of spontaneous AP firing in SAN myocytes due to a greater contribution from NPR-C. In agreement with this observation, CNP and cANF can also decrease $I_{Ca,L}$ in SAN myocytes in the presence of maximum doses of ISO (Rose et al., 2004). These changes in ion channel function and AP firing properties correlate with changes in SAN activation and conduction patterns that are also differentially modulated by the GC-linked NPRs and NPR-C (Azer et al., 2014). Collectively, these studies demonstrate that NPs can modulate SAN function via the NPR-A/B receptors (stimulatory) and NPR-C (inhibitory) and that these receptors elicit opposing effects. Because of this, NPs can increase HR and SAN function in some conditions, but decrease HR in others and this is dependent on the extent of β-AR activation and the relative contribution of each NPR in different physiological conditions (Azer et al., 2012, 2014; Moghtadaei et al., 2016b). In addition to these effects of NPs on SAN myocyte function, NPs also regulate collagen deposition, and hence electrical conduction, in the SAN. Consistent with this, genetic loss of NPR-C results in SAN dysfunction in association with enhanced fibrosis and slowed conduction in the SAN (Egom et al., 2015).

Mechanical Effects on the SAN

Mechanical effects on SAN activity (Quinn and Kohl, 2012) were first recognized in 1915 by Francis Bainbridge, who found that intravenous fluid injections in anesthetized dogs resulted in right atrial distension and increased beating rate (Bainbridge, 1915) (similar effects have been observed since in a wide variety of vertebrates (Pathak, 1973), including human (Donald and Shepherd, 1978)). Stretch-induced increases in beating rate also occur in the isolated (decentralized) heart (Blinks, 1956), SAN tissue (Blinks, 1956; Deck, 1964), and SAN cells (Craelius et al., 1988; Iribe et al., 2007), and are insensitive to ablation of intracardiac neurons (Wilson and Bolter, 2002), block of neuronal Na^+ channels (Chiba, 1977; Wilson and Bolter, 2002), and adrenergic and cholinergic blockade (Blinks, 1956; Brooks et al., 1966; Chiba, 1977; Wilson and Bolter, 2002), indicating that nonneuronal, intracardiac mechanisms must be involved.

Microelectrode recordings in cat and rabbit right atrial tissue have demonstrated that stretch increases SAN MDP, reduces maximum systolic membrane potential, and increases DD rate (Deck, 1964). Patch-clamp recordings in axially stretched isolated rabbit SAN cells, combined with computational modeling, have established that this can be explained by a mechanosensitive whole-cell current with a reversal potential of ∼10 mV (Cooper et al., 2000). This current is presumably carried by cation nonselective stretch-activated channels, which are rapidly activating, with a reversal potential between 0 and −20 mV in cardiac cells (Craelius et al., 1988; Guharay and Sachs, 1984), and whose block causes a reversible reduction of stretch-induced changes in beating rate (Cooper and Kohl, 2005). In fact, even if located outside of SAN cells in electrically coupled nonmyocytes (Quinn et al., 2016), cation nonselective stretch-activated channels can account for stretch effects, as they are found in fibroblasts (Stockbridge and French, 1988), which are coupled to SAN cells in rabbit (Camelliti et al., 2004), and are depolarized by stretch (Kohl and Noble, 1996; Kohl et al., 1994).

Stretch may also affect fundamental components of SAN function. When expressed in oocytes, current amplitude (Calloe et al., 2005) and activation and deactivation rate (Lin et al., 2007) of HCN channels are increased with mechanical stimulation. Similarly, Ca^{2+} ($Ca_V1.2$) (Calabrese et al., 2002; Lyford et al., 2002) Na^+ ($Na_V1.5$ and $Na_V1.6$), and K^+ (Kv1, Kv3, Kv7, and KvCa) (Morris, 2011) channels are mechanically modulated in various expression systems. Axial stretch of guinea-pig (Iribe and Kohl, 2008) or rat (Gamble et al., 1992; Prosser et al., 2011, 2013; Iribe et al., 2009) ventricular myocytes, on the other hand, causes an acute increase in Ca^{2+} sparks (spontaneous Ca^{2+} release from the SR). This effect apparently occurs via augmented ryanodine receptor open

probability (Iribe et al., 2009), which may involve local activation by mechanically induced mitochondrial Ca^{2+} release through the mitochondrial NCX (Belmonte and Morad, 2008), a channel whose current alters beating rate in isolated rabbit SAN cells by affecting Ca^{2+} spark frequency (Yaniv et al., 2012). Thus, if any of these effects occurs also in SAN cells, they may contribute to stretch-induced changes in beating rate.

In the whole heart, stretch of SAN tissue will vary regionally and with time. The SAN is structurally heterogeneous, resulting in regional differences in stiffness. Changes in beating rate during externally applied force correlate best with maximum tissue deformation, which occurs at the periphery of the SAN, a region more distensible than the central node (Kamiyama et al., 1984). This difference could be important for transmission of electrical activity from the SAN to atrium (Garny et al., 2003), as the SAN periphery is where (the possibly mechanosensitive) HCN channels are thought to play the largest role in SAN activity (due to the more negative diastolic potential in that region) (Kreitner, 1985; Nikmaram et al., 1997). At the same time, SAN stretch will be greatest at the end of atrial filling, which is when SAN cells are moving toward AP initiation. In this way, mechanical priming of SAN cells could adjust function on a beat-by-beat basis to diastolic load, contributing to the matching of cardiac output (beating rate × stroke volume) to venous return (and may also play a role in respiratory sinus arrhythmia, as it continues to exist in the decentralized, transplanted heart (Bernardi et al., 1989)). Moreover, it appears that physiological loading may be essential to SAN automaticity, as slack tissue often shows no or irregular rhythm, while moderate stretch can restore normal activity (Brooks et al., 1966) (although excessive stretch can induce arrhythmias (Brooks et al., 1966; Hoffman and Cranefield, 1960), so pathophysiological loading during atrial overload may unbalance pacemaker mechanisms). This apparent requirement for a minimum mechanical stimulus is also apparent during ontogenetic initiation of the first heartbeat, as fluid pressure build-up in the quiescent cardiac tube appears to be required for the initiation of its spontaneous activity (Rajala et al., 1977).

There is also evidence supporting an interaction between mechanical and autonomic beating rate modulation. In intact rabbit (Bolter and Wilson, 1999; Bolter, 1994), and isolated atria of rabbit (Bolter, 1996), guinea-pig (Wilson and Bolter, 2001), and rat (Barrett et al., 1998), an increase in right atrial pressure induces both beating rate acceleration and a significant reduction in the percentage response to vagal stimulation. Vice versa, when beating rate is reduced by vagal stimulation the stretch-induced beating rate response is augmented, an interaction that may be mediated by I_{KACh}, as it is reduced with increased right atrial pressure (Han et al., 2010). In this way, parasympathetic control of beating rate may be continuously modified by atrial loading, further contributing to the stretch-induced adjustment of BR to fluctuations in venous return, and helping prevent excessive slowing and diastolic distension, while maintaining cardiac output and adequate circulation (Brooks and Lange, 1977).

Conclusions

Since the discovery of the SAN in 1907 and the AVN in 1906 substantial progress has been made in our understanding of the cellular and molecular mechanisms for spontaneous activity in these specialized pacemaker regions of the heart. These discoveries have been instrumental in facilitating a comprehensive understanding of cardiac electrical conduction. While progress has been considerable, there is still much to be learned, as noted throughout the preceding text. Continuing to unravel the precise determinants of cellular function in the SAN and AVN, and how each is regulated, is of paramount importance because dysfunction in each of these regions can contribute in important ways to cardiovascular disease.

Current treatments for SAN and AVN disease often involve implantation of artificial pacemakers which, while effective, come with a number of attendant risks and limitations. Because of this, and in parallel with the continued generation of novel insight into the mechanisms for automaticity, the field of biological pacing, which seeks to engineer zones of focal spontaneous electrical activity in regions of myocardium that are normally quiescent, has blossomed (Cho, 2015; Rosen et al., 2011). Such approaches, if successful, would free the patient from all hardware associated with traditional artificial pacemakers. Given the challenges associated with correcting SAN and/or AVN disease, motivation should remain high to continue to improve our understanding of the basis for automaticity in the heart with the hopes that this knowledge will inform the next generation of therapeutics targetting these specialized regions of the heart.

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Chronic Infections of the Heart

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Chronic Infective Endocarditis

Definition

Most infective endocarditis (IE) can be classified as acute. However, there are a number of patients, mainly ones with prosthetic valve IE, who develop chronic IE. These patients are often older with multiple comorbidities that preclude definitive removal of their prosthetic valves. Chronic suppressive antibiotics are often prescribed after these patients complete the usual course of intravenous therapy.

Etiologies

In the past, infective endocarditis was classified as either acute or subacute based on the usual progression of untreated disease. This definition is now felt to be mainly of historical interest; most clinicians prefer to classify IE based on organism and type of valve. Native versus prosthetic valve is also of importance (Bennett et al., 2015). The pathophysiology, causative etiologies, diagnostics, and treatment recommendations of acute IE are not discussed in this chapter.

Treatment

Regarding treatment of chronic infective endocarditis, the Infectious Diseases Society of America recommends oral suppressive antimicrobials for fungal endocarditis. It is reasonable to continue this lifelong given the high rate of relapse with fungal infective endocarditis (Baddour, 2001). Similar treatment strategies are employed following acute bacterial endocarditis in patients with prosthetic material such as vascular grafts, artificial or bioprosthetic valves, or prosthetic joints.

Chronic Pericarditis

Definition

Pericarditis is usually diagnosed with the triad of pericardial chest pain, friction rub on auscultation, and diffuse ST-segment abnormalities in the setting of PR depression. However, this triad may be rare in certain disease states such as tuberculous (TB) pericarditis (Mutyaba and Ntsekhe, 2017). Although infectious pericarditis can be classified as acute, recurrent, or chronic these different presentations are driven by etiologies that can result in any of these presentations. Chronic pericarditis, therefore, is a disease state on a spectrum with any of the acute infectious pericarditis syndromes. This discussion will be an overview of the various infectious etiologies of pericarditis with a special focus on the chronic manifestations of these diseases. Chronic presentations can include relapse of pericarditis or progression to development of constrictive or effusive pericarditis.

Approximately one in four patients with acute pericarditis will have at least one episode of relapse (Khandaker et al., 2012). Chronic presentations can include relapse of pericarditis or progression to constrictive or effusive pericarditis.

Etiologies

Viral

Viral pericarditis tends to be a benign and self-limited disease. Most common pathogens include polio, enteroviruses, coxsackie A and B. Constrictive pericarditis is a rare complication of viral pericarditis (Imazio et al., 2007).

The term myopericarditis more appropriately describes viral infections of the heart, as the same viruses are implicated in myocarditis as well as in pericarditis (Smith, 1966). Of these, the most commonly associated with pericarditis are the enteroviruses, especially coxsackie viruses (CV). These infections account for at least one third of all cases of acute viral pericarditis. In these cases, sampling of the pleural fluid for testing presents significant logistical challenges. However, proof of causation exists for all CV-B serotypes along with several A and E serotypes (Guarner et al., 2007). In other serotype infections, the diagnosis of coxsackie myopericarditis is made by establishing infection at an alternative site, for example through stool sampling.

The spread of viruses to the pericardium is usually via a hematogenous route. This triggers an inflammatory process and can lead to an effusion. Many times, patients with acute pericarditis develop recurrent episodes. The mechanism for this has yet to be established but is likely related to immunologic processes rather than active infection.

Clinical manifestations

Enteroviral myocarditis can affect all ages, but particularly places young physically active adolescents and young adults at risk with increased incidence in males compared to females (Sainani et al., 1968). Many of these cases are preceded by a prodrome of upper respiratory infectious symptoms, usually 7–14 days prior to the onset of pericarditis. The clinical syndrome of pericarditis is consistent with presentation of idiopathic pericarditis. This is characterized by a sharp pleuritic chest pain and may be accompanied by friction rub in 35%–80% of cases. 50% of cases may be characterized evidence of pericardial effusion on by enlarged cardiac silhouette on chest X-ray. Gallops or signs of congestive heart failure may be observed in approximately 20% of cases. ECG can support the diagnosis and ST segment changes may accompany associated myocardial disease. Many times, cardiac enzymes may be elevated.

Diagnosis

CV have often been isolated from pericardial fluid during biopsy or autopsy procedures surrounding pericarditis, but this is not a practical clinical test. Many times, cardiac tissue is insufficient for viral culture. The diagnosis must be made through oropharyngeal or stool sampling, if able to be made at all. Real time polymerase chain reaction (RT-PCR) for CV is a promising new diagnostic method, although not yet widely available.

Management, course, and prognosis

The treatment of acute viral pericarditis is largely supportive, with medical management of the potential complications including heart failure and arrhythmias (Feldman and McNamara, 2000). Myopericarditis may be associated with persistent electrical abnormalities. Cardiomegaly with heart failure may develop as a result of myocardial damage and scarring. Chronic constrictive pericarditis is also a potential complication. Multiple infections and noninfectious cardiac insults may progress to dilated cardiomyopathy (DCM) (Codd et al., 1989). There is emerging evidence that enteroviral infection may be detected months to years after the onset of DCM, suggesting a mechanism for the development of a particular subset of DCM (Chapman and Kim, 2008).

Bacterial

Bacterial etiologies usually present as purulent pericarditis. These are rare and usually represent an extension of infection from elsewhere, usually pneumonia or mediastinitis. The exception is mycobacterium tuberculosis (TB) which can occur separately from pulmonary tuberculosis.

Etiologies are often undetermined; 40%–84% of patients with acute pericarditis are listed as idiopathic (Bennett et al., 2015). This continues to be true even when histology, cytology, viral PCR, bacterial, and fungal sequencing are performed (Imazio et al., 2007). Pericardiocentesis may assist in diagnosis, but is negative in most patients. Pericardial biopsy or pericardiotomy may increase diagnostic yield (Bennett et al., 2015).

Tuberculous pericarditis

Tuberculous pericarditis may affect up to 1%–2% of patients with active pulmonary tuberculosis (Larrieu et al., 1980). The HIV epidemic has led to a substantial increase in tuberculous pericarditis (Mutyaba and Ntsekhe, 2017).

A recent series investigated nearly 300 cases of acute pericarditis without clear initial source. Four percent of these cases were eventually diagnosed with tuberculous pericarditis (Sagrista-Sauleda et al., 1988). Constrictive pericarditis developed in six out of seven patients and all of these required pericardiectomy.

Clinical manifestations

While TB primarily manifests as pulmonary disease, it may also develop in extrapulmonary sites including the skin, central nervous system, bones, joints, lymph nodes, liver, and heart. Cardiac involvement can be nearly as devastating as CNS disease in terms of morbidity and mortality.

Cardiac involvement can include TB pericarditis, myocardial TB with or without aneurysm, and TB aortitis with or without aneurysms (Mutyaba and Ntsekhe, 2017). Tuberculosis invades the pericardium via lymphatic, hematogenous, or direct contiguous spread. Pericarditis usually occurs in the setting of very few bacteria. These trigger an aggressive hypersensitivity response with TH-1 helper cells which leads to inflammatory exudative effusion, potentially with tamponade effects. In immunocompromised hosts, the bacterial load is much higher and pericarditis symptoms are often only a portion of diffuse disease.

Diagnosis

Diagnosis of pericarditis itself is often made with chest X-ray, EKG, and echocardiogram. Tuberculous pericarditis can be confirmed by sending pericardial fluid for acid-fast bacterial culture/stain, TB PCR, or via unstimulated interferon gamma (uIFN-γ). Adenosine deaminase (ADA) levels also correlate with disease (Mutyaba and Ntsekhe, 2017). Unstimulated interferon gamma has a 95% sensitivity and 96% specificity, but is more expensive than using acid-fast bacterial (AFB) stains/culture or ADA (Pandie et al., 2014). A scoring system has been developed that uses six clinical and laboratory values with differing weights. These include night sweats, weight loss, fever >38C, leukocytosis less than 10×10^9 (Feldman and McNamara, 2000), and serum globulin >40 g/L. A score of six or more has 36% sensitivity and 85% specificity for TB pericarditis (Reuter et al., 2006).

Management, course, and prognosis

The natural history of TB pericarditis varies widely. Complications can include tamponade, but long-term complications of fibrotic fusion and development of constrictive pericarditis are most concerning. The natural history of this is demonstrated in a recent study. Sixty percent of the control patients required pericardiocentesis despite receiving the standard anti-TB chemotherapy. Of those alive at 12 months, 8% had progressed to constrictive pericarditis and 4% had reaccumulation leading to tamponade (Mayosi et al., 2014). There is evidence to suggest that HIV-positive patients are less likely to progress to constrictive pericarditis.

In some cases, underlying myocardium can become inflamed. This is more likely in HIV-positive patients with CD4 counts less than 100 cells/μL (Syed et al., 2014).

Treatment of TB pericarditis, usually with at least six months of four-drug anti-TB chemotherapy, should be promptly started once alternative diagnoses have been excluded. This is especially true if biomarkers of TB are present and if TB is confirmed at other extrapulmonary sites. Experts suggest treating for TB in endemic areas when no obvious other diagnosis are apparent [KT1] (Mayosi et al., 2005).

Adjunctive steroids reduce hospitalization and may decrease progression to constrictive pericarditis; however, this does not change mortality (Mayosi et al., 2014).

The causative agent of constrictive pericarditis is difficult to diagnose as most patients present with established constriction. An empiric course of anti-TB chemotherapy may be reasonable in patients from high burden areas with concerning recent symptoms of TB and when postoperative pericardial tissue reveals acute inflammation (Mayosi et al., 2005). Unfortunately, the only definitive treatment of tuberculous constrictive pericarditis is surgical pericardiectomy. Mortality rates are significant and can range from 6% to 14% (Mutyaba et al., 2014; Bertog et al., 2004).

Fungal

Fungal causes of chronic pericarditis are infrequently recognized. Disseminated histoplasmosis and coccidiomycosis have been described as causes of acute pericarditis (Bennett et al., 2015). Fungal pericarditis has been reported as a complication of cardiothoracic surgery.

Treatment

Treatment of idiopathic and viral relapsing pericarditis includes high-dose aspirin or other nonsteroidal antiinflammatory drugs (NSAIDs) in combination with colchicine (Imazio et al., 2005). Glucocorticoids are often avoided unless symptoms are clearly refractory to NSAIDs and colchicine. Administration of steroids has been associated with an increased risk of recurrent pericarditis (Lotrionte et al., 2010).

Surgical drainage is essential in patients with purulent (bacterial) pericarditis (Bennett et al., 2015). Pericardiectomy is usually reserved for severe constrictive pericarditis, but can be considered in patients with severe relapsing pericarditis. A recent review of patients from Mayo revealed improvement in symptoms (Khandaker et al., 2012) Although there is little data, experts recommend activity restrictions until symptom resolution (Pelliccia et al., 2006).

Chronic Myocarditis

Definition

The acute presentation of myocarditis is covered elsewhere in this publication. Briefly, most patients present with unexplained heart failure, chest pain, or arrhythmias, often preceded by febrile or respiratory tract illness. Chronic myocarditis is often clinically described as ongoing symptoms for more than 14 days.

General Pathophysiology

Much of our understanding of chronic myocarditis comes from mouse models using coxsackievirus (Bennett et al., 2015). Acute changes involve widespread edema and only a few inflammatory cells. This progresses to loss of striation and fragmentation of

myofibers. Immune cells including NK cells, macrophages, T lymphocytes, and polymorphonuclear cells infiltrate the tissue (Beschorner et al., 1990; Chow et al., 1989). Occasionally interstitial fibrosis occurs and there is loss of myofibers. Ongoing viral or bacterial replication is associated with persistent infiltration and worsening of cardiac function (Kuhl et al., 2005a).

Inability to establish effective immune responses can lead to increased replication and may lead to viral or bacterial persistence. Chronic infection and ongoing replication may lead to DCM. Conversely, excessive immune activation may also play a role in progression to DCM as a result of epitope mimicry and damage of healthy cells (Kuhl et al., 2005a).

Around day 14 of acute myocarditis, fibroblasts and myofibroblasts begin proliferating. M2 macrophages produce collagen to form a permanent scar. In cases that progress to chronic myocarditis, the immune system is unable to completely clear all infected cells. In other cases auto-reactive immune cells persist, causing chronic inflammation. This activates matrix metalloproteases that digest collagen and elastin. In conjunction with profibrotic factors, these facilitate progression to DCM (van den Hoogen et al., 2015).

Etiologies

Most organisms that cause acute myocarditis are also associated with chronic myocarditis. Progression to chronic myocarditis is most often documented in infections involving coxsackieviruses, HIV, and Chagas disease (*Trypanosoma cruzi*).

Viral

A multitude of infectious agents have been reported to cause myocarditis. While bacterial and fungal causes exist, the bulk of acute and chronic myocarditis is virally mediated. Key viruses include coxsackieviruses (members of the enterovirus family), influenza, mumps, measles, polio, and enteroviruses. Adenovirus and parvovirus B19 have also been frequently isolated as causes of myocarditis. Real-time PCR has improved our ability to isolate viral causes, and about two thirds of patients with cardiomyopathies previously designated as idiopathic harbor viral genomes such as Parvovirus, human herpes virus 6 (HHV-6), enteroviruses, adenovirus, EBV, and CMV (Kuhl et al., 2005b). Although these viruses are also found often in samples from asymptomatic patients, viral RNA replicative intermediates were only found in patients with ongoing inflammation.

Diagnosis

Diagnosis begins clinically, usually with a young patient who presents with rapidly progressive cardiomyopathy, arrhythmias, or symptoms of cardiac ischemia in the setting of normal coronary arteries (Dec et al., 1992). In subacute and chronic myocarditis, anticardiac autoantibodies and immunoglobulins can be detected in blood samples (Caforio et al., 2013)

Endomyocardial biopsy (EMB) can be used to obtain tissue for viral pathogen identification. Histopathologic analysis is based on the "Dallas criteria" but this can be open to interpretation and does not provide prognostic assistance (Caforio et al., 2013). The European Society of Cardiology recommends EMB as early as possible while the American College of Cardiology/American Heart association advises EMB when "a specific diagnosis is suspected that would influence therapy" (Fung et al., 2016; Yancy et al., 2013)

Cardiac magnetic resonance (CMR) imaging now provides a noninvasive diagnosis of myocarditis. Hallmarks include edema, early and late enhancement. These can differentiate between myocarditis and myocardial infarction (Childs and Friedrich, 2011). CMR was found to be more accurate in suspected acute myocarditis than in chronic myocarditis (Lurz et al., 2012).

Management, course, and prognosis

Prognosis has been linked to immunohistological signs of inflammation and lack of beta-blocker therapy, but not to histology or detection of viral genomes (Kindermann et al., 2008). Recently, a number of micro RNAs were found to be involved in the progression of viral myocarditis toward DCM (Xu et al., 2014).

Unfortunately the current treatments for advanced DCM involve heart transplantation or mechanical assist devices. Investigations are ongoing regarding treatment via pathogen inhibition, immune modulation, and heart failure therapy (Fung et al., 2016). In mice, novel antiviral agents including astragaloside have been shown to decrease fibrosis and increase survival rate. Other therapies using recombinant proteins and microRNAs have been limited to animal models thus far (Chen et al., 2011). Recent murine study suggests that ivabradine may prevent the progression from viral (coxsackievirus B3) myocarditis to DCM by inhibiting p38 MAPK pathway, downregulating inflammatory responses, and decreasing collagen expression (Yue-Chun et al., 2016).

In a recent double-blinded phase II study in humans, immunomodulatory IFN-β-1b treatment led to more rapid virus elimination, improvement in NYHA functional class, and improved quality of life without increase in adverse events when compared to placebo (Schultheiss et al., 2016). Other promising therapies include immunosuppressants. In one trial, use of azathioprine and prednisone improved left ventricular ejection fraction and New York Heart Association functional class in patients with symptoms for more than 6 months (Wojnicz et al., 2001). NSAIDs have shown to increase myocardial inflammation in viral myocarditis. Intravenous immune globulin has been given, but data is not conclusive. While immunomodulatory drugs appear beneficial for subacute stages of viral myocarditis, use in early stages may have detrimental effects (Fung et al., 2016).

Other viral causes: HIV

HIV has been well documented as a cause of chronic cardiac disease with most data collected from the pre-antiretroviral (ART) time period. It is still not clear what role HIV versus treatment for HIV plays in the development of coronary artery disease. Protease

inhibitors and specific nucleoside reverse transcriptase inhibitors have been associated with an increased risk of cardiovascular disease, but a clear causal relationship has not yet been established (Llibre and Hill, 2016). Even in subjects with suppressed viral loads, cardiac MRI revealed myocardial fibrosis in 82.1% of HIV-infected patients, but only in 27.3% of healthy controls (Luetkens et al., 2016).

Early studies during the AIDS epidemic reported that up to 50% of patients would develop a cardiomyopathy during their lifetimes (Milei et al., 1998). However, it is not fully known whether HIV directly infects the cardiac myocyte leading to dysfunction. In addition, other cardiotropic viruses have been isolated from AIDS patients with cardiomyopathy (Bennett et al., 2015). Other HIV-positive patients demonstrate autoantibodies against myocardial proteins (Currie et al., 1998).

Bacterial

The broad topic of rheumatic heart disease will not be reviewed in this chapter.

Most bacterial cardiac infections manifest as endocarditis. Bacteremia from any pathogen can result in cardiac abscess. Usual pathogens include streptococci, staphylococci, meningococci, salmonella, campylobacter, brucellosis, tularemia, and bartonella. However, myocarditis secondary to toxin production was the most common cause of death in diphtheria. More rare etiologies include legionella, mycoplasma, chlamydia, and rickettsial infections (Bennett et al., 2015). Lyme disease may present as conduction system disturbances, but usually resolves with appropriate treatment and does not result in chronic cardiac changes.

Tuberculosis has been implicated in sudden cardiac death, but this organism usually infects the pericardium rather than the myocardium (Liu et al., 2012). However, there have been reports of extension of pericardial inflammation to myocardial disease. Following the HIV epidemic, TB myopericarditis is most often described in HIV-positive patients from Africa.

Fungal

Fungal myocarditis is not well documented. There have been reports of myocardial involvement in the setting of invasive candida disease, but isolated fungal involvement of the myocardium is rare (Nosanchuk, 2002).

Protozoa

The major protozoan known to cause chronic cardiac disease is *Trypanosoma cruzi*. The clinical manifestations of infection, known as Chagas disease, were described by Carlos Chagas in 1909 (Kropf and Sa, 2009).

An estimated 8 to 12 million people are infected with *T. cruzi* with 10,000 deaths annually. These flagellated protozoa are transmitted mostly by triatominae insects, but transmission also occurs via contaminated blood transfusion, contaminated organ transplant, accidental needlestick, consumption of insect-contaminated foods, or congenital exposure (Mensah et al., 2015). Although Chagas disease is endemic only to South and Central America, changes in human migration are quickly making this a global disease. For example, the CDC estimates that more than 300,000 people with *T. cruzi* infection live in the United States. Screening blood transfusions, kidneys, and livers for *T. cruzi* is recommended if potential donors were born in Mexico, Central America, or South America (Chin-Hong et al., 2011).

Clinical manifestations

Chagas disease follows three phases: acute, indeterminate, and chronic. Approximately 20%–30% of those infected will develop chronic cardiovascular Chagas disease (Stanaway and Roth, 2015). Many patients with the chronic form remain asymptomatic for years. Organ damage occurs as a result of both parasitemia and immune response to parasites. Immune-mediated injury occurs when mononuclear cells infiltrate and release damaging cytokines. Often these are triggered by persistent parasite presence, but autoimmune mechanisms including molecular self-mimicry, polyclonal activation, and shared epitopes also contribute (Rassi et al., 2017). Initial myocarditis from Chagas can be diffuse with necrosis, interstitial edema, vasculitis, and inflammatory cell infiltration. This differs from chronic Chagas cardiomyopathy which is characterized by extensive reactive fibrosis in the setting of few inflammatory infiltrates and mild parasitemia. Although often not seen by histology, *T. cruzi* antigens have been identified via PCR in cardiac tissues of patients with chronic Chagas (Rassi et al., 2017).

Host factors such as genetic polymorphisms may result in changing expression of immune modulators. Patients with enhanced expression of regulatory CTLA-4 system often do not progress to severe Chagas cardiomyopathy (de Oliveira et al., 2015).

Chronic Chagas cardiomyopathy can manifest as abrupt onset heart failure, atrial and ventricular arrhythmias, sudden cardiac death, and thromboembolic events (Mensah et al., 2015). Historically, the heart often demonstrates saccular apical ventricular aneurysm or an overall "flabby" appearance (Johnson et al., 1971; Bennett et al., 2015). In addition, inflammatory markers that are elevated in chronic Chagas disease are also associated with increased risk of stroke. Most patients with chronic Chagas disease die of heart failure, but there is emerging data that cerebral vascular accidents may also be associated with Chagas (Guedes et al., 2016).

Diagnosis

Chagas disease can be diagnosed by microscopic observation of the parasite in a blood smear. However, this is more accurate in the acute rather than the chronic phase of disease. Diagnosis of chronic Chagas disease is often made using two different serological tests: indirect fluorescent antibody and commercial enzyme immune assay. Sensitivity and specificity are variable due to cross reactivation with leishmaniasis antigens. Molecular diagnosis is reserved for cases of transfusion related disease, for acute disease when parasitemia is expected to be high, or when there is concern for reactivation in the setting of immunosuppression (Parasites, n.

d.). ADA and serum C-reactive protein have been shown to increase linearly in regard to disease phase and may serve as surrogate markers for inflammation (Bravo-Tobar et al., 2015).

Electrocardiogram (EKG) is recommended when evaluating potential chronic Chagas disease. In patients with normal EKG and normal radiography of chest and abdomen, patients are listed as "indetermined chronic form." EKG findings include ventricular repolarization, prolonged QTc, left atrial enlargement bundle branch blocks, low QRS voltage, sinus tachycardia, or atrial fibrillation (Dias et al., 2015). Echocardiogram is also recommended. Holter monitoring may also be indicated depending on the level of ventricular dysfunction or prior episodes of ectopy (Dias et al., 2015).

Management, course, and prognosis

Two drugs, benznidazole and nifurtimox, are effective against *T. cruzi*. However, these are not approved by the United States Food and Drug Administration and are only available via investigational protocol from the Center for Disease Control. Treatment is strongly recommended for all children and for adults up to age 50 in the absence of advanced Chagas cardiomyopathy. After age 50, individual factors such as age, clinical status, and overall health should be considered prior to prescribing. Both medications have been shown to have adverse side effects. Benznidazole may cause dermatitis, peripheral neuropathy, and weight loss while nifurtimox commonly causes polyneuropathy, nausea, vomiting, dizziness, and anorexia. Both are contraindicated in severe hepatic or renal disease and have not been studied in breastfeeding infants (Parasites, n.d.). Antiparasitic treatment has not been shown to be beneficial in patients with severe chronic phase Chagas disease.

In terms of other treatments for Chagas disease, trypanocidal therapy with benznidazole in patients with established Chagas' cardiomyopathy significantly reduced serum parasite detection but did not significantly reduce cardiac clinical deterioration through 5 years of follow-up (Morillo et al., 2015).

Ventricular arrhythmias associated with Chagas disease are usually treated with amiodarone. Arrhythmic focus catheter ablations, surgery, and implantable cardioverter defibrillators have been used with success in patients with SVT and those who recover from sudden cardiac death. The treatment of bradyarrhythmias is similar to standard care. Likewise, heart failure due to chronic Chagas cardiomyopathy responds to current heart failure pharmacotherapy (Dias et al., 2015).

Prevention efforts began in the 1990s and included the Southern Cone Initiative, the Andean Pact Initiative, and the Central America Initiative. These programs encouraged efforts to eradicate vectors and reservoirs, monitor blood donations, and screen pregnant women. (Mensah et al., 2015). As a result, transmission of *T. cruzi* was certified as "interrupted" in Uruguay, Chile, Brazil, Guatemala, Honduras, El Salvador, and Nicaragua. The vectors have been eliminated from homes in Brazil, Chile, Argentina, Bolivia, Paraguay, and Uruguay (Mensah et al., 2015). The number of deaths attributed to *T. cruzi* infection also decreased, from 45,000 deaths a year in the 1980s to 14,000 in 2001 to 10,000 most recently (Schmunis, 2007).

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Chronic Kidney Disease as a Risk Factor for Cardiovascular Disease

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Introduction

Cardiovascular disease is a leading cause of mortality and morbidity worldwide (Prevention WicwtUCfDCa, n.d.). According to the World Health Organization, cardiovascular disease was estimated to be responsible for approximately 17.5 million deaths in 2012, or 31% of all global deaths in that year. Of these deaths, an estimated 7.4 million were caused by coronary heart disease and 6.7 million were due to stroke (World Health Organization, 2014). It has been well acknowledged that the traditional cardiovascular risk factors—namely, hypertension, diabetes, dyslipidemia, tobacco use, unhealthy diet, obesity, physical inactivity, and harmful use of alcohol—contribute to the etiology of cardiovascular disease (Expert Panel on Detection, 2001; Asia Pacific Cohort Studies Collaboration, 2005; Woodward et al., 2003). Therefore, the optimal management of these risk factors is known to be effective for preventing the development of cardiovascular disease.

Chronic kidney disease (CKD) is now recognized as a significant and rapidly growing global health burden. CKD is chiefly defined as either kidney damage (e.g., albuminuria >30 mg/d, urine sediment abnormalities and electrolyte and other abnormalities secondary to tubular disorders) or a glomerular filtration rate (GFR) of <60 mL/min/1.73 m^2 for a period of 3 months or more. The prevalence of CKD has been estimated to be more than 10% of the population in many countries worldwide (Eckardt et al., 2013). CKD is caused by lifestyle-related disease such as hypertension, diabetes, obesity, and metabolic syndrome, and various less common diseases that mainly affect the kidney (e.g., glomerulonephritis) (Gansevoort et al., 2013). Since CKD patients have a high prevalence of cardiovascular risk factors (Goldstein et al., 2006), it may be reasonable to consider CKD patients to be a high-risk population for cardiovascular disease. Indeed, growing evidence from numerous epidemiological studies suggests that the lower estimated glomerular filtration rate (eGFR), even in patients with mild-to-moderate kidney impairment, is associated with an increased risk of mortality and cardiovascular morbidity in the general populations (Matsushita et al., 2010). Since CKD patients have a greater prevalence of traditional cardiovascular risk factors, it is important that appropriate treatments, such as blood-pressure-lowering and glucose-lowering therapy and use of statins, be administered to those with CKD in order to reduce the risk of cardiovascular disease (Gansevoort et al., 2013; Goldstein et al., 2006).

In this article, we discuss the current status of epidemiological evidence of the excess risk of cardiovascular diseases, such as coronary heart disease, congestive heart failure, arrhythmia, and cerebrovascular disease (including subclinical cerebral abnormalities), among patients with CKD, taking its prevention and management into account.

Epidemiological Evidence of Excess Cardiovascular Risk in People With CKD

Growing epidemiological evidence suggests that patients with CKD have a greater incidence of cardiovascular disease (Herzog et al., 2011). It is well acknowledged that high cardiovascular mortality is the major cause of reduced life expectancy in dialysis patients (Ritz and Bommer, 2009). According to the United States Renal Data System (USRD), cardiovascular disease contributed to 40% of all deaths among patients who received dialysis in 2009–11 (Collins et al., 2011). The risk of CVD mortality or incidence in patients undergoing hemodialysis or peritoneal dialysis is approximately 10–20 times higher than in the general population (Foley et al., 1998; Seliger et al., 2003). Kidney disease and cardiovascular disease share traditional cardiovascular risk factors, such as aging, diabetes, hypertension, and hyperlipidemia(Goldstein et al., 2006). Intriguingly, it has been reported that stroke events early after starting dialysis therapy were more likely to develop among patients with kidney failure caused by either nephrosclerosis or diabetes mellitus than patients with chronic glomerulonephritis, most of which experienced stroke events more than 36 months after starting dialysis therapy (van der Sande et al., 2004). Supportive findings from the Chronic Kidney Disease Japan Cohort (CKD-JAC) study of 2977 patients with eGFR of 10–59 mL/min/1.73 m^2 showed that patients with diabetic nephropathy or nonchronic glomerulonephritic kidney disease had a

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higher brachial-ankle pulse wave velocity, which is a marker of atherosclerotic disease than those with chronic glomerulonephritis (Imai et al., 2010). This finding supports the hypothesis that kidney impairment in combination with other cardiovascular risk factors accelerates atherosclerosis and raises the risk of cardiovascular disease in the predialysis and dialysis stages.

The increased cardiovascular risk in patients with kidney disease was reported not only among the dialysis patients, but also among patients with all-stage CKD. Go et al. revealed that the multivariable-adjusted hazard ratio (HR) for cardiovascular events was 1.4 (95% confidence interval [CI] 1.4–1.5) for eGFR of 45 to 59 mL/min/1.73 m^2, 2.0 (95% CI 1.9–2.1) for eGFR of 30–44 mL/min/1.73 m^2, 2.8 (95%CI 2.6–2.9) for eGFR of 15 to 29 mL/min/1.73 m^2, and 3.4 (95%CI 3.1–3.8) for eGFR of $<$15 mL/min/1.73 m^2 in a large-scale population-based study involving 1,120,295 adults from a large, integrated system of health care delivery in whom serum creatinine had been measured between 1996 and 2000 and who had not undergone dialysis or kidney transplantation (Go et al., 2004). Similar inverse associations have been observed for the risk of premature death and hospitalization. In a pooled analysis using 22,634 participants from community-based longitudinal studies including the Atherosclerosis Risk in Communities (ARIC) Study, Cardiovascular Health Study, Framingham Heart Study, and Framingham Offspring Study, subjects with eGFR below 60 mL/min/1.73 m^2 had a higher incidence of cardiovascular disease than those with eGFR of $\geq$60 mL/min/1.73 m^2 (42.1 vs. 16.4 events per 1000 person-years). This significant association remained unchanged after adjusting for traditional cardiovascular risk factors (HR 1.19, 95%CI 1.07–1.32) (Weiner et al., 2004). Similarly, in a pooled analysis using the individual data from 30,657 participants from 10 community-based cohort studies in Japan, the multivariable-adjusted HR for the development of cardiovascular disease increased gradually with lower eGFR levels: the HR was 1.57 (95% CI 1.14–2.15) in subjects with eGFR of $<$60 mL/min/1.73 m^2, as compared to those with eGFR of $\geq$90 mL/min/1.73 m^2 (Ninomiya et al., 2008).

In addition to lower eGFR, proteinuria and albuminuria are also good indicators for cardiovascular risk assessment in the general population. A literature-based systematic review from community-based cohort studies demonstrated that the presence of proteinuria or albuminuria was significantly associated with 1.5–2.0 times greater risk for coronary heart disease and stroke (Perkovic et al., 2008; Ninomiya et al., 2009). In addition, the Chronic Kidney Disease Prognosis Consortium (CKD-PC) performed a collaborative meta-analysis of the association between CKD and cardiovascular mortality in a general population cohort that included 105,872 participants from 14 studies employing urine albumin-to-creatinine ratio (ACR) measurements and 1,128,310 participants from seven studies that used urine protein dipstick measurements (Matsushita et al., 2010). This study clearly demonstrated that lower eGFR levels and higher ACR levels were log-linearly associated with the greater risk of cardiovascular mortality. The association between ACR level and cardiovascular mortality was significant within the normal range of ACR (i.e., ACR $\geq$ 1.1 mg/mmol) without threshold effects. In addition, eGFR and ACR were multiplicatively associated with the risk of cardiovascular mortality without evidence of interaction. Similar findings were recorded in the studies with dipstick measurements. Overall, the findings of this study provided strong evidence that eGFR and albuminuria (or proteinuria) are significant independent risk factors for cardiovascular disease in the general population, and both indicators must be evaluated for the early detection and management of the high-risk population for cardiovascular disease.

Possible Mechanisms Underlying the Relationship Between CKD and Cardiovascular Disease

There are several possible explanations for the association between CKD and cardiovascular disease (**Fig. 1**) (Sarnak et al., 2003; Toyoda and Ninomiya, 2014). As mentioned earlier, CKD is associated with a high prevalence of traditional cardiovascular risk factors, such as aging, hypertension, diabetes, smoking habits, and dyslipidemia. Several meta-analyses assessing the association between CKD and cardiovascular disease found that the magnitude of the risk estimates was reduced after adjusting for known traditional cardiovascular risk factors as compared with age-adjusted risk estimates. These findings suggest that an accumulation of traditional cardiovascular risk factors in patients with CKD increases the risk of stroke. In addition, large-scale meta-analyses demonstrated that CKD was a significant risk factor for cardiovascular disease, independent of known cardiovascular risk factors. CKD may also be associated with an increase in "novel" risk factors such as hyperhomocysteinemia, inflammation, asymmetric dimethyl arginine, oxidative stress, anemia, thrombogenic factors, left ventricular hypertrophy, endothelial dysfunction, and arterial stiffness (Sarnak and Levey, 2000; Lau et al., 2016). These traditional and novel risk factors may also play a role of developing process of systemic atherosclerosis. As another possible explanation, impaired kidney function may reflect the duration and severity of hypertension, diabetes or other diseases giving rise to the cardiovascular diseases. Lastly, the results from several previous studies may imply either that CKD itself is a causal factor in the pathogenesis of cardiovascular disease, or that CKD is simply a marker of systemic vessel pathology. The kidney, heart, and brain share unique susceptibilities to vascular injury, so that some vascular risk factors such as hypertension, diabetes, and heart disease may cause similar vascular injuries in these organs.

Association Between CKD and the Subtypes of Cardiovascular Disease

Coronary Heart Disease

Patients undergoing dialysis have been acknowledged to have a greater risk of coronary heart disease (Collins et al., 2011). Some autopsy studies have shown that coronary artery plaques are common in patients undergoing dialysis (Ansari et al., 1993; Schwarz

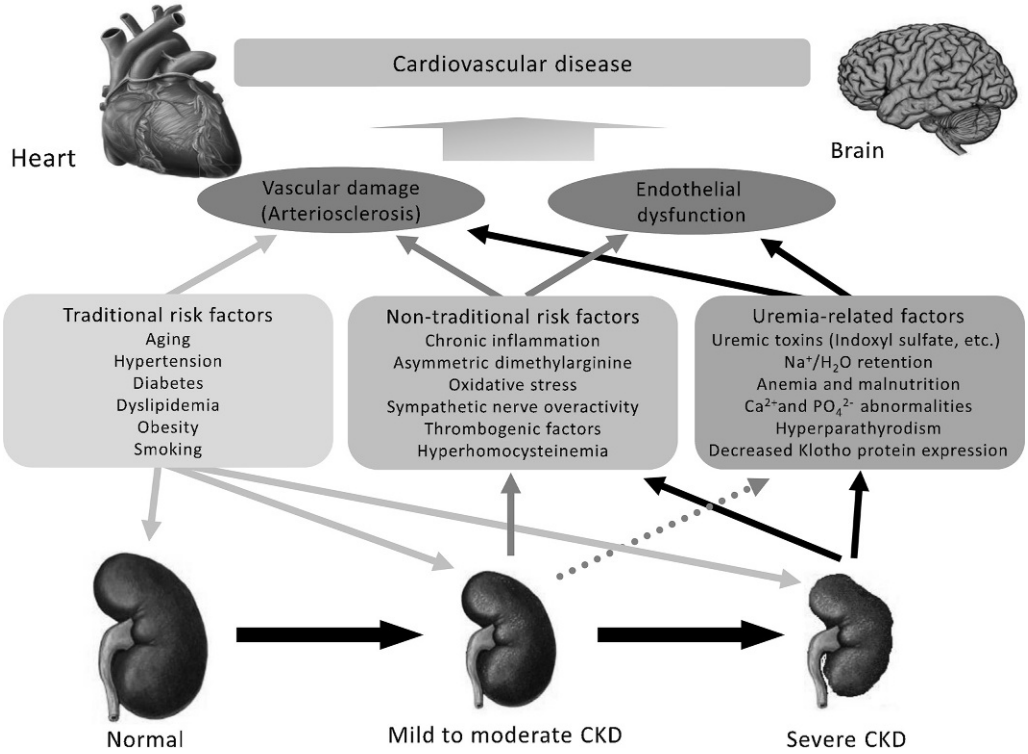

Fig. 1 Traditional and nontraditional risk factors for stroke and kidney disease. Modified from Toyoda, K., Ninomiya, T. (2014). Stroke and cerebrovascular diseases in patients with chronic kidney disease. *Lancet Neurology* **13**, 823-833.

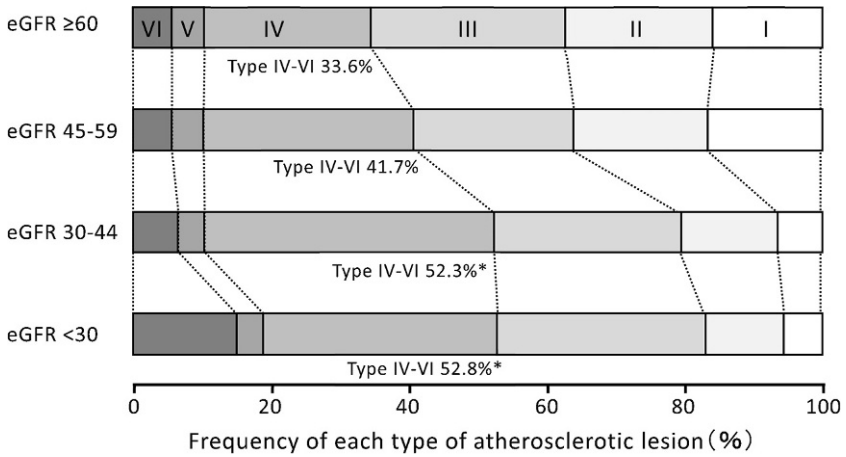

Fig. 2 Frequencies of each type of coronary atherosclerotic lesion according to estimated eGFR level in 126 community-based autopsy samples. The types of atherosclerotic lesion were determined according to the American Heart Association classification. Advanced atherosclerotic lesions were defined as types IV–VI. The frequencies were adjusted for age and sex. $^*P < 0.05$ vs. eGFR ≥ 60. Modified from Nakano, T., Ninomiya, T., Sumiyoshi, S., Fujii, H., Doi, Y., Hirakata, H., Tsuruya, K., Iida, M., Kiyohara, Y., Sueishi, K. (2010). Association of kidney function with coronary atherosclerosis and calcification in autopsy samples from Japanese elders: The Hisayama Study. *American Journal of Kidney Diseases* **55**, 21–30.

et al., 2000). Moreover, an increased risk of coronary events among patients with CKD not undergoing dialysis has been reported in several epidemiological studies (Weiner et al., 2004; Ninomiya et al., 2005, 2008). In the aforementioned pooled analyses from community-based longitudinal studies in the United States and Japan, subjects with CKD had an approximately 2.0–2.5 times greater risk of coronary heart disease than those without CKD after adjusting for traditional risk factors (Weiner et al., 2004; Ninomiya et al., 2008). In support of this finding, a clinical study using coronary angiography found that patients with CKD were more likely to have at least one significant coronary lesion and severe coronary lesions (i.e., three-vessel and/or left main disease) than those without CKD (Chonchol et al., 2008). A cross-sectional study using community-based autopsy samples in Japan has

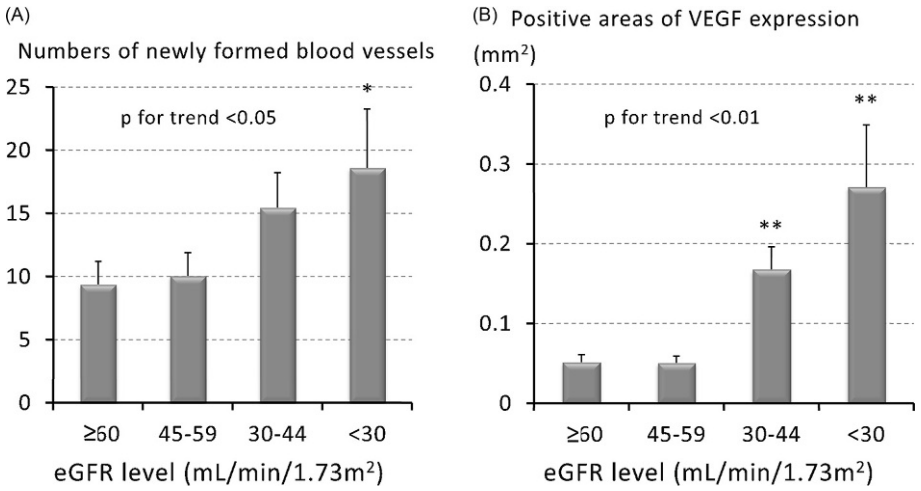

Fig. 3 Numbers of newly formed blood vessels and positive areas of VEGF expression in the intima of coronary arteries according to estimated eGFR level in 126 community-based autopsy samples. *eGFR*, estimated glomerular filtration rate; *VEGF*, vascular endothelial growth factor. The estimates were adjusted for age, sex, hypertension, HbA1c, LDL cholesterol, HDL cholesterol, calcium, phosphate, hemoglobin, smoking, and drinking. $^{*}P<0.05$, $^{**}P<0.01$ vs. eGFR $\geq$60. Modified from Nakano, T., Ninomiya, T., Sumiyoshi, S., Onimaru, M., Fujii, H., Itabe, H., Nakashima, Y., Sueishi, K., Tsuruya, K., Oda, Y., Kitazono, T., Kiyohara, Y. (2013). Chronic kidney disease is associated with neovascularization and intraplaque hemorrhage in coronary atherosclerosis in elders: Results from the Hisayama Study. *Kidney International* **84**, 373–380.

been reported that the severity of coronary atherosclerotic lesion increases with GFR decline (Nakano et al., 2010, 2013). The frequency of advanced atherosclerotic lesions of coronary arteries (defined as the American Heart Association classification types IV–VI) increased gradually with lower eGFR levels (33.6%, 41.7%, 52.3%, and 52.8% for eGFRs of $\geq$60, 45–59, 30–44, and <30 mL/min/1.73 m^2, respectively; *P* for trend = 0.006) (**Fig. 2**) (Nakano et al., 2010). Lower eGFR levels were significantly associated with greater positive areas of oxidized low-density lipoprotein (oxLDL) and vascular endothelial growth factor (VEGF) expression and increased numbers of newly formed blood vessels in the intima (**Fig. 3**) (Nakano et al., 2013). These relationships remained substantially unchanged after adjustment for confounding factors. Together, the results suggest that patients with CKD have intimal neoangiogenesis and an increased risk of intraplaque hemorrhage in the coronary arteries, possibly due to the local accumulation of oxLDL and VEGF. CKD is also known to be prognostic for poor outcomes after percutaneous coronary intervention for coronary heart disease. A hospital-based study revealed that patients with moderate CKD or end-stage kidney disease (ESKD) undergoing PCI have an approximately three times greater risk of in-hospital mortality than those with preserved kidney function (odds ratio [OR] 2.92, 95%CI 1.91–4.46 for moderate CKD; OR 3.68, 95%CI 1.62–8.36 for ESKD) (Parikh et al., 2012). These findings suggest that coronary atherosclerotic lesions must be evaluated carefully in patients with CKD for prevention of premature cardiac death by the early detection and management of coronary heart disease and its risk factors.

Congestive Heart Failure

Cardiac morbidity and mortality rates are high in patients with CKD. Patients with CKD have an increased prevalence of coronary artery disease, silent myocardial ischemia, complex ventricular arrhythmias, atrial fibrillation, left ventricular hypertrophy, mitral annular calcium, and aortic valve calcium compared to those without it (Das et al., 2006). On the other hand, there is limited evidence of the prevalence and incidence of congestive heart failure and the mortality from heart failure in patients with CKD. According to a report from the USRD, the event rate of heart failure was higher in elder subjects with eGFR of <60 mL/min/1.73 m^2 than in those with normal eGFR (176 vs. 56 events per 1000 person-years): the relative risk for heart failure was 1.68 (Shiba and Shimokawa, 2011). The 1-year mortality of subjects with eGFR of <60 mL/min/1.73 m^2 was approximately 25%. The ARIC study also reported showing that the incidence of heart failure in middle-aged US adults was threefold higher for subjects with eGFR of <60 mL/min/1.73 m^2 than those with eGFR $\geq$90 mL/min/1.73 m^2 (18 vs. 6 per 1000 person-years) during a mean follow-up of 13.2 years (Kottgen et al., 2007). The multivariable-adjusted hazard ratio of developing heart failure was 1.94 (95%CI 1.49–2.53) for subjects with eGFR of <60 mL/min/1.73 m^2 as compared to those with eGFR $\geq$90 mL/min/1.73 m^2. The excess risk of heart failure has been recognized to be attributable to hemodynamic factors (e.g., pressure and volume overload) and CKD-related nonhemodynamic factors (e.g., inappropriate activation of the renin–angiotensin system, increased oxidative stress, inflammation, anemia, and raised production of growth factors for collagen and muscle cells) (Cerasola et al., 2011; McCullough, 2004). Pressure overload is largely caused by long-standing hypertension and arterial stiffness (McCullough, 2004). Therefore, cardiac function should be managed carefully in patients with CKD, considering the similar pathophysiologic nature between the kidneys and heart (Shiba and Shimokawa, 2011).

Atrial Fibrillation

Patients with CKD are known to experience a wide spectrum of arrhythmias ranging from premature atrial contraction to atrial fibrillation and flutter, premature ventricular contraction, and ventricular tachycardia, with atrial fibrillation being the most common. According to the data from the international Dialysis Outcomes and Practice Patterns Study (DOPPS), atrial fibrillation was present in 13% (ranging from 6% in Japan to 25% in Belgium) of patients undergoing dialysis, and its prevalence increased with age and was higher in all age categories than in the general population, and new-onset atrial fibrillation developed in 1.0% of the study population during follow-up (Wizemann et al., 2010). This excess risk of atrial fibrillation is also observed in patients in the predialytic stage of CKD. In the Chronic Renal Insufficiency Cohort Study (CRICS), the prevalence of atrial fibrillation in subjects with CKD was 18% in the study population, which was similar to that in patients undergoing dialysis, and was two to three times higher than that reported in the general population of the United States (Soliman et al., 2010). A community-based cross-sectional survey in Japan revealed that multivariable-adjusted OR for prevalent atrial fibrillation was 2.66 (95%CI 1.07–6.60) in subjects with eGFR of <60 mL/min/1.73 m^2, as compared to those with eGFR of ≥ 90 mL/min/1.73 m^2 (Ohyama et al., 2013). With regard to the incidence of atrial fibrillation, the ARIC study clearly demonstrated that lower eGFR levels were associated with the risk of new-onset atrial fibrillation after adjusting for potential confounding factors during a median follow-up of 10.1 years in a general US population: the multivariable-hazard ratios of new-onset atrial fibrillation were 1.27 (95%CI 1.02–1.58), 1.57 (1.19–2.07), and 2.84 (1.70–4.73) for subjects with eGFR of 60–89, 30–59, and 15–29 mL/min/1.73 m^2, respectively, as compared with subjects with eGFR of 90 mL/min/1.73 m^2 (Alonso et al., 2011). Similarly, higher ACR levels were significantly associated with the risk of incident atrial fibrillation, which was 1.89 (95%CI 1.49–2.41) and 2.69 (95%CI 1.82–4.00) for ACR of 30–299 and 300 mg/gCr, as compared to ACR of <30 mg/gCr (Alonso et al., 2011). In addition, the risk of incident atrial fibrillation increased multiplicatively with lower eGFR and higher ACR levels without evidence of interaction (**Fig. 4**) (Alonso et al., 2011). The cases of patients with CKD are likely to be complicated by various metabolic disorders, alterations in the cardiovascular system, and impaired autonomic nervous dysfunctions which facilitate the incidence of atrial fibrillation.

Stroke

There is conflicting evidence as to whether lower eGFR is a risk factor for stroke independent of traditional cardiovascular risk factors. In the aforementioned pooled analyses from community-based longitudinal studies in the United States and Japan, subjects with eGFR of <60 mL/min/1.73 m^2 had a higher incidence of stroke, but this excess risk of stroke in lower eGFR was attenuated, with the result that the association did not quite attain the conventional levels of significance after adjusting for the traditional risk factors (Weiner et al., 2004; Ninomiya et al., 2008). However, these nonsignificant associations between lower eGFR and the risk of stroke arise from insufficient statistical power. A recent meta-analysis of 21 articles derived from 33 prospective studies among 284,672 people experiencing 7863 stroke events, in which multivariate-adjusted relative risks were pooled, suggested that the risk of incident stroke was increased by 43% (95% CI 31%–57%) in subjects with eGFR of <60 mL/min/1.73 m^2 (**Fig. 5**) (Lee et al., 2010). Lower eGFR was a risk factor for both ischemic and hemorrhagic stroke. In addition, 11 of the 33 prospective studies reported both age- and sex-adjusted estimates and the risk estimates adjusted for other known cardiovascular risk factors. A sensitivity analysis using this subset of data revealed that the age- and sex-adjusted summary estimate was 1.64 (95%CI 1.45–1.85), which after further

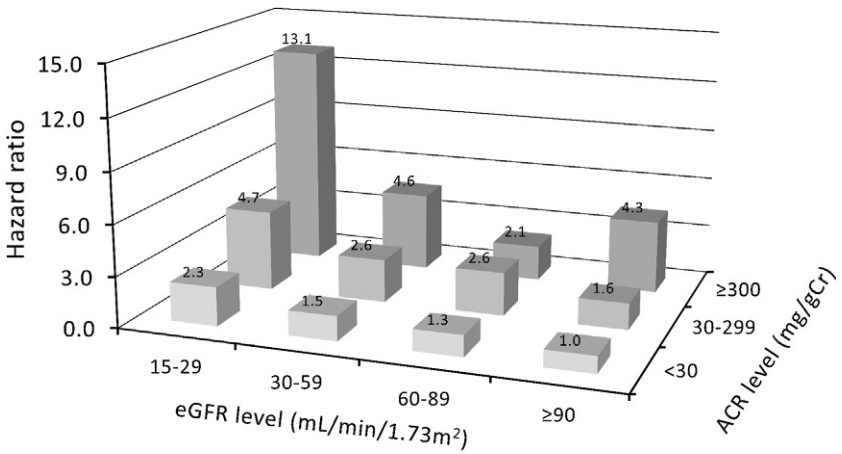

Fig. 4 Multivariable-adjusted hazard ratios for new-onset atrial fibrillation according to estimated eGFR level and ACR level. *eGFR*, estimated glomerular filtration rate; *ACR*, urine albumin-to-creatinine ratio. Hazard ratios were adjusted for age, sex, race, study site, education, income, height, smoking, drinking status, diabetes mellitus, systolic blood pressure, use of antihypertensive medication, body mass index, high-sensitivity C-reactive protein, prevalent coronary heart disease, and prevalent heart failure. Modified from Ohyama, Y., Imai, M., Kurabayashi, M. (2013). Estimated glomerular filtration rate and proteinuria are separately and independently associated with the prevalence of atrial fibrillation in general population. *PLoS One* **8**, e79717.

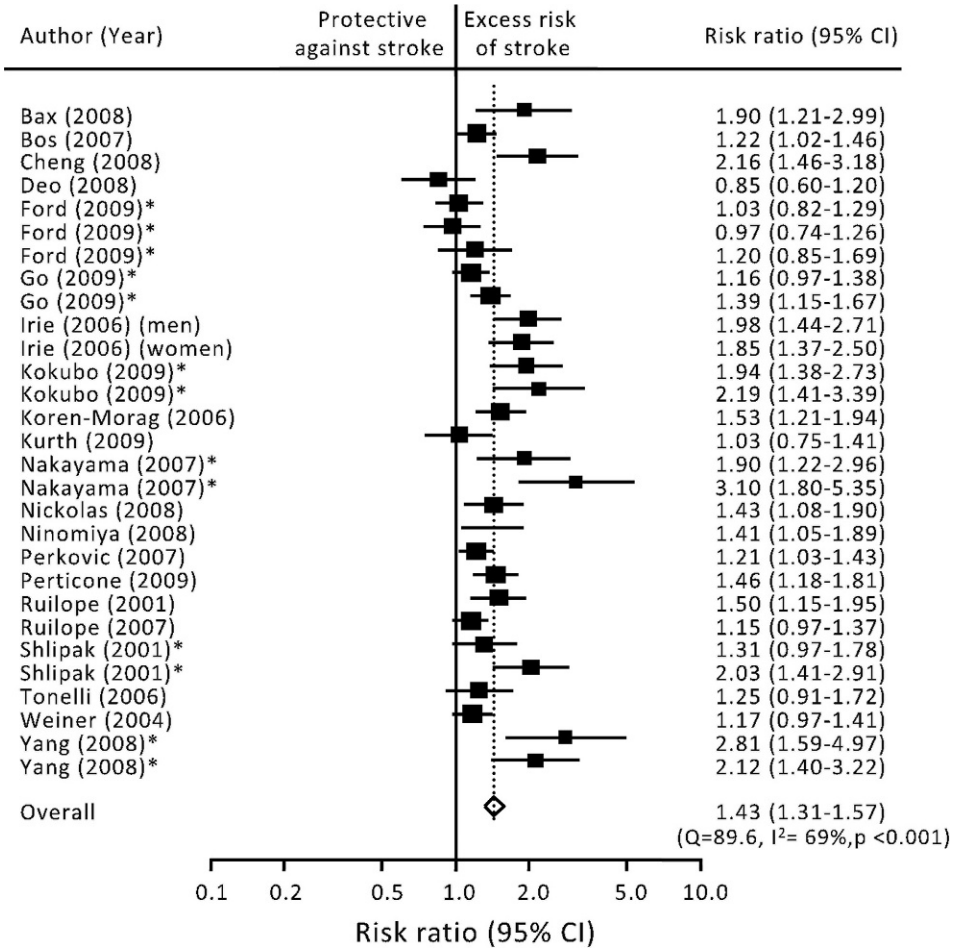

Fig. 5 Overall risk ratio for association of an estimated glomerular filtration rate of <60 mL/min/1.73 m^2 with the risk of stroke in prospective cohort studies. CI, confidence interval. *Subgroups of estimates with eGFR of <60 mL/min/1.73 m^2. Modified from Alonso, A., Lopez, F. L., Matsushita, K., Loehr, L. R., Agarwal, S. K., Chen, L. Y., Soliman, E. Z., Astor, B. C., Coresh, J. (2011). Chronic kidney disease is associated with the incidence of atrial fibrillation: The Atherosclerosis Risk in Communities (ARIC) study. *Circulation* **123**, 2946–2953.

adjustment for other cardiovascular risk factors was reduced to 1.45 (95%CI 1.26–1.68). These findings provide epidemiological evidence that not only an accumulation of traditional cardiovascular risk factors, but also the additional "novel" risk factors play a certain role in the excess risk of stroke among patients with CKD.

Subclinical Cerebral Abnormalities

Subclinical cerebral abnormalities—namely, white matter lesions and silent brain infarcts in the brain, are considered to represent the damaging process of a small vessel disease mediated by endothelial dysfunction, ischemic arteriosclerosis, low perfusion, neurovascular coupling, and diffuse blood–brain barrier disruption (Wardlaw et al., 2009; Fazekas et al., 1993). Several population-based cross-sectional studies have demonstrated that subjects with lower eGFR had a greater volume of white matter lesions and an increased prevalence of silent brain infarcts, as evaluated by MRI (Khatri et al., 2007; Ikram et al., 2008; Seliger et al., 2005; Wada et al., 2008). In the Northern Manhattan Study, the association between CKD and the volume of white matter lesions was investigated in 615 stroke-free participants who underwent magnetic resonance imaging (MRI) (Khatri et al., 2007). eGFR of 15–60 mL/min/1.73 m^2 was significantly associated with an increased log-transformed volume of white matter lesions (β 0.322 [95%CI 0.080–0.564]) after adjusting for traditional cardiovascular risk factors. The Rotterdam study also revealed that subjects with lower eGFR had a greater volume of white matter lesions (difference per 1-SD decrement: 0.14 [95%CI 0.03–0.25]) in the elderly (Ikram et al., 2008). Additional adjustment for traditional cardiovascular risk factors yielded similar findings. Similarly, silent brain infarcts are common in patients with CKD. A cross-sectional survey in the Cardiovascular Health Study found a negative linear association between 1/cystatin C and the prevalence of silent brain infarcts (per 1-SD decrement: multivariable-adjusted OR 1.20 [95%CI 1.09–1.32]) in the elderly (Seliger et al., 2005). The hospital-based studies also indicated that lower eGFR was significantly associated with silent brain infarcts, and patients with more advanced stages of CKD had a greater prevalence of silent brain infarcts (Wada et al., 2008; Shima et al., 2011; Kobayashi et al., 2004). The microvasculature of the brain and kidney has a

similar anatomic as well as functional vasoregulatory (Mogi and Horiuchi, 2011). Both organs share a low vascular resistance system that allows continuous high-volume perfusion and common vascular risk factors including hypertension and diabetes (O'Rourke and Safar, 2005). Kidney impairment is characterized by glomerular endothelial dysfunction and lipohyalinosis, both of which are features of white matter lesions and silent brain infarcts in the brain (Kang et al., 2002). Therefore, it is reasonable to suppose that CKD is a risk factor for cerebrovascular small vessel diseases (white matter lesion and silent brain infarcts) and microangiopathy in the brain.

Prevention and Management of Cardiovascular Disease in Patients With CKD

Strategies for the prevention and management of cardiovascular disease specific to patients with CKD have not been determined. There is limited evidence to inform efforts to prevent new-onset cardiovascular disease or premature cardiovascular mortality among patients with CKD, especially the advanced stage of CKD, because these patients are often excluded from trials. Therefore, we have little choice but to extrapolate preventive goal and management strategies in this population from available information for the general population, small clinical studies, and subgroup analysis of major trials. The first step for the prevention and management of cardiovascular disease in patients with CKD should be similar to that for the general population; that is, intensive control of cardiovascular risk factors—namely hypertension, diabetes, dyslipidemia, smoking, and less exercise (Herzog et al., 2011; Cai et al., 2013). In particular, optimal blood pressure control and glycemic control are of clinical importance in patients with CKD.

For optimal blood pressure control, life-style modifications (i.e., dietary salt reduction, exercise, weight loss, and smoking cessation) and antihypertensive agents together with angiotensin converting enzyme inhibitors or angiotensin receptor blockers are reasonable interventions for any stage of CKD (Herzog et al., 2011). Certainly, these interventions are likely to show benefit against not only atherosclerotic disease, but also heart failure and arrhythmia. On the other hand, evidence of the target level of blood pressure-lowering specific to patients with CKD is still limited. Nevertheless, if we are focused mainly on the prevention of cardiovascular disease, blood pressure lowering itself may be a more pressing concern than the classes of antihypertensive agents. The Blood Pressure Lowering Treatment Trialists' Collaboration, which was a meta-analysis of 26 clinical trials of blood pressure-lowering therapy, revealed that blood pressure lowering significantly reduced the risk of cardiovascular disease by 17% (95%CI 10%–24%) per 5 mm Hg decrement in systolic blood pressure in patients with moderately reduced eGFR, but there was little evidence to support the preferential choice of particular drug classes for the prevention of cardiovascular disease in CKD (Ninomiya et al., 2013).

Diabetes is the primary cause of kidney failure worldwide. Optimal glycemic control can reduce the risk of cardiovascular disease and premature death (Patel et al., 2008; Turnbull et al., 2009). Every effort should be made, therefore, to maintain an HbA1c of <7.0% among patients with CKD, while avoiding hypoglycemia, as recommended by the American Diabetes Association (American Diabetes Association, 2015). At the same time, it is necessary to pay attention to the choice of antiglycemic agents. Dose adjustment of insulin is frequently required along with the progression of kidney impairments, because insulin clearance in the kidney decreases with such progression. Use of metformin and the first-generation sulfonylurea agents should be avoided among patients with advanced CKD, in light of the increased risk of metabolic acidosis and prolonged hypoglycemia (Snyder and Berns, 2004). Careful choice and dose adjustment of antiglycemic agents and frequent monitoring to avoid hypoglycemic events may be necessary, due to the impaired gluconeogenesis and compromised dietary intake as kidney impairment progresses.

The benefit of lipid-lowering therapy in patients with advanced CKD is an area of controversy. Several large-scale clinical trials, such as the 4D, AURORA, and SHARP trials, have presented conflicting results: some trials have shown benefit, while others have shown no effect, in patients undergoing dialysis (Wanner et al., 2005; Fellström et al., 2009; Walker et al., 2011). Although these results are difficult to interpret, they suggest that the patterns of cardiovascular pathology may vary in advanced CKD, with vascular stiffness and calcification, structural heart disease, and sympathetic overactivity contributing to an increasing risk of cardiovascular disease (Foley et al., 1998). However, a recent systematic review of clinical trials demonstrated that statin therapy reduced the relative risk for major cardiovascular events by 23% (95%CI 16%–30%) (Hou et al., 2013). Significant risk reductions were also observed for coronary heart disease and cardiovascular death. Intriguingly, the magnitudes of the relative risk reduction of statin therapy were significantly decreased with the lower eGFR level (P for trend <0.001). This study thus provided strong evidence that statin therapy is beneficial for reducing cardiovascular risk across a broad range of CKD, but the evidence in patients undergoing dialysis is limited.

Finally, the evidence is sparse regarding the effects of antiplatelets and anticoagulants therapy on the development of cardiovascular disease in patients with CKD. A post-hoc analysis of the Hypertension Optimal Treatment (HOT) trial demonstrated that aspirin reduced the risk of major cardiovascular disease by 66% (95%CI: 33%–83%) in hypertensive patients with eGFR of <45 mL/min/1.73 m^2 than in normal kidney function, although the incidence of bleeding was also raised (Jardine et al., 2010). With regard to anticoagulants therapy, this modality has been proven effective for the reduction of morbidity and mortality from stroke and other thromboembolisms among patients with a mild stage of CKD and atrial fibrillation, but its benefit in patients undergoing dialysis is still controversial (Lau et al., 2016). Since both greater thromboembolic and bleeding risks exist among patients with advanced-stage CKD requiring dialysis, it is necessary to carefully choose the treatments and types of agents appropriate for individual patients, while striking a balance between harm and benefit. Large-scale randomized control trials are needed to provide recommendations for antiplatelets and anticoagulants among patients with CKD.

Conclusions

Epidemiological evidence suggests that CKD is a significant risk factor for cardiovascular disease. This excess risk worsens with the severity of kidney impairments. Therefore, patients with CKD should be considered a high-risk population for cardiovascular disease. As the number of elderly increases, the identification of this high risk population will become more important for the prevention and management of cardiovascular comorbidities and related risk factors among patients with CKD or dialysis.

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Clinical Assessment of the Cardiac Arrhythmia Patient

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Introduction

Clinical cardiac electrophysiology, a subspecialty of cardiology, focuses on patients with symptoms of, and evidence for, cardiac arrhythmias. It is based on a large body of evidence, observations, and clinical and basic science that consider arrhythmia mechanisms, evaluation of symptoms, approaches to diagnoses, and, ultimately, treatment. A wide range of outcome measures, including amelioration of symptoms, improvement in functionality and cardiovascular physiology, reduction in risk of arrhythmic, sudden and total mortality, and others, occur with effective arrhythmia management. While many specific recommendations are incorporated into guidelines and consensus documents, clinical assessment remains a challenge (Epstein et al., 2008, 2013; European Heart Rhythm A et al., 2006; Gillis et al., 2012; January et al., 2014; Page et al., 2016; Sheldon et al., 2015).

The approach to a patient with arrhythmias is far from simple as arrhythmias are diverse, occur with wide range of conditions, and have various clinical cardiac and extracardiac manifestations. Symptoms related, or due to, cardiac arrhythmias can be nonspecific, vague, difficult to diagnose, or even absent. Alterations in autonomic tone, or other medical conditions, may explain symptoms that appear to be due to an arrhythmia. These, and other, issues have expanded the reach of management outside the realm of arrhythmia assessment and treatment alone. Frequently, there is a direct connection between autonomic perturbations, comorbidities, and arrhythmia manifestations. Therefore, diagnostic considerations, and therapeutic interventions, are complex, difficult, and challenging making clinical assessment individualized rather than an exact science extending beyond the reach of arrhythmia management alone.

Clinical assessment thus involves more than just evaluation of the arrhythmia. It involves understanding the relationship between the arrhythmia and underlying comorbidities and processes that trigger, or exacerbate, the arrhythmia. As such, autonomic triggers are often considered part of the assessment. Furthermore, comorbidities, without even a primary cardiac arrhythmia diagnosis, can be in the forefront of the assessment of the cardiac patient. Additionally, nonspecific symptoms suspicious for an arrhythmia may be due to other causes for which the clinician must be vigilant and utilize scrutiny.

This introductory chapter focuses on the clinical assessment of the patient with suspected cardiac arrhythmias.

Arrhythmias—What Are We Talking About? (Table 1)

Identification of a suspected rhythm disturbance is only one first step in managing the patient with an arrhythmia. The clinical presentation affects the approach (**Table 2**). Are symptoms severe? Is an arrhythmia present? Is the arrhythmia harmful, does it portend worse things to come, or will it progress to a more serious problem in the future? Does it indicate a serious cardiac or other underlying problem? Is it symptomatic and, if so, does it require intervention? Will the intervention eliminate symptoms? Is the problem debilitating? Is it recurrent?

Arrhythmias range from potentially life-threatening ventricular fibrillation/cardiac arrest to benign, but annoying, premature atrial contractions (PACs). Even premature beats, however, can cause problems. PACs can potentially initiate atrial fibrillation (and cause stroke) (Jensen et al., 2004; Larsen et al., 2015), and premature ventricular contractions (PVCs) and nonsustained ventricular tachycardia may be premonitory signs of more serious or life-threatening arrhythmias (Frolkis et al., 2003; Hohnloser et al., 1999). Frequent PVCs can cause an arrhythmia-induced cardiomyopathy (Gopinathannair et al., 2015).

Sinus tachycardia, the most common and ubiquitous, supraventricular tachycardia, can be due to catecholamine excess, exercise, or various underlying medical problems. Inappropriate sinus tachycardia is a condition in which the sinus rate is out of proportion to physiological demands and otherwise unexplainable (Olshansky and Sullivan, 2013a). Postural orthostatic tachycardia syndrome is an autonomic disorder in which there is sinus tachycardia in a standing position due to orthostatic intolerance (Nwazue et al., 2014; Raj, 2013).

Other forms of supraventricular tachycardia include: AV node reentry, AV reentry (due to an accessory pathway), atrial tachycardia, atrial flutter, sinoatrial reentry, and, the most common pathologic supraventricular tachycardia, atrial fibrillation (Page et al., 2016).

Table 1 Arrhythmia types

- Sinus bradycardia
  - Vagal effect
  - Sick sinus syndrome
  - Tachy-brady syndrome
- AV block
  - First degree
  - Second degree
    - Mobitz Type I ("Wenckebach")
    - Mobitz Type II
    - High-grade
  - Third degree—complete
- AV dissociation
- Tachycardia
  - Sinus tachycardia
    - Appropriate
    - Inappropriate
    - Postural orthostatic tachycardia syndrome
  - Supraventricular tachycardia
    - AV nodal reentry
    - AV reentry and other accessory pathway tachycardias
    - Atrial
    - Sinoatrial reentry
    - Atrial flutter (right or left)
    - Atrial fibrillation
  - Ventricular tachycardia
    - Polymorphic
      - Long QT at initiation—Torsade des pointes
      - Normal QT
    - Monomorphic—single or multiple morphologies
  - Ventricular fibrillation
- Escape rhythms
  - Atrial
  - Junctional
  - Ventricular
- Ectopy
  - Atrial ("PAC")
  - Junctional ("PJC")
  - Ventricular ("PVC")

Table 2 Clinical presentation of arrhythmias

Acute, new-onset, long-standing recurrent, chronic
Predictable, nonpredictable
Paroxysmal—nonsustained, intermittent
Persistent—sustained
Permanent

Ventricular tachycardias can be idiopathic or associated with underlying structural disease including valvular, ischemic, non-ischemic, or infiltrative cardiomyopathies (European Heart Rhythm A et al., 2006). Ventricular tachycardias are classified as monomorphic or polymorphic and can be nonsustained or sustained. Ventricular tachycardia can degenerate into ventricular fibrillation dependent on the rate and underlying cardiac condition with which it is associated. Often, urgent and long-term preventive therapy is required.

Bradycardias manifest as asystole, sinus pauses, sinus bradycardia, and/or AV block may or may not be pathologic. An athlete can have marked sinus bradycardia, a normal physiological response to intermittent intense exercise, that elevates vagal tone and yet for some, sinus bradycardia due to sinus node dysfunction can be so debilitating that a pacemaker is required. However, all bradycardia, including episodic asystole caused by a vagal response and manifesting as syncope, may not necessarily respond to pacing. Thus, as part of the evaluation, it is necessary to identify the arrhythmia, correlate it with symptoms, and understand the risk for serious outcomes and base treatment on the causal mechanism, the risk for recurrence and the expected consequences.

Specific Rhythm Disturbances

Ectopic Beats

Ventricular or atrial ectopic beats (PVCs or PACs) may cause episodes of hard heartbeats or extra beats or feel as though there is a skipped or missed heartbeat. Frequent episodes can initiate dyspnea, chest discomfort, and/or weakness. Ventricular ectopic beats can cause low cardiac output and physiologic bradycardia as the ventricles might not fill or eject blood during the ectopic beat.

Bradycardias

Several mechanisms explain bradycardia. A slow heart rate, by itself, may or may not cause symptoms necessarily. It can be difficult to determine for some individuals if symptoms are due to bradycardia or not. Bradycardia can cause dizziness, lightheadedness, and weakness, even syncope. The critical issue is to correlate the symptoms with the arrhythmia. Athletes, and those in excellent physical condition, may have chronic slow heart rates that do not cause any symptom whatsoever. Chronic bradycardia, without symptoms, is associated with robust long-term survival.

For those who have symptomatic bradycardia, the type of bradycardia must be identified, and then, consideration is required for therapy based on whether bradycardia is due to a reversible cause (such as, a medication) or not. If not, a pacemaker may be indicated to assuage severe symptoms. Bradycardia, especially of an intermittent nature, and, especially at night, may be due to autonomic perturbations that do not require, or respond well to, pacing necessarily.

Tachycardias

Several mechanisms can explain tachycardias and, while some if not many of these tachycardias are benign and even physiologic, pathologic tachycardias, whether they be sustained or nonsustained may have prognostic significance and cause a variety of recurrent and severe cardiac symptoms including collapse and cardiac arrest in some instances. Depending on the type of tachycardia whether it is supraventricular or ventricular, long-term therapy is likely to be indicated and various therapies are possible depending on the problem. Treatments can be curative with ablation for some patients, but other patients require long-term prophylactic preventative measures such as an implantable defibrillator to prevent the risk of sudden cardiac death.

Symptoms and Conditions Due to or Associated With Cardiac Arrhythmias

One important reason to evaluate arrhythmias is due to the symptoms that may occur. Arrhythmias can cause various bothersome but often nonspecific symptoms and problems including: (1) palpitations, (2) dyspnea, (3) chest pain, (4) dizziness, (5) lightheadedness, (6) weakness, (7) syncope or collapse, (8) fatigue or loss of energy, and (9) cardiac arrest (**Table 3**). In some instances, it can be difficult to distinguish whether symptoms are due to an arrhythmia, due to fluctuations in autonomic tone, or due to some other cause entirely.

Symptoms can be severe even if the arrhythmia is benign. Occasionally, ventricular or atrial ectopic beats fit that category. Even sinus tachycardia may be symptomatic and, yet, of little clinical or physiological consequence. Alternatively, severe cardiac arrhythmias may be under recognized and create tremendous long-term problems. Asymptomatic atrial flutter or fibrillation

Table 3 Symptoms suspicious of an arrhythmia

Syncope
Falls
Dizziness
Weakness
Lightheadedness
Fatigue
Chest discomfort
Palpitations
Dyspnea
Exercise intolerance/inactivity

with rapid ventricular response rates ultimately leading to cardiomyopathy is an example. Part of the clinical assessment, therefore, involves distinguishing benign conditions from those that are more malignant even if symptoms are not present.

It is important to correlate the arrhythmia and the symptom. In some instances, an arrhythmia may appear asymptomatic but really is not and, yet, symptoms may be severe when no rhythm disturbance is diagnosed. Symptoms can be nonspecific or difficult to correlate. Long-standing, persistent atrial fibrillation may appear asymptomatic but, after cardioversion, a patient may feel markedly improved. Alternatively, a patient may have severe palpitations but have only normal sinus rhythm.

Unfortunately, for rhythm disturbances in general, there is no specific scoring system that is used to gauge the severity of the rhythm disturbance for a specific arrhythmia. One has been developed for atrial fibrillation several years ago, the Canadian Cardiovascular Society (CCS) Severity of Atrial Fibrillation (SAF) Scale (Dorian et al., 2006). The CCS-SAF score assesses possible atrial fibrillation-related symptoms (palpitations, dyspnea, dizziness/syncope, chest pain, weakness/fatigue), determines a symptom–rhythm correlation, and assesses the effect of these symptoms on patient daily function and quality of life. The type of atrial fibrillation is also assessed. Despite its availability for more than a decade, it has not achieved widespread use and it is not become available for measuring symptoms due to other rhythm disturbances. Similarly, the European Heart Rhythm Association has developed a methodology to gauge symptoms (Wynn et al., 2014).

There can be an association between an arrhythmia and a clinical condition such as atrial fibrillation and congestive heart failure, in which it is not clear if the two are related, associated, or independent or whether treatment of one condition will improve the other (Dickinson et al., 2014). In some instances, one can cause the other; a chronic rapid ventricular response rate in atrial fibrillation can cause an arrhythmia-induced cardiomyopathy (Nerheim et al., 2004). Alternatively, atrial fibrillation can be simply a consequence of the congestive heart failure, not contribute to its progression, and treatment may have little value to the outcome of the patient. Relationships between the rhythm, the symptoms, and other medical conditions determine what therapies are required for the arrhythmia (and its consequences) and other medical conditions such as heart failure.

A medical condition may be present and suspected to be due to an arrhythmia even if an arrhythmia is not diagnosed. Examples include a stroke of undetermined etiology (Brachmann et al., 2016) or congestive heart failure. Asymptomatic nonsustained arrhythmias may be of concern as premonitory signs of something worse to come and may require careful evaluation even if the arrhythmia is infrequent (Hohnloser et al., 1999; Buxton et al., 1999).

Symptoms and Clinical Presentations

Cardiac Arrest/Transient Loss of Consciousness

A patient who loses consciousness transiently may have had syncope or have had an aborted cardiac arrest due to a life-threatening ventricular arrhythmia and may be at risk for recurrence. This is a critical symptom for which careful consideration regarding an arrhythmia, an autonomic mechanism, or both, is required. Recent guidelines carefully delineate an approach to evaluation and management of the patient who loses consciousness due to syncope (Shen, 2017; The European Society of Cardiology, 2009), but several caveats are in order for this rather common problem: (1) It is important to distinguish transient loss of consciousness due to syncope from other causes for loss of consciousness; (2) the evaluation is clinical and based on the episode itself, specific circumstances of the patient, the number and severity of episodes, and the underlying comorbidities (Raj and Sheldon, 2002); (3) the cause can be difficult to ascertain; (4) there is no gold standard to evaluate syncope aside from an excellent clinical evaluation; (5) treatments have not been well tested for many of the conditions causing syncope; (6) it is important to consider cardiac causes for syncope due to potentially severe consequences (Olshansky and Sullivan, 2013b, 2015; Gopinathannair et al., 2008; Olshansky et al., 2008).

Most episodes of syncope are due to transient autonomic perturbations that may not recur and are often benign. However, evaluation is required to rule out a potentially serious cardiac rhythm disturbance and to assess the potential mechanism of the episode to determine if there is likelihood for recurrence. For a patient who collapses, even without a pulse, the episode could be a vasodepressor response with marked vasodilatation, an episode of sustained ventricular tachycardia or marked bradycardia. Abrupt speeding or slowing of the heart rate is more likely to cause hemodynamic collapse than a chronically slow or fast heart rate. By better understanding the mechanisms responsible for syncope, long-term treatment can be assessed and may be required.

For the patient who has documented cardiac arrest due to ventricular fibrillation, evaluation for recurrence and for underlying cardiac causes becomes critical. If cardiac arrest was due to a problem that is nonreversible, long-term protection is required, but the evaluation must first rule out the possibility of an underlying problem that could be at its core.

Palpitations

Palpitations are a sensation of rapid or hard heartbeats (Barsky, 2001). Palpitations are a common reason to consider arrhythmia evaluation. They could be due to supraventricular tachycardia, a PVCs, PACs, sinus tachycardia, ventricular tachycardia, junctional rhythm, or an ischemic event. Palpitations are also commonly due to anxiety or catecholamine excess and not due to an arrhythmia at all. Palpitations may be due to a psychiatric condition such as panic attacks (Barsky, 1992). There is a wide variation in the sensitivity of any individual to an arrhythmia; whereas some fell every ectopic beat, others feel nothing at all even for sustained tachycardias (Barsky et al., 1994).

Palpitations have various clinical presentations (Zimetbaum and Josephson, 1998). The history of palpitations can help guide the subsequent evaluation. The urgency to treat may depend upon how long episodes have been present and how the sensation of palpitations actually is felt. Not all palpitations are the same. Single ectopic beats may be felt as irregularities are skipped beats, whereas sustained tachycardias may present as rapid fast rates associated with near syncope. For episodes of sustained ventricular arrhythmias, there may be a history of underlying heart disease or abnormalities otherwise noted or seen in the history. For sustained episodes of supraventricular tachycardia, there may be a long history of episodic and paroxysmal rapid heart action that begins abruptly with sympathetic stimulation and ends abruptly with vagal activation. AV node reentry is often associated with a sensation of chest pain, shortness of breath, and palpitations in the neck, whereas AV reentry tachycardia may not be to do the atrial ventricular timing differences. Atrial flutter can have its own specific clinical presentations depending on the rate of the atria and of the ventricles. The rhythm may be perceived as regular and rapid or only rapid with minor activity. There may be a sensation of rapid pulsations in the neck.

Atrial fibrillation tends to be associated with irregular and rapid palpitations. Symptoms may be similar, but frequently those who complain about episodic atrial fibrillation notice a very irregular and often rapid ventricular response rate.

A junctional rhythm may present with episodic weakness dizziness and the sensation of a prolonged hard heartbeat that is not fast.

Weakness, Dizziness, Lightheadedness, Fatigue, Falls

Nonspecific symptoms are not unusual for the clinician to address as they may be arrhythmia related. Unfortunately, the symptoms can be due to a wide range of issues making it difficult to evaluate and then treat the problem. If symptoms are due to assuming an upright position, it may be that the patient has orthostatic hypotension or a specific subset of that problem, neurogenic orthostatic hypotension, that is related to an autonomic disorder that causes orthostatic intolerance with major drop on blood pressure and little change in heart rate (Gibbons et al., 2017).

Weakness and fatigue may be due to atrial fibrillation, but this is also complex since patients with atrial fibrillation may be completely asymptomatic, particularly if they are elderly. However, one of the most common complaints of patients or elderly who have atrial fibrillation is fatigue, and this may be passed off as "just getting older" when, in fact, treatment of the problem may improve symptoms. In some instances, the only way to know if a patient is really symptomatic in atrial fibrillation without specific complaints is to return the patient back to normal sinus rhythm to determine whether improvement is possible.

Symptoms may not be due to an arrhythmia alone but due instead to autonomic fluctuations causing hemodynamic alterations. For example, vasovagal syncope is associated with relative bradycardia and hypotension, but the bradycardia is not to do underlying sinus node disease necessarily. Rather, it is due to high vagal activation, and therefore, treatment would be different than would expect if the problem were due to the sinus node alone.

Additionally, some patients have what appears to be bradycardia, but symptoms of dizziness, lightheadedness, weakness, or even syncope instead are due to neurogenic orthostatic hypotension or something else. The relationship between the symptoms and any suspected arrhythmia or blood pressure fluctuation is critical.

Additional History and Comorbidities

To understand and evaluate the patient with an arrhythmia better, it is important to recognize the context in which the suspected rhythm disturbance exists. Presence of underlying heart disease, myocardial infarction, ventricular hypertrophy, valvular heart disease, etc., can set the stage for, or act as a prognosticator of, the specific arrhythmia diagnosed. For example, nonsustained ventricular tachycardia can have prognostic meaning in a patient with ventricular dysfunction or a patient with a channelopathy, such as the long QT syndrome, but may not have meaning in a patient without any heart disease at all.

Similarly, the presence of disease condition not directly related to the heart may be a trigger of the rhythm disturbance, such as atrial fibrillation in a patient with a pulmonary embolism or pneumonia. Other cardiac and noncardiac comorbidities can also act as a benchmark upon which further therapies may need to be undertaken, for the arrhythmia problem itself and for its long-term risks. For example, the CHA2DS2VASc score is used to determine the need for anticoagulation in patients with atrial fibrillation as specific factors and comorbidities increase the risk of stroke and mortality when atrial fibrillation exists (January et al., 2014; Larsen et al., 2012).

Regarding further evaluation, it is critical to understand how the arrhythmia affects long-term risk of mortality and recurrent severe symptoms. Thus, while nonsustained ventricular tachycardia that is asymptomatic and not associated with any underlying structural heart problem may not require treatment, in the presence of severe left ventricular dysfunction, it will have prognostic importance.

Similarly, a severe symptom, such as syncope, without any underlying structural heart disease, may have a benign long-term prognosis. Syncope in the presence of severe ventricular dysfunction raises major concerns. Thus, it becomes important to link the clinical conditions, the medications, and the comorbidities with the arrhythmia itself to determine what the best long-term treatment plan may be. Therefore, the arrhythmia alone is not the only deciding factor with regard to requirements for treatment nor is the need to reduce symptoms the only deciding factor as a patient who is even asymptomatic may be at great risk for sudden cardiac death due to the presence of an arrhythmia in lieu of underlying cardiovascular abnormalities that are present.

Physical Evaluation (Table 4)

The physical examination can provide insights into the diagnosis of the rhythm disturbance in the acute setting and can provide a roadmap to further evaluate and treat the patient. Acutely, physical findings can help identify ectopy, atrial flutter, ventricular tachycardia, and other rhythm disturbances. Chronically, the need for further curative therapy can be assessed based on the underlying conditions and state of the patient.

The physical examination also helps guide the evaluation and the approach to the best long-term therapy. An otherwise healthy, young-appearing individual with highly symptomatic atrial fibrillation is a better candidate for a curative ablation than is an older-appearing demented patient even at the same age with similar comorbidities. Similarly, a patient with long-standing atrial fibrillation who denies any symptoms but presents with substantial peripheral edema and neck vein distention requires careful consideration for aggressive treatment, especially if there is no other explanatory cause as patients may underestimate the severity of their condition.

The physical examination depends, in part, on the place and circumstances under which the evaluation occurs. If the patient is acutely decompensated due to an arrhythmia, the approach is different from in the patient who is coming in for a casual evaluation. In either case, the initial approach begins with the vital signs. For those with orthostatic type symptoms, vital signs should be measured in various positions. This includes blood pressure and heart rate. For the patient who presents acutely and is potentially experiencing a rhythm disturbance, clues in the physical examination can help diagnose the arrhythmia. A variable S1 in a patient who is tachycardic potentially indicates ventricular tachycardia. Flutter waves may be seen in the neck veins. Acutely, for patients who are experiencing a rhythm disturbance, the most pressing issue is the presence of hemodynamic collapse that would require acute treatment of the rhythm disturbance.

For the patient who has had a rhythm disturbance the evaluation should be careful to consider cardiac and noncardiac issues. The overall appearance the patient becomes important with regard to perfusion and evidence for congestive heart failure. There may also be evidence for medical conditions that may interact with symptoms that are caused by an arrhythmia. A patient may be markedly obese and have difficulty breathing suggesting the possibility of sleep apnea. A patient may have muscle weaknesses consistent with myotonic dystrophy indicating the presence of a potentially severe bradycardia or tachycardia with life-threatening process. There may be evidence for valvular heart disease, congestive heart failure, fluid overload, diabetes, severe hypertension, vascular disease, and peripheral edema, for example.

Clinical Acumen

More important than obtaining a history and performing a physical examination is the overall assessment piecing together relationships between the arrhythmia, the condition, and the symptoms. Listening carefully to the patient to understand relationships between symptoms and events is often underemphasized. In one such example, a patient who had an extensive evaluation for syncope and multiple unnecessary hospitalizations turned out to have deglutition syncope that was diagnosed by having him drink a cold carbonated beverage while being monitored. Simply by hearing the patient's story, it was possible to avoid unnecessary, extensive, and repeated evaluations (Olshansky, 1999).

Table 4 Physical evaluation

Heart rate and rhythm
Blood pressure
Orthostatic vital signs
General—overall appearance
Neck—internal jugular distention and pulsations
Cardiovascular—murmurs, rhythm, and gallops
Peripheral—edema, perfusion

Diagnostic Tools (Table 5)

The Electrocardiogram (Table 6)

After the history and physical examination, the first clinical tool to evaluate the arrhythmia patient is the electrocardiogram (ECG). It can be used to diagnose the arrhythmia, if present at the time of symptoms, but it can also determine the presence of accessory pathways, conduction system abnormalities, repolarization abnormalities, and the presence of myocardial damage or infarction. An ECG is required for all patients who have suspected cardiac arrhythmia problems. The ECG may not be diagnostic if the rhythm abnormality is not present, and it may be difficult to determine the arrhythmia mechanism even if it is present.

Electrocardiographic Monitoring (Table 7)

For the patient not at imminent risk of death or sudden death or who is mildly or moderately symptomatic, monitoring is often the next step (Rosero et al., 2013; Mittal, 2014). This can be telemetry in the hospital or outpatient monitoring. The type of monitoring used depends on the arrhythmia suspected and its frequency and severity (**Table 8**) (Mittal, 2014; Zimetbaum and Josephson, 1999; Ruwald and Zareba, 2013). For those patients who have daily symptoms, a 24-h Holter monitor may be all that is required whereas for those patients who are intermittent episodes every several days, long-term external loop recorders may be valuable. For patients who have intermittent syncope, an implantable loop recorder may be required to diagnose the potential cause (Krahn et al., 2001, 2003).

Table 5 Diagnostic tools

Single-lead monitor recording
Implanted device interrogation
Electrocardiogram
External monitor
Holter monitor
Event recorder
Loop recorder
Implantable loop recorder
Tilt-table test
Electrophysiology test
Autonomic test
Exercise test

Table 6 The electrocardiogram

Arrhythmias recorded during symptoms
Bradycardias
AV relationships
Heart block
Wide and narrow QRS complex tachycardias
Bundle branch blocks
Myocardial infarction
Repolarization abnormalities
Depolarization abnormalities (including preexcitation)
Hypertrophy
Brugada syndrome

Table 7 Arrhythmia monitoring—reasons

Unexplained syncope
Symptoms suspicious of an arrhythmia (e.g., palpitations)
Rate assessment—during atrial fibrillation/flutter
Determination of atrial/ventricular arrhythmia burden
Evaluating episodes bradycardia/AV block
Correlating a symptom to an arrhythmia
For prognosis (heart rate variability, nonsustained ventricular tachycardia, etc.)

Table 8 Arrhythmia monitoring—types

Holter monitor
External event recorder
Long-term external patch recorders
Mobile cardiac outpatient telemetry (MCOT)
Implantable loop recorder
Implantable pacemakers/defibrillators

Tilt-Table Testing

For patients with syncope, the evaluation must be directed at the suspected cause for syncope. If the history is not enough to make a diagnosis and the symptoms are severe and/or recurrent enough, aside from monitoring, other tests may be considered. For patients who have a suspected neurocardiogenic causes for syncope, a tilt-table test may prove valuable, but the test is not a "gold standard" to make the diagnosis of vasovagal syncope (Sutton, 2015; Moya et al., 2001).

Exercise Testing

The exercise test may help diagnose a symptomatic arrhythmia triggered by physical activity, but the test itself may not necessarily reproduce the clinical or physiological circumstances under which an arrhythmia, or symptom, develops. The exercise test may also be helpful to rule out other conditions such as ischemia or valvular abnormalities that could potentially exacerbate or create symptoms or even act as triggers for arrhythmias. For patients who have exercise-induced syncope and are suspected to have an arrhythmia, exercise testing may be valuable. Any provocative test attempting to initiate the symptom and/or the arrhythmia has a sensitivity and specificity that makes the test by itself not perfectly diagnostic.

Electrophysiology Testing (Table 9)

Electrophysiology testing can be diagnostic, prognostic, and potentially therapeutic. A clinically significant arrhythmia can be initiated by premature extrastimulus testing delivered during a train of paced beats (induced), and its mechanism can be determined by analysis of electrogram relationship and "mapping." The tissue responsible for a rhythm disturbance can be better understood, and mapping procedures can help localize specific sites that can be treated with ablation techniques and cured. Electrophysiology testing is used occasionally to determine location of block when there is an AV conduction defect or to assess sinus node function.

Electrophysiology testing is used primarily in patients who have an arrhythmia diagnosed. Occasionally, if a patient presents with palpitations that appear to be consistent with or highly suspicious of a supraventricular tachycardia, then an electrophysiology test may be useful without further diagnostic evaluation. For the most part, electrophysiology testing is used as a means to induce a tachycardia that has already been detected but for which more insight is required to determine its mechanism or an approach to treatment. It is more likely to be used to evaluate and treat patients with ventricular tachycardias, supraventricular tachycardias (AV nodal reentry, AV reentry, sinoatrial reentry, atrial tachycardia, atrial flutter, and atrial fibrillation), and PVCs.

Electrophysiology testing can assess the prognosis of patients at risk for sustained ventricular tachycardia and/or ventricular fibrillation cardiac arrest (Buxton et al., 1999; Thomas and Josephson, 2008; Nazari et al., 2014). In years past, it was used more for this purpose but now for select patients with coronary disease who have moderate ventricular function (ejection fraction between 35% and 40%) and nonsustained ventricular tachycardia, it still has a role.

The electrophysiology test may be useful to initiate some supraventricular and ventricular arrhythmias, but a negative electrophysiology test does not completely determine the presence or absence of an arrhythmia in a patient with symptoms that are consistent with an arrhythmia. The test has its own sensitivity and specificity dependent upon the rhythm disturbance, medical therapy, autonomic tone, and the underlying type and degree of heart disease present.

Additional Testing

Any arrhythmia analysis may require assessment of the presence of structural heart disease. This may include echocardiography, exercise testing, cardiac catheterization, noninvasive and an invasive evaluation for coronary artery disease, MRI scanning, or even CT scanning.

Part of the evaluation of the patient and the arrhythmia is to understand if the arrhythmia is a prognostic marker of something worse. Patients with an arrhythmia require an understanding of the presence or absence of structural or genetic conditions that could have consequences. The diagnosis of the underlying process becomes critical. No set evaluation is required regarding the prognosis. This depends up on the arrhythmia, the symptoms, and the conditions being evaluated.

For a middle-aged man with syncope and a left bundle branch block, the evaluation may include an echocardiogram to look for left ventricular dysfunction, a cardiac catheterization to rule out coronary artery disease, and even an electrophysiology test, whereas

Table 9 Reasons to consider electrophysiology testing

Diagnosis
Arrhythmia induction for patients with highly suspicious symptoms
Determining cause for a narrow QRS complex tachycardia/rhythm
Determining cause for a wide QRS complex tachycardia/rhythm
Assessment of the mechanism of a tachycardia
Mapping activation of and pathways responsible for a tachycardia
Evaluating level of block in the AV conduction system
Evaluating sinus node function
Prognosis
Induction of clinically significant ventricular arrhythmias
Assessment of refractoriness of accessory pathways
Assessment of speed of AV conduction during induced atrial arrhythmias

for a young woman with syncope who has delayed activation of the QRS and T-wave inversions in ECG leads V1–V3, an MRI scan may be useful to rule out the presence arrhythmogenic right ventricular dysplasia. Similarly, for a young male who has an ECG consistent with hypertrophy and syncope and there is a family history of sudden cardiac death or for a young woman with syncope who has a borderline long QT syndrome and a similar family history, genetic screening may provide some useful data even though there are no specific randomized controlled clinical trials demonstrating the need to undertake genetic screening under specific circumstances.

Many clinical decisions are difficult as they are in a gray zone, but the proper choice of therapy can have life or death consequences. Differences of opinions for aggressive therapeutic intervention can exist; therapies have their own serious consequences. Furthermore, the treatment, such as an implantable defibrillator, may not prevent the symptom itself (e.g., syncope) but even if the risks of implant are low, the necessity of its implantation is based upon its value in prolonging life in lieu of the difficulties in living with the device.

Evaluation of Patients With Implanted Devices

Interrogation of implantable devices (pacemakers and implantable cardioverter defibrillators) in the clinic or remotely can provide important information regarding the arrhythmia diagnoses (Varma et al., 2014). For a patient with an implantable defibrillator who presents with syncope, one of the first maneuvers is to interrogate the device to determine if there was an arrhythmia present at the time and what that arrhythmia may be. Furthermore, interrogation of the devices will determine if there was a failure of the pacemaker or defibrillator to act appropriately.

Device interrogation will provide some evidence for the "burden" of the arrhythmia and determine rates that may be important with regard to the need for further therapy. For example, in a patient with a high burden of atrial fibrillation, anticoagulation may be required but for a patient who is status post ablation of atrial fibrillation in whom no atrial fibrillation has been detected subsequently, the need for long-term anticoagulation may be reconsidered.

Genetic Testing

Genetic testing does not yet have an established role in the management of arrhythmia diagnosis or treatment but for select cases of patients with channelopathies or hypertrophic cardiomyopathy, for example, prognostic and even diagnostic information may be gleaned in patients with symptoms or who have borderline phenotypic expression of a condition that may be present.

Treatment (Table 10)

Once a patient is properly assessed and an arrhythmia is diagnosed or ruled out, treatment must be contemplated using drugs (for rate and rhythm control) (Epstein et al., 2016; Van Gelder et al., 2016), cardioversion (Botkin et al., 2003), device implantation (Epstein et al., 2008, 2013) (and the type (Gillis et al., 2012)), and/or ablation techniques (January et al., 2014; Page et al., 2016; Calkins et al., 2012). Specific acute (cardioversion, drugs) and chronic medical therapy (drugs, device implants, or ablation) is available for most tachycardias and to prevent adverse thromboembolic outcomes (anticoagulation and atrial appendage occlusion devices in atrial flutter and atrial fibrillation). Patients with autonomic conditions that affect blood pressure and heart rhythm are also treatable with conservative and medical therapy as well.

Treatment varies by the rhythm diagnosed, the severity and frequency of symptoms, the risk of the symptom to the patient, the risk of sudden death, the presence of underlying heart disease, the presence of comorbidities, and the general health of the patient. Options vary but treatments can include complete cure with catheter ablation technology for tachycardias, pacemakers for bradycardias, cardiac resynchronization therapy for heart failure (in patients with left bundle branch block), and defibrillators to interrupt a potentially life-threatening tachycardia or sudden cardiac death due to ventricular fibrillation based on calculated risk of reducing total mortality.

Table 10 Arrhythmia treatments

Ablation
AV junctional ablation/pacemaker
Atrial fibrillation
Atrial flutter
AV node reentry
Atrial tachycardias (including sinoatrial reentry)
Accessory pathways
Ventricular tachycardia
Bundle branch reentry
Ventricular ectopy
Ventricular fibrillation
Device implantation
Pacemaker—single, dual, triple chamber
Implantable defibrillators—single, dual, triple chamber
Drugs
Antiarrhythmic drugs (by Vaughan Williams Classification)
Class I
Ia—quinidine, procainamide, dispyramide
Ib—mexiletine, lidocaine, diphenylhydantoin
Ic—flecainide, propafenone
Class II—beta-adrenergic blockers
Class III—dofetilide, amiodarone, sotalol, dronedarone, ibutilide
Class IV—calcium channel blockers
Other antiarrhythmics—digoxin, adenosine, ivabradine, atropine
Drugs for autonomic dysfunction—droxidopa, fludrocortisone, pyridostigmine, midodrine
Anticoagulants—warfarin, rivaroxaban, apixaban, dabigatran, edoxaban, heparin

Conclusion

The evaluation of the patient who may have an arrhythmia involves careful assessment of the symptoms, consideration of underlying cardiovascular and associated comorbidities, correlation of the symptoms with the arrhythmia, risk stratification based on clinical diagnosis and arrhythmia type, and decision regarding the need to treat the symptom and reduce the risk for serious adverse outcomes. Although the field of arrhythmia management has become much more expansive and inclusive of issues related to autonomic disturbances and clinical circumstances that may or may not be due to arrhythmias, the approach to the patient starts with a careful and complete assessment based on the history and physical followed by appropriate testing that has now become fairly well established.

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Clinical Management of Inherited Arrhythmias

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Introduction

There are a number of inherited cardiac diseases that can cause sudden death. These can be broken up into two broad groups: the cardiomyopathies which are usually associated with a structural abnormality and the ion channelopathies which typically do not have cardiac structural abnormalities (Fernández-Falgueras et al., 2017). This article focuses on the inherited arrhythmia syndromes caused by ion channelopathies. While channelopathies are rare, a basic knowledge and understanding of these inherited syndromes by the general clinical professional will lead to earlier diagnosis and, potentially, prevention of sudden death. This article focuses on a review of the diagnosis and treatment of the clinically important inherited arrhythmias as well as the role of genetic testing. Clinical management in light of the 2013 expert consensus statement on inherited arrhythmia syndromes is discussed (Priori et al., 2013). Ion channelopathies are typically due to abnormalities in cardiac action potentials which are created by gradients formed by ionic currents. These predominantly involve inward currents of Na^+ and Ca^{2+}, and the main outward current of K^+. Changes in genes encoding for ionic channels can alter action potentials, electrical impulse propagation, and lead to malignant arrhythmias and death. The most common channelopathies related to sudden death are Brugada syndrome (BrS), long QT syndrome (LQTS), short QT syndrome (SQTS), and catecholaminergic polymorphic ventricular tachycardia (CPVT) (Nerbonne and Kass, 2005). Although a long list of genes have been attributed to each of these conditions, some have more supporting evidence for linkage than others and the yield of genetic testing varies significantly across these conditions. In addition, some genes have been linked to multiple clinical syndromes (**Fig. 1**), sometimes within the same families.

Inherited arrhythmia syndromes are usually suspected after a presentation with syncope, aborted cardiac arrest, or a family history of sudden death. Some patients are diagnosed based on incidental findings on electrocardiograms. These conditions, however, are often misdiagnosed, particularly when the diagnostic testing is difficult to interpret (Taggart et al., 2007; Viskin et al., 2005). There are current diagnostic criteria which have been identified to help in the diagnosis of these conditions. At our institution, patients are referred for evaluation of sudden cardiac arrest or sudden death in a family member; our evaluation is summarized in **Table 1**.

One of the most important first steps in the diagnostic workup in a patient with a suspected inherited arrhythmic condition is to rule out underlying cardiomyopathy. At the Stanford Center for Cardiovascular Diseases, we have found that patients with hypertrophic cardiomyopathy, for example, have a 25% likelihood of having a corrected QT interval greater than 480 ms (unpublished data).

If there are abnormalities in the initial workup, then further testing is usually obtained which may include an MRI and genetic testing. As a general rule, targeted genetic testing is performed after the clinical diagnosis of a disease process has been made. However, in cases of cardiac arrest or sudden death where an inherited arrhythmia syndrome is suspected but cannot be definitively diagnosed, broader genetic testing can be considered. When considering genetic testing, referral to an inherited arrhythmia center where genetic counseling can be an integral part of the evaluation is recommended. Genetic testing can be useful but the results should be interpreted carefully and in context of the clinical presentation. A well-established pathogenic variant can occasionally be helpful in establishing a clinical diagnosis, but its utility and cost-effectiveness are greatest in the subsequent screening of family members (Perez et al., 2011). For most other genetic abnormalities, interpretation must be carefully made in context of the clinical presentation and the evidence for pathogenicity in existing literature and population datasets.

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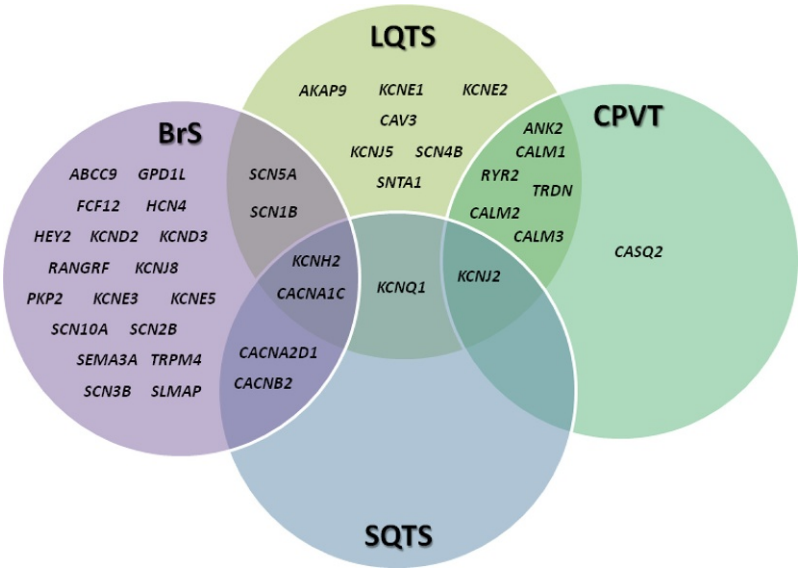

Fig. 1 Overlap in the link between different genes and inherited arrhythmias. (Used with permission, Fernández-Falgueras, A., Sarquella-Brugada, G., Brugada, J., Brugada, R. and Campuzano, O. (2017). Cardiac channelopathies and sudden death: Recent clinical and genetic advances. *Biology (Basel)* **6**(1), 7. http://dx.dy.org/10.3390/biology6010007.

Table 1 Stanford Center for Inherited Cardiovascular Disease: family screening recommendations following a sudden unexplained cardiac arrest or sudden unexplained death

	Children	*Adults*
Family members at 50% risk, Obligate carriers	*Initial evaluation* 0–18 years old – Medical history, physical exam, family pedigree – Non-fasting or fasting lipid panel with direct LDL – Resting EKG, stand-up EKG, Brugada-protocol EKG – Echo – Exercise test – Ambulatory telemetry (24 Hour Holter) *Reflex Tests* – If any abnormalities on echo, reflex to MRI – If death was during sleep, SE Asian ancestry, or suspicious *SCN5A* variant, perform procainamide challenge (even if resting EKG is normal) – If Type 2 or 3 Brugada pattern on resting EKG, reflex to procainamide challenge – If suspect ARVC (based on imaging, or high PVC burden), perform signal average EKG *Ongoing Screening* Tests: – Resting EKG, stand-up EKG, Brugada-protocol EKG – Echo – Exercise test Interval: Every 2–3 years For competitive athletes: Yearly For families with multiple unexplained sudden cardiac deaths: Every 2 years	*Initial evaluation* >18 years old – Medical history, physical exam, family pedigree – Nonfasting or fasting lipid panel with direct LDL – Resting EKG, stand-up EKG, Brugada-protocol EKG – Echo – Exercise test – Ambulatory telemetry (24 Hour Holter, or 2-week Zio Patch if symptoms) – Signal average EKG *Reflex Tests* – If any abnormalities on echo, reflex to MRI – If death was during sleep, SE Asian ancestry, or suspicious *SCN5A* variant, perform procainamide challenge (even if resting EKG is normal) – If Type 2 or 3 Brugada pattern on resting EKG, reflex to procainamide challenge *Ongoing Screening* Tests: – Resting EKG, stand-up EKG, Brugada-protocol EKG – Echo – Exercise test Interval: Every 5 years For competitive athletes: Yearly through college, then every 2 years For families with multiple unexplained sudden cardiac deaths: Every 2 years

Long QT Syndrome

LQTS was first identified in the 1950s in a family with siblings who were deaf and had a prolonged QT interval. This was termed Jervell–Lange-Nielson syndrome and later found to be a recessive form of the disorder (Jervell and Lange-Nielsen, 1957). LQTS is characterized by a prolongation of the QT interval associated with an elevated risk of sudden cardiac death. Fifteen genes have been linked to LQTS, with most families following an autosomal dominant pattern of inheritance. LQT1, LQT2, and LQT3 linked to rare genetic variants in KCNQ1, KCNH2, and SCN5A, respectively, make up more than 92% of patients with the diagnosis of LQTS.

The yield of genetic testing in clinically definitive cases of LQTS is approximately 75%, which is the highest among the inherited arrhythmic disorders (Priori et al., 2013). There is variable penetrance within family members with the same gene mutation. LQTSs are numbered to reflect the order in which new genes were linked to the condition. However, LQT types are sometimes referred by the researchers who identified them: Jervell and Lange-Nielson syndrome (long QT associated with deafness), Andersen–Tawil syndrome (neurologic/musculoskeletal manifestations associated with long QT), and a general term of Romano–Ward syndrome (long QT without deafness) (**Table 2**) (Kaufman, 2011).

Patients typically present with an incidentally identified prolonged QT interval, an arrhythmic event, or a family history of LQTS. Patients who present clinically often have multiple episodes of syncope which place patients at risk for cardiac arrest and sudden death.

Identifying clinical triggers of syncope can suggest a specific genotype of LQTS. LQT1 is classically associated with syncope/arrhythmic events during exercise or stress, LQT2 is associated with syncope/arrhythmic events with sudden noise, and LQT3 is associated with arrhythmic events during acoustic arousal from sleep (Kaufman, 2011). Higher arrhythmic risk correlates with the length of the QT interval, particularly above a corrected QT of 500 ms (Priori et al., 2003).

Morphology of the EKG can also give clues to cardiac risk and suggest genetic variants. The T-wave of LQT1 is typically broad based and monophasic. The T-wave of LQT2 is typically a broad, notched T-wave. LQT3 is associated with a long, flat ST segment and a relatively normal appearing T-wave (Zhang et al., 2000) (**Fig. 2**). The EKG of LQT3 and hypocalcemia can appear very similar. In addition, T-wave alternans is a worrisome finding and denotes electrical instability (Paulussen et al., 2004; Schwartz and Malliani, 1975).

Clinical Diagnosis

LQTS can, at times, be challenging to diagnose. There is an overlap between the higher range of QTc values in normal subjects, and the lower end of values in patients with LQTS. In family members of patients with LQTS, a normal QTc does not exclude the inherited abnormality as up to 25% of those affected can have a normal QTc (Schwartz and Crotti, 2011). The normal QTc range is different for men and women and may be due to hormonal differences. The 99th percentiles for men and women are 470 and 480 ms, respectively (Fernández-Falgueras et al., 2017). However, LQTS is a spectrum and there are those with the inherited defect at risk who have QTc less than the 99th percentile, making it more difficult to diagnose. In the absence of a secondary cause for QT prolongation, LQTS can be diagnosed with a QT_C greater than 500 ms on repeated EKGs. When LQTS is suspected, but the QT_c is less than 500 ms, a LQTS risk score such as the Schwartz score can be used (**Table 3**). This accounts for EKG findings, clinical history, and family history. A score greater than 3.5 meets the criteria for diagnosis (Schwartz et al., 1993; Schwartz and Crotti, 2011)****. Presence of a clearly pathogenic rare genetic variant in one of the LQTS-related genes can also be diagnostic.

Table 2 Subtypes of LQTS and their associated genes, proteins and functional effects

LQT subtype	*Gene*	*Protein*	*Current*
LQT1	*KCNQ1*	KCNQ1 (Kv7.1)	$\downarrow I_{Ks}$
LQT2	*KCNH2*	hERG (Kv11.1)	$\downarrow I_{Kr}$
LQT3	*SCN5A*	Na_v 1.5	$\uparrow I_{Na}$
LQT4 (ankyrin-B syndrome)	*ANK2*	Ankyrin-B	Multichannel interactions
LQT5	*KCNE1*	KCNE1 (minK)	$\downarrow I_{Ks}$
LQT6	*KCNE2*	KCNE2 (MiRP1)	$\downarrow I_{Kr}$
LQT7 (Andersen-Tawil syndrome type 1)	*KCNJ2*	K_{ir}2.1	$\downarrow I_{K1}$
LQT8 (Timothy syndrome)	*CACNA 1C*	Ca_v 1.2	$\uparrow I_{Ca}$
LQT9	*CAV3*	Caveolin 3	$\uparrow I_{Na}$
LQT10	*SCN4B*	Na_v 1.5 β4	$\uparrow I_{Na}$
LQT11	*AKAP9*	AKAP-9 (yotiao)	$\downarrow I_{Ks}$
LQT12	*SNTA1*	α1-Syntrophin	$\downarrow I_{Na}$
LQT13	*KCNJ5*	K_{ir}3.4 (GIRK4)	$\downarrow I_{KACh}$
LQT14	*CALM1*	Calmodulin	Multichannel interactions
LQT15	*CALM2*	Calmodulin	Multichannel interactions

(Used with permission, Bohnen, M. S., Peng, G., Robey, S. H., et al. (2017). Molecular pathophysiology of congenital long QT syndrome. *Physiological Reviews* **97**(1), 89–134. https://doi.org/10.1152/physrev.00008.2016.)

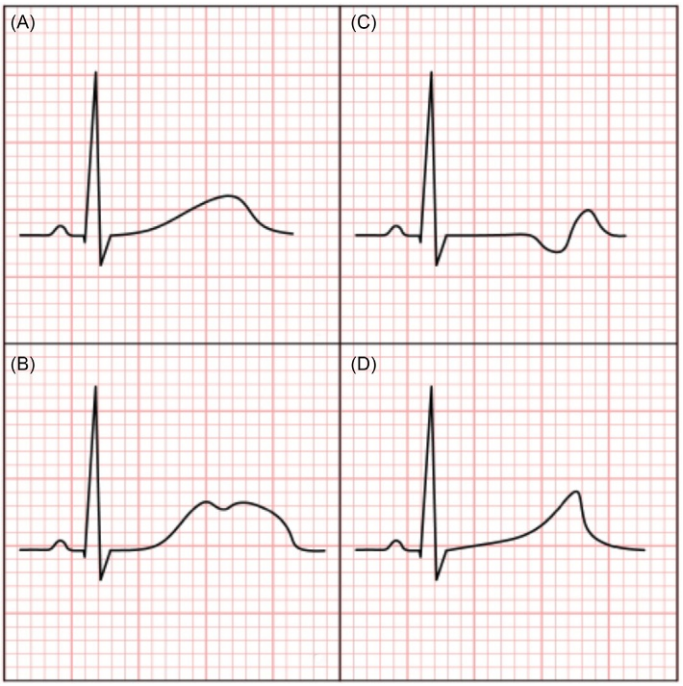

Fig. 2 T-wave configurations in the most common LQTS (A) LQT1 associated with a broad-based T-wave. (B) LQT2 with bifid or notched T-waves. LQT3 is associated with (C) biphasic or (D) asymmetrical and peaked T-waves. (Used with permission, Gomez, A. T., Prutkin, J. M. and Rao, A. L. (2016). Evaluation and management of athletes with long QT syndrome. *Sports Health***8**(6), 527–535. doi: 10.1177/1941738116660294; Chorin, E., Havakuk, O., Adler, A., et al. (2015). Diagnostic value of T-wave morphology changes during "QT stretching" in patients with long QT syndrome. *Hear Rhythm***12**(11), 2263–2271. https://doi.org/10.1016/j.hrthm.2015.06.040.

Table 3 1993–2011 LQTS diagnostic criteria

	Points
Electrocardiographic findings[a]	
A. QTc[b]	
≥480 ms	3
460–479 ms	2
450–459 ms (in males)	1
B. QTc[b] 4th minute of recovery from exercise stress test ≥480 ms	1
C. Torsade de pontes[c]	2
D. T-wave alternans	1
E. Notched T-wave in three leads	1
F. Low heart rate for age[d]	0.5
Clinical history	
A. Syncope[c]	
With stress	2
Without stress	1
B. Congenital deafness	0.5
Family history	
A. Family members with definite LQTS[e]	1
B. Unexplained sudden cardiac death below age 30 among immediate family members[e]	0.5

[a]In the absence of medications or disorders known to affect these electrocardiographic features.
[b]QTc calculated by Bazett's formula where QTc = QT/√RR.
[c]Mutually exclusive.
[d]Resting heart rate below the 2nd percentile for age.
[e]The same family member cannot be counted in A and B.
SCORE: ≤1 point: low probability of LQTS; 1.5–3 points: intermediate probability; ≥3.5 points high probabilty.
(Used with permission, Schwartz, P. J. and Crotti, L. (2011). QTc behavior during exercise and genetic testing for the long-QT syndrome. *Circulation* **124**(20), 2181–2184. https://doi.org/10.1161/CIRCULATIONAHA.111.062182.)

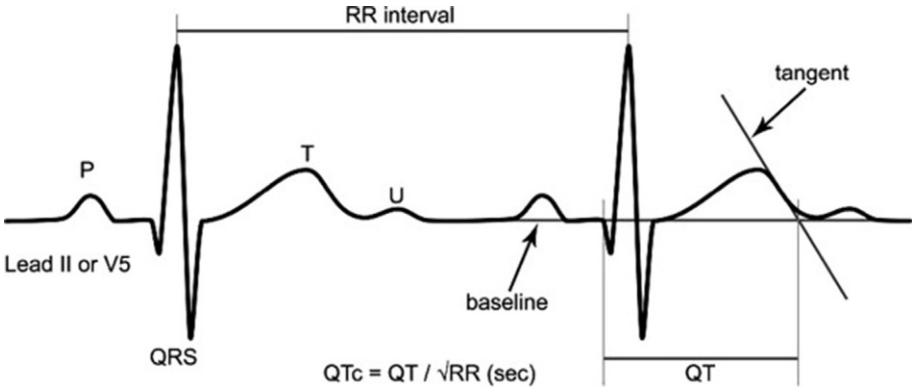

Fig. 3 Measuring the QT interval using the intersection of the tangent method. (Used with permission, Postema, P. G., De Jong, J. S. S. G., Van der Bilt, I. A. C. and Wilde, A. A. M. (2008). Accurate electrocardiographic assessment of the QT interval: Teach the tangent. *Hear Rhythm* **5**(7), 1015–1018. http://dx.dy.org/1018.10.1016/j.hrthm.2008.03.037.)

The mainstay for diagnosis of LQTS is, of course, measuring a prolonged QT on EKG. While in theory this seems relatively easy, there remains some controversy regarding the best way to measure the QT and the best heart-rate-correction formula to use. The 2009 consensus statement on interpretation of EKGs recommends using lead V2 as the mainstay of measuring the QT (Rautaharju et al., 2009). Many of the studies on LQTS have used lead V5 and lead II when measuring the QT and most use Bazzett's formula for correction. For these reasons, this is predominantly the strategy we use at our institution. Nevertheless, in some EKGs, particularly when the T-wave is flat, it can be unclear where the T-wave begins and ends. In these instances, it is sometimes necessary to use a contiguous lead in which the T-wave is more prominent, though this has not yet been validated. The most reliable and reproducible approach in measuring the QT interval is to measure from the beginning of the QRS to the point where the tangent of the initial downward slope of the T-wave meets the horizontal line of the TP segment (**Fig. 3**), and to exclude any U-waves (Postema et al., 2008). Since the QT may be dynamic, it is often helpful to take an average over several beats and to perform ECG measurements on multiple encounters, particularly when the QT prolongation is borderline.

Identifying a prolonged QT interval in the presence of bundle branch blocks and interventricular conduction delay becomes more difficult and problematic. There is controversy on the best approach. One strategy is to use the measurement of the JT interval and identify patients with JTc greater than 360 ms as abnormal. Another is to use the standard QT_c, but use 500 ms as the upper limit of normal. Similarly, challenges remain in correctly measuring the QT interval during atrial fibrillation, without consensus on the most accurate approach. New methods are being devised and validated and it remains to be seen which will become the standard.

To help unmask a prolonged QT, provocative maneuvers can be performed. In patients with LQTS, particularly LQT1, the absolute QT interval tends to remain fixed while the RR interval shortens during tachycardia, resulting in an exaggerated prolongation of the QTc interval. Exercise stress test and measuring the QT interval 3–5 min into recovery, while the heart rate is still elevated, can be helpful. An EKG performed while lying and followed by one after standing for several minutes can also raise the heart rate and can bring out evidence for LQTS (Adler et al., 2012; Wong et al., 2010).

Treatment

One of the hallmarks of treatment is to avoid medications that can prolong the QT. Online resources that maintain updated lists of QT-prolonging medications are available (https://www.qtdrugs.org). Electrolyte abnormalities can exacerbate long QT, correcting these imbalances is important. B-Blockers are the mainstay of medical therapy, even for asymptomatic patients with inherited LQTS to help reduce the patient's sudden death risk. Adrenergic surges modulate repolarization and affect potassium, especially IKs, and calcium handling. Blunting these surges is, at least partially, responsible for the effectiveness of B-blockers and that is why B-blocker therapy is most effective in LQT1, fairly effective in LQT2, but less effective in LQT3. Risk reduction is as high as 67% in LQT1 males and 71% in LQT2 females (Bohnen et al., 2017). A family history of sudden death does not necessarily convey higher risk. ICD implantation is generally indicated in patients who have survived cardiac arrest or who have had recurrent syncope despite B-blocker therapy. As adrenergic drive is a driving trigger, in those who decline ICD therapy or are unable to tolerate beta blocker therapy, left cardiac sympathetic denervation is recommended. Finally, there is mounting evidence that sodium channel blockers can shorten the QT and reduce symptoms in those with a diagnosis of LQT3 (Mazzanti et al., 2016). This is one of the few examples in cardiovascular medicine where genotype can be used to guide medical intervention.

Brugada Syndrome

Brugada syndrome was first described in 1992 by Dr. Brugada (Brugada and Brugada, 1992). The syndrome was characterized by the EKG finding of right bundle branch block with persistent ST elevation and sudden death. Although the prevalence of Brugada syndrome is higher in Southeast Asian populations, it is not limited to this group. There the prevalence of the syndrome is approximately 1–5 in 10,000 in Europe and 15 in 10,000 in Southeast Asia (Gallagher et al., 2008; Rattanawong et al., 2017). The prevalence of the EKG pattern in a meta-analysis was 0.2% in North America and 0.9% in Asia with a worldwide prevalence of 0.4% (Quan et al., 2016). It is found predominantly in the male population with an estimated 9:1 male-to-female ratio (Gallagher et al., 2008; Wilde et al., 2002). Some suggest the male predominance is related to testosterone levels and the more prominent Ito channel found in males (Shimizu et al., 2007; Di Diego et al., 2002).

Clinical Diagnosis

The diagnosis of Brugada syndrome relies primarily on the characteristic EKG morphologies seen in the precordial leads. Although it was previously necessary to manifest a Brugada ECG pattern in the setting of symptoms such as syncope or ventricular arrhythmias to meet diagnostic criteria for Brugada *syndrome*, there was a shift in definition with the 2013 inherited arrhythmia scientific statement. A type I Brugada *pattern* is now necessary and sufficient for diagnosis of BrS. Importantly, there are a number of conditions that can present with Brugada-like patterns. Therefore, when the pattern is present, other conditions should be excluded before a diagnosis of Brugada syndrome is made. Some of these conditions include atypical RBBB, early repolarization, aortic aneurysm dissections, electrolyte disorders, mechanical distortion of the RVOT as it can occur with tumors, pneumothorax, pectus excavatum, muscular dystrophy, myotonic dystrophy, pulmonary embolism, and rarely angina (Shimizu, 2005).

To meet the diagnostic criteria for Brugada syndrome, a patient must demonstrate a type 1 morphology which either occurs spontaneously or is induced with sodium channel blockers and exclusion of other causes of an abnormal EKG as listed above. A type 1 pattern is defined as greater than or equal to 2 mm of ST elevation in leads V1 or V2 with a coved pattern with the leads positioned in the second, third, or fourth intercostal space (**Fig. 4**). Type 2 or 3 Brugada patterns are nonspecific findings, however, in patients with symptoms or family history suspicious for BrS, a provocative test with sodium channel blockers to induce a type one pattern can be performed to establish the diagnosis. A type 2 or 3 Brugada pattern without inducibility of a type one pattern has not been shown to increase risk for sudden death. Other findings that can favor Brugada syndrome include abnormal signal averaged EKG, history of atrial fibrillation, the presence of AV block, and ventricular effective refractory periods less than 200 ms (Priori et al., 2013).

Worrisome clinical symptoms associated with this syndrome include aborted sudden cardiac death, ventricular fibrillation, and syncope. Patients may also complain of symptoms related to ventricular arrhythmias that include palpitations and chest pain. These symptoms typically arise during febrile states or vagotonic conditions and rarely happen during periods of exercise where the adrenergic drive is elevated. In some cases, BrS may be associated with structural abnormalities (Juang and Lin, 2016).

Brugada syndrome is inherited in an autosomal dominant fashion with multiple genes linked to its phenotypic expression. However, the yield of clinical genetic testing is approximately 30%, which is one of the lowest among the inherited arrhythmic syndromes. Nevertheless, genetic testing is recommended in patients with an established history of BrS (Ackerman et al., 2011). The gene with rare variants most commonly attributed to BrS is *SCN5A*, found in up to 18% of individuals with this diagnosis. This gene

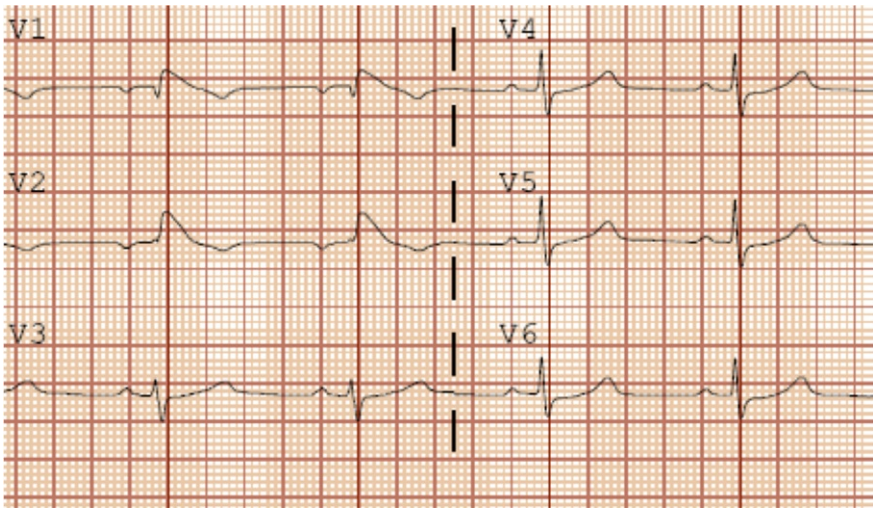

Fig. 4 Precordial leads from patient with a Type 1 Brugada pattern, inducible ventricular tachycardia and a pathogenic variant in *SCN5A* p.Glu1784Lys (c.5350G>A).

encodes the alpha subunit of the cardiac sodium channel and causes a loss or decrease in function (Berne and Brugada, 2012). The use of sodium channel blockers, like procainamide, will exacerbate the typical EKG patterns.

Treatment

The basic treatment for Brugada syndrome, as with all of the channelopathies, is focused on ameliorating sudden death risk. The highest risk marker is a history of sudden cardiac arrest or syncope due to ventricular arrhythmia with a yearly risk of SCD of 10% and 5%, respectively. Consensus recommendations are for these patients to have an ICD placed (Gourraud et al., 2017). ICD therapy can be useful in patients with Brugada syndrome and history of syncope without clear cause which is judged to be potentially due to ventricular arrhythmias. Patients who are otherwise asymptomatic have less than 1% annual risk and defibrillators are not recommended in these individuals.

Electrophysiologic testing is controversial with regard to helping risk stratify asymptomatic patients with a Brugada pattern. While the strategy of placing ICDs in patients who have inducible VF at EPS can be considered, there are conflicting studies arguing for its utility (Gourraud et al., 2017; Priori et al., 2012). Family history of sudden death alone lacks prognostic significance in Brugada syndrome in part due to highly variable phenotypic penetrance (Eckardt et al., 2005). A class III recommendation has been made, stating that an ICD should not be placed based on a family history of sudden death without any of the other high risk markers as stated above (Priori et al., 2013).

Although there is no ideal medical therapy for this syndrome, quinidine may be considered in patients with recurrent ventricular arrhythmias or those who are unable to have a device implant. This medication, however, is not well-tolerated by most patients. For those who present in arrhythmic storms, which may be related to enhanced vagal tone, isoproterenol may be helpful acutely. Epicardial ablation of the RVOT has shown some promise as an adjuvant therapy in patients with refractory ventricular tachycardias (Belhassen, 2016).

Catecholaminergic Polymorphic Ventricular Tachycardia

CPVT is an adrenergically mediated syndrome causing ventricular tachycardia and ultimately ventricular fibrillation leading to sudden death. Typically, structural evaluation demonstrates a normal heart with the first description of this entity described in the 1960s (Berg et al., 1960). The hallmark of this disease process is the presence of premature ventricular beats which increase in frequency with exercise and have more than one morphology. A finding of bidirectional ventricular tachycardia during exercise is highly suggestive of this condition (**Fig. 5**), though this has also been reported in patients with digitalis toxicity or Anderson Tawil syndrome (LQT7).

The true prevalence is unknown but estimated at 1:10,000 individuals. Typically, patients present in early youth around age 10 typically have structurally normal hearts. However, the first presentation can occur in the third and fourth decades of life (Liu et al., 2008). There is an autosomal dominant inheritance pattern with rare variation in the ryanodine receptor accounting for the majority of cases. An autosomal recessive inheritance pattern has been reported with rare variation in cardiac calsequestrin. The yield of genetic testing in clinically definitive CPVT is approximately 50%. A diagnosis of CPVT can be made with evidence of a clearly pathogenic mutation and in family members of diagnosed individuals with exercise-induced premature ventricular contractions. While it is uncommon to diagnose this in individuals older than the age of 40, if patients have a structurally normal heart and polymorphic exercise-induced premature beats, without other clear etiology, then they would meet criteria for the diagnosis.

Treatment

Current treatment focuses on decreasing adrenergic stimulation. Therefore, beta-blockers are recommended in all symptomatic individuals and should be considered for those who are not symptomatic. Nadolol is the typical B-blocker used due to the long-acting nature of the drug and its lack of intrinsic sympathomimetic activity. Therapy should be intensified if ventricular couplets or more are seen on exercise testing as some studies show an association with arrhythmic events (Hayashi et al., 2009). It is also recommended to avoid strenuous activity and stressful environments to decrease the adrenergic stimulation. If patients have

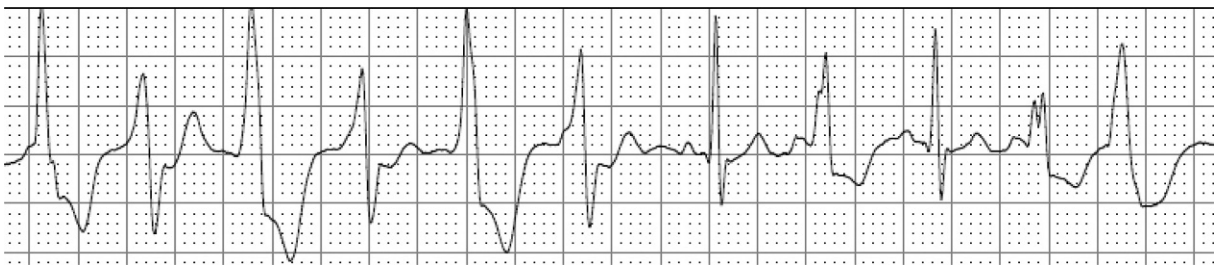

Fig. 5 Bi-directional ventricular tachycardia noted in a patient who had a history of cardiac arrest during rhinoplasty after epinephrine infusion.

experienced cardiac arrest, syncope, or VT on beta blocker therapy, then an ICD is indicated (Priori et al., 2013). However, some question the role of ICD as defibrillation can lead to catecholamine surges which in turn increase the arrhythmia and can lead to poorer outcomes (Miyake et al., 2013). There is mounting evidence that flecainide should be added to B-blocker therapy in those with persistent arrhythmias on B-blocker therapy due to improved outcomes (Roston et al., 2017). For those refractory to or intolerant of therapy, then left cardiac sympathetic denervation can be considered.

Short QT Syndrome

SQTS is a relatively newly defined inherited channelopathy. At the turn of this century, a few families who had a history of early sudden cardiac death were found to also have a short QT (Gaita et al., 2003; Gussak et al., 2000). This syndrome, too, has an autosomal dominant inheritance. The estimated prevalence of this syndrome is less than 1 in 10,000 with a short QT_c defined as less than 330 ms, well below two standard deviations of normal. The diagnosis can be made with a slightly longer QTc (360 ms or less) with one of the following markers: family history of short QT, family history of sudden death younger than 41 years old, a pathogenic mutation or survival of ventricular tachycardia, or ventricular fibrillation arrest. Gollob *et al*. have proposed a scoring system for diagnosis of short QT which has not yet been included in current guidelines (Gollob et al., 2011). The most common forms of SQTS have been shown to be related to potassium channel genes which are also associated with LQTS. As opposed to loss of function causing long QT, a gain of function in these genes causes SQTS (Bellocq et al., 2004; Priori et al., 2005; Brugada et al., 2004). However, loss of function mutations has also been identified in the L-type calcium channel which are also associated with shortening of the QT (Antzelevitch et al., 2007). In contrast to LQTS, SQTS does not appear to have focal triggers like exercise, noise, or sleep (Giustetto et al., 2006).

Treatment

ICDs are indicated in patients with a short QT and who are survivors of cardiac arrest or have documented episodes of sustained VT. Given the small cohort of patients with this syndrome, it becomes challenging to study. Other treatments may be helpful but are not well validated. Although ICD may be considered in patients with short QT and family history of sudden death, this has not been validated. At this point, flecainide, sotalol, ibutilide, and hydroquinidine have all been studied in SQTS. Of those, only hydroquinidine showed some promise in effectiveness (Giustetto et al., 2011).

Conclusion

Technological advances and improvement in genotyping have improved the ability to identify individuals at risk for sudden cardiac death from arrhythmias. Identification of these patients can still be problematic as the inherited channelopathies are relatively rare. More genes continue to be identified and treatment options continue to be refined. Once diagnosis is made, often questions arise regarding screening of relatives. Once identified, first-degree relatives and second-degree relatives engaged in athletic competition should be screened. While specific genetic markers are helpful, a pathogenic variant is not identified in all cases. When an inherited arrhythmia is suspected, such as in cases of sudden cardiac arrest or death, our current practice is to evaluate relatives with an initial screening to include exercise EKG, lying and standing EKG, modified Brugada ECG, signal averaged EKG, and a structural evaluation. Targeted genetic testing is done if family members are found to be affected, otherwise, broad genetic testing is recommended in the proband. If no gene has been identified, interval follow-up is recommended to evaluate for future phenotypic expression.

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Clinical Trials for Atrial Fibrillation—What Do We Know?

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Key Messages

- Rate control is not inferior to pharmacologic rhythm control with respect to mortality, heart failure, and stroke outcomes. However, pharmacologic rhythm control is only at most 70% successful at maintaining sinus rhythm. Ongoing trials are assessing the efficacy of catheter ablation strategies with respect to "hard" cardiovascular outcomes.
- Regardless of whether a rate- or rhythm-control strategy is chosen, the most important aspect of atrial fibrillation management remains stroke prevention.
- The development of direct oral anticoagulants (DOACs) has significantly advanced the management of AF patients. The four currently available DOACs (i.e., dabigatran, rivaroxaban, apixaban, and edoxaban) have at least equal efficacy as warfarin with respect to stroke prevention. In addition, dabigatran (at a dose of 150 mg twice daily) and apixaban are superior to warfarin. All four DOACs have a significantly lower risk of developing intracranial hemorrhage.
- The goal of upstream therapies is to delay or even reverse the natural progression of AF by targeting factors leading to atrial remodeling. There is randomized control trial evidence supporting lifestyle modification, such as weight loss, in reducing the frequency and burden of atrial fibrillation.

Introduction

The breadth and depth of atrial fibrillation (AF) clinical research have grown exponentially, advancing our understanding and management of patients with AF (**Fig. 1**) (Nishida and Nattel, 2014). Over this past decade alone, clinical trials testing direct oral anticoagulants and left atrial appendage (LAA) closure devices have dramatically increased the therapeutic options for stroke prevention. Other studies have helped to refine the role of and approach to catheter ablation for rhythm management (Gillis et al., 2013).

Yet despite this accumulating body of knowledge, the management of patients with AF continues to be challenging with many clinical questions still unanswered. Fortunately, advancement in our understanding of the basic mechanisms underlying AF and the development of new technologies promise for unprecedented opportunities, which will likely revolutionize AF management in the decades ahead (Nattel and Harada, 2014; Iwasaki et al., 2011).

In this article, we will review the landmark clinical trials supporting the current management of AF. In particular, we will discuss (a) stroke prevention in AF; (b) rate control versus rhythm control; and (c) upstream therapies for preventing the progression of AF including recent evidence supporting exercise and lifestyle modification. We will also discuss current knowledge gaps in the management of AF and ongoing clinical trials that hope to address areas of clinical equipoise.

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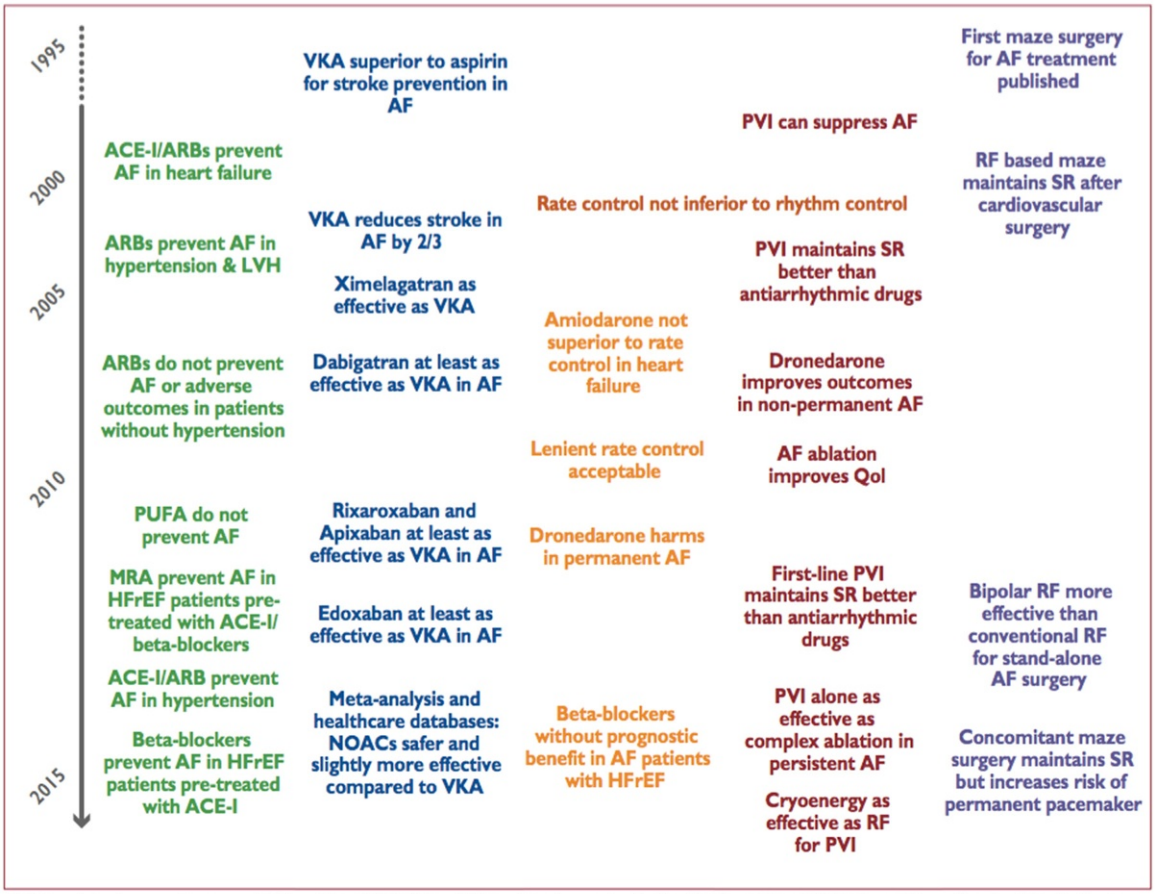

Fig. 1 Timeline of landmark trials in atrial fibrillation. Major findings of landmark trials include treatment of concomitant conditions and upstream therapies (*green*), anticoagulation (*blue*), rate-control therapy (*orange*), rhythm-control therapy (*red*), and atrial fibrillation surgery (*purple*). Reproduced from Kirchhof et al. (2016).*European Heart Journal 37*, 2893–2962 by permission of the European Society of Cardiology.

Anticoagulation for Stroke Prevention in Nonvalvular AF

The association between AF and thromboembolism has long been known (Fisher, 1972). The risk of AF-associated ischemic stroke or systemic embolism is quite variable based on known and emerging risk factors (Gage et al., 2001, 2004; Lip et al., 2010; Hijazi et al., 2013; Oldgren et al., 2016), but appears to be largely independent of the pattern of AF episodes. The substantial benefits of antithrombotic therapy for reducing the occurrence of stroke, systemic embolism, and mortality in patients with nonvalvular AF have been demonstrated in a series of trials of various agents beginning in the late 1980s.

Acetylsalicylic Acid

Low-dose acetylsalicylic acid (ASA) was the first agent to be tested in multiple large, well-designed clinical trials. A 2007 metaanalysis of these studies found that ASA, compared to placebo or no therapy, did not significantly reduce the incidence of stroke or systemic embolism relative risk reduction (RRR) 19% (95% CI −1.0 to 35.0) (Hart et al., 2007). Further, ASA had little effect on reducing the risk of disabling stroke. However, when the analysis was limited to placebo-controlled trials, there was a statistically significant 22% RRR (95% CI 2%–39%) of ischemic and hemorrhagic stroke in patients receiving ASA compared to placebo.

In contrast to warfarin and DOACs, to be discussed later, there appears to be a diminishing role for ASA monotherapy for the prevention of thromboembolic events in patients with nonvalvular AF. Whether ASA is a reasonable antithrombotic monotherapy in very low-risk AF patients (i.e., $CHADS_2 = 0$) has not been well addressed, given that this population is underrepresented in the available clinical trials (Hart et al., 2007; Cooper et al., 2006). This clinical equipoise is reflected in the slightly varying guideline recommendations by major cardiovascular societies in North America and Europe (Kirchhof et al., 2016; Skanes et al., 2012;

January et al., 2014). For example, the 2016 Canadian Cardiovascular Society guidelines for the management of AF recommends use of ASA monotherapy only for patients who are at low to intermediate risk of stroke and have an independent indication for its use such as coronary artery disease (Macle et al., 2016).

Warfarin

Previously, the vitamin K antagonist warfarin was suggested before cardioversion in some patients, but its long-term use was not advocated unless patients had persistent AF in the setting of rheumatic heart disease (Prystowsky, 2008; Wood, 1968). However, the early 1990s witnessed the publication of five landmark trials establishing the benefit of long-term warfarin for stroke prevention in nonvalvular AF: the Atrial Fibrillation, Aspirin, Anticoagulation study (AFASAK), Stroke Prevention in Atrial Fibrillation studies (SPAF I and II), the Boston Area Anticoagulation Trial for Atrial Fibrillation (BAATAF), the Canadian Atrial Fibrillation Anticoagulation study (CAFA), and the Stroke Prevention in Nonrheumatic Atrial Fibrillation study (SPINAF) (BAATAF Investigators, 1990; Investigators SPiAF, 1991, 1994; Ezekowitz et al., 1992; Connolly et al., 1991; Petersen et al., 1989). In a collaboration among the authors of these five trials, the Atrial Fibrillation Investigators pooled their data to conduct a patient-level metaanalysis in 1994 (Investigators, 1994). Together, these trials enrolled about 3000 AF patients with a mean age of 69 years at time of randomization, and prevalence of hypertension and diabetes was 46% and 14%, respectively. Few patients (4.5%) had a prior transient ischemic attack (TIA) or stroke. The annual stroke rate of 4.5% in the control group was reduced to 1.4% in the warfarin group, a RRR of 68% (95% CI, 50%–79%; $p < 0.001$). Furthermore, warfarin was associated with a reduction in all-cause mortality compared to controls (3.6% vs. 5.4%, $p = 0.01$). The annual rate of major hemorrhage (defined as intracranial bleeding or a bleed requiring hospitalization or transfusion of two or more units of blood) was 1.3% for the warfarin group compared a rate of 1.0% for the controls. In summary, warfarin resulted in clinical significant reductions in stroke and death that was not offset by a small absolute increase in hemorrhagic stroke (Investigators, 1994).

The efficacy of dose-adjusted warfarin was also compared with ASA and other antiplatelet agents in several subsequent trials. In a metaanalysis of these studies, warfarin was the superior agent in reducing stroke or systemic embolism, conferring an additional RRR of 52% (95% CI 41%–62%) (Hart et al., 2007).

Subsequent studies assessed the benefit of warfarin in important high-risk subgroups that were underrepresented in the initial Atrial Fibrillation Investigator studies. For example, only 6% of patients enrolled by the Atrial Fibrillation Investigators had a history of TIA or ischemic stroke. The European Atrial Fibrillation Trial (EAFT) ($n = 1007$) sought clarify the efficacy of warfarin or ASA compared to placebo in patients with nonrheumatic AF who had experienced a TIA or stroke within the preceding 3 months (EAFT, 1993). Warfarin led to a highly significant RRR of 66% in annual stroke rate compared to placebo (i.e., an almost identical RRR as in the Atrial Fibrillation Investigator pooled cohort), with an 8% absolute risk reduction in this higher risk group.

Another area of uncertainty that was not addressed by the Atrial Fibrillation Investigators was the benefit of anticoagulation in the elderly AF population (Investigators, 1994). The Birmingham Atrial Fibrillation Treatment of the Aged (BAFTA) Trial was a randomized, open-label study of warfarin compared to ASA in 973 AF patients who were aged 75 years and greater (mean 81.5 years) (Mant et al., 2007). Over a mean follow-up of 2.7 years, warfarin reduced the annual risk of the primary composite endpoint (i.e., fatal or disabling stroke, intracranial hemorrhage, and clinically significant arterial embolism) from 3.8% to 1.8% ($p = 0.003$). There was no difference in prevention of nonstroke vascular events or overall mortality between the warfarin and ASA treatment arms. Surprisingly, the annual risk of major hemorrhage was similar between warfarin (1.4%) and ASA (1.6%). However, it is possible that greater adherence to randomized therapy may have demonstrated a significant difference in bleeding; one third of patients in the anticoagulation treatment arm discontinued warfarin.

Alternatives to Adjusted-Dose Warfarin, Act 1: Low-Dose Warfarin or Clopidogrel

Prior to the randomized trials of direct oral anticoagulants (DOACs) agents, several trials explored alternative anticoagulation strategies with the intent of minimizing the risk of major bleeding, yet maintaining the benefits of stroke and thromboembolism prevention. Various alternative strategies were studied including low-dose warfarin plus ASA, and ASA plus clopidogrel.

The Stroke Prevention in Atrial Fibrillation (SPAF) III trial enrolled 1044 patients with nonvalvular AF and at least one thromboembolic risk factor (Investigators SPiAF, 1996). Patients were randomized to low intensity fixed-dose warfarin plus ASA 325 mg daily, or to adjusted-dose warfarin (INR 2.0–3.0). The trial was terminated early due to the excess of ischemic stroke and systemic embolism in the combination therapy group (7.9% vs. 1.9% per year, $p < 0.0001$). The rates of major and intracranial bleeding were similar in the two treatment groups. After additional similar studies assessing failed to show benefit, this strategy was abandoned (Hellemons et al., 1999; Gullov et al., 1998).

The safety and efficacy of dual antiplatelet therapy (i.e., combination of clopidogrel and ASA) in patients with nonvalvular AF were also assessed in a two large randomized trials by the Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events (ACTIVE) program: ACTIVE-A and ACTIVE-W (Investigators AWGotA et al., 2006; Investigators et al., 2009a). The ACTIVE-W trial used a noninferiority design to compare the effect of the combination of clopidogrel 75 mg daily plus ASA to dose-adjusted warfarin (target INR 2.0–3.0) on the composite endpoint of stroke, non-CNS systemic embolus, myocardial infarction, and vascular death (Investigators AWGotA et al., 2006). ACTIVE-W enrolled 6706 patients with AF plus one or more risk factor for stroke, however, the trial was stopped early due to evidence of warfarin's superiority. After a median follow-up of 1.3 years, the annual risk of the composite outcome was 5.6% in the clopidogrel and ASA group compared to 3.9% with oral anticoagulation (RR

1.44; 95% CI, 1.18–1.76; $p = 0.0003$). Furthermore, there was a higher annual rate of stroke in the combination of clopidogrel and ASA arm compared to oral anticoagulation, without a significant difference in major bleeding.

The ACTIVE-A trial compared the combination of clopidogrel 75 mg daily plus ASA to ASA alone in 7554 patients with AF at increased risk of stroke and for whom vitamin K antagonist therapy was contraindicated (Investigators et al., 2009a). After a mean 3.6 years, the primary composite of any major vascular event (i.e., stroke, myocardial infarction, non-CNS systemic embolism, or death from vascular causes) occurred at an annual rate of 6.8% in the clopidogrel plus ASA treatment arm compared to 7.6% in the placebo group (RR 0.89; 95% CI, 0.81–0.98; $p = 0.01$). The difference was primarily due to a reduction in the rate of stroke in the dual antiplatelet therapy group. However, the rate of major bleeding was increased (2.0% per year with clopidogrel, and 1.3% ASA alone, RR 1.57; 95% CI, 1.29–1.92; $p < 0.001$).

Alternatives to Adjusted-Dose Warfarin Act 2: Direct Oral Anticoagulants

Despite its proven advantages over placebo, ASA monotherapy, and combination antiplatelet therapy, observational studies continued to show that in clinical practice rates of warfarin use in eligible patients remained below 60% (Ogilvie et al., 2010). Barriers to warfarin uptake include its narrow therapeutic window and requirement for ongoing dose adjustment and monitoring. The long search for safe and effective oral anticoagulants that overcome these barriers has culminated in the development and marketing of the direct oral anticoagulants (DOACs), which represent a significant evolution in stroke prevention therapy. Below, we will summarize the landmark studies that compared each of dabigatran, rivaroxaban, apixaban, and edoxaban with warfarin (Connolly et al., 2009; Patel et al., 2011; Granger et al., 2011; Giugliano et al., 2013). **Table 1** compares the trial designs, baseline patient characteristics, and outcomes in these four studies.

The Randomized Evaluation of Long Term Anticoagulation Therapy (RE-LY) trial was a noninferiority trial that assessed the efficacy of dabigatran for the prevention of thromboembolism in nonvalvular AF compared to warfarin (Connolly et al., 2009).

Table 1 Summary of landmark trials comparing direct oral anticoagulants to warfarin for nonvalvular atrial fibrillation

	RE-LY	*ROCKET-AF*	*ARISTOTLE*	*ENGAGE-AF*
DOAC	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
Target	IIa (thrombin)	Xa	Xa	Xa
Half-life (h)	12–17	5–13	~12	9–11
Renal excretion	80%	33%	25%	50%
N	18,113	14,264	18,201	21,105
Study design	Open-label	Double-blind	Double-blind	Double-blind
Median follow-up (years)	2.0	1.9	1.8	2.8
Mean $CHADS_2$	2.1	3.5	2.1	2.8
Dosing	110 mg bid 150 mg bid	20 mg daily 15 mg daily (renal)	5 mg bid 2.5 mg bid**	60 mg OD[a] 30 mg OD[a]
Warfarin time in therapeutic range	64% (mean)	55% (mean)	62% (mean)	65% (mean)
Stroke/embolism (/year)	Warfarin: 1.7% 110 mg: 1.5% (noninferior) 150 mg: 1.1% (superior)	Warfarin: 2.2% Rivaroxaban: 1.7% (noninferior)	Warfarin: 1.6% Apixaban: 1.3% (superior)	Warfarin: 1.5% 30 mg: 1.6% (noninferior) 60 mg: 1.2% (noninferior)
Major bleeding (/year)	Warfarin: 3.4% 110 mg: 2.7% ($p = 0.003$) 150 mg: 3.1% (ns)	Warfarin: 3.4% Rivaroxaban: 3.6% (ns)	Warfarin: 3.1% Apixaban: 2.1% ($p < 0.001$)	Warfarin: 3.4% 30 mg: 1.6% ($p < 0.001$) 60 mg: 2.8% ($p < 0.001$)
Intracranial hemorrhage (/year)	Warfarin: 0.4% 110 mg: 0.1% ($p < 0.001$) 150 mg: 0.1% ($p < 0.001$)	Warfarin: 0.7% Rivaroxaban: 0.5% ($p = 0.02$)	Warfarin: 0.8% Apixaban: 0.3% ($p < 0.001$)	Warfarin: 0.5% 30 mg: 0.2% ($p < 0.001$) 60 mg: 0.3% ($p < 0.001$)
Mortality (/year)	Warfarin: 4.1% 110 mg: 3.8% (ns) 150 mg: 3.6% (ns)	Warfarin: 4.9% Rivaroxaban: 4.5% (ns)	Warfarin: 3.9% Apixaban: 3.5% ($p = 0.047$)	Warfarin: 4.4% 30 mg: 3.8% ($p = 0.01$) 60 mg: 4.0% (ns)

[a]Dose reduction of edoxaban by 50% if one of weight <60 kg, creatinine clearance 30–50 mL/min, or concomitant use of verapamil or quinidine (i.e., potent P-glycoprotein inhibitors) (Connolly et al., 2009; Patel et al., 2011; Granger et al., 2011; Giugliano et al., 2013; Levy et al., 2014).

**Dose reduction of apixaban by 50% if 2 or more of: <= 60 kg, age >= 80 kg creatinine >= 1.5 mg/dL.

RE-LY was an open-label study that randomized 18,113 patients with AF (and ≥ 1 risk factor of previous stroke or TIA, LVEF $< 40\%$, or $\geq$ NYHA II heart failure symptoms) to receive fixed doses of dabigatran (110 or 150 mg twice daily) or open-label warfarin (target INR 2.0–3.0) in a 1:1:1 fashion. After a median 2-year follow-up, the rate of the primary outcome of stroke or systemic embolism was similar in the lower dose dabigatran (110 mg) group compared with warfarin (RR with dabigatran, 0.90; 95% CI, 0.74–1.10; $p < 0.001$ for noninferiority). However, the higher dose dabigatran 150 mg was superior to warfarin with significantly fewer primary outcome events (1.71% in the warfarin group vs. 1.11% in the dabigatran 150 mg group; RR 0.65; 95% CI, 0.52–0.81; $p < 0.001$ for superiority) (Connolly et al., 2010).

In RE-LY, the safety endpoint of major bleeding occurred in 2.9% of the low-dose dabigatran group, 3.3% in the high-dose dabigatran group, and 3.6% in the warfarin group ($p = 0.01$ compared to low-dose dabigatran, and $p = 0.32$ compared to high-dose dabigatran) (Connolly et al., 2010). Importantly, the annual rate of hemorrhagic stroke was significantly decreased with both dabigatran doses compared to warfarin (0.12% with 110 mg of dabigatran, 0.10% with 150 mg of dabigatran, and 0.38% with warfarin). The only adverse event occurring significantly more often in patients receiving dabigatran was dyspepsia (Connolly et al., 2009). Acknowledging the potential interaction between renal function and study results (n.b. dabigatran is 80% renally excreted), RE-LY conducted a prespecified analysis, which found the efficacy and safety of both dosages of dabigatran were consistent with the overall trial irrespective of renal function when stratified by estimated glomerular filtration rates (i.e., eGFR >80, 50–80, and <50 mL/min/1.73 m^2) (Hijazi et al., 2014).

The rivaroxaban-once daily, oral, direct factor Xa inhibition compared with vitamin K antagonist for prevention of stroke and Embolism Trial in Atrial Fibrillation (ROCKET-AF) trial was a large, randomized, double-blind, double-dummy noninferiority trial that enrolled 14,264 (mean age 73 years) patients with nonvalvular AF to receive rivaroxaban (20 mg daily, or 15 mg daily in patients with a creatinine clearance of 30–49 mL/min) or dose-adjusted warfarin (Patel et al., 2011). Baseline stroke risk in patients enrolled in ROCKET-AF was higher than in RE-LY: 87% of the patients had a $CHADS_2$ score of 3 or higher, and 55% had a prior TIA or stroke. Over a median follow-up of 24 months, the primary end of stroke or systemic embolism occurred in 188 patients in the rivaroxaban group (1.7% per year) and in 241 patients in the warfarin group (2.2% per year) (HR 0.88; 95% CI, 0.74–1.03; $p < 0.001$ for noninferiority, $p = 0.12$ for superiority). There was no significant difference in the risk of major bleeding between the two groups ($p = 0.44$), although intracranial and fatal bleeding occurred less often in the rivaroxaban group. The trial concluded that rivaroxaban was not inferior to warfarin for the prevention of stroke or systemic embolism in patients with AF.

The Apixaban for Reduction In STroke and Other ThromboemboLic Events in atrial fibrillation trial (ARISTOTLE) study compared the safety and efficacy of apixaban with warfarin in 18,201 patients with AF and at least one additional risk factor for stroke (Granger et al., 2011). Patients were randomized to either adjusted-dose warfarin or apixaban 5 mg twice daily (or 2.5 mg twice daily in patients meeting two or more of the following criteria: age ≥ 80 years, body weight ≤ 60 kg, serum creatinine level ≥ 1.5 mg/dL). Over a median follow-up of 22 months, the rate of the primary outcome of stroke and systemic embolism was 1.27% per year in the apixaban group compared with 1.60% per year in the warfarin group (HR 0.79; 95% CI, 0.66–0.95; $p < 0.001$ for noninferiority; $p = 0.01$ for superiority). The rate of major bleeding was significantly less in the apixaban group (2.13% per year vs. 3.09% per year with warfarin; HR 0.69; 95% CI, 0.60–0.80; $p < 0.001$). Additionally, ARISTOTLE demonstrated a statistically significant mortality benefit of apixaban over warfarin; the rates of all-cause mortality were 3.52% in the apixaban group and 3.94% in the warfarin group (HR, 0.89; 95% CI, 0.80–0.99; $p = 0.047$). Although the absolute risk reduction was small, apixaban was the first DOAC to reduce risk of death from any cause in comparison to warfarin (Granger et al., 2011).

Apixaban was also studied in nonvalvular AF patients for whom warfarin therapy was unsuitable due to difficult-to-control INR, patient refusal, or increased bleeding risk (i.e., a population reminiscent of the ACTIVE-A trial comparing combination ASA and clopidogrel to ASA monotherapy, Investigators et al., 2009a). The Apixaban versus acetylsalicylic acid to prevent StROke in atrial fibrillation PatiEntS who have failed or are unsuitable for vitamin K antagonist treatment (AVERROES) study randomized 5550 AFs patients (average $CHADS_2$ score of 2.1) in whom warfarin therapy was unsuitable, to apixaban (5 mg or 2.5 mg twice daily) or ASA (81–324 mg daily). The study was stopped prematurely because of the clear advantage of apixaban over ASA in reducing the risk of stroke or systemic events by >50%. There was no increased risk of major bleeding with apixaban over ASA (Connolly et al., 2011). Although there have been no trials comparing apixaban to combination of clopidogrel and ASA in AF patients intolerant of warfarin, an indirect comparison of the AVERROES and ACTIVE-A trials would suggest that apixaban is the preferred agent given the superior efficacy compared to ASA, but without the increased major bleeding risk conferred by combination of clopidogrel and ASA.

Finally, the Effective aNticoaGulation with factor xA next GEneration in Atrial Fibrillation (ENGAGE AF-TIMI 48) was a noninferiority trial ($n = 21{,}105$) designed to compare the efficacy of edoxaban (30 or 60 mg daily) and warfarin in AF patients with respect to the primary outcome of stroke and systemic embolism (Giugliano et al., 2013). During a mean follow-up of 34 months, both daily doses of edoxaban were noninferior to warfarin. The annualized rate of major bleeding events was 3.43% with warfarin compared to 2.75% with high-dose edoxaban (HR 0.80; 95% CI, 0.71–0.91; $p < 0.001$) and 1.61% with low-dose edoxaban (HR 0.47; 95% CI, 0.41–0.55; $p < 0.001$). In addition to a lower risk of major bleeding, there was significantly less hemorrhagic stroke in both edoxaban doses compared to warfarin. Finally, edoxaban-treated patients had lower annualized rates of death from cardiovascular causes compared to warfarin: 3.17% with warfarin versus 2.74% with high-dose edoxaban (HR 0.86; 95% CI, 0.77–0.97; $p = 0.01$) and 2.71% with low-dose edoxaban (HR 0.85; 95% CI, 0.76–0.96; $p = 0.008$).

Although clinical trials have demonstrated the efficacy of DOACs as compared to warfarin in reducing the risk of stroke in AF patients, several concerns have hindered their universal uptake in the clinical community (Mohanty et al., 2014). One of these concerns include the lack of a reversal agent, although recently one of the DOAC antidotes was approved for use in Canada, the United States, and Europe. Idarucizumab, a monoclonal antibody fragment, was designed to reverse the anticoagulant effects of

dabigatran. In an interim analysis of the ongoing REVERSE-AD prospective study (Study of the RE-VERSal Effects of Idarucizumab on Active Dabigatran), idarucizumab was found to be effective at reversing the anticoagulant effect of dabigatran based on the determination of direct thrombin time or ecarin clotting time (Pollack et al., 2015). Furthermore, the results of phase III studies are highly anticipated for andexanet alfa, a reversal agent for the direct factor Xa inhibitors (i.e., apixaban, rivaroxaban, and edoxaban) (Ghadimi and Welsby, 2016; Siegal et al., 2015).

Other challenges of DOACs include the need for dose adjustment in both renal and hepatic impairment. Furthermore, DOACs are contraindicated in patients with end-stage renal disease (with or without renal replacement); patients with creatinine clearance 30 mL/min were excluded from the seminal DOAC clinical trials (Connolly et al., 2009; Patel et al., 2011; Giugliano et al., 2013), with the exception of ARISTOTLE which excluded patients with a serum creatinine of 2.5 mg/dL or calculated creatinine clearance of <25 mL/min (Granger et al., 2011).

Furthermore, it is important to note that DOACs are not indicated in valvular AF (i.e., AF related to rheumatic valvular disease or prosthetic heart valves) as this population was excluded from the trials comparing DOACs and warfarin. RE-ALIGN (Randomized, phase II study to Evaluate the sAfety and pharmacokinetics of oral dabIGatran etexilate in patients after heart valve replacement) was the one randomized clinical trial that explored the use of a DOAC, specifically dabigatran, in patients with mechanical valves compared to warfarin. Although not exclusively an AF trial (i.e., only 28% of patients had AF), dabigatran was found to be inferior warfarin in terms of efficacy and safety; RE-ALIGN was terminated prematurely due to an excess of thromboembolic and bleeding events among patients in the dabigatran group (Eikelboom et al., 2013).

Left Atrial Appendage Closure Devices

Percutaneous LAA occlusion is a nonpharmacological alternative to oral anticoagulation which has been studied in a few clinical trials. The rationale behind LAA occlusion comes from anatomic and echocardiographic findings identifying the LAA as the site of thrombus formation in over 90% of patients with nonvalvular AF (Blackshear and Odell, 1996). Several percutaneous devices have been developed for the purpose of occluding or excluding the LAA from the circulation.

The WATCHMAN™ LAA closure device (Boston scientific) is the most rigorously studied LAA occlusion device currently available, with two randomized clinical trials supporting its use: PROTECT-AF (the Watchman LAA closure device for embolic PROTECTion in patients with Atrial Fibrillation) and PREVAIL (prospective randomized evaluation of the watchman LAA closure device in patients with atrial fibrillation versus long term warfarin therapy) (Holmes et al., 2009; Whisenant et al., 2014).

PROTECT-AF was a noninferiority trial with an adaptive Bayesian design that randomized 707 patients AF and $CHADS_2 \geq 1$ to either the Watchman device or warfarin (target INR 2.0–3.0) in a 2:1 ratio. Following device implantation, patients were continued on warfarin and ASA for 45 days, and then switched to clopidogrel plus ASA to 6 months, followed by ASA alone indefinitely. After 1065 patient-years of follow-up, percutaneous LAA closure with the Watchman device was noninferior to warfarin with respect to the primary composite endpoint of ischemic stroke, hemorrhagic stroke, cardiovascular death, and systemic embolism; the event rate was 3.0 per 100 patient-years in the Watchman group and 4.9 per 100 patient-years in the warfarin group (probability of noninferiority >99.9%) (Holmes et al., 2009). Subsequent analyses, allowed by the Bayesian design, extended the follow-up to 2621 patient-years and demonstrated a consistent noninferiority of the Watchman device compared to warfarin with respect to the primary outcome (Reddy et al., 2013, 2014).

Several concerns, raised by the U.S. Food and Drug Administration regarding patient selection criteria (i.e., inclusion of patients with $CHADS_2$ score of 1) and early complication rate, necessitated the subsequent PREVAIL trial (Whisenant et al., 2014). PREVAIL was designed with three coprimary endpoints. One of the efficacy endpoints, that evaluated late-ischemic efficacy, found that Watchman implantation was noninferior to warfarin in preventing ischemic events >7 days after implantation. The safety endpoint also achieved its prespecified performance goal with a low rate of procedural complications. However, PREVAIL missed its third coprimary endpoint (composite of stroke, systemic embolism, and cardiovascular death) and failed to show noninferiority to warfarin at 18 months (Holmes et al., 2014). An individual patient-level data metaanalysis of these two trials using a traditional superiority design was conducted by the original investigators (Holmes et al., 2015). They reported that the risk of the primary endpoint of all-cause stroke or systemic embolism was similar among patients with the Watchman versus patients on warfarin (HR 0.79, $p=0.22$). Interestingly, Watchman significantly increased the risk of ischemic stroke compared with warfarin (HR 1.95, $p=0.05$), but significantly decreased the risk of both hemorrhagic stroke (HR 0.22, $p=0.004$) and cardiovascular or unexplained death (HR 0.48, $p=0.006$).

Other LAA occlusion devices are available such as the AMPLATZER™ Amulet™ (St. Jude Medical), the LARIAT® system (SentreHeart, Inc), and the WaveCrest® device (Coherex Medical); however, to date the efficacy and safety of these devices have not yet been evaluated in randomized clinical trials. However, the safety and efficacy of the Amulet device will soon be evaluated in the Amplatzer Amulet Investigational Device Exemption (Amulet IDE) trial, which is an upcoming noninferiority trial against the Watchman LAA closure device (ClinicalTrials.gov identifier: NCT02879448). Nonetheless, the development and clinical study of these newer generations offer a promising future for AF patients, especially those who are unable to tolerate chronic anticoagulation.

In summary, the last quarter century has seen dramatic developments in evidence-based antithrombotic therapy for nonvalvular AF. Oral anticoagulation, either with warfarin or a DOAC, is among the most effective therapies in cardiovascular medicine, and is rightly considered the cornerstone of therapy in patients with elevated stroke risk. Future research is required to refine the optimal agents in specific populations, to maximize adherence to recommended therapy, and to further explore the role of percutaneous therapies.

Rate Control Versus Rhythm Control

There are two strategies used to manage AF symptoms: a "rhythm-control" strategy involves re-establishing and maintaining sinus rhythm with antiarrhythmic medications or AF ablation, whereas a "rate-control" strategy involves controlling ventricular rate with atrioventricular (AV) nodal antagonists or AV nodal ablation.

Rate Control: A Reasonable Approach for Most Patients

In the 1980s and 1990s, the prevailing paradigm was in favor of a rhythm-control approach with the belief that maintenance of sinus rhythm circumvent the potential long term adverse consequences of AF: the deterioration in hemodynamics secondary to increased heart rate and loss of AV synchrony, an increased risk for stroke from left atrial thrombi, and progressive left ventricular dysfunction (Falk, 2005; Waldo, 1999).

This paradigm was challenged by several randomized controlled trials (Carlsson et al., 2003; Hohnloser et al., 2000; Opolski et al., 2004), including the AF Follow-up Investigation of Rhythm Management (AFFIRM) (Wyse et al., 2002) and the Rate Control versus Electrical Cardioversion (RACE) (Van Gelder et al., 2002). These landmark studies failed to conclusively show that one strategy is superior to the other in terms of morbidity and mortality (**Table 2**) (de Denus et al., 2005).

AFFIRM, the largest trial comparing rate- versus rhythm-control strategies, randomized 4060 patients with recurrent AF to either rate control (using digoxin, beta-blockers, and calcium channel antagonists) or rhythm control with the most effective antiarrhythmic drug (primarily amiodarone, sotalol, or propafenone). All patients were initially anticoagulated, but those in the rhythm-control arm who maintained sinus rhythm could stop warfarin. Over a mean follow-up of 3.5 years, all-cause mortality, the primary endpoint of the study, was not significantly different between the two treatment arms, although there was a trend toward an increased risk of death in the rhythm-control arm compared to rate control at 5 years (23.8% vs. 21.3%, $p = 0.08$) (Wyse et al., 2002).

The RACE trial was a smaller trial ($n = 522$) that also compared rate- and rhythm-control approaches. Patients in persistent AF were randomized to a strategy of repeat cardioversion and antiarrhythmic drugs to maintain sinus rhythm, or to a rate-control approach. At a mean follow-up of 2.3 years, there was no difference in the primary composite endpoint of cardiovascular death, heart failure, thromboembolic complications, bleeding, pacemaker insertion, or severe adverse effects of drugs.

AFFIRM and RACE were practice-changing studies of their time for several reasons. Firstly, they both challenged the belief that rhythm control conferred additional benefit over rate control. Not only was there no difference in mortality, neither trial revealed a difference in quality of life measures (Hagens et al., 2004; Jenkins et al., 2005). Secondly, AFFIRM and RACE added to the growing body of literature that questioned the notion that maintenance of sinus rhythm obviated the need for anticoagulation (Hart et al., 2000). The rates of stroke were not significantly different between treatment arms, but the majority of thromboembolic events occurred due to subtherapeutic INR or after discontinuation of anticoagulation therapy allowed by study protocol after normal rhythm had been achieved and maintained (Hagens et al., 2003; Sherman et al., 2005).

Table 2 Summary of landmark trials comparing rate versus rhythm strategies in atrial fibrillation

Trial	*N*	*Age (years)*	*Mean follow-up*	*Primary outcome*	*Primary outcome (rate vs. rhythm)*	*Mortality*[a] *(rate vs. rhythm)*	*Thromboembolism (rate vs. rhythm)*
PIAF (2000)	252	60.5	1.0	Symptomatic improvement	60.8% vs. 55.1% (ns)	na	na
AFFIRM (2002)	4060	69.7	3.5	All-cause mortality	25.9% vs. 26.7% (ns)	25.9% vs. 26.7% (ns)	5.5% vs. 7.1% (ns)
RACE (2002)	522	68.0	2.3	CV death, HF, bleeding, pacemaker implantation, thromboembolism, AAD adverse effects	17.2% vs. 22.6% (ns)	6.8% vs. 7.0% (ns)	5.5% vs. 7.9% (ns)
STAF (2003)	200	65.8	1.6	All-cause mortality, cerebrovascular event, CPR, systemic embolism	10.0% vs. 9.0% (ns)	8.0% vs. 4.0% (ns)	1.0% vs. 5.0% (ns)
HOT-CAFÉ (2004)	205	60.8	1.7	All-cause mortality, systemic embolism, major bleeding	2.0% vs. 5.8% (ns)	1.0% vs. 2.9% (ns)	1.0% vs. 2.9% (ns)
J-RHYTHM (2008)	823	64.7	1.6	All-cause mortality, stroke, systemic embolism, major bleeding, HF hospitalization, physical/psychologic disability relating to treatment	22.0% vs. 15.3% ($p = 0.01$)	0.7% vs. 1.0% (ns)	2.9% vs. 2.3% (ns)

Abbreviations: *CV*, cardiovascular; *HF*, heart failure; *CPR*, cardiopulmonary resuscitation; *na*, not available; *AAD*, antiarrhythmic drug (Carlsson et al., 2003; Hohnloser et al., 2000; Opolski et al., 2004; Wyse et al., 2002; Van Gelder et al., 2002; Ogawa et al., 2009).

[a]Percentages reported are for all-cause mortality, except in the RACE trial, which reported CV mortality.

Rate Control for Patients With Heart Failure

Between 30% and 50% of patients with AF have concomitant heart failure (Chiang et al., 2012). The pathophysiology of AF and heart failure are closely aligned, and the development of both conditions results in a poorer prognosis (Kotecha and Piccini, 2015; Wang et al., 2003). Since few patients with heart failure were included in the prior rate versus rhythm trials, the optimal approach in managing these high-risk patients remained unclear. The Atrial Fibrillation Congestive Heart Failure (AF-CHF) trial (Roy et al., 2008) was an open-label, randomized trial comparing pharmacologic rate- and rhythm-control strategies in 1376 patients with symptomatic heart failure and a left ventricular ejection fraction of 35% or less. For patients randomized to rate control, a resting heart rate of <80 beats per min and <110 beats/min during 6-min walk test were targeted using primarily beta-blockers and digoxin. For patients randomized to the rhythm-control arm, aggressive therapy to prevent AF and maintain sinus rhythm was recommended, which included electrical cardioversion within 6 weeks of randomization, and repeated at 3 months if sinus rhythm was not achieved. The majority of patients in this arm were started on amiodarone (82%), but sotalol and dofetilide were also used in selected cases.

Over a mean follow-up of 37 months, there was no difference between the two treatment groups in the primary outcome of cardiovascular death (25% for rate control and 27% for rhythm control; hazard ratio [HR] 1.06; 95% confidence interval [CI], 0.86–1.30; $p=0.59$. Further, there were no significant differences in prespecified secondary outcomes of all-cause mortality, stroke, or worsening heart failure. However, there was a trend toward increased hospitalization in the rhythm-control group (64% vs. 59%, $p=0.06$) due to recurrent AF and bradyarrhythmias. Therefore, the AF-CHF trial showed that the paradigm of adopting an initial strategy of rate control in patients without severe AF symptoms, introduced by the AFFIRM and RACE trials, could be extended to those with heart failure. However, it is important to note that 21% of patients crossed over to rate control because sinus rhythm could not be maintained despite antiarrhythmic drug therapy and serial cardioversions.

Rate Control: What is the Optimal Target Heart Rate in Permanent AF?

As rate-control became more widely adopted, it remained unclear whether the intensity of rate control affected outcomes. Intuitively, the use of strict rate control was thought to reduce symptoms from AF and improve quality of life, reduce the incidence heart failure and improve survival. On the other hand, the enthusiasm for strict rate control was tempered by the required increase in drug doses that had the potential for adverse effects such as bradycardia, syncope, and pacemaker implantation. A post hoc analysis pooling the AFFIRM and RACE cohorts showed no benefit of strict versus lenient rate control, although neither study was designed specifically to address this issue (Van Gelder et al., 2006).

Thus far, only one randomized clinical trial has evaluated different rate-control strategies. The Race Control Efficacy in Permanent Atrial Fibrillation: a comparison between Lenient and Strict Rate Control II (RACE II) trial enrolled 614 patients with permanent AF, younger than 80 years of age, and had a resting heart rate greater than 80 beats/min (Van Gelder et al., 2010). Patients were randomized to strict rate control (defined as a resting heart rate of <80 beats/min and <110 beats per min during moderate exercise) or lenient rate control (defined as <110 beats per min at rest).

RACE II revealed that a lenient rate-control approach was noninferior compared to a strict rate-control approach with respect to the primary composite outcome of cardiovascular death, hospitalization for heart failure, stroke systemic embolism, major bleeding, and life-threatening arrhythmic events at 3 years of follow-up. There were also no differences in symptom burden, quality of life, or hospital admissions (Van Gelder et al., 2010; Groenveld et al., 2011). However, it is important to note that the target heart rates were achieved only 67% of the strict rate control group compared to 98% of the lenient group, resulting in a modest difference in mean achieved heart rate (approximately 10 beats/min) during follow-up. To address this concern, an exploratory analysis was conducted where the strict rate control group was stratified by achievement of target heart rate, finding no difference in the primary outcome between patients in the lenient control arm compared to patients who actually achieved strict rate control targets (Groenveld et al., 2013).

Catheter Ablation: A New Hope for the Rhythm-Control Approach

Although prior trials have not demonstrated a benefit of rhythm control over a rate-control strategy, this may be due to the limited efficacy of current antiarrhythmic therapies at maintaining sinus rhythm (**Fig. 2**). Only two-thirds of patients are able to maintain sinus rhythm at 12–16 months with amiodarone, the most effective antiarrhythmic currently available (Roy et al., 2000). Other currently available antiarrhythmic agents are even less effective, with only 30%–50% of patients maintaining sinus rhythm at 12–16 months (Roy et al., 2000; Nichol et al., 2002). Thus, the failure of the rhythm-control strategy may indicate more about the inadequacies or toxicities of pharmacological antiarrhythmic therapy than it does about the rhythm-control strategy itself.

With the advent of catheter ablation, there has been a renewed interest in exploring the potential benefits of rhythm-control strategy. A metaanalysis of 11 studies comparing catheter ablation to antiarrhythmic therapy found that there was a 36% absolute risk reduction in the recurrence of atrial tachyarrhythmias in patients treated with catheter ablation (RR 0.40; 95% CI, 0.31–0.52; $p<0.001$). Catheter ablation appeared to be superior to antiarrhythmic drug therapy in patients with AF who were drug resistant or intolerant; however, there was a twofold increased risk of major adverse events in the catheter ablation group (Khan et al., 2014).

Several studies examined the utility of ablation as first-line therapy including the Medical Antiarrhythmic Treatment or Radiofrequency Ablation in Paroxysmal Atrial Fibrillation (MANTRA-PAF) and the Radiofrequency Ablation versus Antiarrhythmic

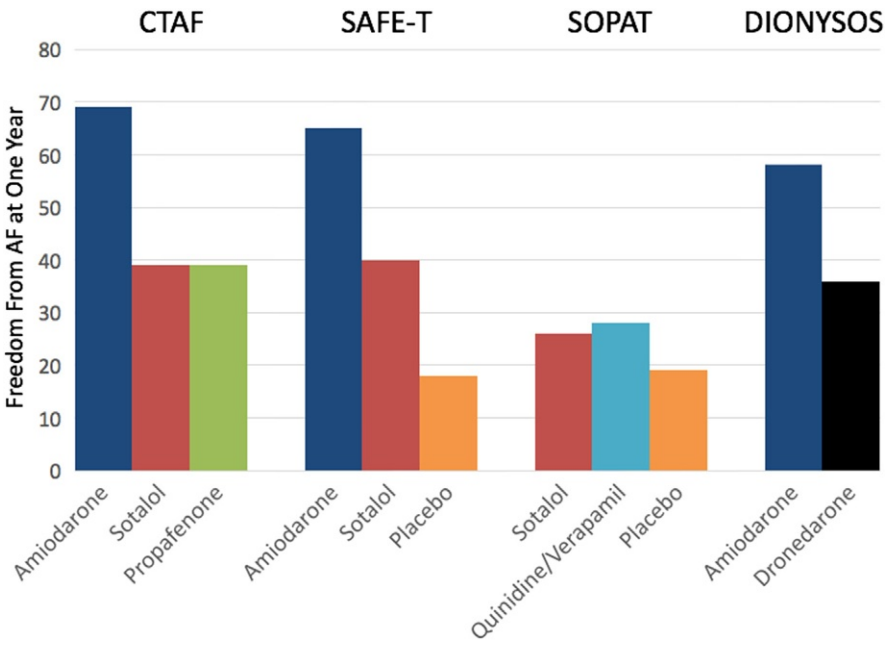

Fig. 2 Selected studies of comparative efficacy of antiarrhythmic drugs for maintenance of sinus rhythm: Abbreviations: *AF*, atrial fibrillation; *CTAF*, Canadian Trial of Atrial Fibrillation; *SAFE-T*, Sotalol Amiodarone Atrial Fibrillation Efficacy Trial; *SOPAT*, Suppression of Paroxysmal Atrial Tachyarrhythmias; and *DIONYSOS*, efficacy and safety of dronedarone versus amiodarone for the Maintenance of Sinus Rhythm in Patients With Atrial Fibrillation (EAFT, 1993; Roy et al., 2000; Le Heuzey et al., 2010; Patten et al., 2004; Singh et al., 2005).

Drugs as First-Line Therapy of Atrial Fibrillation 2 (RAAFT-2) (Cosedis Nielsen et al., 2012; Morillo et al., 2014). MANTRA-PAF randomized 294 antiarrhythmic drug-naive patients with paroxysmal AF to an initial strategy of catheter ablation or therapy with class IC or class III antiarrhythmic agents. There was no significant difference in the primary endpoint of cumulative burden of AF (i.e., percentage of time in AF on Holter monitor); however, at 24 months, significantly more patients in the ablation group were free from any AF (85% vs. 71%, $p=0.004$) and from symptomatic AF (93% vs. 84%, $p=0.01$) (Cosedis Nielsen et al., 2012). Similarly, RAAFT-2 randomized 127 treatment-naïve patients with paroxysmal AF to either antiarrhythmic therapy or ablation. At 2 years, the primary efficacy outcome (i.e., recurrence of any atrial tachyarrhythmia greater than 30 s) occurred more often in the antiarrhythmic drug group compared to ablation groups (72.1% vs. 54.5%, $p=0.02$). Although ablation resulted in a significantly lower rate of recurrent atrial tachyarrhythmias at 2 years, recurrence was frequent in both groups (Morillo et al., 2014). It is currently not known whether catheter ablation of AF reduces outcomes such as mortality or stroke, which is currently being explored by ongoing trials, including: CABANA (Catheter Ablation Versus Antiarrhythmic Drug Therapy for Atrial Fibrillation) trial, which recently completed enrolment (ClinicalTrials.gov identifier: NCT00911508); and EAST (Early Therapy of Atrial Fibrillation for Stroke Prevention) Trial (ClinicalTrials.gov identifier: NCT01288352). Furthermore, the results of two studies assessing the utility of catheter ablation in patients with heart failure are highly anticipated: CASTLE-AF (Catheter Ablation Versus Standard Conventional Treatment in Patients With Left Ventricular Dysfunction and Atrial Fibrillation) (ClinicalTrials.gov identifier: NCT00643188) and RAFT-AF (A Randomized Ablation-based Atrial Fibrillation Rhythm Control Versus Rate Control Trial in Patients With Heart Failure and High Burden Atrial Fibrillation) (ClinicalTrials.gov identifier: NCT01420393).

Alternate Energy Sources for Ablation: Hot or Cold?

Catheter ablation most commonly uses radiofrequency (RF) energy to isolate pulmonary veins (PVI) with a "point-by-point" approach. Novel balloon-based and multielectrode-based ablation technologies have been developed as an alternative to RF ablation, with the advantage of achieving PVI with a single circumferential energy application. The most widely available alternative is the cryoballoon (Arctic Front, Medtronic) which uses cryothermal energy rather than RF. Evidence over the past few years has supported the use of the cryoballoon as a viable alternative to RF ablation. The first randomized, multicenter trial assessing the efficacy of the cryoballoon was the STOP-AF trial (A Clinical Study of the Arctic Front Cryoablation Balloon for the Treatment of Paroxysmal Atrial Fibrillation). STOP-AF reported a superior efficacy of first generation cryoballoon ablation compared with antiarrhythmic medications in patients with paroxysmal AF (Packer et al., 2013).

The largest trial to date directly comparing RF ablation to cryoballoon was the FIRE and ICE trial, which enrolled 762 patients with drug refractory paroxysmal AF to determine if cryoballoon ablation was noninferior to RF ablation (Kuck et al., 2016). After a mean follow-up of 1.5 years, cryoballoon was noninferior to RF ablation with respect to first documented clinical failure (i.e., recurrent of atrial tachyarrhythmias, use of antiarrhythmic drugs, or repeat ablation) within 90 days after index ablation.

Furthermore, there was no significant difference between the two methods with regard to overall safety (Kuck et al., 2016). However, cryoballoon ablation led to an increased risk of phrenic nerve palsy (2.7% vs. 0%), though the majority of cases were transient.

In addition to the large outcome studies mentioned previously, much work remains in optimizing the safety and effectiveness of AF catheter ablation. Active areas of research include the impact of AF catheter ablation on stroke risk, the role of imaging and other biomarkers in optimizing patient selection, the role of novel AF mapping algorithms in identifying key targets, such as rotors or focal sources, for AF substrate ablation, and identifying the most effective ablation strategy in patients with persistent AF or AF that recurs after complete PVI.

Upstream Therapies

Although there has been much progress in elucidating the basic mechanisms underlying AF over the past several decades, our understanding remains incomplete. AF is thought to result from the interaction between triggers (i.e., initiating electric stimuli from the PVI) and substrate (i.e., vulnerable tissue allows AF to be induced and sustained) (Woods and Olgin, 2014). Furthermore, substrate is rendered vulnerable as a result of continuous remodeling of the atria, which involves both electrical and structural changes, altered metabolism, and fibrosis (Kourliouros et al., 2009; Goette and Lendeckel, 2008; Burstein and Nattel, 2008).

There has been a great interest in "upstream therapies" that intend to delay or even reverse the natural progression of AF by targeting factors leading to atrial remodeling (Savelieva et al., 2011a,b). A variety of pharmacologic therapies have been explored, including renin–angiotensin inhibitors (Goette et al., 2012; Investigators et al., 2009b; Yamashita et al., 2011), aldosterone antagonists, statins (Liu et al., 2008; Almroth et al., 2009; Negi et al., 2011), and $n-3$ polyunsaturated fatty acids (Macchia et al., 2013; Nodari et al., 2011). Unfortunately, most of these therapies have not proven beneficial at reducing the recurrence or burden of AF in patients with paroxysmal AF when directly assessed in randomized clinical trials.

One of the largest of these studies was the GISSI-AF (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Atrial Fibrillation) trial, which examined the role of valsartan (compared to placebo) in AF prevention in 1442 patients with documented symptomatic AF and in sinus rhythm at time of randomization (Investigators et al., 2009b). At one-year of follow-up, there was no significant difference between valsartan and placebo in the proportion of patients who had more than one episode of AF (51 vs. 52%) or in the median time from randomization to the first recurrence of AF (295 vs. 291 days).

Although pharmacologic therapies for the prevention of AF have not been efficacious when assessed in randomized trials, lifestyle interventions, such as exercise and weight loss, are emerging as a promising upstream therapy. In a single-center, partially blinded, randomized control trial, 150 patients were assigned to either a physician-led weight loss program (intervention group) or general lifestyle advice (control group) (Abed et al., 2013). Both groups underwent intensive management of cardiometabolic risk factors. Over a follow-up period of 15 months, the intervention group showed a significantly greater reduction in weight when compared to control (14.3 kg and 3.6 kg, respectively; $p<0.001$) and in AF symptom burden scores (11.8 and 2.6 points; $p<0.001$), and symptom severity scores (8.4 and 1.7 points; $p<0.001$). Additionally, there was a significant reduction in number of AF episodes in follow-up (-2.5 vs. no change; $p=0.01$) and duration of episodes when monitored by 7-day Holter monitor (692 min decrease and 419 min increase; $p=0.002$). The authors concluded that weight reduction with intensive risk factor management resulted in reduction of both AF symptom burden and severity. The postulated mechanism was thought to be mediated by favorable cardiac remodeling inferred based on significant reductions in interventricular septal thickness and left atrial area on serial transthoracic echocardiography (Abed et al., 2013). While all patients should be encouraged to adopt a healthier lifestyle, these promising results need verification in independent studies before the intensive risk factor modification program used in these studies can be broadly recommended.

Conclusions

Our understanding of AF has significantly advanced over the past few decades. Landmark studies have disrupted underlying assumptions in areas of clinical practice such as the selection of rate- or rhythm-control approaches, and the decision to initiate anticoagulation in patients with paroxysmal, persistent, or permanent AF. Furthermore, the development of technologies, such as RF and cryoballoon ablation, has provided new opportunities for the management of patients with AF.

Yet despite our accumulating body of knowledge, the management of patients with AF continues to be challenging. The debate of rate control versus rhythm control has re-emerged with the development of catheter ablation of AF due to the potential for more durable maintenance of sinus rhythm than antiarrhythmic drugs. Furthermore, advancement in our understanding of basic AF mechanisms promises for more efficient and successful ablation strategies. Research into targeting localized sources of AF, such as complex fractioned atrial electrograms and rotors, by computed signal analysis represents an important step in this direction (Woods and Olgin, 2014; Haissaguerre et al., 2004).

Additionally, we have much to learn about the role of anticoagulation in high-risk populations such as patients with end-stage renal disease. Despite the prevalence of AF in this population, patients with end-stage renal disease have not been well represented in the major anticoagulation trials. The relative paucity in the literature is reflected in the discordance among various guidelines regarding anticoagulation in patients on dialysis (Kirchhof et al., 2016; January et al., 2014; Verma et al., 2014). Our strategies for anticoagulation also remain unclear in other patient populations with a significant AF prevalence, such as patients with recent acute

coronary syndrome on dual antiplatelet therapy, and patients with postoperative AF following cardiac and noncardiac surgery. Nevertheless, if the pace of progress we have seen in the past few decades of research continues, we can be optimistic that solutions to these problems will emerge, and the care of patients with AF will continue to improve.

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Complications of Pregnancy and Future Cardiovascular Risk

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Introduction

Cardiovascular disease (CVD) is the single most important noncommunicable disease and causes the greatest number of deaths worldwide. The World Health Organization estimates that 17.6 million people died from CVD in 2012 accounting for 31.43% of global mortality (McAloon et al., 2016). Of this, ischemic heart disease accounts for approximately 7.4 million deaths which is 13.2% of deaths due to CVD (McAloon et al., 2016). Contemporary research on coronary heart disease (CHD) in women shows that the popular belief "heart disease is a man's disease" is wrong. CHD has often been considered to predominantly affect males due to the earlier onset of symptoms, a greater clustering of typical clinical manifestations, and traditional risk factors. In contrast, CHD in women has long been ignored due to atypical symptoms and nontraditional risk factors. However according to current statistics, CHD is the leading cause of death among women in every major developed country and most developing nations (Wenger, 2012). The generally accepted "norm" is that age-dependent decline in health status coupled with postmenopausal decline in estrogen levels contribute to morbidity and mortality from CHD in older women (Savonitto et al., 2016). However, recent studies demonstrate that young women have poorer prognoses after myocardial infarction compared with their male peers (D'Onofrio et al., 2015). A common key factor underlying CHD in both sexes is metabolic syndrome. This is a spectrum of metabolic and vascular derangements including obesity, insulin resistance, dyslipidemia, and hypertension which collectively identify individuals at risk of CHD (Wilson et al., 1999; Isomaa et al., 2001). Pregnancy can be considered a cardiovascular stress test for women because the normal maternal physiological response to pregnancy represents a transient excursion into metabolic syndrome (Fig. 1).

Maternal Physiological Adaptation to Pregnancy

Pregnancy is a period of unique hemodynamic and metabolic adaptation with maternal physiology changing throughout gestation to support the demands of the growing fetus. These changes pose a substantial burden on blood pressure regulation, glucose and lipid metabolism, and immune tolerance. During pregnancy, under the influence of estrogen and progesterone, the maternal vascular system undergoes many changes. There is an increase in plasma volume of 45%, red cell mass increases by 20%, heart rate by 25%, stroke volume by 25%, and consequently the cardiac output increases by 35%–50% toward the third trimester (Heidemann and McClure, 2003). Increased circulating estrogen and progesterone and the increase of the angiogenic factors including vascular endothelial growth factors and placental growth factor cause vasodilatation leading to a reduction in systemic vascular resistance which in turn will reduce systolic and diastolic blood pressures by mid-pregnancy which gradually return to prepregnancy levels by term (Heidemann and McClure, 2003; Troisi et al., 2008). Pregnancy is also characterized by resetting of glucose and lipid homeostasis that manifests as decreased insulin sensitivity, increased circulating triglycerides, and low-density lipoproteins mimicking the pathological changes seen in metabolic syndrome (Heidemann and McClure, 2003). In addition,

pregnancy is a prothrombotic and hypercoagulable state with an increase in fibrinogen and most clotting factors (Heidemann and McClure, 2003). Normal pregnancy also involves upregulation of the inflammatory cascade and an increase in the white cell count which are recognized as additional risk factors for cardiovascular disease (Chandra et al., 2012). Markers of inflammation including C-reactive protein, interleukin-6, and raised white cell count are independent predictors of cardiovascular events and diabetes (Haffner, 2001). These multiple hemodynamic and metabolic responses in pregnancy add further to the notion that pregnancy is a stress test for the female cardiovascular system.

Pregnancy Complications and Risk for Vascular Disease

In the last decade, an accumulating body of research has shown that several pregnancy complications are associated with subsequent CVD morbidity and mortality. Most of this evidence comes from large data linkage studies that have assessed the relationship between adverse pregnancy outcomes and CVD. A summary of the findings is detailed in **Table 1**.

Hypertensive Disorders of Pregnancy

Hypertensive disorders of pregnancy (HDP) are a leading cause of maternal and infant morbidity and mortality and can be divided into gestational hypertension (GH) and preeclampsia. Worldwide, several definitions are used in the diagnosis of HDP. According to the International Society for the Study of Hypertension in Pregnancy, GH is defined as a systolic blood pressure $\geq$140 mmHg or a diastolic blood pressure $\geq$90 mmHg measured at least on two occasions after 20 weeks of gestation in a previously normotensive

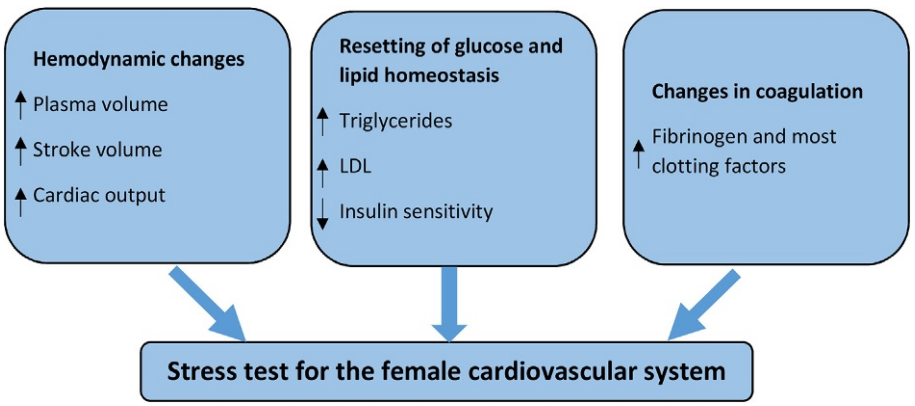

Fig. 1 Pregnancy as a stress test for the female cardiovascular system.

Table 1 Relative risk for vascular diseases after pregnancy complications

Pregnancy complication	*Developing a CV disease*	*Ischaemic heart disease*	*Stroke*	*Hypertension*	*Type 2 diabetes*	*Dyslipidaemia*
Preeclampsia (overall)	2.15 (1.76–2.61)	2.06 (1.68–2.52)	1.53 (1.21–1.92)	2.76 (1.63–4.96)	2.27 (1.55–3.32)	1.07 (0.74–1.54)
Early preeclampsia	9.5 (3.5–20.3)					
Gestational hypertension	1.89 (1.31–2.72)	1.44 (1.30–1.60)	1.41 (1.20–1.65)	2.87 (0.84–9.77)	2.10 (1.45–3.04)	1.07 (0.74–1.54)
SGA pregnancy	1.66 (1.26–2.18)	1.68 (1.31–2.14)	1.62 (1.51–1.74)	1.23 (1.16–1.30)	0.96 (0.86–1.06)	
Spontaneous preterm birth	2.06 (1.58–2.68)	1.38 (1.22–1.57)	1.71 (1.53–1.91)	1.29 (1.24–1.35)	1.77 (1.46–1.15)	
Recurrent miscarriage		1.99 (0.94–4.19)	0.98 (0.80–1.21)			

The above results are from the most recent systematic review and metaanalyses by Heida et al., 2016; Hypertensive diseases of pregnancy (preeclampsia and gestational hypertension)—metaanalysis of 28 cohort and 20 case–control studies, sample size of individual studies ranged from 40 to 1030,000, follow-up ranged from 3 to 37 years; SGA (small for gestational age)—metaanalysis of 9 studies, sample size of individual studies ranged from 6608 to 1,400,000, follow-up ranged from 11.8 to 37 years; Spontaneous preterm birth—metaanalysis of 11 cohort studies, sample size of individual studies ranged from 446 to 923,686, follow-up ranged from 12 to 35 years (results are after excluding or correcting for preeclampsia and SGA); Recurrent miscarriage—metaanalysis of 3 cohort and 2 case–control studies, sample size of individual studies ranged from 123 to 267,000, follow-up ranged from 11 to 19 years.

woman. Preeclampsia is defined as the same pattern of elevated blood pressure but with proteinuria of ≥300 mg/24 h or any multisystem complication of preeclampsia or with fetal growth restriction (Tranquilli et al., 2014). Preeclampsia is also classified according to time of onset with early onset being regarded as <34 weeks of gestation and late onset >37 weeks of gestation. A recent systematic review and metaanalysis that has assessed studies published until the end of 2012 demonstrates that preeclampsia is associated with an increased risk of developing or dying from overall CVD (relative risk; RR 2.15, 95% CI 1.76–2.61), ischemic heart disease (RR 2.06, 95% CI 1.68–2.52), and stroke (RR 1.53, 95% CI 1.21–1.92). The likelihood of developing risk factors for CVD including hypertension (RR 2.76, 95% CI 1.63–4.69) and diabetes (RR 2.27, 95% CI 1.55–3.32) is also increased among women who develop preeclampsia compared to women who have normotensive pregnancies (Heida et al., 2016). These findings are comparable with the results of a previous metaanalysis (Bellamy et al., 2007). The risk factors for CVD are evident 10–14 years after a preeclamptic pregnancy, therefore, increasing the risk for premature CVD among these women (Bellamy et al., 2007). Only a few studies have stratified preeclampsia into early versus late disease. Based on these studies, early preeclampsia is associated with nearly an 8-fold increased risk of ischemic heart disease (RR 7.71, 95% CI 4.4–13.52) compared with normotensive pregnancies (Bellamy et al., 2007). In addition women with severe preeclampsia (blood pressure >160/110 mmHg plus proteinuria >0.3 g/24 h or diastolic blood pressure >110 mmHg plus proteinuria >5 g/24 h) have a greater risk of later ischemic heart disease (RR 2.86, 95% CI 2.25–3.65) compared with women with mild preeclampsia (RR 1.92, 95% CI 1.65–2.24) (Bellamy et al., 2007). According to the same systematic review, GH is also shown to associate with an increased risk of developing or dying from overall CVD (RR 1.89, 95% CI 1.31–2.72), ischemic heart disease (RR 1.44, 95% CI 1.30–1.60), and stroke (RR 1.41, 95% CI 1.20–1.65) (Heida et al., 2016). Therefore, women who develop gestational hypertensive diseases are at approximately twice the risk of subsequent CHD compared to women who have uncomplicated pregnancies. Interestingly, in a Norwegian cohort of 836,147 women, Skjaerven and colleagues showed that the rate of cardiovascular mortality among women with preterm preeclampsia was 9.2% after having only one child, the rate falling to 1.1% for women with two or more children (Skjaerven et al., 2012). Among women with only one lifetime pregnancy, the increase in risk of cardiovascular death was higher than for those with two or more children after term and preterm preeclampsia (Skjaerven et al., 2012). The finding that cardiovascular death in women with preeclampsia in their first pregnancy is concentrated mainly in women with no additional births could be due to health problems or the experience of a pregnancy complication that discourage or prevent subsequent pregnancies rather than to preeclampsia itself (Skjaerven et al., 2012).

Pregnancies Complicated by Small for Gestational Age Infants

Small for gestational age (SGA) is defined as birth weight below the 10th percentile of the referent population. SGA can complicate a normotensive pregnancy or can occur as a complication of a hypertensive pregnancy. The link between birth weight and adult cardiovascular health was first highlighted by David Barker and colleagues in the late 1980s. It was shown that infants born with a low birth weight had a higher mortality rate due to cardiovascular disease compared to infants born with a normal birth weight (Barker et al., 1989). Since then this finding has been confirmed by many other large studies (Eriksson et al., 2001; Lawlor et al., 2005). In addition to the risk for the child, women who deliver SGA infants are at an increased risk of later life CVD (Ngo et al., 2015). Delivery of a SGA infant is associated with an increased risk of developing or dying from overall CVD (RR 1.66, 95% CI 1.26–2.18), ischemic heart disease (RR 1.68, 95% CI 1.31–2.14), and stroke (RR 1.62, 95% CI 1.51–1.74) (Heida et al., 2016). The risk of CVD is also shown to increase in a stepwise manner with delivering one SGA infant is associated with a RR of 1.41 (95% CI 1.36–1.46), two SGA infants with a RR of 1.74 (95% CI 1.58–1.93), and three or more SGA infants with a RR of 1.86 (95% CI 1.35–2.57) (Nilsson et al., 2009). Therefore, women who give birth to SGA infants appear to be at 1.5 times higher risk of CVD compared to women who have uncomplicated pregnancies.

Spontaneous Preterm Birth (sPTB)

Spontaneous preterm delivery is defined as delivery before 37 weeks of gestation and complicates 5–11% of pregnancies in the developed world (Zeitlin et al., 2013). Worldwide, sPTB is the commonest cause of neonatal and early-life morbidity and mortality (Liu et al., 2015). Recent evidence demonstrates an increase CVD risk among women who deliver preterm. sPTB was associated with an increased risk of developing or dying from overall CVD (RR 2.06, 95% CI 1.5–2.68), ischemic heart disease (RR 1.38, 95% CI 1.22–1.57), and stroke (RR 1.71, 95% CI 1.53–1.91) (Heida et al., 2016). Therefore, women who deliver preterm are at twice the risk of developing CVD compared to women who deliver at term.

Recurrent Miscarriage

Miscarriage is a common pregnancy complication with a risk of 12%–15% in each pregnancy and is defined as the premature loss of a fetus up to 20 weeks of gestation in most countries. Recurrent miscarriage has been defined as three or more consecutive miscarriages, but this is increasingly being revised to two or more miscarriages. The incidence of recurrent miscarriage is 1%–3%. Women with a history of miscarriage are shown to be at a higher risk of CHD compared to women who have not experienced miscarriage. Women with a history of miscarriage are shown to be at 45% risk of CHD, while those with a history of recurrent miscarriage have a 2-fold increased risk of CHD (Oliver-Williams et al., 2013). Although a link between recurrent miscarriage and CHD has been reported, most studies on miscarriage have not classified miscarriage phenotypically, and a recent systematic review

and metaanalysis failed to show an association between recurrent miscarriage and CHD (Heida et al., 2016). Since a large proportion of early miscarriage may be due to anembryonic losses, it is difficult to draw conclusions from the above findings. Furthermore, whether miscarriage occurs in first trimester or second trimester may be influential.

Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) complicates approximately 5.8% of pregnancies (Lavery et al., 2016). In most cases, the blood glucose returns to prepregnancy levels after delivery. However, there is a strong relationship between GDM and subsequent type 2 diabetes mellitus (Bellamy et al., 2009). In addition, women who develop GDM are also more likely to experience other CHD risk factors including obesity, hypertension, and hyperlipidemia (Fraser et al., 2012). Current evidence suggests that GDM is associated with an increased risk of CHD but whether this association is independent of the increased risk due to type 2 diabetes is inconclusive due to limited data (Archambault et al., 2014).

Link Between Pregnancy Complications and CHD

The above shows robust evidence that women who develop these pregnancy complications are at a higher risk of CHD. But whether these complications are independent risk factors for CHD or whether a shared risk factor profile contributes to both pregnancy complications and subsequent CHD remains to be determined.

Shared Risk Factors Leading to Pregnancy Complications and Subsequent CHD

Established cardiovascular risk factors including obesity, hypertension, hyperlipidemia, and insulin resistance are also risk factors for pregnancy complications. Women who embark on a pregnancy with these preexisting risk factors and a superimposed genetic predisposition could develop pregnancy complications. If the risk factors persist after pregnancy, these women could develop CHD in later life. Therefore, pregnancy complications and CHD can be considered a spectrum of disorders that manifest at different time points during the life course. Hence, the concept that pregnancy can be considered as a vascular and metabolic stress test for women, which some pass (have an uncomplicated pregnancy) and some fail (develop one or more pregnancy complications), has been proposed (Williams, 2003).

Pregnancy Complications as the Cause for CHD

An alternative hypothesis is that pathophysiological changes that occur during complicated pregnancies may persist predisposing to later life vascular diseases, a theory that has mainly been conceived with respect to preeclampsia. There are many theories regarding the cause of preeclampsia, and current literature supports a multifactorial etiology. The most likely underlying pathogenic mechanisms include abnormal development of the placenta and an imbalance between proangiogenic and antiangiogenic proteins with a predominance of antiangiogenic factors. This angiogenic imbalance is proposed to result in maternal systemic endothelial dysfunction which could persist after pregnancy predisposing to later life vascular diseases. This hypothesis was recently tested in a mouse model of preeclampisa (Pruthi et al., 2015). A preeclampsia-like state was induced in pregnant CD1 mice by overexpressing soluble fms-like tyrosine kinase-1, a circulating antiangiogenic protein that induces hypertension and glomerular disease resembling human preeclampsia. Two months postpartum, features of preeclampsia were completely resolved. The mice were then challenged with unilateral carotid injury. "Preeclampsia"-exposed mice had significantly enhanced vascular remodeling with increased smooth muscle cell proliferation and vessel fibrosis compared to mice with uncomplicated pregnancies (Pruthi et al., 2015). These suggest that vessels exposed to preeclampsia retain a persistently enhanced vascular response to injury despite resolution of preeclampsia after delivery which may contribute to increased subsequent cardiovascular risk among preeclamptic women (Pruthi et al., 2015).

Placenta as a Link Between Pregnancy Complications and CHD

A successful pregnancy requires the development of an optimal uteroplacental vasculature. In normal pregnancy, the process of placentation is initiated when the blastocyst adheres to the maternal uterine endometrium leading to the formation of a lineage of epithelial cells called the invasive extravillous cytotrophoblasts (EVT). These cells invade the uterine decidua, the inner third of the myometrium (interstitial invasion), and the spiral arteries (endovascular invasion), a process that is highly controlled so that the depth of invasion of the uterus is sufficient but not excessive (Gude et al., 2004). During endovascular invasion, the endothelium and the underlying vascular smooth muscle cells are replaced by EVT resulting in the conversion of narrow-calibre high-resistance vessels into wide-calibre low-resistance vessels capable of supplying enough maternal blood to the placenta to accommodate the increasing demands of the growing fetus. To facilitate this process, cytotrophoblasts make a "switch" in their cell surface adhesion molecules from an epithelial to endothelial phenotype in a process called "pseudovasculogenesis" (Damsky et al., 1994). Failure of

these processes results in "decidual vasculopathy" with small, poorly developed spiral arteries and compromised blood flow to the placenta (Zhou et al., 1993).

Initially, impaired invasion and spiral artery remodeling were thought to lead to defective uteroplacental circulation and subsequent placental ischemia. More recently, it has been shown that true ischemia probably only occurs in more advanced stages and that stressors including inflammation and oxidative stress contribute to the release of soluble factors including soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) from the placenta that gain entry into the maternal circulation (Andraweera et al., 2012). sFlt-1 is a splice variant of the vascular endothelial growth Factor (VEGF) receptor and binds to both VEGF and placental growth factor (PlGF) preventing their interaction with the endogenous receptors located on vascular endothelial cells (Andraweera et al., 2012).

Both VEGF and PlGF are potent angiogenic factors that play critical roles in maintenance of a healthy vascular endothelium throughout the body (Andraweera et al., 2012). Endoglin is typically expressed on the surface of placental syncytiotrophoblasts and endothelial cells and acts as a coreceptor for the proangiogenic transforming growth factor β (Andraweera et al., 2012). Endoglin regulates nitric oxide-dependent vasodilatation. Proteolytic processing of the membrane-bound endoglin results in soluble endoglin which inhibits TGF β signaling and deleteriously affects vascular tone by blocking the activation of endothelial nitric oxide synthase (Andraweera et al., 2012). Increased sFlt-1 and sEng along with decreased serum VEGF and PlGF have been demonstrated to precede the clinical onset of preeclampsia and is used as biomarkers of preeclampsia (Andraweera et al., 2012). During a preeclamptic pregnancy, sFlt-1 and sEng produced by the placenta gain entry into the maternal circulation resulting in systemic maternal endothelial dysfunction (Andraweera et al., 2012) (**Fig. 2**). In theory, this endothelial injury may persist predisposing to later life vascular disease. However, recent evidence suggests that the risk for CVD after a preeclamptic pregnancy may be attenuated by a subsequent pregnancy (Morris et al., 2015).

Pregnancy-induced physiological changes to the cardiovascular system are vital for a successful pregnancy. Women who develop preeclampsia are considered to have failed the cardiovascular stress test of pregnancy and are considered to represent a subpopulation in whom the cardiovascular response was inadequate to meet the demands of normal pregnancy (Craici et al., 2008). The risk for preeclampsia is highest for first pregnancies, decreasing with subsequent pregnancies (van Rijn et al., 2006) assuming the woman maintains the same partner (for a detailed account of the role of the father in preeclampsia see Dekker et al., 2011).

A longer time interval between subsequent pregnancies is associated with an increasing risk for preeclampsia suggesting a protective effect of pregnancy that decreases overtime (Mikolajczyk et al., 2008). A recent study examined women prior to the first pregnancy and postpartum up to 33 months to evaluate the effect of pregnancy on cardiovascular physiology and compared women with a subsequent pregnancy to a time matched group of women who did not have a subsequent pregnancy (Morris et al., 2015). Women with a subsequent pregnancy were shown to have significantly decreased blood pressure and increased arterial compliance compared to their prepregnancy evaluation, when compared to those without a subsequent pregnancy (Morris et al., 2015). This suggests a beneficial effect of pregnancy on cardiovascular remodeling that may contribute to the attenuation of risk for preeclampsia in a subsequent pregnancy and is compatible with the findings of Skjaerven and colleagues that the risk for CVD after preeclampsia is mainly concentrated among women who have no subsequent births (Skjaerven et al., 2012).

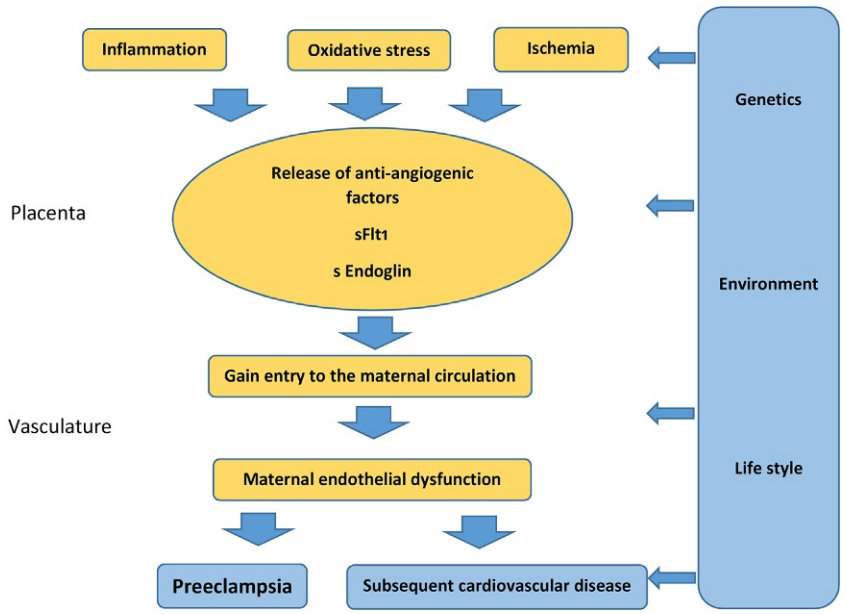

Fig. 2 Placenta as a link between preeclampsia and subsequent cardiovascular disease.

Endothelial Dysfunction Linking Preeclampsia With Subsequent CVD

Endothelial dysfunction is believed to play a critical role in preeclampsia, and the antiangiogenic molecules secreted by the placenta are thought to be key regulators of this process (Andraweera et al., 2012). Endothelial dysfunction precedes the clinical manifestations/clinical detection of preeclampsia. A recent systematic review assessed studies that examined endothelial dysfunction using flow-mediated dilation before, during, and after preeclampsia (Weissgerber et al., 2016). This review demonstrates that compared with women who did not have preeclampsia, women who had preeclampsia have lower flow-mediated dilation before the development of preeclampsia, at the time of preeclampsia, and for 3 years postpartum (Weissgerber et al., 2016). These suggest that vascular dysfunction predates preeclampsia and may contribute to its pathogenesis and may represent the mechanistic link for increased CVD risk subsequent to a preeclamptic pregnancy. This also suggests that similar risk factors may lead to preeclampsia and CVD.

Pregnancy Complications and Subsequent CVD: Common Antecedents

The link between pregnancy complications and increased risk for later life CHD is now well established. However, the unanswered question is whether the increased risk is attributed to factors that originate during a complicated pregnancy or whether prepregnancy risk factors associate with both groups of disorders. At present, the paucity of literature limits our ability to answer this question. A Norwegian study that combined longitudinal population-based data with medical birth records demonstrated that adjustment for prepregnancy classic cardiovascular risk factors including BMI, blood pressure, and lipid profiles attenuated much of the increased risk for future vascular disease after a gestational hypertensive disorder, suggesting that women already programmed to develop vascular diseases also develop placenta-mediated pregnancy complications (Romundstad et al., 2010). As with most multifactorial diseases, a genetic susceptibility can also contribute to both groups of disorders. A recent metaanalysis of microarray data, obtained from blood samples of patients with CVD versus controls and placental samples from women with preeclampsia versus healthy women with uncomplicated pregnancies, demonstrated that 22 differentially expressed genes were common between preeclampsia and CVD (Sitras et al., 2015). Bioinformatic analysis of these genes revealed that among others oxidative stress, inflammation and VEGF signaling pathways were involved in the pathophysiology of both preeclampsia and CVD (Sitras et al., 2015). Genome-wide association studies and candidate gene association studies also show that some gene variations in the above pathways are associated with both preeclampsia and CVD and to a lesser extent other pregnancy complications and CVD (Andraweera et al., 2011; Johnson et al., 2013; Andraweera et al., 2016).

Clinical Implications

The bulk of evidence linking pregnancy complications with CVD has resulted in preeclampsia being recognized by the American Heart Association as a female-specific cardiovascular risk factor (Mosca et al., 2011). Despite the gains that have been made in overall cardiovascular disease prevention, death rates due to CVD among women 35–54 years of age actually appear to be increasing (Mosca et al., 1997; Fuster et al., 2011), the most likely reason being the obesity epidemic. This emphasizes the urgent need for early prevention in target populations. Primary prevention, if applied to risk populations, can reduce the incidence of CVD. However, there is growing concern that many preventive efforts are implemented too late in order to be effective. Therefore, a strategy for "primordial prevention"—prevention of CVD risk factors themselves may prove more effective. In this context, pregnancy provides a unique opportunity to identify women who would most benefit from early preventive efforts.

Approximately, 80% of women in developed countries and 90% of women in developing countries give birth to at least one child (Rich-Edwards et al., 2014). The probability of a woman experiencing any one of the complications during pregnancy including gestational hypertension, preeclampsia, intrauterine growth restriction, gestational diabetes, or preterm delivery is 2%–12% (Rich-Edwards et al., 2014). In the developed world, 36% of first pregnancies and 29% of subsequent pregnancies are complicated by at least one of the above pregnancy complications (Rich-Edwards et al., 2014). Based on recent systematic reviews and metaanalyses, each pregnancy complication is associated with approximately double the risk of CVD. If 80% of women bear children and 30% of them have a pregnancy complication, then approximately 20%–30% of women are at increased risk of future CVD (Rich-Edwards et al., 2014).

This calls for the development of a postpartum referral system where the obstetrician who cared for the woman when she developed a pregnancy complication should initiate a follow-up process where the woman is subsequently cared for by her general practitioner and, when required, by a cardiologist. However, in order to prevent overburdening staff and the health budget, appropriate guidelines need to be formulated on the referral criteria.

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Comprehensive Lifestyle Modification for Hypertension and Lifestyle-Related Disease Under the New Guidelines

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Hypertension is one of the strongest risk factors for cardiovascular disease (CVD) worldwide (Rapsomaniki et al., 2014). Recent studies suggested that prevention of hypertension effects not only prevention of CVD but also the management of other diseases, such as dementia, cancer, and periodontal disease (Kokubo and Iwashima, 2015). In light of that concept, when lifestyle modifications recommended by hypertension guidelines are followed, we could expect that these diseases other than CVD could be prevented or ameliorated. Hypertension guidelines have recently been updated in many countries. The components of the various countries' hypertension guidelines include both pharmacotherapy- and nonpharmacotherapy-based recommendations, and the latter are also known as lifestyle modifications.

In this brief review, we organize the reported results that form the basis for the lifestyle modification recommendations for preventing and controlling hypertension that are provided in the major hypertension guidelines, and we indicate how these guidelines' lifestyle modification recommendations resemble those of other countries' guidelines.

U.S., Europe, and Japanese Guidelines

The Eighth Joint National Committee (JNC8) have reported 2014 evidence-based guidelines for the management of high blood pressure in adults (James et al., 2014). However, lifestyle recommendations are shown in a different report (Eckel et al., 2014), which is based on the target for reducing CVD risk factors, such as blood pressure and lipids. We therefore use the previous version from the seventh Joint National Committee (JNC7) (Chobanian et al., 2003), which consists of recommendations regarding weight reduction, adopting the DASH eating plan, dietary sodium reduction, physical activity, and the moderation of alcohol consumption in the present review (**Table 1**). The 2013 guidelines on hypertension from the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) (Mancia et al., 2013) follow the guidelines jointly issued by these two societies in 2003 (European Society of Hypertension-European Society of Cardiology Guidelines Committee, 2003), and 2007 (Mancia et al., 2007). The lifestyle modifications described by the ESH/ESC are similar to those noted by the JNC7.

In addition, an effective approach to high blood pressure control was approved by a science advisory committee of member of the American Heart Association (AHA), the American College of Cardiology (ACC), and the U.S. Centers for Disease Control and Prevention (CDC) (Go et al., 2014). "Clinical Practice Guidelines for the management of Hypertension in the Community" were also reported by the American Society of Hypertension (ASH) and the International Society of Hypertension (ISH) (Weber et al., 2014). These Guidelines' lifestyle modifications are similar to those defined in the Japanese Society of Hypertension Guideline in 2014 (Shimamoto et al., 2014), although cutoff points differ slightly. These lifestyle modifications consist of appropriate diet (reducing salt intake, eating more vegetables and fruits, reducing saturated fatty acid [e.g., by eating more fish]), moderate drinking, quitting smoking (including avoiding passive smoking), and increasing physical activity.

All of the guidelines systematically summarize the details of the lifestyle modifications needed to prevent and manage hypertension, and they emphasize the importance of comprehensive lifestyle modification. Among the above-mentioned guidelines, only the JSH 2014 guideline promotes adequate guidance/education for the prevention of obesity in children, which may be the one of the most important approaches to prevent hypertension at the population level. In addition, the JSH 2014 recommends modifying not only conventional risk factors for hypertension but also other modifiable lifestyle risk factors such as emotional stress, cold, and other daily activities (e.g., bathing, sexual intercourse) in daily life. However, these lifestyle factors have not been established as risk factors for hypertension, and further evidence must be established to test the effects of modifying these lifestyle risk factors for preventing hypertension.

Table 1 Lifestyle recommendations for the management and prevention of hypertension in the US, European, and Japanese guidelines

	JNC7 (Chobanian et al., 2003)	*2013 ESC/ESH (Mancia et al., 2013)*	*JSH 2014 (Shimamoto et al., 2014)*	*ASH/ISH (Weber et al., 2014)*
Weight reduction	18.5≦BMI≦24.9 kg/m^2	• Reduction of BMI to 25 kg/m^2 • Waist circumference; <102 cm (men), <88 cm (women)	BMI <25 kg/m^2	Weight loss in overweight and obese patients
Nutrients and food	Adopting DASH diet; rich in fruits, vegetables, and low fat dairy with a reduced content of saturated and total fat	• Increase intake of vegetables/fruits (300–400 g/day), low-fat dairy products, dietary and soluble fiber, whole grains, protein from plant sources, and fish (≥2/week) • Reduced intake of saturated fat and cholesterol	• Increased intake of fruit/vegetable and fish • Reduced intake of cholesterol/saturated fatty acid	
Dietary sodium reduction	Dietary sodium intake <100 mEq/L (2.4 g sodium or 6 g salt)	Salt intake <5–6 g/day	Salt intake <6 g/day	Reducing salt intake
Physical activity	Regular aerobic physical activity (>30 min) most days	• Moderate-intensity dynamic aerobic exercise (>30 min) on 5–7 days/week • Resistance exercises on 2–3 days/week	Daily primary periodic (≥30 min) and aerobic exercise	Regular aerobic exercise
Alcohol consumption	≤2 drinks/day (most men), ≤1 drink/day (women and lighter weight persons)	• ≤20–30 mL/day (men), ≤10–20 mL/day (women) • Total alcohol consumption <140 g/week (men), 80 g/week (women)	≤20–30 mL/day (men), ≤10–20 mL/day (women)	≦2 drinks/day (men), ≦1 drink/day (women)
Smoking	Stop smoking for cardiovascular risk reduction	Giving up smoking	• Smoking cessation • Avoiding passive smoking	Stop smoking
Others		Mediterranean type diet DASH diet	• Avoiding exposure to cold • Managing emotional stress	

Other Western Countries Guidelines

Table 2 shows lifestyle recommendations for the management and prevention of hypertension guidelines in Western countries, that is, the guidelines from Canada (Dasgupta et al., 2014), United Kingdom (National Institute for Health and Care Excellence, 2016), France (Blacher et al., 2014), and Australia (Guideline for the diagnosis and management of hypertension in adults, 2016). The basic lifestyle recommendations by these four countries are the same as the major guidelines summarized in **Table 1**, such as weight reduction, nutrients and food types, sodium reduction, physical activity, moderate alcohol consumption, and quitting smoking. Only the Australian guideline recommends physical activity levels according to age category (cutoff 65 years old), and the dietary sodium reduction recommendation is less than 4 g.

Other Countries/Regions Guidelines

Table 3 shows lifestyle recommendations for the management and prevention of hypertension guidelines in China (Kjeldsen et al., 2014; Liu, 2011; Wang, 2015), Taiwan (Chiang et al., 2015), South Korea (Shin et al., 2015), South Africa (Hypertension Guideline Working Group et al., 2014), and Latin America (Sanchez et al., 2009). The basic lifestyle recommendations in these five countries' guidelines are the same as those of the major guidelines in **Table 1**, that is, weight reduction, nutrients and food, dietary sodium reduction, physical activity, alcohol consumption, and smoking. Only Taiwan's guidelines states the possible harm of extreme

Table 2 Lifestyle recommendations for the management and prevention of hypertension in the Canadian, UK, French, and Australian guidelines

	Canada (CHEP) (Dasgupta et al., 2014)	*United Kingdom (NICE) (National Institute for Health and Care Excellence, 2016)*	*France (Blacher et al., 2014)*	*Australia (Guideline for the diagnosis and management of hypertension in adults, 2016)*
Weight reduction	● 18.5 ≦ BMI ≦ 24.9 kg/m^2 ● Waist circumference: <102 cm (men), <88 cm (women)		● Reducing weight if over weight	● BMI <25 kg/m^2 ● Waist circumference; <94 cm (men), <90 cm (Asian men), <80 cm (women)
Nutrients and food	● Emphasizes intake of fruits, vegetables, low-fat dairy products, dietary and soluble fiber,whole grains, and protein from plant sources reduced in saturated fat and cholesterol	● Ascertaining patients' diet patterns ● Promoting lifestyle change (offering appropriate guidance and written/audiovisual materials) ● Discouraging excessive consumption of coffee and other caffeine-rich products	● Adapting a diet rich in fruit and vegetables	● Limiting total fat intake to 20%–25% of energy intake ● 5 servings of vegetables and 2 servings of fruit daily
Dietary sodium reduction	● Sodium intake <2000 mg/day (5 g/day salt)	● Lower dietary sodium intake	● Reducing excessive salt consumption	● <4 g/day salt intake
Physical activity	● 30–60 min of moderate intensity dynamic exercise 4–7 days/week	● Ascertaining patients' exercise patterns ● Promoting lifestyle change; offering appropriate guidance and written/audiovisual materials	● Regular physical activity	(18–64 years old patients) ● Accumulation of 150–300 min of moderate intensity activity/week ● Accumulation of 75–150 min of vigorous activity/ week ● Muscle strengthening activities ≥2 days/ week (>65 years old patients) ● Some form of physical activity ● Accumulation of ≥30 min of moderate intensity physical activity on most days
Alcohol consumption	● ≤2 drinks/day ● ≤14 standard drinks/week (men), ≤9 drinks/week (women)	● Avoiding excessive alcohol intake	● Reducing excessive consumption of alcohol	● ≤2 drinks/day (men), ≤1 drink/day (women)
Smoking		● Stop smoking	● Smoking cessation	● Cessation of smoking
Others	● Stress management			

sodium intake restriction, based on several studies that reported a J-shaped relationship between sodium intake and the composite outcomes (death, major cardiovascular events).

All of the listed guidelines systematically summarize the modifiable lifestyle risk factors based on research evidence. However, most of the guidelines do not describe ways to implement their lifestyle modifications practically, and although the Australian

Table 3 Lifestyle recommendations for the management and prevention of hypertension guidelines in the China, South Korean, Taiwan, South Africa, and Latin American guidelines

	China (Kjeldsen et al., 2014; Liu, 2011; Wang, 2015)	*Taiwan (Chiang et al., 2015)*	*South Korea (Shin et al., 2015)*	*South Africa (Hypertension Guideline Working Group et al., 2014)*	*Latin America (Sanchez et al., 2009)*
Weight reduction	Reduction of body weight	$22.5 \leqq BMI \leqq 25.0$ kg/m^2	Reduce by 1 kg	$18.5 \leqq BMI \leqq 24.9$ kg/m^2	Weight reduction and stabilization
Nutrients and food	Increasing dietary potassium	Recommendation of the DASH diet	Vegetable-based healthy diet habit	● Recommendation of the DASH diet ● Reduced intake of saturated fat and total fat	● Increase in fruits/ vegetables ● Decrease in saturated ant total fat intake ● Increase of potassium intake >6 g/ day
Dietary sodium reduction	Sodium reduction	● Sodium intake of 2–4 g/day ● Alerting possible harmless of too aggressive sodium restriction to <2 g/day	Salt intake; <6 g/ day	<100 mmol or 6 g NaCl/day	Salt intake <6 g/ day
Physical activity	Increasing physical activity	Aerobic, at least 40 min/day, and at least 3–4 days/week	30–50 min/day for more than 5 days in a week	30 min brisk walking most days	Recommending physical exercise
Alcohol consumption	Reduction of alcohol intake	<30 g/day for (men), <20 g/day (women)	<2 glasses/day	$\leqq$2 drinks/day	Reduction of excessive alcohol intake
Smoking	Smoking cessation	Smoking cessation	Smoking cessation	Complete smoking cessation	Smoking cessation
Others			Controlling of emotional stress		

guidelines attempt to show how to modify one's lifestyle in practice, the evidence for this modification is lacking. For both healthcare providers and patients, the methods that can be used for achieving lifestyle modifications are of interest, and a further accumulation of evidence regarding efficient methodology for lifestyle interventions is needed.

Appropriate Diet

Many epidemiological studies have shown that salt intake reduction is directly related to decreasing blood pressure. The Dietary Approaches to Stop Hypertension (DASH) diet, which was investigated in a randomized trial comparing the effects of three total salt intake levels (high, intermediate, and low salt intakes) on blood pressure, resulted in significantly lower systolic (SBP) and diastolic blood pressures (DBP) at each salt level, respectively (Sacks et al., 2001). The DASH diet and salt reduction lowered SBP and DBP independently. In addition, a meta-analysis of randomized controlled trials demonstrated that the decreases in blood pressure for a 100-mmol/day reduction in daily sodium excretion were 3.7 and 1.0 mmHg for SBP ($P < 0.001$) and 0.9 and 0.1 mmHg for DBP ($P > 0.05$) in the hypertensive and normotensive trials, respectively (Midgley et al., 1996). A systematic review and meta-analysis showed that reduced sodium intake reduced blood pressure and had no adverse effect on blood lipids, catecholamine levels, or renal function, and moderate quality evidence in children showed that a reduction in sodium intake reduced the children's blood pressure (Aburto et al., 2013).

A meta-analysis of 17 randomized controlled trials evaluated the current evidence on the effect of healthy dietary patterns such as the DASH diet, Nordic diet, and Mediterranean diet on blood pressures in adults (Ndanuko et al., 2016). In this meta-analysis, significant reductions of 4.26 mmHg in SBP and 2.38 mmHg in DBP were observed. When the authors of the meta-analysis considered the nature of intervention, they found that the studies in which weight loss, exercise, and sodium restriction were kept constant had a significant reduction of 4.25 mmHg in SBP and 2.27 mmHg in DBP. The consumption of dietary patterns

characterized by a high consumption of fruit, vegetables, whole grains, legumes, seeds, nuts, fish, and dairy and a low consumption of meat, sweets, and alcohol resulted in significant reductions in blood pressure.

Weight Reduction and Physical Activity

A meta-analysis of 25 randomized controlled trials from different ethnic populations showed a blood pressure reduction of −4.4/−3.6 mmHg for an approximately 5 kg weight loss by means of energy restriction, physical activity, or both (Neter et al., 2003). Larger blood pressure reductions were achieved in populations that included subjects taking antihypertensive drugs. A recent systematic review and meta-analysis of randomized control trials with a meta-regression of potential effect modifiers revealed that exercise was associated with a reduction in SBP of −4.40 mmHg and in DBP of −4.17 mmHg at 3–6 months (Williamson et al., 2016). The observed intervention effect was lost after 12 months of follow-up with no reported benefit over control. These findings indicate that continuing exercise is necessary for lowering blood pressure.

Moderate Alcohol Consumption

Drinking alcohol is associated with increased blood pressure in many epidemiological studies. A meta-analysis of randomized controlled trials showed that a reduction in alcohol intake among heavy drinkers significantly reduced their systolic and diastolic blood pressure (Xin et al., 2001); overall, alcohol reduction was associated with a significant reductions of −3.31 mmHg in SBP and −2.04 mmHg in DBP. A prospective study indicated that moderate drinking reduced the risk of CVD in hypertensive patients and that there was a U-shaped relationship between the volume of alcohol and the risk of CVD (Higashiyama et al., 2013).

Smoking Cessation

Smoking is a risk factor for many diseases including cancer and CVD. Considering that the purpose of hypertension treatment is to prevent CVD, quitting smoking is important regardless of the influence of smoking on blood pressure. Both normotensive and untreated hypertensive smokers present higher daily blood pressure levels compared to nonsmokers (Minami et al., 1999; Groppelli et al., 1992). Even passive smoking increases the risks of cancer and CVD (Makris et al., 2009).

Prevention of CVD

Table 4 shows the lifestyle recommendations for the prevention and management and prevention of CVD from the United States (James et al., 2014), Europe (Piepoli et al., 2016), Japan (Teramoto et al., 2013), Australia (National Vascular Disease Prevention Alliance, 2012), and New Zealand (New Zealand Guidelines Group, 2012). The basic skeleton of the CVD guidelines is almost the same as that of the hypertension guideline. A characteristic feature of the CVD guidelines is that they recommend fish intake (in the guidelines from Europe, Japan, and Australia), and the recommendation encouraging fish eating is also part of the hypertension guidelines of these countries. In the Japanese guidelines, an increased intake of unrefined grains, seaweed, and soy products is part of the JAS other than the hypertension guideline.

Conclusions

The updated hypertension guidelines in major countries consist of the following elements: weight reduction, appropriate food intake (dietary patterns characterized by a high consumption of fruit, vegetables, whole grains, legumes, seeds, nuts, fish, and dairy and a low consumption of meat and sweets), dietary sodium reduction, increasing physical activity, moderate alcohol consumption, and quitting smoking (including avoiding passive smoking). The hypertension guidelines are essentially the same for each country or region, beyond race and culture. The CVD guidelines are very similar to the hypertension guidelines, and the JAS guidelines add the promotion of soy products in the hypertension guideline. Therefore, the various hypertension guidelines mirror the basic skeletons of other disease guidelines. Toward the prevention of cardiovascular diseases, it is of primary importance to improvement the lifestyle modifications described in the hypertension guidelines.

Table 4 Lifestyle recommendations for the management and prevention of cardiovascular disease in the JNC8, ESC, JAS, and Australian guidelines

	JNC8 (James et al., 2014)	*ESC (Piepoli et al., 2016)*	*JAS (Teramoto et al., 2013)*	*Australia (National Vascular Disease Prevention Alliance, 2012)*	*New Zealand (New Zealand Guidelines Group, 2012)*
Weight reduction	• $18.5 \leqq BMI \leqq 24.9$ kg/m^2 • Waist circumference: <102 cm for men, <88 cm for women	• $20 < BMI < 25$ kg/m^2	Maintaining an ideal body weight	• BMI <25 kg/m^2 • Waist circumference <94 cm in men (<90 cm in Asian men), <80 cm (women, including Asian women)	Reducing risks of excessive body weight
Nutrients and food	Emphasizes intake of fruits, vegetables, low-fat dairy products, dietary and soluble fiber, whole grains, and protein from plant sources reduced in saturated fat and cholesterol	• SFA to account for <10% of total energy intake, through replacement by PUFAs • Trans unsaturated fatty acids: as little as possible, preferably no intake from processed food, and <1% of total energy intake from natural origin • 30–45 g/day, preferably from wholegrain products • Fruits ≥200 g/day (2–3 servings) • Vegetable ≥200 g/day (2–3 servings) • Fish 1–2/week, one of which to be oily • Unsalted nuts 30 g/day • Sugar-sweetened soft drinks and alcoholic beverages consumption must be discouraged	• Increase intake of vegetables, fruits, unrefined grains, seaweed, fish, and soy products • Reducing intake of meat fat, dairy products, and egg yolk	• Consume a varied diet rich in vegetables, fruits, wholegrain cereals, lean meat, poultry, fish, eggs, nuts and seeds, legumes and beans, and low-fat dairy products • Limit foods containing saturated and trans fats	Low energy, low glycogenic index/load, and macronutrient approach
Dietary sodium reduction	Sodium intake <2000 mg/day (5 g/day salt)	Salt intake <5 g/day	Reduced intake of food containing too much salt	Salt intake <6 g/day (~2300 mg sodium)	
Physical activity	30–60 min of moderate intensity dynamic exercise 4–7 days/week	• For healthy adults of all ages; ≥150 min/week (moderate intensity) or 75 min/week (vigorous intensity) aerobic PA or an equivalent combination thereof • For healthy adults of all ages; a gradual increase in aerobic • PA to 300 min/week (moderate intensity), or 150 min/week (vigorous intensity), or an equivalent combination thereof	Aerobic exercise ≥30 min daily	At least 30 min physical activity on most or preferably every day of the week	• Increasing periods of physical activity ≥60 min every day • Reducing screen time
Alcohol consumption	• ≤2 drinks/day • Limiting 14 standard drinks/week (men), 9 drinks/week (women)	<20 g/day (men), <10 g/day (women)	Avoiding excessive alcohol consumption	≤2 standard drinks per day	
Smoking		• Smoking cessation • Avoiding passive smoking	• Stop smoking • Avoiding passive smoking	Stop smoking using counseling, and if required nicotine replacement therapy or other medication	
Others	Stress management	Prescribing psychological therapy for psychosocial risk factors and coping with illness			

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Computational Systems Biology for the VEGF Family in Angiogenesis

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Angiogenesis Overview

The Angiogenic Process

Angiogenesis is the physiological process where new microvessels form from preexisting microvessels (Ribatti and Crivellato, 2012; Djonov et al., 2000). Similarly, arteriogenesis is where new collateral arteries form from preexisting arteries (Helisch and Schaper, 2003; Heil et al., 2006). As angiogenesis and arteriogenesis are similar processes (Heil et al., 2006), albeit at different scales, we use either term interchangeably for the purpose of this review. Angiogenesis occurs in two different forms: sprouting or intussusceptive angiogenesis (Ribatti and Crivellato, 2012; Djonov et al., 2000). Sprouting angiogenesis involves preexisting blood vessels to sprout and form new blood vessels. Sprouting angiogenesis is initiated by extracellular growth factor binding to endothelial cell (EC) surface receptors (Ucuzian et al., 2010). This ligand-receptor binding has dual action: (1) it initiates enzyme secretion from ECs, which break down the basement membrane and (2) it promotes directed EC migration and proliferation (Ucuzian et al., 2010). The migrating ECs result in tube formation and fusion, which are stabilized by pericyte recruitment in microvessels, or smooth muscle cell recruitment in arteries, to result in new, functional blood vessels (Eilken and Adams, 2010; Ribatti and Crivellato, 2012). The majority of current angiogenesis research focuses on sprouting angiogenesis, due to its prevalence in wound healing (Honnegowda et al., 2015) and cancer progression (Welti et al., 2013).

Intussusceptive angiogenesis is the splitting of an existing blood vessel into two blood vessels (Burri et al., 2004; Mentzer and Konerding, 2014; Djonov et al., 2000). Intussusceptive angiogenesis occurs by blood vessel walls continuously extending into the

lumen, forming an intravascular pillar, which eventually splits a single tube into two tubes. Unlike sprouting angiogenesis, intussusceptive angiogenesis is ineffective at vascularizing regions lacking blood vessels, instead primarily adding additional vessels to regions already containing blood vessels (Djonov et al., 2000; Burri et al., 2004; Mentzer and Konerding, 2014). Additionally, intussusceptive angiogenesis does not require EC migration or proliferation (Mentzer and Konerding, 2014). While intussusceptive angiogenesis can be initiated by growth factor stimulation, it also results from mechanical stress produced by blood flow (Makanya et al., 2009). Intravascular pillars seem to specifically form at vessel bifurcations when hemodynamics are altered to cause high flow velocity, but low shear stress (Styp-Rekowska et al., 2011; Jain et al., 2014). As such, intussusceptive angiogenesis is difficult to regulate, as hemodynamics cannot be easily altered and requires invasive procedures (Jain et al., 2014). Further research investigating chemical cues, including any mechanotransduction pathways activated through shear stress, is necessary to develop efficient, noninvasive methods for regulating intussusceptive angiogenesis.

Growth Factor-Directed Angiogenesis

Sprouting and intussusceptive angiogenesis are both critical to normal physiological processes, such as wound healing and embryonic development. Moreover, over 70 diseases, including cancer and occlusive vascular disease, are angiogenesis dependent (Egginton, 2010; Carmeliet and Jain, 2000). In 1971, Judah Folkman hypothesized that tumor growth depends on angiogenesis initiated by a tumor angiogenesis factor (Folkman, 1971). This hypothesis was derived from studies showing that tumors only grow to a dormant state, at 2–3 mm in diameter, in the absence of neovascularization (Folkman, 1970; Gimbrone et al., 1969; Greene, 1941), tumor implantation induces EC proliferation (Cavallo et al., 1972; Folkman, 1971) and formation of new capillaries (Warren and Shubik, 1966; Warren, 1966; Algire et al., 1945), and tumor growth is limited by the rate of EC proliferation (Tannock, 1968, 1970). Since this hypothesis, many studies have been conducted to arrive at our current understanding of tumor angiogenesis: tumor cells promote sprouting angiogenesis to provide the necessary nutrients for further tumor growth and metastasis, reviewed in (Hillen and Griffioen, 2007; Weis and Cheresh, 2011; Carmeliet and Jain, 2000). Inhibiting sprouting angiogenesis is therefore a promising approach to prevent transition of tumors from a benign to malignant stage (Samant and Shevde, 2011; Moreira et al., 2007).

Vascular Normalization

In 2005, Rakesh Jain put forth an alternative hypothesis on tumor angiogenesis: rather than destroying tumor vasculature to deprive the tumor of oxygen and nutrients, antiangiogenic therapies are most effective by normalizing the abnormal tumor vasculature to allow more efficient drug delivery (Jain, 2005). This hypothesis was derived from studies showing that tumor vasculature is structurally and functionally abnormal (Jain, 2004; McDonald and Choyke, 2003; Baluk et al., 2003), that this structural abnormality impairs blood flow and compromises the ability for drug delivery to tumors (Jain, 1998; Ma et al., 2001; Padera et al., 2004), and that normalizing tumor vasculature allows drug delivery deeper into tumors to cause tumor regression (Tong et al., 2004; Wildiers et al., 2003; Winkler et al., 2004). Studies have continued to provide support for this hypothesis, reviewed in (Huang et al., 2013; Goel et al., 2012); a recent clinical trial shows that vascular normalization, measured by pericyte coverage, is associated with improved pathological response to the antiangiogenic drug bevacizumab (Tolaney et al., 2015). Understanding the mechanisms through which antiangiogenic drugs normalize tumor vasculature, and optimizing treatment regimens to best regulate sprouting angiogenesis, is a primary challenge for preventing tumor angiogenesis and tumor progression (Cao, 2016; Vasudev and Reynolds, 2014).

Occlusive vascular diseases stem from a lack of blood flow, resulting in tissue ischemia, loss of limb function, and death (Peach et al., 2012). For occlusive vascular diseases, promoting either sprouting or intussusceptive angiogenesis to reestablish proper blood flow is therefore a promising approach to prevent tissue ischemia (Berger and Hiatt, 2012; Rasmussen et al., 2002). Overall, the ability to control angiogenesis would allow for the prevention and treatment of pathologies: preventing cancer mortality by inhibiting tumor angiogenesis, and treating vascular diseases by promoting angiogenesis.

VEGF Family Overview

The vascular endothelial growth factor-A (VEGF-A) is a key growth factor that promotes angiogenesis. The existence of VEGF-A was first hypothesized by Judah Folkman in 1971, who characterized VEGF-A as an unknown tumor angiogenesis factor (Folkman, 1971). Senger et al. identified this unknown factor as vascular permeability factor (VPF) in 1983 (Senger et al., 1983), and Leung et al. characterized this factor, and termed it VEGF, in 1989 (Leung et al., 1989). Keck et al. showed in 1989 that VPF and VEGF are the same molecule (Keck et al., 1989), demonstrating that this single factor has multiple functions. In 1993, Napoleone Ferrara's laboratory demonstrated for the first time that inhibiting VEGF suppresses tumor growth (Kim et al., 1993) (**Fig. 1**). Since these studies, VEGF has been studied as a promising therapeutic target for cancer and vascular disease, reviewed in (Majesky, 1996; Carmeliet and Jain, 2000).

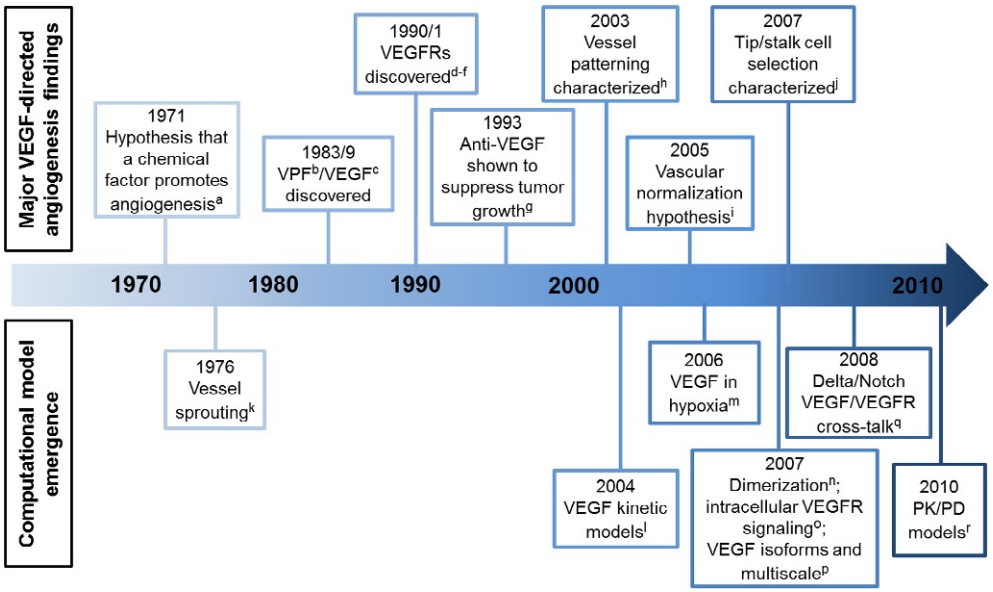

Fig. 1 *Timeline of VEGF-directed angiogenesis research.* Timeline highlighting the major discoveries and emergence of computational models in VEGF-directed angiogenesis. Superscripts refer to references of the discovery or the first known study to develop a computational model for that specific topic. References are: [a](Folkman, 1971); [b](Senger et al., 1983); [c](Leung et al., 1989); [d](Shibuya et al., 1990); [e](Matthews et al., 1991); [f](Terman et al., 1991); [g](Kim et al., 1993); [h](Gerhardt et al., 2003); [i](Jain, 2005); [j](Hellstrom et al., 2007); [k](Liotta et al., 1977); [l](Mac Gabhann and Popel, 2004); [m](Qutub and Popel, 2006); [n](Feilim Mac Gabhann and Popel 2007); [o](Alarcón and Page, 2007); [p](Mac Gabhann and Popel, 2007a,b); [q](Bentley et al., 2008); [r](Stefanini et al., 2010).

VEGF Ligands

VEGF-A is now known as one of five related growth factors expressed in humans that make up the VEGF family: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF) (Ferrara et al., 2003). There are two additional VEGF ligands: viral VEGF (VEGF-E) (Ogawa et al., 1998) and snake venom VEGF (VEGF-F) (Takahashi et al., 2004); these ligands are not expressed in humans, and as such, shall not be discussed in detail in this review. The VEGF growth factors bind with high affinity to three tyrosine kinase receptors, VEGFR1, VEGFR2, and VEGFR3. Many VEGF ligands also contain a heparin-binding domain, in addition to binding neuropilins, coreceptors to the VEGFRs. VEGF-A, often referred to simply as VEGF, promotes angiogenesis through interaction with VEGFR1 and VEGFR2. Conversely, all other VEGF growth factors and VEGFR3 exhibit weak angiogenic potential. VEGF-B and PlGF specifically bind VEGFR1, and have been identified as key promoters in neurogenesis and embryogenesis. VEGF-C and VEGF-D promote lymphangiogenesis through VEGFR3 (**Table 1**).

The VEGF ligands and receptors are also expressed in isoform variants, each having specific interactions and functions. VEGF-A has seven currently known splice variants, in addition to full-length VEGF-A, which are distinguished by amino acid length: VEGF-A_{121}, VEGF-A_{145}, VEGF-A_{148}, VEGF-A_{165}, VEGF-A_{183}, VEGF-A_{189}, and VEGF-A_{206}. A VEGF-A_{110} isoform is also created through proteolytic cleavage of longer VEGF isoforms by plasmin (Keyt et al., 1996). VEGF-regulated angiogenesis research typically focuses on VEGF-A_{165}, the predominant VEGF-A isoform (Ferrara et al., 2003). For this reason, VEGF-A_{165} is often referred to simply as VEGF, a notation we adopt henceforth in this review.

The aforementioned splice variants have recently been typified as the VEGF-A_{xxxa} isoforms, as secondary VEGF-A_{xxxb} isoforms containing the same number of amino acids, but different sequences and function, have emerged. Currently, four VEGF-A_{xxxb} isoforms have been identified: VEGF-A_{121b}, VEGF-A_{145b}, VEGF-A_{165b}, and VEGF-A_{189b}, fully reviewed in (Qiu et al., 2009; Hilmi et al., 2012). Key points to know about these isoforms include: VEGF-A_{165b} is the best studied VEGF-A_{xxxb} isoform; VEGF-A_{165b} binds to VEGFR2 with the same kinetics as VEGF-A_{165}, but does not activate VEGFR2 nor the signaling pathways that VEGF-A_{165} activates (Woolard et al., 2004). Subsequently, the VEGF-A_{xxxa} isoforms are characterized as pro-angiogenic, whereas the VEGF-A_{xxxb} isoforms are antiangiogenic.

Likewise, multiple isoforms of VEGF-B have also been discovered (Olofsson et al., 1996a,b). VEGF-B is considered to primarily be a neuroprotective factor (Falk et al., 2009); VEGF-B has also been identified to act as a myocardium-specific angiogenic factor (Kivelä et al., 2014; Bellomo et al., 2000) and a regulator of energy metabolism by modulating fatty acid uptake (Hagberg et al., 2010), reviewed in (Li, 2010; Bry et al., 2014). The two discovered VEGF-B isoforms are VEGF-B_{167} and VEGF-B_{186}, differentiated by amino acid length (Olofsson et al., 1996a,b). VEGF-B_{167} has been identified as the predominant isoform, with over 80% total VEGF-B being expressed as VEGF-B_{167} (Li et al., 2001). However, other than the fact that VEGF-B_{167} contains a heparin-binding domain while VEGF-B_{186} does not (Li, 2010), the functional differences between VEGF-B_{167} and VEGF-B_{186} are currently unknown.

Conversely, VEGF-C does not exist in multiple isoforms. VEGF-C is considered to primarily promote lymphangiogenesis through interaction with VEGFR3, reviewed in (Su et al., 2007). VEGF-C also interacts with VEGFR2, although VEGF-C/VEGFR2

Table 1 The VEGF family proteins

Protein		*Family interactions*	*Primary function*	*Discovery*
VEGF-A		VEGFR1, VEGFR2	Proangiogenic	(Senger et al. 1983; Leung et al. 1989)
	$VEGF\text{-}A_{110}$	VEGF-A (homodimer)		(Burchardt et al. 1999)
	$VEGF\text{-}A_{121}$	PlGF		(Tischer et al. 1991)
	$VEGF\text{-}A_{145}$			(Poltorak et al. 1997)
	$VEGF\text{-}A_{148}$		Unknown	(Whittle et al. 1999)
	$VEGF\text{-}A_{165}$		Proangiogenic	(Tischer et al. 1991)
	$VEGF\text{-}A_{183}$			(Lei et al. 1998)
	$VEGF\text{-}A_{189}$			(Tischer et al. 1991)
	$VEGF\text{-}A_{206}$			(Houck et al. 1991)
	$VEGF\text{-}A_{121b}$		Antiangiogeneic	(Woolard et al. 2004)
	$VEGF\text{-}A_{145b}$			(Perrin et al. 2005)
	$VEGF\text{-}A_{165b}$			(Bates et al. 2002)
	$VEGF\text{-}A_{189b}$			(Woolard et al. 2004)
VEGF-B		VEGFR1	Neurogenesis and embryogenesis	(Olofsson et al. 1996b)
	$VEGF\text{-}B_{167}$	VEGF-B (homodimer)		(Olofsson et al. 1996b)
	$VEGF\text{-}B_{186}$			(Olofsson et al., 1996a)
VEGF-C		VEGFR3, VEGFR2 VEGF-C (homodimer)	Lymphangiogenesis	(Joukov et al. 1996)
VEGF-D		VEGFR2, VEGFR3 VEGF-D (homodimer)	Lymphangiogenesis	(Achen et al. 1998)
PlGF		VEGFR1	Proangiogenic	(Maglione et al. 1991)
	PlGF-1	PlGF (homodimer)		(Maglione et al. 1993)
	PlGF-2	VEGF-A		(Maglione et al. 1993)
	PlGF-3			(Cao et al. 1997)
	PlGF-4			(Yang et al. 2003)
VEGFR1		VEGF-A, VEGF-B, PlGF	Angiogenesis	(Shibuya et al. 1990)
	sVEGFR1	VEGFR1 (homodimer) VEGFR2	Antiangiogenic	(Kendall and Thomas 1993)
VEGFR2		VEGF-A VEGFR2 (homodimer)	Angiogenesis	(Matthews et al. 1991; Terman et al. 1991)
	sVEGFR2	VEGFR1, VEGFR3	Antiangiogenic Antilymphangiogenic	(Ebos et al. 2004)
VEGFR3		VEGF-C	Lymphangiogenesis	(Aprelikova et al. 1992)
	sVEGFR3	VEGFR3 (homodimer) VEGFR2	Antilymphangiogenic	(Singh et al. 2013)

Currently characterized ligands, receptors, and their isoforms in the VEGF family. Inter-family interactions, function, and discovery of each VEGF family protein are given. I list the general, primary function for each protein; note that specific function may differ depending on cell type or physiological context.

interactions do not appear sufficient to promote lymphangiogenesis (Kodera et al., 2011). VEGFR2 might have an indirect modulatory role in VEGF-C lymphangiogenesis: VEGF-C induces VEGFR2/VEGFR3 heterodimerization, unlike VEGF-A, which differentiates VEGF-C signaling from VEGFR3 homodimers (Deng et al., 2015; Nakao et al., 2011).

Similarly, VEGF-D does not exist in multiple isoforms. VEGF-D is considered to primarily promote lymphangiogenesis through VEGFR3, as reviewed in (Krebs and Jeltsch, 2013). VEGF-D also binds VEGFR2 (Achen et al., 1998), implying that VEGFR2/VEGFR3 heterodimerization might be important for VEGF-D signaling. However, unlike with VEGF-C, lymphatic development does not appear to be affected by VEGF-D deletion (Baldwin et al., 2005). As such, VEGF-D signaling and function remains questionable and additional research is necessary to make any additional assertions about VEGF-D signaling.

PlGF contains four known isoforms, termed PlGF1-4 (Maglione et al., 1993; Cao et al., 1997; Yang et al., 2003). Similar to VEGF, PlGF isoforms result from alternative splicing, each containing a different number of amino acids: 131, 152, 203, and 224. Like the VEGF-B isoforms, PlGF-2 and PlGF-4 contain heparin-binding domains, while PlGF-1 and PlGF-3 do not (De Falco, 2012). Also like the VEGF-B isoforms, the functional difference between PlGF isoforms is not currently known.

VEGF Receptors

Similar to the VEGF ligands, the VEGFRs are also expressed in variant isoforms. Soluble isoforms, truncated full-length receptors without the transmembrane or intracellular domains, were identified for all three VEGFRs (Kendall and Thomas, 1993; Pavlakovic et al., 2010; Singh et al., 2013). These soluble isoforms are considered to contain no signaling properties, acting to sequester free VEGF (Hornig et al., 2000; Pavlakovic et al., 2010). The soluble VEGFR isoforms can dimerize with full-length membrane VEGFRs, which may additionally direct VEGFR signaling (Kendall et al., 1996). Intracellular VEGFR isoforms also exist; intracellular VEGFR1 isoforms containing either the full or partial intracellular domain of full-length VEGFR1 were identified (Mezquita et al., 2010,

2014). It stands to reason that other VEGFR isoforms may yet be undiscovered. Identifying all VEGFR isoforms and functions may be necessary to achieve complete control of angiogenesis.

Dimerization, the binding of two receptor monomers to form a receptor dimer, is a critical step to VEGFR phosphorylation and signal transduction. VEGFR1, VEGFR2, and VEGFR3 all form homodimers: two VEGFR1 monomers bind to form a VEGFR1-VEGFR1 homodimer, etc. Heterodimerization, where two different VEGFR monomers bind, also occurs. VEGFR2 forms hetero-dimers with both VEGFR1 and VEGFR3, whereas VEGFR1 and VEGFR3 are not able to heterodimerize. These homodimer and hetero-dimer pairs can activate different intracellular signaling pathways, leading to differential cell responses.

Overall, this VEGF family overview showcases the large VEGF signaling network. The multiple ligand types, receptor types, isoforms, and dimers complicate our ability to understand and predict how angiogenesis occurs. Furthermore, VEGF signaling cooperates with signaling from other receptors to direct angiogenesis: VEGF-VEGFR and Delta-Notch signaling interact to direct tip/stalk cell selection in sprouting angiogenesis, reviewed in (Blanco and Gerhardt, 2013). Thus, our ability to effectively regulate angiogenesis for cancer and vascular disease therapeutics has relied on methods that delineate this complex VEGF signaling axis to identify key signaling features and targets.

Here, we discuss how systems biology has been used to provide this delineation of VEGF signaling, to identify key VEGF signaling features and targets, in angiogenesis. Systems biology is an iterative approach between mathematical or computational modeling with quantitative experimentation to understand the entire biological system (Chen et al., 2014). Systems biology is also advantageous by being quantitative and predictive in nature, allowing features such as model-directed experiments to quicken discovery of key angiogenesis nodes. Systems biology also has the power to isolate and examine subsystems within angiogenesis, such as receptor signaling pathways to identify critical signaling nodes in angiogenesis (Weddell and Imoukhuede, 2015a). As such, systems biology can examine a system at various scales: angiogenesis can be examined macroscopically, such as sprout formation, or microscopically, such as VEGFR signal propagation.

In this review, we provide an overview of systems biology techniques that have been employed to mathematically or computationally explore angiogenesis (**Table 2**, **Fig. 2**). We review studies employing these systems biology techniques to examine the VEGF family in angiogenesis to provide new biological insights, and to design pro-angiogenic or antiangiogenic therapies. Lastly, we provide a brief overview on the current challenges in manipulating VEGF signaling and angiogenesis and future research directions to achieve complete angiogenic control.

Systems Biology Approaches

Deterministic Kinetic Modeling

Chemical reactions describing the kinetic reaction network are modeled using the law of mass-action: the rate of a reaction is directly proportional to reactant concentration (Eq. 1):

$$[\mathrm{A}] + [\mathrm{B}] \underset{k_r}{\overset{k_f}{\rightleftarrows}} [\mathrm{C}] \tag{1}$$

Table 2 Systems biology modeling approaches

Computational method	*Scale*	*Functional outputs*	*Angiogenesis applications*	*Reference*
Kinetic: deterministic	Molecules in continuum	Temporal concentration	Protein interactions Protein transport Drug PK/PD	(Resat et al. 2009)
Kinetic: stochastic	Molecules	Temporal concentration	Protein interactions Protein transport Drug PK/PD	(Resat et al. 2009)
Agent based	Molecular Cell	Spatiotemporal agent dynamics	Protein or cell motility Protein or cell interactions Cell proliferation	(An et al. 2009)
Molecular modeling	Molecular	Binding potential	Structural analysis Inhibitor identification	(Aloy and Russell 2006)
Finite element	Tissue Fluids	Continuum mechanics	Hemodynamics Vessel sprouting Drug delivery	(Vanegas-Acosta and Garzón-Alvarado 2014)
Multivariate	Cell Tissue	Signal-to-response	Stimuli to cell response	(Kirwan et al. 2012)

Typical computational models used in systems biology, the scale(s) of the quantities they model (i.e. molecules, cell, tissue), functional output(s) given by the model, specific applications to angiogenesis, and references describing the methods in detail.

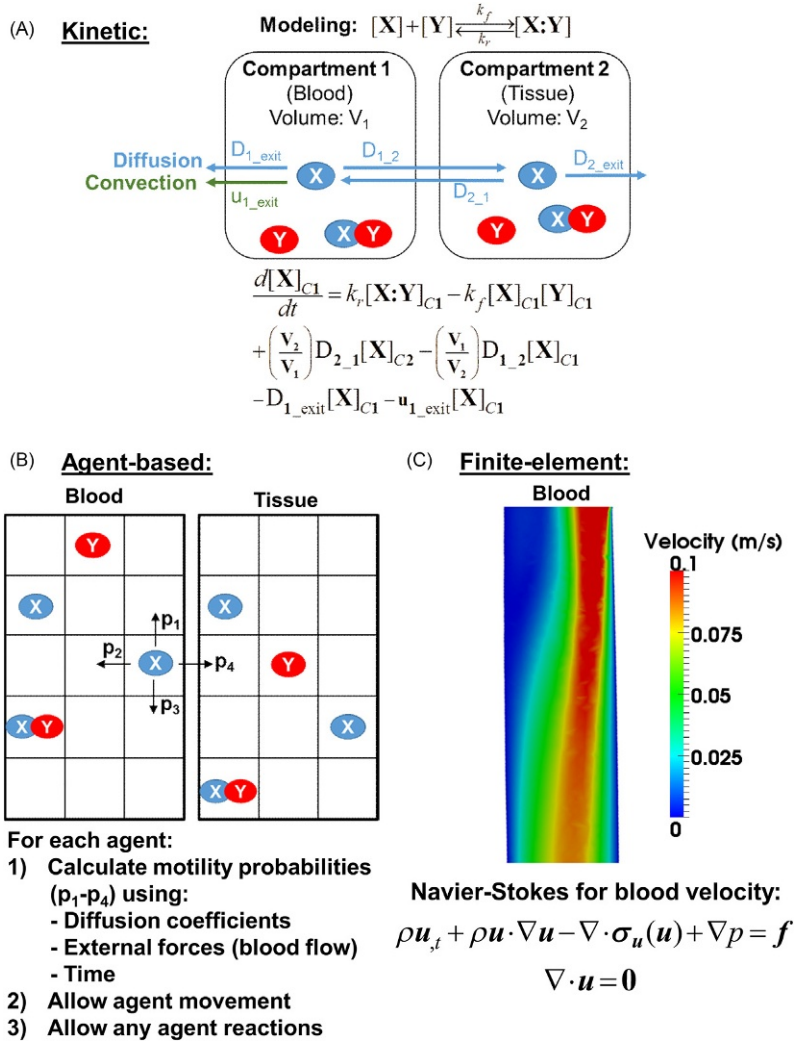

Fig. 2 *Example systems biology techniques to model protein transport and interactions.* (A) A deterministic kinetic compartmental model containing a single chemical reaction involving two molecules [X] and [Y] binding to form [X:Y], all with units of M. The reaction is defined by the forward rate k_f (M^{-1} s^{-1}) and reverse rate k_r (s^{-1}). In this example, X is a free molecule able to move across compartments, while Y is anchored within the compartment. Compartment 1 is blood that is spatially close enough to interact with tissue defined by Compartment 2, both with units of L. *Blue arrows* indicate diffusion, while the *green arrow* indicates convention from blood flow. For this kinetic model, diffusion and convection terms are assumed to have units of s^{-1}. An example ordinary differential equation governing [X] in Compartment 1 is shown. (B) An agent-based model using a grid for spatial discretization. Pseudo-rules are given for directing agent motility and interactions. (C) Example of finite element modeling to determine blood flow velocities, taken from simulations performed in (Weddell et al., 2015). The blood velocity field can be integrated with kinetic or agent-based models to provide more physiologically relevant convection rates or movement probabilities, respectively. Conversely, the tissue could also be modeled with finite elements, and advection–diffusion-reaction could be solved.

Here A, B, and C are species concentrations, A and B interact to form C with forward rate k_f, and C dissociates to form A and B with reverse rate k_r. For systems biology applications, reactions describe interactions between reactants, modeled as biological species such as proteins or genes. For deterministic kinetic models, species are assumed to be contained in a continuous molecular concentration. One typical deterministic kinetic modeling application is to quantify temporal species concentrations using ordinary differential equations (Eq. 2):

$$\frac{d[\mathrm{A}]}{dt} = k_r[\mathrm{C}] - k_f[\mathrm{A}][\mathrm{B}] \tag{2}$$

Eq. (2) indicates the temporal concentration of species A ([A]) defined by the chemical reaction in Eq. (1). Kinetic models are also often employed as compartmental models, where species reactions are bounded within a physical space (compartment), but may transport between other compartments that are physically separated (Fig. 2). In systems biology, a microscale compartmental example is modeling the extracellular and intracellular spaces, which are physically separated by the cell membrane. A macroscale example is modeling compartments as different tissues, such as bloodstream and skeletal muscle tissue, which are physically

separated by the blood vessel walls. A thorough review on kinetic modeling of signaling networks at micro- and macro-scales can be found in Janes and Lauffenburger (2013).

A second typical deterministic kinetic modeling application is to quantify spatial or spatiotemporal species concentrations are using the advection–diffusion-reaction equation modeled with partial differential equations (Eq. 3):

$$\frac{\partial[\mathrm{A}]}{\partial t} = \mathrm{D}\nabla^2[\mathrm{A}] - \vec{v} \cdot \nabla[\mathrm{A}] \pm \mathrm{R} \tag{3}$$

where [A] is the concentration of a species A, D is the diffusion coefficient of species A, ∇ is the spatial partial derivative, $\vec{v}$ is the convective velocity field, and R is any reactions involving species A. In purely kinetic models, modeling species diffusion and convection typically involves compartmental modeling, where species transport between compartments is defined by either constant D and $\vec{v}$ terms, or D and $\vec{v}$ terms that are altered algorithmically.

One primary limitation to kinetic modeling is it requires model parameterization, species concentrations, and reaction kinetics to be defined from biological data. Model parameterization is an essential step to develop physiologically relevant models (Le Novere, 2015; Brodland, 2015; Janes and Lauffenburger, 2013). Species concentrations, such as membrane receptor levels, can be accurately parameterized with quantitative flow (qFlow) cytometry (Pannu et al., 2001; Wang et al., 2006; Imoukhuede et al., 2013; Chen et al., in press), a high-throughput approach that detects receptor expression with a fluorescent affinity probe and quantifies absolute receptor concentrations using fluorescent calibration standards (Pannu et al., 2001; Imoukhuede and Popel, 2011; Chen et al., in press). Recently, qFlow cytometry has been used to obtain data on VEGFRs on ECs in vitro (Chen et al., 2015; Imoukhuede and Popel, 2011), ex vivo (Imoukhuede and Popel, 2012), and from pathological tissues (Imoukhuede et al., 2013; Imoukhuede and Popel, 2014). These VEGFR concentration data have been used to advance several computational models (Yen et al., 2011; Finley et al., 2011, 2013, 2015; Finley and Popel, 2012; Weddell and Imoukhuede, 2014; Tan et al., 2013a,b). Prior to the adoption of this emerging tool for systems biology, most kinetic models contained concentration parameters that were either estimated or generalized across multiple reactions; one such methodology for estimating kinetic parameters from network connectivity is given in (Ko et al., 2009). This limitation in parameters is also seen in the need for kinetic parameters. Due to this limitation, researchers have recently been developing methods for quantifying reaction rates for specific biological processes: Bose and Janes recently developed a method for high-throughput quantification of signal molecule dephosphorylation kinetics via phosphatase activity (Bose and Janes, 2013). Similarly, surface plasmon resonance (SPR) has recently been used to identify new interactions between PDGF ligands and VEGFRs, implicating cross-family signaling in angiogenesis, while simultaneously providing quantitative interaction kinetics that can seed computational models (Mamer et al., 2016). While SPR is low throughput natively, SPR on a chip technology has recently been developed to allow high-throughput, array-based SPR identification of interactions and kinetic rate quantifications (Li et al., 2017; Zhang et al., 2015). Development of such high-throughput methods to completely parameterize kinetic models, from species concentrations to specific kinetics for every reaction, would provide additional insights into the biological system and how it can be manipulated, while maintaining high physiological relevancy.

Stochastic Modeling

Deterministic kinetic modeling always gives the same results given the same reactions, concentrations, and kinetics. However, biological processes have elements of randomness; deterministic modeling particularly fails at low species concentrations, where the assumption that species are contained in a continuous molecular concentration does not hold, and reactions occur stochastically (Buiatti and Longo, 2013; Pilpel, 2011). Stochastic kinetic models incorporate this random element into deterministic kinetic models to predict biological randomness and noise (Wilkinson, 2009). Systems biology typically applies stochastic modeling through the Gillespie algorithm or Monte Carlo simulations. Briefly, the Gillespie algorithm simulates time-dependent trajectories of the species in a chemical reaction network (Wilkinson, 2009). Monte Carlo simulates stochastic reactions by introducing probability distributions for the occurrence of each reaction (Kerr et al., 2008).

Agent-Based Modeling

Agent-based models represent each individual species (i.e., cell or protein) as a discrete agent that follows a certain set of rules. Similar to kinetic modeling, agent-based models in systems biology are typically used to quantify spatiotemporal species information (An et al., 2009). Unlike kinetic modeling, agent-based models do not require kinetic or concentration information; rather, rules define species interactions and transport, which may or may not include kinetic or concentration information (Chen et al., 2014). Cellular automaton is one primary example of agent-based modeling: creating a two- or three-dimensional spatial grid, where each lattice on the grid contains an agent of interest, and simulating the spatiotemporal agent movements and interactions across the grid.

Agent-based models are advantageous as they incorporate stochasticity, and provide spatiotemporal information on individual agents, without requiring complex mathematical equations (such as Eqs. 2, 3) relationships to be defined and solved. However, defining the rules for agents to follow requires knowledge of the system mechanisms, simulating many agents is highly expensive computationally (Cosgrove et al., 2015). Thus, agent-based models are primarily useful for quantifying the behavior of a system with known mechanisms, whereas kinetic modeling can be useful for uncovering the system mechanisms (An et al., 2009).

Molecular Modeling

Molecular modeling simulates the three-dimensional structural interactions between atoms and molecules (Aloy and Russell, 2006). For the purposes of this review, we focus on molecular modeling in the context of computational drug screening: identifying potential VEGF inhibitors (Hoi et al., 2015). Computational drug screening is an approach to identify novel therapeutics for targeting signaling proteins. Potential drugs targeting the signal protein of interest are predicted by screening through different molecules, and quantifying their binding strength to the signal protein. Binding strength is typically determined through docking analysis, predicting the ability of a molecule to bind the signal protein through preferred orientation, size, flexibility, predicted interaction kinetics, and atomic structure. The therapeutic efficacy of these drugs is then examined in vitro or in vivo (Hoi et al., 2015).

Finite Element Modeling

Finite element modeling is based on similar principles of cellular automaton: a spatial domain is bounded and discretized to calculate the quantity of interest within each lattice on the grid temporally (Vanegas-Acosta and Garzón-Alvarado, 2014). Finite element modeling differs from agent-based modeling in two primary ways: (1) finite element models quantify materials in continuum, such as fluid velocities or temperature fields and (2) finite element models are defined from conservation laws. A typical finite element application is to quantify hemodynamic forces, velocity, pressure, and shear stresses, through the Navier–Stokes equations (Weddell et al., 2015):

$$\rho \boldsymbol{u}_{,t} + \rho \boldsymbol{u} \cdot \nabla \boldsymbol{u} - \nabla \cdot \boldsymbol{\sigma}_{\boldsymbol{u}}(\boldsymbol{u}) + \nabla p = \boldsymbol{f} \tag{4}$$

$$\nabla \cdot \boldsymbol{u} = 0 \tag{5}$$

where ρ is the fluid density, $\boldsymbol{u}$ is the velocity field, $\boldsymbol{u}_{,t}$ is the time derivative of the velocity field, $\boldsymbol{\sigma}_{\boldsymbol{u}}$ is the viscous stress, p is the pressure, and $\boldsymbol{f}$ is the external forces. Eqs. (4), (5) are defined by conservation of momentum and mass respectively. Finite element models could be used to calculate physiologically relevant velocity fields for advection–diffusion-reaction simulations (Eq. 3), allowing multiscale VEGF modeling. Some disadvantages are that finite element models are computationally expensive, and fluid properties must be carefully chosen to ensure accurate velocity fields (Weddell et al., 2015). For angiogenesis applications, finite element modeling is typically used to examine how blood flow stress directs vessel growth or intravenous angiogenic drug delivery.

Multivariate Models

The above computational techniques require no experimental data training for model development—granted such models are typically trained to ensure physiological accuracy. However, these models require high parameterization when the number of reactions and species becomes large and not all species or variables related to the system are typically incorporated into these models. To overcome these challenges, multivariate models seek to provide signal-to-response statistical models derived directly from experimental datasets, which do not require explicit definition of system mechanisms. A commonly used multivariate model in systems biology is partial least squares regression (PLSR). PLSR is a regression technique that correlates independent variables to dependent variables within the system (Kreeger, 2013). An example is building a PLSR model to correlate ligand stimuli (independent variable) to cell response (dependent variable) using experimental observations, and then applying the PLSR model to predict what cell responses will occur from untested ligand stimuli (Kreeger, 2013).

Statistical modeling is another commonly used multivariate approach in systems biology, where the probability of observing some response from a system of interest is calculated given a probability model (Bartocci and Lió, 2016). Statistical distribution mixture modeling is one such approach useful for quantifying biomarker heterogeneity (Chen et al., in press), which can provide insight such as which patient subpopulations are resistant to a drug (Weddell and Imoukhuede, 2014). Bayesian statistics is one such commonly used statistical model; Bayesian statistics infers posterior probabilities of model parameters by model training with empirical data (Eddy, 2004). An example Bayesian model application is predicting receptor signaling cross-talk involved in drug resistance, using empirical gene expression profiles from drug-resistance and drug-nonresistant patients (Azad et al., 2015). Machine learning is a similar statistical modeling approach, which describes a system from empirically derived sample inputs through processing algorithms (Tarca et al., 2007). Machine learning differs from Bayesian statistics in that machine learning does not describe biological mechanisms of a system, rather providing an optimized fit of input data to response. An example machine learning application is mapping tissue gene expressions to disease groups, to allow predictive disease classification from future tissue gene expression screenings (Sommer and Gerlich, 2013).

While multivariate models are powerful at predicting signal-to-responses, they are empirical-based models that are not capable of describing mechanisms of a biological system. Since this review focuses on computational systems biology for understanding angiogenesis mechanisms, we do not review multivariate approaches for angiogenesis.

In the following section, we provide an overview of computational systems biology studies that explore angiogenesis mechanisms and methods for regulating angiogenesis.

Systems Biology for Studying Angiogenesis

Sprouting Angiogenesis

Computational modeling, as a tool to understand angiogenesis, has been applied hand-in-hand with experimental investigation since the field of angiogenesis first emerged in the early 1970s, when Judah Folkman discovered the tumor angiogenic factor (Folkman, 1971). The earliest angiogenesis computational model examined vessel sprouting and network formation by diffusion modeling (Liotta et al., 1977). As VEGF and VEGFRs were not characterized until the late-1980s to early 1990s (Table 1), these initial angiogenesis models examined vessel sprouting in response to the uncharacterized molecule tumor angiogenic factor (Folkman, 1971). Despite not modeling sprouting directly by VEGF, these early computational models offered many important insights into growth factor-directed angiogenesis. Such computational models determined that the presence of an angiogenic factor is necessary to develop high density tumor vascularization, a concept that was contentious for its time (Deakin, 1976; Liotta et al., 1977). Later sprouting models highlighted the importance of an angiogenic factor, finding that directed vessel growth (Stokes et al., 1990) and vessel loop formation (Stokes and Lauffenburger, 1991) require a growth factor gradient. Cellular automaton and random walk approaches were applied to track individual cells throughout sprouting (Chaplain and Anderson, 1996), which captured the proliferative phenotype of cells behind the sprouting tip (Anderson and Chaplain, 1998).

As the roles of VEGF and other factors became defined in angiogenesis, computational models began to examine sprouting as a system comprising multiple driving factors or cell types. Some such recent sprouting models have predicted that basic fibroblast growth factor enhances VEGF-directed angiogenesis by upregulating VEGFR2 (Connor et al., 2015), and that VEGF and angiopoietins coordinate angiogenesis through EC migration and vessel maturation by pericytes (Zheng et al., 2013). Finite element modeling has also been used to identify that traction forces employed by cell growth controls matrix deformation and additional angiogenic growth and remodeling (Edgar et al., 2014). Additional computational systems biology models that have been specifically studied sprouting angiogenesis are reviewed in (Peirce et al., 2012). Overall, such sprouting models have advanced our understanding of how single or multiple growth factor gradients direct angiogenesis. Sprouting models also offer a powerful, macroscopic framework to examine specific subsystems within angiogenesis; as such, sprouting models have been extended to understand how VEGF-mediated tip/stalk cell selection directs angiogenesis.

Tip/Stalk Cell Selection and Vessel Sprouting

Gerhardt et al. defined vessel patterning for the first time in 2003, characterized by tip cells responding to VEGF with guided migration, and stalk cells responding with proliferation (Gerhardt et al., 2003). Vessel patterning has since been well characterized in VEGF-directed sprouting angiogenesis, identified as an important feature for VEGF signaling and lumen formation to create functional blood vessels, reviewed in (Bautch, 2012). As VEGF/VEGFR and Delta/Notch signaling cross-talk was characterized as a key feature in tip/stalk cell selection and vessel patterning (Hellstrom et al., 2007; Hellström et al., 2007), agent-based computational models worked hand-in-hand with experimental investigations to explore this relationship. Insights gained from such agent-based models include identifying that Delta like ligand (Dll)-4 and VEGFR2 expression oscillate to direct sprouting (Bentley et al., 2008), and the validated prediction that tip/stalk cell selection is driven through tip cell filopodia extension (Bentley et al., 2009). Perhaps the most important insight into tip/stalk cell selection given by computational models is that this process is reversible; Bentley et al. first reported that DII4/Notch lateral inhibition between ECs during loop formation causes cell fates to flip (Bentley et al., 2009), a process now validated through further model-directed (Jakobsson et al., 2010) and exploratory (Siemerink et al., 2012) experiments. Recent agent-based modeling, integrated with in vivo experiments, identified that the rate of tip cell selection defines a trade-off between sprout extension and vessel branching, dictating vessel network density (Kur et al., 2016). Model-derived experiments also found that reversible tip/stalk cell selection is present in embryonic neural crest cells, accurately predicting gene expression patterns that different tip and stalk cells (McLennan et al., 2012, 2015). Some examples of inferences recent tip/stalk cell sprouting models have made include: tip cells migrate back and forth to dynamically alter the leading cell based on VEGFR2 expression (Boas and Merks, 2015), stalk cell proliferation is dependent on traction forces applied by tip cell migration (Santos-Oliveira et al., 2015), and that tip cell polarization and directed movement is mediated by the VEGF-VEGFR binding distribution on the cell surface (Jain and Jackson, 2013).

VEGF-VEGFR Kinetic Models

VEGF-VEGFR interaction models at the single cell scale seek to understand how the kinetics of the ligand-receptor interactions dynamically alters protein and complex concentrations. Typically, these concentrations are taken as the functional output of VEGF-VEGFR interaction models, providing inference to angiogenic potential (i.e., higher phospho-VEGFR2 concentrations imply more angiogenesis will occur). VEGF-VEGFR interaction models are powerful as they quantify protein and complex concentrations that are difficult to probe or differentiate experimentally, and allow perturbations (such as ligand or receptor concentration effects) to be easily examined. While ligand-receptor kinetic models were first introduced in the early 1970s (Bell, 1974), VEGF-VEGFR interactions would not be modeled until 2004 (Mac Gabhann and Popel, 2004). This model predicted that the experimental hypothesis that PlGF displaces VEGF from VEGFR1, enhancing VEGF signaling through VEGFR2, was incorrect, and suggested a

functional VEGFR1 signaling role. Later experimental evidence backed up this model result, showing that PlGF upregulates pro-angiogenic factors and induces metastasis (Marcellini et al., 2006; Roy et al., 2005). This initial model showcases the predictive power of VEGF-VEGFR interaction models. VEGF-VEGFR interaction models have been continuously developed throughout the years to explore VEGFR signaling dynamics. We provide an overview of VEGF-VEGFR interaction models based on the subsystems they explore.

VEGF Expression in Hypoxia

Hypoxia inducible factor-1α (HIF-1α) is one of the primary molecules that directs vascularization in response to hypoxic environments by promoting VEGF expression (Masoud and Li, 2015; Ziello et al., 2007), leading to increased tumor cell invasiveness (Du et al., 2008). Computational systems biology has thus investigated HIF-1α activation in response to oxygen concentration, and subsequent VEGF expression for promoting angiogenesis. An initial hypoxia kinetic model developed by Qutub and Popel identified that HIF-1α activation from hypoxia either directs steep, switch-like or gradual cell responses; this dual cell response may be an important consideration for HIF-1α targeting therapeutics (Qutub and Popel, 2006). Another kinetic model examined how VEGF expression is mediated through HIF-1α degradation by two enzymes, prolyl hydroxylase and asparaginyl hydroxylase (Yucel and Kurnaz, 2007). This model identified that prolyl hydroxylase alone is sufficient at abolishing HIF-1α activity, and that regulating prolyl hydroxylase activity may be an effective method for controlling the angiogenesis response to hypoxia (Yucel and Kurnaz, 2007). A recent kinetic model examined the role of miRNAs in hypoxia-induced HIF-1α activity and VEGF expression, identifying that argonaute 1 overexpression decreases VEGF production (Zhao and Popel, 2015). These potential therapeutic targets identified by hypoxia-induced VEGF expression models offer potential options for controlling angiogenesis, and require further investigation.

VEGFR Dimerization Models

VEGF signaling can lead to differential signaling outcomes based on whether it signals through VEGFR homodimers or heterodimers (Roskoski, 2008). VEGFR dimerization formation is difficult to examine experimentally, making the effects of dimerization parameters, such as dimerization rates or ratio of dimer formation, difficult to elucidate. VEGF computational models have provided VEGFR dimerization to be probed, with such findings as that dimerization does not affect complex formation at membrane patches dominated by stochastic VEGF-VEGFR binding (Mac Gabhann et al., 2005). A generalized receptor model showed that homo- versus heterodimer formation is predominantly dependent on relative receptor concentrations (Mamer and Imoukhuede, 2015a), providing therapeutically relevant receptor concentration spaces for tuning dimer formation. This model further found that ligand-receptor binding kinetics also direct homo- versus heterodimer formation (Mamer and Imoukhuede, 2015a), which may be especially important for angiogenesis given the differential binding kinetics of VEGF with VEGFR1/VEGFR2 and the ability for PDGFs to compete for VEGFR binding (Mamer and Imoukhuede, 2015b). While these computational models elucidated how VEGFR dimers form, understanding functional differences in VEGF signaling through VEGFR1 homodimers, VEGFR2 homodimers, and VEGFR1/VEGFR2 heterodimers remains a challenge that systems biology may yet answer.

VEGF Isoform-VEGFR Kinetic Modeling

Similar to VEGF signaling being directed by VEGFR dimer formation, VEGFR signaling is directed by the ligand isoform that binds (Table 1). While computational models have examined VEGF isoforms primarily in the context of pathology (described later), here we highlight studies that have examined VEGF isoforms in normal physiology. An early model examining $VEGF_{165}$- and $VEGF_{121}$-VEGFR binding distributions in skeletal muscle tissue found that NRP potentiates $VEGF_{165}$-VEGFR2 binding, and removing NRP causes equal $VEGF_{165}$- and $VEGF_{121}$-VEGFR2 binding (Mac Gabhann and Popel, 2007a,b). A two compartment blood-tissue model examined $VEGF_{121}$ and $VEGF_{165}$ binding distributions with luminal and abluminal receptors, finding that abluminal VEGF predominantly binds VEGFR1, whereas luminal VEGF predominately binds VEGFR2 (Stefanini et al., 2009). Another study elucidated that VEGF isoform patterning observed in vivo (Grunstein et al., 2000; Ruhrberg et al., 2002) is directed by isoform specific sequestration and degradation through heparan sulfate proteoglycan binding (Vempati et al., 2011). Note that these computational models examined $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$ binding distributions with VEGFRs; no other $VEGF_{xxxa}$ isoforms have been modeled, and no $VEGF_{xxxb}$ isoform models exist to the best of our knowledge. $VEGF_{165b}$ has recently been shown to bind VEGFR2 with higher affinity than $VEGF_{121a}$ (Wittenkeller et al., 2015; Mamer et al., 2016), a commonly studied isoform, indicating that modeling VEGFR binding to $VEGF_{xxxb}$ isoforms may be an essential next step toward understanding angiogenesis.

Kinetic Modeling of VEGFR Intracellular Signaling

While these extracellular models provide a template for identifying key extracellular nodes and processes mediating VEGF-VEGFR interactions, they do not characterize how intracellular nodes mediate angiogenesis. To overcome this limitation, VEGF computational models were extended to examine how extracellular factors and VEGF-VEGFR binding couple with intracellular signaling to direct angiogenesis. The earliest VEGFR intracellular signaling model we identified, developed in 2007 by Alarcon and Page, provides the mathematical basis for modeling VEGF binding a generalized VEGFR, VEGFR internalization, and coupling of a generalized src-homology 2 (SH2) containing kinase to the VEGFR (Alarcón and Page, 2007). Such mathematical techniques have

been applied to examine specific signaling molecules: Mi et al. use model-directed experimentation to show that VEGFR2-PLC_δ directs intercellular Ca^{2+} signaling, mediating cell–cell communication in wound closure (Mi et al., 2008). Napione et al. show through model and experimentation that PLC_γ and Akt phosphorylation depend on VEGFR2 expression, mediated by cell density (Napione et al., 2012). A recently established modeling approach translating adapter phosphorylation to global cell response (Weddell and Imoukhuede, 2015a) showed that VEGFR1 actively promotes cell migration and proliferation through PLC_γ and PI3K activation (Weddell and Imoukhuede, 2015b, 2016b,c), advancing the previous characterization of VEGFR1 as a decoy receptor (Simons et al., 2016; Claesson-Welsh, 2016).

Kinetic Modeling of Internalized VEGFR Signaling

Computational analyses have also identified an important role for receptor internalization in intracellular signaling; computational models quantifying receptor signaling localization throughout endocytosis found that receptors primarily signal from intracellular endocytic vesicles (Weddell and Imoukhuede, 2016a, 2017). Another model indicated that phosphorylation of specific receptor sites may depend on intracellular compartmentalization (Clegg and Mac Gabhann, 2015). Anderson et al. showed through combined model and experimentation that heparin-bound VEGF increases VEGFR2 phosphorylation by slowing and lengthening the endocytosis process (Anderson et al., 2011). Together, these VEGF-VEGFR interaction models provide systemic information on the VEGF signaling axis: mapping entire extracellular and intracellular processes that mediate VEGF signaling and subsequent angiogenesis.

Multiscale VEGF Kinetic Models

VEGF interaction models have been expanded from the cell surface to macroscale. These systemic VEGF computational studies model the same VEGF-VEGFR interactions as at the cell scale, but expand the model scope to and interactions to examine VEGF distribution and binding at tissue or whole-body scales. At the tissue scale, $VEGF_{165}$ and $VEGF_{121}$ binding distributions to VEGFRs and NRP1 were modeled in skeletal muscle tissue, providing tissue scale findings such as that $VEGF_{165}$ concentrations in interstitial space does not affect steady-state VEGF binding distributions (Mac Gabhann and Popel, 2007a,b). VEGF interactions are also modeled at the whole-body scale, using compartmental modeling to simultaneously quantify VEGF interactions and transport between biological compartments (Weddell and Imoukhuede, 2014). Whole-body VEGF models first emerged by examining VEGF in tissue and blood compartments, providing the notable insights that unbound VEGF primarily localizes to tissue compartments (Yen et al., 2011), but that soluble VEGFR1, which sequesters unbound VEGF, does not decrease VEGF signaling potential in those tissue compartments (Wu et al., 2009). These macroscopic VEGF-VEGFR interaction models are also regularly used to explore angiogenesis in pathology: understanding both how VEGF signaling is important to pathology, and testing VEGF therapeutics. In the following section, we review modeling approaches to explore and optimize VEGF therapeutics, specifically pro-angiogenic therapeutics for vascular disease, and antiangiogenic therapeutics for cancer.

VEGF Models for Exploring Therapeutic Approaches in Pathology

Pro-Angiogenic Therapies

Pro-angiogenic treatments have exhibited continuous success at vascularizing ischemic tissue in animal models, but such treatments have not translated to clinical benefits (Cao, 2010). Computational models for pro-angiogenic therapies seek to optimize VEGF signaling to vascularize ischemic tissue and provide clinically effective options for treating vascular diseases. Pro-angiogenic computational models first examined VEGF gradients in rest and exercise, as exercise is the most effective preventer of vascular disease (Myers, 2003). Some key findings from these computational studies include that skeletal muscle VEGF gradients result in heterogeneous VEGFR activation, which may define the mechanism for stochastic sprout locations (Mac Gabhann et al., 2007b), and that VEGF signaling and subsequent tissue vascularization is most effective within the first week of starting exercise regimes (Ji et al., 2007). Unfortunately, patients with progressed vascular disease are unable to exercise; thus, computational models also examined other pro-angiogenic therapies in severe artery diseases. Such models suggested that injecting myoblasts overexpressing VEGF may effectively promote angiogenesis, and although identified to be less effective than exercise, it may be a promising therapeutic for patients unable to exercise (Mac Gabhann et al., 2007a). A recent model suggests that targeting miRNA, specifically inhibiting miR-15a, may effectively increasing VEGF synthesis and function in peripheral artery disease (Zhao and Popel, 2015). Hemodynamic forces are also an important consideration in the progression of peripheral artery disease (Weddell et al., 2015), and multiscale models quantifying hemodynamics with molecular interactions may provide new insight into vascular disease. Further exploration into computationally derived therapeutic options for vascular diseases may overcome the barrier currently preventing clinical efficacy of pro-angiogenic therapies.

PK/PD Models for Antitumor Angiogenesis

Whole-body pharmacokinetic/pharmacodynamics VEGF interaction models have been developed to systemically quantify VEGF-targeting therapeutic efficacies to inhibit tumor angiogenesis. These tumor angiogenesis models extend compartmental models of VEGF interactions with VEGFRs and extracellular proteins to account for drug administration to the blood stream, absorption into

healthy and diseased tissue, and drug-target interactions. Compartmental models examined VEGF dynamics following anti-VEGF injection (Stefanini et al., 2010; Wilde and Damian, 2014; Finley et al., 2013), identifying that $VEGF_{121}$ inhibition is more effective at reducing tumor angiogenic potential than $VEGF_{165}$ inhibition (Finley and Popel, 2012) and predicted that anti-VEGF efficacy is sensitive to VEGFR levels on tumor cells (Finley et al., 2011). Further investigation into physiological VEGFR heterogeneity identified that high VEGFR1 levels result in ineffective anti-VEGF therapy (Weddell and Imoukhuede, 2014), implicating VEGFR heterogeneity as a drug resistance mechanism. Pharmacokinetic modeling has also identified potential drug interaction mechanisms: the anti-VEGF drug aflibercept may bind NRP-bound VEGF, in addition to free VEGF (Finley et al., 2015). Overall, these systemic VEGF models offer a powerful platform for testing antitumor angiogenesis therapies, which can be applied to study patient-specific therapeutic efficacy, in addition to elucidating mechanisms of drug interactions and resistance.

Computational Drug Screening for VEGF Therapeutics

Computational drug screening has recently been applied to identify possible molecular compounds that selectively inhibit VEGFR2. These screening approaches typically iterate through compounds available in molecular databases, and identify potential novel VEGFR2-inhibitors through a computational structural comparison to an established VEGFR2 inhibitor (Ragno et al., 2015). The compounds exhibiting the greatest therapeutic potential are then tested experimentally. Such structural screening studies have identified a compound, termed HP-14, that exhibits a four-fold higher reduction in HUVEC proliferation than the established VEGFR inhibitor Vatalanib (Schmidt et al., 2008; Nitzsche et al., 2010). Other screening studies have identified compounds that significantly inhibit VEGFR1 and VEGFR2 phosphorylation to prevent HUVEC tube formation in vitro (Gautier et al., 2016), inhibit VEGFR2 kinase activity and HUVEC wound closure without affecting HUVEC proliferation (Kankanala et al., 2012), and inhibit vessel sprouting ex vivo (Latham et al., 2014). Further review of antiangiogenic VEGFR2-targeting therapies identified through computational screening can be found in (Shi et al., 2016). This computational screening approach, linked with experimental validation, offers rapid identification of promising VEGF inhibitors that may allow optimizing patient-specific therapeutics.

Challenges and Future Directions

Overcoming Resistance of VEGF-Targeting Therapeutics

Overall, computational studies and systems biology have driven angiogenic research fundamentally and to direct angiogenic therapeutics. Many challenges remain to be overcome to obtain complete control of angiogenesis. Overcoming anti-VEGF drug resistances is a large challenge in providing effective cancer treatment by inhibiting angiogenesis (Meadows and Hurwitz, 2012; Kubota, 2012; Lu and Bergers, 2013). Such therapeutic resistance was connected with heterogeneity in EC protein profiles (Aird, 2012), leading to systems biology studies that provided mechanistic insight into anti-VEGF resistance: high VEGFR1 cell subpopulations result in ineffective anti-VEGF treatment (Weddell and Imoukhuede, 2014), a result observed clinically (Paiva et al., 2015; Dhakal et al., 2012; Lambrechts et al., 2013). Despite such advances, anti-VEGF and other VEGF-targeting therapeutics are still met with resistance in many patients (van Beijnum et al., 2015). Adopting the approach to connect cell heterogeneity with therapeutic resistance (Weddell and Imoukhuede, 2014) may allow such drug resistances to be overcome. However, such an approach requires effective isolation of single cell types and cell biomarker quantification to characterize patient subpopulations and provide accurate model parameterization (Chen et al., 2014). These hurdles may be overcome by applying newly established technologies: the secondary anchor targeted cell release system for effective cell isolation (Ansari et al., 2015, 2016), and quantitative flow cytometry (qFlow) to quantify cell biomarkers (Lee-Montiel and Imoukhuede, 2013; Imoukhuede and Popel, 2014; Chen et al., 2015). The secondary anchor targeted cell release system quickly, efficiently, and gently isolates specific cell types without altering biomarker expression, while qFlow cytometry detects biomarker expression with a fluorescent affinity probe and quantifies absolute receptor concentrations using fluorescent calibration standards (Pannu et al., 2001). These high-throughput approaches ensure accurate model parameterization, an essential step to develop physiologically relevant models (Le Novere, 2015; Janes and Lauffenburger, 2013; Brodland, 2015), while also providing data specific to the cell, biological system, or patient to be modeled.

Quantifying VEGFR Signaling Throughout Endocytosis

One primary challenge in achieving complete angiogenic control is to understand the relationship between endocytosis and VEGFR signaling. Recently, intracellular-based receptors have emerged as key signal transducers (Irannejad et al., 2015; Tomas et al., 2015). Recent computational models have quantitatively shown that receptors primarily signal intracellularly (Weddell and Imoukhuede, 2017), and undergo significant nuclear translocation (Weddell and Imoukhuede, 2016a), underscoring a need to better understand the function of internalized receptors. Furthermore, the high intracellular expression of VEGFR1 and VEGFR2 (Zhang et al., 2010) indicates that intracellular VEGFRs endocytosis may have a crucial role in mediating VEGFR signaling.

Mapping the VEGF Isoform Functions

Another primary challenge in controlling angiogenesis is elucidating the function of all VEGF proteins. Specific functions for most VEGF isoforms remain undefined. While systems biology has identified differential $VEGF_{165}$ and $VEGF_{121}$-VEGFR binding and function, few of the other VEGF isoforms have been studied computationally or experimentally. $VEGF_{xxxb}$ functions in particular remain undefined, but may be important for angiogenesis as at least one binds VEGFR2 with higher affinity than $VEGF_{xxxa}$ isoforms (Mamer et al., 2016). Additionally, a recent study identifying that $VEGF_{165b}$ alters Dll4 expression (Ye et al., 2016), together with evidence that targeting Delta-Notch signaling may be effective anticancer therapeutic (Briot and Iruela-Arispe, 2015), implies an important $VEGF_{xxxb}$ role for tumor angiogenesis. $VEGF_{xxxb}$ may also have higher expression than $VEGF_{xxxa}$ in certain diseases (Lim et al., 2016), further highlighting the necessity to understand $VEGF_{xxxb}$ functions. Unlocking the mechanisms that mediate VEGF isoform expression, binding, and signaling may be the key to overcoming VEGF therapeutic resistance.

Uncovering the VEGFR1 Signaling Role

Classically, VEGFR1 has generally been ignored due to its decoy status in angiogenesis; VEGFR1 is thought to exhibit no intracellular signaling, serving to bind VEGF with high affinity to module VEGF binding and signaling through VEGFR2. However, a recent modeling approach (Weddell and Imoukhuede, 2015a), coupled with experimental investigation, showed that VEGFR1 natively functions to promote the angiogenesis hallmarks of cell migration and proliferation through PLC_{γ} and PI3K pathways (Weddell and Imoukhuede, 2015b, 2016b,c). This modeling approach can next be applied to quantify VEGFR1 signaling in different cancers and vascular disease. Indeed, emerging evidence implies quantifying VEGFR1 signaling is especially important in these diseases: membrane VEGFR1 is upregulated during vascular reperfusion stages in ischemic tissue (Imoukhuede et al., 2013), hypoxic tumor cells, and tumor ECs (Imoukhuede and Popel, 2014), and VEGFR1 tyrosine kinase-deficient mice exhibit reduced angiogenesis (Murakami et al., 2008). Furthermore, PlGF stimulates EC growth and migration (Fischer et al., 2008; Adini et al., 2002), and inhibiting PlGF prevents tumor growth and metastasis (Fischer et al., 2007).

VEGF Signaling Models for Clinical Applications

Toward using systems biology to guide angiogenic therapeutics, developing clinically relevant models that allow patient-specific investigation are essential (Neal and Kerckhoffs, 2010; Chen and Snyder, 2012). Developing such personalized models is a nontrivial task (Walpole et al., 2013), necessitating multiscale modeling approaches to capture all clinical features relevant to angiogenesis, such as VEGF interactions at the microscale and hemodynamics at the macroscale (Galie et al., 2014; Baeyens et al., 2015; Weddell et al., 2013). Integrating macroscale blood flow stress with microscale VEGFR signaling may be an important clinical consideration as well, since shear stress induces VEGFR signaling (dela Paz et al., 2012) and directs vessel patterning (Anderson et al., 2015). Choosing which modeling approach to use also must be balanced between computational complexity and physiological accuracy; take hemodynamic modeling as an example: while modeling blood properties as Newtonian is less mathematically complex than modeling the shear thinning properties of blood, Newtonian models do not provide physiologically relevant hemodynamics (Weddell et al., 2015). Comprehensive angiogenesis computational models that guide therapeutic development for clinicians in an accessible, clinically relevant way is a large challenge in systems biology today, but would provide a platform for effective personalized medicine that no other approach can.

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Congenital Heart Valve Disease in the Adult

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Introduction

Congenital valvular disease has an incidence of around 2% (Rose, 1996). However, half of the individuals born with a congenital heart abnormality will avoid surgical intervention in early childhood. A minority of patients recognized early will eventually need treatment, while others will only be diagnosed in adulthood. Congenital valvular abnormalities can involve any one of the four valves (Figs. 1–5). Congenital valvular abnormalities identified in adulthood most commonly affect the aortic valve (AV). This chapter will discuss a variety of congenital valvulopathies, their clinical management, pathology, and indications for treatment.

Bicuspid Aortic Valve and Aortic Stenosis

Definition

Congenitally bicuspid AV (BAV) occurs when only two valve leaflets arise in aortic valvulogenesis. This abnormality is usually well tolerated unless adulthood is associated with other structural abnormalities. A unicuspid AV is characterized by a single cusp and a small (usually stenotic) central orifice. It is often found with a single commissural attachment to the aortic wall. Although these valves tend to be narrow at birth, symptomatic obstruction may not present until later in life (Fig. 6).

Epidemiology

Four post-mortem studies provide only epidemiology data of the BAV in the adult population. Only 15%–28% of those with a BAV found post mortem are normally functioning (Roberts, 1970; Osler, 1886). The others are associated with aortic stenosis (AS)

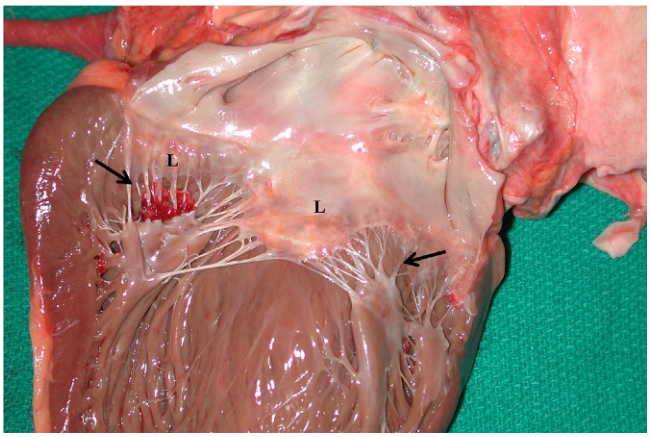

Fig. 1 A normal mitral valve. Note attachments of the leaflets (L) and of the chordae tendinae (*arrows*).

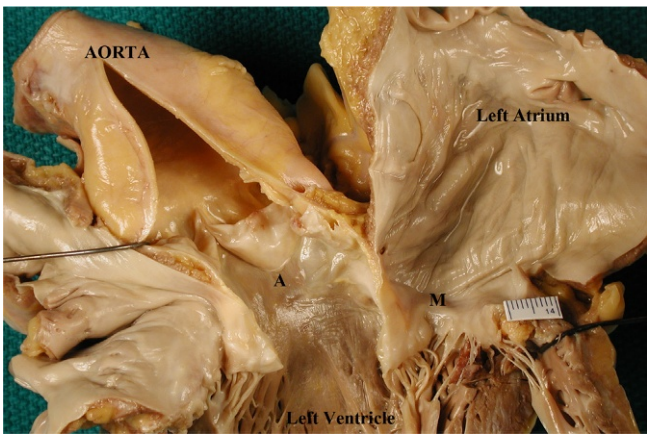

Fig. 2 Normal 3-cusp aortic valve. Note the left ventricular (LV) inflow tract and mitral valve (M) and the LV outflow tract and aortic valve (A).

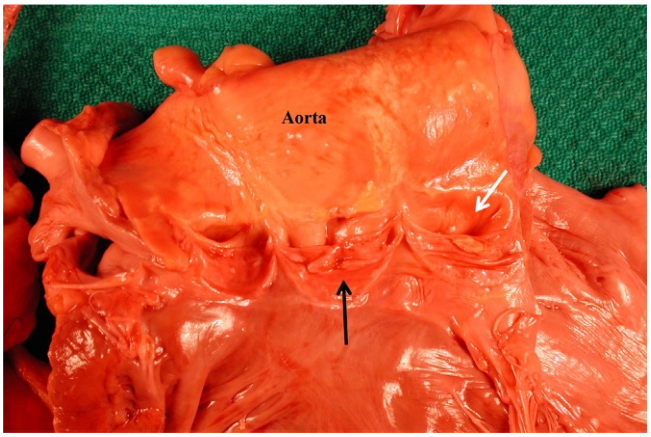

Fig. 3 Normal aortic valve. The three semilunar cusps are seen (*arrow*) and the coronary arteries arise from the sinuses of valsalva (*white arrow*).



Fig. 4 Surgically excised Mitral Valve, anterior mitral leaflet with chordae tendinae (*arrow*). The leaflet is normal, but was excised because of dysfunction related to nonvalvular causes, related to a previous myocardial infarction and papillary muscle dysfunction.

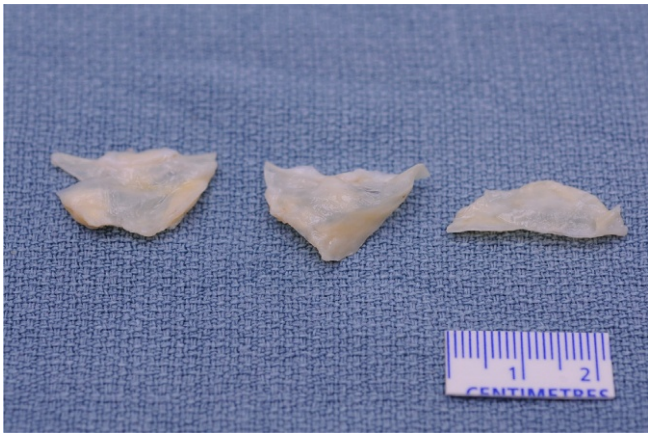

Fig. 5 Surgically excised aortic valve. 2 left cusps show the flow surface, while the 3rd shows the nonflow surface. The semilunar cusps are largely normal in structure, and were excised because of valvular incompetence, related to aortic and ascending aortic aneurysmal dilatation.

(15%–71%) (Roberts, 1970; Grant et al., 1928), aortic regurgitation (1.5%–3%) (Roberts, 1970; Grant et al., 1928), infective endocarditis (9.5%–40%) (Roberts, 1970; Julian et al., 1996), and aortic dissection (5%) (Julian et al., 1996). Based on this data, the accepted overall incidence is quoted as 1%–2% in the general adult population (Ward, 2000). Even though BAV is the most common cause of isolated valvular AS in adults, it can also be associated with dilation, aneurysm, dissection, or rupture of the aorta as it has been shown that there is abnormal maturation of the vascular wall in these patients (Grewal et al., 2016).



Fig. 6 (A) Congenitally bicuspid aortic valve. Surgically excised aortic valve showing the sinus surface of two cusps with marked thickening and nodular calcification. In addition one cusp (*the larger one*) shows a ridge (*raphe*) of calcification (*arrow*) which starts at about half way up the cusp and extends beyond the base of the cusps. The flow surface of the cusps was fairly smooth. The larger cusp had the appearance of a "birds wings in flight," typical of many congenitally bicuspid aortic valves.

Clinical Presentation

Most common clinical feature of BAV is AS with or without concomitant regurgitation. The disease can be asymptomatic if mild. Symptoms may develop with progression of disease and include angina, presyncope or syncope with exercise, or dyspnea on exertion, mostly due to valve calcification and/or thickening with subsequent narrowing. Often, these symptoms develop at the time when AS becomes severe. In the early stage, detection of AS can first be found by a thorough physical examination. In a patient with severe AS, the murmur is typically in the 2nd–3rd intercostal space on the right side, with mid-late peaking harsh systolic ejection murmur. Along with a soft and single or paradoxical S2, an S3 or S4 may be present. Carotid upstroke is of small volume and weak, termed "parvus et tardus." Other findings include apical-carotid and brachial radial delay, which also suggests severe AS.

Investigations

An electrocardiogram (ECG) often shows nonspecific findings. As a result of chronic pressure load on the left ventricle (LV), left ventricular hypertrophy is a common finding. If aortic stenosis is suspected, transthoracic echocardiography (TTE) with Doppler measurements can provide information regarding valve morphology, ascending aorta, aortic sinuses, aortic annulus, and degree of stenosis and regurgitation. In addition, degree of LV dilation and LV systolic function are important measures, which may assist with timing of intervention.

When noninvasive strategies are unable to delineate degree of severity, or if clinical assessment is discordant, cardiac catheterization can be another modality to assess valve severity. Concurrent angiography should be performed to identify flow limiting coronary artery disease. If a patient is found to have severe AS, and is asymptomatic, exercise testing could be used to assess for symptoms. A dobutamine stress echocardiography can be used in the select patient population with LV ejection fraction <40%, if there is a question regarding the true severity of AS and the presence of contractile reserve. There is limited use of computed tomography (CT) or magnetic resonance imaging (MRI) in this patient population in assessment of severity of stenosis, but could be used for more accurate measurements of the aortic sinus, sinotubular junction (STJ), and ascending aorta (Nishimura et al., 2014).

Management

In all patients with AS, optimizing hemodynamics remains the standard of care. Managing hypertension as well as optimizing volume status is most helpful when patients become symptomatic. Though there is data to support the use of beta-blocker and ACE inhibitor therapy in patients with Marfan syndrome to prevent aortic root dilation (Lacro et al., 2014), there is no data to support this in patients with BAV. In addition, rate control of atrial arrhythmias is also instrumental in allowing adequate cardiac output.

If the patient is symptomatic with evidence of severe AS by either history of exercise testing, intervention is indicated (class of recommendation (COR) I, level of evidence (LOE) B). In addition, in asymptomatic patients with severe AS and LVEF <50%, or those undergoing other cardiac surgery, intervention is also indicated (class of recommendation I, level of evidence B). The other indications with variable COR and LOE can be found elsewhere (Nishimura et al., 2014). If the patient is an acceptable surgical candidate, AV replacement is the mainstay of treatment, with documented improvement of symptoms, and improving life expectancy. The type of prosthetic valve used is based on discussions with the patient and the cardiovascular surgeon, and is often a balance between patient age, comorbidities, and ability for safe anticoagulation. If the root also needs to be replaced, then

other surgical considerations need to be considered. Typically, this is indicated when the aortic sinuses or ascending aorta measures >5.5 cm or if >5.0 cm in conjunction with a family history of aortic dissection or a rate of increase in diameter of ≥0.5 cm/year (Nishimura et al., 2014).

If a patient is deemed to be too high risk for surgery, transcatheter aortic valve implantation (TAVI) allows AV implantation without a sternotomy, or cardiopulmonary bypass and is associated with a shorter "in-hospital" stay. Though these patients were not originally included in the early TAVI studies (Leon et al., 2010), due to the elliptical shape of the annulus, its use in BAV patients has been reported with similar mortality rates compared to those with a tricuspid AV (Phan et al., 2015; Kochman et al., 2014). The rate of perivalvular leaks was also not significantly higher in patients with BAV (Phan et al., 2015).

Balloon aortic valvuloplasty is an alternative option to surgery in some patients with a BAV with AS. Its use can be applied to young patients with commissural fusion without significant leaflet calcification. Indications include those with symptoms and a peak-to-peak gradient of >50 mmHg at the time of catheterization or if asymptomatic with peak-to-peak gradient >60 mmHg with ECG changes of ST or T segments in the lateral leads (Nishimura et al., 2014). However the recurrence of the clinical symptoms has to be kept in mind as the need for repeat intervention is common.

If a patient does not meet criteria for intervention either due to the degree of stenosis or aortic dilation, annual evaluation is recommended in patients with aortic dilation (>4.5 cm), a rapid rate of change in aortic diameter, and in those with a family history of aortic dissection. If the aortic root measures between 4 and 4.5 cm, less frequent reimaging is suggested (Nishimura et al., 2014).

Patients with valvular AS are at increased risk of bacterial endocarditis. Antibiotic prophylaxis is not recommended in recent guidelines regarding the management of these diseases. Good oral hygiene with regular dental assessments is standard of care (Nishimura et al., 2014; Wilson et al., 2007).

Pathology

Inequality of cusp size, presence of a raphe (fibrous ridge) typically found on the sinus surface between the two portions of the V-shaped cusp (often resembling a "birds wings in flight"), and smooth cusp margins are the three hallmarks of the BAV (Roberts, 1970; Peacock, 1865). In the early stages, the cusps may be near normal, structurally, except for the presence of the raphe and the accompanying fibrosis around it. As the patient ages, the valve leaflets undergo progressive changes especially around the areas of the raphe, with resulting grossly evident fibrocalcific nodules resulting in stiffened cusps and in irregular cusp surfaces. These nodules may rupture or ulcerate, allowing for deposits of thrombus and development of inflammation as well as a potential for infection. The valve tissue around the calcific nodules often shows neoangiogenesis (new thin-walled vessels usually with no supporting smooth muscle) as well as chronic inflammation. A frequently seen additional and interesting feature is the presence of varying degrees of osteocartilagenous metaplasia. The reasons underlying these morphologic changes include the structural abnormality (absence of one or more raphe), the flow abnormalities, and the altered and high mechanical stresses on a bicuspid AV leading to valvular endothelial cell dysfunction, possibly with deposition of low-density lipoprotein particles that trigger inflammation and the deposition of platelets. This results in fibrosis and osteoblastic transformation. Close inspection of excised valves will usually reveal one raphe or at times two narrow strand-like or chord-like raphe or partial commissures with the latter believed to be a stage in the development of the raphe. The raphe may sometimes be difficult to appreciate, in the presence of "massive calcification," and the nodular calcium may make the placement of the usual models of TAVI prostheses difficult. The cuspal tissue around the commissures often shows a moderate degree of fibrosis and thickening.

The congenitally unicommissural valve has a "linear or exclamation mark"-shaped orifice, two raphe, and one commissure (Fig. 7). This congenital abnormality usually presents earlier in life. Another congenital abnormality is the "acommissural or dome"-shaped aortic valve, which has only one sheet of aortic tissue with no commissures and often manifests in the first or second decades of life.

Subvalvular and Supravalvular Aortic Stenosis

Definition

A small proportion of patients with subvalvular aortic stenosis (SAS) and supravalvular aortic stenosis (SVAS) will present for the first time as adults. They are more commonly seen in the symptomatic adult after the recurrence of a previously treated lesion. Although the physical findings of SAS and SVAS are similar, as they both are along the spectrum of left ventricular outflow tract (LVOT) obstruction, their epidemiology, natural history, and treatment are markedly different.

Subvalvar Aortic Stenosis

Epidemiology

SAS, a type of LVOT obstructive lesions is found in 6% of children with congenital heart disease (Hoffman and Christianson, 1978). It can be a fixed or a dynamic lesion. It typically consists of a membranous or a fibromuscular ring beneath the AV generally at the most convex part of the interventricular septum (IVS). The membrane becomes a ring when it extends onto the ventricular aspect of



Fig. 7 A nonflow surface of a surgically excised aortic valve from a 45-year-old male. This valve, a congenitally unicommissural valve, shows diffuse fibrosis and thickening with 2 incomplete commissures or raphe (*arrows*). Tissue around and between the raphe shows marked thickening and calcification. The changes are consistent with significant dysfunction. (B) Flow surface of the valve.

the anterior mitral leaflet. Although it can be found as an isolated lesion, it has been found to coexist with other abnormalities in 50%–65% of cases, which include, but not exclusively, patent ductus arteriosus, ventricular septal defect, aortic coarctation, and pulmonary stenosis (PS) (Leichter et al., 1989).

Clinical Presentation

If this lesion occurs in isolation, it is not often detected in childhood. Given its incidence with more significant defects, it is most often found on echocardiography. When present, most patients present when the lesion is severe, when a murmur is appreciated on clinical examination. In severe cases, exertional dyspnea, chest pain, or syncope may be present. On auscultation, a systolic ejection murmur can be appreciated on the left sternal border in most cases. Valsalva maneuvers could differentiate a fixed lesion (softer with maneuver) from a dynamic lesion (louder with maneuver).

Investigations

An ECG often shows LV hypertrophy (LVH), akin to the changes seen in valvular AS. Other findings are typically nonspecific. TTE remains the standard modality for its identification. Visualization of the membrane or ridge can be seen. The aortic and mitral valves should be assessed for attachment points. LV dimensions and function should be assessed to gauge the degree of compensatory LVH. In some cases, an area of muscular thickening (tunnel type) may be seen. Continuous wave (CW) Doppler should be used to assess the gradient through the lesion, if any, and provocative maneuvers should be employed to assess for a dynamic lesion. If patients have exertional symptoms, gradients can be assessed post exercise. If visualization is difficult with TTE, transesophageal echocardiography (TEE) can be employed. Similar to AS, cardiac catheterization can be employed if there is discordance between clinical examination and imaging.

Management

In adults, management is controversial. Some groups advocate for more aggressive surgery earlier as to circumvent damage to the AV and the rate of recurrence. One group studied patients who underwent surgical repair and dichotomized the group based on peak gradients of either $\leq$40 or >40 mmHg. The authors found that early repair could prevent recurrence and the need for redo surgery as well as progressive aortic disease (Brauner et al., 1997). Other groups argue that surgical repair should be completed when the gradient is >40 mmHg or if $\leq$40 mmHg, in the presence of symptoms (Stassano et al., 2005).

If surgery is indicated, the nature of the lesion dictates the type of surgical repair. For a discrete lesion, a "membranectomy" can be performed with a myectomy if needed. If the AV is affected, the surgery would also include repair or replacement of the aortic valve. Though there is much debate regarding the addition of a myectomy in preventing recurrence, one study found that only preoperative LVOT gradient, tunnel-type lesion, and younger age predicted those who would have recurrence (Brauner et al., 1997). Mortality in the adult population has been quoted at 0%–3.8% (Brauner et al., 1997; Stassano et al., 2005). In patients with significant narrowing, a Konno procedure may be needed. This surgery involves replacement of the AV and widening of the ventricular septum in the region of the valve with a patch.

Recurrence has been found to occur in 20%–30% of patients, with redo operation in 15%–20% of these patients. Intervention prior to the gradient exceeding 40 mmHg has been found to reduce the rate of recurrence and has been found in one study (Brauner et al., 1997). Others have found that close proximity to the AV, preoperative Doppler gradients $\geq$60 mmHg, and involvement of leaflet tissue requiring "surgical peeling" increase risk of recurrence of obstruction (Geva et al., 2007).

Patients with documented SAS do not require infective endocarditis precautions as per the most recent AHA guidelines (Nishimura et al., 2014; Wilson et al., 2007).

Pathology

The most common clinical presentation is as a fibrous crescent or ring that completely encircles the LVOT to produce a discrete obstructive lesion (type I). The obstruction can also be caused by a tunnel-like fibromuscular band (type II). Histological examination reveals a "hood"-like layer of fibroelastic tissue (collagen and elastic fibers) with the deeper layers showing muscle fibers. Most resections do include a rim of myocardium.

If the AV is involved due to "jet lesions" from flow through the narrowed and obstructed LVOT, the leaflets can be thickened and show focal calcification and with fibrous attachments connecting them to the membrane itself (Teis et al., 2010).

Supravalvar Aortic Stenosis

Epidemiology

Supravalvar AS is the least common among the lesions that occur in the LVOT. It is seen in 1:20 000 live births (Metcalfe et al., 2000). SVAS is classified as either discrete (type I) where the narrowing is a localized hourglass constriction diffuse (type II) where there is narrowing at the STJ junction and ascending aorta and may extend to the aortic arch and descending aorta (Deo et al., 2013). The most common anatomic variant is the type I. Its occurrence is thought to be due to a genetic abnormality in the elastin gene (7q11.23) which causes an aortopathy (Merla et al., 2012). This genetic abnormality has been reported in patients with the Williams syndrome, either by a familial form or in sporadic cases. Similar to the SAS, SVAS is often found with other defects, and includes abnormal AV morphology, coarctation of the aorta with coronary artery stenosis, and pulmonary artery stenosis (Stamm et al., 1997; Stamm et al., 2001; Wren et al., 1990). Those with Williams syndrome can have either type of SVAS.

Clinical Presentation

Patients with Williams syndrome often present during childhood since it is associated with distinctive facial abnormalities, termed "elfin facies," which include an upturned nose, long philtrum, wide mouth, full lips, and micrognathia. In some cases, a white lacy pattern can be seen on their iris. These children are often of low stature, and present with hypertension and hypercalcemia along with developmental delay (Collins, 2013).

Symptoms of SVAS are similar to AS and SAS (dyspnea, angina, and syncope), but given its involvement with other vascular beds, patients can present with a variety of findings. Hypertension is usually present due to lack of vessel distensibility but occasionally, this can also be associated with concurrent renal artery stenosis (Merla et al., 2012).

Coronary artery abnormalities can also be found in these patients, and can include ostial and diffuse coronary artery stenosis, coronary artery dilation due to long standing hypertension, or obstruction to the coronary vessel from an abnormal AV (Collins, 2013). A patient presenting with symptoms of ischemia should have urgent angiography.

In patients with Williams syndrome, sudden cardiac death can occur and its incidence is 25–100 times greater than the general population (Bird et al., 1996). Its pathophysiology is not known, but it has been proposed that it is a result of LVOT obstruction, coronary artery disease, and prolongation of the QT interval on ECG (Collins, 2013).

Supravalvar stenosis can be detected by typical findings in cardiac auscultation. A systolic ejection murmur can best be heard at the 1st intercostal space on the right side. The second heart sound may be louder due to higher pressures proximal to the narrowing.

Thrills can also be heard in the suprasternal area. If there are other arterial involvements, a patient can have asymmetric carotid pulsation or a significant blood pressure differential between the arms (seen in two thirds of patients) (Flaker et al., 1983).

Investigations

If there is a suspicion of William's syndrome, genetic testing should be undertaken to confirm the abnormality (Collins, 2013).

To delineate the extent of stenosis, similar to SAS, TTE can identify the degree of obstruction using Doppler, the degree of LV hypertrophy and dilation, and the presence of other congenital lesions. Similarly, if there are limitations to a surface study, catheterization can be completed. If other vascular beds are involved, magnetic resonance angiography (MRA) can provide more detailed assessment.

Management

Management of these patients is not clear as no guidelines for surgical intervention exist given the rarity of the abnormality. In the infant, surgical intervention is typically done when the gradient >30 mmHg (Jonas et al., 1994). Others have found that earlier intervention, when the gradient exceeds 20 mmHg, is helpful to avoid progression post surgery (Wessel et al., 1994). Surgical repair consists of enlarging the aortic root and STJ with a patch repair, as well as correction of any other concomitant abnormality (coronary involvement, AV repair, or right ventricular outflow tract repair). Other methods of surgical repair have been developed and can be found elsewhere (Deo et al., 2013). Though balloon dilation and stenting has been reported in the literature, long-term outcomes do not exist (Pinto et al., 1994; de Lezo et al., 1996). The operative mortality has been quoted to be as high as 9% (Stamm et al., 1999). Patients with diffuse obstruction and those with other congenital abnormalities often do worse (van Son et al., 1994).

Patients with SVAS do not require infective endocarditis prophylaxis as per the most recent AHA guidelines (Nishimura et al., 2014; Wilson et al., 2007).

Pathology

Supravalvar stenosis can be present as an hourglass deformity superior to the sinuses of Valsalva, or as a more diffuse disease in the aorta. The defining feature of this condition is a fixed aortic narrowing at the level of the STJ, which typically produces an hourglass narrowing of the aorta. A wide range of other anatomic variations are seen, including hypoplastic SVAS and membranous SVAS. The obstruction may extend a variable distance along the aorta, and a generalized arteriopathy affecting both systemic and pulmonary arterial systems may be seen. Histologically, there is a fibrotic, thickened intima with disorganized medial elements (thick, irregular elastic fibers, swirling collagen, and hypertrophied smooth muscle cells) (O'Connor et al., 1985).

Ebstein's Anomaly

Definition

Ebstein's anomaly (EA) occurs when the posterior and septal leaflets of the right atrio-ventricular or tricuspid valve (**Fig. 8**) are malformed and attached lower down in the right ventricle (RV), closer to the apex, so that these leaflets are significantly larger than is usual (Ebstein, 1866). It is believed to occur due to a failure of the leaflets to delaminate in utero and results in adherence of the leaflets to the myocardium partially or wholly (Lamers et al., 1995), though the entire mechanism is not well elucidated (**Fig. 9**). It is

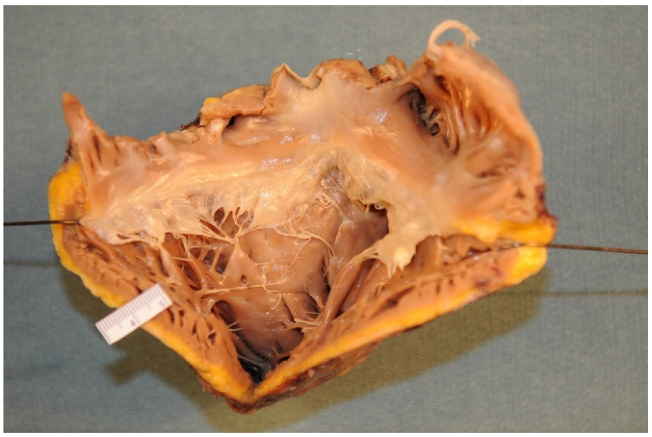

Fig. 8 A normal right atrio-ventricular or trileaflet valve.

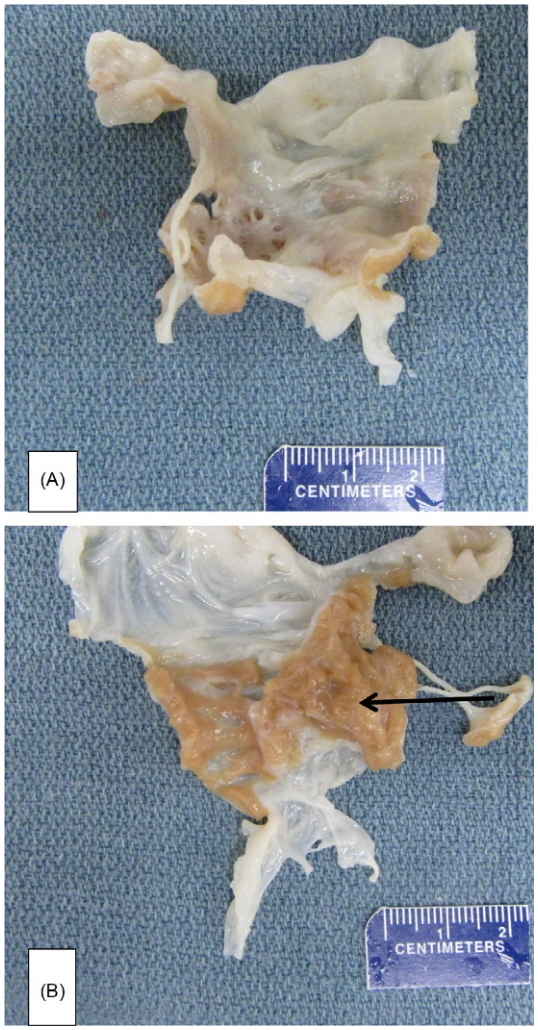

Fig. 9 A surgically excised tricuspid valve leaflet (A) from a 23-year-old lady who had an Ebstenoid tricuspid valve, consistent with significant dysfunction. This anterior leaflet is much larger and voluminous than normal and reached virtually to the right ventricular apex. (B). The nonflow surface of the same leaflet shows the same features and in addition shows attached tan-colored muscle from the areas at which the leaflet was adhesed to the endomyocardium (*arrow*).

also believed to be associated with a sporadic mutation(s) of chromosomes 1p36 and 8p23.1 (Digilo et al., 2011). Its clinical presentation varies dramatically. If severe, this malformation can be discovered in a newborn, and if less severe, can be found in the adult population.

Epidemiology

EA has been reported in 1 in 200,000 live births and accounts for <1% of congenital heart disease (Nora et al., 1974). There is no sex predilection and it is often found with other cardiac anomalies. These include patent foramen ovale (PFO)/atrial septal defect (ASD) which has been reported to occur in 80% of patients (Brickner et al., 2000). Others include pulmonary atresia/stenosis, aortic coarctation, ventricular septal defect, tetralogy of Fallot, atrioventricular septal defect, and mitral valve prolapse (Osiro et al., 2013).

Clinical Presentation

Clinical presentation is typically dictated by the severity of the anomaly, which can range from asymptomatic to severely symptomatic during infancy. If diagnosed in infants, they often present with cyanosis, once the ductus arteriosus closes. In most patients, clinical symptoms can occur in their teen and adult life. The most common includes arrhythmias and right-sided heart failure (Safi et al., 2016).

Investigations

An ECG is often abnormal in this patient cohort, and at times, can be diagnostic in adults. Most often, there are tall and broad p waves due to right atrial enlargement, along with first-degree AV-block and right bundle branch block. There could be a fragmented QRS complex due to abnormal conduction through the AV node. Atrial arrhythmias are common, found in 20%–30% of patients, and often are the cause of adults seeking medical attention (Vacca et al., 1958). In addition, type B Wolff Parkinson White syndrome has been reported to occur in 5%–25% (Hebe, 2000).

TTE remains integral to the diagnosis as well as to assess severity of disease. Assessment should include the tricuspid valve leaflets, insertion points to the RV, the RV, as well as the right ventricular outflow tract. The echocardiogram is most useful to look at the septal leaflet as the tricuspid valve. MRI can also be used, and can visualize the posterior leaflet of the tricuspid valve and assess RV size and function, if TTE proves to be difficult (Attenhofer Jost et al., 2012). If arrhythmias are suspected, a Holter monitor would be helpful to identify these.

Management

Adult patients can benefit from follow-up with regular testing for signs of deterioration of cardiac function. Mild symptoms can be treated with medication for right-sided heart failure (HF). Surgical intervention is suggested if patients have symptoms or decreased exercise capacity, cyanosis (O2 saturation <90%), paradoxical embolism, or progressive RV dilation or dysfunction (Warnes et al., 2008). Most experienced centers prefer valvular repair with plication of the atrialized RV over bioprosthetic valve replacement (Safi et al., 2016). If arrhythmias are present, one can consider a concurrent MAZE procedure or ablation of accessory pathways. If an interatrial communication is present, closure during the surgery can also be performed. Peri-operative mortality remains low at experienced centers, at 2.7% at 30 days (Brown et al., 2008). Redo surgery is advised in a symptomatic patient with reduced exercise tolerance (NYHA class III/IV), severe tricuspid regurgitation leading to RV dilation and dysfunction, bioprosthetic valve stenosis (gradients >12 mmHg) in the absence of reduced functional status or symptoms (Warnes et al., 2008).

Pathology

Most of the pathology of Ebstein's anomaly has been studied in 15 cases from the Mayo clinic (Anderson and Lie, 1978). In the tricuspid valve, when visualized from the atrial side, the anterior leaflet is attached to the atrio-ventricular (AV) junction in the anatomically correct position. The septal leaflet is seen displaced toward the apex and is often attached to the interventricular septal endocardium rather than to the AV junction. A portion of the ventricle is then exposed (often triangular shaped). The posterior leaflet is often attached to the ventricular surface where the septal leaflet lies. Its opposite side then meets the anterior leaflet at the AV junction. Though the attachment points of the anterior leaflet are normal, the leaflet is often larger and its attachment points to the papillary muscles are not normal. The septal and posterior leaflets vary in shape and are dependent on the degree of displacement toward the apex. Their chordae are also tethered to the ventricular wall.

The RV was then divided based on the insertion of the posterior and septal leaflets, and are called the proximal (atrialized) and distal RV. The proximal portion is often thick, fibrous, and smooth. The posterior portion was often aneurysmal and microscopically, would have sparse muscle fibers. The distal RV is often dilated and thin walled and in some cases, there was RV hypertrophy. The latter features are thought to be physiologic and secondary to the valvular abnormality and are thought to correlate with advanced disease. A PFO or ASD can also be found.

Pulmonary Valvular Stenosis

Definition

Similar to AS, PS can occur in the supravalvular, valvular, or subvalvular position. In this section, we will only discuss valvular pulmonic stenosis, as the others are commonly seen in infants and children, in association with other significant congenital cardiac conditions, such as the tetralogy of Fallot. In these patients, the valve is typically trileaflet, but has been reported to be bileaflet in <20% of cases (Keith et al., 1978). The narrowing is believed to be due to thickening of the leaflets and fusion of the commissures, thereby restricting flow during systole. This has been associated with tetralogy of Fallot, Noonan syndrome, and congenital rubella syndrome.

Epidemiology

This is a common congenital defect that has been quoted to occur in 10% of children born with congenital heart disease (Rocchini and Emmanouilides, 1995). The incidence of valvular stenosis compared with the other two (sub and supravalvular) is not known. Children with isolated congenital valvular pulmonic stenosis often survive into adulthood without any interventions. If unrecognized during childhood, the adult patient often presents with this isolated anomaly.

Clinical Presentation

Once the severity of stenosis increases (typically peak gradient >30 mmHg), patients can present with mild dyspnea to symptoms of severe right-sided heart failure, which include dyspnea with exertion, fatigue, abdominal bloating secondary to ascites, and marked peripheral edema. Physical exam findings are often helpful to delineate the severity of stenosis. S2 is often split due to the delay in ejection through the stenosed valve, with degree of split correlating with severity. A pulmonary ejection click and a right-sided S4 could also be appreciated as well. The murmur is a systolic ejection murmur best heard at the left sternal border, with its duration signaling the degree of severity. Late peaking systolic murmurs typically occur when the stenosis is severe. In addition, there could be an RV heave secondary to RV hypertrophy.

Investigations

TTE remains the preferred investigation for the management of PS (Nishimura et al., 2014). The peak and mean gradients measured by pulse and CW wave Doppler are integral for planning for intervention if needed. The valve leaflets are often thickened and immobile. Along with this, the degree of RV hypertrophy, dilation, and dysfunction are also an important part of the ultrasound assessment. Presence of other congenital anomalies would be useful as well. The use of TEE is not useful for evaluation of PS.

Other tests including an ECG can document RV hypertrophy or a right bundle branch block pattern. Cardiac catheterization may be a useful adjunct if the clinical symptoms or examination are discordant with the findings on TTE. It can also be useful to differentiate between valvular and subvalvular stenosis. Lastly, MRI can be useful for a more thorough assessment of the RV.

Patients with mild disease should be followed every 5 years, whereas patients with moderate disease should be examined every 2–5 years if asymptomatic (Warnes et al., 2008).

Management

Management for valvular s is typically driven by the presence of symptoms and the peak gradients measured by TTE. The 2008 AHA/ACC guidelines suggest balloon valvotomy in asymptomatic patients with a domed pulmonic valve and peak instantaneous Doppler gradient >60 mmHg or a mean Doppler gradient >40 mmHg or symptomatic patients with a domed pulmonic valve and peak instantaneous Doppler gradient >50 mmHg or a mean Doppler gradient >30 mmHg (both, in association with <moderate pulmonic valve regurgitation). In other cases where there are other congenital anomalies, which can include an associated hypoplastic pulmonary annulus, severe pulmonary regurgitation, subvalvular PS, or supravalvular PS, surgery is indicated (Warnes et al., 2008).

Pathology

Congenital pulmonary stenosis is either categorized as dome-shaped acommissural or dysplastic. In the domed-shaped type, the valve is typically dome shaped with a central aperture. The ridges that are seen are the areas of malformed commissures. In the dysplastic type, the cusps are thickened and rubbery. The valve annulus is small and the commissures are not fused (Waller et al., 1995). On microscopic examination, there are extensive, irregularly arranged aggregates of acid mucopolysaccharide staining material and fibrous tissue (Waller, 1984a; Waller, 1984b).

Congenital Mitral Stenosis

Definition

Congenital anomalies of the mitral valve (MV) are very rare. The valve is either malformed or malpositioned, or there may be anomalies of the papillary muscles. Most are associated with other congenital abnormalities such as the ostium primum ASD and present in infancy. MS may be due to valvular, subvalvular, or supravalvular causes, though we will only be discussing valvular MS. Congenital valvular mitral stenosis (MS) is a rare condition and may be due to one or more of several causes, such as hypoplasia of the MV annulus, double orifice mitral valve (DOMV), abnormal chordae tendinae (usually short and thick), or a parachute MV, where there is one papillary muscle supplying chordae tendinae to both leaflets. These lesions may occur by in isolation, or may be found with other congenital cardiac lesions such as aortic coarctation, AS, and SVAS.

Epidemiology

Abnormalities of the MS have an incidence of lower than 0.1% among individuals with congenital heart disease. Echocardiograms of 13,400 individuals showed an incidence of 0.5% (Bannerjee and Kohl, 1995). If severe or if associated with other major congenital cardiac lesions, manifest during infancy or childhood and are managed at that time. We will consider those conditions, which are relatively mild and therefore not operated in infancy or childhood. There is no mention of any gender difference in their incidence or of any ethnic predilection.

Clinical Presentation

In any of the conditions associated with MS, the presenting symptoms are usually those of heart failure and include dyspnea, orthopnea, and nocturnal dyspnea. Dilatation of the left atrium, which occurs may lead to palpitations, atrial fibrillation, thrombus formation in the atrial appendage and subsequent thromboembolism. If MS is undetected, pulmonary hypertension can occur which can lead to hemoptysis. Later effects of biventricular failure include hepatic congestion and hepatomegaly, and tenderness can be found. On physical examination, the murmur of MS is best heard at the apex with the patient lying on his/her left side. The murmur is pan-diastolic with presystolic accentuation if the patient is in sinus rhythm. It is low pitched and described as a "rumbling" sound. The murmur is typically preceded by an opening snap.

Investigations

Echocardiography (TTE) is the main modality to highlight MS severity. Focus on hemodynamic severity (mean gradient, mitral valve area, and pulmonary artery pressure) is important to record. In addition, the size of the left atrium and ventricular function should be analyzed as well. If atrial thrombus needs to be excluded or if the severity of MR is not well assessed by TTE, TEE would be necessary. Exercise testing can also be employed if clinical symptoms are not in keeping with echocardiographic findings. If symptoms are discordant to severity seen on TTE/TEE, cardiac catheterization can be used to assess hemodynamics. If surgical intervention is planned, coronary angiography should be done to ascertain coronary anatomy.

Management

General symptomatic treatment of heart failure and pulmonary edema would include adequate decongestive therapies. If left atrial thrombus is detected, anticoagulants are needed to prevent systemic embolization. Hypoplastic mitral valve would likely need surgical replacement with a mechanical or biological prosthesis. Once patients become symptomatic, mitral valve surgery is indicated if they are NYHA class III-IV with severe MS (defined as mitral valve area <1.5 cm^2 with a transmitral mean gradient >5–10 mmHg at a normal heart rate). In addition, surgery is indicated in asymptomatic patients with severe MS if undergoing cardiac surgery for another indication (Nishimura et al., 2014). Class IIa and IIb indications can be found elsewhere (Nishimura et al., 2014). Percutaneous options are not ideal as the anatomy is quite complex.

Pathology

The normal mitral valve is a complex structure comprised of the mitral annulus, the leaflets, the chordae tendinae, the papillary muscles, and the two left-sided cardiac chambers. These must function appropriately and synchronously for the valve to function well and normally. The mitral leaflets are unequal structures, which control blood flow from the pulmonary veins to the systemic circulation. Surgically the leaflets are divided into the P1, P2, P3, and A1, A2, A3 segments, which surgeons use as landmarks for mitral valve repair (Charpentier et al., 1998). The subvalvular structures are comprised of the chordae tendineae and the anterior and posterior papillary muscles. The chordae tendinae are attached to the base (strut chordae), rough zone or zone of coaptation, and the free margin (marginal chordae). All chordae "arise"from the papillary muscle tips (Bharati and Lev, 1996a).

Congenital mitral valve stenosis is rare and is associated with thickening of the mitral leaflets, especially the anterior leaflet (Bharati and Lev, 1996b). The mitral orifice will be smaller than usual. In addition most cases will show left atrial hypertrophy and dilatation, RV hypertrophy, and dilatation. The papillary muscles may be fused into one rim of muscle and therefore give rise to a "parachute" papillary muscle. The chordae tendinae may be short and in fact, the papillary muscle tips may be very close to or flush with the edge of the leaflets.

Cor Triatriatum or Double Left Atrium

Definition

This is strictly speaking, not a congenital valvular disease. However its presentation and symptoms may be those of mitral stenosis and thus, the reason for mentioning it here. In this lesion, there is a fibromuscular membrane across the left atrium, in essence dividing the LA into an upper chamber into which the pulmonary veins open and a lower chamber which has the mitral valve at its distal end. There may or may not be any other associated congenital cardiac lesion.

Epidemiology

This is a rare condition, being seen in 17 hearts out of 6300 congenitally malformed hearts (Bharati and Lev, 1996b). In this series, the male to female incidence was approximately 2:1.

Clinical Presentation

The membrane may have 1–2 large perforations or several small perforations, in which case the patient may have no symptoms. Auscultation may not reveal any murmurs and an echocardiogram may show the membrane. The patient may remain asymptomatic and the diagnosis made incidentally or present with features of mitral stenosis.

Management

The TTE or TEE would likely pick up the lesion incidentally or in a symptomatic patient. The management would depend on the severity of the lesion and could include surgical excision of the membrane.

Mitral Valve Prolapse

Mitral valve prolapse (MVP) and resulting mitral valve incompetence are said to be present when the mitral valve leaflets extend at least 2 mm beyond the long axis annular plane of the mitral valve during systole (Abadir et al., 2009). It is usually secondary to degenerative changes and is the most common form of mitral valve dysfunction in developed countries. However, it does occur within patients with Marfan syndrome and is associated with other congenital cardiac lesions including as ostium secundum ASD, Ebstein's anomaly, and hypertrophic cardiomyopathy.

Cleft Mitral Leaflet

This anomaly is rare, but would be associated with mitral valve incompetence.

The presence of a division (cleft) in a mitral leaflet is typically found on the anterior leaflet. This may occur in isolation or may occur with AVSD (Abadir et al., 2009). Isolated cleft in the posterior leaflet is even rarer and usually occurs in segment P2 (Kent et al., 2001).

The lesion can be seen on TTE or TEE. The appearance is that of a slit hole in the AML. Surgical repair of the MV cleft is the preferred mode of definitive treatment. At times, this may not be feasible (i.e., leaflet edges markedly thickened and retracted) and a replacement has to be performed with an appropriate prosthesis.

Conclusion

In this section we have highlighted and discussed some of the commoner congenital valvular abnormalities seen in adults. The impact of valvular abnormalities is usually progressive and significant, at times culminating in congestive heart failure. Their symptoms are often similar, though the signs can be different. Most valvular lesions are fairly easily treated with currently available prosthetic devices. Most, though not all, devises today are fairly long lasting.

Mechanical heart valves have great durability but need lifelong anticoagulation, while tissue prostheses will fail, one day, but do not usually need any anticoagulants. Today it is the surgeon and the patient who decide which type of prosthetic heart valve is best suited to a patient.

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Coronary Anatomy

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Introduction

Coronary circulation is unique to vertebrates; no invertebrates have a coronary circulation. Among the vertebrates, mammals, reptiles, and avians possess coronary circulations with both arterial and venous systems.

The coronary arteries are the first branches of the aorta, arising usually from two of the three aortic sinuses of Valsalva.

Embryology of the Coronary Circulation

Initially, coronary circulation does not exist; the nutrients and oxygen, which nourish the endocardium and myocardium, are extracted by diffusion from the blood flowing through the lumen of the heart tube, which is initially extensively trabeculated. However, with increase in thickness of the heart tube, by the third week, a dedicated vascular system begins to form from the epicardial surface. Coronary vasculature forms by an elaborate process involving vasculogenesis, angiogenesis, and arteriogenesis (Tomanek, 2015; Bernanke and Velkey, 2002).

The proximal ends of the coronary arteries are formed when strands from a peritruncal ring of vascular structures penetrate the aorta by ingrowth, at the right and left sinuses of Valsalva.

Epicardial cells are derived from the proepicardial organ, which lies in the region of the sinus venosus. The initial channels are formed predominantly within the epicardial covering but are scattered throughout the myocardium mediated by the ventricular epicardial growth factor. At this time, there is no continuity between the separate vascular structures, and they are not connected with systemic circulation. However, subsequently, the vessels coalesce to form the early vascular plexus (in a process termed epithelial-to-mesenchymal transformation), which is initially seen within the subepicardial layers of the atrioventricular and interventricular grooves, and around the ventricular outflow tract. Following this, a complex network develops throughout the myocardium by sprouting from the primary plexus, driven by the hypoxic gradient.

Relatively late during the embryonic stage, the network gains access to the aortic root by ingrowth, subsequent to the process of aortopulmonary rotation. The sudden exposure to pulsatile flow and improved perfusion initiate the arterialization of this vascular network (embryonal arteriogenesis). This arteriogenesis includes (a) the migration of smooth muscle cells and pericytes from the epicardium and the aortic root, (b) the assembly of these cells with the primitive vascular structures to form stable vessels, and (c) radial growth and regression along with (d) apoptosis. This process occurs in a base-to-apex direction.

The precursors of the coronary veins have been seen to develop similar to the coronary arteries, as a capillary plexus, which then grows toward and into the wall of the sinus venosus. The connection to the coronary sinus or atrial lumen occurs only during the last stage of development. The muscularization of coronary veins, like that of arteries, is dependent on the establishment of coronary flow and is a late feature. The venous side of the capillary plexus precedes the arterial side in making connection with the luminal circulation. The left coronary artery formation precedes that of the right.

Thus, coronary blood vessels are a developmental mosaic, including cells derived from different embryonic sources. Sinus venosus endocardium mostly accounts for the makeup of coronary veins, whereas ventricular endocardial sprouts and epicardial-derived endothelial cells are the main contributors of the arterial components of the coronary tree.

Histology of Coronary Arteries

Like all other arteries, coronaries have three layers—intima, media, and adventitia. The endothelial cells of the intima lie on the subendothelial layer of connective tissue and smooth muscle cells. They are aligned to the long axis of the artery and are attached by occluding and communicating junctions. The intimal layer is separated from the media by the internal elastic lamina. The media consists of multiple layers of smooth muscle cells (about 40 layers) and connective tissue (consisting of elastic fibers and collagen). External elastic lamina separates the media from the adventitia and is composed of elastin and is considerably thinner than the internal elastic lamina. The adventitia is composed of fibrous tissue surrounded by vasa vasorum, nerves, and lymphatics (Waller et al., 1992a).

Normal Coronary Anatomy

Normal coronary anatomy is discussed in the articles by Loukas et al. (2009a, 2013) and Patel (2008).

Aortic Origin (Ostia) of the Coronaries

The two sinuses adjacent to the pulmonary trunk give rise to the major coronary arteries, the right coronary artery and the main stem of the left coronary artery. The origins of these arteries can vary significantly in relation to the sinotubular junction and the aortic valve commissures. It is exceedingly rare for a coronary artery to originate from the aortic sinus that is not adjacent to the pulmonary root (noncoronary sinus). The coronary arteries and their branches are normally embedded within the loose connective tissue and the adipose tissue of the subepicardial space (Almuwaqqat et al., 2012).

The right coronary artery arises from the right coronary aortic sinus of the right anterior surface of the aortic root. In many instances, two arterial ostia are present in this sinus, with the second ostium most often giving rise to the infundibular (conal) artery, but less frequently to the artery of the sinus node. The right coronary ostium is at the level of the free margin of the cusp in majority (>70%) of cases, above in about 20% and below the sinotubular junction in the rest.

The main stem of the left coronary artery arises from the left sinus of Valsalva just below the sinotubular junction, but in some, it has a high takeoff.

The right and left coronary ostia are placed under the sinotubular junction in about 55%; right ostium below and the left above in about 30%; right ostium above and left below in 8% and both ostia above in 6% of cases. The radial separation of the right and left coronary ostia in humans is about 123 degrees.

The ostia that are within 1 cm of the sinotubular junction in the adult heart are considered normal variations, whereas origins >1 cm relative to the junction are known as ectopic origins, or high takeoffs. It is very unusual for a major coronary artery to take origin from the aortic sinus distant from the pulmonary trunk (noncoronary sinus). A takeoff that is >2.5 cm above the sinotubular junction is considered an ectopic ostium. The combination of high takeoff with atherosclerotic changes in the coronary artery can be associated with an increased incidence of sudden cardiac death. When the arterial orifice is within 5 mm of the zone adjacent to the peripheral attachment of the aortic leaflets at the sinotubular junction, it is termed commissural origin.

Abnormal ostia due to slit-like openings, acute angle of takeoff, or fibrous valve-like ridges can cause flow impairment.

Right Coronary Artery

The right coronary artery occupies the right atrioventricular groove (Adams and Treasure, 1985). Its first part extends to the acute margin of the ventricular mass, where it gives rise to the acute marginal artery, giving rise to many atrial branches from its cranial

surface. It extends to the apex forming anastomoses apex between the anterior interventricular (left anterior descending) artery. It also gives rise in this part of its course to the infundibular artery (in half the population), unless it arises directly from the aorta, and in just over half the population, to the artery supplying the sinus node (in two-fifth of the population, it arises from the circumflex vessel). In some, the infundibular artery anastomoses with an infundibular branch of the anterior descending artery—called arterial circle of Vieussens.

The right coronary artery itself then continues to encircle the tricuspid valve, reaching the crux. It gives rise to the right inferior ventricular branches, which supply the diaphragmatic wall of the right ventricle from this segment. In nine-tenths of the population, having reached the crux, the right coronary artery gives rise to the inferior interventricular (posterior descending) artery and the artery to the atrioventricular node—right dominance. Atrioventricular nodal artery forms a U-loop forming the floor of the triangle of Koch. This artery, along with the septal branches from the anterior descending artery, supplies the proximal portion of the atrioventricular conduction apparatus. The right coronary artery then continues to supply variable portion of the diaphragmatic wall of the left ventricle.

The posterior descending (inferior interventricular) artery arises from the right coronary artery in all of the 90% of individuals with right coronary arterial dominance, and from the circumflex artery in the remaining one-tenth (more common in males). There are anastomoses from this artery with parallel branches of the right marginal artery and perpendicular branches of the anterior descending artery in the inferior atrioventricular groove and at the apex. Perforating branches from the artery supply the inferior part of the muscular interventricular septum, the adjacent ventricular walls, and the posteromedial papillary muscle of the mitral valve in those individuals with right coronary arterial dominance. Rarely, the anterior descending artery can extend into the inferior interventricular groove, wrapping over the apex, supplying the territory usually tended by the posterior descending artery.

There are variations to the origin of the posterior descending artery; it can originate at the crux as in most instances (approximately 60%) or can branch off before the crux runs along the acute margin of the right ventricle before reaching the posterior interventricular groove (approximately 30%). In some instances, the acute marginal artery itself reaches the distal part of the posterior interventricular groove.

The Left Main Stem

As the left main stem exits from the aortic sinus, it enters the left margin of the transverse sinus, between the left atrial appendage and the pulmonary trunk. It has a length rarely more than 1 or 2 cm in the adult, after which it divides into the circumflex and anterior descending branches. The main stem usually measures between 5 and 10 mm in diameter (Medrano-Gracia et al., 2016).

It supplies a larger mass of myocardium—most of the left ventricle, the muscular ventricular septum, and the anterolateral papillary muscle of the mitral valve, the left atrium, and in about half the population gives rise to the sinus nodal artery. In around one-quarter of the population, the main stem also gives rise to an intermediate branch and rarely to two intermediate arteries.

When there are separate origins of the anterior descending and circumflex arteries, the main stem is evidently absent. This exists in 0.41%–0.52% of individuals and carries no hemodynamic consequences.

The left main bifurcation angle is 89 ± 21 degrees for cases with and 75 ± 23 degrees for those without an intermediate (ramus) artery.

The Left Anterior Descending Artery

The left anterior descending artery emerges on the anterosuperior surface of the heart, coursing around the base of the pulmonary trunk to lie in the anterior interventricular groove. It then runs to the ventricular apex, in some individuals continuing around the apex and extending to variable distance within the inferior interventricular groove. It supplies the apex of both right and left ventricles. It gives rise to two groups of vessels, the diagonal arteries and the septal perforators; both sets vary in their number and course. During its course, the anterior descending artery can give rise to as many as three to four diagonal branches, coming off at acute angles to supply the lateral wall of the left ventricle. There are also a variable number of septal perforating branches. The deep septal perforators branch at right angles to the anterior descending artery and supply the anterior two-thirds of the muscular ventricular septum. The first and rarely the second or third of these also supply the atrioventricular conduction axis. The anterior descending artery can also give rise to an infundibular branch, leading to an anastomotic circle around the infundibulum.

The Circumflex Artery

The circumflex artery passes posteriorly from the main stem, running in the posterior atrioventricular groove. In most individuals, it runs for a short distance within the groove before ending as the obtuse marginal artery or branches. In minority (10%), it reaches the crux of the heart, giving rise to the atrioventricular nodal artery, and occasionally beyond the crux to supply the diaphragmatic surface of the right ventricle—left coronary arterial dominance. The circumflex artery hence supplies the lateral and posterior walls of the left ventricle and the anterolateral papillary muscle of the mitral valve.

The Intermediate Artery

In some individuals, the left main stem branches into three rather than two arteries. The third branch is called the intermediate or the ramus branch and is of varying size.

Coronary Arterial Dominance and Perfusion Territories

In the majority, it is the right coronary artery that supplies the inferior wall of the right ventricle and the inferior portion of the muscular ventricular septum (Adams and Treasure, 1985), whereas the branches of the left coronary artery supply the majority of the anterolateral walls and the obtuse margin of the left ventricle. In addition, in up to half the population, the dominant right coronary artery also supplies a significant part of the diaphragmatic wall of the left ventricle. In right dominance, it is the branches of the right coronary artery that typically supply the posteromedial papillary muscle of the mitral valve. In case of an extremely dominant right coronary artery (with hypoplastic circumflex artery), the branches of the right coronary artery can supply the entire inferior wall of the left ventricle.

Coronary circulation is right dominant when the posterior descending artery and posterolateral branches originate from the right coronary and left dominant when they both originate from the circumflex vessel. A balanced coronary circulation is defined when the posterior descending artery originates from the right and all posterolateral branches from the circumflex. However, a more accurate definition of dominance is the arterial origin of the atrioventricular nodal artery. The typical right dominant circulation is found in 71%—in this pattern, the RCA supplies ≈15% and the left coronary artery ≈85% of the left ventricle. A left dominant circulation pattern is present in 10%–12% of cases in which the left coronary artery supplies all the flow to the left ventricle, and the circumflex gives rise to the posterior descending artery. The remaining 17% of hearts have balanced circulation.

Despite the earlier generalization, normal variations exist. A long left anterior descending artery that wraps around the left ventricular apex can supply a variable portion of the inferior wall. The circumflex artery usually supplies a variable extent of the lateral wall, depending on the number and size of the obtuse marginal branches, the number and size of diagonal branches from the LAD, the presence of posterolateral branches from the right, and the presence of an intermediate branch. The inferior wall demonstrates the largest variability of vascular supply.

The AHA 17-segment model recommends the following: Segments 1, 2, 7, 8, 13, 14, and 17 are assigned to the left anterior descending artery distribution (Donato et al., 2012). Segments 3, 4, 10, and 15 are assigned to the right coronary when it is dominant, and segments 5, 6, 11, 12, and 16 are generally assigned to the circumflex artery. However, the normal variations in coronary perfusion distribution make these assigned segments inaccurate (Cerci et al., 2012). For example, circumflex artery can be the main artery for the blood supply of the inferior wall segments even if the origin of the posterior descending artery is from the right coronary (Detre et al., 1975).

Coronary Anomalies

The concept of normal variant versus anomaly is based on a definition of coronary anomalies as patterns observed in less than 1% of the population (Waller et al., 1992a,b,c; Pérez-Pomares et al., 2016; Ripley et al., 2014; Altin et al., 2015; Angelini and Flamm 2007; Shriki et al., 2012). In the normal population, there is a wide variation in the absolute origin and course of a particular coronary artery. Coronary anomalies come in various forms—anomalous origins of the arteries, anomalous epicardial course, or other abnormal features such as myocardial bridging or abnormal communications. Clinically relevant variations in the origin and course of the coronary arteries in normal hearts (without congenital defects) are rare. Reported incidence of such anomalies is about 0.3% in an autopsy series and higher at 1.6% in a larger angiographic cohort. Anomalies carry a higher risk for atherosclerosis and could present with cardiac symptoms or sudden death. The task force of the American Heart Association on sudden death attributes one-fifth of such instances in young athletes to coronary arterial anomalies.

Anomalies are more commonly found in patients with congenital cardiac diseases such as tetralogy of Fallot, transposition, and bicuspid aortic valves.

Classification of anatomic variations
Appropriate sinus origin
High
Low
Commissural
Anomalous sinus origin
Right coronary artery arising from left coronary sinus
Main stem of left coronary artery arising from right coronary sinus
Circumflex artery arising from right coronary sinus
Anterior descending artery arising from right coronary sinus

Classification of anatomic variations
Right or left coronary artery arising from noncoronary (posterior) sinus
Ectopic origin of coronary artery
From pulmonary trunk
From right pulmonary artery
From left pulmonary artery
From brachiocephalic artery
Solitary coronary artery
Abnormal distal connections (Fistulas)
Connections with a cardiac cavity
Coronary arteriovenous fistulas
Coronary to extracardiac arterial or venous connections
Anomalies of intrinsic anatomy
Coronary ostial stenosis or atresia
Coronary ectasia or aneurysm
Absent coronary or hypoplasia
Miscellaneous anomalies of intrinsic anatomy
Myocardial bridging
Duplication of coronary arteries

Whatever sinus they originate from, there are three possible proximal routes of the coronaries: (a) retroaortic, (b) interarterial, or (c) prepulmonary. Some have described a fourth pattern, a so-called transseptal course. This so-called transseptal course is a variant of the interarterial route. Here, either the left main stem or the anterior descending artery courses toward the subpulmonary infundibulum but then emerges, entering the basal muscular septum.

Certain combinations of sinus origins and proximal coronary courses stand out as being frequent.

Gender Variations

In a retrospective analysis of nearly 7800 coronary angiograms (Aydar et al., 2011), females were noted to have a higher incidence of specific coronary anomalies than males (4.4 vs. 2.9%). They also presented with a higher incidence of a separate origin of the circumflex artery and left anterior descending artery from the left sinus of Valsalva compared with males (3.1 vs. 1.1%, although this may not be considered as an anomaly by most). There was no difference in the frequencies of the second most common anomaly, that is, circumflex from the right sinus or the right coronary artery, between males and females. Males were found to have a higher incidence of myocardial bridges (1.5 vs. 0.85%). No differences in gender were detected for fistulas.

High-Takeoff Coronary Arteries

A high-takeoff coronary artery is defined as arising at least 1 cm in adults or 20% the depth of the sinus in children above the sinotubular junction. The prevalence of high-takeoff coronary arteries in the general population is about 0.20%. Right coronary arteries make up 85% of reported cases (it is also reported with increased frequency with bicuspid aortic valve). About 4% were noted to have either an acute angulation from the aorta, an interarterial, or an intramural course. In these cases, reduced coronary flow results from (a) intramural intussusception, (b) coronary hypoplasia (including ostial hypoplasia or slit-like aortic ostium), (c) external compression, or (d) combination of factors (Loukas et al., 2015).

The Circumflex Artery Taking Its Origin From Right Sinus

The circumflex artery taking its origin from right sinus most frequently takes a retroaortic course, passing through the transverse sinus of the pericardium to emerge into the posterior atrioventricular groove between the left atrial appendage and the pulmonary trunk. This course is of no clinical significance, unless the origin is intramural. Circumflex arising from the right sinus or right coronary artery, with retroaortic course, accounts for about half of all reported anomalous arrangements—between 0.45% and 0.67% in patients undergoing coronary angiography.

Retroaortic course of a solitary coronary artery is much rarer but may be of clinical significance, as it may preclude to premature atherosclerotic disease. A prepulmonary course running anteriorly across the subpulmonary infundibulum from their anomalous sinus origin is considered benign and is seen most frequently when the left main stem or one of its branches arises from the right sinus or the right coronary artery from the left sinus. The prepulmonary course is of far greater significance in the setting of congenitally abnormal hearts, such as tetralogy of Fallot or transposition.

The interarterial course is of greatest clinical significance, as this "malignant" anomalous pattern is most frequently associated with sudden cardiac death. There are many anomalous sinus origins that can be associated with such an interarterial course. However, the most frequent are those where the coronary artery originates from the opposite sinus, such as the left main stem arising from the right sinus, or the right coronary artery arising from the left sinus. Frequently, the coronary artery itself also takes an intramural course as it exits from the aortic sinus leading to further luminal compromise. When the LAD originates from the RCA or the right sinus of Valsalva, it runs between the aorta and the pulmonary artery in 75% of the cases. Anomalous left coronary connection to the right sinus is considered more malignant because of the larger amount of myocardium at ischemic risk. Causes of myocardial ischemia include (i) increased cardiac output and dilation of the aorta and pulmonary artery, with compression of the anomalous vessel coursing in between; (ii) the acute angle takeoff of the anomalous vessel with further stretch during exercise leading to a flap-like closure of the ostium; (iii) spasm or kinking of the anomalous vessel; and (iv) the course within the aortic wall (intramural) of the proximal segment of the anomalous vessel. Higher rates of major adverse cardiac events have also been reported with a high interarterial course (right coronary ostium located above the level of the pulmonary valve) as opposed to low interarterial course (ostium below pulmonary valve).

Transseptal Course

When a coronary artery arises from the contralateral artery or cusp and takes a course through the septal myocardium, it has a transseptal route. A transseptal artery has a lower position than an interarterial artery and is located caudad to the crista supraventricularis. Usually, the transseptal artery is the left anterior descending artery or the left main vessel.

Intramural Course of Coronaries

Normally, coronary arteries arise at right angles to the wall of the aortic sinus. In the intramural variant, the artery runs for varying lengths within the aortic wall, taking course obliquely before its own discrete walls cover its lumen. Intramural origin can be the result of high takeoff but more commonly is in the plane of the sinotubular junction, where the peripheral aspects of the aortic leaflets are attached; consequently, this part of the aortic wall is thicker. Intramural and/or intussuscepted portions of anomalously coronary arteries are generally hypoplastic, with an ovoid rather than a circular cross-sectional profile.

Ectopic Origins of Coronary Arteries

The most frequent ectopic origin is from the pulmonary trunk or one of its branches although origins from the brachiocephalic or subclavian arteries are reported (more commonly with the right coronary). The pulmonary origin is potentially fatal unless diagnosed and treated surgically, because the coronary artery, and the myocardium it supplies, is fed with desaturated blood. In 90% of cases, the left main is the anomalous artery. The extent of the collateral circulation between the two coronaries is the major determinant of the degree of ischemia, severity of clinical presentation, and outcome.

Solitary Coronary Arteries

Solitary coronary arteries can cause sudden cardiac death and has the potential to precipitate severe ischemic heart disease, including myocardial infarctions, in the young. Their incidence is reported to be approximately 0.024%–0.044% of the general population. Such arteries can originate from the left sinus and divide into an anterior interventricular artery and left circumflex coronary artery as normal. The circumflex that is dominant gives rise to the posterior descending artery and continues through the anterior atrioventricular groove to supply the area usually tended by the right coronary artery. A solitary right coronary artery can take one of two forms. It can continue beyond the crux, run through the posterior atrioventricular groove, and terminate as the anterior descending artery, or alternatively, it can give rise to the left main stem, which can then take either retroaortic, interarterial, or prepulmonary course before branching into the anterior descending or circumflex arteries. The interarterial pattern is the most pathologically significant.

Coronary ostial stenosis or atresia is a rare condition, with most of the reported cases discovered at angiography and involving the left main. Clinical presentation is usually early in the first year of life.

Abnormal Distal Connections

Coronary Artery Fistulas

Coronary artery fistulas are rare and lead to a direct communication between a coronary artery and (a) one of the cardiac chambers, (b) the coronary sinus, (c) a venous structure, or (d) a pulmonary artery. Fistulas between the coronary arteries and either the

coronary venous system or the cardiac chambers are seen in up to 0.5% of individuals. They are of functional importance as the abnormal communication bypasses the myocardial capillary bed frequently (in 90%) causing a left-to-right shunt. With larger fistulas, a diastolic "coronary steal" may exist, drawing blood away from the normal coronary tree, leading to myocardial ischemia. They can be remarkably tortuous, and the right coronary artery is more commonly involved than the left. The most common fistulous sites occur in the right ventricle in 41%, right atrium in 26%, pulmonary artery in 17%, coronary sinus in 7%, left ventricle in 3%, and superior vena cava in 1% of cases.

About half of the patients remain asymptomatic; the other half develop complicating features such as infective endocarditis, myocardial ischemia, pulmonary hypertension, high-output cardiac failure, and rupture. Most fistulas grow over time, and large fistulas may lead to heart failure associated with high-flow shunting.

Collaterals

Collateral arterial circulation typically occurs as a response to ischemic myocardium in the setting of severe atherosclerosis (Loukas et al., 2009b), suggesting the development of the collateral arteries to be strongly associated with slowly developing coronary arterial obstruction. It is only when circulation is impaired that the collateral vessels, through the process of arteriogenesis, attain functional role. The process of arteriogenesis converts the preexisting collateral arterioles to mature into functional muscular arteries. There is increase in luminal pressure, proximal to the collateral branch as vascular obstructions develop. This rise in pressure widens the lumen of the collaterals, allowing for physiologically relevant flow. This shear stress essentially induces the formation of collateral arteries from a preexistent network.

The collateral arteries arise from the same tissue components as the native coronaries during development, and as such are histologically indistinguishable. However, the native coronary arteries develop from base to apex, along the direction of blood flow—larger arteries develop first, and the branching follows. The collateral arteries do not follow this pattern, and these bridging vessels can allow flow in both directions.

Endomural collateral arteries are found between coronary arteries, whereas extracardiac collateral arteries take origin most commonly from the bronchial or internal thoracic arteries. Collaterals from the bronchial arteries typically occur at sites of pericardial reflections and communicate most frequently with the circumflex artery and can be found in up to one-fifth of normals. Collaterals originating from the internal mammary arteries typically occur between the right internal mammary and right coronary arteries or between the left internal mammary and the posterior descending artery, in response to myocardial ischemia.

Research suggests a strong protective role for a well-developed collateral coronary arterial circulation. During acute coronary occlusion, long-term cardiac mortality is reduced to 25% when compared with patients without collateral supply. Well-developed collaterals are ready to resupply the myocardium within minutes to hours if reocclusion occurs.

Myocardial Bridging

Myocardial bridge is the second most common type of the coronary artery anomaly. During their passage in the epicardium, epicardial portions of the coronary arteries can dive to form an intramyocardial course, reemerging more distally—known as myocardial bridging (seen in up to one-third of patients on CT). The anterior descending artery has been reported by some to take a partially transmural course in up to four-fifths of individuals. Bridging of arteries other than the anterior descending branch, nonetheless, should probably be considered as anomalies rather than normal variations. The length of the myocardial bridges in the males is noticeably longer than that of the females. Symptoms and ischemia tend to occur specifically when the bridging segments are long (2–3 cm) and deep (2–3 mm) within the myocardium.

Three main conditions can lead to ischemia in myocardial bridging: (a) the "spillover" phenomenon—persistence of narrowing in early diastole—the more severe the systolic narrowing, there is higher likelihood of "spillovers" occurring; (b) periods of tachycardia, in which systole occupies a greater percentage of the cardiac cycle due to shortening of the diastolic period; and (c) microvascular compression. The tunneled segment is often spared from atherosclerotic disease, although the presence of atherosclerosis is frequently seen in the segment proximal to the bridge.

Duplication of Coronary Arteries

Any of the three coronary arteries can be duplicated, but most frequently, it is the anterior descending artery that has this pattern—in up to 1% of the population. It is frequently seen as a short artery that terminates proximally within the anterior interventricular groove, and a longer one that extends to the apex.

It is important to note that any of the indicated coronary anomalies can coexist with congenital heart malformations.

Age-Related Changes in the Coronaries

In fetal coronaries, the intima and the adventitia are less well developed. However, the media is well developed with a circular layer of smooth muscle cells and fine elastic fibers. The intima is a continuous single layer of endothelial cells. After birth, the internal elastic lamina fragments with proliferation of fibroblasts and increase in the ground substance in the subendothelium. This leads to a "musculoelastic" layer between the intima and the media. The external elastic lamina forms by 6 months of age (Waller et al., 1992b).

In normal human coronaries, the amount of smooth muscle cells and fibroelastic tissue is a function of age. Intima progressively thickens, and by late adolescence, it is as thick as the media; beyond this age group, it becomes thicker than the media. By the middle age, the intima shows evidence of atherosclerosis, and the overlying media thins with discontinuation, fragmentation, and/or disappearance of the internal elastic lamina. Degenerative changes such as calcium deposition take place in the intima but rarely in the media. With older age, the arteries become tortuous, and the calcific deposits increase.

Coronary Blood Flow

Coronary anatomy alone is never sufficient to predict physiological status at the level of the myocardial bed (Schelbert, 2010; Gould, 2009). The coronary circulation has in effect two compartments, the large epicardial conduit vessels and the resistance vessels, typically less than 300 μm in diameter. Whereas the conduit vessels exert little if any resistance to flow, resistance to flow progressively increases as the vessel diameter of the resistance vessels declines from about 300 μm in the small arteries to less than 100 μm in the arterioles. Intracoronary pressures decline along the microvasculature (with most of the pressure dissipating in the 300–100 μm diameter vessels) until reaching a pressure of 20–30 mmHg, still adequate to maintain a gradient across the capillaries.

Myocardial flow increases are initiated by a metabolically mediated decrease in microvascular resistance, possibly involving adenosine as a metabolite of adenosine monophosphate and causing vascular smooth muscle relaxation. The resulting flow increase is augmented by endothelium-dependent factors; higher flow velocities exert greater shear-stress forces upon the endothelium with stimulation of the endothelial nitric oxide synthase and release of the smooth muscle relaxing nitric oxide (NO). Endothelial cells interact with vascular smooth muscle cells to regulate the vessel diameter to changes in flow velocities ("flow-mediated dilation"), both at the level of the microvessels and the epicardial conduit vessels. In addition, both endothelial and vascular smooth muscle cells have adrenoreceptors and thus respond to regional and systemic sympathetic stimuli. This finely tuned interaction between vasoconstrictive (vascular smooth muscle-dependent) and vasodilator forces (mostly endothelium-related) regulates the vessel diameter against the flow velocity to minimize resistance to flow, thereby increasing myocardial flow.

Myocardial flow reserves of less than 2.0 or 2.5, based on findings with intracoronary flow velocity probes, are now accepted as the lower limit of normal. Flow reserves of <1.5 are predictive of poor long-term outcomes (increase in mortality and progression to heart failure). Flow responses to vasodilator stress in coronary artery disease may differ from those during physical exercise. Instead of an attenuated or absent flow-mediated conduit vessel dilation, physical stress may, as mentioned earlier, actually lead to a decrease in the vessel lumen or the stenosed vessel diameter, possibly due to increased α-adrenergically stimulated smooth muscle constriction (local release of norepinephrine from adrenergic nerve terminals in the coronary arteries and release of catecholamines from the adrenal glands into the circulation) or release of vasoactive substances from the endothelium.

Flow limitation depends not only on the degree of luminal narrowing but also on the length of the stenosis, its eccentricity, its in- and outflow angles, and surface properties. In addition, functional adaptations to structural disturbances do happen.

Microvascular disease in the absence of apparent coronary vessel disease can occur. Conditions that cause this include diffuse luminal narrowing without discreet coronary stenosis, systemic vasculopathies associated with inflammatory disorders, transplant vasculopathies, and microangiopathies (e.g., in diabetes), or in hypertrophic cardiomyopathy.

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Coronary Artery Bypass Grafting

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Introduction

The purpose of this article is to give the reader, a scientist or a scientist in training, an overview of coronary artery bypass grafting from the clinician's point of view. Accordingly, it is written and edited with that in mind explaining some of the clinical terms in what is close to lay terms, while taking liberties at times with some of the fascinating science behind the clinical events, using terms more familiar to scientists. The article starts with a brief look at the embryology of the human heart, a description of the anatomy, especially with regard to the myocardial circulation, and the coronary arteries. After an introduction to the pathophysiology of coronary artery disease, specifically atherosclerosis, the discussion of CABG begins, starting with the history of CABG, cardiopulmonary bypass, current thinking about the evidence for indications and selection, some basic science aspects of vascular biology that support decision-making, the technique of CABG, and an overview of perioperative management.

Background

Over the past half century, restoring the circulation to the heart (myocardial revascularization) has evolved into a standard of care that has changed and saved and improved the lives of millions of people worldwide. In the context of the changes in the practice of medicine since the industrial revolution, restoring the circulation to the heart when the myocardial blood supply has been compromised has been one of the pivotal advances in medicine and surgery. One tool in that armamentarium has been coronary artery bypass grafting (CABG). While the acronym for this procedure, CABG (pronounced "cabbage"), has been decried by some as degrading as it is the name of a common vegetable, it has stuck and has been accepted over its nearly 50 year history.

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Embryology and Anatomy

The heart is a four-chambered dual pump (Fig. 1). It is a double pump responsible for blood flow to both the lungs (the pulmonary circulation for gas exchange) and the rest of the body (the systemic circulation primarily for delivery of oxygen and nutrients).

Embryologically, in keeping with the concept that ontogeny recapitulates phylogeny, the human heart begins with a tube (much like that of a fish) that eventually folds and compartmentalizes into two separate hearts (Fig. 2). Those two hearts ultimately fuse into a four-chambered interdependent muscular pump supplying two separate and connected circulations, pulmonary and systemic. In cardiac hemodynamics, this tandem arrangement is truly interdependent, as the flow through the pulmonary circulation (right side of the heart) in the normal healthy person must equal that of the systemic circulation (left side of the heart).

The right side of the pump generates flow to the pulmonary circulation, while the left side pumps to the systemic circulation. Approximately five liters of blood each minute courses through this double pump as it generates blood flow through both the pulmonary and systemic circuits. The left (systemic) side of the heart pumps oxygen (O_2)-rich blood through the arterial system to the tissues of the body to support aerobic metabolism. Having delivered its load of O_2, the deoxygenated red blood cells along with a plasma load of dissolved carbon dioxide return to the right (pulmonic) side of the heart to be pumped to the lungs for gas exchange then return to the left heart to repeat the process (Fig. 3).

While this huge volume of blood courses through the chambers of the heart each minute, the heart's share of that blood is delivered to the myocardium, the heart muscle, via its own circulation, the coronary arteries. The coronary arteries are so named as they course over the top and sides of the surface of the heart much like a crown named by the early anatomists who used the Latin for crown (corona). While the anatomical pattern is generally consistent, there is usually normal and occasionally abnormal variability (Fig. 4).

Atherogenesis

The coronary arteries in some individuals develop atherosclerosis, commonly called hardening of the arteries. In fact, calcified atherosclerotic plaques feel hard to the touch. Touching of the heart was limited to postmortem examinations in early days, and since the advent of cardiac surgery, expanded to the operating room.

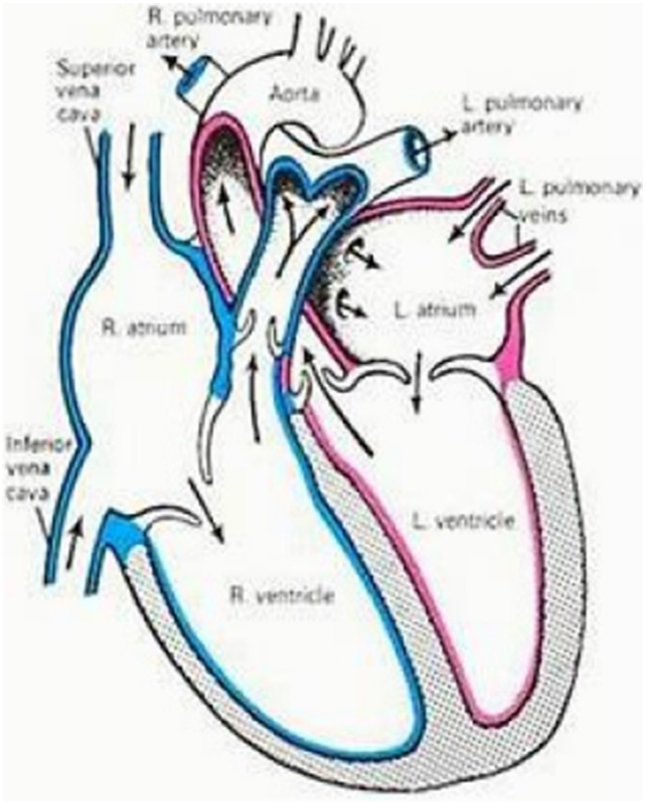

Fig. 1 The four chambers of the heart and the tandem nature showing the direction of blood flow and depicting the chambers and vessels with oxygenated blood in red and those with deoxygenated blood in blue.

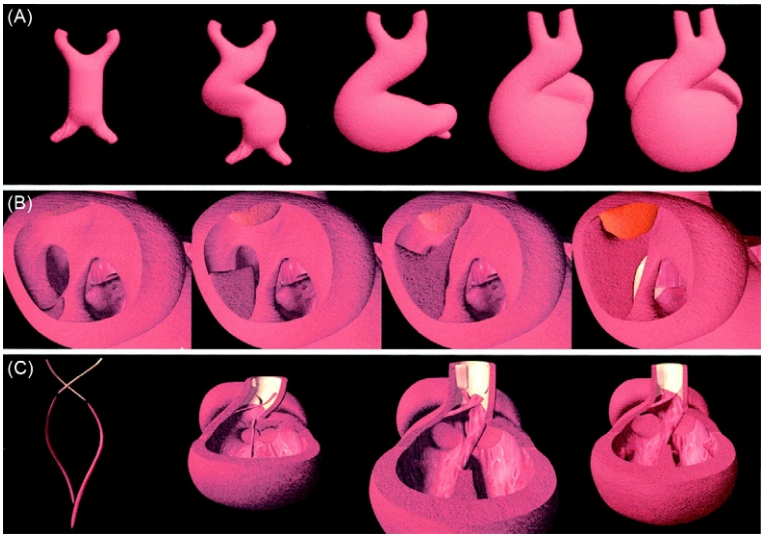

Fig. 2 Embryogenesis from days 21 to 28: (A) The cardiac loop is formed. The heart tube is folded into an S-shaped dextro-ventral convexity. (B) The atria are partitioned. The septum primum (*in brown*) grows from the inferior part of the atria to the top, leaving a foramen called the ostium primum. The septum secundum (*in orange*) comes from the top. The ostium primum will be closed at the end of the fifth week by an expansion of tissue coming from the endocardial cushions (*in yellow*). (C) The conus and the truncus are partitioned. The dextrodorsal and sinistroventral conus ridges, which are isolated in the first picture, partition the conus by a helical outgrowth into two cavities: the subpulmonary and the subaortic coni. The truncus is partitioned from the bottom upward from aorticopulmonary swellings, leading to the formation of the aorta and pulmonary arteries. Reproduced with permission from Wolters Kluwer Health Inc. Schleich, J.-M. and Dillenseger, J.-L. (2001). *Circulation* **104**, e134.

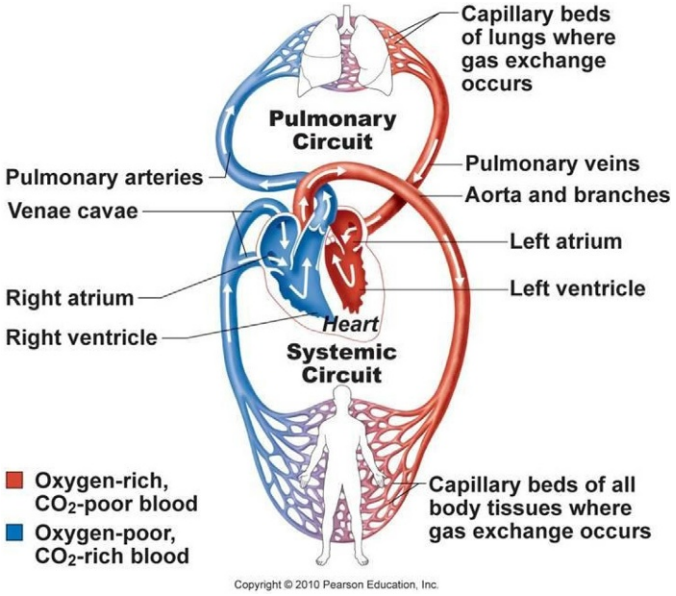

Fig. 3 This illustration depicts the two circulations supplied by the tandem heart, showing the four cambers, direction of flow, and colors representing oxygenated and deoxygenated blood. From Pearson Education (Copyright 2010).

The term atherosclerosis is derived from Latin and Greek roots meaning "gruel or pasty" (athero) and hard (scler), ultimately gaining acceptance as the lay term, "hardening of the arteries." The deposition of this pasty material in the coronary arteries, a consequence of the metabolism of some types of cholesterol, ultimately becomes calcified with time, and becomes firm or hard. In its early stages atheromas consist of soft plaque that can rupture and promote thrombosis, occluding the coronary artery, interrupting myocardial blood supply, and potentially causing a myocardial infarction (heart attack). In its more chronic and progressive form, the atheromatous plaque results in coronary insufficiency, a supply-demand problem, where the blood supply through narrowed coronary arteries to a segment of the heart is not adequate to meet metabolic demands (Figs. 5 and 6).

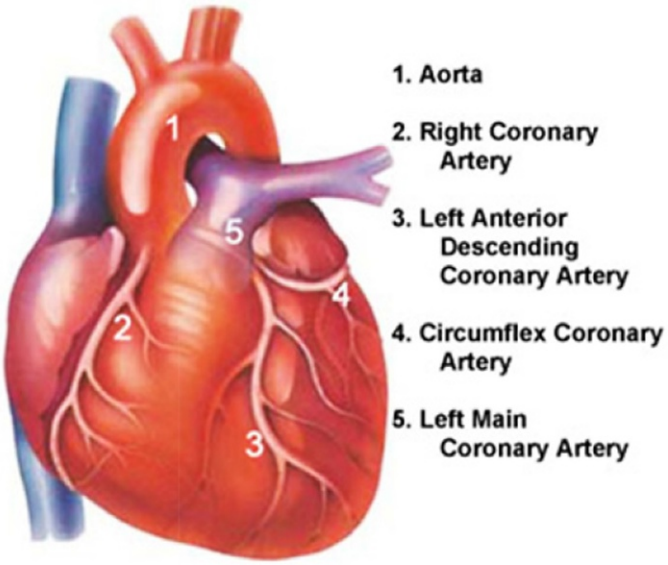

Fig. 4 This illustration depicts the usual distribution of the coronary arteries. Note that the major vessels are epicardial, coursing over the surface of the heart, making them easily accessible to the cardiac surgeon for revascularization. From daviddarling.info.



Fig. 5 Plaque is shown beneath the lining of the artery, showing how the artery's lumen is accordingly narrowed, and the lining of the vessel is still intact.



Fig. 6 Plaque rupture is shown from the vantage point of the cellular elements coursing through the artery with platelets aggregating at the rupture site to begin the formation of a clot. From Crystalgraphics.

The clinical presentations of coronary artery disease range from patients who are virtually asymptomatic to those who experience sudden death with little or no warning. On one end of the spectrum, patients may present with chest discomfort (angina) with exertion or at rest to more extreme acute coronary syndromes, including myocardial infarction. Treatment that consisted of medical management with rest and medications such as nitroglycerine was all there was to offer to these patients until the late 1960s and early 1970s, the time when CABG surgery was conceived and implemented. During the first half of the 20th century, innovative surgical strategies to restore blood supply to those hearts were suboptimal. By the early 1960s there were a few reports of success surgically bypassing occluded and stenotic (narrowed) vessels. The advent of a reliable method to visualize the coronary artery anatomy (coronary angiography) in that decade led to the beginning of the CABG era.

Diseases addressed by cardiac surgery are divided into two main categories: congenital/genetic and acquired. Congenital and genetic cardiac disorders are driven by events that result in structural abnormalities that become apparent very early in life, even in utero, or genetically acquired defects that may effect cardiac and great vessel structure later in life. Clarity in categorizing diseases is often challenging because of overlap. While coronary artery disease (CAD) is considered to be in the category of acquired cardiac disease, there is a clear genetic component with offspring of parents with premature CAD (Khurram et al., 2007) having a higher predilection for acquiring CAD than others. The multifactorial etiology of CAD is apparent as atherogenesis is driven by multiple factors: genetics, lifestyle (smoking, diet, and exercise), and metabolism (such things as diabetes and obesity).

Atherogenesis (the formation of atherosclerosis from the root "gen," meaning to form) is a disorder of the artery wall that may be the result of a chronic inflammatory fibroproliferative process that has become excessive and in its excess this protective response becomes the disease state, a maladaptive response. The process starts with the adhesion of monocytes and lymphocytes (white blood cells) to the luminal side of the endothelial cell lining of the coronary arteries. Those monocytes migrate into the subendothelial space and differentiate into macrophages. Once those macrophages ingest low-density lipoproteins (LDL), the LDL is modified in such a way (such as oxidation) that cholesterol esters accumulate inside and form "foam cells," which are really dead macrophages engorged with LDL. When foam cells combine with T lymphocytes the fatty streak is formed, the beginning of the atherosclerotic plaque is formed by migration of vascular smooth muscle cells from the media of the vessel to the intima. Eventually a fibrous cap is formed, completing the plaque. Ironically, the body's effort to manage this fatty endovascular deposition results in the proliferation of atherosclerotic plaques. This exuberant response is regulated by inflammatory cytokines released by the foam cells and growth regulatory peptides released by platelets, and in the case of coronary artery disease, the body's protective intent becomes the disease (Ross and Agius, 1992) (**Fig. 7**).

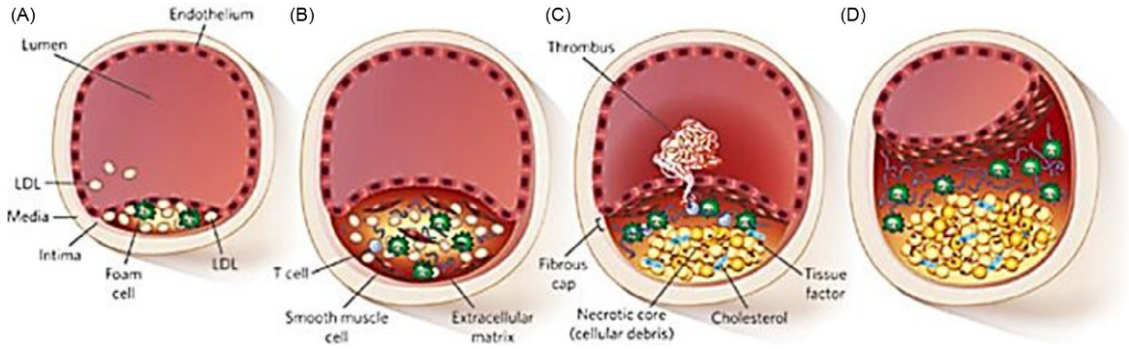

Fig. 7 Atherosclerosis occurs at sites in the arterial tree where laminar flow is disrupted. A lesion begins as a fatty streak (A) and can develop into an intermediate lesion (B), and then into a lesion that is vulnerable to rupture (C) and, finally, into an advanced obstructive lesion (D). A more detailed description of this process is as follows: (A) Atherogenic lipoproteins such as low-density lipoproteins (LDLs) enter the intima, where they are modified by oxidation or enzymatic activity and aggregate within the extracellular intimal space, thereby increasing their phagocytosis by macrophages. Unregulated uptake of atherogenic lipoproteins by macrophages leads to the generation of foam cells, which are laden with lipid. The accumulation of foam cells leads to the formation of fatty streaks, which are often present in the aorta of children, the coronary arteries of adolescents, and other peripheral vessels of young adults. Although they cause no clinical pathology, fatty streaks are widely considered to be the initial lesion leading to the development of complex atherosclerotic lesions. (B) Vascular smooth muscle cells—either recruited from the media into the intima or proliferating within the intima—contribute to this process by secreting large amounts of extracellular-matrix components, such as collagen. The presence of these increases the retention and aggregation of atherogenic lipoproteins. In addition to monocytes, other types of leukocyte, particularly T cells, are recruited to atherosclerotic lesions and help to perpetuate a state of chronic inflammation. As the plaque grows, compensatory remodeling takes place, such that the size of the lumen is preserved while its overall diameter increases. (C) Foam cells eventually die, resulting in the release of cellular debris and crystalline cholesterol. In addition, smooth muscle cells form a fibrous cap beneath the endothelium, and this walls off the plaque from the blood. This process contributes to the formation of a necrotic core within the plaque and further promotes the recruitment of inflammatory cells. This nonobstructive plaque can rupture or the endothelium can erode, resulting in the exposure of thrombogenic material, including tissue factor that stimulates the formation of a thrombus in the lumen. If the thrombus is large enough, it blocks the artery, which causes an acute coronary syndrome or myocardial infarction (heart attack). (D) Ultimately, if the plaque does not rupture and the lesion continues to grow, the lesion can encroach on the lumen and result in clinically obstructive disease. Above quoted from the following article and illustration taken from Rader, D. J. and Daugherty, A. (2008). Translating molecular discoveries into new therapies for atherosclerosis. *Nature* **451**, 904–913. https://doi.org/10.1038/nature06796.

The CABG Era

Currently, CABG surgery is performed on over 400,000 patients in the United States annually, and since its inception has provided myocardial revascularization world-wide to millions. During the first half of the 20th century, strategies to restore cardiac blood supply were manifold and included scarifying the surface of the heart or applying muscle flaps or omentum to induce collateral vessel ingrowth, cervico-dorsal sympathectomy, coronary sinus constriction or arterialization, and internal mammary artery (IMA) ligation. Some of these procedures would occasionally bring symptomatic relief of angina, encouraging proponents, but following an historic randomized trial comparing IMA ligation with a control sham operation of sternotomy alone, the procedure of IMA ligation was shown to be ineffective (Cobb et al., 1959).

The Vineberg Procedure

In 1945, Arthur Vineberg, MD, from Montreal, noting the sinusoidal microcirculation peculiar to the myocardium, postulated that a systemic artery, imbedded in the myocardium, might find adequate runoff into these sinusoids to remain patent and to increase blood flow to the myocardium (Vineberg, 1958) (**Fig. 8**).

During the next four years, he pursued his idea in the animal lab and discovered that, if he rendered the myocardium ischemic by obstructing the flow in the principle artery to the left ventricle (left anterior descending coronary artery or LAD), the implanted left internal mammary artery (LIMA) would not only maintain flow and remain open, but would, over time establish connections to arterial branches of the coronary circulation. In 1950, he performed this procedure for the first time on a patient with severe coronary insufficiency. The patient survived the procedure but died a short time later, before the effect of the procedure could be ascertained. The procedure was repeated on a second patient who lived for ten years and was significantly improved. Dr. Vineberg continued to perform these operations and many of his patients enjoyed dramatic improvement. Many of his colleagues around the world remained skeptical, doubting that meaningful connections were actually being formed to the coronary arteries. There were some advocates of this procedure, including Dr. Bigelow in Toronto and Dr. DeBakey in Houston, who were able to show flow in the heart muscle from Vineberg's operation by using the then new technology of coronary angiography (Bigelow et al., 1966). At this point in history, cardiac surgeons were on the cusp of the CABG era when direct anastomoses of both the mammary artery and saphenous vein graft became feasible and successful, with instant revascularization. The knowledge from coronary angiography that the Vineberg procedure did actually revascularize the myocardium came late and was eclipsed by CABG surgery and has remained so since (Thomas, 1999).

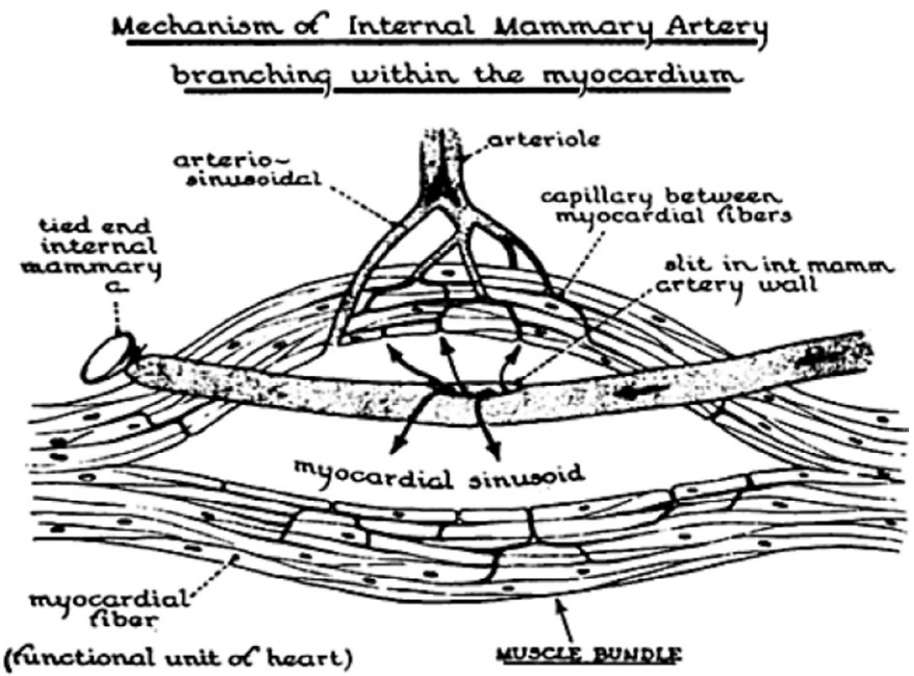

Fig. 8 Drawing of implanted internal mammary artery in left ventricle with opening on one side of vessel. Blood escapes from internal mammary artery into myocardial sinusoids, which is why the implanted vessel remain open until its own branches join the coronary arterioles. From Vineberg, A. (1958). Coronary vascular anastomoses by internal mammary artery implantation. *Canadian Medical Association Journal***78**, 871–879. Copyright © by the Canadian Medical Association.

Coronary Angiography: The Beginning

In 1958, pediatric cardiologist, Mason Sones, at the Cleveland Clinic, while performing a heart catheterization on a 27-year-old patient with rheumatic heart disease, accidentally injected a bolus of dye into his right coronary artery, an error known to be fatal. The result was cardiac arrest and a beautiful picture of the coronary artery. The heart was restarted with a cough, and coronary angiography was born, albeit in the face of vigorous opposition from others in the cardiac field who thought the technique was ethically irresponsible (Sones et al., 1959). Sones and his cardiology fellow, Earl Shirley, perfected the technique and began performing angiograms on hundreds of patients from all over the country, including many patients who had had previous Vineberg procedures, many of which showed dramatic flow from the implanted IMAs into the branches of blocked coronary arteries. These findings stimulated renewed interest in the Vineberg procedure, particularly at the Cleveland Clinic, and in 1968, they published a report on 1100 IMA implants, 92% of which were open and 54% had significant visible connections to the coronaries (Fergusson et al., 1968; Cineangiography, n.d.; Effler et al., 1963).

Peripheral Vascular Surgery Foreshadowed CABG

Beginning in the 1950s, great advances were made in arterial surgery, stimulated by the wartime injuries of WWII and the Korean conflict. Led largely by the advances of Michael DeBakey (DeBakey et al., 1958) in Houston, artificial arterial substitutes were created out of nylon and later Dacron and PTFE (Teflon), and used to replace arterial aneurysms and to bypass occluded or stenotic vessels. Initially, most arterial obstructions were treated by endarterectomy, in which the obstructing cholesterol-laden plaque was surgically removed from the artery. This technique worked well when the obstruction was localized to a short segment of an artery but was less effective when the occlusive disease involved longer arterial segments. For these situations, DeBakey developed the arterial bypass technique, in which an artificial tube graft was connected to the side of the obstructed artery above and below the obstruction, thus increasing blood flow distally while leaving the native circulation intact, in the event that the artificial graft failed and became occluded.

The technique was used first to bypass obstructed iliac arteries, using a bifurcated Dacron graft from the aorta to the common femoral arteries. Later, obstructed superficial femoral arteries were bypassed with Dacron bypass grafts from the common femoral to the popliteal artery. Grafts to these smaller arteries and particularly to those below the knee did not remain open consistently, and surgeons began using the long saphenous vein (from the patient's leg) for the graft, with much better results. While the greater saphenous vein that is harvested for use in CABG, the rest of the venous system that is left in place is more than adequate to provide venous drainage to the leg.

Over time, as surgical techniques improved, vein bypasses were constructed to smaller and smaller arteries further down the leg. These were reasonably successful until, close to the ankle, the recipient artery was no more than 2–3 mm in diameter, and then the graft would usually not stay open. That was, therefore, considered the size limit for a saphenous bypass graft. For this reason, at that time, bypassing the coronary arteries was not considered feasible.

The First CABGs

Between 1962 and 1967, human CABG using autogenous saphenous vein grafts were performed by Sabiston (1962), Garrett (1964), Kahn (1966), and Favaloro (1967). In 1964, surgeon Edward Garrett, working with Dr. Michael DeBakey in Houston, encountered a patient with a relative large, but severely stenotic LAD coronary artery (Cooke, 2015). He performed a vein bypass graft from the ascending aorta to the obstructed artery and it remained open and relieved the patient of his angina (**Fig. 9**).

Subsequent follow-up showed that the graft remained open for at least ten years. At the time, Garrett believed that this was an unusual and lucky case and that it was unlikely that it could be repeated reliably and did not justify the risk, which was then considerable, of subjecting these clinically fragile patients to the hazards of cardiopulmonary bypass. He did not publish a report of this case. It was not appreciated at the time that the patency of these grafts was more dependent on blood flow through the graft as well as the size of the recipient vessel. A few years later, Rene Favaloro, at the Cleveland Clinic, performed the same procedure, in a similar situation to a large right coronary artery, which was also a success. He subsequently reported on his series and ignited an industry (Favaloro et al., 1969).

In the late 1960s, Dr. Favaloro was a cardiothoracic surgery fellow at the Cleveland Clinic. Before he published the series of cases where he was able to revascularize the heart using saphenous veins from patients' legs, many preceding events had to occur and come together for this relatively straightforward solution to evolve. These events included technologic advances in cardiac surgery, peripheral vascular surgery, and diagnostic cardiology. By the 1960s, the heart–lung machine or cardiopulmonary bypass (CPB) was in common use, having been invented in the early 1950s. At the beginning of the CABG era, CPB had improved to the point where it was being used regularly in cardiac surgery centers for congenital heart surgery and valve surgery. In addition, the Sones technique of coronary angiography allowed practitioners to see precisely where occlusions and stenoses were in the coronary arteries. At that point in history, cardiovascular surgeons had much experience in peripheral arterial surgery, in particular, restoring circulation to extremities by using the patient's own veins (autologous) to bypass occluded peripheral vessels, particularly in the legs. With the

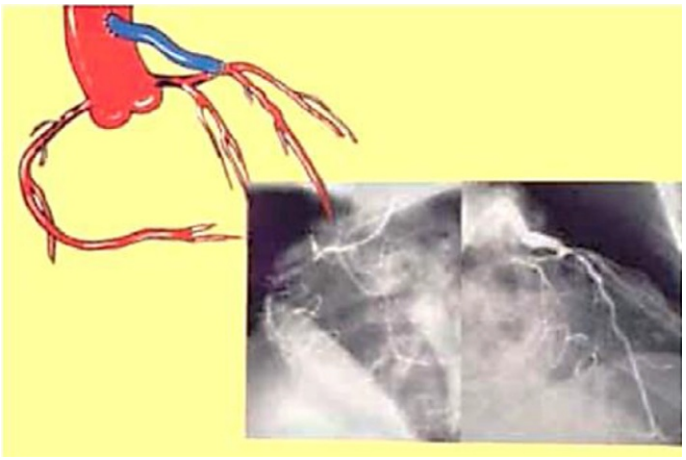

Fig. 9 Images of first coronary artery bypass performed by Doctors Ed Garrett and Jimmy Howell. Image and description from DeBakey, M. E. (2015). Houston hearts: A history of cardiovascular surgery and medicine at Houston Methodist Debakey Heart & Vascular Center. *The Methodist DeBakey Cardiovascular Journal* **11** (3 Suppl.), 5–17.

experience of operating on the heart using the heart–lung machine, the knowledge that vessels could be bypassed with autologous vein, and having a roadmap supplied by the angiographer, CABG became feasible (Fig. 10).

Cardiologists and vascular surgeons were poised and ready for this long-awaited step in the discovery process, and coronary surgery virtually exploded across the western world. Initially, bypass grafts were done to only the largest coronaries with localized proximal disease in relatively young patients. For each distal anastomosis of the vein to the coronary artery, the ascending aorta was cross-clamped and the heart allowed to stop from ischemic arrest. The vein was then carefully sewn to an opening in the side of the coronary artery using magnifying loupes, after which the clamp was removed from the aorta to allow the heart to recover. After a few minutes of recovery time, the process was repeated for the next distal anastomosis to a second coronary, using another segment of vein. When the last distal attachment had been completed, a partially occluding clamp was placed on the ascending aorta (Fig. 11), and the opposite ends of the vein grafts (proximal) were sewn to small openings made in the wall of the aorta. Over time, many

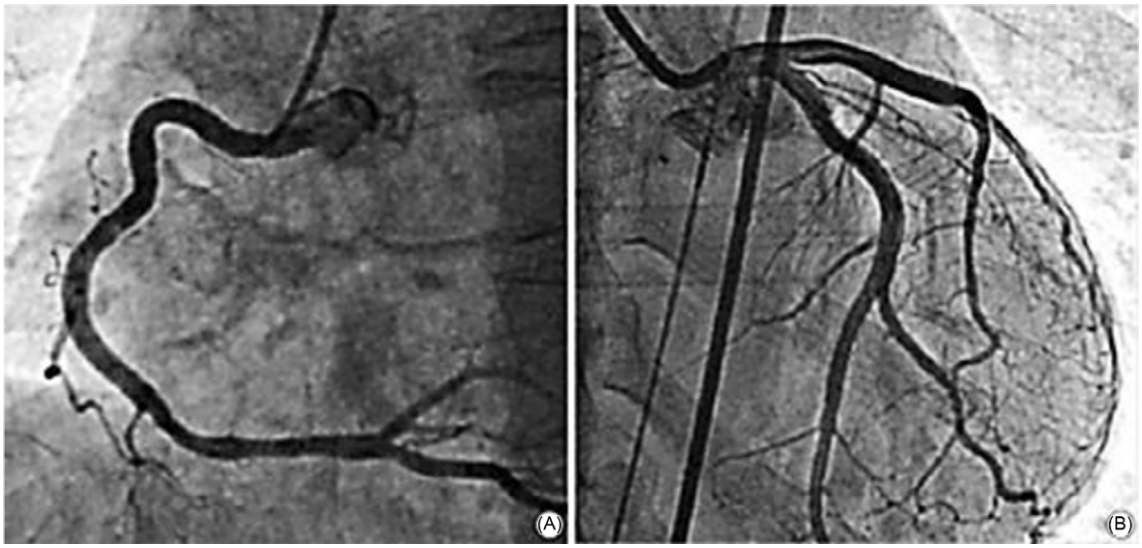

Fig. 10 The roadmap: Frames from cineangiography of normal coronary arteries. (A) Radiocontrast dye has been injected from the catheter positioned in the root of the aorta at the origin of the right coronary showing a view of a normal vessel and its branches. (B) The dye has been injected into the left main coronary artery revealing its branches, the left anterior descending, and circumflex coronary arteries. From: © Copyright Policy—open-access Open-i service of the National Library of Medicine enables search and retrieval of abstracts and images (including charts, graphs, clinical images, etc.) from the open source literature, and biomedical image collections. Searching may be done using text queries as well as query images. Open-i provides access to over 3.7 million images from about 1.2 million PubMed Central® articles; 7,470 chest x-rays with 3,955 radiology reports; 67,517 images from NLM History of Medicine collection; and 2,064 orthopedic illustrations.

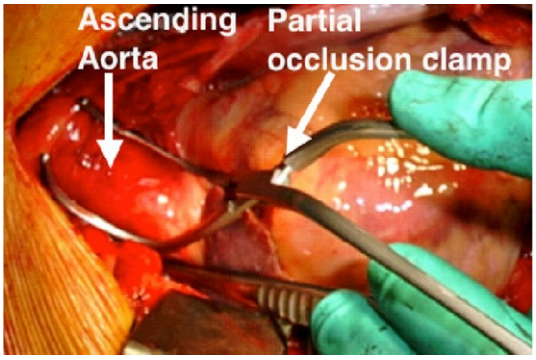

Fig. 11 This illustration depicts the application of a partially occluding clamp on the ascending aorta allowing flow through the native vessel while giving the surgeon the opportunity to occlude part of it to sew a proximal anastomosis to the depressurized segment of the aorta. From Royse, A. and Royse, C. (2009). Epiaortic ultrasound assessment of the thoracic aorta in cardiac surgery. *Best Practice & Research Clinical Anaesthesiology* **2**3, 335–341.

improvements were made in all phases of the procedure, including local and systemic cooling, myocardial preservation, more precise suturing techniques, and improvements in cardiopulmonary bypass equipment and technology.

The concept of temporarily inducing cardiac arrest with cold potassium rich solution injected antegrade into the coronary arteries was introduced in the late 1970s. This solution, coined "cardioplegia" (literally meaning to paralyze the heart muscle) was infused into the coronaries to arrest the heart, literally causing the cells of the heart to hibernate, preserving its function. The high concentration of potassium in the solution literally paralyzes the cells by influencing its electrical properties (by eliminating the potential difference across the cell membrane), and the low temperature induced decreases the metabolic demands of the cells, the combination of which is termed "myocardial preservation" (Yamamoto and Yamamoto, 2013). The surgeon could now work on these tiny vessels in a still, bloodless field. With these improvements, it was possible to do all the distal anastomoses during a single cross clamping of the aorta and significantly shorten the length of the procedure and the time on the heart lung machine. This was particularly important since over time, as surgical techniques were improving, the patients were getting older with more advanced disease, and the number and difficulty of the grafted arteries was increasing.

In 1968, shortly after the onset of the beginnings of coronary bypass surgery, Kolessov, in Russia performed the first bypass from the left internal mammary artery (LIMA) to the left anterior descending coronary artery (Kolessov, 1967). Initially this did not attract much attention, because the vein grafts were quicker and easier and seemed to work as well, at least in the short term. Green, at NYU, was the first in the United States to perform and publish about the use of the LIMA. Over the years, evidence began mounting that the vein grafts were slowly developing atherosclerotic obstructions of their own, and the LIMA grafts, which Green and about 5%–10% of the other surgeons preferred, were remaining free of disease and lasting longer. In 1985, a paper from Montreal reported on a large series of coronary bypass patients who had follow up angiograms 10 years after their initial surgery (Bourassa et al., 1985; Lytle et al., 1983). They found that about 30% of the vein grafts had occluded and that 95% of the LIMA grafts were open and free of disease. These observations led to a dramatic increase in the use of LIMA grafts, particularly to the LAD coronary artery with a noticeable improvement in short- and long-term results, regardless of the age of the patient or associated disease.

Concurrently, a number of surgeons extended this technique to include the right internal mammary (RIMA) and occasionally the gastroepiploic artery anastomosed to the inferior or posterior coronary branches. The gastroepiploic artery courses along the surface of the greater curvature of the stomach. By extending the median sternotomy incision inferiorly and opening the upper abdomen, the stomach can be exposed, the gastroepiploic artery mobilized, and can be extended above the diaphragm to the heart, long enough to reach vessels on the inferior surface of the heart. Radial artery grafts were also employed, particularly when satisfactory veins are not available. To date, radial arterial grafts and free IMA grafts have not consistently demonstrated superiority over the combination of LIMA and saphenous grafts, particularly in combination with cholesterol lowering drugs.

Indications and Selection for CABG Surgery, Shared Decision-Making, and the Heart Team

Modern management of ischemic coronary artery disease and the role of CABG surgery have been in a continual state of flux over the past 50 years. Initial enthusiasm for the broad application of surgical revascularization was tempered by the results of three important prospective randomized trials that were carried out from 1972 to 1979 (VA Coronary Artery Bypass Surgery Cooperative Study Group, 1992; Varnauskas, 1988; Killip et al., 1985). In these studies, patient disease patterns and outcomes were compared between medical and surgical therapy allowing for the stratification of patients with coronary artery disease into low-, medium-, and high-risk groups. The impact of surgical revascularization on these groups proved a clear benefit for CABG in patients with severe left main coronary artery and three-vessel coronary artery disease, while the benefits of CABG surgery were shown in patients with double- and single-vessel disease only if the proximal LAD was involved. In patients with double- and single-vessel coronary artery disease not involving the LAD, medical management was found to be equal to or superior to CABG. As surgical management became standard for specific disease patterns, new medications as well as interventional technologies (percutaneous balloon

angioplasty, bare metal, and drug-eluting stents) were introduced and dramatically impacted decision-making for patients with ischemic heart disease. The RITA, BARI, and SYNTAX Trials provided the evidence for this decision-making (Henderson et al., 1998; Investigators, 2007; Serruys et al., 2009).

The development of the Society of Thoracic Surgeons (STS) database in 1989 (Shroyer, 2015) has further aided surgeons in determining patient risk for CABG and allowed for refinement of individual patient treatment plans based on both the degree of coronary disease and the severity of a number of comorbidities. With the sophistication of current medical, interventional, and surgical options that are available today, a multidisciplinary heart team approach that allows for regular communication between the primary cardiologist, interventionalist (cardiologist that is trained to do interventions such as angioplasties and stents), and cardiothoracic surgeon provides the best opportunity for optimal patient management.

The technological advancements in CABG surgery over the past 50 years have also provided today's surgeon with a number of options in surgical planning. These range from the performance of a standard "on-pump" procedure with a combination of differing conduits to "off-pump" techniques and or hybrid approaches that combine surgical and interventional technologies. The cardiopulmonary bypass machine is often referred to as the "pump." The proper selection from these many operative choices is predicated upon a high-quality, systematic, preoperative assessment of the patient. Such a process is greatly aided by the deployment of a team of supporting nurses, physician assistants, residents, and/or fellows under the guidance of the attending surgeon. The modern reality of practicing medicine in the age of electronic medical records allows providers rapid access to not only the historical details of the patient as documented by their referring physicians during any number of prior encounters but also to their laboratory and imaging studies. The obvious goal of such a record has been to give the provider an efficient source of information that should theoretically decrease the time required to thoroughly evaluate and formulate a proper plan for his or her patient. Though this often works out quite nicely, electronic medical records are only as good as the data that has been uploaded into them and thus are not impervious to collecting inaccurate or incorrect patient information. With that in mind, the most efficient use of the consultant's time is to first thoroughly review the imaging and laboratory studies in the contest of the patient's historical and physical findings.

After reviewing the recent history and physical and progress notes, it is imperative that the consulting surgeon confirm with the patient the details of their history and conduct a thorough review of systems and physical examination. It is incumbent upon the operating surgeon to perform his or her own cardiovascular physical examination with close attention to the presence or absence of cardiac murmurs and vascular bruits, clinical signs of heart failure, discrepancies in brachial (arm) blood pressures, character of peripheral pulses, and the quality of potential primary and secondary conduit choices (greater or lesser saphenous veins and radial arteries). With this approach, the surgeon can confidently determine the patient's angina and heart failure scores, confirm the presence of serious comorbidities, potentially identify missed diagnoses, calculate the risk for CABG surgery (STS or EuroScore) (Shahian et al., 2009; Roques et al., 1999; Roques et al., 2003), and start formulating the surgical plan tailored to the patient's health status, including the patient and family in a shared decision-making process (Lin and Fagerlin, 2014).

Failure to prepare in this manner can result in suboptimal operative planning, avoidable complications, avoidable deaths, and the performance of operations on patients who would have been better off with medical or interventional management. Once the decision for surgery has been made, the details of the operative plan are formulated including selection of arterial targets, grafting strategy, determination of the cardiac support plan, and selection of conduit. In general, the selection of target arteries is based on findings of the coronary angiogram (a cine radiograph of coronary arteries injected with radio-opaque dye through a catheter) in combination with information from any viability scans (imaging using markers that indicate the presence of living myocardial cells in a nonfunctioning area of the heart) that may have been performed. The cardiac support plan is generally based upon the surgeon's preference, status of the ascending aorta, and the presence or absence of major comorbidities.

The most commonly used plan for cardiac support during CABG continues to be cardiopulmonary bypass with cardioplegic arrest. The next most common technique is Off-Pump CABG (OPCAB) in which coronary anastomoses are performed on the beating heart with the aid of special stabilizing devices and occasional intracoronary shunts. In the mid-1990s, as a response to advances in percutaneous revascularization, surgeons developed several different strategies to make direct coronary bypass surgery less invasive. The first was coined Minimally Invasive Direct Coronary Artery Bypass or MIDCAB. In this technique, the LIMA was harvested and anastomosed to the LAD via a left anterior thoracotomy incision through the fourth intercostal space with or without cardiopulmonary bypass support. Though this technique had many successes, revascularization was limited to the LAD, pain from the thoracotomy incision was significant, hemodynamic instability with need for conversion to full sternotomy was not uncommon, and inadvertent grafting of the LAD at the wrong site occurred (Vicol, 2003).

Around the same time, an Off-Pump approach via a full sternotomy with mechanical stabilization techniques (OPCAB) was developed that allowed for complete revascularization with a lower incidence of grafting errors. Both techniques had the goal of not exposing patients to the deleterious effects of CPB. Over time, some centers practicing both techniques reported OPCAB to be the superior method. Several prospective randomized studies have proven the safety of OPCAB, and there are a number of surgeons who prefer OPCAB as their primary method of CABG. Other surgeons feel that their ability to perform a sutured anastomosis is far superior while On-Pump with cardioplegic arrest and selectively utilize Off-Pump CABG in cases of a "porcelain aorta" (an ascending aorta that has so much dense, calcified atheromatous plaque that it feels as firm as porcelain, risky to manipulate for fear of breaking of particles and causing embolic strokes) or severe pulmonary disease. Conflicting inferiority/superiority outcomes from several studies allow for the support of both positions (Hattler et al., 2012; Lamy et al., 2012; Puskas, 2009; Diegeler, 2013; Bakaeen and Puskas, 2014; Taggart et al., 2016).

Over the nearly 50 years of the "CABG Era," evidence has been gathered through these databases, observational analyses, and trials that has, in the aggregate, provided the surgeon with guidance as to what the indications are for surgery and who should be

selected as each patient provides a different context depending on age, comorbidities, and other factors. Armed with the evidence provided by these studies, the first management decision would be to decide whether to offer the patient medical therapy, CABG or "PCI" a percutaneous intervention (angioplasty and stenting). The major drivers of that decision include the patient's coronary anatomy, the presence of diabetes mellitus, the clinical syndrome (stable angina, acute coronary syndrome, acute myocardial infarction (STEMI or NSTEMI)), and left ventricular dysfunction and mitral valve disease. The terms "STEMI" or ST-segment elevation myocardial infarction or "NonSTEMI" define electrocardiographic features of myocardial infarctions that have been found to distinguish the extent of injury and treatment decisions in the context of the coronary anatomy and the other features outlined in **Fig. 12**. These evidence-based factors and decision points are well summarized by Alexander and Smith from their 2016 review article on CABG surgery (Alexander and Smith, 2016).

Indications for CABG that are associated with a survival benefit over medical therapy with or without PCI

Acute STEMI

- Coronary anatomy not amenable to PCI
- Mechanical complications (e.g., ventricular septal defect, rupture of the free wall of the ventricle, or papillary-muscle rupture with severe mitral regurgitation)

Coronary artery disease other than acute STEMI

- Left main coronary artery disease (≥50% stenosis) and high complexity for PCI (SYNTAX score ≥33)
- Three-vessel coronary artery disease (≥70% stenosis) and intermediate or high complexity for PCI (SYNTAX score ≥23)
- Two-vessel coronary artery disease (≥70% stenosis) involving the LAD artery and intermediate or high complexity for PCI (SYNTAX score ≥23)

Factors increasing the survival benefit of CABG

Left ventricular dysfunction (ejection fraction ≤45%)

Diabetes mellitus

Ischemic mitral regurgitation

PCI failure with or without acute myocardial infarction

Indications for CABG when PCI is noninferior to CABG and when PCI or CABG is preferred over medical therapy

Left main coronary artery disease (≥50% stenosis) and low-to-intermediate complexity for PCI (SYNTAX score ≤32)

Three-vessel coronary artery disease (≥70% stenosis) and low complexity for PCI (SYNTAX score ≤22)

Two-vessel coronary artery disease (≥70% stenosis) involving the LAD artery and low complexity for PCI (SYNTAX score ≤22)

Factors increasing the benefit of PCI over CABG

Elevated risk of death with CABG

Elevated risk of stroke

Extreme frailty

Prior CABG

Acute STEMI at presentation

Other indications for CABG

Clinically significant coronary artery disease (≥70% stenosis) in ≥1 vessel and refractory angina despite medical therapy and PCI

Clinically significant coronary artery disease (≥70% stenosis) in ≥1 vessel in survivors of sudden cardiac arrest presumed to be related to ischemic ventricular arrhythmia

Clinically significant coronary artery disease (≥50% stenosis) in ≥1 vessel in patients undergoing cardiac surgery for other indications (e.g., valve replacement or aortic surgery)

Fig. 12 A summary of the indications for CABG surgery from Alexander JH, Smith PK. Coronary-Artery Bypass Grafting N Engl J Med 2016;374:1954-64. The SYNTAX scoring system, developed as part of the Synergy between PCI with Taxus and Cardiac Surgery study, classifies the extent and complexity of coronary artery disease, with a score of 22 or lower indicating low complexity, a score of 23 to 32 indicating intermediate complexity, and a score of 33 or higher indicating high complexity. CABG denotes coronary–artery bypass grafting, LAD left anterior descending, PCI percutaneous coronary intervention, and STEMI ST-segment elevation myocardial infarction (Ong et al., 2006; Sianos et al., 2005). From: Yusuf, S. et al. (2016). Cholesterol lowering in intermediate-risk persons without cardiovascular disease. *New England Journal of Medicine* **374**, 2021–2031.

Coronary Angioplasty and Stenting

In 1977, Andreas Gruentzig, a German cardiologist living in Zurich, first employed a balloon on the end of a cardiac catheter (Matthias et al., 2014; Gruentzig et al., 1979) (**Fig. 13**) to dilate a stenotic lesion in a patient's LAD coronary artery.

Later that year, Dr. Gruentzig presented the results this technique of four such successful balloon angioplasties at the American Heart Association Meeting, which led to widespread acknowledgement of this procedure, and, with successive refinements in technique and devices, by 1990, percutaneous angioplasty became a commonly used method of treating coronary disease by revascularization. Unfortunately, a significant number of these dilated arteries developed recurrent stenosis due to reaccumulation of plaque a few months to a year after balloon angioplasty, requiring another angioplasty or a bypass graft. During the next decade, an expandable wire stent was developed to prevent restenosis of the vessel. Later these stents were coated with antiproliferative drugs to slow or prevent plaque growth (**Fig. 14**).

Several large randomized clinical trials, most notably the SYNTAX Trial (Gulati et al., 2009; Serruys et al., 2009), have helped to define the relative effectiveness of the various revascularization techniques currently available in different situations and, in

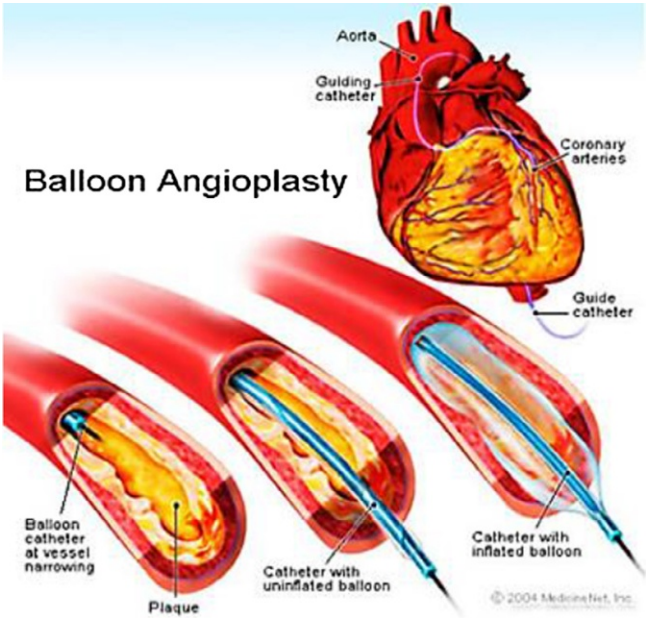

Fig. 13 Illustration depicting a balloon angioplasty where a tiny catheter with an integrated balloon is guided into a coronary artery, the balloon positioned into the area of stenosis, inflated, thereby improving blood flow through that segment. This original procedure has subsequently been enhanced by the addition of drug-eluting stents that discourage restenosis of the vessel. From: MedicineNet.com.

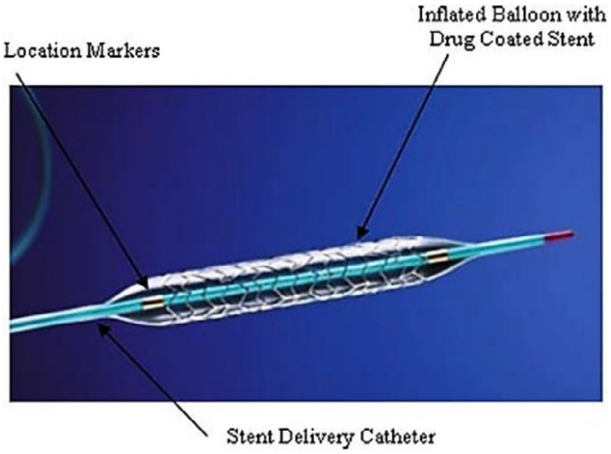

Fig. 14 This is an example of a drug-eluting stent. It is the TAXUS Express Paclitaxel-Eluting Coronary Stent System, which releases paclitaxel, the stent that was used in the SYNTAX Trial. From: Food and Drug Administration—http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm081189.htm, Public Domain, https://commons.wikimedia.org/w/index.php?curid=973844.

particular, which patients are better served by surgery versus balloon angioplasty. Long-term results have shown that diabetic patients and those with multiple coronary blockages appear to do better with coronary surgery, where grafts can be constructed to three or more coronary branches at once, often creating multiple distal anastomoses with a single vein graft. Revascularization is more frequent with stents as opposed to CABG, while strokes are more common with CABG. At this writing, 5-year results from SYNTAX have been published and data on 10-year mortality is currently being analyzed.

As technology has evolved in both surgery and interventional cardiology, the cardiac surgeons who perform the CABG surgery and the cardiologists who perform coronary angioplasties and place stents both have technologies that have been found to suit different subsets of patients requiring coronary revascularization. This commonality has brought the two specialties closer together, forming "Heart Teams" to address coronary revascularization and other complex cardiac issues such as advanced valve therapy and heart failure.

Cardiopulmonary Bypass

The concept of cardiopulmonary bypass (CPB), commonly known as the heart–lung machine, became a reality in the early 1950s when it was used in a succession of patients to correct congenital cardiac problems such as atrial septal defect, the failure of the completion of the formation of a partition between the left atrium and the right atrium. CPB is a form of extracorporeal circulation, meaning circulation outside of the body. Substituting a machine for the heart and lungs to pump the blood and provide gas exchange allowed surgeons to stop the heart and stop ventilation so as to have an opportunity to open the heart and work on its interior, correcting and reconstructing congenital defects. As CPB began to evolve and improve in the first decade of its use, pioneering medical centers and surgical teams began to use it for acquired cardiac problems, such as valve surgery, in addition to correcting congenital defects. By the 1960s, valve replacement surgery became a reality, and at the beginning of the CABG era in the early 1970s, CPB was used as well.

With the ability of the surgeon to stop the heart and work on its interior during this era, the potential to apply lessons learned in peripheral vascular surgery to the coronary arteries became real. The coronary arteries course over the surface of the heart (epicardial) and are visible and palpable. The use of the principles of peripheral aterial bypass to the coronary arteries was applied in the early days of CABG, and with CPB and cardioplegia, the surgeon was now able to work in a still, bloodless field, using familiar techniques in this new setting.

The heart and lungs provide gas exchange and perfusion of more than 37 trillion living cells. The idea of designing a machine that could take over the function of the heart and lungs was conceptualized in 1813 by Le Gallois; however, the first attempts to actually apply the concept occurred in the early 1950s (Groom and Fitzgerald, 2017). The first successful use of cardiopulmonary bypass was in 1953 by a surgeon, John H Gibbon Jr., and his team at Jefferson Hospital in Philadelphia, Pennsylvania (Stammers, 1997). Gibbon used his heart–lung machine to "perfuse the patient" allowing her heart to be stopped while he and his team opened her right atrium and sutured closed her atrial septal defect. The early years following that landmark case were not so promising. Five subsequent procedures at Jefferson Hospital where cardiopulmonary bypass was used by Gibbon were not successful as all five patients did not survive, and he abandoned the use of the heart–lung machine. At least seventeen other cases were performed in the early 1950s using some form of extracorporeal circulation, and only Dr. Gibbon's patient survived. Nonetheless, his one successful case served to inspire others, including John Kirklin at The Mayo Clinic, C. Walton Lillihei at the University of Minnesota, and Denis Melrose at Hammersmith Hospital in London, to press with their research and application the heart–lung machine with CPB techniques that were crude compared to today's standards. (**Fig. 15**).

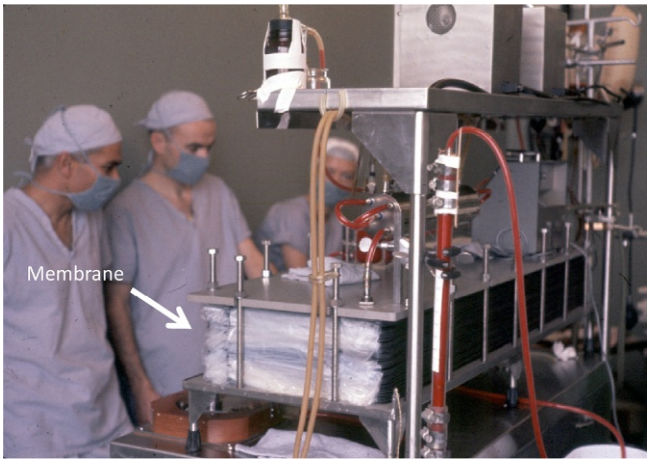

Fig. 15 The Mark-Clowes heart–lung machine with a membrane oxygenator in use at Maine Medical Center in 1959. Sixteen units of donor blood were needed to prime this heart-lung circuit. With permission from Archives of Maine Medical Center.

The accomplishments of these early pioneers in cardiac surgery have been described as being "one of the boldest and most successful feats of man's mind." (Eloesser, 1970).

Since the 1950s, CPB has steadily improved in terms of survival and reduction in complications such as hemorrhage, stroke, and kidney injury. CPB techniques are used for temporary or longer-term support, known as extracorporeal membrane oxygenation (ECMO).

CPB has been designed as an integrated system that provides nutritive solutions with an appropriate hemodynamic driving force to maintain whole-body homeostasis, protecting the patient from ischemic injury. Over the last three decades options for the use of CPB for coronary artery revascularization have been developed such as percutaneous interventions (angioplasty and stenting), and by surgical procedures not utilizing CPB, collectively called off-pump CABG surgery (OPCAB) and procedures using hybrid miniaturized circuits to support the circulation during grafting.

The benefit of CPB was validated in a randomized clinical trial, the Randomized Off-pump or On BYpass (ROOBY) trial, involving 2203 elective or urgent coronary artery bypass grafting (CABG) patients randomized to either off- or on-pump surgery is a testament to the efficacy and safety of CPB as currently practiced. At one year, the on-pump group had significantly better composite outcomes (death, myocardial infarction, or repeat revascularization) than the off-pump group (9.9% vs. 7.4%; $P=0.04$). The overall rate of graft patency was lower in the off-pump group than in the on-pump group as well (82.6% vs. 87.8%; $P<0.01$).

The ROOBY Trial and other trials demonstrated that off-pump CABG yielded no advantage in clinical outcomes and cost over on-pump CABG (Shroyer et al., 2009). A metaanalysis which included 12 randomized clinical trials reported a 38% increased rate of early revascularization with off-pump surgery (Takagi et al., 2013). Moller and colleagues published a systematic review including over 15,000 subjects that reported no clinical benefit in mortality, stroke, or myocardial infarction or increased survival benefit of OPCAB (Møller et al., 2012).

A cardiovascular perfusionist is a specialized healthcare professional who operates the heart–lung machine during cardiac surgery and other surgeries that require cardiopulmonary bypass to manage the patient's circulatory physiology (**Fig. 16**). After a patient's circulation is accessed thru large cannulae (tubing providing connection to the CPB circuit) in the right atrium in the ascending aorta, the perfusionist, under the direction of the heart surgeon, has the ultimate aim of providing satisfactory gas exchange and metabolic needs at the cellular level (Galletti and Brecher, 1962). CPB is being used to provide perfusion for about 1.4 million patients each year around the globe. It has become evident that efforts should be directed toward measuring the adequacy of perfusion at the cellular level and at the end organ level is where we should be focusing our attention as we try to improve patient support with CPB (Ranucci et al., 2005; Demers et al., 2000; de Somer et al., 2011).

The principal component of the Heart–lung machine includes pumps, reservoirs, tubing, and filters. The CPB circuit consists of a number of components for gas exchange, temperature regulation, filtration, monitoring, and safety mechanisms. **Fig. 17** is a diagram of the various components of the CPB circuit (Kim et al., 2013).

Blood drains by gravity or with the use of gentle suction into the oxygenators venous reservoir labeled (B). (A) represents the arterial pump that pumps the blood from the venous reservoir (B) and delivers blood to the membrane oxygenator which is attached to the lower part of the venous reservoir. Once oxygen, carbon dioxide, and heat exchange have occurred the blood is directed thru an arterial blood filter (C). A purge line to the upper most part of the filter and serves for the removal any microemboli that may have been introduced into the blood during its passage through the circuit. The oxygenated blood is introduced back into the patient's circulatory system through a cannulae (a large tube connected to the circuit) placed in the ascending aorta. The line attached to intravenous bags labeled (D) provides a method for priming the CPB circuit with electrolyte fluid or a port for adding blood during bypass. Four roller pumps labeled (E) in the diagram are auxiliary pumps. The one on the far left is used to pump cardioplegia solution with a mixture of blood and additives, labeled (H), used to arrest the heart. This solution is cooled with a separate heat-exchanger labeled (F).



Fig. 16 S-5 Heart-lung machine (LivaNova, Houston, TX). The cardiovascular perfusionist operating the pump is seated at the lower left. With permission from RC Groom, CCP, Director of Cardiopulmonary Perfusion, Maine Medical Center, Portland Maine, USA.

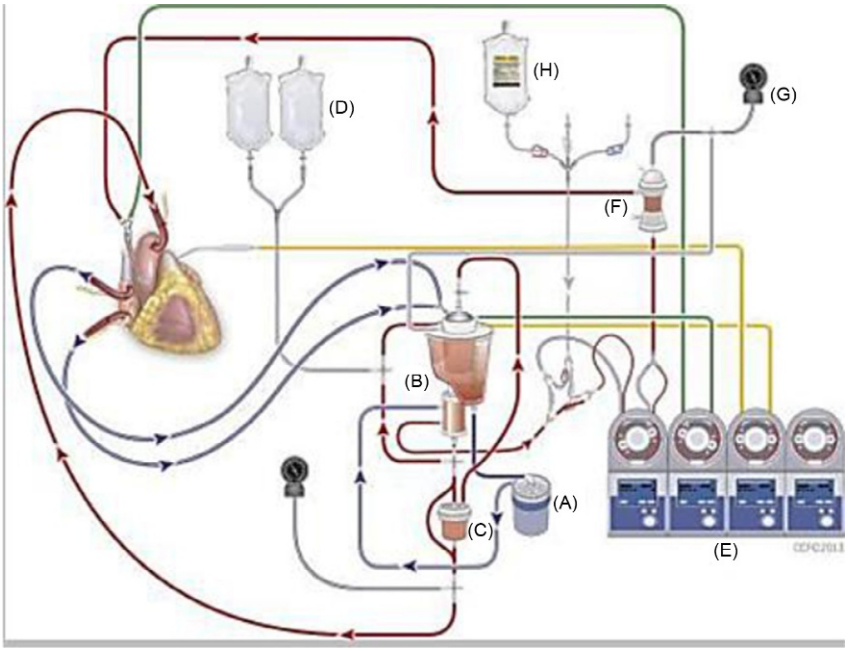

Fig. 17 Diagram of a cardiopulmonary bypass circuit. (A) Centrifugal pump, (B) reservoir and oxygenator, (C) solution, (E) heart–lung machine, (F) cardioplegia heat exchanger, (G) manometer for the cardioplegia system, (H) del Nido cardioplegia solution. Reprinted with Permission *J Extra Corp Technol* 2014 46(4): 317–323.

Pumps

Two principle types of pumps are used to propel blood thru the extracorporeal circuit and into the patient's circulation, roller pumps, and centrifugal pumps. These pumps, unlike the heart, provide continuous flow rather than pulsatile flow. Numerous studies over the past three decades have sought to determine if pulsatile flow improved organ perfusion and patient outcomes; however, the evidence is inconclusive (Alghamdi and Latter, 2006; Likosky, 2009). Metaanalysis by Nam (Nam et al., 2015) and Seivert (Sievert and Sistino, 2012) both showed improvements in renal function with pulsatile flow. Sievert further reported that the studies with Intra Aortic Balloon Pump (IABP) generated pulsatile flow had the best results and also noted significant reduction in lactate levels in the pulsatile groups. Zangrillo carried out a metaanalysis of preoperative use of IABP in 625 high-risk CABG patients and reported a significant reduction in 30-day mortality (risk ratio 0.38 p for effect 0.004) (Zangrillo et al., 2015). More recently Lim and colleagues undertook a metaanalysis to look at pulmonary function with pulsatile perfusion and found a significant improvement in pulmonary function and ICU length of stay (Lim et al., 2015). Clearly pulsatile flow is an area ripe for future study. Although the two types of pumps available for arterial blood pumping, the centrifugal pump and roller pump, are capable of producing pulsatile flow, at this juncture pulsatile flow is not commonly utilized.

The dual roller pump is the most common type of pump used for cardiopulmonary bypass. It operates by occluding tubing between a stationary raceway and rotating rollers (**Fig. 18**). The pumping mechanism is referred to as the "pump head," and the tubing that traverses the raceway is referred to as the "pump header." Roller pumps were first proposed for use in cardiovascular medicine in the 1930s by Gibbon (Gibbon, 1937). The mechanism for flow with the roller pump is the displacement of fluid, in a progressive fashion, from suction to discharge, with the capacity of the displacement dependent both on the volume of the tubing occluded by the rollers and on the number of revolutions per minute (rpm) of the roller. The volume displaced per revolution is multiple times the revolutions/minute to calculate the estimated blood flow. This is displayed on a digital readout and is referred to as the output (flow) of the pump. It is measured in liters per minute (**Fig. 19**).

Another common pump used for arterial blood flow is the centrifugal pump (**Figs. 20** and **21**). Although nearly all of the randomized trials show significant benefit to systems designed with centrifugal pumps, it is difficult to separate the improved performance conferred from other characteristics, such as lower prime volume, surface coating, more limited surface area, and reduced air-to-blood contact. A metaanalysis that included 18 randomized, controlled trials comparing centrifugal and roller pumps in adult cardiac surgery suggests no significant difference for hematological variables, postoperative blood loss, transfusions, neurological outcomes, or mortality (Saczkowski et al., 2012).

Centrifugal pumps produce less blood damage; however, this improvement appears to be inconsequential. Nevertheless, recently published Guidelines on Perioperative Blood Transfusion and Blood Conservation in Cardiac Surgery, endorsed by the Society of Thoracic Surgeons and the Society of Cardiovascular Anesthesiologists state that, "It is not unreasonable to select a

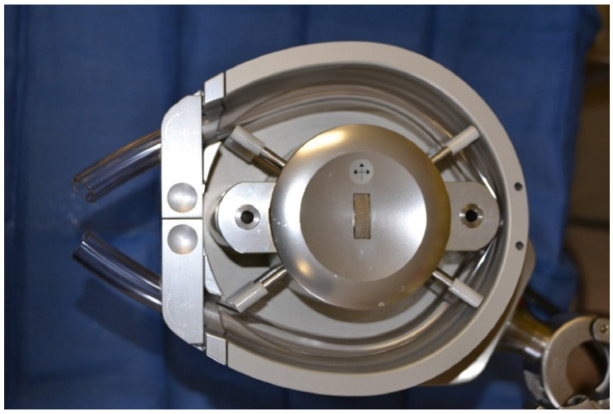

Fig. 18 Twin (dual) roller pump. Occlusiveness of the pump is adjusted by the thumb adjustment knob at the center of the roller assembly. Roller guides hold the tubing in place in the pump race way. With permission from RC Groom, MS, CCP.



Fig. 19 Roller Pump control module displays flow (3.04 l/min) and Revolutions/Minute (117 RPM). With permission from RC Groom, MS, CCP.

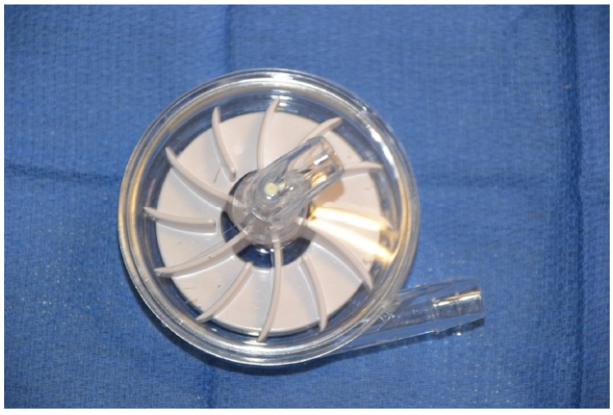

Fig. 20 Disposable component for a centrifugal pump (Revolution by LivaNova, Houston, TX). A magnetic internal rotor is coupled to a motor that rotates the rotor creating centrifugal force that propels the blood though the outlet of the disposable centrifugal head. With permission from RC Groom, MS, CCP.



Fig. 21 Centrifugal disposable head magnetically coupled to a motor to produce fluid flow. With permission from RC Groom, MS, CCP.

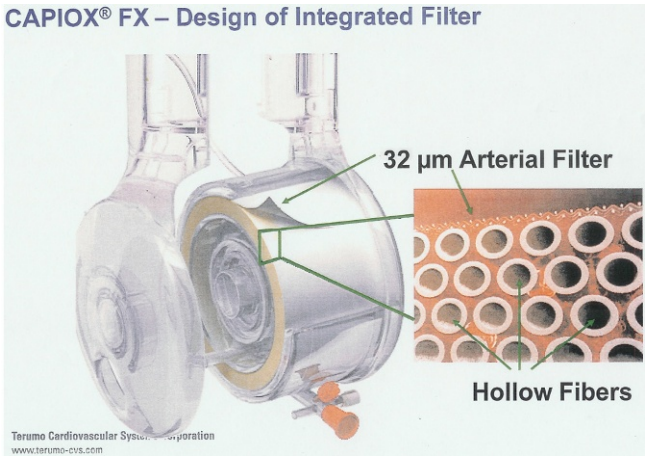

Fig. 22 Terumo FX Oxygenator Hollow fiber oxygenator. Exploded view shows hollow fibers where gas flows through the fiber and blood flows around the fiber. Gas transfer occurs across the semipermeable hollow fiber membrane surface. The FX model has an integrated 32 µm filtration surface encapsulated into the oxygenator. Reprinted with Permission Terumo Cardiovascular Inc. Ann Arbor, MI.

centrifugal pump rather than a roller but more so for safety reasons rather than blood conservation" (American Heart Association/ American College of Cardiology Class IIb level of evidence B) (Society of Thoracic Surgeons Blood Conservation Guideline Task Force, 2011).

Oxygenators

Membrane oxygenators operate by diffusion of oxygen and carbon dioxide across a microporous hollow fiber bundle. The gas exchange occurs by diffusion of the gases across microporous fiber surface, oxygen moving from the interior of the hollow fiber into the blood and carbon dioxide diffusing from the blood cross into the interior of the fiber and swept away by the regulated gas flow through the hollow fiber. Blood oxygenators have integrated heat exchangers that allow the circulating blood to be cooled or warmed. The heat exchangers are comprised of stainless steel or polypropylene tubes that are not permeable. Water from a regulated water supply flows through the fibers and heat is transferred from the blood flowing around the tubes into the circulating water. While there have been major improvements in the materials and surfaces that the blood is exposed to in the extracorporeal circuit, there remains hematological changes including the liberation of inflammatory mediators (Hammerschmidt et al., 1981; Chenoweth et al., 1981). Platelets and leukocytes that elicit a complex series of inflammatory and hemostatic reactions that ultimately increase the risk for postoperative complications are activated.

The oxygenator (Fig. 22) represents the largest source of nonendothelialized surface area in the extracorporeal circuit, ranging in size between 0.5 and 2.5 square meters. Other issues identified include the occurrence of gaseous microemboli during cardiac

surgery with CPB (Dickinson et al., 2006; Likosky et al., 2009) that have been managed with in-line filters. These arterial line filters significantly reduce the load of gaseous and particulate emboli. A dose–response relation between these emboli and subtle neurologic injury has been reported, and some studies have demonstrated a protective effect of arterial line filtration on neurologic outcomes. The use of a leukocyte-depleting arterial line filter has been shown to reduce cerebral embolic count and demonstrated a trend toward improved postoperative psychometric test scores and should be used in CPB circuits. Transfer of volatile (inhalational) anesthetic agents does not readily cross this membrane. The inhaled anesthetic during the course of CPB is delivered via the membrane oxygenator, rather than the patient's lungs that are not ventilated during the procedure. Noninhalational agents such as narcotics adequately supplement the inhaled anesthetic during the conduct of the operation.

While many advances in CPB have been realized since the first successful procedure in 1953, there remains a vast areas of research opportunities related to cardiopulmonary bypass including: (1) issues related to material–blood interaction specifically inflammation and coagulation-related issues; (2) patient and organ system responses to cardiopulmonary bypass; (3) the use of hypothermia, brain and myocardial protection; and (4) improving safety.

CABG Technique

As previously mentioned, the fundamental technique for performing CABG surgery was developed by peripheral vascular surgeons who had developed procedures to replace or bypass vessels peripheral to the heart and great vessels. In particular, the ability to bypass lower extremity vessels was developed using the greater saphenous vein. The venous component of the circulatory system is a low-pressure system, while the arterial part of the circuit is pressurized by the contractions of the left side of the cardiac pump. The venous circuit is necessary for the return of blood to the heart without a pump to pressurize it. Venous return to the heart relies on other mechanisms for propulsion such as gravity, in the case of the head, and external muscle pumping, redundancy and valves, in the case of the lower extremities. As seen in the illustration showing the deep and superficial venous circulation, the huge capacity of the venous system allows for the return of as much blood each minute as is being pumped out, roughly five liters per minute. This "forward flow," under normal conditions, allows for adequate oxygen delivery and nutrients to end organs and other tissues.

The large capacity of the venous compartment creates redundancy allowing the reconstructive vascular and cardiac surgeons to relocate some of these veins, the greater saphenous vein being a good example as a conduit for restoring blood flow to both the heart the periphery such as the legs. While it was not designed to function as an artery, the saphenous vein, when reversed because of its valves, provides a useful and readily available conduit for the surgeon. It is the right size, handles easily, and has no issues regarding rejection or foreign body reaction, as it is the patient's own tissue. Thus, with the advent of CABG surgery, the reversed saphenous vein graft became the workhorse conduit of choice for the cardiac surgeon and has remained so after nearly 50 years. As it is designed to work in a low-pressure system, graft patency and longevity is not ideal in this vein graft that was not originally designed to function under such high internal pressures. Early in the experience of CABG surgery, it became apparent that the internal mammary artery (LIMA) was a superior alternative. The LIMA became the favored choice for grafting the left anterior descending coronary artery (**Fig. 23**)

Saphenous Vein Graft: Its Position as Conduit of Choice in CABG Surgery

During the early period of CABG surgery in the United States, the saphenous vein graft was the conduit of choice for coronary bypass surgery. It soon became apparent that the saphenous vein graft was prone to progressive disease and eventual failure as illustrated earlier. During the early experience with myocardial revascularization, no interventional techniques, such as balloon angioplasty, were available to rescue a diseased graft territory, thus patients with early vein graft failure were at risk for a repeat CABG operation if medical management was not successful. In an attempt to alleviate vein graft-related problems, cardiac surgeons adopted and popularized the use of the left internal mammary artery (LIMA) with anastomosis to the left anterior descending coronary artery (LAD).

Use of the LIMA remained a relatively controversial technique until the surgeons from Cleveland clinic (Loop et al., 1986; Loop, 1996; Cameron et al., 1996; Burfeind et al., 2004) and others published papers establishing the evidence that the LIMA was superior to the saphenous vein graft when used in the LAD position in both freedom from recurrence of angina, myocardial infarction, and death. In spite of these landmark papers, universal application was slow. By the early 2000s, the STS data showed still only 90% of cases being done in the country were utilizing a LIMA. In the 1980s and 1990s, studies of the use of bilateral IMAs showed decreased need for reoperation and improvements in survival. Currently, the use of the LIMA is now nearly universal with CABG surgery, while utilization of bilateral IMAs has not been as widely adopted (Puskas et al., 2014; Lytle, 2001, 2013; Buxton et al., 2014; Taggart et al., 2016; Lytle et al., 1985).

The Saphenous Vein Graft: An Imperfect Yet Remarkable Conduit

Though the use of the saphenous vein as a conduit has been shown to be inferior to the left internal mammary artery (Lytle et al., 1985; Loop et al., 1986; Sabik et al., 2003, 2005) it remains a central part of the surgeons assortment of conduit choices in the

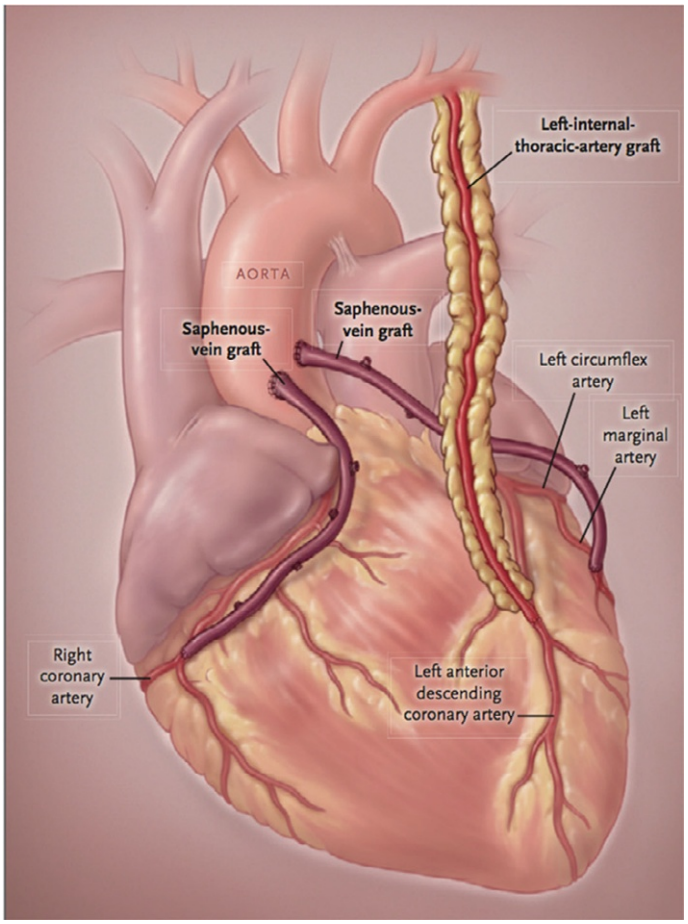

Fig. 23 A depiction of a left internal mammary (LIMA) graft to the left anterior descending coronary artery and saphenous vein grafts to the left marginal and right coronary arteries. From: *N Engl J Med* 2016;374:1955.

performance of modern CABG surgery. In fact, it remains the most commonly utilized conduit by surgeons today (Alexander and Smith, 2016). By the early 1970s, vein graft failure was already well recognized and a pathophysiologic process involving the intima was being described. Endothelial injury that occurs during vein graft harvest followed by and/or additionally caused by exposure of the vein graft to arterial pressure has been associated with platelet deposition, subsequent intimal hyperplasia, atherosclerotic plaque formation, and eventual stenosis and closure. A series of studies over the past 40 years have proven the benefit of antiplatelet therapy with low to intermediate dose aspirin as well as high-intensity statin therapy for lipid management in the interruption of this pathophysiologic process and improvement in vein graft patency. Unfortunately, studies of agents that directly interrupt intimal hyperplasia at the DNA level such as Edifoligide in the PREVENT IV study (2002–03) have not been as successful (Lopes et al., 2012a).

Despite these adverse findings, there are a number of the greater saphenous vein characteristics that make it such a good conduit and help to explain its continued status as workhorse in CABG surgery. These include its length, ease of use, ease of harvest, reasonably matched size in comparison to the targeted coronary artery, immediate capacity for high flow to the target vessel, capacity for sequential grafting, and suitability to the construction of a proximal anastomosis to the ascending aorta. During the early experience with CABG surgery, vein harvesting was universally carried out with either a full open technique or via a series of skip incisions. Dissatisfaction with the rate of wound complications/infections, delayed healing, pain, and cosmetics (appearance) along with advances in minimally invasive surgery led to the development of endoscopic vein harvesting (EVH) techniques by the mid to late 1990s.

Initially, EVH was criticized for a number of drawbacks including increased time of harvest, increased risk of conduit injury, increased potential for retained clot in the harvested vein, increased risk of vein stenosis or graft occlusion, risk of CO_2 (carbon dioxide) embolism, and operator dependence for graft quality. Several prospective studies carried out from 2000 to 2009 produced conflicting results regarding noninferiority of the endoscopically harvested vein graft. Other studies during this era revealed that the clot formation associated with EVH is most likely a result of combined local trauma and stagnant blood (Lopes et al., 2009, 2012b; Poston, 2006; Dacey et al., 2009, 2011). As a result of continued technological advances in operative equipment, increased surgical

experience, earlier intraoperative use of heparin, and associated improvement in the quality of harvested veins, EVH has become the preferred method of greater saphenous vein procurement over the past 10 years (Raja and Sarang, 2013).

During preoperative planning, the surgeon determines the use of conduits based on the number, size, and location of diseased the coronaries. Generally speaking, the LIMA will be placed to the LAD. Based on patient comorbidities, severity of coronary obstruction, acuity/severity of patient illness, and presence of adequate ulnar collateral flow, the surgeon will determine the appropriateness of the use of secondary arterial grafts such as the RIMA as a pedicled (attached to its native source) or free graft, and the left/right radial artery. If appropriate for a particular case, those conduits will be placed to the second or third tier target vessels. Severity of stenosis of the native coronary has been associated with superior saphenous vein graft longevity over arterial conduit choices due to the negative effects of competitive flow. In the case of the RIMA, SVG is superior when grafted to the distal right coronary artery when the stenosis is less than 80–90% because of size compatibility and consequent flow characteristics. Likewise, if the native stenosis is less than 70 or 80%, saphenous vein graft patency is superior to radial artery grafts (Yie, 2008; Taggart, 2013; Athanasiou et al., 2011; Deb, 2012; Sabik, 2008). The practical result of such operative planning is that the distal coronaries that are not going to be grafted by arterial conduits will need to be grafted with saphenous vein.

The Internal Mammary Artery: The Physiology of Arterial Grafting

The internal mammary artery grafted to the left anterior descending coronary artery is a major quality indicator in CABG surgery and gives superior short- and long-term outcomes when compared with saphenous vein grafts (Hillis et al., 2011). Linguistically, the Latin root of the word mammary is "mamma" or breast. Further underscoring the physiologic importance of the breast is the use of the same root in Taxonomy, or the scientific classification of organisms. In this classification system, Mammals represent a major Class within the Animal Kingdom. As the name of this Class implies, its members share a critical survival advantage: The ability to sustain their offspring by providing nutrition in the form of milk from maternal mammary glands. Though the title "internal thoracic artery" is admittedly more anatomically accurate, it can give the impression that the artery has the functional importance of a redundant tributary vein from the superficial venous system of the leg or of a vestigial structure such as the appendix (Conti, 1991).

The title "internal mammary artery" reinforces the concept that this artery serves as a critical support element of Mammalian breast systems and by extension, species survival. Moreover, given the importance of this vascular structure in supporting the level of lactation required for the continuing generation of mammalian species, it is not surprising to find that internal mammary arteries have some unique characteristics when compared with other arteries in the body. From studies of human physiology, we know that a nursing human mother forms about a liter and half of milk per day (Hall, 2016.). In order to support this rate of production, the cardiovascular system provides arterial support to each breast from two main arteries: the internal mammary and the lateral thoracic arteries. A recent study of the blood flow to the lactating human breast revealed that an average of 126 L of blood was supplied to each breast over a 24-h period with 70% of that flow being derived from the left internal mammary artery the artery often destined to revascularize the heart (Geddes et al., 2012). In pregnancy, such flow augmentation can present as a continuous murmur over the front of the chest called the "mammary souffle of pregnancy" (Scott and Murphy, 1958). The capacity of the internal mammary arteries to increase blood flow in response to end organ demand during the pregnant/lactating state has proven to be an excellent conduit characteristic when providing blood flow to an ischemic myocardial zone.

In addition to the ability to increase blood flow, studies have shown that IMAs have specific anatomic and physiologic properties that account for their superiority when used as a coronary bypass graft. These arteries have been shown to be impervious to the transfer of lipoproteins. This characteristic seems consistent with its primary support role as a conduit for optimal oxygen and nutrient delivery, especially lipoproteins, to the lactating breast for production of calorie dense milk and is also likely associated with its remarkable resistance to the development of atherosclerosis. Anatomically, this resistance to the transfer of lipoproteins may be related to an endothelial layer containing fewer fenestrations and lower intercellular junction permeability. Physiologically, IMA endothelial cells (the cells that line the blood vessels) have continuing high levels of heparan sulfate, tissue plasminogen activator (a protein involved in the breakdown of blood clots), and endogenous nitric oxide (a vasodilator) production (Osuka, 2013).

The internal mammary artery (IMA) graft's superior long-term patency could also be partly attributed to the fact that the endothelium of the IMA graft can release prostacyclin. Prostacyclin (also called prostaglandin I2 or PGI_2) is a potent vasodilator that can also inhibit platelet aggregation. Lin et al (Lin et al., 1991). demonstrated that endothelium of IMA grafts could release prostacyclin either in a basal condition or upon stimulation of hypoxia. Subramian et al (Subramanian et al., 1986). found in vitro that segments of the mammary artery (IMA) had a high capacity for PGI_2 synthesis and diminished inhibition of PGI_2 after aspirin was demonstrated for IMA compared with saphenous vein tissue and may be a factor in the improved patency of IMA grafts. Homolay (Homolay et al., 1993) studied the prostacyclin-producing capacity of the IMA using a platelet aggregation bioassay. He concluded that the synthesis of prostacyclin was significantly higher in the internal mammary artery than that was in the saphenous vein.

Many of the factors that contribute to the long-term patency of the LIMA graft are summarized in **Fig. 24** (Bruce Ferguson, 2016). The proinflammatory environment of the diseased heart is "normalized" as the graft stays open for many years by virtue of its endothelial production of endogenous vasodilators (nitric oxide), prostacyclin (PGI_2), along with the production of epoxyeicosatrienoic acids (EET). EETs are cardioprotective signaling molecules formed within various types of cells by the metabolism of arachidonic acid.

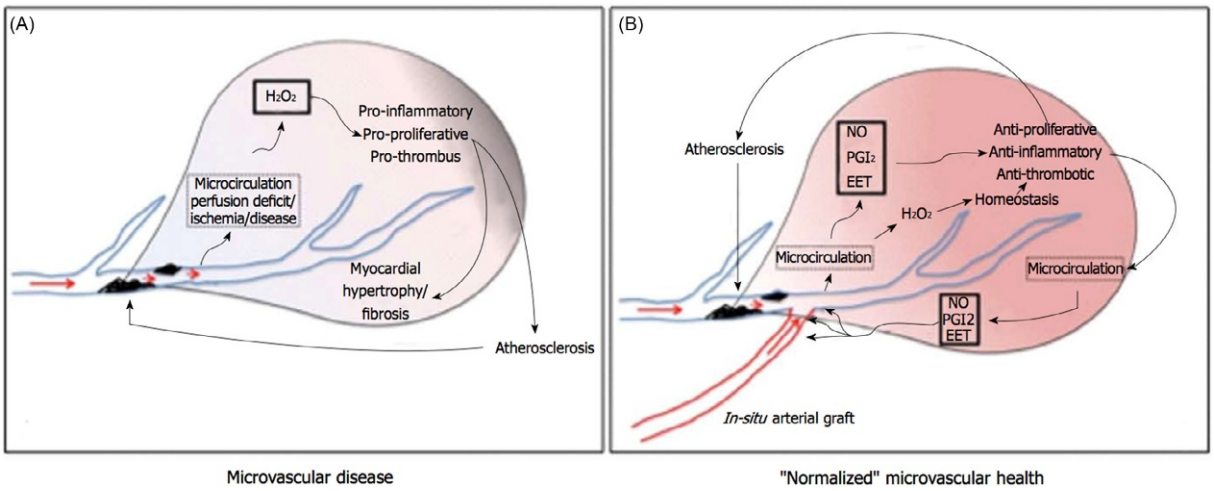

Fig. 24 In a healthy heart, arteriolar endothelium produces NO (nitric oxide), prostacyclin (PGI2, and EETs) as well as low levels of hydrogen peroxide, which support a quiescent nonproliferative state. With the onset of disease (A), flow through the microvasculature releases hydrogen peroxide, creating a proinflammatory environment throughout the organ, potentially leading to hypertrophy, fibrosis, and atherosclerosis. In (B), with bypass grafting of ischemic myocardium, the microvascular health of the myocardium is "normalized." *NO*, nitric oxide; *PGI2*, prostacyclin; *EET*, epoxyeicosatrienoic acids. From: *World J Cardiol* 2016; 8(11): 623–637.

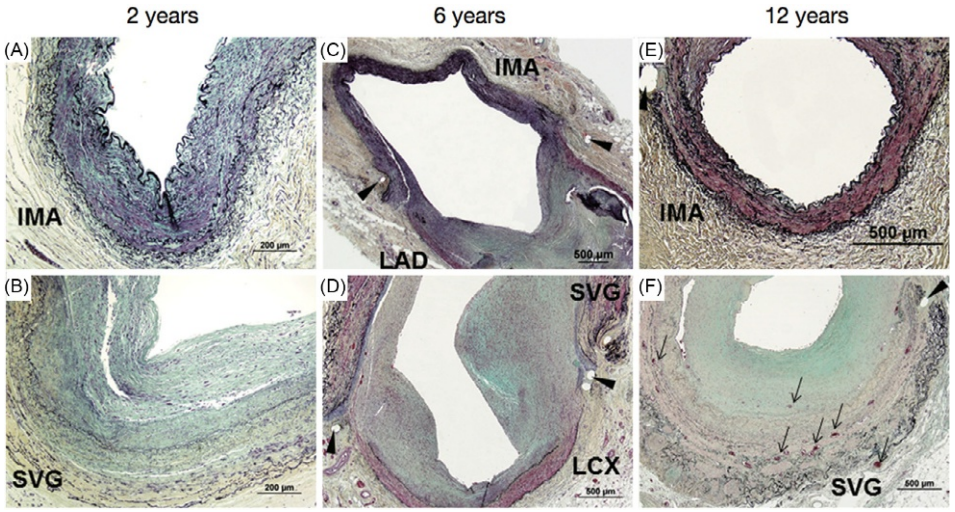

Fig. 25 Histologic changes observed in the internal mammary artery (IMA) graft as compared to the saphenous vein graft (SVG). (A, B). Histologic sections showing IMA and SVG obtained from a 76-year-old man who underwent coronary artery bypass graft (CABG) surgery 2 years antemortem. IMA shows no or rare intimal smooth muscle cells (SMCs), whereas SVG exhibits moderate neointimal growth with few SMCs but rich in matrix which consists of proteoglycans and collagen. (C, D). Histologic sections showing IMA and SVG obtained from a 69-year-old man who underwent CABG surgery 6 years antemortem. Note the absence of intimal thickening in IMA. (C) versus the presence of moderate neointimal thickening in SVG from SMCs and proteoglycan-collagenous matrix at the site of anastomosis (arrow heads indicate suture sites) with left anterior descending artery (LAD) or left circum ex artery (LCX) (D). (E, F) IMA graft and SVG from a 77-year-old woman who underwent CABG surgery 12 years antemortem. While the IMA shows minimal intimal thickening, SVG exhibits moderate to severe neointimal growth with proteoglycan–collagen matrix and angiogenesis (*arrows*). All sections were stained with Movat pentachrome. Illustration From: Osuka, F. (2013). Why as the mammary artery so special and what protects it from atherosclerosis? *Annals Cardiothoracic Surgery* **2** (4), 519–526.

The combination of these attributes along with an ideal size match to target coronary arteries and associated decrease in turbulent flow helps to account for the superior performance of the IMA as a bypass graft relative to the saphenous vein graft as illustrated in Fig. 25.

The Radial Artery

The utilization of a radial artery free graft was first described by Dr. Alain Carpentier, in Paris, France, one of the pioneers of modern cardiac surgery (Carpentier et al., 1973). After initial intense interest, reports of radial artery graft failures with profound narrowing

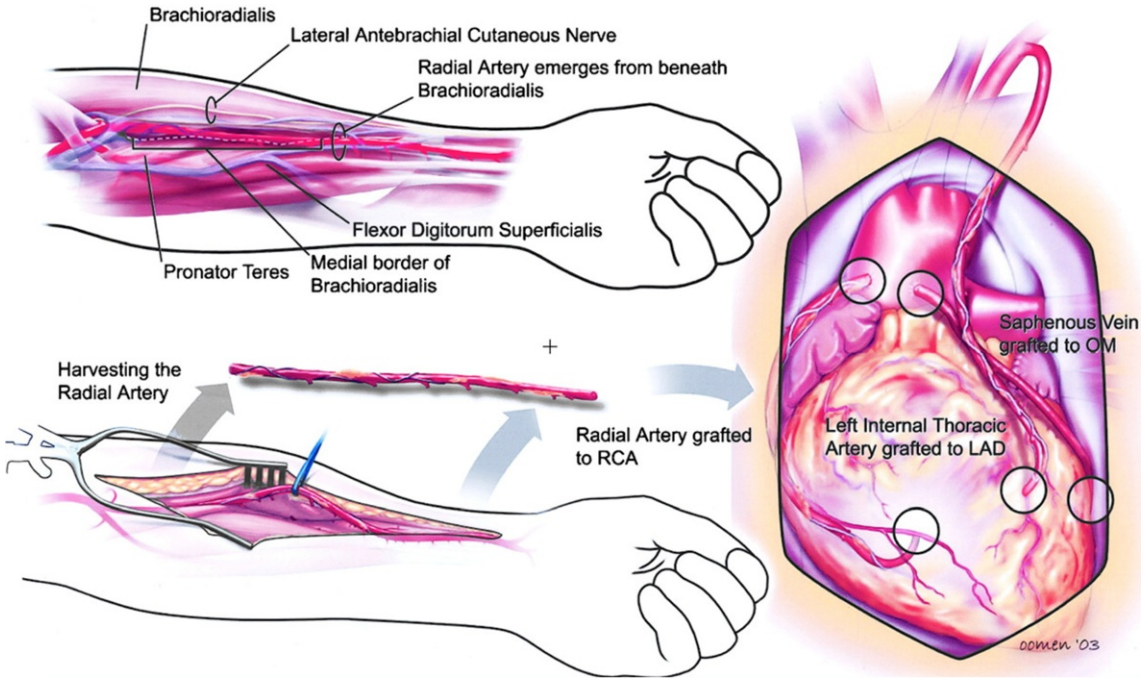

Fig. 26 The radial artery (RA), harvested as a pedicle, is dissected through a skin incision starting 3 cm distal to the elbow crease lateral to the biceps tendon, stopping 1 cm before the proximal crease at the wrist level, centered over the radial pulsation, between the flexor carpi radialis and the radial tubercle. Care is taken to avoid damaging the lateral antebrachial cutaneous nerve (sensory), which crosses obliquely from medial to lateral. After incision of the skin and fatty tissue, the RA can be seen in the distal third of the forearm beneath the fascial layer and is completely exposed by lateral mobilization of the brachioradialis. All side branches are ligated and the artery is irrigated topically with dilute papaverine solution during the dissection. Compared with the LIMA, the RA has a much thicker muscular media that likely contributes to its increased propensity to go into spasm. The RA may be proximally anastomosed to the LIMA, forming a composite graft, or to the ascending aorta to bypass all coronary territories. Illustrated is the use of a free RA graft anastomosed proximally to the ascending aorta to bypass a lesion in the right coronary artery, the LIMA anastomosed to the LAD, and a saphenous vein graft to the first obtuse marginal branch (OM). From: Verma et al Circulation. 2004;110:e40–e46.

of the graft (so-called angiographic string sign) discouraged utilization of the radial artery for CABG surgery. However, long-term follow-up of these arteries revealed that vessels that had at one point shown a string sign showed with reassessment progression to be widely patent (Verma et al., 2004). As arterial spasm might be a contributing factor, the use of the radial artery was resurrected along with intraoperative and postoperative vasodilator therapy to decrease vasospasm in the vessel. As long-term vasodilator therapy could result in atrophy of the muscular component of the radial artery, the vasodilators are given for a limited amount of time (Verma et al., 2004). Fig. 26 depicts the harvesting and use of the radial artery.

In some centers, all arterial grafting has become the ideal operation, while others have not adopted that philosophy. In fact, most patients do not receive all arterial grafts as most surgeons have not adopted all arterial grafting as a primary myocardial revascularization strategy. These competing philosophies reflect one of the current major controversies in surgical myocardial revascularization.

Conduct of the CABG Operation

Shortly after induction of general anesthesia, the greater saphenous veins are assessed and proposed incision sites are marked in the leg(s). Ultrasound can be used to augment physical exam information and accurately predict sites of branching, varicosity, and duplication. The patient is then "prepped" with a topical antiseptic from the chin down to the level of both ankles and then covered with sterile drapes, leaving only the surgical incision sites exposed. A longitudinal midline sternotomy is made, aided by a surgical saw, cutting in the midline of the sternum, allowing for a precise closure, securely repairing the bone and the rest of the wound at the close of the procedure (Fig. 27). The midline has no muscle to cut through and at the completion of the procedure the sternum is repaired with sternal wires or other methods of firm secure closure, allowing the bone to heal much like any other orthopedic repair of bone with various types of hardware. The discomfort during healing is well tolerated in general and affords the patient less pain than other major incisions such as thoracotomies (between the ribs) or laparotomies (large abdominal incisions)

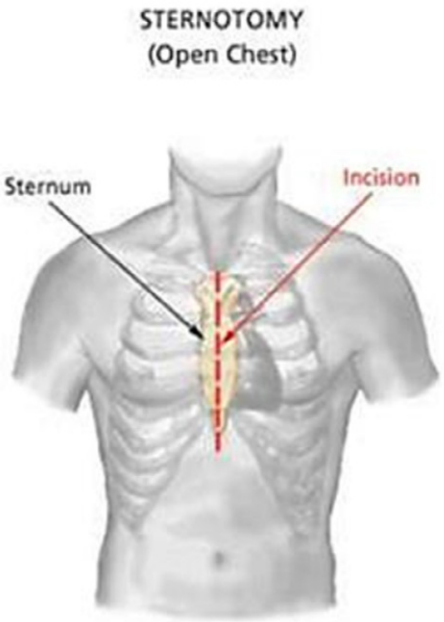

Fig. 27 The sternotomy incision that is typically used in CABG surgery and other types of cardiac surgery. From www.surgery.usc.edu. IAC Publishing, LLC ("Ask") provides the Ask.com site (the "Site") and the offered services and features (the "Services") subject to these Terms of Service ("Terms of Service").

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Once the sternum is opened, the mammary artery is harvested after which the sternum is separated with a sternal retractor (Fig. 28), and the pericardium (sac around the heart) is exposed and divided. At this time the heart is exposed with all of its structures, epicardial vessels, and the large veins leading to and great arteries exiting the pumping chambers.

If a radial artery is going to be harvested, the anesthesia team will start either a low dose of intravenous nitroglycerin or a calcium-channel blocker to inhibit potential vessel spasm. The entire operation is performed under conditions of ideal illumination such as an intense headlight, and magnification (surgical loupes that magnify 2.5 to 3.5 times). While the left internal mammary artery (LIMA), right internal artery (RIMA), or both and/or radial arteries are being harvested, a small incision is made over the greater saphenous vein either above or below the knee and eventually exposing the vein. Once the vein has been identified, the patient is given a low dose of intravenous heparin. Endoscopic vein harvesting (EVH) equipment is then deployed to bluntly dissect out the vein creating a carbon dioxide (CO_2) insufflated tunnel that allows for a minimal touch technique, safe branch management, and tunnel hemostasis (control of bleeding) (Fig. 29) (Accord and Maessen, 2011).

The near and far ends of the vein are accessed via additional small incisions and the vein is ultimately removed from the leg. It is then reversed (as it has one-way valves) and flushed with a solution of heparin and saline in order to remove clots and to identify branches, varicosities, defects, or injuries. If the vein is not felt to be adequate, remaining vein on the same leg the other leg is then harvested. In general, branches are clipped or tied at the level of the vein though some surgeons prefer to preserve 2–3 mm of the length on the larger branches for potential use when performing sequential distal anastomoses, allowing more than one coronary artery to be grafted with one section of saphenous vein. Once the vein has been attended to, the leg wounds are inspected for hemostasis, irrigated, and carefully closed in layers. If an inadequate length of vein conduit is available, then a backup plan for harvesting the RIMA or radial arteries is activated. Alternatively, though not ideal, short segments of usable vein from each leg can be sewn together end-to-end in order to create a composite graft of adequate length. A prognostic saphenous vein scoring system that also takes into account the runoff status (size and quality with regard to the amount of atherosclerosis) of the target coronary artery has been described (Sarzaeem et al., 2010). In circumstances in which a compromised vein conduit has to be used in CABG, and/or

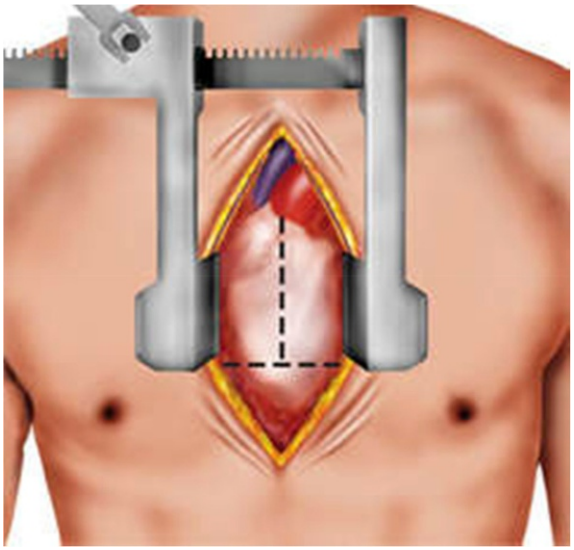

Fig. 28 The sternotomy has been performed and a sternal retractor has been inserted and spread to expose the pericardium with the dotted lines depicting the planned typical pericardial incisions that will expose the heart. From ht.edwards.com. IAC Publishing, LLC ("Ask") provides the Ask.com site (the "Site") and the offered services and features (the "Services") subject to these Terms of Service ("Terms of Service").

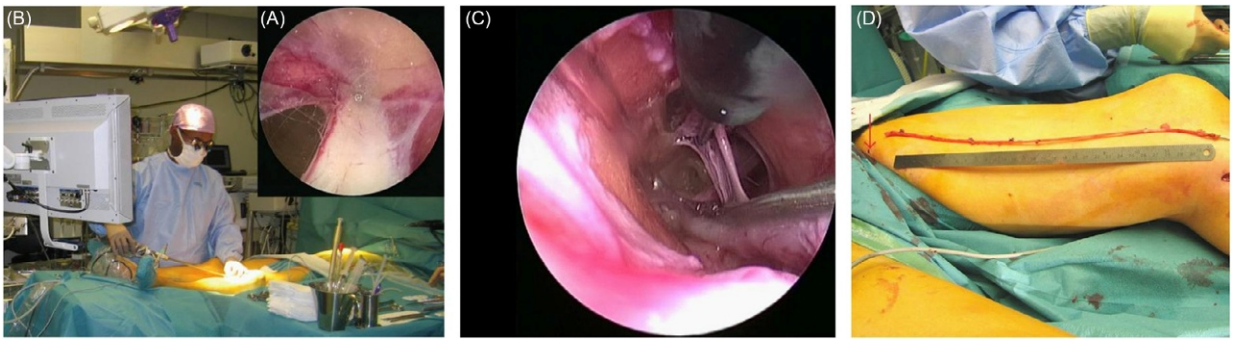

Fig. 29 Endoscopic vein harvesting. (A) The first step is the identification of the great saphenous vein (GSV) through a 3-cm incision near the knee. (B) The next step is the dissection of the vein from the surrounding subcutaneous tissue. (C) After creating a tunnel by means of CO_2 insufflation, all side branches of the GSV are identified, coagulated using diathermy, and transected. (D) The final result is demonstrated, 35 cm of vein harvested through a 3-cm incision near the knee and a 3-mm counter-incision in the groin (*arrow*). From: https://www.hindawi.com/journals/crp/2011/813512.fig.001a.jpg Copyright © 2011 Ryan Accord and Jos Maessen. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

the runoff is not ideal, antiplatelet therapy (medications such as aspirin or clopidogrel that decrease platelet aggregation or "stickiness" to discourage the initiation on intraarterial clotting) is reasonable and evidence-based (Une et al., 2014).

A crucial aspect of long-term management of this newly create anatomy is termed "secondary prevention." All of the efforts to revascularize coronary arteries do not cure coronary artery disease, and a comprehensive postsurgical management program must be instituted that includes high-intensity statins, antiplatelet medications such as low-dose aspirin, beta blockers, aldosterone antagonists (if heart failure or left ventricular systolic dysfunction), angiotensin receptor blockers (if diabetes or left ventricular systolic s dysfunction), and lifestyle changes that include regular aerobic exercise, a diet low in saturated fats and carbohydrates and abstention from smoking (Kulik et al., 2015; Fihn et al., 2014).

Once all conduits are prepared and the patient is systemically heparinized, the heart and ascending aorta are inspected. Distal coronary artery targets are confirmed and grafting sites can be marked. Once grafting sites are determined, the length of conduit that will be required to reach the aorta is determined as well as the distance between any sequential anastomoses (the practice of using one conduit for multiple distal connections). Once it is confirmed that adequate conduit is available, the surgeon may use natural branches that may be available for sequential grafting or can fashion an arterial "Y" configured graft with a combination of arterial

or venous conduits. If there are any concerns about quantity or quality of the graft material, the surgical assistant can harvest more saphenous vein as the surgeon initiates bypass and begins to perform distal anastomosis with conduit that has been deemed adequate at this point in the procedure.

CABG Using Cardiopulmonary Bypass

If the CABG is going to be performed with CPB support and cardioplegic arrest as described in the section on CPB, aortic and right atrial cannulation sutures are placed, after which aortic and right atrial cannulae are inserted and secured. A retrograde cardioplegia cannula is inserted into the coronary sinus and an antegrade cannula is placed in the ascending aorta. Appropriate connections are made with the tubing from the CPB and the patient is then placed "on bypass" and usually cooled (actively or passively) using systemic hypothermia (cooling the entire body) while cardioplegia is being prepared. The distal ascending aorta is cross-clamped between the aortic cannula and the antegrade cardioplegia line and induction antegrade/retrograde cardioplegia is delivered (Fig. 30). Depending on surgeon preference, the metabolic rate of the myocardium can be further reduced with topically with chilled saline or ice slush.

Once cardioplegia has been delivered, the heart is ready for grafting. With the heart now flaccid and cooled on CPB, each distal coronary artery or branches thereof (targets) is adequately exposed, the visceral pericardium covering each vessel is dissected away, and the vessel is opened. A bloodless, still, clear sewing field has been created, and the surgeon is now ready to sew the conduit to the coronary artery at a point beyond the stenosis (narrowing) or obstruction. The appropriate conduit (vein or artery) is now tailored to fit the opening in the recipient artery as sutured with a very fine monofilament suture using fine microvascular instruments and intense lighting (headlight) and magnification (surgical loupes). Prior to completion, adequate patency is confirmed, the conduit is deaired and tested for leaks which are repaired if present. More cardioplegia is now given, and this cycle is repeated until each distal anastomosis is completed.

Rewarming of the patient is initiated using the heat exchanger on the CPB machine prior to the performance of the final anastomosis and preparations are made for construction of the proximal anastomoses. Once all of the anastomoses have been completed and with the aortic cross clamp having been removed, the heart begins to warm and regain its usual metabolism and electromechanical properties. Once the patient is adequately rewarmed and the heart is recovered, the patient is weaned from CPB with varying levels of inotropic/pressor support, decannulated, and the surgical wound closed. At this time, the team is dealing with other details of weaning from CPB, primarily hemodynamic support, fluid, and electrolyte balance and management of coagulation abnormalities when they arise. Graft flows are checked with flow probes for adequacy with Doppler flow signals, the most commonly used being transit-time flow meters.

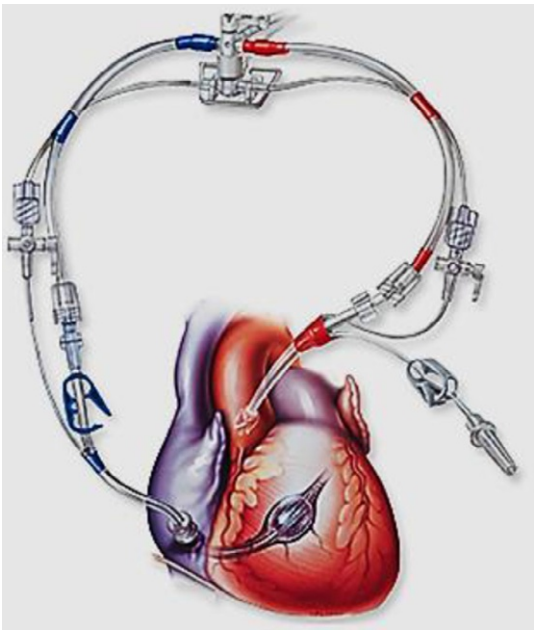

Fig. 30 This illustration depicts the heart with both antegrade (aortic) and retrograde (coronary sinus) catheters placed in the heart and ready to be attached to the CPB circuit for administration of cardioplegia. From: criticalunit.com critical-care-medicine.becoming a better Clinician.

CABG Without Cardiopulmonary Bypass: Off-Pump CABG (OPCAB)

If the operation is going to be performed off-pump, the room is kept warm and intravenous amiodarone (an antiarrhythmic) and low-dose norepinephrine infusions are initiated. Temporary pacemaker wires are placed and tested. All of these measures are taken in order to be prepared for rhythm and hemodynamic abnormalities that must be managed in the course of the operation as the surgeon does not have the advantage of the mechanical support of CPB. Exposure and stabilizing devices are now deployed to optimize exposure on a beating heart with blood continuing to flow in all of its vessels as opposed to the still, bloodless field afforded by the use of CPB, and cardioplegic arrest. Exposure is gained with a combination of deep pericardial retraction sutures, an apical suction universal retractor and judicious use of surgical pads. Communication between the anesthesiologist and the surgeon regarding hemodynamic stability is a top priority to maintain safe conduct of the operation. The role of the cardiac anesthesiologist is demanding and includes constant hemodynamic monitoring, transesophageal echocardiographic (TEE) assessment for ischemia, and quick responses for maintenance of blood pressure and cardiac output and allows the surgeon to safely perform the distal anastomoses. Once the target artery is exposed, it is immobilized with a stabilizing device(s), the visceral pericardium is dissected away, and preparations are made to perform the distal anastomosis. Control of blood flow at the site of the intended anastomosis is afforded by a proximal occlusion method. When the artery is opened, a clear anastomotic site is gained by a combination judicious use of a device that blows CO_2 in a gentle mist. In circumstances where hemodynamic stability is difficult to maintain because of ischemia, an intracoronary shunt can be placed into the anastomosis to provide a blood free zone. Construction of the anastomosis is the same as described in the on-pump CABG section. Once the anastomosis is completed, the conduit is flushed and the proximal occlusion technique is released to ensure deairing and the suture line is tied down. This cycle is completed until all distal anastomoses are performed.

The proximal anastomoses can be performed on the unclamped aorta with a device such as the Heartstring (Fig. 31) or with the aid of a partial occlusion clamp. Bypass grafts can now be tested with a transit time flow probe (Doppler technology), and once grafts are determined to be acceptable, protamine can be administered to reverse the effects of heparin, and the chest closed.

When surgeons are confronted with ischemic coronary beds that are not amenable to CABG due to poor distal targets, hybrid techniques of managing those ischemic zones are available (Gosev and Leacche, 2014). For example, if numerous small branches that arise from a larger artery that is amenable to more proximal percutaneous intervention populate the ischemic zone, then a multidisciplinary approach of traditional CABG and PCI with a drug eluting stent can be performed simultaneously in a hybrid operating room or as separate procedures utilizing both an OR and a cardiac catheterization laboratory.

When an ischemic zone has targets that are not amenable to either CABG or PCI, then transmyocardial laser revascularization (TMR) can be utilized as an adjunct to CABG (Horvath, 2008; Bhimji and Sheridan, 2015). This technique utilizes a carbon dioxide or YAG laser to create multiple full-thickness channels in an ischemic zone that cannot otherwise be revascularized. Though it was initially hypothesized that the laser-created channels would develop into sinusoidal-type structures with direct perfusion of the myocardium from the left ventricle, this has never been found to occur. The relief of angina may be due to an augmented development of collaterals or to a local sympathectomy of the treated visceral epicardium and myocardium. The Society of Thoracic Surgery recommendation is that such an approach is reasonable; however, further investigation is needed as several studies to date have returned conflicting angina benefit and survival results (Bridges et al., 2004; Allen, 2000).



Fig. 31 The Heartstring® is an example of a novel device that allows the surgeon to sew a graft onto a hole created in the aorta for that purpose without having to clamp or manipulate the aorta in a cast of beating heart surgery (OPCAB) or when the aorta is so diseased that minimal aortic manipulation is desired to minimize the stroke risk from dislodging particulate matter from the aorta that could embolize (move from one place to another in the circulation). From: Boston Scientific Corporation.

Controversy exists with regard to many aspects of the practice of medicine and surgery and the ideal technique for the performance of CABG surgery is no exception, subject to conflicting evidence, longstanding practices, and beliefs. While different practices have varying outcomes in different sets of hands, the evidence and the science behind it seem to be sending a message with a common theme: With some exceptions, an all-arterial operation done on CPB is likely to achieve the best short- and long-term outcomes (Puskas et al., 2014; Lytle, 2001; Buxton et al., 2014).

Perioperative Management, Complications

CABG surgery as described is performed in cardiac surgery centers with experienced teams and access to all of the supporting multidisciplinary specialties to guide the patient safely through a defined pathway that is well described, frequently assessed, and provides as much reliability as possible while reducing variability. Decision-making in this context is based upon checklists and algorithms that are agreed upon by the team based on current evidence. Once the patient is referred to the system and placed on the pathway, defined processes are initiated starting with a readiness for surgery checklist that includes everything from demographics, socioeconomic factors, comorbidities, and evidence-based indications. Thinking as far ahead of postacute care considerations is done at this point, especially with so-called complex discharges that may require skilled nursing facilities or rehabilitation. All decisions cannot be based on checklists and algorithms alone and require more complex thinking, many times aided by the multidisciplinary team, especially in the selection process. Checklists and algorithms help to drive preoperative orders that include diagnostic tests such as laboratory studies and imaging. The results of those tests continue to be fed back into the decision-making process. The involvement of family and patient is crucial in the informed consent as they develop an understanding of expectations as best they can.

The care team is extensive, requiring the involvement of specialty nurses, outpatient and inpatient, advance practice providers (nurse practitioners and physician assistants), physicians in training (residents), respiratory therapists, cardiopulmonary perfusionists, physical and occupational therapists, pharmacists, registered dieticians, critical care specialists (surgeons and anesthesiologists), care managers, and an extensive administrative support team usually in the context of a tertiary care hospital.

The CABG operation may take up to 4 or 5 h, including 1–2 h of CPB time and 5 or 6 days in the hospital before discharge to a postacute care facility of home. Full recovery in an uncomplicated case can take up to 12 weeks. All of this timing is dependent on whether there are complications or not. Nearly every feature of the process is carefully documented during the patients hospitalization in the electronic medical record and subsequently entered into an extensive database that is submitted to regional and national databases allowing centers to see their teams' performance compared with centers like theirs. Surgical site infections, strokes, and mortality are key markers as well as each center's ability to rescue patients from multiple other complications that may arise.

In summary, patients are selected after shared decision-making in the context of evidence accumulated through many observational and randomized trials with this operation being done in the setting of an experienced center with a heart team and a multidisciplinary team of caregivers. That center is constantly scrutinizing data compared with like centers in a national database. Outcomes are fed back to front line workers in the multidisciplinary team, establishing and maturing pathways to minimize variability in care and achieve the best outcomes possible.

Summary

CABG surgery has a central place in the effort to revascularize the myocardium, gained after over more than a 50-year history. The knowledge gained by the world experience of cardiac surgeons performing millions of operations using evidence available to guide appropriate selection of patients, the technologic advances gained by the cardiopulmonary perfusionists, understanding of myocardial physiology, myocardial protection, vascular biology, secondary prevention, heart teams, and multidisciplinary teams of perioperative caregivers has and will improve, save and prolong productive lives. As less invasive techniques evolve and translational research leads us to a better understanding of the exuberant, maladaptive systemic inflammatory response with strategies to mitigate it, CABG surgery will continue to evolve to be even safer and valuable.

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Cyanotic Congenital Heart Disease

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Introduction

Congenital heart disease occurs in approximately 1% of live births, and approximately 15% of these have cyanotic heart disease (Hoffman and Kaplan, 2002; Mitchell et al., 1971). Infants born with cyanotic congenital heart disease are particularly vulnerable to morbidity and mortality, often requiring intervention in the neonatal period for survival. Prior to 1945, no surgical treatments existed for patients with cyanotic heart disease, and thus, most of these babies did not survive infancy. Advances in surgical techniques have led to dramatic improvements in survival. Today, treatments are available for nearly all infants with cyanotic congenital heart disease. The improvement in survival in recent decades has led to there being more adults than children with congenital heart disease.

Cyanotic congenital heart disease has often been presented as a list of lesions, often aided with mnemonics such as "T"s and "P"s. However, this article will present a framework for understanding congenital heart disease that focuses on principles of physiology and anatomic features that lead to cyanosis, grouping lesions by their common pathophysiologic features. We will discuss representative cyanotic heart lesions, along with available medical, catheter-based, and surgical interventions.

Physiologic Principles of Cyanotic Congenital Heart Disease

An understanding of cyanosis as well as fetal and transitional circulatory physiology aids in understanding the pathophysiology of cyanotic congenital heart disease.

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Cyanosis

The eye is not very sensitive in detecting cyanotic heart disease. Detection of cyanosis or blueness of the skin requires the presence of approximately 3 $g dL^{-1}$ of deoxyhemoglobin in arterial blood (Lees, 1970; Nadas, 1992). For a newborn, with a total hemoglobin of 20 $g dL^{-1}$ (just above the normal range), cyanosis would be detectable at an arterial oxygen saturation of about 85% (17 $g dL^{-1}$ of oxyhemoglobin, 3 $g dL^{-1}$ of deoxyhemoglobin), whereas for a 2-month old infant with a total hemoglobin of 10 $g dL^{-1}$ (just below the normal range), cyanosis would not be detectable until the arterial oxygen saturation reached about 70% (7 $g dL^{-1}$ of oxyhemoglobin, 3 $g dL^{-1}$ of deoxyhemoglobin). It is important to recognize that when infants are cold, vasoconstriction decreases flow to the skin, particularly of the hands and feet, resulting in the appearance of blueness of the skin caused by lowering of venous oxygen saturations. This normal phenomenon, known as acrocyanosis, occurs even when arterial saturations are normal (close to 100%), and it does not indicate the presence of cyanotic heart disease.

Cyanosis may be the result of lung disease or heart disease. Often, this distinction is clinically apparent. Infants with cyanosis from lung disease often demonstrate signs of respiratory distress on physical examination, including dyspnea, tachypnea, and retractions, whereas infants with cyanosis from heart disease usually breathe comfortably. Arterial blood gases often show an elevation of P_{CO_2} in infants with lung disease, while those with cyanosis from heart disease often have normal or low P_{CO_2}. Although not directly measurable without cardiac catheterization, the pulmonary venous oxygen saturation is low in infants with pulmonary causes of cyanosis, while it is normal in isolated cyanotic congenital heart disease. As a result of this decreased pulmonary venous oxygen saturation in infants with cyanosis from lung disease, there is often a marked improvement in arterial P_{O_2} while breathing 100% oxygen (FiO_2 of 1.0), while in infants with cyanotic heart disease and normal pulmonary venous oxygen saturations, there is little improvement in arterial P_{O_2} with 100% oxygen, as the pulmonary venous saturation was already nearly 100%. There may be some effect of oxygen on infants with cyanotic heart disease with supplemental oxygen, as a small amount of oxygen can be dissolved in blood, and oxygen is also a pulmonary vasodilator, but it is extremely rare for the arterial P_{O_2} to exceed 150 torr in infants with cyanotic heart disease, even on 100% oxygen. Other clues suggesting congenital heart disease as the cause of cyanosis in neonates include the presence of a murmur, abnormal cardiac silhouette on chest x-ray, and presence of risk factors for congenital heart disease including chromosomal abnormalities, multiple congenital anomalies, maternal diabetes mellitus, and a family history of congenital heart disease (Fig. 1).

Fetal Circulation

In order to understand the pathophysiology of cyanotic congenital heart disease in the hours and days after birth, one must understand the fetal and transitional circulation. In utero, the right ventricle (RV) and left ventricle (LV) work in parallel, both pumping blood primarily to the systemic fetal circuit (from the LV directly to the ascending aorta, and from the RV through the ductus arteriosus to the descending aorta). The fetal RV contributes about twice as much flow to the fetal circulation as does the fetal LV. Lesions affecting inflow or outflow of only one ventricle do not often compromise the fetal circulation, as the unaffected ventricle is able to maintain the circulation.

Fig. 2 depicts the fetal circulation just before birth (Taussig, 1947). The umbilical vein carries oxygenated blood from the placenta to the fetus, draining through the liver via the ductus venosus to the inferior vena cava (IVC). IVC blood flow streams toward the patent foramen ovale (PFO), allowing the most oxygenated blood in the fetus to pass to the left heart and out the ascending aorta, where it supplies the coronary arteries and developing brain. Deoxygenated superior vena cava (SVC) blood is directed through the right heart, much of it flowing from the pulmonary artery through the patent ductus arteriosus (PDA) to the

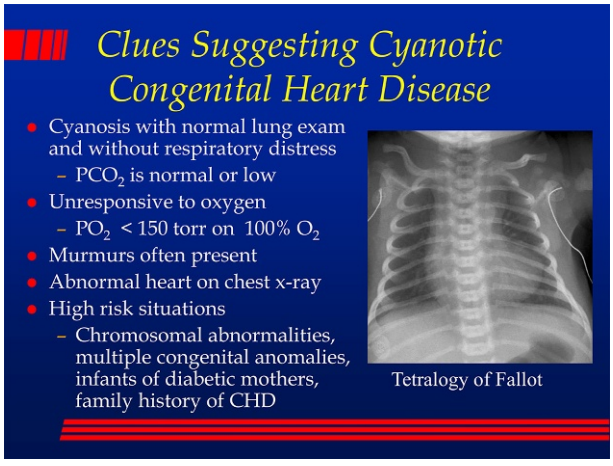

Fig. 1 Clues suggesting cyanotic congenital heart disease. Clinical clues may help differentiate cardiac and pulmonary causes of cyanosis in infants. The chest radiograph of an infant with tetralogy of Fallot demonstrates decreased pulmonary vascular markings, an upturned apex because of right ventricular hypertrophy, and a concave main pulmonary artery segment because of pulmonary stenosis.

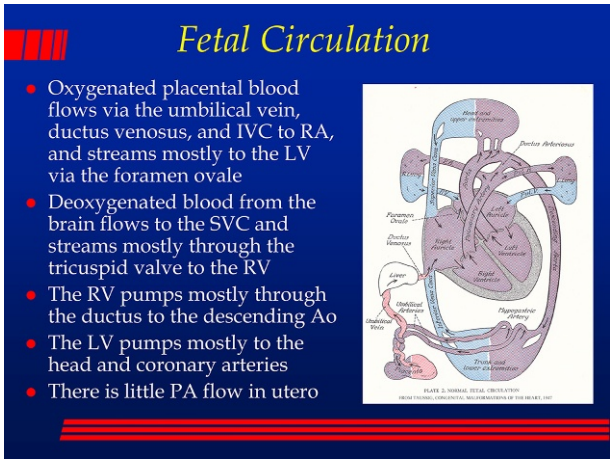

Fig. 2 Fetal circulation. An understanding of the fetal circulation just before birth informs the expected changes in physiology during transition to extrauterine life. The ductus venosus, foramen ovale, and ductus arteriosus serve to maximize blood and oxygen delivery to vital organs and minimize pulmonary blood flow in utero. Pathophysiologic changes allowing recognition of underlying structural heart defects often appear when these structures close after birth (Taussig, 1947).

descending aorta and the umbilical arteries back to the placenta. The fetal lungs receive only a small portion of the combined ventricular output, as they are not the organ of oxygenation of the fetus and do not require a large amount of flow.

Transitional Circulation

With the newborn's first breath, the aeration of the alveolae causes a fall in the pulmonary vascular resistance (PVR), and the increased pulmonary venous return to the left atrium pushes the flap of the foramen ovale (known as septum primum) toward the more muscular superior portion of the atrial septum (known as septum secundum), limiting left-to-right atrial shunting. In the first hours to days of life, the PDA usually closes. Maintaining these communications between the right and left heart is important for the postnatal survival in many infants with cyanotic congenital heart disease. PVR, which is high in utero, usually continues to drop dramatically in the first days of life and continues to fall throughout the first several weeks of life.

An understanding of the transitional physiology informs the expected clinical presentation for most infants born with cyanotic congenital heart disease. Often, these infants appear well as long as the ductus arteriosus allows a source of pulmonary blood flow, even in the absence of normal antegrade flow from the RV to the lungs. In left-sided obstructive lesions (not discussed in detail in this chapter), systemic perfusion may be dependent on flow from the RV through the ductus. It is often at the time of ductal closure, usually 24–48 h after birth, that infants with ductal-dependent pulmonary or systemic blood flow decompensate, prompting evaluation for congenital heart disease. On the other hand, neonates may be profoundly unstable within minutes of birth if their congenital heart disease is dependent on an atrial communication that is not adequate in size and restricts atrial level mixing.

A Framework for Understanding Cyanotic Heart Disease

In cyanotic congenital heart disease, desaturation occurs because deoxygenated systemic venous blood reaches the aorta without first passing through the pulmonary capillary bed (Fig. 3). A site of mixing is necessary but not sufficient for cyanosis to occur. Sites of mixing include abnormal venous connections, atrial septal defects (ASDs), ventricular septal defects (VSDs), and the ductus arteriosus. A site of mixing is not sufficient to cause cyanosis, because in the otherwise structurally normal heart, the pressures in the right heart are lower than those in the left heart, resulting in primarily left-to-right shunting. Thus, there must also be something that makes it easier for systemic venous (deoxygenated) blood to go to the body than to the lungs. We present representative cyanotic heart lesions in three groups:

1. Right heart obstructive lesions
2. Lesions with unfavorable streaming
3. Complete mixing lesions, if the relative pulmonary blood flow is significantly less than systemic blood flow

Representative Lesions With Right Heart Obstruction

Right-sided obstruction makes it hard for deoxygenated caval blood to get to the pulmonary arteries. The authors find it helpful to think of right-sided obstruction in the order of flow through the heart, so we will discuss representative right heart obstructive lesions in order of site of obstruction, from the atrial level to the great arterial level.

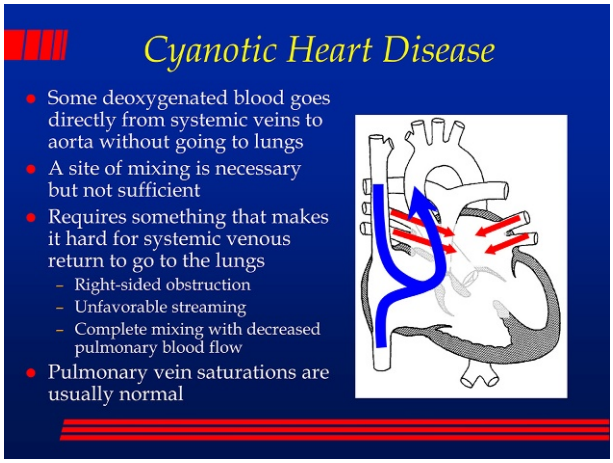

Fig. 3 Cyanotic heart disease. In cyanotic congenital heart disease, some deoxygenated caval blood reaches the aorta without first passing through the lungs.

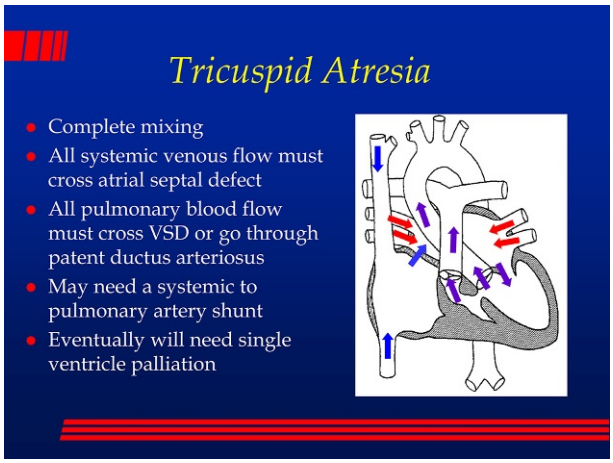

Fig. 4 Tricuspid atresia with normally related great arteries. In tricuspid valve atresia, the systemic and pulmonary venous return mixes in the left atrium before being pumped by the ventricles to the aorta and pulmonary artery, resulting in cyanosis from complete intracardiac mixing.

Tricuspid Atresia

Tricuspid atresia comprises 1%–3% of all congenital heart diseases (Dick et al., 1975; Hoffman and Kaplan, 2002). The cause of tricuspid atresia is unknown. Since tricuspid atresia prevents flow across the tricuspid valve, deoxygenated caval blood preferentially crosses the foramen ovale to the left atrium (Fig. 4).

Arterial oxygen saturation in tricuspid atresia is governed by the relationship of the great arteries, the presence and size of a VSD, and the degree of pulmonary/subpulmonary obstruction. In a little more than half of cases of tricuspid atresia, the aorta and pulmonary artery are normally related, but in the remainder, they are transposed (reversed), with the pulmonary artery arising from the morphologic LV, and the aorta arising from the diminutive RV (Tandon and Edwards, 1974). In tricuspid atresia with normally related great arteries, pulmonary blood flow crosses from the LV across the VSD to the RV and can be limited by restriction at the VSD, the subpulmonary region, and/or at the pulmonary valve. When the great arteries are transposed, there is often little obstruction to pulmonary blood flow. This physiology results in higher oxygen saturations in the short term but leads to pulmonary overcirculation, edema, and congestive heart failure (CHF) over the long term. In tricuspid atresia with transposed great arteries, restriction to flow at the VSD or the right ventricular outflow tract (RVOT) results in limitation of systemic arterial flow, with pathophysiology similar to that seen in other left heart obstructive lesions such as hypoplastic left heart. When there is obstruction either to pulmonary blood flow (as in tricuspid atresia with normally related great arteries) or to systemic blood flow (as in tricuspid atresia with transposition), maintaining patency of the ductus arteriosus allows an alternate route for perfusion of the affected circulation.

Tricuspid Insufficiency and Ebstein's Anomaly

Just as obstruction to forward flow through the tricuspid valve leads to cyanosis from right-to-left shunting of deoxygenated caval blood through the foramen ovale, significant tricuspid valve insufficiency can act as a functional barrier to forward flow to the lungs,

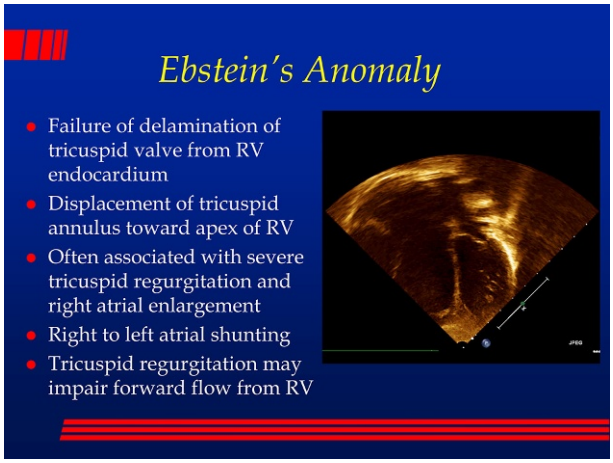

Fig. 5 Ebstein's anomaly. Significant tricuspid regurgitation can result in cyanosis from right-to-left atrial shunting. The echocardiogram on the right shows an apical four-chamber view of an infant with severe displacement of the tricuspid valve nearly into the apex of the right ventricle.

also leading to right-to-left atrial level shunting and cyanosis. Many cases of congenital tricuspid insufficiency are caused by a valve deformity termed Ebstein's anomaly, characterized by failure of delamination of one or more tricuspid valve leaflets from the endocardium during valve development, producing displacement of the septal and inferior valve leaflets in the direction of the right ventricular apex (Fig. 5). Fetuses and infants with Ebstein's anomaly often have massive cardiomegaly before and after birth because of enlargement of the right atrium (RA) and the portion of the RV proximal to the displaced tricuspid valve, known as the atrialized RV. Maternal exposure to lithium, used in treatment of bipolar disorder, is a cause of Ebstein's anomaly, but in the current era, it is not a frequent cause of the anomaly.

Pulmonary Atresia and Stenosis

Pulmonary valve abnormalities are common in patients with congenital heart disease. Pulmonary stenosis (PS) and atresia are collectively quite heterogeneous with respect to their associated lesions and presentation. Although patients with mild-to-moderate PS typically have normal oxygen saturations, severe RVOT obstruction may result in right-to-left shunting of deoxygenated blood at the atrial or ventricular level, resulting in cyanosis.

Pulmonary atresia with ventricular septal defect

In pulmonary atresia with VSD, also described as tetralogy of Fallot (TOF) with pulmonary atresia, the right-to-left shunting occurs at the VSD. In this form of pulmonary atresia, the pulmonary arteries often do not develop normally and may or may not be confluent. Often, there are multiple aorticopulmonary collateral arteries (MAPCAs) that perfuse segments of the lung, and flow through the ductus arteriosus may not be necessary for survival. In that case, the true confluent pulmonary arteries are often very diminutive and may be difficult to see using imaging techniques such as echocardiography. However, these MAPCAs have a tendency to narrow over time, resulting in worsening cyanosis from decreasing pulmonary blood flow. The management of diminutive pulmonary arteries with MAPCAs can be quite challenging, often requiring multiple repeat percutaneous and surgical interventions for rehabilitation after the collateral vessels are unifocalized into reconstructed branch pulmonary arteries. In other cases, the pulmonary arteries may be well formed and confluent, although sometimes, there is a narrowing where ductal flow bifurcates to the right and left pulmonary arteries, and ductal closure may result in discontinuous pulmonary arteries. The management of pulmonary atresia with VSD and well-formed pulmonary arteries is similar to that of TOF, as described in the following text.

Tetralogy of Fallot

Classic TOF is the most common form of cyanotic congenital heart disease, affecting about 1 in 2000 live births (Hoffman and Kaplan, 2002). It was first described as a constellation of four cardiac abnormalities leading to obstruction of pulmonary blood flow and cyanosis. These include PS, a VSD, dextroposition of the aortic valve such that it overrides the ventricular septum, and right ventricular hypertrophy. More recently, these findings have all been attributed to an anterior and cephalad deviation of the outlet septum, resulting in RVOT obstruction and the malalignment of VSD, with hypertrophy of the RV that is the result of the RVOT obstruction (Fig. 6).

The degree of cyanosis in TOF depends on the degree of RVOT obstruction, which affects the degree of right-to-left shunting across the VSD. Neonates with severe RVOT obstruction are cyanotic at birth and sometimes require the patency of the ductus arteriosus to maintain adequate pulmonary blood flow. On the other hand, neonates with mild-to-moderate obstruction may have

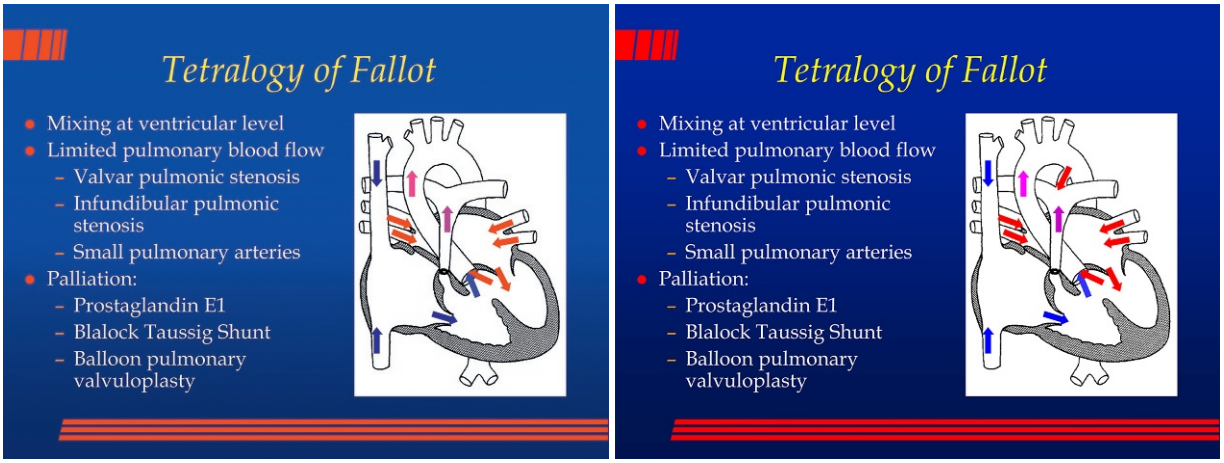

Fig. 6 Tetralogy of Fallot. Cyanosis results from right-to-left ventricular shunting caused by obstruction of the right ventricular outflow tract. Reopening the ductus arteriosus with prostaglandin infusion can allow left-to-right ductal shunting, increasing pulmonary blood flow, and increasing the amount of oxygenated blood in the mixture of systemic and pulmonary venous blood flowing out the aorta, thus improving systemic arterial oxygen saturation.

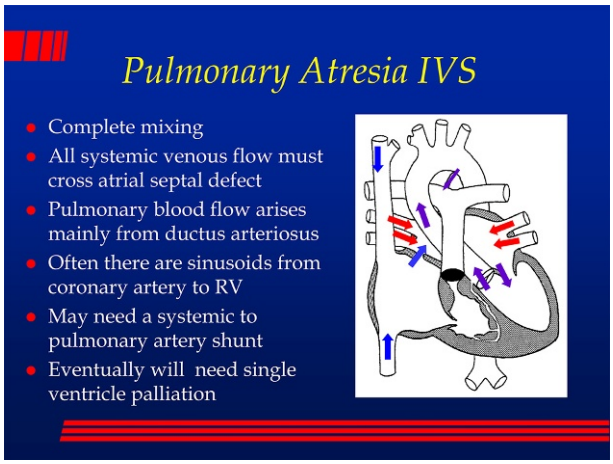

Fig. 7 Pulmonary atresia with intact ventricular septum. Complete mixing occurs at the atrial level because of the absence of forward flow across the pulmonary valve. Obstruction of the pulmonary valve results in high right ventricular pressures, often resulting in right ventricular sinusoids and coronary artery fistulae. Pulmonary blood flow prior to intervention is dependent on ductal patency for left-to-right ductal flow.

no visible cyanosis at rest and are instead diagnosed after detection of a heart murmur or cyanosis with crying. As the PVR declines in the first few days of life, pulmonary blood flow may increase, right-to-left shunting may decrease, and cyanosis may become less apparent. Over weeks to months, however, progressive right ventricular hypertrophy often leads to worsening of subpulmonary infundibular obstruction and increased cyanosis, as well as increased likelihood of acute hypercyanotic episodes known as "tet spells." These episodes are thought to be related to a combination of decreased preload of the RV, increased afterload of the RV, perhaps related to dynamic obstruction from an increased contractile state of the outflow tract, and decreased systemic vascular resistance. Beta-blocker therapy to slow the heart rate, increase diastolic ventricular filling, and decrease dynamic RVOT obstruction may prevent or decrease these spells, but surgical intervention is generally indicated when cyanosis is observed.

Pulmonary atresia with intact ventricular septum

Pulmonary atresia with intact ventricular septum (PA/IVS) occurs in approximately 1 in 15,000 live births (Daubeney et al., 1998; Ferencz et al., 1985). In PA/IVS, the absence of a VSD precludes ventricular right-to-left shunting, resulting in significantly elevated right ventricular pressures, impaired right ventricular diastolic filling, and right-to-left shunting at the atrial level. Pulmonary blood flow is achieved through the PDA (Fig. 7). When the tricuspid valve is competent, the RV is usually diminutive and often quite hypertensive, sometimes suprasystemic (higher than the left ventricular pressure). When there is significant tricuspid insufficiency, the right ventricular systolic pressure is usually not suprasystemic. It is thought that pulmonary atresia that occurs early in right ventricular development results in a diminutive RV, while pulmonary atresia that occurs late in development may be associated with a right ventricular size that is closer to normal size.

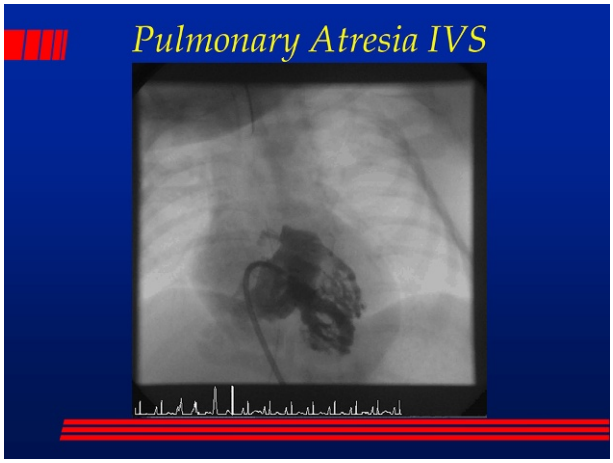

Fig. 8 Pulmonary atresia with intact ventricular septum. An anterior–posterior projection of a right ventriculogram demonstrates retrograde flow through right ventricular sinusoids to the coronary arterial system.

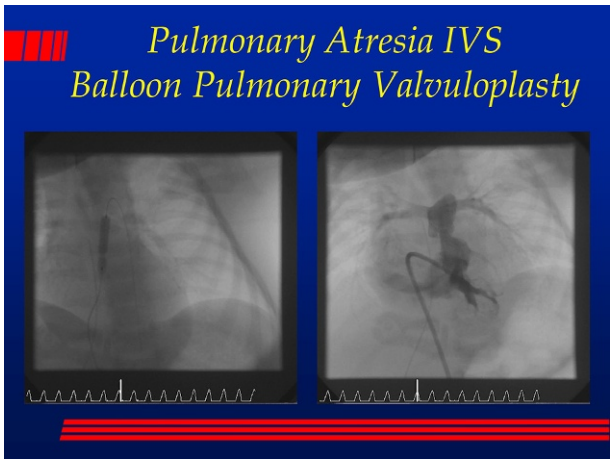

Fig. 9 Pulmonary atresia with intact ventricular septum balloon pulmonary valvuloplasty. Left panel: Following radiofrequency perforation of an atretic pulmonary valve, a balloon is positioned over a wire across the valve and expanded to dilate the pulmonary valve. Right panel: Following valvuloplasty, contrast flows from the right ventricle to the pulmonary arteries.

In utero, the absence of right ventricular outflow through the pulmonary valve may result in retrograde flow through sinusoids connected to the coronary arterial system (Fig. 8). These may be associated with absent aorticocoronary connections, coronary stenoses or interruption of a coronary artery at its aortic origin, as well as coronary-cameral fistulae, in which coronary arteries connect directly to the RV, potentially leading to a steal phenomenon. In these scenarios, coronary arterial flow is dependent on the high-pressured RV, and interventions to relieve the right ventricular pressure may result in myocardial ischemia and death. When there are RV-dependent coronary arteries, heart transplantation is often preferable to surgical palliation or balloon dilatation of the atretic pulmonary valve (Fig. 9).

Critical pulmonary stenosis

The physiology of critical PS is similar to that of PA/IVS, except that elevated right ventricular pressures typically do not lead to the coronary artery abnormalities discussed above. In critical PS, pulmonary blood flow is limited by an abnormal pulmonary valve, often thickened and with decreased valve leaflet mobility (Fig. 10). Similar to TOF, progressive right ventricular hypertrophy also may contribute to RVOT obstruction below the valve. If the RVOT obstruction is severe, impaired diastolic function with decreased ventricular relaxation will lead to impaired ventricular filling and increased right atrial pressure, causing right-to-left atrial shunting across the foramen ovale, and cyanosis.

Obstruction at the Pulmonary Arteries and Arterioles With PDA

When a PDA is present in combination with downstream obstruction to pulmonary blood flow, right-to-left ductal shunting can lead to differential cyanosis, in which blood flow to the upper body is normally saturated but that to the lower body is desaturated.

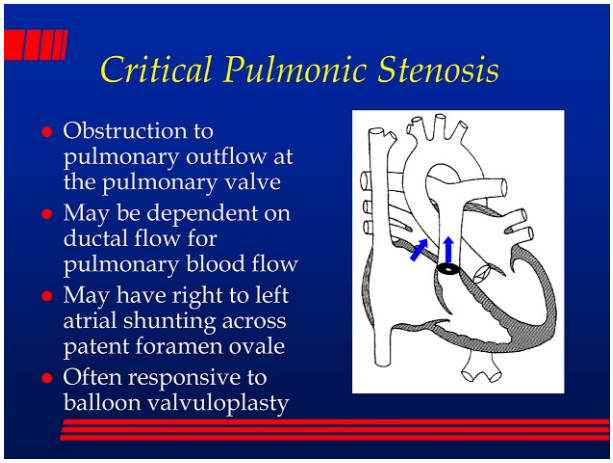

Fig. 10 Critical pulmonary stenosis. Obstruction to pulmonary blood flow results in cyanosis from right-to-left atrial shunting. This lesion is often responsive to balloon valvuloplasty.

Peripheral pulmonary artery stenosis may be seen in Williams syndrome, Alagille syndrome, or congenital rubella infection, among other systemic diseases. If the stenosis is bilateral, peripheral pulmonary artery stenosis can manifest with cyanosis, owing to elevated pressure in the proximal pulmonary artery that leads to right-to-left PDA shunting. However, if the stenosis is unilateral, cyanosis will generally not be present, because flow will simply increase to the unaffected branch pulmonary artery. PDA shunting will be left-to-right in this case.

Elevated PVR in noncardiac causes of pulmonary artery hypertension (PAH) may result in cyanosis from right-to-left shunting across the PDA caused by obstruction of flow within the arterioles of the pulmonary vascular bed itself. There are many causes of PAH, including idiopathic PAH, heritable PAH, persistent pulmonary hypertension of the newborn (PPHN), chronic lung disease, interstitial lung disease, and developmental lung diseases. Premature infants are particularly prone to PPHN early in life and chronic lung disease over time. In addition to right-to-left ductal shunting in infants with PAH, right atrial hypertension is common in these infants, causing right-to-left atrial shunting across the foramen ovale.

Representative Lesions With Unfavorable Streaming

Some relationships of cardiac structures can favor streaming of deoxygenated systemic venous blood to the systemic arterial circulation. This phenomenon is referred to as unfavorable streaming. Transposition of the great arteries (TGAs) is the prototype lesion for this phenomenon, but "transposition physiology" can be seen in other congenital heart lesions.

d-Transposition of the Great Arteries

TGAs account for 5% of all congenital heart defects, with an incidence of approximately 1 per 30,000 live births (Ferencz et al., 1985; Hoffman and Kaplan, 2002). By definition, TGA consists of ventriculoarterial discordance, with the morphologic RV giving rise to the aorta, and the morphologic LV giving rise to the pulmonary artery. In d-TGA, sometimes known as "simple" transposition, the systemic veins return normally to the right-sided systemic venous atrium and the pulmonary veins return normally to the left-sided pulmonary venous atrium, there is atrioventricular concordance with the RA filling the right-sided morphologic RV and the left atrium filling the left-sided morphologic LV (d-looped ventricles), but there is ventriculoarterial discordance. This discordance results in the pulmonary artery and aorta arising from the LV and RV, respectively, resulting in two parallel circulations (Figs. 11–13).

Survival of infants with d-TGA after birth requires mixing between the systemic and pulmonary circulations, as without mixing, oxygenated blood cannot get from the lungs to the systemic circulation. Fortunately, most infants with d-TGA have both atrial mixing at the foramen ovale and great arterial mixing at the ductus. In d-TGA, at steady state, there cannot be any net flow from the systemic circulation to the pulmonary circulation (or vice versa), as net shunting in either direction would result in all of the blood accumulating in one of the two parallel circulations. The shunting from the pulmonary circuit to the systemic circuit must be exactly equal to the shunting from the systemic circuit to the pulmonary circuit. Because the two shunts must be equal, a single site of mixing is usually not adequate, as there would have to be bidirectional shunting at different times in the cardiac cycle. Usually, a VSD or PDA alone is much less efficient in allowing mixing of oxygenated and deoxygenated blood than is an ASD alone, and the most efficient mixing is the combination of a PDA and an atrial communication, with the PDA allowing shunting of deoxygenated blood from the aorta to the pulmonary arteries, and the ASD allowing shunting of oxygenated blood from the left atrium to the RA, RV, and aorta. In the absence of an adequate atrial communication, it is often necessary to perform a balloon atrial septostomy (BAS), discussed in more detail in the following section on interventions (Fig. 14).

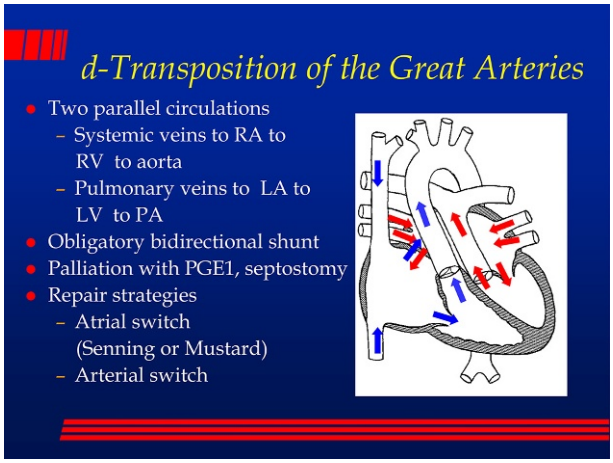

Fig. 11 d-Transposition of the great arteries. Two parallel circulations result when the great arteries are transposed. Because there can be no net right-to-left or left-to-right shunting with parallel circulations, multiple sites of mixing are typically needed for adequate systemic oxygen delivery. Patency of the ductus arteriosus can be maintained with a prostaglandin infusion, and emergent balloon atrial septostomy can improve atrial mixing.

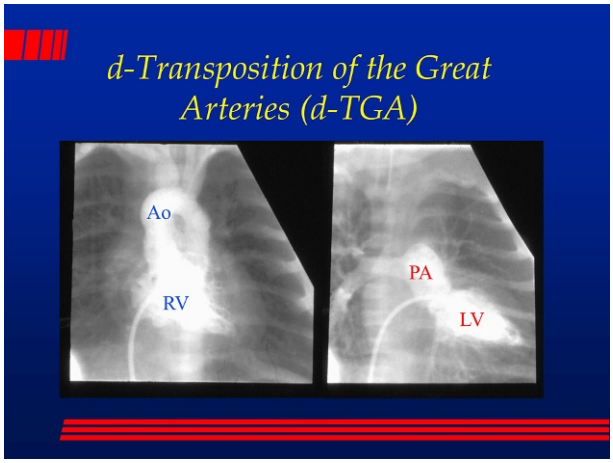

Fig. 12 d-Transposition of the great arteries. Right and left ventriculograms reveal ventriculoarterial disconcordance. Left panel: The right ventricle (RV) gives rise to the aorta (Ao). Right panel: The left ventricle (LV) gives rise to the pulmonary artery (PA).

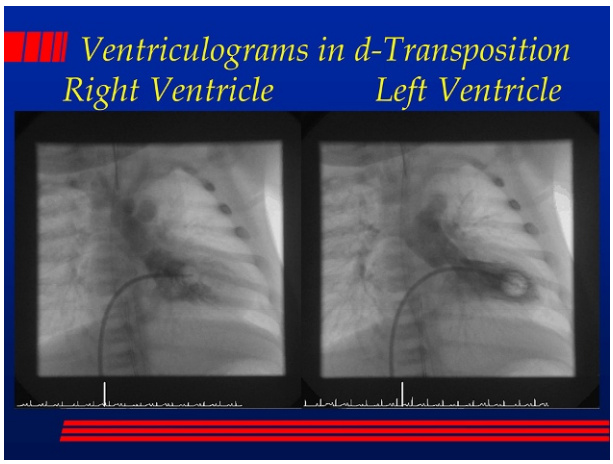

Fig. 13 Ventriculograms in d-transposition. Right and left ventriculograms reveal ventriculoarterial disconcordance. Left panel: The right ventricle gives rise to the aorta, with filling of the pulmonary artery through the patent ductus arteriosus. Right panel: The left ventricle gives rise to the pulmonary artery. In levophase (after the contrast has returned from the pulmonary circulation to the left atrium), there is filling of the right atrium through the atrial septal defect, with subsequent filling of the right ventricle.

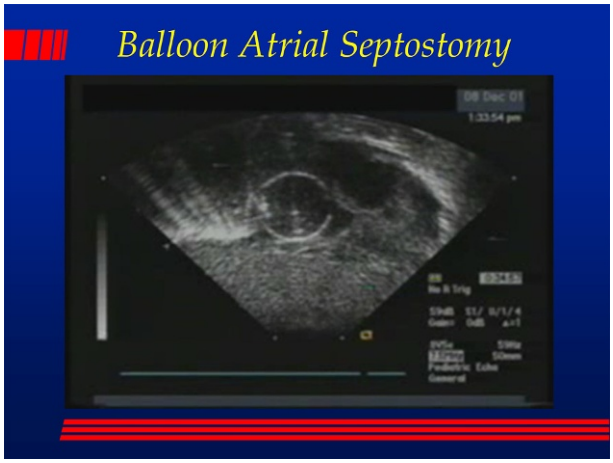

Fig. 14 Balloon atrial septostomy. In a Rashkind balloon atrial septostomy, a balloon tipped catheter is positioned from the femoral or umbilical vein, through the right atrium, across the small atrial septal communication into the left atrium. Under echocardiographic guidance, the balloon is inflated with fluid and is abruptly pulled back to the right atrium, tearing the atrial septum in the process. Dr. William Rashkind, the developer of the procedure, was said to have commented that the most important thing about a balloon septostomy was the "jerk on the end of the catheter."

d-TGA may result in "reverse differential cyanosis," with desaturation and cyanosis of the upper body and higher saturations in the lower body. This phenomenon typically occurs while PVR is high shortly after birth, when oxygenated blood flows from the pulmonary artery to the descending aorta, because PDA flow early on is bidirectional. With a combination of d-TGA and aortic arch obstruction, either from coarctation or from interrupted aortic arch, the flow from the RV through the ductus is the main source of blood flow to the descending aorta, making the reverse differential cyanosis more prominent. Obtaining saturation levels in the right arm and in the legs can be diagnostically useful in suggesting d-TGA when the lower body saturation are higher than the upper body. The other situation in which one may see higher oxygen saturations in the lower body is in infants with totally anomalous pulmonary venous return to the SVC, as SVC flow preferentially streams to the tricuspid valve, resulting in higher oxygen saturations in the pulmonary artery than in the ascending aorta, as discussed in the following.

Other Lesions With Unfavorable Streaming

Other scenarios with unfavorable streaming are relatively rare. For example, persistence of the left cardinal vein in embryologic development results in a left superior vena cava (LSVC) that drains to the RA via the coronary sinus (CS). When the CS is partially or completely "unroofed" to the LA, a left-to-right shunt may occur, similar in physiology to an ASD. However, if the LSVC flow is vigorous and the CS flow is directed into the left atrium rather than through the CS or to the RA, deoxygenated systemic venous blood may enter the systemic cardiac output, resulting in cyanosis.

Representative Lesions With Complete Mixing and Decreased Pulmonary Blood Flow

Although there is conceptual overlap between right-sided obstructive lesions with right-to-left shunting and complete mixing lesions, the authors find it helpful to discuss complete mixing lesions separately.

Atresia of Any Valve

Atresia of any valve results in complete mixing of systemic and pulmonary venous blood, with all of the cardiac output crossing the corresponding nonatretic valve. For example, in tricuspid atresia, all flow must cross the mitral valve, and in pulmonary atresia, all flow must cross the aortic valve. Complete mixing occurs with atresia of left-sided valves as well. Infants with complete mixing lesions may or may be cyanotic depending on the relative pulmonary and systemic blood flows. In complete mixing lesions, the determinants of the arterial oxygen saturation are the pulmonary venous saturation (How red is the red blood?), the systemic venous saturation (How blue is the blue blood?), and the relative amounts of each, expressed as the Q_P/Q_S, or the ratio of pulmonary to systemic blood flow, as illustrated in Fig. 15. Even without changes in the underlying cardiac anatomy, there can be changes in these parameters that dramatically change arterial oxygen saturation, resulting in development or resolution of cyanosis.

For example, in an infant with tricuspid atresia with a restrictive VSD, the oxygen saturation may fall precipitously with ductal closure. One day after birth, when the ductus arteriosus is still patent, there may be much more pulmonary blood flow than systemic blood flow, sometimes resulting in a Q_P/Q_s of about 3/1. Assuming a mixed venous saturation of 60% and a pulmonary venous

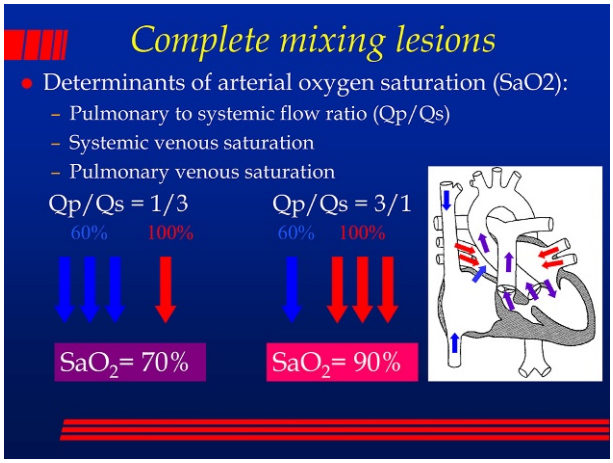

Fig. 15 Complete mixing lesions. Systemic arterial oxygen saturations in complete mixing lesions are dependent on the relative amounts of systemic (Qs) and pulmonary (Qp) blood flow. In this example with 100% pulmonary venous saturation and 60% systemic venous saturation, a systemic arterial saturation of 70% would reflect restricted pulmonary blood flow, with a Qp/Qs of 1/3. A systemic arterial saturation of 90% would reflect exuberant pulmonary blood flow, with a Qp/Qs of 3/1.

saturation of 100%, in this scenario, there would be three parts pulmonary venous blood and one part systemic venous blood in the mix, resulting in an arterial saturation of 90%. When the ductus closes, there would be much less pulmonary blood flow, perhaps resulting in a Q_P/Q_s of 1/3. In that scenario, there would be three parts systemic venous blood and one part pulmonary venous blood, resulting in an arterial oxygen saturation of 70%. This is actually an oversimplification, as the systemic venous saturation would also fall, so the arterial saturation would probably actually be even lower after ductal closure. These principles apply to all variants of valve atresia, with right-sided atresia (tricuspid and/or pulmonary atresia) being more likely to result in cyanosis, and left-sided atresia (mitral and/or aortic atresia) resulting in decreased systemic perfusion with only mild cyanosis.

Truncus Arteriosus, Double Inlet LV, and Other Mixing Lesions With Favorable Streaming

In some lesions, such as truncus arteriosus or double inlet LV, there may not be complete mixing, as there may be favorable streaming of pulmonary venous return to the aorta and systemic venous return to the pulmonary artery. For this reason, truncus arteriosus without branch pulmonary artery stenosis does not usually present with clinical cyanosis because of favorable streaming and increased Q_P/Q_s. Similarly, infants with single ventricle, such as double inlet LV, may have favorable streaming and minimal cyanosis. Although infants with mitral and/or aortic atresia have complete mixing, they often have a high Q_P/Q_s.

Total Anomalous Pulmonary Venous Return

Total anomalous pulmonary venous return (TAPVR) to the systemic veins results in nearly complete mixing, with the presence and degree of cyanosis determined by the Q_P/Q_s. In TAPVR, the confluence of the pulmonary veins (also known as the common pulmonary vein) fails to integrate into posterior wall of the fetal left atrium. Thus, the pulmonary veins typically return to a confluence that drains into one of the systemic veins. If the pulmonary venous drainage is obstructed at any level, a life-threatening scenario develops, wherein there is little benefit to medical intervention, requiring emergent surgical intervention to relieve the obstruction and direct the pulmonary venous return to the left atrium.

Three variants of TAPVR are commonly seen. The most common form is supracardiac TAPVR, in which the pulmonary venous confluence drains cephalad via a vertical vein to the left innominate vein (Fig. 16). The vertical vein is usually unobstructed, but external compression can lead to obstruction of pulmonary venous drainage as it passes between the left bronchus and left pulmonary artery. In the second TAPVR variant, the confluence drains directly to the CS. This connection is almost universally unobstructed. The final variant involves caudal drainage from the retrocardiac confluence via a vertical vein to the umbilicovitelline system, typically to the portal vein (Fig. 17). This form of TAPVR almost always involves obstructed pulmonary venous drainage.

In TAPVR, the foramen ovale must remain patent to allow right-to-left shunting to the left atrium. Often, the ductus arteriosus also remains patent, but this may or may not help with hemodynamic stability after birth. With falling PVR in the first few days of life, pulmonary blood flow increases dramatically and commonly exceeds systemic blood flow, sometimes giving a Q_P/Q_s of 3/1 or more. Therefore, when the pulmonary venous drainage is unobstructed, systemic oxygen saturations may exceed 90%. However, neonates with obstructed TAPVR typically present with profound cyanosis as a result of the combination of decreased pulmonary blood flow from the downstream obstruction as well as pulmonary edema from high pulmonary venous pressures. This pulmonary edema does not improve with oxygen therapy. Indeed, pulmonary vasodilators (such as oxygen) can lead to rapidly worsening pulmonary edema by allowing arteriolar dilation with ongoing downstream obstruction (Fig. 18).

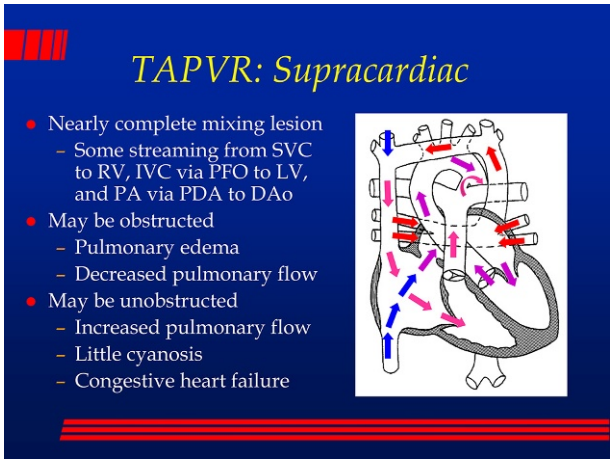

Fig. 16 Supracardiac total anomalous pulmonary venous return (TAPVR). An ascending vertical vein drains the oxygenated pulmonary venous blood to the innominate vein. Preferential streaming of superior vena caval blood through the tricuspid valve to the right ventricle and then through the ductus arteriosus to the descending aorta, and streaming of IV blood across the foramen ovale to the left ventricle may result in reverse differential cyanosis, with higher arterial oxygen saturations in the lower body than in the upper body.

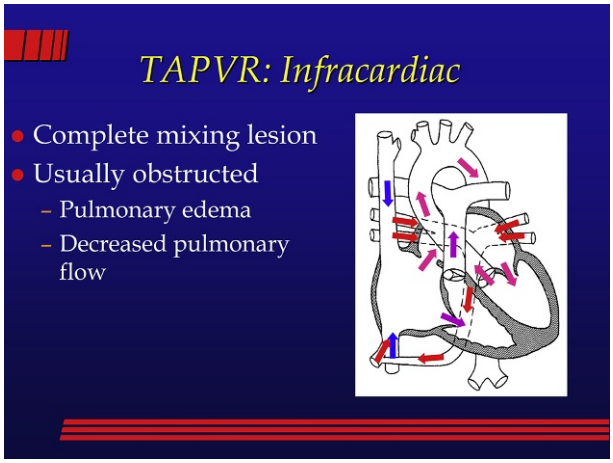

Fig. 17 Infracardiac total anomalous pulmonary venous return (TAPVR). A descending vertical vein drains the pulmonary venous confluence through the liver. This drainage is almost always obstructed, resulting in pulmonary venous congestion.

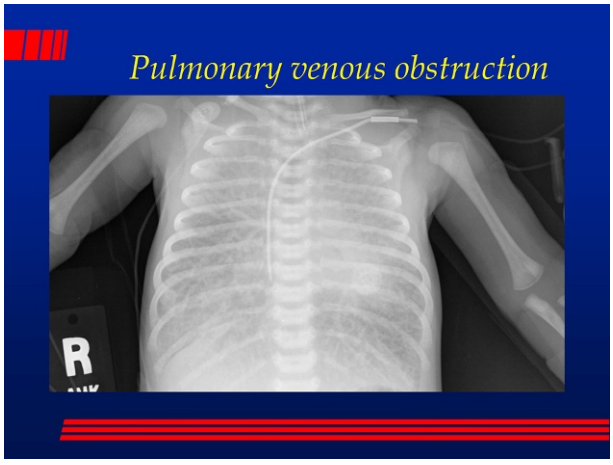

Fig. 18 Chest radiograph showing pulmonary venous obstruction. When total anomalous pulmonary venous return is obstructed, pulmonary blood flow is reduced, and pulmonary edema may further worsen cyanosis because intrapulmonary oxygen diffusion is impaired, causing pulmonary venous desaturation.

Just as d-TGA can lead to reverse differential cyanosis, so can supracardiac TAPVR. In this scenario, highly oxygenated SVC flow that includes the anomalous pulmonary venous return is preferentially directed toward the right heart through the tricuspid valve, and deoxygenated IVC flow is preferentially directed toward the left heart through the PFO. In this scenario, when the PVR is high, oxygenated blood from the pulmonary artery shunts across the PDA from right to left into the descending aorta, resulting in higher oxygen saturations in the lower body than in the upper body.

Interventions for Congenital Heart Disease

Supplemental Oxygen

Often, cyanosis in a newborn infant is the result of lung disease that results in pulmonary venous desaturation. However, infants with lung disease often normalize their arterial oxygen saturations with supplemental oxygen, as the increased partial pressure of oxygen in the alveoli can overcome diffusion barriers and normalize the pulmonary venous saturation. When a newborn with cyanosis does not respond to supplemental oxygen, cyanotic congenital heart disease must be suspected.

Because pulmonary venous blood is normally nearly 100% saturated in the absence of lung disease, in patients with cyanotic heart disease and right-to-left shunting of desaturated systemic venous blood, supplemental oxygen only slightly increases pulmonary venous oxygen content by increasing dissolved oxygen. Even a 300 torr increase in the P_{O_2} results in an increase of only 0.9 mL dL^{-1} (300 torr $\times$ 0.003 mL dL^{-1} $torr^{-1}$) in the oxygen content. For an infant with a normal hemoglobin of 17 g dL^{-1}, a pulmonary venous oxygen saturation of 100%, and an oxygen content of 23 mL dL^{-1} (1.36 mL g^{-1} of hemoglobin $\times$ 17 g hemoglobin dL^{-1}), the pulmonary venous oxygen content increases less than 4%, even with a marked 300 torr increase in the pulmonary venous P_{O_2}.

Since the shunting of systemic venous blood in infants with cyanotic heart disease is minimally affected by supplemental oxygen, there is little change in the arterial oxygen saturation with supplemental oxygen in these infants. This observation is the basis for the "hyperoxia test," in which the P_{O_2} of an arterial blood gas is compared before and after administration of 100% oxygen, with a $P_{O_2} < 150$ torr on 100% oxygen (FiO_2 of 1.0) strongly suggesting the presence of congenital heart disease.

Maintaining Ductal Patency

Often, ductal closure reduces pulmonary blood flow in infants with cyanotic heart disease, and it is at the time of ductal closure that cyanosis is first recognized. Fig. 19 illustrates the impact of ductal closure in tricuspid atresia. If an infant is suspected or confirmed to have cyanotic congenital heart disease, the PDA can be artificially maintained by administration of intravenous prostaglandin E_1 (PGE_1). Commonly observed side effects of PGE_1 therapy are apnea, fever, hypotension, and pulmonary vasodilatation. However, when these side effects are appropriately managed, maintaining ductal patency is often the simplest strategy for stabilizing an infant with a ductal-dependent circulation. Often, reducing the dose of PGE_1 to the minimal effective dose that maintains ductal patency avoids these side effects. Maintaining ductal patency is an effective short-term strategy for cyanotic heart disease, irrespective of whether the cause is limitation of pulmonary blood flow in right-heart obstructive lesions or inadequate mixing in unfavorable streaming in transposition physiology.

In complete mixing lesions, the management challenge lies in appropriately balancing the pulmonary and systemic blood flows through the transition period. PGE_1 is a potent pulmonary vasodilator, as is oxygen. As the PVR drops, pulmonary blood flow

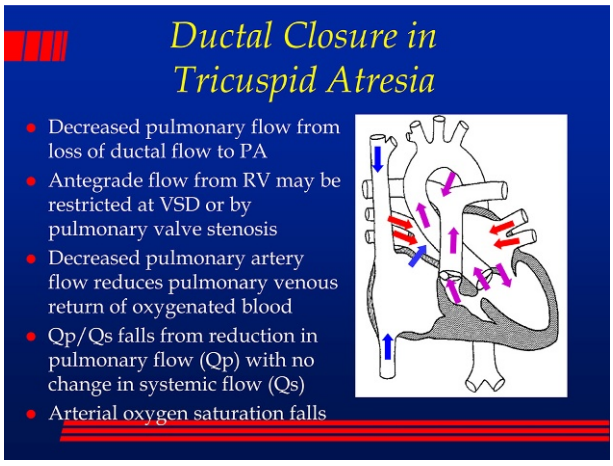

Fig. 19 Ductal closure in tricuspid atresia. When an infant is not prenatally diagnosed with a ductal-dependent cyanotic congenital heart defect, ductal closure results in decreased pulmonary blood flow and systemic arterial saturation. Complete mixing in tricuspid atresia necessitates a careful balance of systemic and pulmonary blood flow, which cannot be maintained if the ductus arteriosus closes.

increases, increasing the overall work of the heart and ultimately leading to CHF. In the presence of a PDA, pulmonary overcirculation not only leads to CHF but also can compromise systemic perfusion of vital organs by allowing shunting of the systemic output to the lungs, decreasing splanchnic perfusion and placing the infant at risk for intestinal ischemia and necrotizing enterocolitis.

Recently, percutaneous placement of a stent in the ductus arteriosus has been used as a more stable alternative to long-term PGE_1 infusions in neonates who require long-term ductal patency and are not anticipated to undergo surgical repair or palliation prior to hospital discharge. However, ductal stenting is not without procedural risk and possibility of complications, for example, from stent migration, misplacement, or subsequent stent thrombosis. In addition, ductal stents may complicate the eventual surgical intervention by distorting growth of the pulmonary arteries.

Balloon Atrial Septostomy

Infants who depend on an atrial communication may require emergent intervention after birth if the ASD or PFO is too small for adequate intracardiac mixing. This is particularly important for transposition physiology and left-sided obstructive disease. In these cases, a BAS procedure enlarges the atrial communication to stabilize the infant. In this procedure, a balloon-tip catheter is placed across the small ASD under fluoroscopic or echocardiographic guidance. The balloon is inflated and pulled back to tear the atrial septal tissue and enlarge the communication (see **Fig. 14**). Over time, the atrial septal tissue may close the artificially enlarged defect. In those infants who require a longer-term solution, a stent may also be placed in the atrial septum to maintain unrestricted atrial level mixing until surgical intervention.

Balloon Pulmonary Valvuloplasty

Balloon pulmonary valvuloplasty is a useful percutaneous intervention for infants with critical PS or a membranous form of pulmonary atresia (see **Fig. 9**). Inflating a balloon positioned in the pulmonary valve annulus tears the valve (typically between fused valve leaflets) to relieve the obstruction to pulmonary blood flow. The major risk of this procedure is valve insufficiency, which over time may cause problems and require further intervention. However, balloon pulmonary valvuloplasty is an effective and well-established procedure to obviate the need for a surgical intervention in the neonatal period.

Select Surgical Interventions

Blalock–Taussig (systemic to pulmonary artery) shunt

Advances in surgical techniques over the last few decades have enabled either palliation or repair of most congenital cardiac lesions. The first noteworthy surgical intervention for cyanotic heart disease is referred to as the Blalock–Taussig (BT) shunt, in reference to the surgeon Dr. Alfred Blalock and cardiologist Dr. Helen Taussig who first described the procedure. Dr. Taussig had sought an intervention to increase pulmonary blood flow in patients with TOF. Dr. Blalock, with the assistance of his lab technician Vivian Thomas, performed the first operation in a human in 1944, effectively launching the field of surgery for congenital heart disease.

The BT shunt is a systemic-to-pulmonary artery shunt, serving as an artificial ductus (**Fig. 20**). Originally performed as a direct anastomosis from the right subclavian artery to the pulmonary artery, it now is generally performed as a "modified" version, using a synthetic tube from the right innominate artery to the proximal right pulmonary artery (RPA). The BT shunt is used either as a bridge

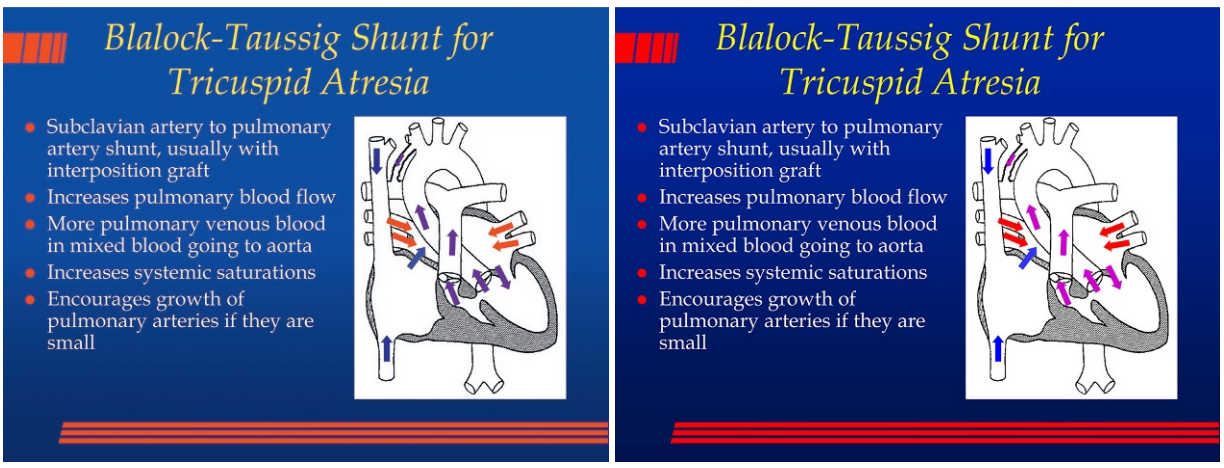

Fig. 20 Blalock–Taussig shunt for tricuspid atresia. If adequate pulmonary blood flow is not achieved without the ductus arteriosus, a Blalock–Taussig shunt may be placed to provide a stable blood supply to the pulmonary arteries. The flow through the shunt increases pulmonary blood flow. With increased pulmonary blood flow, there is more oxygenated blood in the mixture of systemic and pulmonary venous blood, resulting in a higher arterial oxygen saturation.

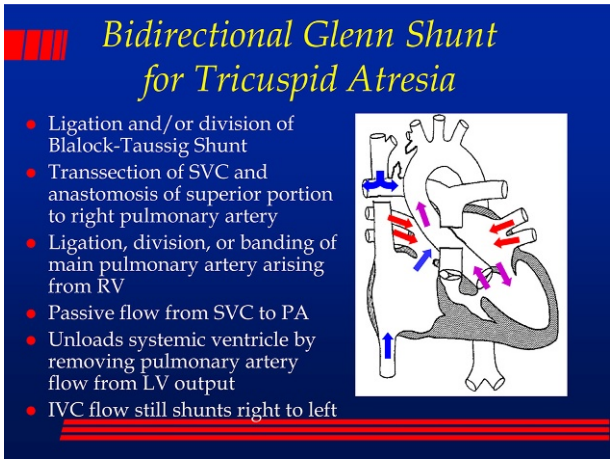

Fig. 21 Bidirectional Glenn shunt for tricuspid atresia. As the second stage of single ventricle palliation, the superior cavopulmonary anastomosis, known as the bidirectional Glenn shunt, allows passive flow from the head and arm veins to the pulmonary arteries.

to more definitive repair or as part of the first operation for single ventricle palliation. BT shunts are not without risk. Thrombosis and shunt occlusion pose a significant risk of sudden death. Therefore, the next operation is typically performed within the first year of life, depending on the patient and the procedure.

Single ventricle palliation

Single ventricle palliation is reserved for patients without two functional ventricles. The operations that follow the initial neonatal intervention (usually the BT shunt with or without another intervention) are typically performed in two additional stages. The operations that comprise staged single ventricle palliation ultimately result in systemic venous blood passively flowing through the pulmonary vascular bed before returning to the heart. Therefore, cardiac catheterization with confirmation of low PVR is typically required prior to each operation. Usually, the superior cavopulmonary anastomosis ("Glenn" shunt) is performed first. Although originally described as a division of the RPA, with an end-to-side surgical anastomosis from the RPA to the SVC, with ligation or division of the connection of the SVC to the RA, it is now generally performed as a "bidirectional Glenn shunt" with division of the SVC, an end-to-side anastomosis of the SVC to the superior surface of the RPA, and closure of the stump of the SVC above its junction with the RA (Fig. 21), around 4–6 months of age. In some circumstances in which the RV is small, but adequate to handle the venous return from the lower body, the bidirectional Glenn shunt may be the final operation, known as a 1½-ventricle repair. However, intrapulmonary arteriovenous malformations tend to form over time in situations where the pulmonary vascular bed is not directly exposed to hepatic venous blood on its first pass.

For this reason, most patients who have undergone the Glenn will undergo connection of the IVC with the pulmonary artery ("Fontan" operation). This operation can be accomplished with an extracardiac synthetic tube or as an intraatrial lateral tunnel (Fig. 22). The same general approach is used in other forms of single ventricle physiology, including double inlet LV, unbalanced atrioventricular septal defect, and hypoplastic left heart syndrome.

Repair of TOF

Patients with TOF typically undergo "complete" repair before 6 months of age. If the pulmonary valve annulus is adequate, a "valve-sparing" repair is used, with the VSD closure and subpulmonary muscle resection performed from a transatrial and transpulmonary approach. However, if the pulmonary valve annulus is too small, repair is usually accomplished with a transannular patch extending from the RVOT to the main pulmonary artery, relieving the RVOT obstruction but resulting in a freely insufficient pulmonary valve (Fig. 23).

Repair of d-transposition of the great arteries

The first successful repairs of d-TGA were atrial switch operations, baffling the systemic venous return to the LV and out to the pulmonary artery, and the pulmonary venous return to the RV and out to the aorta (Fig. 24). There were two common versions of the atrial switch operation: the Senning procedure and the Mustard procedure. While the operative mortality of these operations was quite low, these procedures resulted in the RV serving as the systemic ventricle. This had the disadvantage of an increased risk for ventricular dysfunction developing over time, as well as atrial arrhythmias and caval obstruction. Technical improvements in coronary reimplantation to the "neo-aortic" root in the 1970s and 1980s enabled a shift from the atrial switch to the arterial switch operation (ASO), which is now the standard operation for TGA (Fig. 25). Due to significant heterogeneity in coronary artery anatomy in TGA, the coronary arteries must be well delineated on preoperative evaluation.

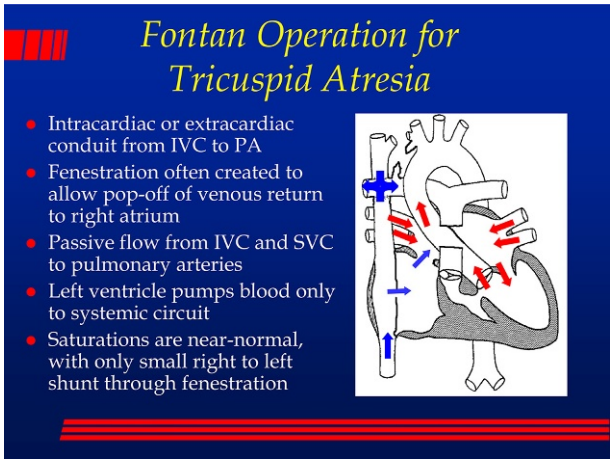

Fig. 22 Fontan operation for tricuspid atresia. Typically the final stage in single ventricle palliation, an inferior cavopulmonary connection is created in the Fontan operation, resulting in almost all systemic venous blood passively flowing through the lungs prior to returning to the heart. The presence of a fenestration allows a small amount of systemic venous return to bypass the lungs, improving stability in the early postoperative period, but causing some cyanosis, usually only mild.

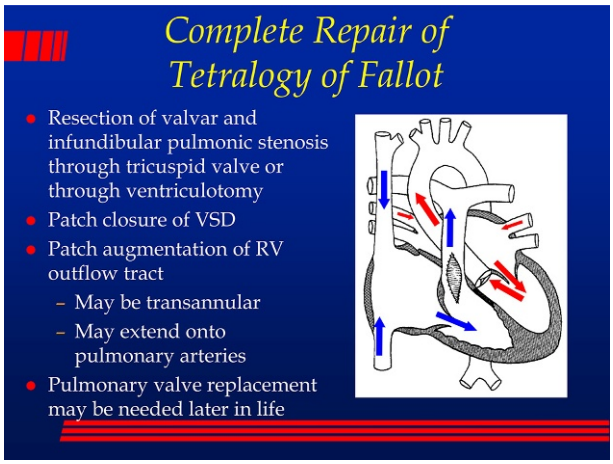

Fig. 23 Complete repair of tetralogy of Fallot. In complete repair of tetralogy of Fallot, the ventricular septal defect is patched closed, and the right ventricular outflow tract obstruction is relieved by resection of infundibular muscle, and patch augmentation of the right ventricular outflow tract, in most cases. Extension of the outflow tract patch across the pulmonary valve results in pulmonary valve insufficiency, which may necessitate pulmonary valve replacement later in life.

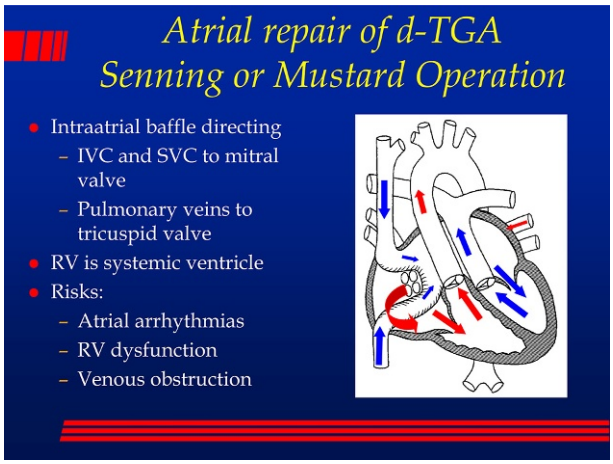

Fig. 24 Atrial repair of d-transposition of the great arteries with the Senning or Mustard operation. Intraatrial baffles are used to direct systemic venous blood to the left ventricle, which pumps it to the pulmonary artery, and to direct pulmonary venous blood to the right ventricle, which pumps it to the aorta. Atrial arrhythmias, right ventricular dysfunction, and venous obstruction are common complications of this approach.

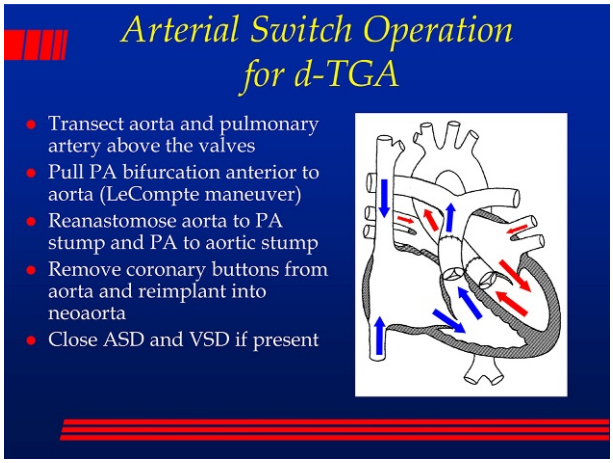

Fig. 25 Arterial switch operation for d-transposition of the great arteries. Advances in surgical technique for manipulation of coronary arteries have more recently enabled the arterial switch operation. This operation results in the systemic ventricle being the left ventricle, which generally has greater longevity than a systemic right ventricle.

Survival and Outcomes

As discussed earlier, advances in medical and surgical interventions have resulted in improved survival. However, even when the surgical repair is considered "complete," many of these children develop long-term sequelae that necessitate lifelong follow-up with a cardiologist.

Long-term complications of single ventricle palliation include arrhythmias, heart failure, protein-losing enteropathy, plastic bronchitis, and liver failure. Survival after Fontan operation depends partly on whether the single functional ventricle is a morphologic LV or RV, with better outcomes in single LVs. Survival after single ventricle palliation for tricuspid atresia is 79%, 62%, and 45% at 10, 20, and 30 years following operation (Pundi et al., 2015).

Outcomes of TOF repair are quite good, with operative survival greater than 96% and 30-years survival above 85% (Alexiou et al., 2001; Murphy et al., 1993). However, progressive right ventricular dilatation from chronic pulmonary insufficiency can lead to right ventricular, and ultimately left ventricular, dysfunction over time. Pulmonary valve replacement is an attractive option in this case, but the optimal timing of this operation is the subject of significant debate, since reoperation is not entirely without risk, and current options for pulmonary valve replacements are unlikely to last as long as patients with TOF are likely to live, necessitating multiple pulmonary valve replacements. The goal of pulmonary valve replacement is to remove the volume load on the RV before there is irreversible impairment of right ventricular function. Over the past 10 years, transcatheter pulmonary valve replacement has become a good option for some patients following repair of TOF.

Outcomes of the ASO for transposition are now excellent, with operative survival approaching 100% in high-volume centers and overall 5-year survival above 96% (Dibardino et al., 2004).

Conclusion

Cyanotic congenital heart disease is a heterogeneous group of abnormalities of cardiac development that result in deoxygenated blood being pumped to the body without first passing through the lungs. This requires both a site of mixing and another factor that preferentially directs blood flow from the systemic veins to the aorta. Obstruction to pulmonary blood flow, unfavorable streaming, and complete mixing with decreased pulmonary blood flow all lead to clinical cyanosis. Fortunately, medical and surgical advances over the past decades have dramatically improved outcomes such that most infants with cyanotic congenital heart disease now have options for interventions that allow survival.

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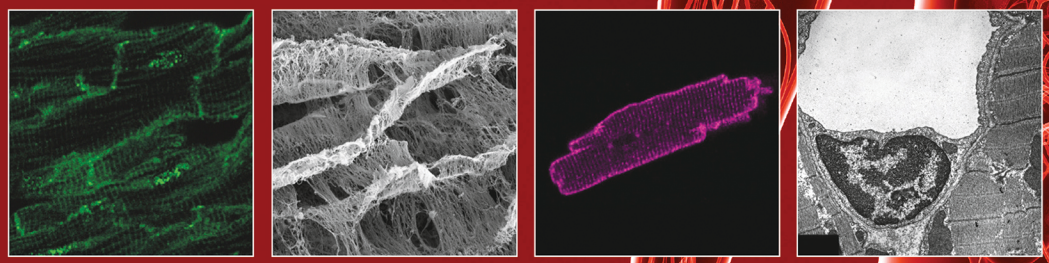

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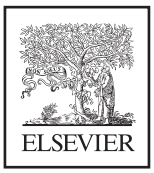

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PREFACE

The burden of cardiovascular disease is growing across the globe as the leading noncommunicable cause of morbidity and mortality. This is despite outstanding research that has improved our understanding of cardiovascular biology, the determinants of disease, and development of new therapies. We are optimistic that the next generation of scientists and clinicians will further these efforts and ultimately bring about changes that will reduce risk and improve outcomes for patients worldwide. We have assembled this encyclopedia with the explicit intent to provide this generation with easy access to past and current wisdom, with the hope that this helps to accelerate innovation in cardiovascular medicine.

This encyclopedia represents both a dream and a labor of love. The dream is to create a scientific compendium that recognizes that the methods and tools used to access and study the scientific literature have changed, with a burgeoning impact of digital technology—the so-called pocket book encyclopedia that is accessible on a smart phone fitting into medical overalls, and that can also be read as a textbook at a desk! These changes in the access of publications have been accompanied by the evolving need for studying different aspects of biology and disease as stand-alone chapters not necessarily read sequentially. Furthermore, as editors we recognize the fundamental importance of conveying knowledge through brilliantly illustrated texts that are profusely embedded with links to key references. The critical importance to use multimedia tools and to provide downloadable slides for educational purposes is reflected in this encyclopedia. Additionally, we want to ensure the feasibility of updating text, figures, and other reading material iteratively even after a publication date, recognizing the rapid advent of information and huge strides made in cardiovascular medicine every day. Thus, this work represents the end of the beginning of a live text that will metamorphose over time, being guided by the rapid strides and advances in both cardiovascular medicine and publication science.

Overall, we conceptualized this work as providing broad coverage of science and also serving the expansive needs of a readership that is quite diverse, that is, encompassing medical students, residents, fellows, and postdoctoral scientists interested in cardiovascular medicine. We hope the labor of love of an outstanding team of editors and a stellar group of authors is readily evident to the broad readership we have targeted.

Assembling this compendium had many challenges. Early on we made the choice to cover the broad range of cardiovascular biology, pathophysiology, epidemiology, and treatment of disease. We sought to assure equal coverage to foundational cardiovascular cellular and molecular biology as well as state-of-the-art treatments. Covering the breadth inevitably led to some overlap between some chapters, and yet we certainly have some residual gaps. We hope to have the opportunity to fill these gaps in future editions. There is also inevitable variation in the style and scope of articles in this multiauthor publication. We and the section editors felt it was more important to allow our expert authors freedom in how they chose to present their topic.

We invite feedback on how this encyclopedia can be improved as a resource for trainees worldwide. Undoubtedly, as noted above, there are gaps in content areas that need to be bridged in future iterations of this encyclopedia. We look forward to hearing from and listening to our readership about how the text can be better organized or better compiled to serve their scholarship better in future years.

This project was made possible through the hard work of many people from across the globe. We were pleased to have section editors and authors from around the world. Drs. Kessler and Schunkert, renowned cardiologists with an outstanding record of accomplishment in genetic research, have compiled a series of chapters on genetics and genomics. Dr. Baliga, a distinguished cardiologist and educator, has conceptualized a tour de force of chapters that capture all aspects of the heart failure syndrome, whereas Dr. DiSalvo, a master clinician and educator, has synthesized a fine tapestry of chapters focusing on diagnostic testing with key sections on cardiac imaging, interventions, and device therapy. Dr. Raj, an outstanding clinical investigator and

electrophysiologist, brought together an exemplary collection of chapters that offer coverage of a broad spectrum of topics in cardiac electrophysiology. Drs. Major and Liaw, both highly accomplished basic scientists and educators, were instrumental in identifying topics of fundamental importance to the field of cardiovascular biology and medicine. Dr. Lenihan, a pioneering clinical scientist and outstanding cardiologist, brought together a special set of articles that represent interactions between cardiovascular and other fields of medicine. Dr. Gale, a celebrated cardiologist with expertise in comparative effectiveness research and clinical cardiology, offers a series of stellar chapters underscoring various aspects of the pathogenesis and management of atherosclerotic cardiovascular disease. Dr. Kokubo, a leading preventive cardiologist with specialization in cardiovascular epidemiology, has orchestrated state-of-the-art chapters on cardiovascular epidemiology.

We would like to thank our colleagues at Elsevier who provided steadfast support of an exemplary nature throughout this long journey. Last but not the least, the editors would like to thank their families, their mentors, and their respective institutions for the constant encouragement, advice, and support over the years.

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Dairy Products and Cardiovascular Diseases

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Preface

What Are People Consuming in the World?

Excess fat intake is known as an important risk factor for cardiovascular disease (CVD). In the United States and Europe, intakes of fat and oil are high with a great portion of animal fat. It is also assumed that animal fats significantly rely on dairy products (Parodi, 2011). On the other hand, lesser dairy products are consumed in the Eastern countries; supply source of fat and oil is primarily vegetables (**Table 1**). In Pakistan and India, however, meat consumption is very low, consumption of milk and dairy products is relatively high.

Japan is one of the following countries consuming dairy products a little; after the World War II, milk has been introduced in school meals and then relatively many Japanese become to consume dairy products (**Fig. 1**). Nevertheless, Japanese elderly consume little dairy products. Two of the authors (Y and A) participated in the Fujiwara-kyo study (Okamoto et al., 2010, 2015), a study of successful aging in the elderly (≧65 years), and conducted a survey of food intakes using diaries for consecutive 7 days. We analyzed these diaries with the "Cooked-Dish Codes" method (Amano and Yanagi, 2010). In 1643 males and 1625 females, the median consumption of all dairy products was only 0.8 portion per day (unpublished data).

China consumes a lot of meat but little milk and dairy products. Despite some exceptions, many regions consuming a lot of rice are consistent with regions consuming little milk and dairy products.

Therefore the following text describing the relationship between dairy products and CVDs should be read with caution; there are great differences in lifestyles between any regional, cultural, and ethnic groups.

Prevalence of Overweight in the World

The relationship of CVD and obesity is widely recognized (World Health Organization (WHO), WHO warns of the rising threat of heart disease . . .). Prevalence of overweight is varying in countries; however, they are generally higher in the Western countries. WHO (Obesity and overweight) says:

Table 1 Compositions of food supply and their ranking among some countries

Ranking	*Cereals*	*%*	*Potatoes*	*%*	*Sugar and confectioneries*	*%*	*Beans*	*%*	*Vegetables*	*%*	*Fruits*	*%*
1	Bangladesh	77.1	Nigeria	21.4	New Zealand	17.5	Kenya	7.0	China	7.3	Iran	7.1
2	Indonesia	63.4	Kenya	9.1	Switzerland	16.2	Nigeria	5.6	Republic of Korea	5.2	Philippines	5.9
3	Egypt	62.6	Indonesia	6.2	United States of America	15.4	Brazil	5.5	Iran (Islamic Republic of)	4.5	Italy	4.9
4	Vietnam	58.6	Poland	6.1	Mexico	15.4	India	5.4	Egypt	4.0	Egypt	4.8
5	India	57.1	Russian Federation	6.0	Argentina	14.7	Japan	4.4	Turkey	3.9	The Netherlands	4.6

Ranking	*Meat and poultry*	*%*	*Eggs*	*%*	*Milk and dairy products*	*%*	*Fish and shellfish*	*%*	*Fat and oil*	*%*	*Miscellaneous*	*%*
1	Argentina	17.9	Japan	2.8	Finland	18.3	Japan	5.1	Spain	24.9	Ireland	10.7
2	Finland	16.1	China	2.4	Sweden	17.3	Malaysia	3.7	Hungary	23.1	Czech Republic	10.7
3	Vietnam	15.2	Mexico	2.1	Pakistan	16.3	Norway	3.3	Italy	20.8	Thailand	10.0
4	China	14.8	Denmark	2.0	The Netherlands	15.0	Republic of Korea	3.3	Canada	20.8	Germany	10.0
5	Australia	14.5	Malaysia	1.9	France	14.9	Spain	2.7	United States of America	20.4	Portugal	9.4

FAOSTAT ("Food Supply", 2015.8.21) modified by Honkawa (Honkawa Data Tribune) and translated by Yanagi.

"Beans" include soy beans and peanut. "Milk and dairy products" include butter and cream.

Comparison among 40 countries: Norway, Sweden, Finland, Denmark, United Kingdom of Great Britain and Northern Ireland, Ireland, Spain, Portugal, France, Italy, Germany, the Netherlands, Switzerland, Czech Republic, Poland, Hungary, Russian Federation, Turkey, Egypt, Kenya, Nigeria, Iran (Islamic Republic of), Pakistan, India, Bangladesh, Thailand, Malaysia, Vietnam, Indonesia, Philippines, China, Republic of Korea, Japan, Australia, New Zealand, Canada, United States of America, Mexico, Brazil, Argentina.

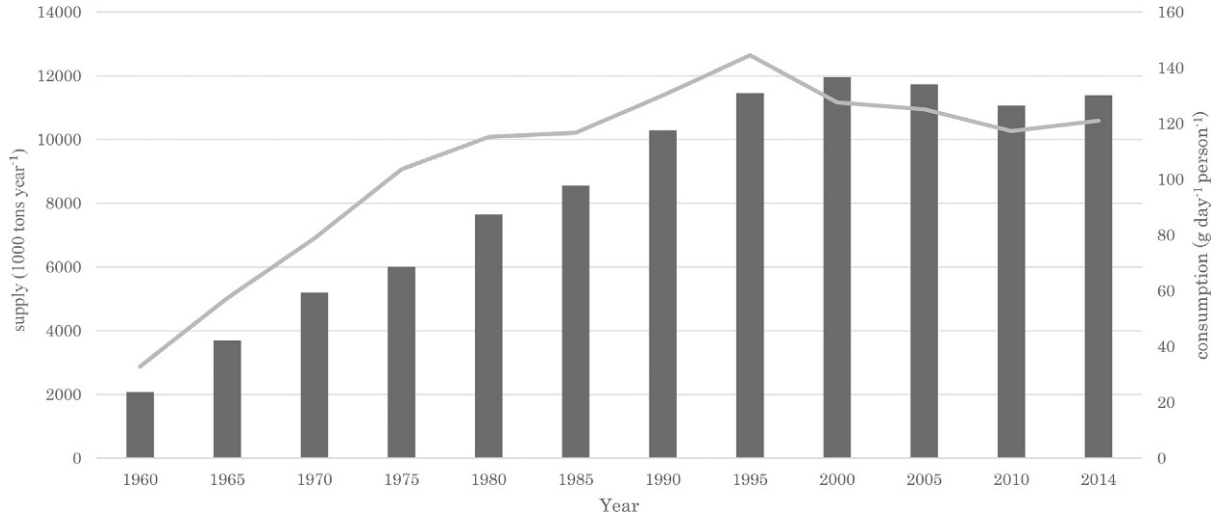

Fig. 1 Trend of milk and dairy supply and consumption in Japan. Bars show supplies and a line shows consumption. Modified from the followings: Table of food supply and demand (2000–14). Ministry of Agriculture, Forestry and Fisheries (MAFF); Milk and dairy product, cumulative long-term data (1960–99). Ministry of Internal Affairs and Communications (MIAC); Food intake by groups, long-term time-series data (1946–2014). Ministry of Health, Labour and Welfare (MHLW).

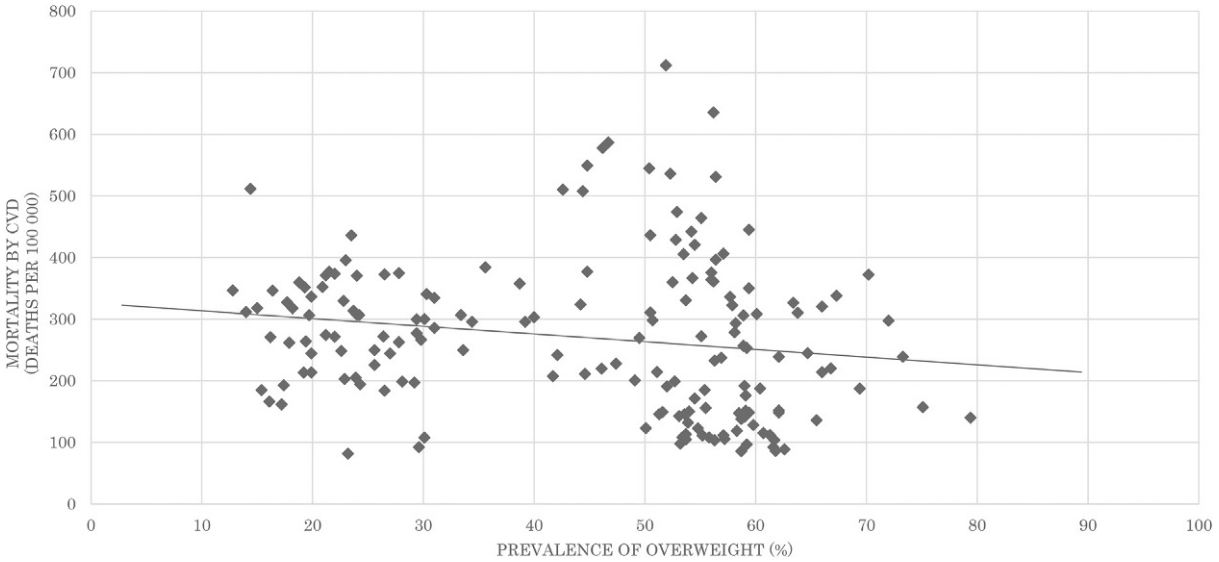

Fig. 2 Relationship between prevalence of overweight and mortality by CVD; 172 countries (modified from Global Health Observatory data. WHO, 2012). $y = -1.251x + 325.9$, $R^2 = 0.029$.

> "The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. Globally, there has been:
> an increased intake of energy-dense foods that are high in fat; and . . ."
> "Raised BMI is a major risk factor for noncommunicable diseases such as:
> cardiovascular diseases (mainly heart disease and stroke), which were the leading cause of death in 2012;
> diabetes; . . .
> The risk for these noncommunicable diseases increases, with increases in BMI."

However, the prevalence of overweight does not positively correlate with the mortality by CVD in 172 countries (WHO, Global Health Observatory data; Fig. 2). This ecological data does not seem to support the relationship between obesity and CVD (the "obesity hypothesis").

Another ecological data (WHO, Overweight Data by WHO region) also does not support the "obesity hypothesis" because the life expectancy seems to be longer in the region with a high prevalence of overweight (Table 2). Therefore, any discrepancy or heterogeneity between ecological data and observational data should be considered cautiously when we discuss the relationship between dairy products and CVDs.

Table 2 Relationship between prevalence of BMI ≥ 25, both sexes, 18+ years (age-standardized estimate) and life expectancy (years)

Region	*Overweight (%, 2014)*	*95% CI*	*Life expectancy (2015)*
Africa	30.8	28.7–32.9	60.0
Americas	61.3	58.7–63.8	76.9
South-East Asia	22.2	19.3–25.4	69.0
Europe	58.6	56.3–60.8	76.8
Eastern Mediterranean	46.8	44.0–49.3	68.8
Western Pacific	33.0	28.7–37.3	76.6

$y = 0.245x + 61.00$, $R^2 = 0.334$.
Modified from WHO regional data (2014, 2015).

Some Reviews of Relationship Between Dairy Products and Prevalence of CVDs

A Review of the Review of Dairy Products and the Incidence of Vascular Disease and Diabetes

According to the review of dairy products and the incidence of vascular disease and diabetes (Elwood et al., 2010):

1. Eight cohort studies analyzing the relationship between dairy products and all-cause mortality were included. A meta-analysis of six studies of them was conducted between the most and the least consuming groups; the adjusted estimates of relative risk (RR) was 0.87 (95% CI 0.77–0.98).
2. Eleven cohort studies analyzing the relationship between dairy products and the incidence of ischemic heart disease were included. A meta-analysis of nine studies of them was conducted between the most and the least groups and RR was 0.92 (0.80–0.99).
3. Eleven cohort studies analyzing the relationship between dairy products and the incidence of stroke were included. They showed a significant heterogeneity. A meta-analysis showed RR was 0.78 (0.68–0.91).
4. Focusing on hemorrhagic stroke, there were five studies showing a significant heterogeneity. The RR was 0.75 (0.60–0.94).

However, this summary should be cautiously interpreted, because;

1. Heterogeneity was not reported in the meta-analyses of all-cause mortality and ischemic heart disease. However, there are seven of eight studies on all-cause mortality that did not show significant differences by themselves, and two of them showed RR exceeding 1.0.
2. In studies on ischemic heart disease, 16 studies were identified and 5 of them were excluded. RRs in the four cohorts of five excluded ones were beyond 1.0 and two of them were statistically significant.
3. The JPHC study, a cohort of Japan (Umesawa et al., 2008), was included in this meta-analysis of incidence of stroke; its RR was 0.70 (0.57–0.86). However, the JPHC study surveyed the association between the dairy calcium intake and stroke but not the consumption of dairy products and stroke. The dairy calcium intake was calculated only from "cheese" and "daily products except cheese" using food frequency questionnaire (FFQ) and there is uncertainty that this calculated dairy calcium reflects the total dairy consumption precisely.

   The other included Japanese cohort, the JACC study (Umesawa et al., 2006) also surveyed the association between the dairy calcium intake and mortality from stroke; multivariate RRs of mortality from stroke were 0.53 (0.34–0.81) for men and 0.57 (0.38–0.86) for women. The dairy calcium intake was also calculated only from milk, yogurt, and cheese using FFQ. Umesawa et al. discussed only calcium intake in this population and there is also uncertainty in the precision of total dairy consumption.

   On the other hand, the Nurses' Health Study (Iso et al., 1999) was included in this meta-analysis. They collected data on usual dietary intake using a semiquantitative FFQ and reported significant inverse association between the total calcium intake and incident ischemic stroke (P for trend: 0.04) but not between the dairy calcium intake and ischemic stroke (P for trend: 0.08). They also reported no association between the total calcium intake and all stroke (P for trend: 0.34).

   Therefore, we would like to say it is difficult to consider an adjustment for calcium intake in the analysis of dairy product as equivalent the analysis of dairy calcium intake.
4. The cohort of Kinjo et al. (1999) was included in the meta-analysis of hemorrhagic stroke. They reported a joint effect of dairy milk, meat, and fish. In the subpopulation of dairy milk consumption one to three times per week or more, meat consumption less than one times per week, and fish consumption less than one times per week, RR was 0.92 (0.82–1.02). They concluded intake of animal protein was considered a major risk factor.

In view of these aspects, it is suggested that protein and calcium intake from sources other than dairy products should be carefully adjusted when discussing the association between dairy products and CVDs.

Results From a Systematic Review of Dairy Consumption and CVD Accompanying Some Problems

A systematic review on dairy consumption and CVD (Alexander et al., 2016) shows summary relative risk estimates (SRRE) as follows:

1. SRRE of total dairy intake and total stroke is 0.91 (0.83–0.99).
2. SRRE of total dairy intake and total CVD is 0.88 (0.75–1.04).
3. SRRE of total dairy intake and total coronary heart disease (CHD) is 0.91 (0.80–1.04).

Only the SRRE of total stroke is statistically significant. However, when you pay attention to some of the included and excluded studies, important problems emerge.

1. Life Span Study (Sauvaget et al., 2003)

   The Life Span Study (LSS) reported a dietary survey using FFQ. The hazard ratio of total stroke mortality between the consuming *dairy products* almost daily versus never groups was 0.75 (0.57–0.90). However, milk and dairy products (butter and cheese, excluding margarine) were interviewed and reported separately (**Table 3**). The subgroup of *dairy products* almost daily in the LSS was a very special population having consumed animal protein a lot.

   We believe the way of citation by Alexander has a clear error. Thus meta-analysis of total daily intake and total stroke should be corrected. The SRRE will be greater than 0.91 and might not be statistically significant.
2. NIPPON DATA80 (Kondo et al., 2013)

   Results in males and females are highly heterogeneous. In the meta-analysis, Alexander described rate ratios (RR) of males (RR 1.49, 95% CI 0.91–2.45) and females (RR 0.60, 0.36–1.00) separately. There were no other studies which showed such heterogeneity between males and females in this review.

   However, Japanese males certainly have a smaller intake of dairy products than the Westerners (**Table 4**), and they also have a lower mortality of CVD (108.0 deaths per 100,000 in 2012) than the Westerners. Moreover, Japanese males in this cohort showed positive relationship between dairy product consumption and CHD incidence (RR 1.33, 0.97–1.82).
3. Some excluded studies from the SRRE

   We would like to comment about some of cohort studies excluded from the SRRE. The Rotterdam study (Praagman et al., 2015) reported 564 strokes and 567 CHD occurred in the 4235 participants whose median intake of total dairy was 397 g day^{-1}. Total dairy, milk, low-fat dairy, and fermented dairy were not significantly related to incident stroke or fatal stroke; incident and fatal CHD were not significantly related, too.

   The Whitehall II study (Soedamah-Muthu et al., 2013) reported 323 CHD occurred in the 4526 subjects whose median intake of total dairy was 378 g day^{-1}. Intakes of total dairy, high- and low-fat dairy, total milk, low-fat milk, fermented dairy products, yogurt, and cheese were not statistically significantly associated with incident CHD.

   There was no clear description why these important studies were excluded from the SRRE; they just commented "Studies required to report a composite variable for total dairy intake and a composite variable for total CVD."

Table 3 Milk and dairy products in the Life Span Study

Food	*Category*	*Cases*	*Hazard ratio*	*All subjects*
Milk	Almost daily	10,371	0.94 (0.79–1.12)	31,832
Dairy products	Almost daily	3690	0.73 (0.57–0.94)	28,662

Dairy products contain butter and cheese but not margarine.

Modified from Sauvaget, C., Nagano, J., Allen, N., Grant, E. J. and Beral, V. (2003). Intake of animal products and stroke mortality in the Hiroshima/Nagasaki Life Span Study. *International Journal of Epidemiology***32**(4), 536–543.

Table 4 Milk and dairy products consumption (g day^{-1}) by tertiles in NIPPON DATA80

	Low	*Moderate*	*High*
Male	18.0 (15.0)	61.5 (11.1)	132.6 (53.3)
Female	22.7 (18.9)	79.0 (14.8)	168.3 (62.2)

Modified from Kondo, I., Ojima, T., Nakamura, M., et al. (2013). Consumption of dairy products and death from cardiovascular disease in the Japanese general population: the NIPPON DATA80. *Journal of Epidemiology***23**(1), 47–54.

As can be seen from the above example, risk estimates of dairy products to CVDs have high heterogeneity. It is also difficult to conclude the dose–response relationship between dairy product consumption and incidence of CVDs. Therefore we would like to summarize that there is no clear evidence which shows benefit or harm for general population to take dairy products ad libitum.

Some Reviews of Relationship Between Dairy Products and Risk Factors of CVDs

Do Dairy Products Have an Antihypertensive Effect?

A review on milk products (Kris-Etherton et al., 2009) concluded calcium in dairy products has an antihypertensive effect. If paying attention to prospective studies in the review, only two of four studies on low-fat milk reported reductions in blood pressure and only one of six studies on whole milk (or simply "milk") reported systolic blood pressure reduction.

We would like to comment about one of included prospective studies showing challenging results. The Women's Health Study (WHS) was an randomized controlled trial (RCT) having used low-dose aspirin and vitamin E. Wang et al. (2008) excluded women with insufficient completion of the semiquantitative FFQ or implausible total energy intake from the WHS population and followed 28,886 women with a mean age of 53.8 years to evaluate the incidence of hypertension. They reported that the RRs of incident hypertension across increasing quintiles of low-fat dairy product intake and showed significant trend of inverse association ($P=0.001$). However, adjustment for dietary calcium significantly attenuated the inverse association of low-fat dairy intake with risk of hypertension (P for trend: 0.61). High-fat dairy product intake showed no significant association (P for trend: 0.17).

Kris-Etherton et al. also reported RCTs loading dairy products; two parallel and three crossover studies. One parallel study showed a significant decrease only in systolic blood pressure (SBP) and the other did not show significant blood pressure reduction. In crossover trials, one showed elevated blood pressure without significance; two other trials showed significant reduction only in SBP.

Summarizing these studies, there is inconsistent evidence to support that dairy products have clear blood pressure lowering effect.

Furthermore, this review mentioned sour-milk drinks containing a lot of peptides (Val-Pro-Pro and Ile-Pro-Pro) and the possibility of an antihypertensive effect. We have a discussion of the sour-milk studies in the next section.

Clinical trials on sour-milk and blood pressure

An RCT of the sour-milk drink was conducted on the blood pressure of 36 hypertensive subjects aged 40–86 years (Hata et al., 1996). However, the decrease in SBP and diastolic blood pressure (DBP) in the sour-milk group tended to be greater than that in the placebo group, there was no statistical analysis of BP between the two groups and these differences probably were not statistically significant.

Another sour-milk study (Itakura et al., 2001) was conducted on the BP of 18 hypertensive (mean age 54 years) and 26 normotensive (mean age 36 years) subjects. In normotensive subjects, both treatments did not affect BP. In untreated hypertensive subjects, SBP decreased significantly from the initial value after ingestion of the sour-milk; however, there was no statistically significant difference between treatment groups.

One more sour-milk study was conducted in Japan (Mizushima et al., 2004) on the BP of 46 borderline hypertensive men aged 23–59 years. In the sour-milk group, change in systolic BP was significant; however, two-way ANOVA did not show significant differences in changes in SBP between the two groups.

Kris-Etherton et al. showed other two studies on milk with peptides that have found no decrease in BP in hypertensive subjects. These studies are enough to discourage you conducting a large RCT on sour-milk drink supplemented with peptides.

Do Dairy Products Induce the Increase of Serum Lipids?

A review on fatty acids and CVD (Parodi, 2011) summarized RCTs of low saturated fat diets and CHD prevention, concluding: "None of the estimates of risk for total and CHD mortality attained statistical significance."

Low saturated fat diets seem to have minimal effect for CHD prevention. However, dairy products contain not a little saturated fatty acid, there is no clear evidence to encourage to avoid dairy products for the prevention of CVD.

Do Dairy Products Induce the Increase of Body Weight?

A meta-analysis on the effects of high- and low-fat dairy foods (Benatar et al., 2013) included 20 RCTs. Primary outcomes were body weight, insulin resistance, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, C-reactive protein, and blood pressure. They concluded that a moderate increase in dairy food consumption has no or small effects on the major cardiovascular and metabolic factors. However, mean weight gain of low-fat dairy food was twice that of whole fat dairy food. Therefore we have no evidence to encourage obese individuals having low-fat dairy foods freely.

Controversy on the Relationship Between Consumption of Dairy Products and Prevalence of Type1 Diabetes

There have been more than 25 years since an ecological study suggested cow's milk may contain a triggering factor for the development of type1 diabetes mellitus (T1DM). In fact, Japanese have low T1DM incidence less than 3 persons $100{,}000^{-1}\text{year}^{-1}$ and consume dairy products less than one-third of the Western populations (Dahl-Jorgensen et al., 1991).

A review (Gerstein, 1994) summarized breastfeeding in T1DM. Thirteen relevant case–control studies were meta-analyzed. The odds ratio (OR) of 12 peer-reviewed studies for T1DM patients exposed to less than 3 months of breastfeeding was 1.37 (95% CI 1.22–1.53). The overall OR of five studies for cow's milk exposure before 3–4 months of age in patients with T1DM was 1.57 (1.19–2.07).

Norris and Pietropaolo (1999) argued that these summary ORs are quite small and would not meet the criterion for causation. They also criticized that two cohort studies did not show the difference in duration of breastfeeding between antibody positive and negative high-risk populations. However, one cohort study suggested marginal association between high milk consumption in childhood (⊠3 glasses daily) and more frequent progression to clinical DM in a high-risk population.

These data suggest quite early exposure to cow's milk is not entirely safe. Populations consuming very little dairy products have no need to be encouraged to increase them.

Summary

Dairy products are important for the Westerners because they are the primary source of protein and minerals. There is no clear evidence which shows benefit or harm for general population to take dairy products ad libitum. On the other hand, people who do not consume dairy products routinely cannot be recommended to increase their dairy consumption. So we would like to conclude:

1. In a population approach, consider and respect the dietary habits and traditions of inhabitants, and it is not safe to force a sudden change for the intake of dairy products.
2. In a high-risk approach, consider the probability that dairy products can lead to weight gain, and dairy products are not recommended for some obese individuals. Comparing with other alternative foods, dietary interventions should be individualized.

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Diabetes Mellitus

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Diabetes Mellitus as a Global Epidemic

Diabetes Mellitus—Definition and Subtypes

Diabetes mellitus is a group of diseases primarily characterized by chronic hyperglycemia due to a lack of sufficient insulin action. Diabetes is roughly divided into type 1 and type 2. Type 1 diabetes, which was formerly called juvenile-onset or insulin-dependent diabetes (IDDM), accounts for less than 5%–10% (depending on areas of the world) of all diabetes. It occurs due to an acute destruction of insulin-producing beta cells in the pancreas through autoimmune disorders that lead to a sudden and total deficiency of insulin, the only hormone that can lower plasma glucose levels.

Type 2 diabetes, which used to be known as noninsulin dependent diabetes (NIDDM) or adult-onset diabetes (but currently is also common among overweight children), accounts for the vast majority of all diabetes mellitus. In this type, secretion of insulin remains but not in quantities sufficient to keep the glycemic level in a normal range, frequently due to not only decreased levels of insulin secretion but also an inappropriately increased demand for insulin because of the response of the body's cells to decreased insulin. This lowered response to insulin is called insulin resistance and occurs in various situations, typically obesity. In patients with type 2 diabetes, insulin resistance (i.e., insufficient action of insulin) and insulin deficiency (i.e., insufficient secretion of insulin) both contribute to raising plasma glucose levels but in different proportions, varying from patient to patient.

Vascular Complications of Diabetes Mellitus

Diabetes, regardless of its type, gives rise to a variety of systemic complications, most of which are vascular diseases and are associated with diminished expectancy and quality of life. Among them are *microvascular* complications mainly comprised of retinopathy, nephropathy, and neuropathy, all of which are specific to diabetes, and cardiovascular diseases (CVDs) consisting of coronary heart disease (CHD), stroke, and peripheral artery disease (PAD), which are called *macrovascular* complications or *macroangiopathy* of diabetes. Hypertension and heart failure are also more common in diabetic individuals than in nondiabetic individuals.

Global and Regional Prevalence of Diabetes Mellitus

Although plateauing in the increase in diabetes mellitus has been reported recently in some developed countries such as in the United States (Geiss et al., 2014), the world prevalence and incidence of diabetes mellitus are still increasing. A recent analysis of pooled data of population-based studies from 146 countries revealed that global age-standardized diabetes prevalence was estimated to have grown from 4.3% (95% CI 2.4–7.0) in 1980 to 9.0% (7.2–11.1) in 2014 in men and from 5.0% (2.9–7.9) to 7.9% (6.4–9.7) in women. The world adult population with diabetes increased by fourfold from 108 million to 422 million during this period. This enormous increase can be explained by the 28.5% due to the rise in prevalence, 39.7% due to population growth and aging, and 31.8% due to interaction of these two factors (NCD Risk Factor Collaboration, 2016). According to the seventh edition of the *Diabetes Atlas* from the International Diabetes Federation (International Diabetes Federation, 2015), 1 in 11 adults (415 million) in the world currently have diabetes (majority of which are type 2) and this figure is estimated to rise to 1 in 10 by 2040.

Regionally, in the United States, 9.3% of adults have diabetes (1.0% out of 9.3% were undiagnosed) according to the National Health and Nutrition Examination Survey 2005–10 (Selvin et al., 2014). However, three quarters of people with diabetes live in low- and middle-income countries although the disease is often misunderstood to occur predominantly in "wealthy" developed countries (International Diabetes Federation, 2015). It also should be noted that 60% of people with diabetes live in Asia, with almost one-third in East Asia including China; these numbers are still rapidly growing (Nanditha et al., 2016).

In addition to overt diabetes, there are 318 million adults with impaired glucose tolerance (IGT) or prediabetes worldwide who are at greater risk for future type 2 diabetes as well as CVD. Because of their asymptomatic nature, it is very difficult to find IGT and early diabetes except by a health check-up or screening. As a consequence, nearly half of the global diabetic population is undiagnosed, which frequently allows silent progression of complications including atherosclerosis.

Role of Diabetes Mellitus in the Progression of CVD

Insulin Resistance and Metabolic Syndrome

Causes of the substantially increased risk of CVD in diabetic patients are complicated. A variety of underlying atherogenic mechanisms associated with insufficient insulin action commonly cluster within an individual with diabetes and act synergistically to increase the risk of CVDs. As a background of this clustering, insulin resistance, which is explained as the reduced response to the hormonal messenger of insulin signal transduction, plays a crucial role.

Insulin resistance is a pathophysiological basis of not only type 2 diabetes but also metabolic syndrome, which is also a predictor of CVD and its mortality. In fact, because of sharing common soil in their mechanisms, obesity, hypertension, and dyslipidemia, all of which are components of metabolic syndrome, are very common comorbidities in type 2 diabetes (Nelson et al., 2002). In fact, the majority of individuals with type 2 diabetes in the U.S. are overweight (Nelson et al., 2002).

Other Mechanisms That Promote Cardiovascular Damage in Diabetes

The increased oxidative stress due to production of superoxide anions and lipid peroxidation products, along with the decreased availability of antioxidants that are seen in diabetes, are considered to play an important role in the development of diabetes complications (Nishikawa et al., 2000). Oxidative stress, as well as sustained hyperglycemia, also promotes the formation of advanced glycation end-products (AGEs). AGEs, which are produced by nonenzymatic glycation of proteins, can affect or alter the physiological functions of normal proteins. Hyperreactivity of platelets in addition to impaired fibrinolysis and decreased activity of antithrombotic factors also enhance the hypercoagulable status seen in diabetes.

Through these mechanisms that have still not been completely clarified, chronic hyperglycemia causes endothelial or mitochondrial dysfunction, metabolic disturbances, and fibrosis of the extracellular matrix, which lead to functional impairment of myocardium even without significant coronary stenosis and is called cardiomyopathy. In fact, risk of heart failure was significantly increased in patients with diabetes; the annual mortality of patients with heart failure was increased more than 10-fold that in diabetic patients without heart failure (MacDonald et al., 2008.

Diabetic Autonomic Neuropathy and Other Complications of Diabetes

That CHD in diabetic patients is often overlooked or found in advanced stages has been explained by the fact that it is frequently asymptomatic or without typical symptoms because of autonomic dysfunction due to diabetic neuropathy. Among patients with type 1 diabetes, those with cardiovascular autonomic neuropathy have a 1.53 (95% CI, 1.01–2.32)-fold higher incidence of any CVD event (Pop-Busui et al., 2017). Autonomic neuropathy might also be a cause of prolongation of the QT interval and arrhythmia, and the heart rate-corrected QT interval was shown to be an independent predictor of all-cause and CVD mortality in patients with type 2 diabetes (Cox et al., 2014). Among patients with type 2 diabetes who were insufficiently controlled with metformin or sulfonylurea monotherapy, unrecognized Q-wave myocardial infarction was found in 1.9%, which accounted for one-third of the prevalence of myocardial infarction (MacDonald et al., 2011).

Probably because of the sharing of common mechanisms, one could be a predictor of subsequent other complications. For example, retinopathy could be a predictor of macrovascular complications in patients with type 2 diabetes. People with mild to moderate nonproliferative diabetic retinopathy had a higher risk of CHD (hazard ratio 1.69, 95% CI, 1.17–2.97) and stroke (2.6, 1.03–4.86) (Kawasaki et al., 2013).

Impact of Diabetes on CVD

Diabetes as a Risk Factor for CVD

As a risk factor for CVD, diabetes has an enormous impact from the clinical as well as public health point of view considering the global epidemic of the disease. Compared with nondiabetic individuals who were similar in CHD risk factors, diabetic patients had a higher prevalence, extent, and severity of CHD (Rana et al., 2012). Diabetes was estimated to confer a CHD risk equivalent to 15 years of aging (Booth et al., 2006).

Type 2 diabetes has been known to be associated with a several-fold greater risk for CVD. A collaborative meta-analysis of 102 prospective studies concluded that diabetes conferred an approximately two-fold excess risk for a wide range of vascular diseases, independent of other conventional risk factors (Emerging Risk Factors Collaboration, 2010). An analysis of a nationally representative database with information from the past two decades in the United States demonstrated large reductions in the incidence of acute myocardial infarction (AMI), stroke, lower-extremity amputation, and end-stage renal disease (Gregg et al., 2014). Relative risk of these four complications between diabetic and nondiabetic individuals in 1990 and 2010 were 3.8 and 1.8 for AMI, 3.1 and 1.5 for stroke, 18.8 and 10.5 for lower-extremity amputation, and 13.7 and 6.1 for end-stage renal disease, respectively. Especially, the number of cases with AMI among 10,000 diabetic patients declined from 37.5 in 1990 to 25.8 in 2010, which was a 31% reduction, while a 68% reduction was seen in nondiabetic persons during the same period. Although epochal improvement was confirmed, a large burden of the disease continues since the relative risks were significant and prevalent diabetes persists.

Diabetes has long been recognized as "CHD risk equivalent" as a predictor of CHD as strong as prior CHD (Juutilainen et al., 2005). This provided a rationale for treating diabetic patients as aggressively as nondiabetic patients with prior myocardial infarction in consideration that the 10-year mortality from CHD was reported to be more than 20%, which is comparable to that in those with prior CHD (Haffner et al., 1998; Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001).

Additional lifetime healthcare expenditures because of diabetes in the U.S. ranged from $75,000–89,000 per patient (Leung et al., 2015), suggesting that diabetes imposes an enormous health and economic burden globally considering the fact that three quarters of people with diabetes live in low- and middle-income countries (International Diabetes Federation, 2015).

Diabetes as a Risk and Cause of Mortality

Excess cardiovascular mortality risk among Swedish patients with type 2 diabetes was 1.14 (95% CI 1.13–1.15) compared to nondiabetic individuals; the degree of excess mortality was greater in younger than older diabetic persons and in those with worse glycemic control and more severe nephropathy (Tancredi et al., 2015). In the United States, mean life-years lost by diabetes were estimated to be 5.5–6.2 years (depending on ethnic origins) (Leung et al., 2015), and 10.6% and 17.7% of cardiovascular mortality in men and women aged 65–79 years, respectively, are theoretically avoidable with the elimination of diabetes (Patel et al., 2015).

Among people with diabetes, CVD is the most common and major cause of death in many countries although in East Asian countries, cancer is a more common cause (Tseng, 2004; Hotta, 2010). Analysis of a large cohort of more than a million Australians with diabetes also demonstrated the absence of a decline in cancer mortality in spite of a significant decline in all-cause, CVD, and diabetes mortality during 2000–11 and warrants urgent attention (Harding et al., 2016)

Fortunately, the situation has improved (hopefully due to recent advances in screening and care) and CV mortality risk has decreased to 10% over 10 years in a more recent cohort (Howard et al., 2006), which depends largely on coexisting CV risk factors including factors other than hyperglycemia. Age-standardized CVD mortality rate decreased each year by 0.35 (type 1) and 0.15 (type 2) per 1000 person-years between 2000 and 2011 (Donahoe et al., 2007).

Characteristics of CVD in Diabetic Patients

CVDs accompanied by diabetes are known to be more severe and to be associated with a poorer outcome and prognosis compared to those not associated with diabetes. Multiple vessel disease is more common (Waller et al., 1980) and death after AMI was found to be approximately 1.5-fold higher in individuals with diabetes than in those without it (Sprafka et al., 1991; Liang et al., 2014). Mortality at 30 days and 1 year after acute coronary syndrome events was also higher in diabetic patients compared to nondiabetic individuals (Donahoe et al., 2007). Even in the recent "stent era," among patients who underwent percutaneous coronary interventions due to myocardial infarction, patients with diabetes had an approximately 2.5-fold greater risk for recurrent myocardial infarction as well as all-cause mortality during the first 30 days or even at 3 years after the percutaneous coronary intervention compared to nondiabetic persons (Jensen et al., 2012).

Because asymptomatic occurrence and progression of type 2 diabetes is common, a substantial proportion of all CVD incidents are seen in patients with preexisting but undiagnosed diabetes. Oral glucose tolerance testing identified the presence of disorders of glucose metabolism in approximately half of ischemic strokes (Urabe et al., 2009) and half of acute coronary syndrome (Hashimoto et al., 2005) patients without previously known diabetes.

CVD Risk in People With Prediabetes

Unlike microvascular complications, which occur only years after the development of diabetes, the risk of macrovascular complications start to rise even during the prediabetic stage. At this moment, there are several definitions of prediabetes. These include impaired fasting glucose (IFG) according to the criteria of the American Diabetes Association (5.6–6.9 mmol/L) and the WHO expert group (6.1–6.9 mmol/L) and impaired glucose tolerance (IGT) (2 h plasma glucose concentration 7.8–11.0 mmol/L during an oral glucose tolerance test) or elevated HbA1c of 5.7%–6.4% (39–47 mmol/mol) according to American Diabetes Association criteria or 6.0%–6.4% (42–47 mmol/mol) according to the National Institute for Health and Care Excellence (NICE) guidelines in the United Kingdom. However, regardless of definitions, prediabetes was shown to significantly increase the risk of CVD and its mortality by 13%–30% (depending on its definition) (Huang et al., 2016).

Random plasma glucose levels were also shown to be associated with CVD even within normal range in a large Chinese cohort without diabetes (Bragg et al., 2016). The 2-h postchallenge glucose level in an oral glucose tolerance test was shown to be more strongly associated with CVD than fasting plasma glucose; i.e., IGT was a stronger predictor of CVD than IFG (Tominaga et al., 1999). This indicates that postprandial hyperglycemia, which is characteristic of pre- or early-stages of diabetes, could promote the atherosclerotic process.

CVD in Women and Younger Adults With Diabetes

Impact of diabetes on the risk of CVD is significantly greater in women than in men. It is known that the protection that premenopausal women have against CVD is almost lost when diabetes is present (Nathan, 1993). Meta-analysis of 37 cohort studies found that the relative risk for fatal CHD in patients with diabetes (vs. nondiabetic persons) was significantly higher among women (relative risk 3.5, 95% CI 2.7–4.5) than among men (2.1, 1.8–2.3) (Huxley et al., 2006). The same group reported that the additional risk for vascular dementia, but not for nonvascular dementia, was greater in diabetic women than in diabetic men (Chatterjee, 2016). A meta-analysis of 64 cohorts demonstrated that the pooled maximum-adjusted relative risk of stroke associated with diabetes was 2.28 (95% CI 1.93–2.69) in women and 1.83 (1.60–2.08) in men, with a 27% significantly higher incidence in women. These findings indicated that women with diabetes had a 1.27 (1.10–1.46)-fold greater risk of stroke than diabetic men (Peters et al., 2014).

Diabetes has a more profound impact on younger adults than on the elderly in terms of comparison with nondiabetic persons. In contrast to the fact that diabetic men aged 51–60 years have approximately a 2.5-fold higher risk of CHD than nondiabetic men of the same age, diabetic men aged 31–40 years have an approximately 17 times higher risk than nondiabetic individuals (Fujihara et al., 2016).

Risk Factors for CVD in Diabetic Patients

Hyperglycemia and its Control

Despite the fact that diabetes is a well-established predictor of CVD, reported effects of hyperglycemia per se on CVD have differed between observational cohorts and clinical trials of hypoglycemic agents.

Influence of hyperglycemia determined by observational studies

In prospective observational studies, hyperglycemia was shown to be an established independent predictor of CVDs regardless of the ethnic group. A meta-analysis of 18 prospective cohort studies demonstrated that each 1% increase in HbA1c corresponded to an 18% or 12%–14% greater risk for CVD events or all-cause mortality, respectively, of which results were similar between type 1 and type 2 diabetes (Selvin et al., 2004). In another large cohort, every 1-mmol/L (18-mg/dL) increase in fasting plasma glucose predicted a 17% increase in the risk of future cardiovascular events or death (Anand et al., 2012).

Effects of glucose lowering in clinical trials

In spite of these associations between hyperglycemia and CVD in observational studies, the clinical evidence that intensive glycemic control lowers the risk for CVD events or death is relatively limited, notwithstanding the well-established reduced risk of microvascular complications such as retinopathy or nephropathy.

The Diabetes Control and Complications Trial (DCCT) (Diabetes Control and Complications Trial Research Group, 1993) comprised of patients with type 1 diabetes and the UK Prospective Diabetes Study (UKPDS) (UK Prospective Diabetes Study, UKPDS Group, 1998) comprised of patients with type 2 diabetes proved the effect of glycemic control for respective intervention periods of approximately 6.5 or 10 years respectively by insulin and/or oral hypoglycemia agents with a marked reduction in

microvascular complications. Nevertheless, neither study failed to demonstrate the expected significant reduction in CVD risk during observational periods of less than 10 years.

Interestingly, a significant reduction in CVD risk in a therapeutic intervention group compared to a conventional therapy group was finally reached during extended observation of these clinical trials long after finishing the therapeutic intervention for hyperglycemia when HbA1c of both groups had already converged (Nathan et al., 2005; Holman et al., 2008). The phenomenon is called "metabolic memory" or "legacy effect," indicating that glycemic control requires a long time to produce an obvious effect on CVD risk but that the effect continues for years. This could also explain the dissociation between observational and intervention studies. The mechanism of this phenomenon is not completely clarified but is speculated to be at least partly due to an epigenetic process (Miao et al., 2014).

However, strangely enough, three subsequent large clinical trials (Action to Control Cardiovascular Risk in Diabetes (ACCORD) (Action to Control Cardiovascular Risk in Diabetes, ACCORD Study Group, 2008), Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) (ADVANCE Collaborative Group, 2008), and Veterans Affairs Diabetes Trial (VADT) Duckworth et al., 2009), all of which were designed to aim for even better results by further stricter glycemic control, not only failed to demonstrate significant reductions in CVD events but also unexpectedly found increased mortality in the intensive intervention group in the ACCORD study.

The reasons why the intensive hypoglycemic intervention failed to reduce or even increased the incidence of CVD were not fully resolved despite rigorous explanatory analysis including possible candidates such as a higher incidence of weight gain and hypoglycemia accompanying the therapeutic intervention or a too-rapid lowering of HbA1c. Hypoglycemia associated with therapeutic use of hypoglycemic agents, including insulin, is one the causes that might explain the dissociation. A meta-analysis suggested that severe hypoglycemia was strongly associated with a higher risk of CVD (relative risk 2.05, 95% CI 1.74–2.42) (Goto et al., 2013)

Meta-analyses of these clinical trials were also repeated many times to integrate the effects of intensive glycemic control on CVD events and mortality but only showed similar results of modest though significant reductions in mainly nonfatal myocardial infarctions but no significant effect on mortality (Turnbull et al., 2009; Ray et al., 2009; Kelly et al., 2009; Boussageon et al., 2011). These results imply that, at least, concentrating on a rapid and intense lowering of only glucose levels would not necessarily result in a reduction of CVD in patients with diabetes.

Blood Pressure and its Control

Hypertension is more frequent in people with diabetes than in those without it, and the frequency of diabetes is known to also be higher in persons with hypertension than in normotensive individuals. In a historical cohort of the Multiple Risk Factor Intervention Trial (MRFIT), systolic blood pressure was shown to be an independent risk factor for CVD in persons with diabetes (Stamler et al., 1993). In an analysis of Framingham participants newly diagnosed with diabetes, hypertension was shown to be associated with a 72% increase in the risk of all-cause death and a 57% increase in the risk of any CV event in individuals with diabetes. The population-attributable risk from hypertension in individuals with diabetes mellitus was 30% for all-cause death and 25% for any cardiovascular event (Chen et al., 2011).

In clinical trials, each 10-mmHg decrease in systolic blood pressure was associated with reductions in risk of 12% (95% CI 10–14) for any complication related to diabetes, 15% (12–18) for deaths related to diabetes, and 11% (7–14) for myocardial infarctions in the UKPDS (Adler et al., 2000). Similarly seen in a meta-analysis of 40 large-scale randomized controlled trials of blood pressure-lowering treatment that did not exclude patients with diabetes, each 10-mmHg lowering of systolic blood pressure was associated with a significantly lower risk of mortality (relative risk 0.87, 0.78–0.96), coronary heart disease (0.88, 0.80–0.98), and stroke (0.73, 0.64–0.83) (Emdin et al., 2015). However, it is noteworthy that the "legacy effect," which was seen in terms of the effect of glycemic control, was not observed in blood pressure control, implying the necessity of continuous control for prevention of CVD in patients with diabetes (ACCORD study group, 2010a)

At the same time, a U-shaped association was reported between blood pressure and stroke risk among diabetic patients who had undergone a pharmacological intervention, suggesting that too-aggressive control of blood pressure $<$110/65 mmHg was associated with a rather higher risk of stroke (Zhao et al., 2013). Also noteworthy is that, according to the recent meta-analyses of 49 clinical trials (Brunström and Carlberg, 2016), antihypertensive treatment reduced the risk of mortality and cardiovascular morbidity in diabetic patients only in those whose systolic blood pressure was $>$140 mmHg; for those with systolic blood pressure $\leq$ 140 mmHg, further treatment was associated with a rather increased risk of cardiovascular death.

Serum Lipids and Their Control

Dyslipidemia is known to be more common in diabetic individuals compared to nondiabetic individuals. As with people without diabetes, dyslipidemia is one of the most potent risk factors for CVD, especially CHD, in people with diabetes. In particular, the association of low-density lipoprotein (LDL) cholesterol and CVD risk is well established regardless of age, sex, or ethnic group notwithstanding the presence or absence of diabetes.

A meta-analysis of 16 clinical trials of patients with type 2 diabetes showed that the pooled relative risk associated with a 1-mmol/L (39 mg/dL) increase in LDL cholesterol was 1.30 (95% CI 1.19–1.43) for incident CVD and 1.50 (95% CI 1.25–1.80) for

CVD mortality, independently of other conventional risk factors (Wang et al., 2013). This is similar to the results for nondiabetic persons (Cholesterol Treatment Trialists' Collaborators (2008, 2010).

Dyslipidemia in type 2 diabetes is known to be characterized by increased levels of triglycerides and decreased levels of high-density lipoprotein (HDL) cholesterol based on being accompanied by obesity and insulin resistance. In Japanese persons with type 2 diabetes, hypertriglyceridemia had an impact on CHD comparable to LDL cholesterol, and both additively affected the CHD risk (Sone et al., 2011). However, clinical trials using fibrates, agents for lowering triglycerides and increasing HDL cholesterol, indicated relatively limited effects on CVD mortality in diabetic patients compared to trials using statins (Keech et al., 2005; Burgess et al., 2010; ACCORD Study Group, 2010b).

Effects of Control of Combinations of Multiple Risk Factors and Lifestyles

It has been established that simultaneous control of multiple risk factors is essential and effective for preventing CVD and its mortality in patients with diabetes, though accomplishing this is not necessarily easy in clinical settings. It also needs to be mentioned that even by the most efficient way to prevent CVD, it takes nearly 10 years for the mortality difference between groups to reach statistical significance. That differences between groups favoring those receiving a multifactorial intervention over traditional treatment become evident long after the intervention has been discontinued suggests that "metabolic memory" or the "legacy effect" exists not only for glycemic control but also for multifactorial interventions.

In the Steno-2 study, though it involved a limited number of participants, 7.8 years of intensified multifactorial intervention for hyperglycemia and hypertension and the use of lipid-lowering agents and aspirin together with lifestyle modifications was shown to successfully reduce the risk of nonfatal CVD (Gaede et al., 2003) and mortality (Gaede et al., 2008) among patients with type 2 diabetes. During 21.2 years of follow-up, including the intervention period in the first 7.8 years, the hazard ratio for CVD events was 0.55 (95% CI, 0.39–0.77) and median time before the first CVD event after randomization was 8.1 years longer in the multifactorial intervention group than in the conventional therapy group. Hazard ratio for all-cause mortality for multifactorial intervention group was also 0.55 (0.36–0.83) (Gæde et al., 2016).

Lifestyle intervention is also effective in preventing CVD in persons with type 2 diabetes and prediabetes. In the Da-Qing study in China, the lifestyle intervention program for the first 6 years in people with impaired glucose tolerance could reduce the incidence of cardiovascular and all-cause mortality even after 23 years of follow-up (Li et al., 2014). In the Japan Diabetes Complications Study (JDCS), an intervention program focusing on a lifestyle intervention, including telephone counseling, successfully decreased the risk of stroke in patients with type 2 diabetes (Sone et al., 2010). In the same cohort, it was shown that a significant reduction in the hazard ratio in patients in the top ($\geq$15.4 metabolic equivalents [MET] h/week) vs. the bottom tertile ($\leq$3.7 MET h/week) of leisure-time physical activity was observed for stroke (HR 0.55, 95% CI 0.32–0.94) and total mortality (0.49, 0.26–0.91) (Sone et al., 2013).

In spite of this clinical evidence, almost half of adults with diabetes in the United States did not meet the goals recommended by therapeutic guidelines (Ali et al., 2013). Among adults with diabetes, those with optimal levels of blood pressure, LDL-cholesterol, and HbA1c comprised only 7.2% of the study population but were associated with the great reduction in cardiovascular event risk of 62% (Wong et al., 2016).

Conclusions

Diabetes mellitus even in its early- or pre-stages confers a substantial burden on CVD morbidity and mortality worldwide. Because of its asymptomatic onset and progression, population-based screening programs are essential for detection of the disease before the development of vascular complications including CVD.

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Diagnosis and Imaging of Congenital Heart Disease

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Introduction

History of Diagnosis in Pediatric Cardiology

Early pediatric cardiologists relied primarily on auscultation, electrocardiography, and chest x-ray for diagnosis. At that time, there were few surgical options available, limiting the need for detailed diagnoses. As surgical techniques advanced, diagnostic methods were forced to keep pace. Cardiac catheterization became the diagnostic method of choice. Catheterization provided valuable hemodynamic data and angiography allowed better delineation of cardiac anatomy. However, catheterization was limited by invasiveness and lack of portability. As ultrasound equipment improved, echocardiography became the modality of choice for diagnosis in congenital heart disease (CHD). While newer techniques, such as cardiac magnetic resonance imaging (CMR) and computed tomography (CT), continue to advance the field, echocardiography remains the mainstay for diagnosis. Other modalities function primarily as adjuncts. As such, this article focuses on diagnosis of various congenital heart lesions using echocardiography. Each section will include a brief overview of various types of cardiac anatomy then review diagnosis by echocardiography. We will then discuss the strengths and weaknesses of CMR, CT, and cardiac catheterization and the situations in which they are most commonly used. This article serves as an overview of cardiac lesions that are fundamental to understanding diagnosis of CHD. For more detailed explanations of cardiac anatomy and to become acquainted with additional lesions not discussed here, please see related articles.

An Overview of Segmental Anatomy

A continued topic of controversy in the world of pediatric cardiology is the correct vocabulary to use when describing complicated cardiac anatomy, such as that of heterotaxy. The two main systems of nomenclature are referred to by the eponyms "Van Praaghian" and "Andersonian." The Van Praaghian method defines the heart based on situs and five cardiac segments. Situs refers to the spatial relationships and chirality of each cardiac chamber. Chirality implies that each cardiac chamber's morphology has fundamental

chiral characteristics that are uniquely "right" or "left." This school of nomenclature divides the heart into five cardiac segments, the three main segments being the atria, ventricles, and great arteries and two connecting segments being the atrioventricular canal and infundibulum. According to Van Praagh, normal cardiac anatomy is labeled as {S, D, S} meaning visceroatrial situs solitis, D-looped ventricles, and great artery situs solitus (Ezon et al., 2015).

The Andersonian system fundamentally differs from Van Praaghian by dividing the heart into three main components, the atria, ventricles, and great arteries. This nomenclature system does not use situs to discuss the spatial relationships of the heart segments. Instead, in this system, both the spatial arrangement of each cardiac component and the way they join to successive components are described. The terms concordant and discordant are used to discuss the relationship of successive components. Using the Andersonian method, a normal heart would be described as usual atrial arrangement, concordant atrioventricular connections, and concordant ventriculoarterial connections (Ezon et al., 2015).

Echocardiography

Physics

While a detailed description of the underlying physics is beyond the scope of this article, a limited understanding can help illuminate the strengths and weaknesses of the modality. Echocardiography uses ultrasound waves to image cardiac structures. An echocardiographic probe emits waves that are reflected, absorbed, or scattered as they contact internal structures. The reflected waves return to the probe and are used to construct an image. The resolution and penetration depend on the frequency of the probe, with higher frequencies providing better resolution but decreased penetration. The larger a patient, the more penetration is needed, decreasing image resolution. Ultrasound waves cannot propagate through air and cannot pass through bone, limiting the available "windows" for visualizing the heart.

Strengths and Weaknesses

Echocardiography has many advantages. It is portable, non-invasive, and fast. Images can be obtained and interpreted in "real-time," allowing rapid diagnosis in critically ill infants and children. Doppler echocardiography (spectral and color flow) allows for accurate assessment of valvar stenosis and insufficiency, vascular obstruction, and ventricular and atrial septal defects (ASDs). Transesophageal echocardiography (TEE) can provide imaging during sterile procedures, such as cardiac catheterizations and cardiac surgery, and provides excellent images with less dependency on imaging windows. Advances in equipment have led to improved image quality, better three-dimensional (3D) imaging, and the ability to assess subclinical dysfunction using myocardial strain. However, echocardiography has many limitations. Accurate diagnosis depends on good image quality, and imaging windows tend to worsen as children and adults grow, an issue exacerbated by the obesity epidemic. In older children, some structures can be extremely difficult to image even with good windows. Quantitation of chamber sizes and function, particularly of the right ventricle, can be difficult and often involves geometric assumptions that limit accuracy. This is somewhat alleviated by 3D imaging, but adequate 3D datasets can be difficult to obtain. Finally, objective hemodynamic assessment is limited. Despite these limitations, the advantages of echocardiography insure that it will remain the modality of choice for diagnosis of CHD for the foreseeable future.

Septal Defects

Atrial septal defects

Background

The atrial septum is the wall between the left and right atria, preventing mixing of blood between chambers. A patent foramen ovale (PFO) is a normal interatrial communication during fetal life that may persist after birth (**Fig. 1**, **Movies 1** and **2**). Usually there is complete anatomic closure of a PFO by adulthood with only about 20%–25% of adults having PFOs (Silvestry et al., 2015). An ASD, on the other hand, is due to a deficiency of atrial septal tissue and is not a normal finding. There are various types of ASDs including the secundum ASD, primum ASD (a variant of AVSD discussed later), sinus venosus defect (often associated with partial anomalous pulmonary venous return), and coronary sinus defect (**Fig. 2**). All types of ASDs lead to left to right shunting across the atrial septum and right-sided chamber dilation. Secundum ASDs are the most common type of ASD comprising approximately 75% of ASDs (Kuijpers et al., 2015) (Yong et al., 2009; Gabriels et al., 2014). This defect is graded as small when less than 6 mm, moderate when 6–12 mm, or large when greater than 12 mm (McMahon et al., 2002).

Diagnosis by ECHO

The goal of transthoracic echocardiography is to determine the location and size of the ASD, measure the defect's margins to determine suitability for transcatheter closure, and assess hemodynamics. This echocardiographic examination relies mainly on 2D imaging and color Doppler. The subcostal acoustic window, which utilizes the orthogonal plane to the atrial septum, is the optimal view to minimize the likelihood of false dropout acoustic signals (**Fig. 3A** and **B**). This contrasts with the apical window, which is at most risk for dropout as a result of the parallel orientation of the ultrasound beam relative to the atrial septum. Hemodynamic assessment should also be completed. This includes color Doppler to assess the directionality of flow across the atrial septum, which

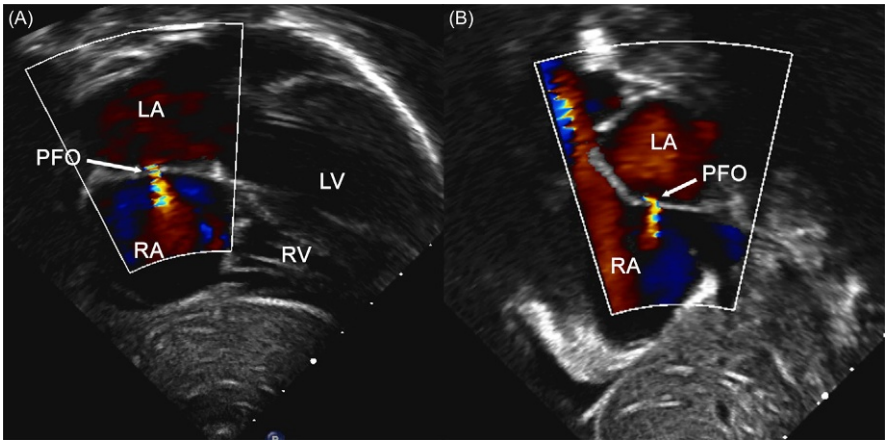

Fig. 1 Subcostal coronal (A) and sagittal (B) views with color flow mapping demonstrating a patent foramen ovale (PFO). Blood flow across the defect, from left atrium (LA) to right atrium (RA), represented by the red jet of color flow moving toward the transducer.

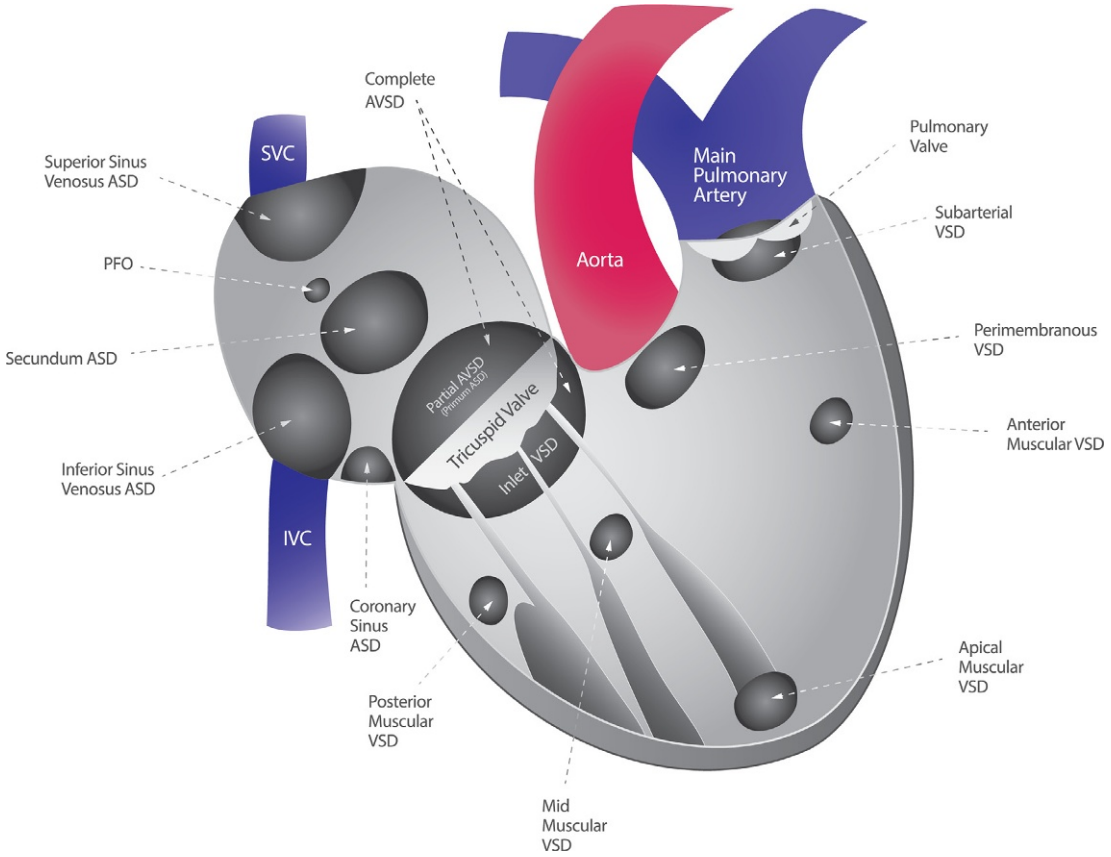

Fig. 2 Drawing of the right atrial and right ventricular surfaces of the atrial and ventricular septae illustrating the multiple names and locations of atrial and ventricular septal defects. Of note, while a primum atrial septal defect (ASD) and an inlet ventricular septal defect (VSD) can each occur in isolation, a complete atrioventricular septal defect (AVSD) is caused by a defect in both. *SVC*, superior vena cava; *IVC*, inferior vena cava.

is usually left to right, and also the degree of right-sided chamber enlargement. Other markers of this increased hemodynamic load include diastolic ventricular septal flattening and increased pulmonary blood flow. TEE can help guide percutaneous or operative closure, and 3D imaging plays an important role in guiding the interventionalist as well as assessing adequate device placement (Fig. 3C and D, Movies 3 and 4).

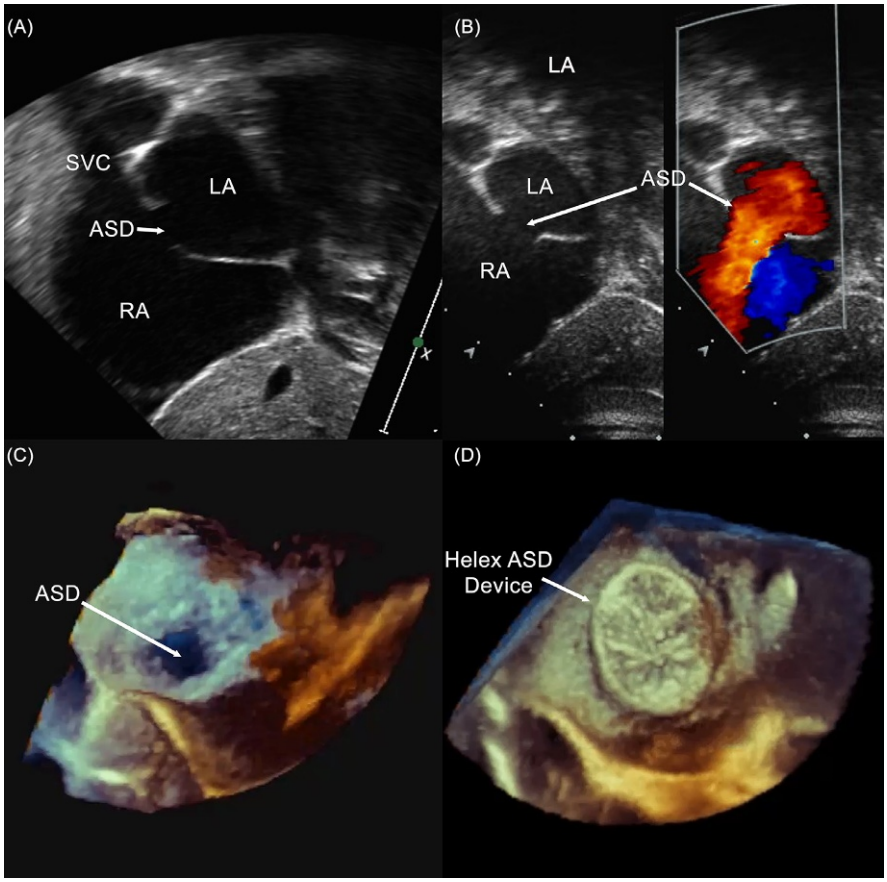

Fig. 3 Atrial septal defect (ASD) as seen in a subcostal sagittal view (A). Similar view with color compare demonstrates left to right shunting from the left atrium (LA) to the right atrium (RA) across the ASD, represented by the red jet of color flow moving toward the transducer (B). Three-dimensional image of an ASD obtained with transesophageal echocardiography (TEE) prior to device closure (C). Three-dimensional image obtained by TEE after device closure with a Helex device demonstrating good placement of the Helex across the ASD (D). *SVC*, superior vena cava.

Ventricular septal defects

Background

The ventricular septal defect is the most common form of CHD in childhood. It occurs in 50% of all children with CHD (Fyler, 1992c; Minette and Sahn, 2006) and in 20% as an isolated lesion (Minette and Sahn, 2006; Moller et al., 1995). Advances in imaging have improved detection of VSDs, increasing the postulated incidence of VSDs to approximately 5 per 100 births from 2 per 1000 births (Minette and Sahn, 2006; Ooshima et al., 1995; Roguin et al., 1995). Following bicuspid aortic valves, VSDs are the most common congenital heart defect in the adult population (Minette and Sahn, 2006; Fyler, 1992b). VSDs can occur in multiple locations, and there are numerous classification methods to describe VSDs (Van Praagh et al., 1989; Soto et al., 1980, 1989; Hagler et al., 1985; Jacobs et al., 2000b). To help decrease confusion related to the multiple naming conventions, the Congenital Heart Surgery Nomenclature and Database Project created a unified nomenclature system (Jacobs et al., 2000b). The following discussion is based on this unified terminology, which divides the ventricular septum into four primary locations: muscular, inlet, perimembranous, and subarterial (**Fig. 2**).

Diagnosis by ECHO

The goals of the TTE examination include clearly identifying the VSD location, size, number of defects, and relationship of the defect to neighboring structures (such as a straddling valve) that may complicate surgical repair. Numerous planes and sweeps are necessary to profile the ventricular septum using both spectral Doppler and color Doppler. A typical VSD echo examination includes the parasternal long- and short-axis views to profile perimembranous, subarterial, and muscular VSDs, the apical four-chamber view sweeping posterior to anterior to examine the inlet septum, trabecular septum, and membranous septum, and the subcostal window to investigate the inlet and membranous septum (**Figs. 4** and **5**, **Movies 5–7**). Finally, the degree of left to right shunting at the level of the VSD can be assessed qualitatively by noting the degree of left ventricular enlargement (**Fig. 5C**), systolic septal flattening, and increased pulmonary blood flow. Careful delineation of VSD location and margins is necessary to guide operative or percutaneous closure.

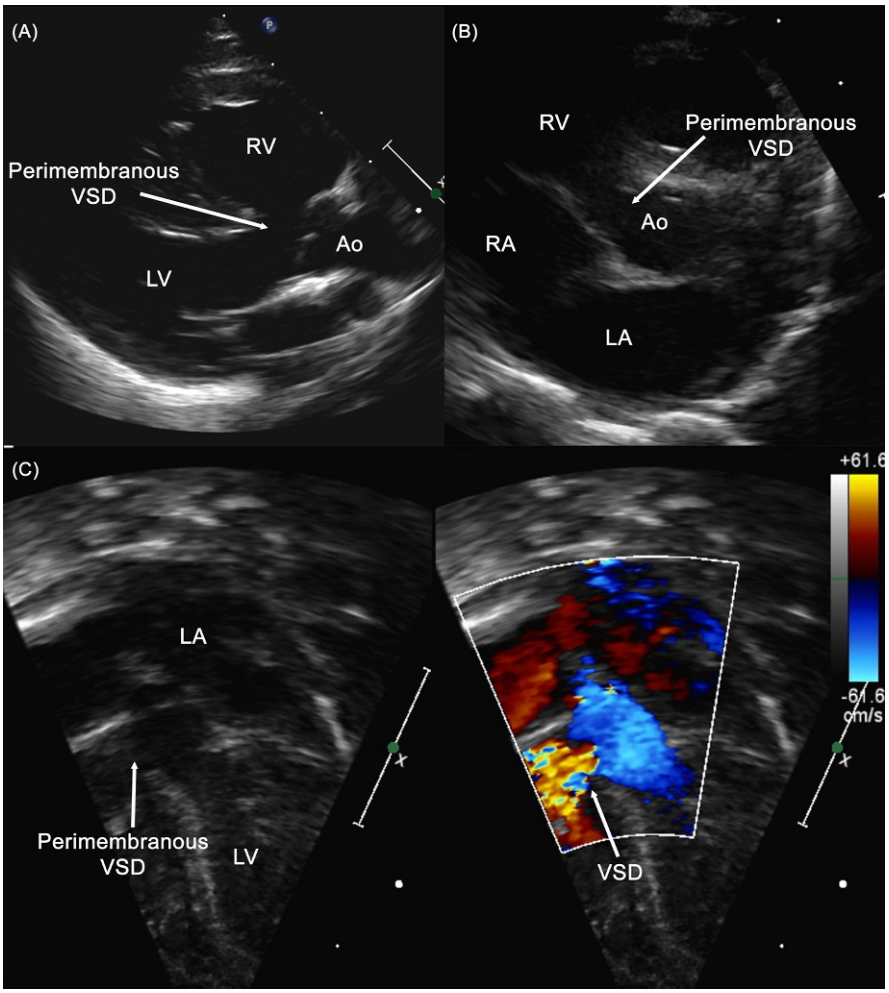

Fig. 4 Long (A) and short (B) axis views of a perimembranous ventricular septal defect (VSD). Apical 4-chamber view with color compare (C) of the perimembranous VSD. The red jet of color flow moving toward the transducer represents left to right shunting across the VSD. *Ao*, aorta; *LA*, left atrium; *LV*, left ventricle; *RA*, right atrium; *RV*, right ventricle.

Atrioventricular septal defects

Background

There are many labels used to name the constellation of defects known as atrioventricular septal defects (AVSDs) including endocardial cushion defects and common atrioventricular canals. Here we will be using the name AVSD. This defect is defined as a deficiency in the formation of the cardiac crux (**Fig. 2**) (Calabro and Limongelli, 2006). AVSDs account for about 3% of congenital cardiac malformations and two out of every 10,000 live births (Calabro and Limongelli, 2006; Fyler, 1992a; Ferencz et al., 1997).

AVSDs are most commonly divided into three subtypes: (1) partial, (2) complete, and (3) transitional (Jacobs et al., 2000a). A partial AVSD is defined as a common atrioventricular junction with two separate orifices, a left AVV with three leaflets (cleft) and a primum ASD (Jacobs et al., 2000a). A complete AVSD is defined as a primum ASD, inlet VSD, and a large common valve that spans the defects (Jacobs et al., 2000a). A transitional AVSD is somewhere in between partial and complete, where the AV valve has two orifices but there are both a primum ASD and an inlet VSD (Jacobs et al., 2000a). Despite these distinctions between the partial and complete AVSD, certain anatomic features are shared in all types of AVSDs. Common features include the following:

- Absence of the muscular atrioventricular septum resulting in a loss of the normal offsetting of atrioventricular valves and leading to the left and right AV valves inserting at the same level
- Anterior displacement of the aortic valve, which usually rests between the mitral and tricuspid valves
- Elongation of the LVOT creating a long narrow LVOT referred to as a "gooseneck" deformity

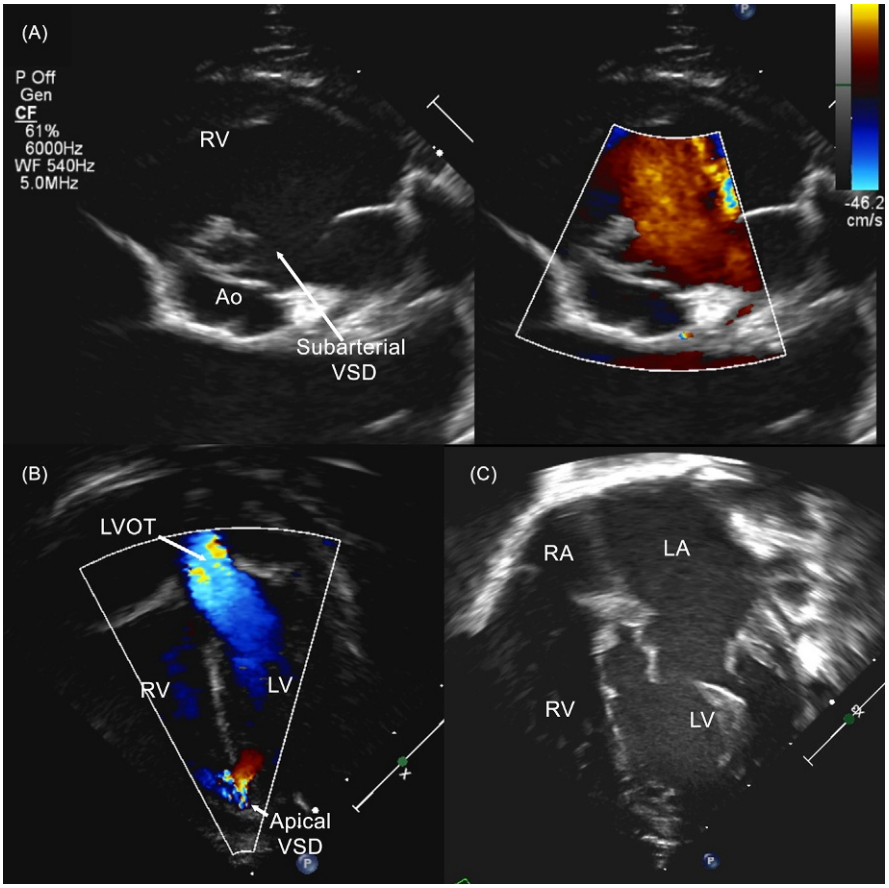

Fig. 5 Short-axis color compare image demonstrating a subarterial ventricular septal defect (VSD) (A). The red jet of flow represents left to right shunting across the VSD. Apical 4-chamber view with color flow mapping demonstrating an apical muscular VSD (B). Apical 4-chamber view in a patient with a large VSD demonstrating significant left-sided chamber dilation (C). *Ao*, aorta; *LA*, left atrium; *LV*, left ventricle; *LVOT*, left ventricular outflow tract; *RA*, right atrium; *RV*, right ventricle.

Diagnosis by ECHO

Evaluation of AVSD anatomy can be broken down into a few echocardiographic goals: determination of the size of the ASD and VSD, assessing the anatomy of the atrioventricular valve and chordal attachments, and understanding the degree of ventricular balance (primarily in complete AVSDs). The apical four-chamber view visualizes the common AV valve (**Fig. 6**, **Movie 8**) and can further assess AV valve anatomy, including chordal attachments crossing the ventricular septal defect that might preclude biventricular repair. Three-dimensional imaging can aid in assessment of the AV valve prior to and after surgery (**Fig. 7**, **Movies 9** and **10**). The same TTE techniques previously described for evaluation of ASDs and VSDs are employed to visualize the primum ASD and inlet VSD. When diagnosing a complete AVSD, it is also vital to clarify the defect as "balanced" or "unbalanced." When the common AV valve is positioned equally over both ventricles it is referred to as "balanced" versus when the common AV valve is unequally positioned over the right or left ventricle it is called "unbalanced" (Cohen et al., 1996). This is best assessed by imaging the AV valve en face in a modified subcostal coronal view (**Fig. 8**). Balanced AVSDs account for approximately 90% of complete AVSDs. (Cohen et al., 1996; Corno et al., 1988). In general, an AVSD is considered unbalanced if more than two-third of the common valve is committed to one ventricle. Unbalanced AVSDs are often associated with ventricular hypoplasia and are unlikely to be candidates for biventricular repair (Cohen et al., 1996).

The severity of atrioventricular valve regurgitation (AVVR) and LVOT obstruction should be clarified as these have hemodynamic implications. Both partial and complete AVSDs can have varying amounts of AVVR, which can be visualized with color Doppler (**Fig. 9**, **Movie 11**). Clarifying the origin and direction of the regurgitant jet(s) may require multiple imaging planes including apical, subcostal, and parasternal windows. With the elongated LVOT "gooseneck" deformity, LVOT obstruction may occur. The apical 5-chamber view and parasternal long-axis views display the LVOT well and allow for appropriate angles of interrogation to use Doppler and locate the level(s) of obstruction.

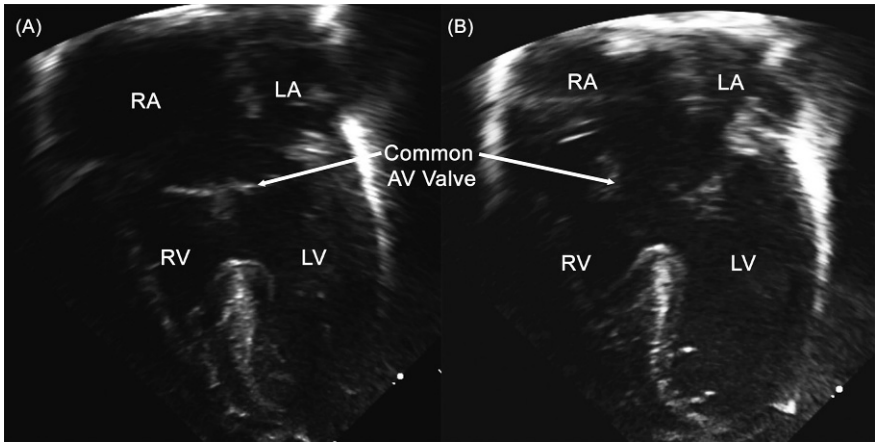

Fig. 6 Apical 4-chamber view demonstrating a complete atrioventricular septal defect with a common AV valve in systole (A) and diastole (B). *LA*, left atrium; *LV*, left ventricle; *RA*, right atrium; *RV*, right ventricle.

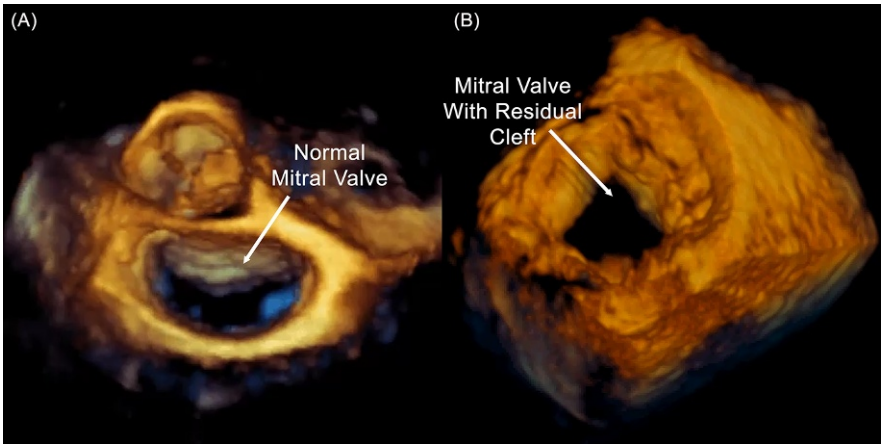

Fig. 7 Three-dimensional transesophageal echocardiography (TEE) of a patient with a normal mitral valve (A) and an atrioventricular septal defect (AVSD) who underwent previous surgical repair and has a residual cleft (B).

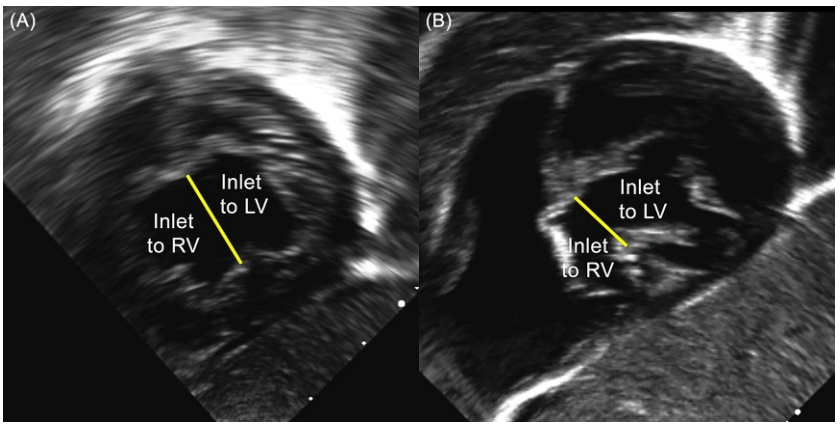

Fig. 8 Subcostal images demonstrating a balanced atrioventricular septal defect (AVSD) (A) and an unbalanced AVSD (B) with the valve shifted over the left ventricle (LV) and a smaller inflow to the right ventricle (RV).

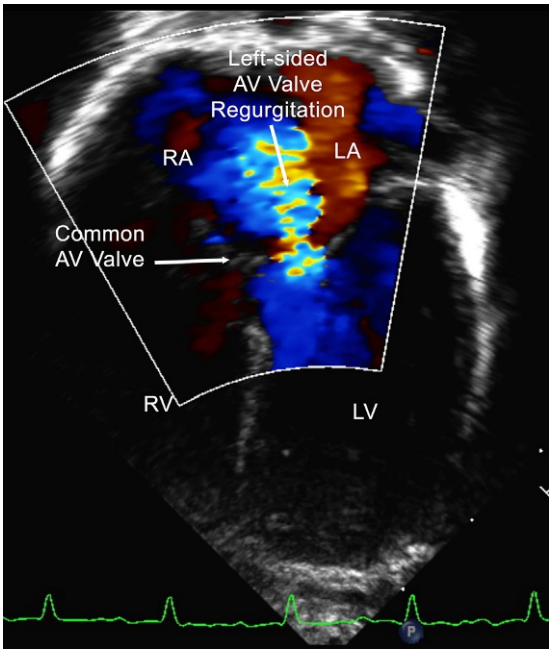

Fig. 9 Apical 4-chamber image demonstrating mild to moderate left-sided atrioventricular (AV) valve regurgitation (blue jet).

Right-Sided Heart Lesions

Tetralogy of Fallot

Background

Tetralogy of Fallot (TOF) was the first formally described cyanotic cardiac lesion (Karl and Stocker, 2016). TOF consists of four anatomic components of variable severity: (1) an anteriorly malaligned VSD, (2) overriding aorta, (3) obstruction of the right ventricular outflow tract (RVOT), and (4) right ventricular hypertrophy (Bailliard and Anderson, 2009). While described as a tetralogy, the displacement of the infundibular septum leads to all four components. TOF occurs in 3 of every 10,000 live births, is the most common cyanotic cardiac disease in patients greater than 1 month of age, and accounts for up to 10% of all congenital cardiac malformations (Bailliard and Anderson, 2009). Patients with TOF are at risk for hypercyanotic episodes (colloquially referred to as "tet spells") where dynamic subpulmonary obstruction and/or pulmonary hypertension lead to decreased pulmonary blood flow, increased right to left shunting across the VSD, and severe cyanosis. Pulmonary atresia VSD (also referred to as TOF with pulmonary atresia) is a more severe variant of TOF with a hypoplastic (or absent) RVOT, atretic pulmonary valve, and pulmonary blood flow supplied exclusively by either a patent ductus arteriosus (PDA) or aorticoopulmonary collateral vessels (Karl and Stocker, 2016). TOF with absent pulmonary valve represents another TOF variant with rudimentary, nonfunctional pulmonary valve leaflets leading to free pulmonary insufficiency (Karl and Stocker, 2016); this lesion is often associated with severe main and branch pulmonary artery dilation and airway obstruction.

Diagnosis by ECHO

TTE is used to both diagnose TOF and investigate for associated anomalies. The parasternal long axis view highlights the malalignment of the conal septum with the overriding aorta (**Fig. 10**, **Movie 12**). The degree of aortic override can be assessed in both the parasternal long- and short-axis views, with greater than 50% of the aorta overriding the right ventricle defined as a double outlet right ventricle. Clear views of the RVOT, dynamic nature of subpulmonary obstruction, size of the pulmonary valve annulus, MPA and branch PAs, and any additional sources of blood flow to lungs are necessary to understand the patient's risk for cyanosis and to appropriately plan the surgical repair (Bailliard and Anderson, 2009). The parasternal short axis is ideal for measuring the size of the pulmonary valve annulus, MPA, and branch PAs (**Fig. 11**). The degree of RVOTO can be assessed in all views using color flow mapping and continuous wave Doppler, though apical 4-chamber and subcostal windows often provide an excellent angle of interrogation (**Fig. 12**, **Movie 13**).

After diagnosis, TOF-associated anomalies must be clarified. Anomalous origins of the coronary arteries occur in one-sixth of patients, the most common being the left anterior descending artery from the right coronary artery. A right aortic arch is present in one-quarter of patients with TOF (Bailliard and Anderson, 2009). After repair, many patients have severe pulmonary valve

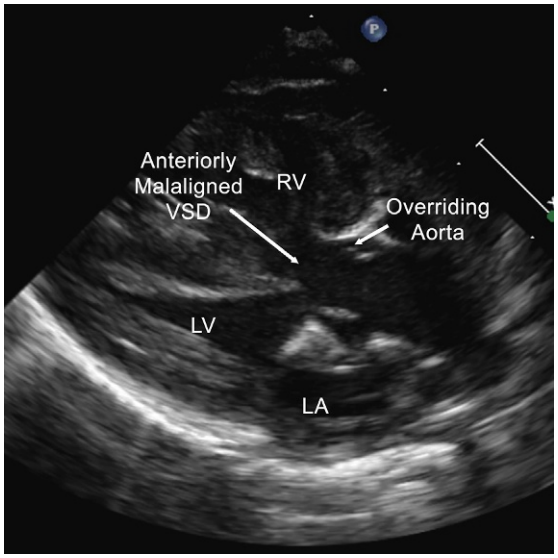

Fig. 10 Parasternal long-axis view in a patient with tetralogy of Fallot demonstrating the overriding aorta and anteriorly malaligned VSD. *LA*, left atrium; *LV*, left ventricle; *RA*, right atrium; *RV*, right ventricle.

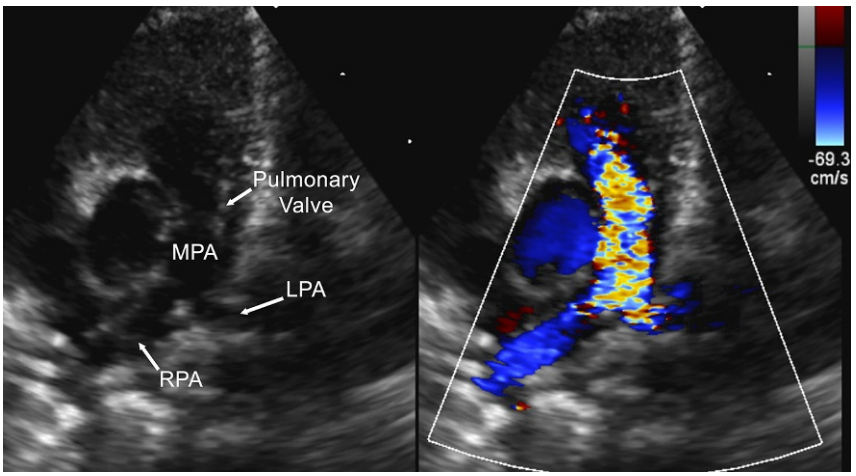

Fig. 11 Parasternal short-axis color compare image in a patient with tetralogy of Fallot demonstrating hypoplasia of the pulmonary valve annulus, main pulmonary artery (MPA), and the right and left pulmonary arteries (RPA and LPA).

insufficiency. These patients need to be followed closely for RV dilation and ventricular dysfunction, and many require pulmonary valve replacement, either in the operating room or using one of the newer percutaneous valves (Valente et al., 2014; Cheatham et al., 2015).

Pulmonary valve stenosis

Background

Isolated pulmonary valve stenosis occurs with an incidence of 1.5–6.5 per 10,000 live births accounting for 2%–13% of all CHD (Waller et al., 1995) (Hoffman and Christianson, 1978). In addition to isolated disease, multiple types of CHD are associated with pulmonary valve stenosis, including TOF, double outlet right ventricle, single ventricle anatomy, and AVSD (Waller et al., 1995). Here we will focus on isolated valvar pulmonic stenosis, though patients can have concurrent subvalvar and supravalvar obstruction and these regions must be assessed carefully. Pulmonary valve stenosis is most commonly associated with commissural fusion but

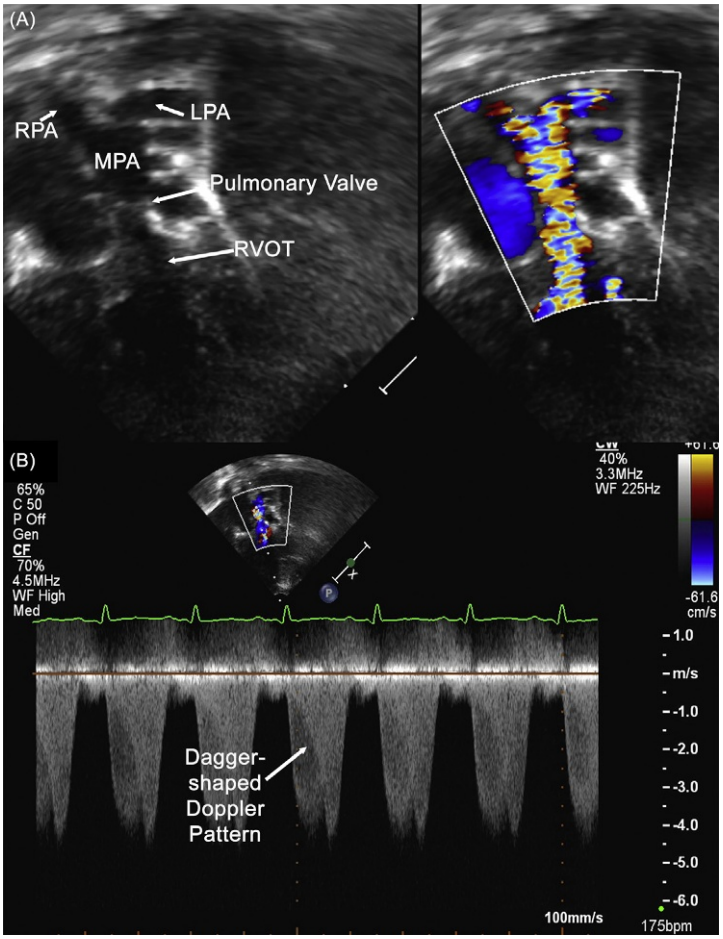

Fig. 12 Apical 4-chamber color compare image in a patient with tetralogy of Fallot demonstrating subvalvar, valvar, and supravalvar obstruction with aliasing throughout the right ventricular outflow tract (RVOT) (A). Continuous wave Doppler across the RVOT demonstrates dagger-shaped pattern, suggesting a component of subvalvar RVOT obstruction (B). *MPA*, main pulmonary artery; *RPA*, right pulmonary artery; *LPA*, left pulmonary artery.

can also be due to pulmonary valve dysplasia. In dysplastic pulmonary valve stenosis, all three cusps of the tricuspid pulmonary valve are thickened and rubbery (Waller et al., 1995; Koretzky et al., 1969); this is often associated with Noonan's syndrome.

Diagnosis by ECHO

Similar imaging planes employed in the diagnosis of TOF are utilized when imaging pulmonary stenosis. The pulmonary valve anatomy, size of the pulmonary valve annulus, size of the main and branch pulmonary arteries, and the subvalvar region must all be documented. Use of both pulse and continuous wave Doppler in the subvalvar, valvar, and supravalvar regions identifies the level(s) and severity of obstruction. Multiple views, including parasternal long and short axis, apical 4-chamber with anterior angulation, and subcostal (coronal and sagittal), should all be employed (**Fig. 13, Movie 14**). Right ventricular hypertrophy may develop in more significant pulmonary obstruction. Estimates of the right ventricular pressure can be made using CW Doppler of the tricuspid insufficiency and the interventricular septal geometry. The presence of an atrial level shunt with flow from right to left is an indicator that there is significantly elevated right ventricular end diastolic pressure.

Truncus arteriosus

Background

Persistent truncus arteriosus (TA) comprises <1% of congenital heart defects (Guenther et al., 2009). This anatomy is a consequence of improper conotruncal septation within the developing heart resulting in a common arterial trunk that gives rise to systemic, coronary, and pulmonary blood flow with an infundibular ventricular septal defect (Rajasinghe et al., 1997). The truncal valve is trileaflet in 70% of cases but can also be quadricuspid (21% of cases), bicuspid (9%), or even more rarely have more than four leaflets (Verhaert et al., 2010). Collett and Edwards (1949) described four types of TA lesions based on the origin of the pulmonary arteries (**Fig. 14**) (Collett and Edwards, 1949). A Type I truncus is defined by a common arterial trunk that gives rise to a short main pulmonary artery that divides into right and left branch pulmonary arteries (48%–68% cases). Type II truncus and Type III truncus are defined by the absence of a main pulmonary trunk. In Type II the left and right pulmonary arteries arise close to one another

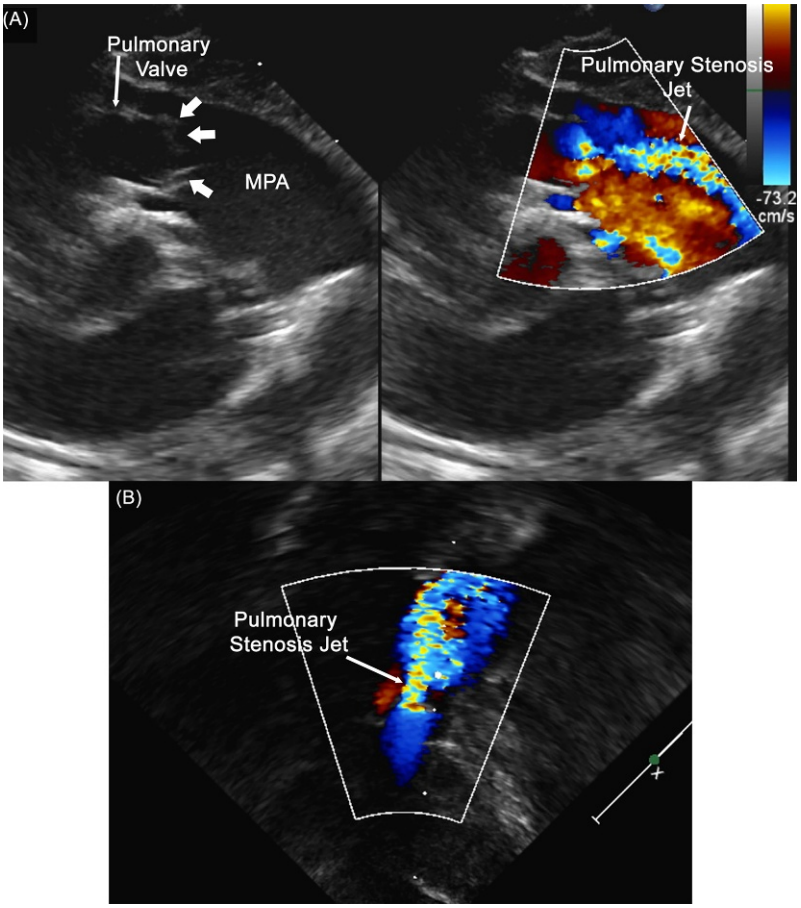

Fig. 13 Modified short-axis color compare image demonstrating the thickened and doming pulmonary valve leaflets (*arrows*) and a systolic pulmonary stenosis jet (*blue*) aimed into the dilated main pulmonary artery (MPA) (A). Apical 4-chamber image with color flow mapping demonstrating a systolic pulmonary stenosis jet (*blue*) (B).

versus in Type III these branch pulmonary arteries arise more distant from one another. Type II is more common than Type III, occurring in 29%–48% versus 6%–10% of cases. Finally, in Type IV truncus the lungs are supplied entirely by aorticopulmonary collaterals with no main pulmonary trunk or branch pulmonary arteries. This last form (Type IV) is now considered to be a variation of pulmonary atresia VSD and no longer considered a form of TA (Verhaert et al., 2010). In the Van Praagh classification of TA, Type A1 is the same as Type I, Type A2 is the same as Types II/III, Type A3 is TA with only one pulmonary artery off the truncal root (the other supplied by a PDA or collateral vessel), and Type A4 is TA with an interrupted arch. After ASDs, coronary artery abnormalities are the most common associated anomaly, occurring in nearly half of the cases (Butto et al., 1986; Mosca, 2010). Aortic arch abnormalities such as right aortic arch and interruption of the aortic arch are also common, occurring 36% and 11% of the time, respectively (Butto et al., 1986).

Diagnosis by ECHO

In order to diagnose TA there should be clear visualization of a dilated single great artery with a single semilunar valve overriding the interventricular septum. The parasternal long-axis view enables visualization of the common great artery overriding the interventricular septum (**Fig. 15**, **Movie 15**). The single great vessel usually overrides an outlet type VSD. The valve morphology should be documented in the short-axis view and the valve should be assessed for stenosis or insufficiency in all views (**Fig. 16**, **Movie 16**). Branch pulmonary artery anatomy should be assessed carefully, primarily in the short-axis and suprasternal notch views, though other views can be useful (**Fig. 17**). Parasternal short axis can also be used to determine coronary anatomy. Arch branching and sidedness should be carefully assessed in the suprasternal notch view.

Ebstein's anomaly

Background

Ebstein's anomaly is a malformation of the tricuspid valve and right ventricle which occurs in approximately 1 per 200,000 live births and accounts for <1% of all CHD (Attenhofer Jost et al., 2007; Dearani and Danielson, 2000). The hallmark of this lesion is the failure of delamination of the septal and posterior leaflets of the tricuspid valve leaving portions adherent to the underlying

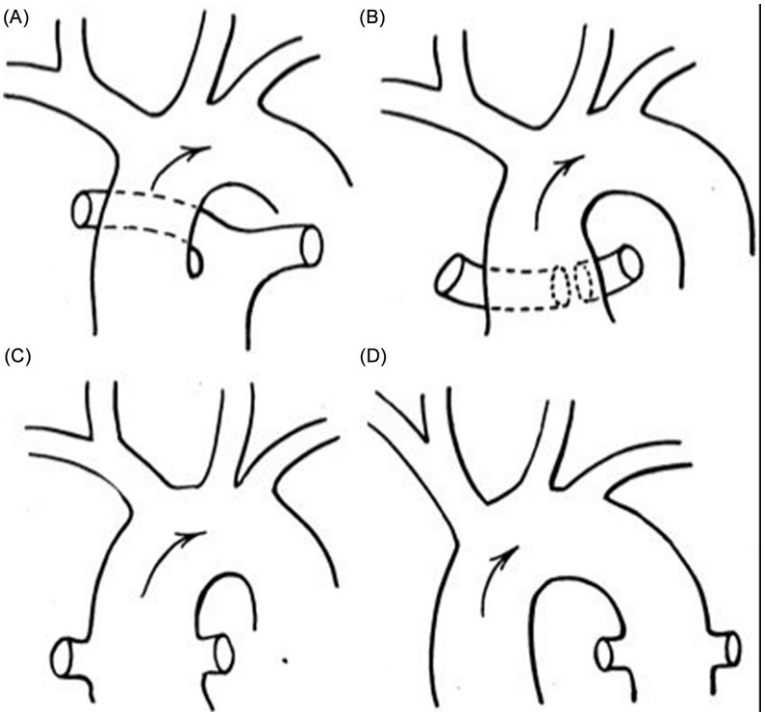

Fig. 14 Original figure published by Collett and Edwards (1949) demonstrating the four types of truncus arteriosus (TA). In Type I TA (A), the common arterial trunk gives rise to a main pulmonary artery that divides into the left and right pulmonary arteries (LPA and RPA). In Type II TA (B), the LPA and RPA arise separately from the common arterial trunk, but in close proximity. In Type III TA (C), the LPA and RPA arise separately from the common arterial trunk and are more distant from each other when compared with Type II. Type IV (D) is no longer considered a variant of TA and is now considered a variant of pulmonary atresia with ventricular septal defect (also called tetralogy of Fallot with pulmonary atresia). Type IV was originally described as TA with both branch pulmonary arteries supplied by aorticopulmonary collateral vessels.

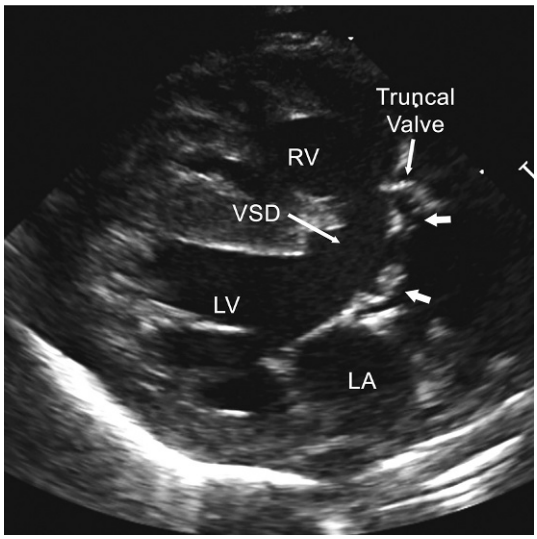

Fig. 15 Long-axis image in a patient with truncus arteriosus. There is a large truncal valve annulus that with thickened and doming truncal leaflets (*arrows*). *LA*, left atrium; *LV*, left ventricle; *RV*, right ventricle; *VSD*, ventricular septal defect.

myocardium. Additionally, there are anomalies of the tricuspid valve anterior leaflet including redundancy, fenestrations, and tethering. With portions of the septal and posterior leaflets adherent to the myocardium there is an overall downward displacement of the functional annulus, dilation of the "atrialized" portion of the right ventricle, and enlargement of the right atrioventricular junction (Attenhofer Jost et al., 2007; van Son et al., 2001; Cohen et al., 1994). Ebstein's anomaly usually results in tricuspid

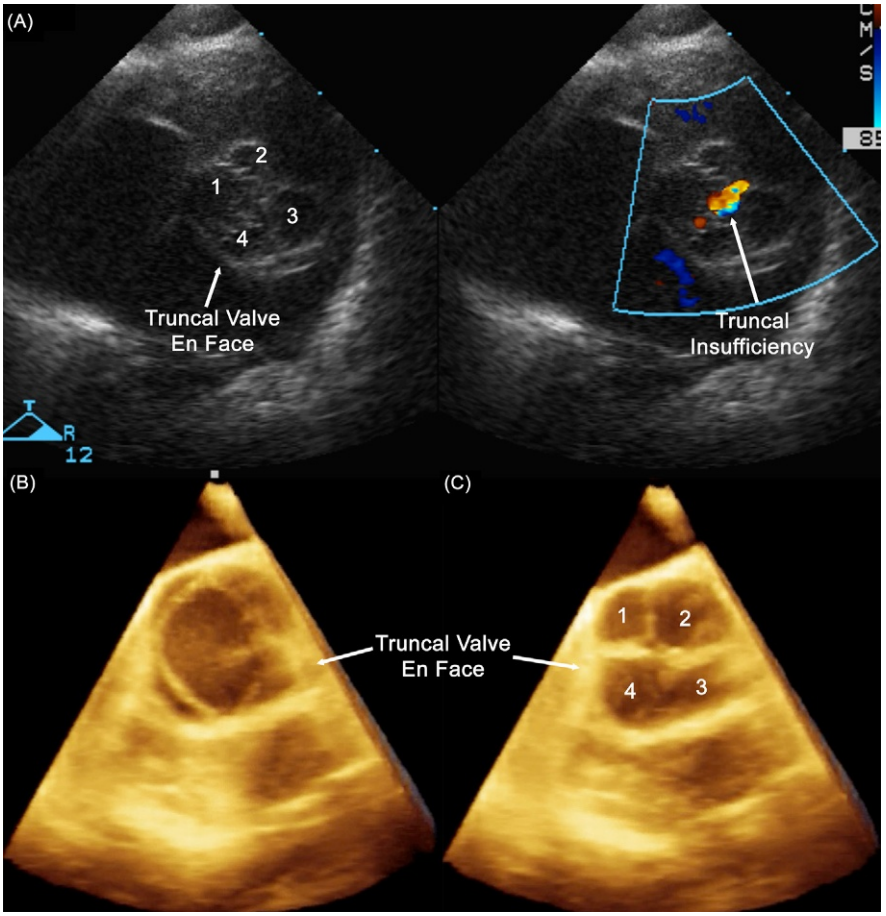

Fig. 16 Short-axis image with color compare demonstrating a quadricuspid truncal valve en face with mild truncal insufficiency, represented by color flow at the center of the valve (A). 3D image of a patient with quadricuspid truncal valve in early systole (B) and diastole (C).

regurgitation and can be associated with pulmonary valve atresia. The severity of disease can be variable, with some infants dying soon after birth (or in utero) and other patients who are essentially asymptomatic.

Diagnosis by ECHO

The echocardiographic examination of Ebstein's anomaly focuses heavily on the apical 4-chamber view which highlights the cardiac crux (**Fig. 18**, **Movies 17** and **18**). This view is used to calculate the displacement index, or distance between the septal insertions of the tricuspid and mitral valves divided by body surface area. Ebstein's anomaly is defined as a displacement index of greater than 8 mm/m^2. Careful attention should be given to the assessment of the complex tricuspid valve anatomy, including location and degree of valvar regurgitation. A thorough understanding of the tricuspid valve anatomy is necessary for appropriate surgical planning. 2D echo should also be used to thoughtfully examine the right ventricle, which is divided into three main regions. The first portion, the inlet portion, is directly involved with the malformation and is functionally integrated into the right atrium. The second and third portions, the trabecular and outlet portions, are not involved and comprise the functional ventricle (Attenhofer Jost et al., 2007). Careful attention should also be given to characterize the pulmonary valve, degree of right atrial enlargement, right ventricular enlargement, and right ventricular dysfunction. Finally, left ventricular anatomy should be assessed closely as left ventricular noncompaction can occur in association with Ebstein's anomaly.

Left-Sided Heart Lesions

Left ventricular outflow obstruction

Left ventricular outflow tract obstruction (LVOTO) includes subaortic obstruction, aortic valve stenosis, and supravalvar stenosis (Aboulhosn and Child, 2006). These lesions often occur concurrently with other types of left-sided obstruction. For example, Shone's complex is a syndrome with multiple left-sided lesions of inflow and outflow obstruction, originally described as a

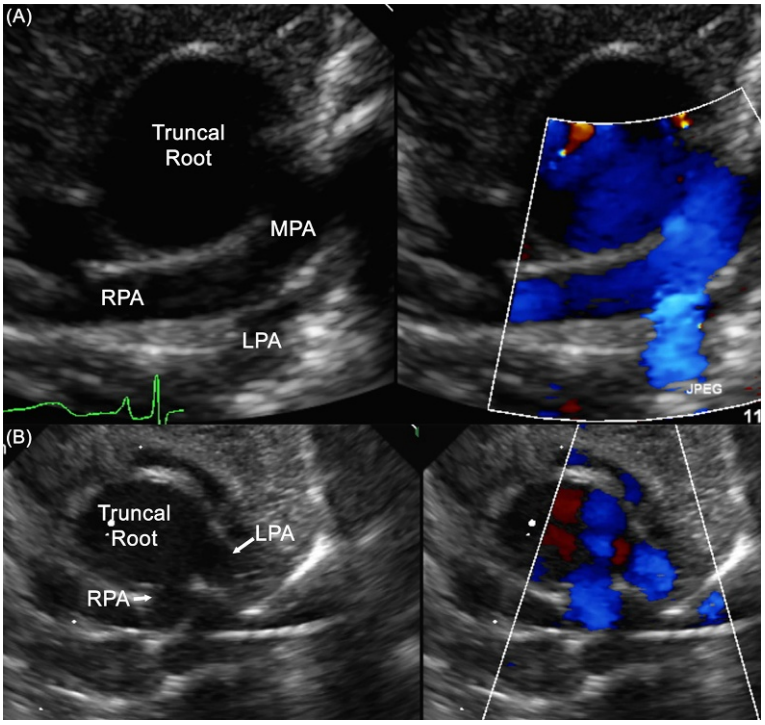

Fig. 17 Suprasternal notch color compare image of patient with truncus arteriosus type I (A) where the branch pulmonary arteries arise from a long main pulmonary artery (MPA) segment; patient with truncus arteriosus type II, where the branch pulmonary arteries arise directly from the common arterial trunk (B). *LPA*, left pulmonary artery; *RPA*, right pulmonary artery.

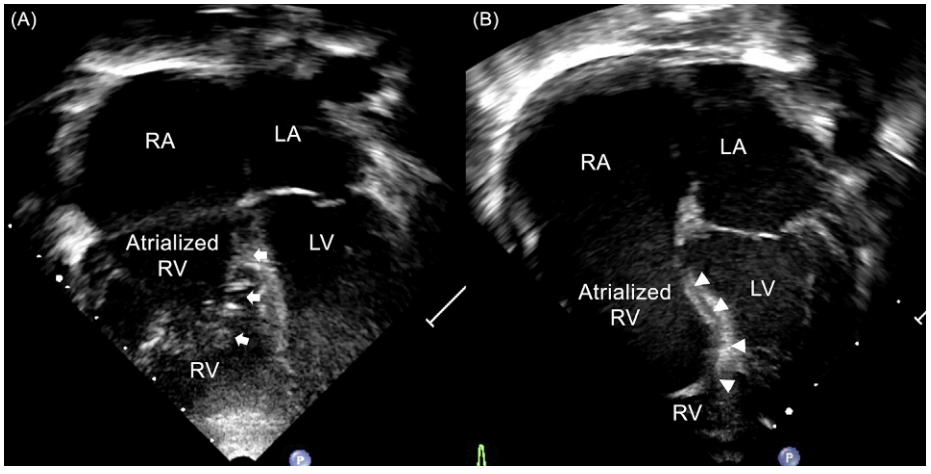

Fig. 18 Apical 4-chamber view in a patient with moderate Ebstein's anomaly (A). *Arrows* delineate the tethered and apically displaced septal leaflet. Apical 4-chamber view in a patient with severe Ebstein's anomaly (B). *Arrowheads* delineate the severely tethered and apically displaced septal leaflet, a significant portion of which had complete failure of delamination from the septum. *LA*, left atrium; *LV*, left ventricle; *RA*, right atrium; *RV*, right ventricle.

supraannular mitral ring, parachute mitral valve, subaortic stenosis, and coarctation of the aorta (Shone et al., 1963). Below we will discuss each LVOT obstructive lesion individually. Later, in the single ventricle section, we will discuss hypoplastic left heart syndrome.

Subaortic stenosis

Subaortic stenosis can be dynamic, usually due to hypertrophic cardiomyopathy, or fixed. As hypertrophic cardiomyopathy is covered in a different chapter, here we will focus on fixed subaortic stenosis. Subaortic stenosis is associated with the presence (with

or without spontaneous closure) of ventricular septal defects. It is due to a discrete membrane in 90% of cases but can also be caused by muscular narrowing or a combination of the two (Aboulhosn and Child, 2006; Brauner et al., 1997).

Aortic valve stenosis

Bicuspid aortic valve is the most common form of CHD and occurs in 1%–2% of the general population (Atkins and Sucosky, 2014). The most common type is due to fusion between the right and left coronary leaflets, occurring 71% of the time (Atkins and Sucosky, 2014). This malformed valve may have elements both of stenosis and insufficiency. Aortic valve stenosis can also occur in the setting of unicuspid, bicuspid, tricuspid, or quadricuspid leaflet morphology (Waller et al., 1994).

Supravalvar stenosis

Supravalvar aortic stenosis is usually a focal narrowing that begins at the level of the sinotubular junction, though it can be a more diffuse narrowing (Aboulhosn and Child, 2006). It is often associated with Williams syndrome.

Diagnosis by ECHO

The TTE examination of LVOTO utilizes multiple imaging planes to clarify the subvalvar, valvar, and supravalvar anatomy. The aortic annulus, aortic root, sinotubular junction, and ascending aorta are usually measured in the parasternal long-axis window (**Figs. 19–21**, **Movies 19–21**). The short axis is used to obtain an en face view of the aortic valve, allowing for assessment of aortic

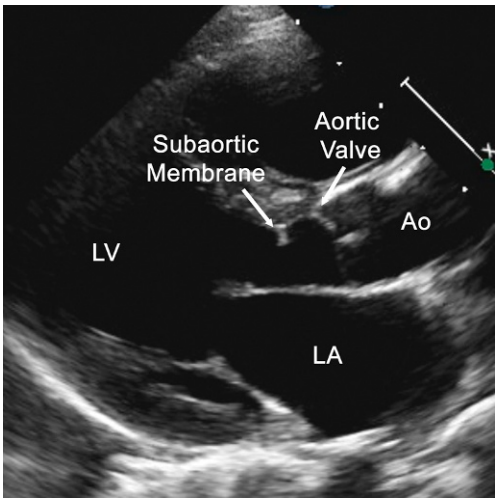

Fig. 19 Parasternal long-axis image in a patient with a subaortic membrane just below the aortic valve annulus. *Ao*, ascending aorta; *LA*, left atrium; *LV*, left ventricle.

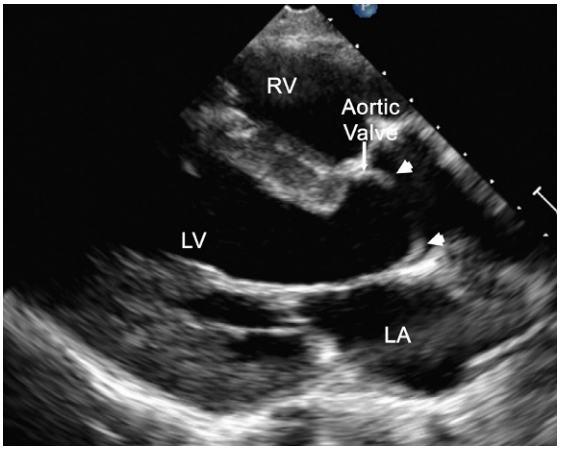

Fig. 20 Parasternal long-axis image in a patient with severe aortic stenosis and insufficiency. The left ventricle (LV) is dilated and hypertrophied. The aortic valve leaflets (*arrowheads*) are thickened and doming. *LA*, left atrium; *RV*, right ventricle.

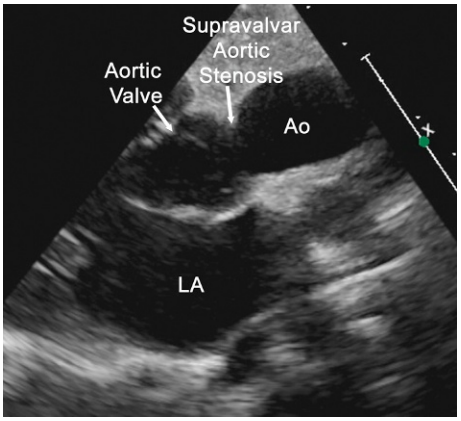

Fig. 21 High parasternal long image in a patient with valvar and supravalar aortic stenosis. The aortic valve leaflets are thickened and doming. There is significant hypoplasia of the ascending aorta at the sinotubular ridge.

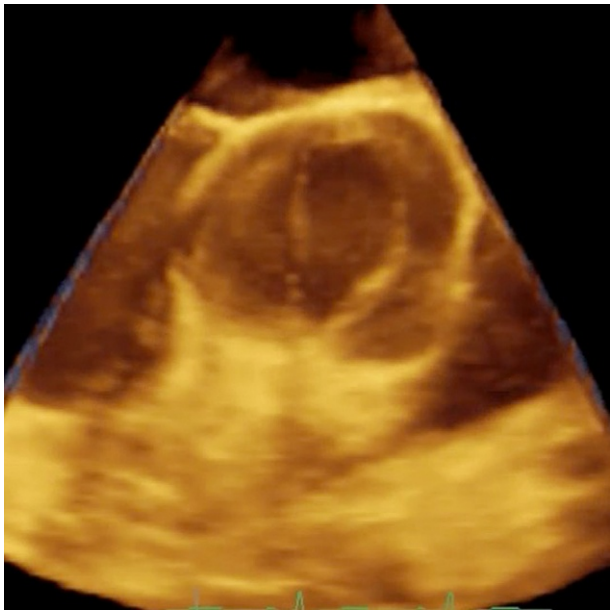

Fig. 22 Three-dimensional transesophageal image of the aortic valve en face demonstrating a bicuspid aortic valve.

valve morphology and estimation of aortic valve regurgitation if present (**Fig. 22**, **Movie 22**). Degree of LVOT obstruction can be assessed using Doppler, most commonly in the apical 4-chamber and suprasternal notch views. Once there is suspicion of LVOTO, all left-sided cardiac structures should be examined carefully. Additionally, hemodynamic effects of obstruction should be evaluated, including left ventricular size and function and presence of endocardial fibroelastosis (Aboulhosn and Child, 2006).

Coarctation of the aorta

Background

Coarctation (CoA) is a narrowing of the proximal thoracic aorta. It most commonly occurs as focal stenosis in the region of the aortic isthmus between the origins of the left subclavian artery and the insertion of the ductus arteriosus (Ho and Anderson, 1979). In addition to the more discrete stenosis, CoA can also be long-segment in nature or result from either transverse arch or abdominal aortic hypoplasia (Torok et al., 2015; Ho and Anderson, 1979; Price et al., 2014; Mullen, 2003). CoA accounts for 5%–7% of all CHD, with an incidence of approximately 3 cases per 10,000 births (Torok et al., 2015; Padua et al., 2012). CoA is also highly associated with a bicuspid aortic valve, with as many as 50%–75% of patients with CoA also having bicuspid aortic valves (Siu and Silversides, 2010; Roos-Hesselink et al., 2003).

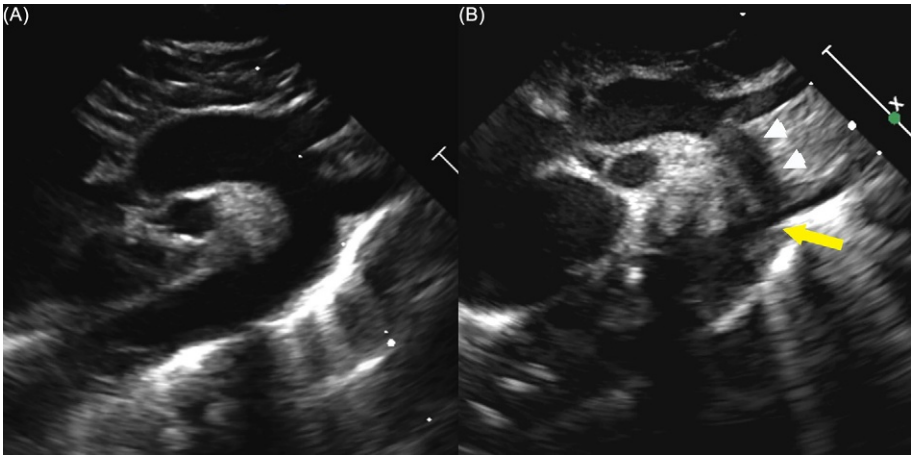

Fig. 23 Suprasternal notch view demonstrating a widely patent aortic arch (A) and long-segment coarctation of the aorta (B) with a posterior shelf (*yellow arrow*) and distal transverse arch hypoplasia (*white arrowheads*).

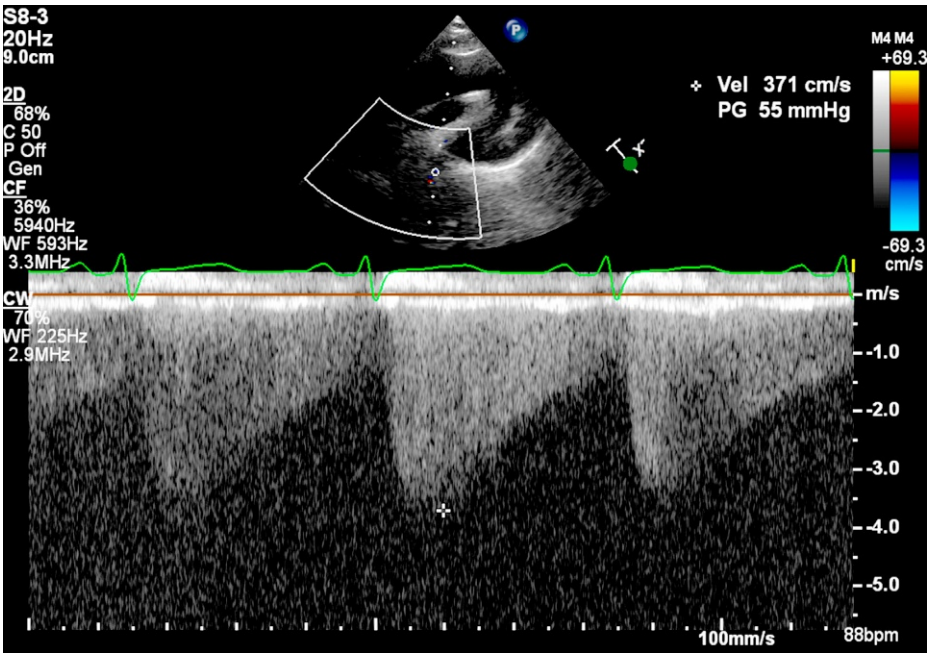

Fig. 24 Continuous wave Doppler in suprasternal notch through descending aorta in a patient with coarctation of the aorta demonstrating a peak gradient of 55 mmHg and a continuous antegrade flow pattern suggestive of obstruction.

Diagnosis by ECHO

The suprasternal notch view allows the best imaging plane of the ascending, transverse, and proximal descending aorta (Fig. 23). 2D imaging allows for careful measurements of each region and identification of features associated with CoA such as a posterior shelf and increased distance between the left common carotid and left subclavian arteries. Color Doppler can be used to demonstrate flow acceleration in the region of hypoplasia, and CW Doppler of the descending aorta can demonstrate continuous low velocity antegrade flow distal to the coarctation (Fig. 24). Subcostal views of the abdominal aorta with CW Doppler may show a loss of pulsatile flow in severe CoA (Fig. 25).

The presence of a PDA can normalize the descending aortic Doppler velocity and aortic arch diameters (Soslow et al., 2013). In these patients, observation until the PDA has closed may be the only option to determine whether or not a CoA is present.

d-Transposition of the great arteries

Background

d-Transposition of the great arteries (dTGA) is a cyanotic congenital heart defect with discordant ventriculoarterial alignment, or the aorta arising from the right ventricle and the pulmonary artery arising from the left ventricle. This anatomy creates two parallel

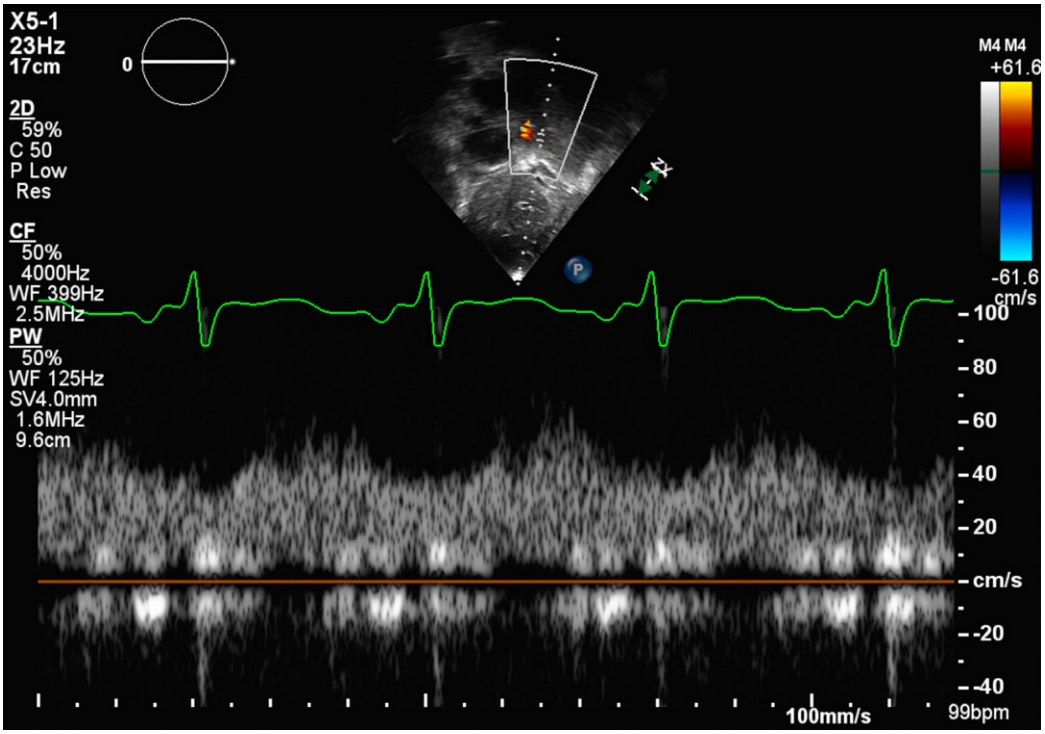

Fig. 25 Pulse wave Doppler in the subcostal sagittal view in a patient with coarctation of the aorta demonstrating poor pulsatility in the descending aorta at the diaphragm suggesting proximal obstruction.

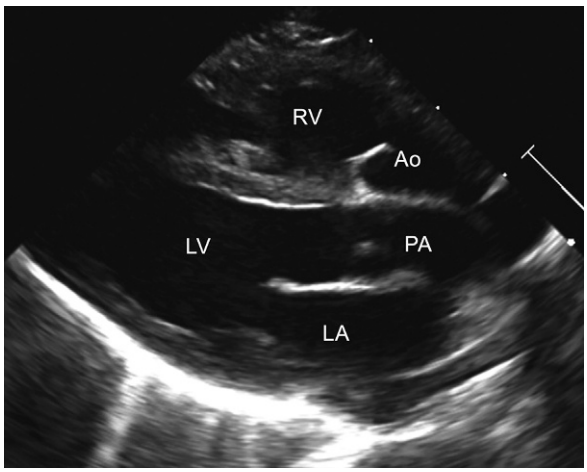

Fig. 26 Parasternal long-axis image in a patient with transposition of the great arteries. The pulmonary artery (PA) arises from the left ventricle (LV) and takes a characteristic posterior course. The aorta (Ao) arises from the right ventricle (RV). The Ao and PA take a parallel course. *LA*, left atrium.

circulations, resulting in significant cyanosis if there is no mechanism for mixing (Cohen et al., 2016). The incidence of dTGA is approximately 3 per 10,000 live births (Hoffman and Kaplan, 2002), and it is the second most common cyanotic heart disease, with TOF being the most common (Cohen et al., 2016; Hoffman and Kaplan, 2002). A myriad of structural abnormalities can occur with dTGA, the most common of which is a VSD, which is present in approximately 40% of patients (Cohen et al., 2016).

Diagnosis by ECHO

The diagnosis of dTGA can be made from either the parasternal long axis or subcostal windows. In the parasternal long axis, the great artery arising from the left ventricle takes a more posterior course than that found in a normal heart (**Fig. 26**, **Movie 23**). In addition, the great arteries spiral around each other as they originate from the two ventricles in a normal heart but take a parallel course in a heart with dTGA (**Fig. 26**) (Cohen et al., 2016). A sweep in the apical 4-chamber or subcostal coronal view demonstrates

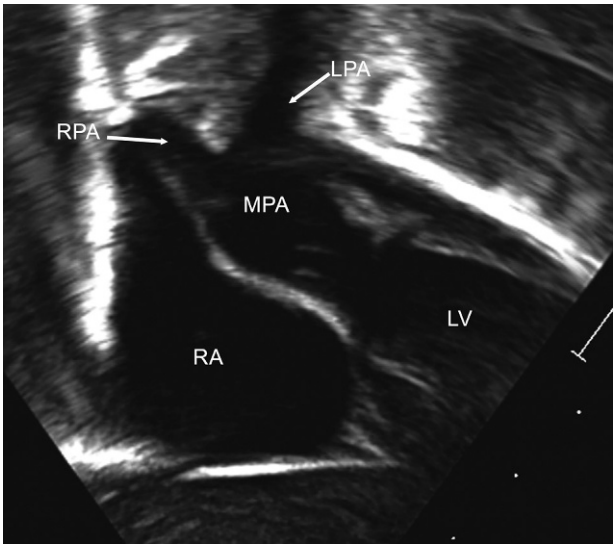

Fig. 27 Subcostal coronal view with anterior angulation demonstrating the main pulmonary artery (MPA) and branch pulmonary arteries arising from the left ventricle (LV). *LPA*, left pulmonary artery; *RA*, right atrium; *RPA*, right pulmonary artery.

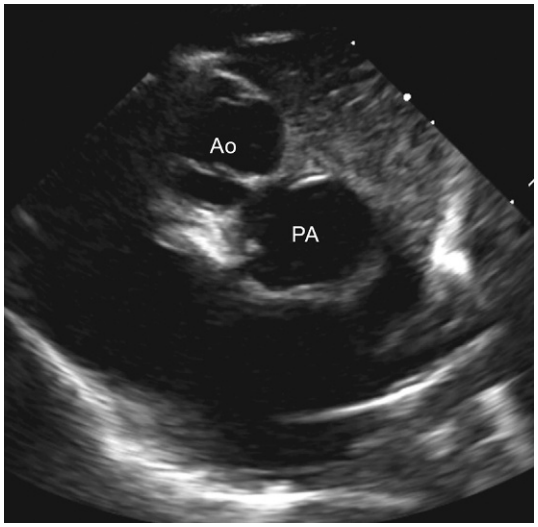

Fig. 28 Parasternal short-axis image of a patient with d-transposition of the great arteries. The aorta (Ao) is anterior and rightward of the pulmonary artery (PA).

branching of the vessel arising from the left ventricle, which establishes this vessel as the pulmonary artery (**Fig. 27**, **Movie 24**). Continuing the sweep anteriorly reveals the aorta originating from the right ventricle (Cohen et al., 2016). The parasternal short-axis view is utilized to determine the positions of the aortic and pulmonary valves relative to each other (**Fig. 28**). The morphology and function of the pulmonary valve requires careful assessment, as this valve will become the "neo"-aortic valve if an arterial switch operation is performed.

Shortly after a diagnosis of dTGA is suspected, it is essential to investigate the atrial septum. Given the parallel circulations, adequate mixing of oxygenated and deoxygenated blood is necessary. This mixing almost always requires a large enough atrial communication, though some mixing occurs across both a VSD and PDA, if present. The subcostal windows allow 2D visualization and measurement of the ASD. Color Doppler and continuous wave Doppler are used to help determine the direction and adequacy of flow across the ASD, though the oxygen saturation is the primary determinant of whether emergent intervention is necessary. If the atrial level mixing is inadequate, then a balloon atrial septostomy with echo guidance will need to be performed (**Movie 25**).

The aorta in dTGA is usually anterior to the pulmonary artery and as a result the coronary arteries are never in the normal position in this lesion (Cohen et al., 2016). This anterior position of the aorta can make visualization of the coronaries difficult. Multiple alternative views are often necessary to clarify the coronary anatomy. Although the most common coronary distribution is the right and left main coronaries arising from the right and left facing cusps, there are many coronary artery variations, including a

circumflex off of the right, single coronary, and one or both coronary arteries with an intramural course. It is important to document variations in this anatomy as these variations, particularly intramural coronary arteries and coronaries arising from a single ostium, can be associated with increased mortality in patients who undergo the corrective arterial switch operation (Pasquali et al., 2002).

Single ventricle

Single ventricle anatomy is potentially the most heterogeneous anatomic group of CHD. This wide anatomic spectrum may involve many of the previously described lesions as associated components. The three prototypical single ventricle lesions, listed from most common to least common, are hypoplastic left heart syndrome, tricuspid atresia, and double inlet left ventricle (**Fig. 29**, **Movies 26–28**). These lesions are rare within the CHD population, with the most common (HLHS) occurring in approximately 2 per 10,000 live births (Khairy et al., 2007; Hoffman and Kaplan, 2002). The most common course for single ventricle physiology is a three-staged palliative surgical route, described in more detail in other articles. The degree of pulmonary and systemic blood flow dictates the design of the first surgical palliation. Usually one or a combination of the following is performed:

- A systemic to pulmonary shunt to augment pulmonary blood flow
- A pulmonary artery band to restrict pulmonary blood flow
- Reconstruction of the left ventricular outflow tract and aortic arch to address concerns of restricted systemic blood flow

The second stage creates a cavopulmonary anastomosis, named the Glenn shunt, connecting the superior vena cava to the right pulmonary artery allowing passive systemic drainage into the pulmonary arteries (Feinstein et al., 2012). Lastly the Fontan procedure completes the total cavopulmonary connection by redirecting the IVC flow into pulmonary arteries and places the systemic and pulmonary circulations in series (Feinstein et al., 2012). Given the significant heterogeneity and the associations with multiple anatomic abnormalities, all of the previously described echo techniques should be employed when evaluating these lesions.

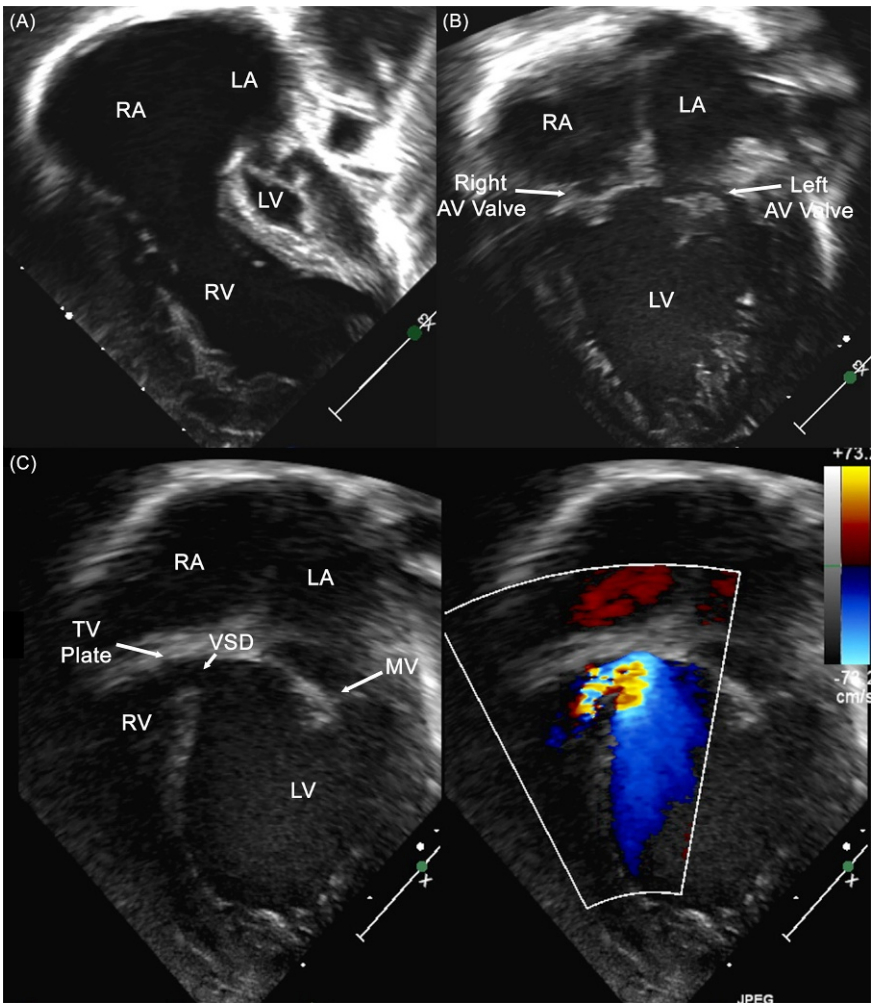

Fig. 29 Apical 4-chamber view of patient with hypoplastic left heart syndrome (A), tricuspid valve atresia (B), and double inlet left ventricle (C). *AV*, atrioventricular; *LA*, left atrium; *LV*, left ventricle; *MV*, mitral valve; *RA*, right atrium; *RV*, right ventricle; *TV*, tricuspid valve; *VSD*, ventricular septal defect.

Fetal Echocardiography

Fetal echocardiography allows new insights into the evolution of CHD in utero and also provides perinatal planning to optimize site of delivery and availability of appropriate resources for children with heart disease diagnosed prenatally (Donofrio et al., 2014). In the current era, multicenter collaborative studies are expanding our understanding of individual lesions, examining the natural history of lesions, and the impact of prenatal and postnatal management strategies (Donofrio et al., 2016; Freud et al., 2015). In addition, fetal echocardiography can be used to diagnose and direct therapy for arrhythmias, screen for development of congenital heart block, and monitor patients during prenatal surgery (Rychik et al., 2015; Strasburger and Wakai, 2010). While in its infancy and still not without controversy, the number of centers performing prenatal cardiac interventions is increasing (Freud and Tworetzky, 2016; Moon-Grady et al., 2015; Araujo Junior et al., 2016).

Although prenatal diagnosis prevents postnatal decompensation, it is unclear whether it truly improves postnatal mortality (Landis et al., 2013); some studies suggest that prenatal diagnosis improves preoperative and/or postoperative mortality in certain types of CHD (Li et al., 2016; Morris et al., 2014; Franklin et al., 2002). Prenatal detection may also decrease the risk of developing postnatal brain injury (Peyvandi et al., 2016). In addition, fetal diagnosis allows families to prepare mentally and financially for complex CHD and may decrease anxiety (Pinto et al., 2016). Although some types of cardiovascular disease cannot be definitively diagnosed prenatally due to fetal circulation, imaging windows, and issues with resolution, most significant lesions can usually be diagnosed prenatally (**Figs. 30** and **31**, **Movies 29** and **30**). It should be noted, however, that an obstetrician must identify a heart abnormality (or have suspicion for a heart abnormality) in order to initiate referral for fetal echocardiography.

Alternative Imaging Modalities

Cardiac Catheterization

While no longer the technique of choice for diagnosis of all forms of CHD, cardiac catheterization remains an integral diagnostic and interventional method. Catheterization has the advantage of supplying both imaging (angiography) and hemodynamic (pressures and saturations) data with the potential for concurrent intervention. Angiography allows for excellent delineation of pulmonary blood flow, including branch pulmonary arteries, surgical shunts, and aorticopulmonary collateral vessels, all of which can be difficult to image using echocardiography (**Fig. 32**). Angiography also allows for assessment of proximal and distal coronary arteries and can be used to assess complex anatomy not otherwise delineated by noninvasive methods (**Fig. 33**). Catheterization remains the gold standard for hemodynamic assessment, including pulmonary vascular resistance, pulmonary to systemic flow ratio (Qp:Qs), cardiac output, and valvar gradients. Early catheterization may be of benefit in critically ill patients with unclear diagnoses, especially those supported by extracorporeal membrane oxygenation (Burke et al., 2017). Limitations of catheterization are that it is invasive, routinely performed under general anesthesia, requires contrast and radiation exposure, and has potential for rare but significant morbidities, including vessel damage, stroke, cardiac perforation, arrhythmia, and death.

Cardiac catheterization has become the interventional method of choice in multiple disease processes as it is less invasive and has a faster recovery than cardiac surgery. If feasible and safe based on the patient size and anatomy, ASDs and PDAs are

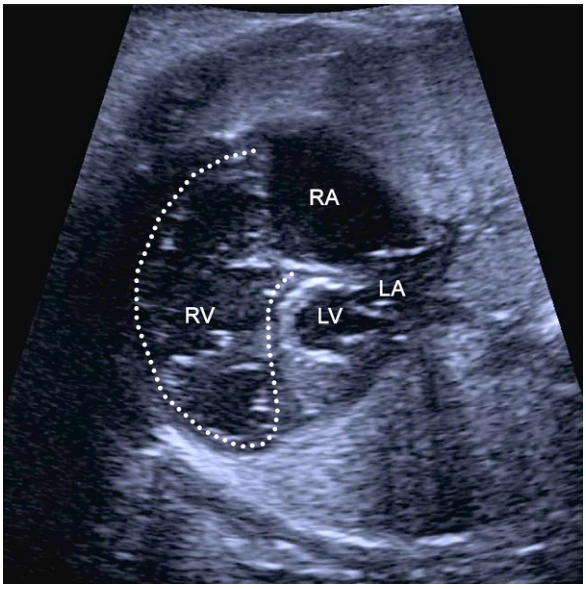

Fig. 30 Fetal echocardiogram of fetus with hypoplastic left heart syndrome with small left atrium (LA) and left ventricle (LV). *RA*, right atrium; *RV*, right ventricle.

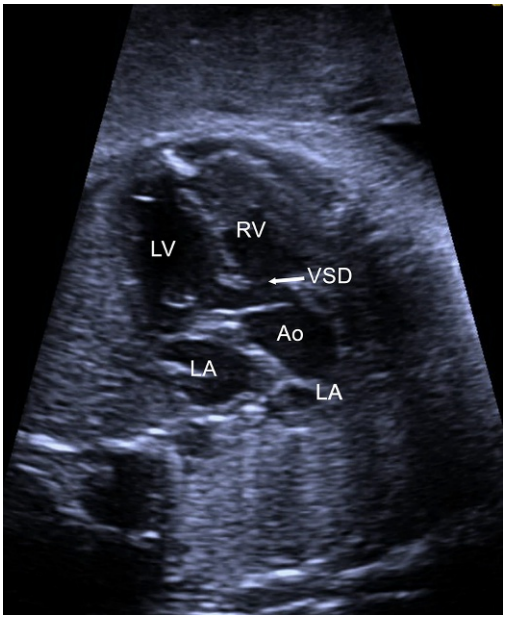

Fig. 31 Fetal echocardiogram in fetus with tetralogy of Fallot. There is a large, overriding aorta (Ao) and large membranous to outlet ventricular septal defect (VSD). *LA*, left atrium; *LV*, left ventricle; *RV*, right ventricle.

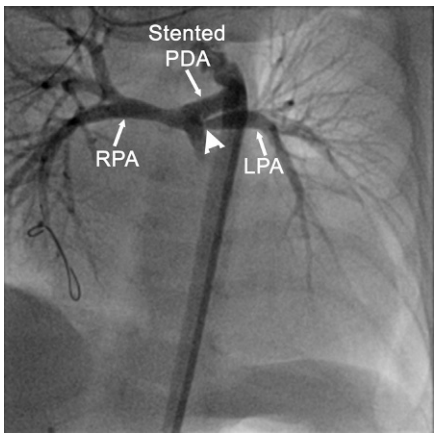

Fig. 32 Angiography in a patient with single ventricle physiology after stenting the patent ductus arteriosus (PDA). There is mild hypoplasia of the proximal left pulmonary artery (LPA) marked by the *arrowhead*. *RPA*, right pulmonary artery.

preferentially closed percutaneously. Pulmonary and aortic balloon valvuloplasty are preferentially performed in the catheterization laboratory. Interventions on extracardiac vessels, including pulmonary artery rehabilitation and coarctation angioplasty or stenting, are now routinely performed percutaneously. Though VSDs can be closed percutaneously, the frequency of this procedure varies by center and careful patient selection is necessary due to the risk of heart block (Bai et al., 2015). Procedures such as PDA stenting in patients with more complex anatomy are becoming more routine as it averts the need for an operative aorta to pulmonary artery shunt (Santoro et al., 2015). Finally, percutaneous pulmonary valve replacements have good long-term outcomes and have become a relatively common intervention in patients with CHD (Cheatham et al., 2015).

Cardiac Magnetic Resonance Imaging

CMR has many advantages, including excellent delineation of endocardial borders, absence of radiation, and no dependence on echocardiographic "windows" or limitations in image quality due to patient body habitus. CMR is the gold standard for assessment of ventricular volume, mass, and systolic function (**Fig. 34**) (Karamitsos et al., 2009); it is integral to the care of patients with repaired TOF, who require frequent scans to evaluate right ventricular size and function, which cannot be as accurately quantified using echocardiography (**Fig. 35**) (Valente et al., 2014; Geva, 2011). CMR can also assess ventricular adequacy in patients with small

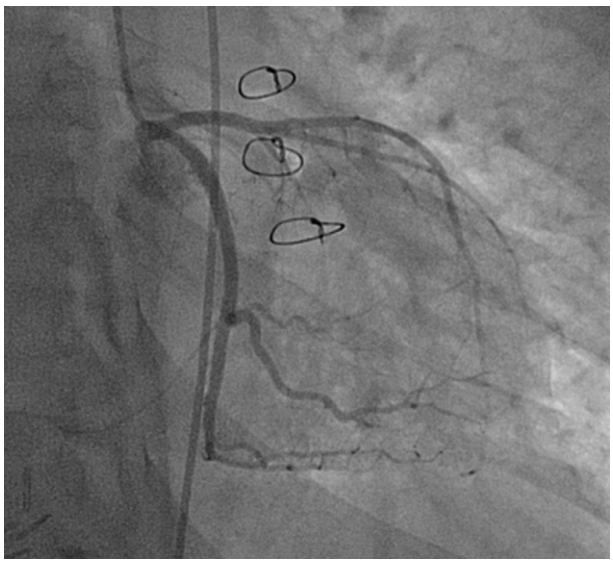

Fig. 33 Coronary artery angiography in the left main coronary artery in a patient with a previous heart transplantation.

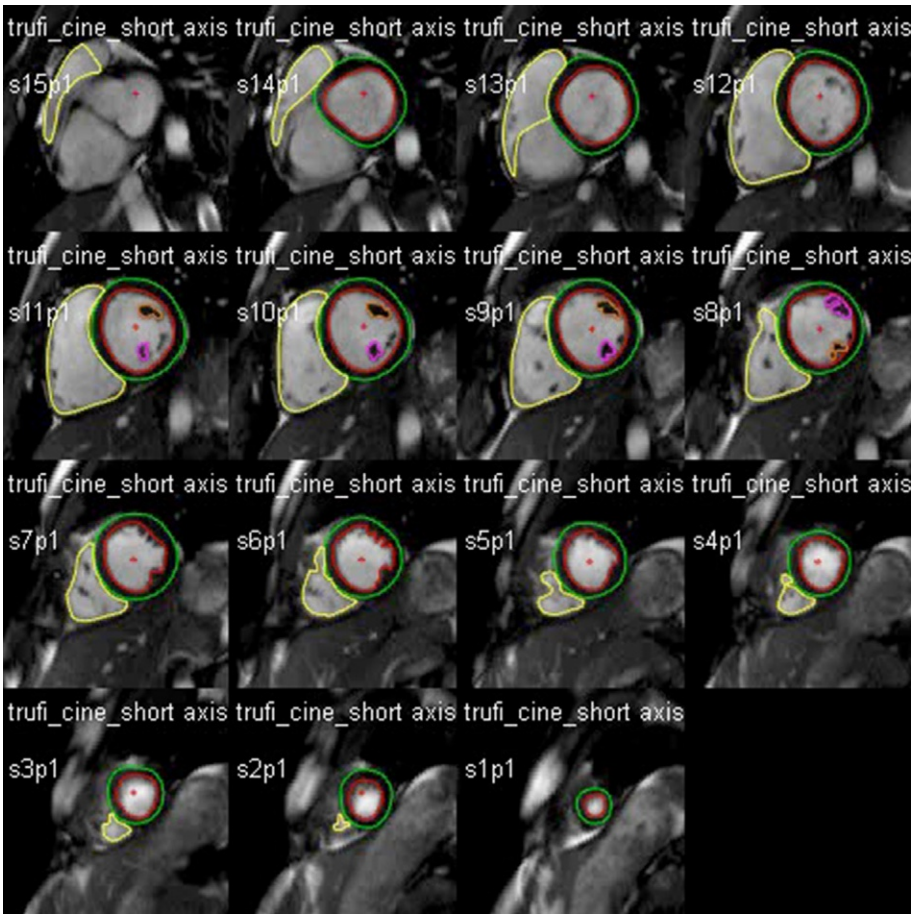

Fig. 34 Example of cardiac MRI contours at end diastole performed on a healthy control patient.

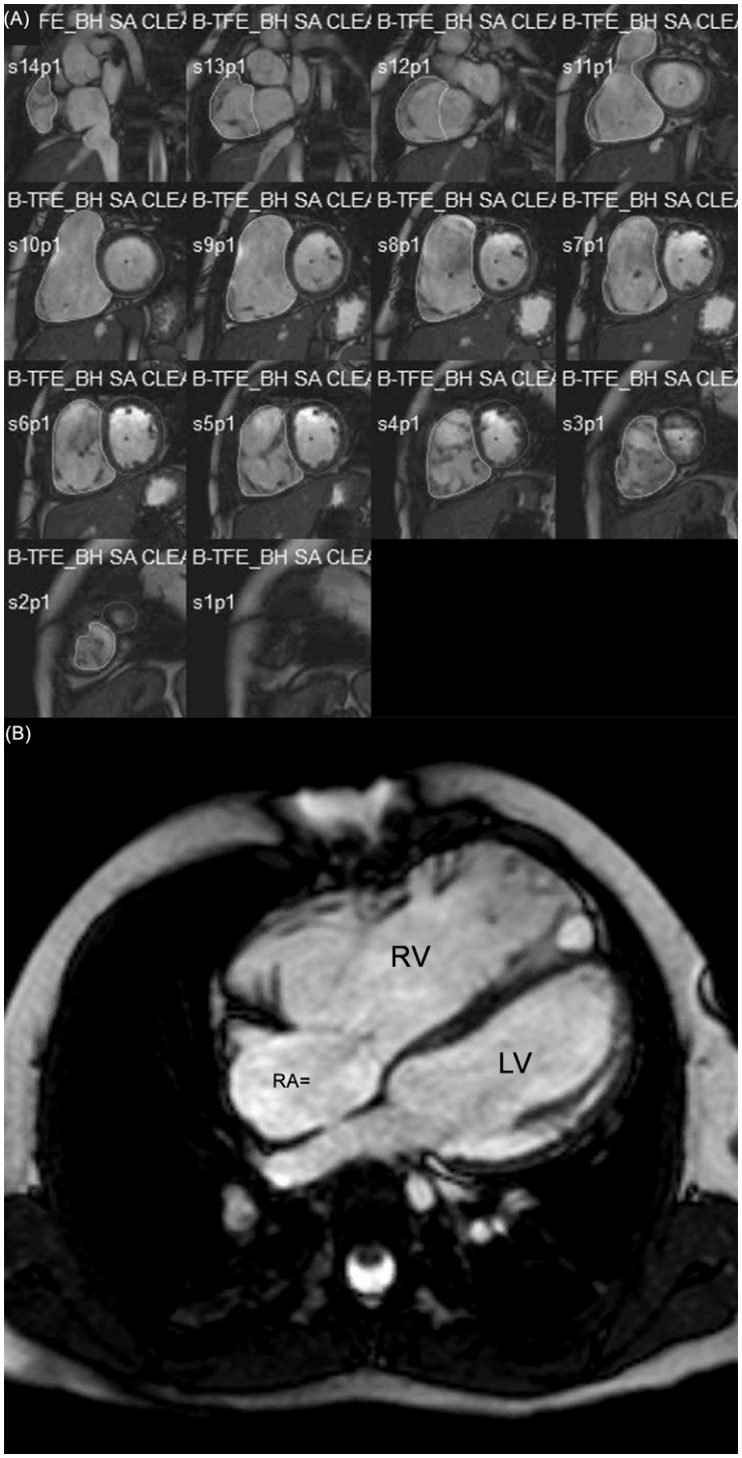

Fig. 35 Example of cardiac MRI contours at end diastole performed on a patient with tetralogy of Fallot and severe RV dilation (A). Four-chamber image in same patient demonstrating severe RV dilation (B). *LA*, left atrium; *RA*, right atrium; *RV*, right ventricle.

left or right ventricles. It provides excellent visualization of extracardiac blood vessel abnormalities, especially with the addition of gadolinium contrast agents. It is particularly useful for patients with pulmonary or systemic venous abnormalities, branch pulmonary artery stenosis, coarctation of the aorta (**Fig. 36**, **Movie 31**), or vascular rings (**Fig. 37**, **Movie 32**). Phase contrast imaging allows for quantification of blood flow, including valvar regurgitation, Qp:Qs, and branch pulmonary artery differential flow.

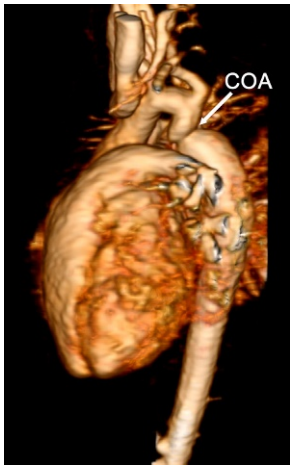

Fig. 36 Three-dimensional reconstruction of magnetic resonance angiography in a patient with coarctation of the aorta (COA) and aberrant right subclavian artery (not shown in still frame, evident in movie).

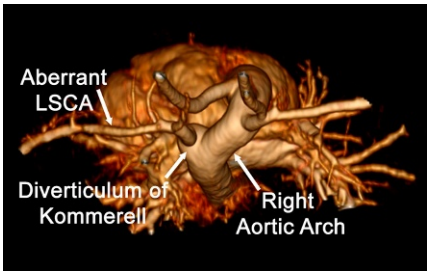

Fig. 37 Three-dimensional reconstruction of magnetic resonance angiography in a patient with a vascular ring consisting of a right aortic arch with an aberrant left subclavian artery (LSCA) and a diverticulum of Kommerell.

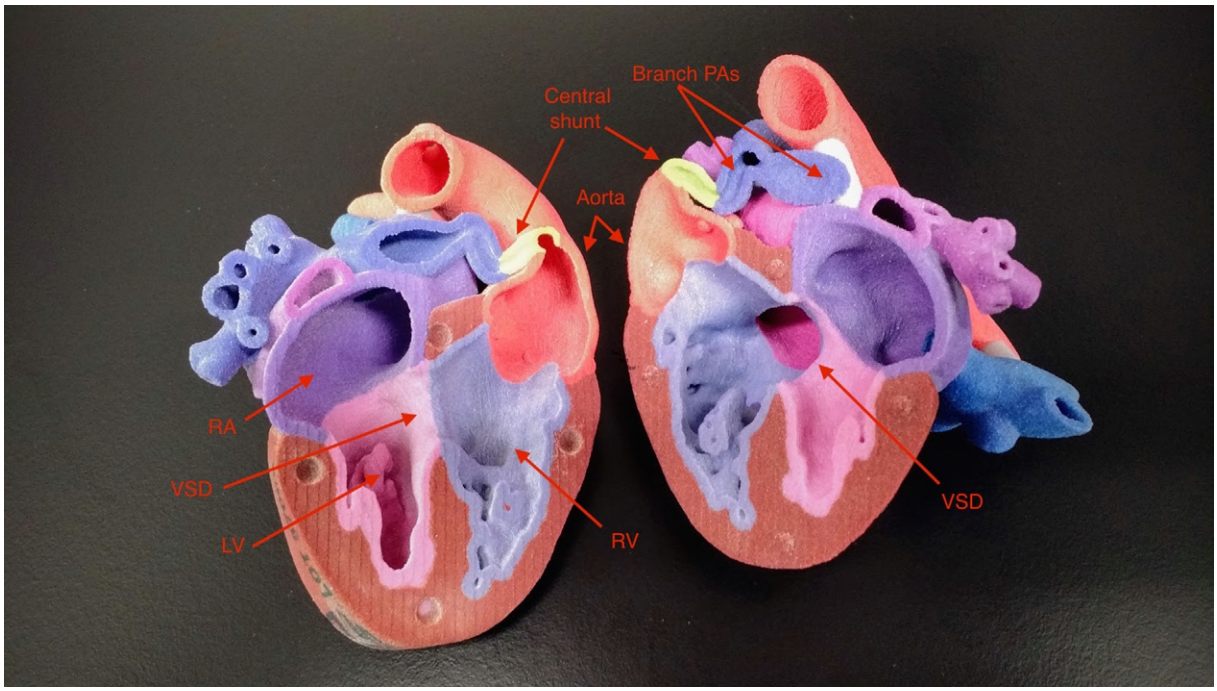

Fig. 38 Three-dimensional print of magnetic resonance angiography obtained in a patient with situs inversus totalis, l-looped ventricles (atrioventricular discordance), large ventricular septal defect (VSD), aorta off of the right ventricle (RV), and pulmonary atresia status postcentral shunt placement. *LV*, left ventricle; *PAs*, pulmonary arteries; *RA*, right atrium. Figure courtesy of Shafkat Anwar, MD, Assistant Professor, Pediatrics, Division of Pediatric Cardiology, Washington University School of Medicine.

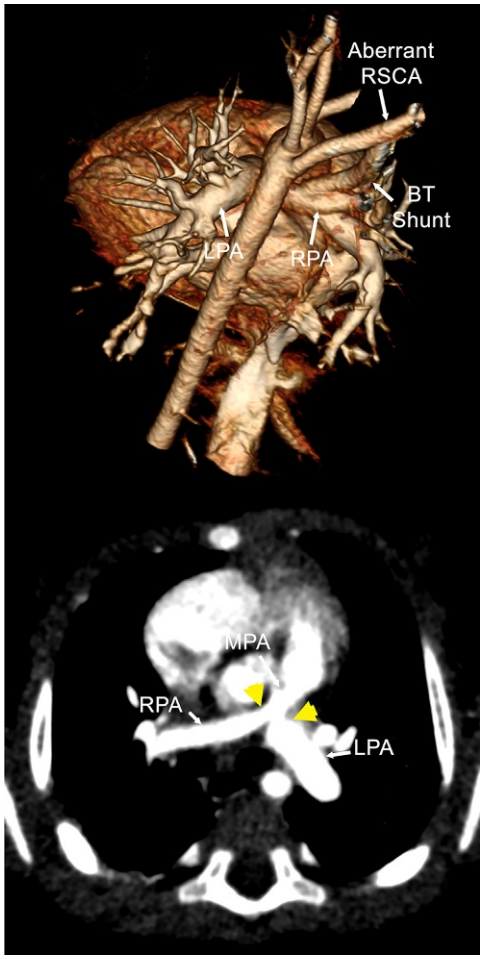

Fig. 39 Three-dimensional reconstruction of a computed tomography angiogram (CTA) in a patient with tetralogy of Fallot status postplacement of a modified Blalock–Taussig (BT) shunt off of an aberrant right subclavian artery (RSCA) (A). The shunt curves from the RSCA and connects into the right pulmonary artery (RPA). Maximal intensity projection of the CTA in the axial plane demonstrating proximal right and left pulmonary artery (RPA and LPA) hypoplasia, as represented by *yellow arrowheads*. The main pulmonary artery (MPA) is also hypoplastic.

CMR is unique in that it permits tissue characterization, including assessment of myocardial edema, fibrosis, and perfusion. Tissue characterization with a combination of T2-weighted imaging and delayed enhancement allows for assessment of edema or fibrosis and is particularly useful for diagnosis of myocarditis or any number of causes of ischemia, including coronary artery disease or hypertrophic cardiomyopathy (Friedrich et al., 2009; Weng et al., 2016; Kim et al., 2000). More advanced parametric mapping quantifies the diffuse myocardial fibrosis found in multiple pediatric disease processes (Soslow et al., 2016; Toro-Salazar et al., 2013; Chen et al., 2016; Riesenkampff et al., 2016). In addition, 3D magnetic resonance angiography can be used to create 3D printed models that allow for advanced surgical planning and training in cases of complex CHD (**Fig. 38**) (Vukicevic et al., 2017; Anwar et al., 2016). However, CMR does have limitations. Chief among these is the need for patients to remain still during the examination and hold their breath frequently; younger children may require anesthesia or sedation. Other limitations include significant artifact related to some metallic implants, inability to image in the setting of most pacemakers, lack of portability, long duration of examinations, the need for gadolinium contrast in certain situations, inferior assessment of valvar stenosis compared with echocardiography, and cost.

Cardiac CT

Cardiac CT has many advantages when compared to echocardiography, cardiac catheterization, and CMR. These include rapid acquisitions that rarely require sedation or anesthesia, high resolution, accurate assessment of ventricular function and volumes comparable to that of CMR, and the ability to safely image patients with metallic implants, including pacemakers, with less artifact. In patients with TOF with a contraindication to CMR, CT is an excellent option to evaluate ventricular volumes and function (Valente et al., 2014). CT angiograms (CTA) are especially useful for evaluating congenital coronary artery anomalies and for assessment of extracardiac abnormalities, including coarctation of the aorta, vascular rings, and branch pulmonary artery stenosis

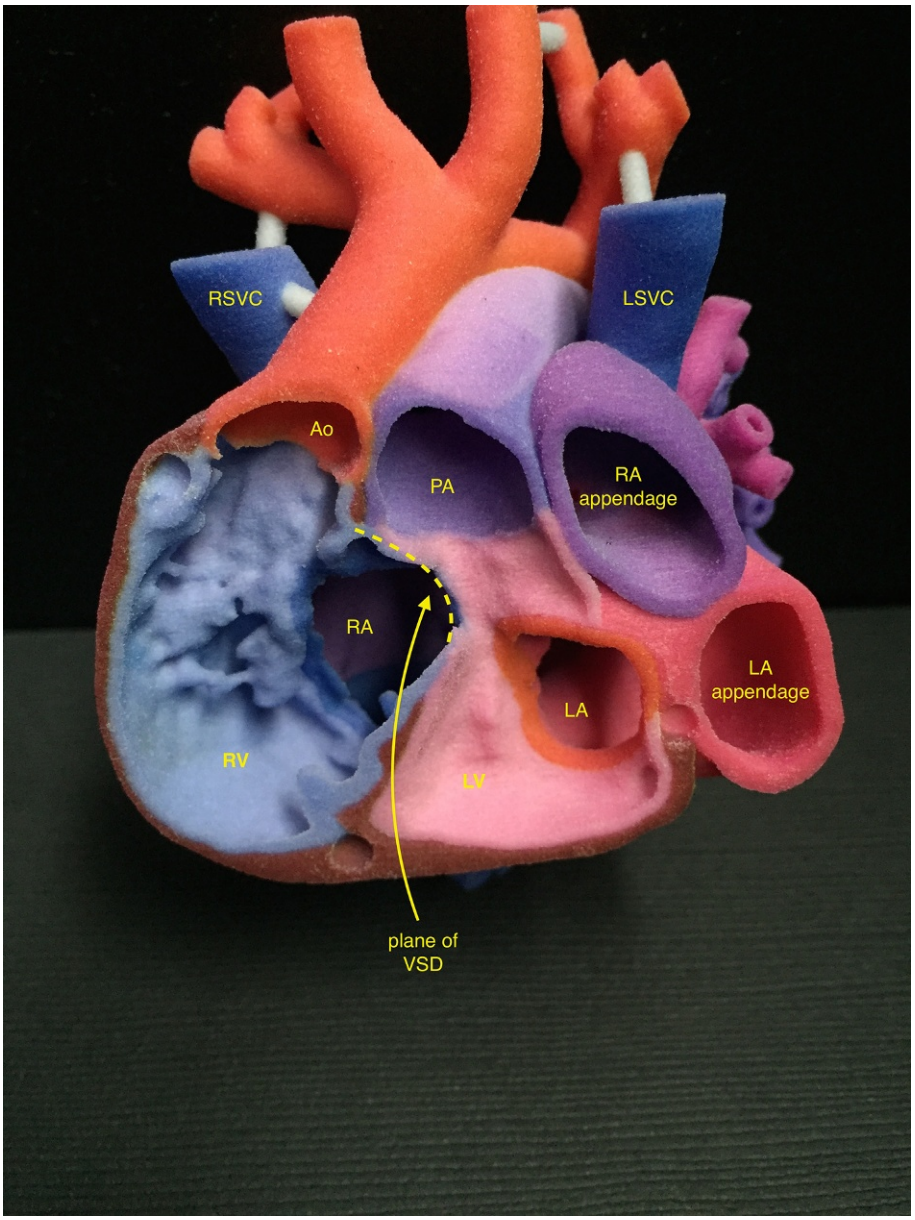

Fig. 40 Three-dimensional print of a neonate with dextrocardia, d-transposition of the great arteries, a ventricular septal defect (VSD), and bilateral superior vena cavae. *Ao*, aorta; *LA*, left atrium; *LSVC*, left superior vena cava; *LV*, left ventricle; *PA*, pulmonary artery; *RA*, right atrium; *RSVC*, right superior vena cava; *RV*, right ventricle. Figure courtesy of Shafkat Anwar, MD, Assistant Professor, Pediatrics, Division of Pediatric Cardiology, Washington University School of Medicine.

(**Fig. 39**, **Movie 33**). The high resolution of CTA makes it an excellent modality for obtaining 3D datasets for 3D printing, which can aid in surgical planning and even surgical training (**Fig. 40**) (Yoo et al., 2017; Anwar et al., 2016; Vukicevic et al., 2017). While new scanners are able to provide excellent data with reduced radiation exposure, radiation remains a concern in children where the lifetime effect is greater than adults (Johnson et al., 2014; Meinel et al., 2015; Gao et al., 2016). Other disadvantages include contrast exposure, inability to assess flows with standard CT, and lower temporal resolution than CMR or echocardiography.

Conclusions

The anatomy of CHD can vary widely and many patients have multiple defects. Accurate diagnosis requires careful assessment using echocardiography. When necessary, echocardiographic imaging can be supplemented with adjuncts including cardiac catheterization, CMR, and CT.

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Diagnosis of Non-ST-Elevation Myocardial Infarction (NSTEMI)

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Introduction

Myocardial infarction (MI) is a major global cause of mortality and is associated with high morbidity. Rates of MI are an indicator of the burden of coronary artery disease (CAD) in various populations and can be the first or repeated manifestation of CAD.

The first definition of MI, proposed by the World Health Organization (WHO) in 1979, was based on symptoms, ECG abnormalities, and cardiac enzymes (1979). Due to several developments such as improvement of biomarker sensitivity and cardiac imaging techniques and improved ECG criteria and the necessity to differentiate between spontaneous and procedure-related MI, a new definition of MI was proposed in 2000 by a joint European and American expert committee (Alpert et al., 2000). An update in 2007 introduced a clinical classification into five types of MI depending on the condition leading to an MI and was endorsed by the European Society of Cardiology (ESC), the American College of Cardiology Foundation (ACCF), the American Heart Association (AHA), and the World Heart Federation (WHF) (Thygesen et al., 2007).

The current 2012 version of the universal definition of MI has been further refined with adapted ECG criteria, a more prominent role of cardiac imaging, slight changes in the classification of the types of MI, and further recommendations on the use of troponin assays (Thygesen et al., 2012).

The World Health Organization (WHO) also recommends the universal MI definition when there are no resource constraints. When measurement of biomarkers and even ECG recordings are not possible due to limited economic resources, the WHO suggests more flexible definitions and standards (Mendis et al., 2011).

An unambiguous and consistent definition of the term MI is necessary, since MI is a frequently used outcome parameter in clinical trials and observational studies and has many legal and psychological implications. Knowledge of the universal definition of MI and current guidelines for treatment of acute coronary syndrome allows clinicians to correctly diagnose or rule out MI.

Definition and Types of MI

For diagnosis of acute MI, objective evidence of myocardial necrosis must be present in the context of acute myocardial ischemia (Thygesen et al., 2012). Cardiac troponin is the preferred biomarker for the diagnosis of MI due to absolute tissue specificity. A rise and/or fall of cTn with at least one value above the 99th percentile upper reference limit (URL) is mandatory to support the presence of an acute event as compared with a chronic process that is indicated by a stable elevation. An MI can only be diagnosed in the presence of myocardial ischemia. Therefore, the criteria for diagnosis of MI are met, when additionally at least one of the following is present:

- Symptoms of ischemia
- New or presumed new significant ST segment–T wave (ST-T) changes or new left bundle branch block (LBBB)
- Development of pathological Q waves in the ECG
- Imaging evidence of new loss of viable myocardium or new regional wall-motion abnormality
- Evidence of intracoronary thrombus by angiography or autopsy

Types of Acute MI

Based on pathophysiological, clinical, and prognostic differences, five types of MI are differentiated (Thygesen et al., 2012, by permission of Elsevier):

Type 1. Spontaneous myocardial infarction—Spontaneous myocardial infarction related to atherosclerotic plaque rupture, ulceration, fissuring, erosion, or dissection with resulting intraluminal thrombus in one or more of the coronary arteries leading to decreased myocardial blood flow or distal platelet emboli with ensuing myocyte necrosis. The patient may have underlying severe CAD but on occasion nonobstructive or no CAD.

Type 2. Myocardial infarction secondary to an ischemic imbalance—In instances of myocardial injury with necrosis where a condition other than CAD contributes to an imbalance between myocardial oxygen supply and/or demand, for example, coronary endothelial dysfunction, coronary artery spasm, coronary embolism, tachy-/bradyarrhythmias, anemia, respiratory failure, hypotension, and hypertension with or without LVH.

Type 3. Myocardial infarction resulting in death when biomarker values are unavailable. Cardiac death with symptoms suggestive of myocardial ischemia and presumed new ischemic ECG changes or new LBBB, but death occurring before blood samples could be obtained, before cardiac biomarker could rise, or in rare cases, cardiac biomarkers were not collected.

Type 4a. Myocardial infarction associated with PCI is arbitrarily defined by elevation of cTn values $>5 \times$ 99th percentile URL in patients with normal baseline values ($\leq$99th percentile URL) or a rise of cTn values >20% if the baseline values are elevated and are stable or falling. In addition, either (i) symptoms suggestive of myocardial ischemia, or (ii) new ischemic ECG changes or new LBBB, or (iii) angiographic loss of patency of a major coronary artery or a side branch or persistent slow or no flow or embolization, or (iv) imaging demonstration of new loss of viable myocardium or new regional wall-motion abnormality is required.

Type 4b. Myocardial infarction related to stent thrombosis—Myocardial infarction associated with stent thrombosis is detected by coronary angiography or autopsy in the setting of myocardial ischemia and with a rise and/or fall of cardiac biomarkers values with at least one value above the 99th percentile URL.

Type 5. Myocardial infarction related to coronary artery bypass grafting (CABG)—Myocardial infarction associated with CABG is arbitrarily defined by elevation of cardiac biomarker values $>10 \times$ 99th percentile URL in patients with normal baseline cTn values ($\leq$99th percentile URL). In addition, either (i) new pathological Q waves or new LBBB, or (ii) angiographic documented new graft or new native coronary artery occlusion, or (iii) imaging evidence of new loss of viable myocardium or new regional wall-motion abnormality.

Type 1 Versus Type 2 Myocardial Infarction

Although some cases may be easily explained retrospectively, the prospective classification of a type 1 MI versus a type 2 MI is challenging without information on coronary anatomy such as the presence of plaque rupture, erosion, fissure, or dissection. Although there may be some differences regarding troponin peak concentrations, kinetic changes, and baseline clinical characteristics, a prospective classification is virtually impossible. Reports on the prevalence of type 2 MI are very heterogeneous. Since the diagnostic criteria include a wide range of clinical conditions, these often vary between studies due to variable interpretations of the universal definition (Collinson and Lindahl, 2015; Chapman et al., 2017). A higher prevalence of type 2 MI has been reported for women (Cediel et al., 2017). There are no data showing that a type 2 MI has to be treated the same way like a type 1 MI, that is, no data on the role of dual platelet inhibition, usefulness of ticagrelor or prasugrel, duration of treatment, or adjunctive therapies including anticoagulation and glycoprotein IIb/IIIa inhibitors. Importantly, mortality rates of patients with type 2 MI seem to be higher than mortality rates in patients with type 1 MI (Cediel et al., 2017; Saaby et al., 2013). Since type 2 MI is the only type of MI unrelated to coronary occlusion, some authors have suggested that future MI definitions should be more restrictive and group current type 2 MI with myocardial injury due to nonischemic causes (Nagele, 2016).

Type 4a MI (MI Associated With PCI)

One important update in the current universal definition of MI takes into account the commonly observed troponin elevations after PCI. In the previous definition of MI, 15% of all patients treated with PCI were classified as having type 4a MI, since any elevation above $3 \times$ of the URL was considered as a periprocedural MI even when patients had no symptoms (Testa et al., 2009). The updated and current criteria had the goal of increasing the prognostic relevance of type 4a MI diagnoses, now demanding both clinical signs for myocardial ischemia and elevation above $5 \times$ of the URL when troponin levels are normal before PCI. In patients with elevated troponin levels before PCI, an increase of 20% is diagnostic if troponin levels were stable or falling beforehand.

It now appears that rates of type 4 a MI have declined after introduction of the revised criteria. Myocardial injury, however, is still detectable in a substantial proportion of patients following elective PCI with or without stenting. Causes for periprocedural cTn elevations are multifactorial with a predominance of side branch occlusion (Park et al., 2013). Monitoring of periprocedural myocardial injury is feasible with cTn. However, it should be remembered that considerably higher increases from ULN are

required using cTn or hsTn instead of CKMB in order to predict the same prognostically equivalent amount of injury due to the substantial higher sensitivity of cTn and particularly hsTn (Katus et al., 1991a; Apple, 2009). The thresholds for cTn defining a type 4a MI were set arbitrarily by convention in light of sparse study findings. In order to detect postprocedural myocardial injury or type 4a MI, serial measurements immediately before and up to 48 h post PCI are recommended (Thygesen et al., 2012). The consequences of relevant myocardial injury or a type 4a MI include extended monitoring for arrhythmias and reevaluation of procedural success.

The Electrocardiogram (ECG): First and Crucial Diagnostic Tool

An electrocardiogram (ECG) should be obtained and interpreted by a physician within 10 min after admission (Roffi et al., 2016; Amsterdam et al., 2014).

When patients present with significant ST elevations in at least two contiguous leads, presumed new left bundle branch block, anterior ST depressions indicative of true posterior MI, or ST elevation in aVR in combination with ST depression in eight or more surface leads suggestive of multivessel or left main coronary artery disease, immediate coronary angiography should be performed.

Depending on the localization of infarction, ST segment elevations may be observed in anterior leads (V1–V6), inferior leads (II, III, and aVF), lateral/apical leads (I and aVL), and in leads representing the free wall of the right ventricle (V3R and V4R) or the inferobasal wall (V7–V9).

In the third definition of MI, minor important modifications have been made to the ECG criteria, acknowledging age- and gender-specific differences in leads V2–V3. In these two leads, ST segment elevations are considered significant when the J point is elevated $\geq$0.15 mV in women, $\geq$0.2 mV in men $\geq$40 years, and $\geq$0.25 mV in men under 40 years. In all other leads, an elevation $\geq$0.1 mV is considered diagnostic (**Table 1**).

True posterior MI may present with ST elevation in posterior chest leads V7–V9 and ST depression in V1–V3, which is why additional obtainment of ECG leads V7–V9 in patients with initial nondiagnostic ECG at intermediate/high risk for ACS is recommended. In the opinion of the authors, routine obtainment of V7–V9 should be considered in all patients with suspected MI.

In patients with inferior MI and suspected right ventricular infarction, right precordial leads (V3R and V4R) should be obtained. In these leads, an ST elevation $\geq$0.05 mV ($\geq$0.1 mV in men <30 years) may support the diagnosis (Lopez-Sendon et al., 1985).

Elevations of lead aVR have been shown to be associated with an adverse prognosis (Wong et al., 2010) and when they present in combination with ST depression >0.1 mV in eight or more surface leads, multivessel or left main coronary artery disease should be suspected (Yan et al., 2007) (**Figs. 1** and **2**).

ECG interpretation can be difficult in patients with left or right bundle branch block, early repolarization, and persisting ST elevations due to a residual aneurysm or due to incorrectly positioned leads. In patients with preexisting left bundle branch block (LBBB), comparison with a previous ECG and concordant ST elevations may help detect acute MI (Lopes et al., 2011).

Prior MI

Regardless of symptoms, Q waves and QS complexes in the absence of left ventricular hypertrophy and LBBB suggest prior MI (Savage et al., 1977). The current universal definition specifies any Q wave in V2–V3 >0.02 s or QS complex in V2 and V3 as significant. Furthermore, Q waves $\geq$0.03 s and $\geq$0.1 mV deep or QS complex in two contiguous leads I, II, aVL, aVF or V4–V6 (or V7–V9) are considered significant.

Prior MI may also be diagnosed in the presence of an R wave $\geq$0.04 s in V1–V2 and R/S $\geq$1 with a concordant positive T wave.

New pathological Q waves in asymptomatic patients may be indicative of previous silent MI.

It is important to remember that ST-T abnormalities are frequent in patients with Brugada syndrome, stress cardiomyopathy and early repolarization syndrome, making diagnosis of acute myocardial ischemia solely with ECG sometimes impossible. However, new ST segment elevations for a prolonged period of time in combination with reciprocal ST segment depressions are usually due to acute coronary occlusion (Wang et al., 2003). Other conditions associated with ST-T abnormalities are peri-/myocarditis, pulmonary embolism, hypothermia, intracranial processes, and electrolyte abnormalities.

Table 1 ECG manifestations of acute myocardial ischemia

ST elevation
New ST elevation at the J point in two contiguous leads with the cut points: $\geq$0.1 mV in all leads other than leads V2–V3 where the following cut points apply: $\geq$0.2 mV in men $\geq$40 years, $\geq$0.25 mV in men <40 years, or $\geq$0.15 mV in women
ST depression and T wave changes
New horizontal or downsloping ST depression $\geq$0.05 mV in two contiguous leads and/or T inversion $\geq$0.1 mV in two contiguous leads with prominent R wave or R/S ratio >1

Thygesen, K. et al. (2012). Third universal definition of myocardial infarction. *Journal of the American College of Cardiology* **60,** 1581–1598, modified by permission of Elsevier.

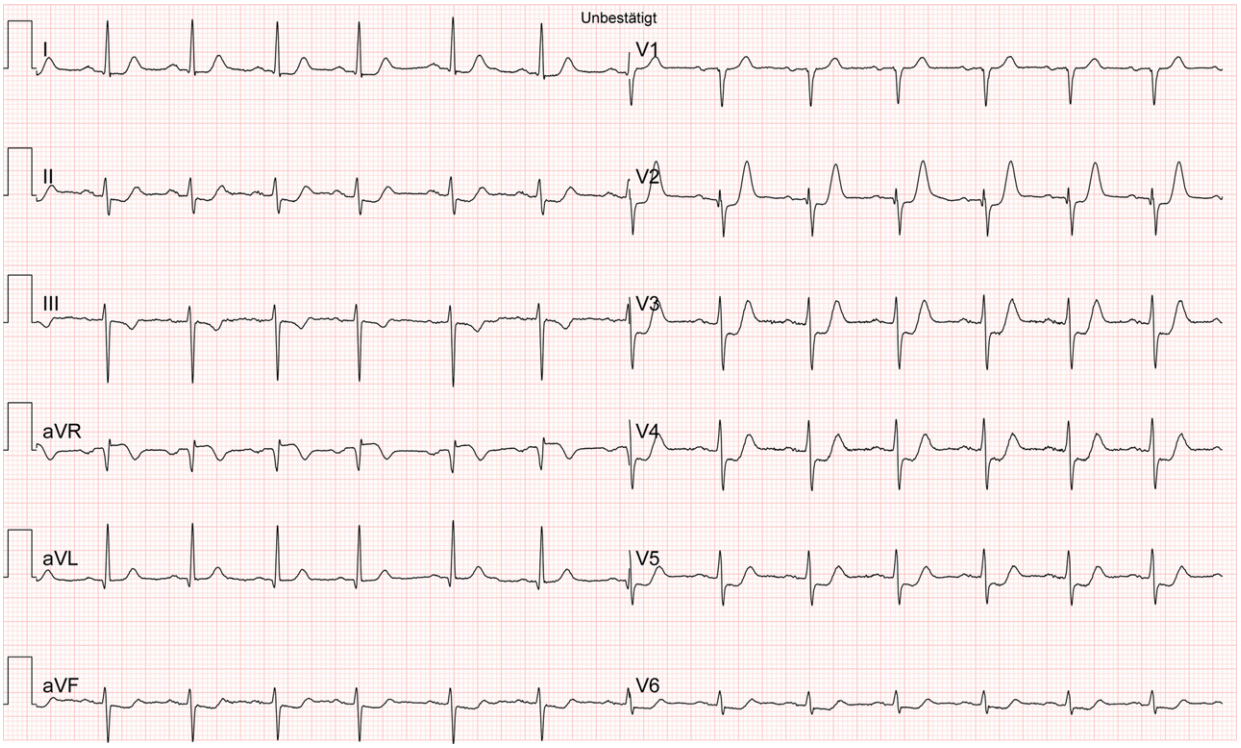

Fig. 1 ECG of a patient with mild ST elevation in lead aVR and accompanying ST segment depressions in eight leads. Immediate coronary angiography revealed occlusion of left anterior descending artery and critical left main stenosis in this patient.

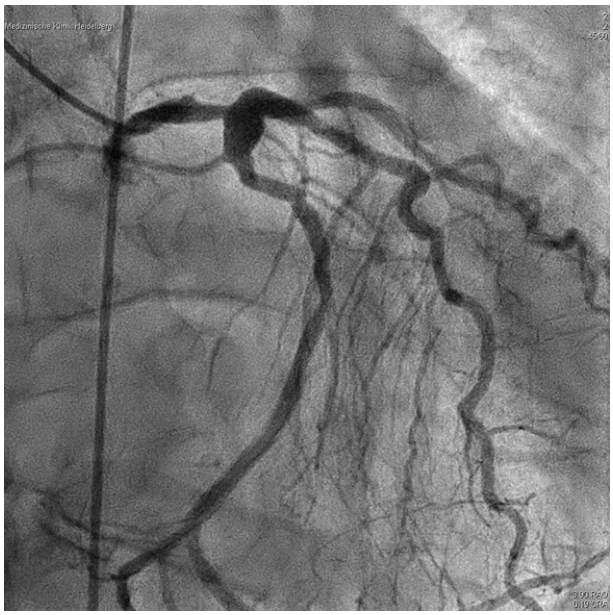

Fig. 2 Coronary angiogram of a patient with left main stem stenosis.

Cardiac Troponin: Preferred and Gold Standard Biomarker

Cardiac troponin is a regulatory protein of the myocardial contractile apparatus. Of its three subtypes (troponin T, I, and C), only cardiac troponin T (cTnT) and troponin I (cTnI) are solely expressed in cardiomyocytes. Elevations of cTnT or cTnI therefore indicate myocardial injury (Katus et al., 1991a). Cardiac troponin is released during the first 24 h from the cytoplasmic pool. A plateau is reached after 48–72 h due to proteolytic degradation of the contraction apparatus (Katus et al., 1991b; Remppis et al., 1994). Rising

and/or falling troponin levels and markedly elevated troponin levels at admission increase likelihood of acute myocardial injury. Stable serial troponin values are indicative of chronic myocardial injury. Laboratories should ensure certain quality standards, including an assay precision $\leq$10% coefficient of variation (CV) at the 99th percentile URL. Several manufacturers improved analytic sensitivities and precision of their assays at the 99th percentile value considerably. By convention, assays are graded by sensitivity and precision. Distinctions are made between guide acceptable, clinically useable, and not acceptable assays. High-sensitivity assays are further classified by their ability to measure cTn in healthy persons. Accordingly, a troponin assay can only be labeled hs if the assay can quantify concentrations in at least 50% of healthy subjects (Apple, 2009).

Cardiac Imaging in Patients with Suspected MI

Diagnosis of MI can be made in the presence of evidence of new regional wall-motion abnormalities in echocardiography, myocardial scars in MRI or in nuclear tests, or an intracoronary thrombus during coronary angiography in combination with a significant rise and/or fall of cardiac troponin even in the absence of clinical symptoms. Transthoracic echocardiography (TTE) should always be available in chest pain units and emergency rooms. TTE allows detection of regional wall abnormalities and helps detect signs of other nonischemic causes of chest pain such as myocarditis, valvular disease, cardiomyopathy, pulmonary embolism, or aortic dissection. Another advantage of echocardiography is the detection of complications of MI such as ventricular wall rupture, secondary mitral valve regurgitation after papillary muscle rupture, or ischemia (Flachskampf et al., 2011). Myocardial disease can best be assessed with cardiac MRI. Multidetector computed tomography for anatomical evaluation of coronary arteries may be helpful in patients with low to intermediate pretest probability for ACS. In such patients, a normal CT scan can exclude coronary artery disease (Samad et al., 2012).

Diagnostic Protocols

Introduction of hsTn assays has reduced the time to collect the second sample from 6–9 h after the first sampling to 3 h (Roffi et al., 2016). In countries and hospitals without access to hsTn, retesting of cTn should be performed 3–6 h after presentation with the use of a contemporary sensitive cTn or after 6–9 h with almost all POCT assays due to the lower analytic sensitivity. More recently, faster diagnostic protocols have been recommended by ESC guidelines as an alternative to the standard 0–3 h protocol. The 0–1 h accelerated diagnostic protocols for rule-out and rule-in are explicitly recommended provided that one of three validated hsTn assays is used (Roche Elecsys hsTnT, Abbott hsTnI Architect STAT or Siemens Dimension Vista hsTnI) (Roffi et al., 2016).

MI can also be ruled out immediately in low-risk patients with undetectable admission levels below limit of detection (LoD) or limit of blank (LoB) without the need for a second sample. As an evolving or small MI may be missed during the short hs troponin blank period, an instant rule-out protocol should not be applied to very early presenters, who are presenting within 1 h after onset of symptoms. Conversely, in patients with very high troponin elevations at time of presentation, the diagnosis of MI is very likely with a positive predictive value of >85%, sometimes obviating the need for a second cTn sample (Mueller-Hennessen et al., 2017). This management cutoff may prompt immediate coronary angiography for suspected large MI in a typical clinical context. However, it is important to always consider other acute myocardial or valvular pathologies or acute endocarditis, which may also present with high initial troponin values and require different management.

In order to increase practicability and permit a safer rule-out and discharge, some algorithms, particularly a 2 h accelerated diagnostic protocol with cTn measurements at 0 and 2 h, recommend implementation of a clinical risk score and ECG criteria (Than et al., 2012). Faster diagnostic algorithms generate an intermediate (or observational) diagnostic zone for patients who neither fulfill criteria for rule-out nor for rule-in. The final adjudicated diagnoses of patients in such an observational zone include NSTEMI but more often myocardial injury due to other reasons than ACS. Such patients often are male, have more comorbidities, are of higher age, have renal impairment, and have underlying cardiovascular disease. Long-term outcomes may be comparable with those of patients with confirmed MI (Nestelberger et al., 2016).

Patients in the observational zone require monitoring, serial cTn testing, and extended diagnostics in order to provide an accurate diagnosis and specific treatment. Cardiac imaging with echocardiography and multislice CT are increasingly important for the diagnostic workup of patients in this subgroup.

When sensitive or high-sensitivity cardiac troponin assays are unavailable, additional measurement of copeptin at admission is recommended. Normal copeptin levels without elevated cardiac troponin levels at presentation allow safe rule-out of MI.

All algorithms should only be used in the context of the overall presentation of the patient. When patients arrive less than 1 h after onset of chest pain, testing after 3 h should be performed. Whenever there is high clinical suspicion or when symptoms persist, additional measurements should be performed.

Age- and Gender-Specific Cutoffs

The introduction of more sensitive cTn assays allows detection of lower concentrations in women than in men, reflecting anatomical and functional differences of the heart between genders. The universal definition of MI recommends the use of

sex-specific cutoffs, but validation in prospective trials is not available, and existing evidence is controversial regarding the relevance of sex-specific cutoffs for diagnostic or prognostic classification. So far, diagnostic protocols recommend a single sex-independent cutoff for the standard 0–3 h algorithm and for the faster alternatives using hsTnT or hsTnI (Roffi et al., 2016). It should be noted that implementation of sex-specific cutoffs may improve diagnosis of MI to a small extent but only at the cost of practicability complicating diagnosis extraordinarily, particularly if other comorbidities such as renal failure, heart failure, or age that can confound the troponin result are considered as well (Mueller-Hennessen et al., 2016).

Conclusion

The current version of the universal definition of MI allows for a precise and consistent diagnosis of MI. Clinicians and researchers are strongly encouraged to study the detailed criteria to correctly diagnose or rule out acute MI.

Conflicts of Interest

MV has received financial support for clinical trials from Bayer Healthcare Germany and has been reimbursed for travel expenses and fees associated with attending seminars and conferences by Bayer Vital, Daiichi Sankyo, Octapharma, Lilly Germany, GlaxoSmithKline, Roche Diagnostics, TEVA, Brahms, Leo Pharma, and Abbott. Dr. Giannitsis has received financial support for clinical trials from Roche Diagnostics Ltd., Switzerland; Mitsubishi Chemicals, Germany; Siemens Healthcare; BRAHMS Biomarkers; Clinical Diagnostics Division; and Thermo Fisher Scientific, Germany. He is consultant to Roche Diagnostics and BRAHMS Biomarkers and has received speaker's honoraria from Roche Diagnostics, Siemens Healthcare, BRAHMS Biomarkers, and Mitsubishi Chemicals.

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Diet in Heart Failure

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Heart Failure

Chronic heart failure (HF) is a growing public health problem associated with significant mortality, morbidity, and health care expenditures (Roger, 2013). It affects approximately 5.7 million Americans and carries a 5-year mortality rate of ~50%. The prevalence of HF is expected to rise 46% from 2012 to 2030, due to improvements in medical and nonpharmacological care, allowing more patients to survive acute myocardial infarction and live to an older age (Mozaffarian et al., 2015). At present, approximately 1.5%–2% of Canadians are affected with HF (Ezekowitz et al., 2011).

Heart failure occurs when the heart is unable to maintain an adequate cardiac output to meet metabolic requirements of the rest of the body (Kemp and Conte, 2012). HF induces a systemic response, leading to the activation of neurohormonal systems characterized by an overexpression of the sympathetic and renin–angiotensin aldosterone system. While this is initially beneficial in maintaining renal and organ perfusion, it becomes maladaptive over time and causes the exacerbation of hemodynamic abnormalities found in HF. This results in further remodeling and neurohormonal release and progressive cardiac impairment, leading to excessive sodium and fluid retention (Colin-Ramirez and Ezekowitz, 2016). This disruption contributes to the signs and symptoms seen in patients with HF, greatly affecting health-related quality of life and physical functioning (Yancy et al., 2013). Common symptoms include dyspnea from pulmonary congestion, peripheral edema from impaired venous return, nausea, lack of appetite, and fatigue (Kemp and Conte, 2012).

Diet in Heart Failure

Dietary Sodium Intake Recommendations

The disruption of various complex compensatory mechanisms in patients with HF alters the patient's ability to effectively balance salt intake and excretion, resulting in a significant amount of sodium and fluid retention. An excessive sodium intake can further increase this retention, causing the exacerbation of HF symptoms. Thus, dietary sodium restriction has been considered a major self-care behavior recommended to these patients. However, due to a lack of conclusive evidence regarding the association between reduced sodium consumption and clinical events in the HF population, there is an inconsistency that exists among current recommendations for sodium intake in HF (Colin-Ramirez and Ezekowitz, 2016). The American College of Cardiology (ACC) and the American Heart Association (AHA) recommend decreasing sodium intake in patients with symptomatic HF, based on the association between sodium intake and hypertension, LV hypertrophy, and cardiovascular disease. It is recommended that stage

A and B HF patients restrict their sodium intake to 1500 mg/day, while stage C and D patients consume <3000 mg/day to reduce HF symptoms (Yancy et al., 2013). The Canadian Cardiovascular Society (Malcon et al., 2008), the Heart Failure Society of America (Lindenfeld et al., 2010), and the National Heart Foundation of Australia and the Cardiac Society of Australia and New Zealand (2011) suggest a sodium intake between 2000 and 3000 mg/day. Lastly, the European Society of Cardiology (ESC) recommends consuming less than 2400 mg/day, although no evidence level has been given to support this recommendation (Ponikowski et al., 2016).

Clinical Studies on Dietary Sodium Intake in HF

Observational data suggested that patients with HF who consume higher amounts of sodium (>2800 mg/day) are at greater risk of an acute decompensated HF (ADHF) event (Arcand et al., 2011), while a recent longitudinal cohort study showed that a sodium intake <2500 mg/day increased risk of HF hospitalization, among patients with HF NYHA class II/III (Doukky et al., 2016). Importantly, generalizability of these results may be limited due to these patients being participants of a RCT of behavioral modification that included several other co-interventions.

Results from previous RCT's have raised concern that sodium restriction may not be beneficial and may even produce detrimental effects in patients with HF (Lennie et al., 2013). Three large-scale RCTs assessing clinical outcomes associated with sodium restriction in patients with HF found that patients with systolic HF following a low-sodium diet (1800 mg/day) had a higher readmission and mortality rate in comparison to those following a moderate-sodium diet (2800 mg/day) in combination with oral furosemide (250–1000 mg/day) and fluid restriction (1 L/day) (Parrinello et al., 2009; Paterna et al., 2008; Paterna et al., 2009). However, it is important to note that the use of this combination treatment may have confounded the association between sodium restriction and clinical outcomes, making it difficult to analyze the direct effect of sodium intake. In contrast to these findings, the results from a recent pilot study suggest that following a low-sodium diet (1500 mg/day) may decrease BNP levels and improve quality of life in ambulatory patients with chronic HF on optimal medical treatment (Colin-Ramirez et al., 2014).

Overall, as a result of conflicting evidence, the optimal level of sodium intake in HF patients remains unclear. A sodium consumption between 2000 and 3000 mg/day appears to be safe and tolerable; however, the effects of reducing intake to less than 1500 mg/day must be elucidated in future large-scale RCTs (Colin-Ramirez and Ezekowitz, 2016).

Fluid Intake

Fluid requirements may vary depending on the stage and severity of HF. According to current major guidelines, fluid restriction of 2 L/day is reasonable in patients with advanced HF to relieve congestive symptoms. However, a routine strict fluid restriction in patients with HF regardless of symptoms or other considerations does not appear to be beneficial (Ponikowski et al., 2016; Yancy et al., 2013).

Macronutrients

Energy and Protein

The cytokine and hormonal derangements associated with the onset of HF leads to a metabolic imbalance favoring catabolism (Rahman et al., 2016). Thus, patients with HF require an increased calorie and protein intake in order to prevent a negative nitrogen balance and tissue breakdown. It has been recommended that clinically stable depleted patients with HF consume a daily intake of 29.5 kcal/kg + 1.1 g protein/kg, while normally nourished patients consume 24.3 kcal/kg + 1 g protein/kg (Aquilana et al., 2003).

Macronutrient Composition of the Diet

There is a growing body of evidence that suggests macronutrient ratios play an important role in minimizing the pathophysiologic disturbances that occur in patients with HF. Results from a recent study found that among 39 patients with HF and right ventricular dysfunction, those following a low-carbohydrate diet (40% carbohydrates, 40% fat, 20% protein) experienced significant weight loss, an increased oxygen saturation, and an improved NYHA functional class, in comparison to the control group (50% carbohydrates, 30% fat, 20% protein) (Olvera et al., 2014). Similar results were found in a study conducted to evaluate the effectiveness of a high-protein diet in promoting weight loss and improving clinical outcomes in 14 obese patients with HF. Patients were assigned to 1 of 3 dietary interventions: a high-protein, low-calorie diet (40% carbohydrates, 30% protein, 30% fat), a standard protein, low-calorie diet (55% carbohydrates, 15% protein, 30% fat), or a conventional, isocaloric diet (55% carbohydrates, 15% protein, 30% fat). After 12 weeks, patients in the high protein, low-calorie group experienced the greatest reduction in body weight and demonstrated improvements in functional status, lipid profiles, and quality of life scores (Evangelista et al., 2009). While these findings are promising, both studies are limited by a small sample size and short duration. A large-scale RCT with a longer follow-up is necessary to determine whether intentional weight loss and favorable outcomes are sustainable and whether there are any adverse consequences, particularly in regard to renal function. Future research evaluating macronutrient ratios in patients with HF must consider common comorbidities within this population, the likelihood of adherence, and patient diversity (Evangelista et al., 2009). Current major guidelines have not provided specific macronutrient

intake recommendations; however, a diet containing 50%–55% carbohydrates, 25%–30% fat, and 15%–20% protein seems to be tolerable in this patient population (Colin-Ramirez et al., 2014).

Omega-3 Supplementation

The role of omega-3 polyunsaturated fatty acids (omega-3 PUFAs) in patients with HF has become a common area of interest in HF management. The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Heart Failure (GISSI-HF) study, a randomized control trial consisting of 6975 patients, examined the effects of omega-3 supplementation in patients with chronic HF. Subjects who received 1 g of omega-3 PUFAs/day showed a 10% reduction in mortality and a 7% reduction in hospitalizations over 3.9 years (Tavazzi et al., 2008; Masson et al., 2013). Additionally, a recent meta-analysis pooled the results from 4 different studies and found omega-3 supplementation to have a favorable effect on cardiac function, remodeling, and functional capacity (Butler, 2016). However, other recent trials and meta-analysis have not found a significant relationship between omega-3 supplementation and decreased mortality in patients with cardiovascular disease (Rauch et al., 2010; Kotwal et al., 2012). As a result, current ACC/AHA guidelines consider omega-3 supplementation to be a safe and tolerable adjunctive therapy for this patient population, while further research is required to determine optimal dosing and formulation, as well as the effects of consuming omega-3 PUFAs through dietary sources alone (Yancy et al., 2013).

Micronutrients

Factors Related to Micronutrient Deficiency in HF

Patients with HF may be prone to micronutrient deficiencies due to several factors, including oxidative stress (Shetty et al., 2015), poor nutrient absorption and the use of loop diuretics, a common drug therapy that causes an excess urinary loss of thiamine, potassium, magnesium, and calcium (Weber, 2004). In terms of malabsorption, patients often experience abdominal and gut edema with dysfunctional intestinal barriers leading to an increased permeability, exposure to endotoxins, and chronic inflammation, all contributing to the impairment of mucosa function and nutrient uptake (Trippel et al., 2013).

In addition to the factors mentioned above, an inadequate dietary intake may increase the risk of nutrient deficiency in HF. Approximately 50% of patients are considered malnourished and consume a less varied diet, poor in key micronutrients (Rahman et al., 2016). Several factors may contribute to a decreased dietary intake, such as lack of hunger, fatigue, nausea, premature satiety, age-related changes in taste and smell, the consumption of foods with a low nutrient density, and clinically recommended dietary restrictions (Grossniklaus et al., 2018; Lennie et al., 2013).

Results from recent studies have suggested that a sodium-restricted diet ($<$2000 mg/day) can lead to a reduced intake of other important nutrients in this patient population already at nutritional risk (Frediani et al., 2013; Jefferson et al., 2015). A prospective trial, requiring patients with HF to limit sodium intake to less than 2000 mg/day for 1 week, found that a 49% sodium reduction lead to several unintentional dietary consequences, including a reduction in calorie, carbohydrate, calcium, thiamine, and folate intakes. There was also a decrease in saturated fat consumption and a trend to lower fat intakes (Jefferson et al., 2015). However, a pilot study consisting of 35 patients following a low-sodium diet for 6 months found that patients who reduced their sodium intake by $\geq$25% showed a greater increase in folate intake and a larger reduction in calcium intake in comparison to the $\leq$25% sodium reduction group. Other relevant nutrient intakes were not significantly affected and no significant weight loss was documented in either group. These findings highlight the importance of utilizing an individualized and comprehensive dietary approach when counseling patients with HF to lower sodium intake. When the whole diet is considered, sodium restriction can be lowered without significantly compromising other aspects of the diet (Colin-Ramirez et al., 2015).

Micronutrient Recommendations

Given the increased risk of nutrient deficiency in this population, it is crucial that patients follow the Dietary Reference Intake (DRI) for micronutrients (Yancy et al., 2013). However it is important to note that potassium replacement is not required when a potassium-losing diuretic is used with an angiotensin-converting enzyme inhibitor (ACE inhibitor) and a mineral corticoid receptor antagonist (MRA) or an angiotensin receptor blocker (ARB). Additionally, when potassium-sparing diuretics or supplements are used in combination with an ACE inhibitor (or ARB) and MRA, serious hyperkalemia can occur (McMurray et al., 2015).

There is currently no clinical evidence to suggest micronutrient supplementation is effective in improving clinical outcomes or survival rates in patients with HF. As a result, the ESC guidelines only list supplements as a possible treatment for cardiac cachexia, with no proved benefits; and ACC/AHA guidelines do not recommend nutritional supplementation in the treatment of HF, aside from the clinical treatment of documented deficiencies (Yancy et al., 2013; Ponikowski et al., 2016).

Cardiac Cachexia

Cardiac cachexia is an inflammation-related form of malnutrition, common to many chronic diseases, including severe HF. Approximately 5%–15% of patients with HF are cachectic, with an annual mortality rate of 20%–30% (Griva, 2015). It is

characterized as a multifactorial metabolic disorder, involving muscle wasting, fatigue, and weakness. Progressive weight loss includes the loss of lean muscle mass, body fat, and bone (Evans et al., 2008).

The pathophysiology is associated with alterations in neurohormonal and immunological status, an imbalance in catabolic and anabolic pathways, and the activation of proinflammatory cytokines. Additionally, inadequate protein intake, poor enteral nutrient absorption, and physical inactivity contribute to the wasting process. An inadequate dietary intake does not usually trigger the onset of cachexia alone; however deficiencies in micronutrients and macronutrients can lead to disease progression (Griva, 2015).

Diagnosis of Cardiac Cachexia

Due to the complex, multifactorial pathophysiology of cachexia, it is often underdiagnosed and rarely treated until the late stages. The consensus panel outlined a set of diagnostic criteria to allow clinicians and researchers to make a definitive diagnosis of cachexia. The criteria include edema-free weight loss of a least 5% within the past 12 months or a body mass index <20 kg/m^2, in addition to at least three of the following: decreased muscle strength, fatigue, anorexia, low muscle (fat-free) mass, and biochemical abnormalities characteristic of inflammation, anemia, or hypoalbuminemia (Evans et al., 2008).

Treatment of Cardiac Cachexia

There is no current standardized therapy for cardiac cachexia, though it is recommended that cachectic patients or those at risk of developing it undergo comprehensive therapy aimed at increasing muscle mass, strength, and body weight (Griva, 2015). Both ACE inhibitors and B-blockers have been shown to prevent weight loss associated with severe HF. ACEIs have shown a direct effect on muscle wasting by preventing protein degradation. Likewise, data from animal studies suggest that B-blockers may decrease total energy expenditure and increase skeletal muscle mass (Rahman et al., 2016). Other drug therapies such as ghrelin receptor agonists (anamorelin) and selective androgen receptor modulators (enobosarm) have shown promising results in the treatment of cachexia, while research surrounding myostatin antagonists is still underway (Griva, 2015).

Nutritional therapy alone has no effect on the catabolic process of cachexia; however when used in combination with other therapies beneficial effects are more likely. Evidence has shown that protein supplementation acts to increase muscle synthesis, particularly when used in conjunction with exercise (Loncar et al., 2016). The PROTAGE Study Group, an international study group aimed at providing recommendations for optimal protein intake by older people, suggests an average daily intake of at least 1.0–1.2 g/kg of protein to enhance muscle synthesis. Protein quality and timing of ingestion may be relevant to treatment; however further research is required to support specific recommendations (Bauer et al., 2013). Additionally, caloric supplementation has led to weight gain and improved quality of life (Loncar et al., 2016). It is beneficial for patients to receive individualized dietary counseling aimed at ensuring they are consuming enough protein and calories to meet their energy needs, as well as sufficient micronutrients to optimize cellular function (Rahman et al., 2016). Other nutritional interventions such as eicosapentaenoic acid, B-hydroxy B-methylbutyrate, and resveratrol may prevent wasting and muscle loss in animal models, without proven effects on humans (Loncar et al., 2016). In regard to exercise, tolerable endurance and resistance programs are suggested to maintain lean body mass and function (Bauer et al., 2013). Aerobic therapy is the most proven to counteract muscle wasting, and is recommended by HF treatment guidelines (Yancy et al., 2013; Ponikowski et al., 2016).

Dietary Patterns

The Dietary Approaches to Stop Hypertension (DASH) Diet

The DASH diet is a dietary pattern found to have a beneficial effect on preventing and managing risk factors associated with CVD, such as hypertension. Such a diet is typically comprised of fruits, vegetables, low-fat dairy products, whole grains, chicken, fish, and nuts. In comparison to the typical western diet, it is significantly lower in saturated fat, total fat, cholesterol, refined sugar, and sodium (Eilat-Adar et al., 2013). The results from recent studies have suggested that in addition to its protective role in preventing CVD, the DASH diet may be an effective therapeutic tool in HF management (Rifai et al., 2013). A trial evaluating the effects of consuming a sodium-restricted DASH diet (1500 mg/2100 cal) in 13 patients with treated hypertension and HF with preserved ejection fraction found that after a 21 day-follow-up period, patients experienced beneficial changes in ventricular diastolic function, arterial elastance, and ventricular-arterial coupling (Hummel et al., 2013). Additionally, results from a recent RCT showed that adhering to the DASH diet for 3 months lead to an improvement in endothelial function, exercise capacity, and quality of life scores in patients with chronic symptomatic (stage C) HF (Rifai et al., 2013). Given these findings, integrating this combination diet into the dietary habits of patients with HF seems safe and reasonable. Importantly, the DASH diet is varied, nutrient dense, and low in sodium and saturated fat, making it highly suitable for the HF population. Further research examining the effects of the DASH diet in these patients is required, especially in those with concomitant renal disease or on potassium-sparing medications, who may need to restrict their potassium intake (Colin-Ramirez et al., 2014).

The Mediterranean Diet

The Mediterranean diet is characterized by a high intake of fruits, vegetables, nuts, legumes, whole grains, fish, olive oil, and a limited consumption of red meat and high-fat dairy products. Several experimental and observational studies have suggested that this dietary pattern plays a protective role against cardiovascular disease. A recent prospective cohort study found the Mediterranean diet to be associated with a lower risk of sudden cardiac death in postmenopausal women in comparison to the DASH diet (Bertoia et al., 2014). These results are consistent with those of the PREDIMED trial, a primary prevention trial which found that following the Mediterranean diet substantially reduced the risk of major cardiovascular events, including strokes, myocardial infarctions, and death (Estruch et al., 2013). Additionally, a study focusing on the effect of the Mediterranean diet in the primary prevention of HF found that the Mediterranean diet for 1 year was associated with a significant reduction in N-terminal pro-brain natriuretic peptide levels among individuals at high risk of CVD (Fito et al., 2014). Lastly, two population-cohort studies examined the beneficial effects associated with a high adherence to the Mediterranean diet. One study examining men specifically found the Mediterranean diet was associated with a lower risk of developing HF and a lower mortality from HF (Tektonidis et al., 2016); and the second study showed that a high adherence to this dietary pattern lowered the risk of myocardial infarction, ischemic stroke and HF, among women (Tektonidis et al., 2015). Collectively, these studies demonstrate a clear association between a Mediterranean diet and improved cardiovascular health; however, a full examination of the role of this dietary intervention in the management of HF is required.

Conclusion

In addition to fluid and sodium restriction, evidence has suggested that other dietary components may play a critical role in the nonpharmacological treatment of HF. However, there is still a strong need for future research to further clarify the beneficial effects of dietary manipulation in patients with HF. While previous studies have shown promising results, many are limited by small sample sizes and a short duration. Larger, high-quality RCTs that account for differences within this patient population such as the cause and severity of HF, concomitant disease, age, race, body size, among others, are required in order to incorporate evidence-based dietary advice into the clinical treatment of HF. Finally, patient education by a dietitian, who is able to provide nutritional counseling according to patient's individual needs, is key to promote an appropriate nutrient intake among these patients and identify those at risk of malnutrition.

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Further Reading

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Dietary Fat and Risk of Cardiovascular Disease

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Types and Source of Dietary Fat

Dietary fat is a macronutrient found in food from plants and animals sources and provides energy for the body. Generally, the higher the content of saturated fatty acids, the more the solid nature of fat at room temperature, thus animal fats are solid, whereas plant fats (oils) are liquid at room temperature, except for some seed oils, like coconut oil and palm kernel oil, which are high in saturated fat and therefore solid at room temperature (Dietary Fats, 2005). In a review of data from 28 countries, the contribution of total fat intake to the total energy intake ranges between 11.1% in China to 50.7% in Nigeria (Elmadfa and Kornsteiner, 2009).

Fatty acids are the major form of dietary fat (mainly as triglycerides), and according to the presence and number of double bonds in their carbon chain, they are classified into saturated and unsaturated fatty acids.

Saturated Fatty Acids

Saturated fatty acids (SFA) contain no double bond; this type of fat can be synthesized by the body and its major dietary source is food from animal sources, such as full-fat dairy products, red meat, and poultry. Moreover, there are numerous types of SFA according to length of their chain (contains 4–16 carbon atoms). SFA intake is generally high in Africa and North America and low in East-Asia; the contribution of SFA intake to the total energy intake ranges between 3.1% in China to 25.4% in Nigeria (Elmadfa and Kornsteiner, 2009).

Unsaturated Fatty Acids

Unsaturated fatty acids contain one or more carbon atom in a double bond. The three major types of unsaturated fatty acids are monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and trans fatty acids.

Monounsaturated fatty acids (MUFA)

MUFA contain one double bond; this type of fat is liquid at room temperature and found in a variety of food and oils, such as olive oil, safflower oil, peanut oil, and corn oil. The contribution of MUFA intake to the total energy intake ranges between 3.5% in China to 22.3% in Greece (Elmadfa and Kornsteiner, 2009).

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Polyunsaturated fatty acids (PUFA)

PUFA contain more than one double bond and found mainly in plant-based food and oils. Omega-3 (*n*-3) and Omega-6 (*n*-6) fatty acids is a classification that depends on the position of the first double bond from the methyl terminus of PUFA. The contribution of PUFA intake to the total energy intake ranges between 3.3% in India to 11.3% in Taiwan (Elmadfa and Kornsteiner, 2009).

Omega-3 (*n*-3) PUFA

Omega-3 PUFAs are found in some types of fatty fish like salmon, tuna, trout, mackerel, and sardines. Moreover, seeds like nuts, walnuts, butternuts, and sunflower and oils of canola, flaxseed, and soybean are rich plant sources of *n*-3 fatty acids. Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid are the three main types of *n*-3 fatty acids involved in human physiology. The contribution of ALA intake to the total energy intake ranges between 0.4% in France to 1.0% in Finland (Elmadfa and Kornsteiner, 2009); while that for EPA and DHA varied between 0.03% in China to 1.05% in Japan (Elmadfa and Kornsteiner, 2009).

Omega-6 PUFA

Omega-6 PUFA, mainly linoleic acid (LA), come from vegetable oils. The contribution of LA intake to the total energy intake ranges between 2.7% in India to 7.2% in Austria (Elmadfa and Kornsteiner, 2009).

Trans fatty acids

Trans fatty acids are unsaturated fatty acids; however, they contain at least one double bond in the "trans" configuration, the thing that makes them more similar to saturated than unsaturated fat. Trans fatty acids are solid at room temperature and occur, in small amounts, naturally in some food. However, most of the solid fats content of trans fatty acids is produced by part hydrogenation of oils, which causes isomerization and relocation of some double bonds giving more solidity to the fat and increasing its trans fatty acids content. Trans fatty acids are high in stick margarine and high content of trans fatty acids can be found in cakes, pastries, doughnuts, and French fries (Litin and Sacks, 1993). The contribution of trans fatty acid intake to the total energy intake ranges between 0.5% and 2.1% in western Europe (Hulshof et al., 1999), 2% the US (Ascherio et al., 1999), and 1%–2% in Netherlands (Oomen et al., 2001).

Function of Dietary Fat

Total Fat

Dietary fats are essential for several biological functions including growth and development via being a major source of energy, working as auxiliary building pieces of the body, conveying fat-soluble vitamins, and involving in crucial physiological procedures in the body (Dietary Fats, 2005; Elmadfa and Kornsteiner, 2009).

Provision of energy

Among the sources of energy in the human diet, fat, carbohydrate, and protein, fat is the most concentrated source providing 9 kcal/1 g consumed, which is more than double the energy content of protein or carbohydrate (4 kcal/g). Fat can be stored in the body's fat tissue, which releases fatty acids when energy is required (Dietary Fats, 2005; Elmadfa and Kornsteiner, 2009).

Structural component

The cell membranes that control the movement of substances in and out of the cells are mainly made of phospholipids, triglycerides, and cholesterol. The human brain is the fattest organ in the body that may consist of at least 60% fat, and the major brain fatty acid is DHA (Dietary Fats, 2005; Elmadfa and Kornsteiner, 2009).

Carrier of vitamins

Fat is a transporter for vitamins A, D, E, and K and enhance their intestinal absorption (Dietary Fats, 2005; Elmadfa and Kornsteiner, 2009).

Other biological functions specific for each type

SFA are a source of body fuel, structural components of cell membranes, and associated with proteins to a degree of being necessary for their normal function. MUFA are important component of cell membrane structure, particularly the myelin sheath of nervous tissue. The human body cannot produce PUFA, for example, LA and ALA, and therefore are called essential fatty acids. Without these essential fatty acids, some vital functions would be compromised, thus they must be provided by the diet. LA and ALA are involved in several physiological processes such as inflammation, blood clotting, and wound healing through engagement with prostaglandins and leukotrienes after being transformed to longer chain fatty acids. LA works as the precursor to arachidonic acid (AA) that involved with regulation of gene expression. Trans fatty acids are not essential and provide no known benefit to human health (Dietary Fats, 2005; Elmadfa and Kornsteiner, 2009).

Associations Between Dietary Fat Intake and Risk of Cardiovascular Disease and Its Risk Factors

Many previous studies have examined the associations of dietary fat intakes and levels of tissue biomarkers of fatty acids with risk of cardiovascular disease (CVD) and the results were inconsistent (**Table 1** for cardiovascular risk factors, **Table 2** for risk of coronary heart disease, **Table 3** for risk of stroke, and **Table 4** for risk of other and total cardiovascular diseases). SFA and cholesterol intakes were associated with higher risk of CVD among women with type 2 diabetes mellitus, while intakes of total fat, MUFA, PUFA, and trans fat were not (Tanasescu et al., 2004). Another study found no association between intakes of total fat, SFA, MUFA, and PUFA with the risk of CVD (Leosdottir et al., 2007); whereas, other studies showed a decreased risk of CVD with higher intake of SFA (Yamagishi et al., 2010, 2013a), MUFA (Guasch-Ferré et al., 2014), PUFA, LA, and ALA (Laaksonen et al., 2005), and with fish and *n*-3 PUFA (Yamagishi et al., 2008a).

The pooled RR of total CVD events reported in meta-analyses were 0.98 (0.92–1.05) for reducing total fat intake from 32% to 20% of energy; 1.00 (0.89–1.11; *P*-trend = 0.95) (Siri-Tarino et al., 2010) and 0.97 (0.84–1.12; *P*-trend = 0.69) (de Souza et al., 2015) for high SFA intake; 0.91 (0.86–0.96; *P*-trend = 0.001) and 0.88 (0.80–0.96; *P*-trend = 0.004) for high MUFA intake (Schwingshackl and Hoffmann, 2014); 0.87 (0.79–0.95; *P*-trend = 0.002) for high *n*-3 PUFA supplementation (Marik and Varon, 2009).

A Scottish study showed that adipose tissue MUFA concentrations were positively associated, while *n*-3 PUFA concentrations were inversely associated, with the risk of incident CVD (Woodward et al., 2011). Neither adipose tissue SFA nor *n*-6 PUFA concentrations were associated with any increased or decreased risk of CVD (Woodward et al., 2011). However, a Swedish study showed that serum concentrations of SFAs, namely, palmitic, palmitoleic, and dihomo-γ-linolenic, were positively and serum concentrations of LA were inversely associated with cardiovascular mortality (Warensjö et al., 2008). Among the biomarkers of dairy fat, concentrations of plasma phospholipid 15:0, but not 14:0 or trans-16:1*n*-7, were inversely associated with the incident CVD (de Oliveira Otto et al., 2013).

Although most CVD share many of the risk factors, the relations of dietary fat with risk of different CVD endpoints are not necessarily the same.

Numerous observational studies have shown a positive association between dietary fat intake and risk of CHD (Esrey et al., 1996; McGee et al., 1984; Kushi et al., 1985; Shekelle et al., 1981), more evident for intakes of SFA (Yamagishi et al., 2013a; Esrey et al., 1996; Ascherio et al., 1996; Hu et al., 1997, 1999; Howard et al., 2006; Kromhout et al., 1995; Jakobsen et al., 2009) and trans fatty acids (Oomen et al., 2001; Ascherio et al., 1994, 1996; Hu et al., 1997; Howard et al., 2006; Kromhout et al., 1995; Oh et al., 2005; Pietinen et al., 1997) intakes; other studies did not find any associations with intakes of total fat (Leosdottir et al., 2007; Hu et al., 1997; Howard et al., 2006; Oh et al., 2005), SFA (Leosdottir et al., 2007; Siri-Tarino et al., 2010; de Souza et al., 2015; Oh et al., 2005; Pietinen et al., 1997), MUFA (Leosdottir et al., 2007; Laaksonen et al., 2005; Schwingshackl and Hoffmann, 2014; Jakobsen et al., 2009; Oh et al., 2005; Pietinen et al., 1997), or PUFA (Tanasescu et al., 2004; Leosdottir et al., 2007; Yamagishi et al., 2008a; Esrey et al., 1996; McGee et al., 1984; Pietinen et al., 1997; Dolecek, 1992); whereas some other studies reported inverse associations with MUFA (Hu et al., 1997) and PUFA (Laaksonen et al., 2005; Howard et al., 2006; Jakobsen et al., 2009; Oh et al., 2005; Iso et al., 2006; Li et al., 2015; Mozaffarian et al., 2010; Farvid et al., 2014). In meta-analyses, the pooled RR for risk of CHD associated with intakes of SFA were 1.07 (0.96–1.19; *P*-trend = 0.22) (Siri-Tarino et al., 2010), 1.15 (0.97–1.36; *P*-trend = 0.10) (de Souza et al., 2015), and 1.02 (0.97–1.07) (Chowdhury et al., 2014); those associated with MUFA intakes were 0.96 (0.90–1.01; *P*-trend = 0.13) (Schwingshackl and Hoffmann, 2014) and 101 (0.96–1.07) (Chowdhury et al., 2014); those associated with PUFA intakes were 0.81 (0.70–0.95; *P*-trend = 0.008) (Mozaffarian et al., 2010) and 0.99 (0.89–1.09) for *n*-6 fatty acids and 0.93 (0.84–1.02) for *n*-3 fatty acids (Chowdhury et al., 2014); while the pooled RR associated with trans fatty acids intakes were 1.28 (1.09–1.50; *P*-trend = 0.003) (de Souza et al., 2015) and 1.16 (1.06–1.27) (Chowdhury et al., 2014).

The associations between plasma cholesterol ester and tissue phospholipids fatty acids composition with risk of CVD should be carefully interpreted. Some fatty acids, such as LA and ALA, cannot be synthesized by the human body and their plasma and tissue levels reflect their dietary intakes; however, other fatty acids, especially SFA, can be endogenously synthesized and their plasma and tissue levels are not valid biomarkers for dietary intakes (Ma et al., 1995; Sun et al., 2007a).

In case-control studies, high plasma, but not erythrocyte, content of biomarkers for dairy fat (C 15:0 and trans 16:1*n*-7) (Sun et al., 2007b) and trans fat (Sun et al., 2007c) was associated with the risk of CHD. Adipose tissue C18:1 trans fatty acids content was not associated with the risk of CHD mortality (Aro et al., 1995). Levels of stearic acid (18:0), dihimo-gamma-linolenic acid (20:3*n*6), and total SFA were associated positively, while AA (20:4*n*6) and total PUFA levels were associated inversely, with CHD risk (Wang et al., 2003). Based on fatty acids' affinity and fluidity, a lipophilic index that summarizes individual fatty acid plasma and erythrocyte levels was associated with risk of CHD (Wu et al., 2013). A meta-analysis showed that lower LA tissue content was associated with increased risk of nonfatal-CHD, while lower DHA and EPA were associated with increased risk of fatal CHD cases (Harris et al., 2007).

As for stroke, some studies reported inverse associations with intakes of total fat (McGee et al., 1985), SFA (Tanasescu et al., 2004; Yamagishi et al., 2013a; McGee et al., 1985), MUFA (Schwingshackl and Hoffmann, 2014), and PUFA (Atkinson et al., 2011; Iso et al., 2001a); others reported no association with intakes of total fat (Atkinson et al., 2011; He et al., 2003), SFA (Atkinson et al., 2011; He et al., 2003), MUFA (Atkinson et al., 2011; He et al., 2003), PUFA (Yamagishi et al., 2008a; He et al., 2003), or trans fat (He et al., 2003), while one study reported positive associations with intakes of trans fat (Kiage et al., 2014).

Table 1 Association between dietary fat intake and some cardiovascular risk factors

Published year/study population/ref no	*Study/cohort name*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/ outcome*	*Outcome*	*Results*
Mutanen et al. (1992)		59 (29 male and 30 female) normotensive		Randomized crossover trial for 10 weeks	10 weeks	High SFA, high PUFA and Low MUFA diets	SBP and DBP	SBP and DBP after high SFA diet were 122.6 ± 11.5 mmHg and 75.4 ± 7.5 mmHg; decreased to 119.6 ± 10.3 and 73.9 ± 7.4 mmHg after PUFA diet, and was 120.1 ± 11.2 and 72.6 ± 6.4 mmHg, with low MUFA diet
Shah et al. (2007)		6 randomized crossover, 1 randomized parallel, and 3 nonrandomized trials		A meta-analysis of intervention studies		High-carbohydrate versus high MUFA diets	SBP and DBP	Carbohydrate diet associated with higher SPB [$x(-)$ difference: 2.6 (95%CI: 0.4, 4.7) mmHg; $P=0.02$] and DBP [1.8 (0.01, 3.6) mmHg; $P=0.05$] than did MUFA diets.
Ferrara et al. (2000)		23 (10 male and 13 female) hypertensive subjects	25–70 years	Randomized crossover trial for 6 months.	6 months	11 patients assigned to an MUFA dietary regimen and 12 to a PUFA-rich diet	SBP and DBP	SBP and DBP decreased with MUFA compared with PUFA diets; (127 [14] versus 135 [13] mmHg; $P=.05$) and (84 [8] versus 90 [8] mmHg; $P=.01$), respectively. Antihypertensives dose was reduced by 48% (95%CI, −25% to −71%) during the MUFA diet and by 4% (95%CI, −24% to 17%) during the PUFA diet, P-difference $=0.005$
Harris (1989)		928 participants of 44 trials		A systematic review		Fish oil and *n*-3 fatty acids intakes	levels of cholesterol, triglycerides, LDL- and HDL-cholesterol	No change in total or LDL-cholesterol, a 25% decrease in triglyceride levels, and a slight rise in HDL-C (+3%).
Harris (1997)		36 placebo-controlled crossover and 29 parallel design studies		A systematic review	≥ 2 weeks	<7 g *n*-3 fatty acids/d	Lipid profile	5%–10% increase in LDL-cholesterol, 1%–3% increase in HDL-cholesterol and 25%–30% decrease in serum triglycerides. No change in total cholesterol
Morris et al. (1993)		1356 subjects of 31 placebo-controlled trials		A meta-analysis		*n*-3 fatty acids in fish oil	Blood pressure	Mean reduction of −3.0, −1.5 mmHg (95%CI: systolic BP: −4.5, −1.5; diastolic BP: −2.2, −0.8)
Mensink and Katan (1992)		682 (474men and 208 women) of 27 clinical trial between 1970 and 1991		A meta-analysis		Replacement of fat by carbohydrate and SFA with PUFA	Lipid profile	Fat by carbohydrate decreased HDL-cholesterol levels. SFA by PUFA decreased HDL-cholesterol by (0.2 mg/dL) per 1%E. SFA elevated while PUFA reduced LDL-cholesterol

(*Continued*)

Table 1 (Continued)

Published year/study population/ref no	*Study/cohort name*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/ outcome*	*Outcome*	*Results*
Becker et al. (1983)		12 healthy men		A Feeding trial	4 weeks	Effect of SFA, MUFA, and PUFA	Lipid profile	Total cholesterol 166 mg/dL and LDL-cholesterol 103 mg/dL decreased to 133 and 81 mg/dL (SAF), 127 and 71 mg/dL (MUFA), and 123 and 65 mg/dL (PUFA). HDL-cholesterol changed from 46 to 40 mg/dL (SAF), 43 mg/dL (MUFA) and 45 mg/dL (PUFA)
Nelson et al. (1997)		12 healthy volunteer		A single-blind crossover feeding study	130 days	AA-rich diet 1.7 g/d	lipid profile	No statistically significant effect
Lopez-Garcia et al. (2005)	the Nurses' Health Study I Cohort	730 women	43–69 years	cross-sectional study		Trans fatty acid intake via FFQ	age, BMI, intake of MUFA, PUFA, and SFA, smoking status, physical activity, alcohol intake, and postmenopausal hormone therapy	Among women in the highest versus lowest quintile of trans fat intake, CRP levels were 73% ($P=0.009$) higher. IL-6 levels were 17% higher, sTNFR-25% ($P=0.002$), E-selectin 20% ($P=0.003$), sICAM-1 10% ($P=0.007$), and sVCAM-1 levels 10% ($P=0.001$).
Mensink et al. (1992)		58 healthy men and women		Experimental feeding trial		Replacing SFA by MUFA or PUFA	Lp[a] concentration	MUFA replacing SFA, the median Lp[a] concentration increased by 7 mg/L. PUFA replacing SFA, the median Lp[a] concentration increased by 3 mg/l.
Summers et al. (2002)		17 subjects (6 Type II diabetics, 6 nonobese nondiabetics and 5 obese nondiabetics)		A crossover design	Two 5-week periods	SFA or PUFA diets	Lipid profile and other metabolic concentrations and Subcutaneous and visceral fat areas	For PUFA versus SFA diets: subcutaneous fat area decreased ($P=0.001$), fasting total and LDL-cholesterol concentrations decreased ($P=0.001$ and $P=0.002$) and insulin sensitivity increased ($P=0.02$). Total and visceral fat, HDL-cholesterol, triglyceride, glucose or other metabolite concentrations did not change.
Hodson et al. (2001)		Trial I, $n=29$; and trial II, $n=42$ volunteers		Human clinical trial		High SFA diet replaced by high MUFA or PUFA diet		Replacing SFA with *n*-6 PUFA lowered plasma total cholesterol by 19%, LDL-cholesterol by 22%, and HDL-cholesterol by 14%. Replacing SFA with MUFA lowered plasma total cholesterol by 12%, LDL-cholesterol by 15%, and HDL-cholesterol by 4%.
Howard et al. (2010)	Women's Health Initiative Dietary Modification Trial	2730 Postmenopausal women	50–79 years	Randomized controlled trial	8.1 years	Reducing intake of total fat to 20% of energy intake	Lipid profile, blood pressure and other metabolic factors	LDL-C level changes between the intervention and control groups were of 1.2, 2.8, and 0.9 mg/dL after 1, 3, and 6 years ($P=0.25$, 0.03, and 0.68, respectively). No statistically significant effects on levels of triglycerides or HDL-C; ratio of total cholesterol to HDL-C; levels of non–HDL-C, lipoprotein(a).

Table 2 Associations of dietary fat intake and serum fatty acids levels with risk of Stroke

Published year/ ref no	*Study/cohort name*	*Sample size/Study population*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/outcome*	*Adjusted variables*	*Results*
Leosdottir et al. (2007)	The Malmö Diet and Cancer Study	28,098 (61% women)	Middle aged	Prospective cohort	8.4 years	Intakes of fat via FFQ	Age, smoking habits, alcohol consumption, socio-economic status, marital status, physical activity, BMI, fibre intake, blood pressure, total fat intake for the ratio between unsaturated- and saturated fats.	HRs of ischemic stroke in the highest quartile of intakes among men were 0.97 for total fat, 1.19 for SFA, 0.82 for MUFA and 1.06 for PUFA, while among women were 0.97, 1.19, 0.82 and 1.06, respectively. No significant associations in multivariable model
Yamagishi et al. (2013a)	The Japan Public Health Center-based prospective Study	38,084 Japanese men and 43,847 women	45–74	Prospective cohort	11.1 years	Dietary SFAs via FFQ	Age; sex; energy intake; cohort; cigarette smoking status; alcohol intake; body mass index; sports at leisure time; walking and standing time; perceived mental stress; energy-adjusted dietary intakes of carbohydrate, protein, cholesterol, vegetables, fruit, and calcium.	HR for total, stroke and ischemic stroke and intraparenchymal haemorrhage in the highest SFA intake were 0.77 (0.65–0.93), 0.84 (0.67–1.06) and 0.61 (0.43–0.86), respectively.
Yamagishi et al. (2010)	The Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC) Study	58,453 Japanese men and women	40–79 years	Prospective cohort	14.1 years	Intakes of SFA via FFQ	Age, sex, a history of hypertension and diabetes, smoking status, alcohol consumption, BMI, mental stress, walking, sports, educational level, and dietary intakes of total energy, cholesterol, *n*-3 and *n*-6 polyunsaturated fatty acids, vegetables, and fruits	RR for total and ischemic stroke and intraparenchmal hemorrhage mortality were 0.69 (0.53–0.89; *P*-trend = 0.004), 0.58 (0.37–0.90; *P*-trend = 0.01) and 0.48 (0.27–0.85; *P*-trend = 0.03), respectively
Yamagishi et al. (2008a)	The Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC) Study	57,972 Japanese men and women	40–79 years	Prospective cohort	12.7 years	Intakes of fish and *n*-3 PUFA via FFQ	Age, sex, a history of hypertension and diabetes, smoking status, alcohol consumption, BMI, mental stress, walking, sports, educational level, and dietary intakes of total energy, cholesterol, and *n*-6 PUFA vegetables, and fruits	RR for total and ischemic stroke and intraparenchmal hemorrhage mortality were 0.91 (0.74–1.13; *P*-trend = 0.40), 0.93 (0.65–1.34; *P*-trend = 0.78) and 0.95 (0.62–1.47; *P*-trend = 0.58), respectively in highest versus lowest quintiles of fish intake, and were 0.93 (0.70–1.22; *P*-trend = 0.46), 1.17 (0.71–1.92; *P*-trend = 0.58) and 0.70 (0.40–1.24; *P*-trend = 0.16), respectively in highest versus lowest quintiles of *n*-3 PUFA intake.
Siri-Tarino et al. (2010)		347,747 subjects of 21 prospective cohort studies published until September 2009		Meta-analysis	5–23 years	Saturated fat intake		The pooled relative risk estimates that compared extreme quantiles of saturated fat intake were 1.07 (95% CI: 0.96, 1.19; *P* = 0.22) for CHD, 0.81 (95%CI: 0.62, 1.05; *P* = 0.11) for stroke, and 1.00 (95%CI: 0.89, 1.11; *P* = 0.95) for CVD.

(*Continued*)

Table 2 (Continued)

Published year/ ref no	Study/cohort name	Sample size/Study population	Age at entry	Study design	Follow-up years	Exposure measures/outcome	Adjusted variables	Results
de Souza et al. (2015)		347,747 subjects of prospective cohort, case-control, nested case-control, or case-cohort studies published until May 2015.		Meta-analysis		SFA and trans fat intakes	With SFA, RR were 0.97 (0.84–1.12) for CVD mortality, 1.15 (0.97–1.36) CHD mortality, 1.06 (0.95–1.17) incident total CHD, 1.02 (0.90–1.15) incident ischemic stroke, and 0.95 (0.88–1.03) for type 2 diabetes. With total trans fat intake RR were (1.28, 1.09–1.50) for CHD mortality, and 1.21 (1.10–1.33) for total incident CHD but were 1.07 (0.88–1.28) for ischemic stroke and 1.10 (0.95–1.27) for type 2 diabetes	
Schwingshackl and Hoffmann (2014)		32 cohort studies (42 reports) of 841,211 subjects		A systematic review and meta-analysis Literature until June 2nd, 2014		MUFA (of both plant and animal origin), olive oil, oleic acid, and MUFA: SFA ratio		High MUFA was associated with a RR of stroke mortality of: 0.83 (0.71, 0.97, $P=0.02$).
Howard et al. (2006)	The Women's Health Initiative Randomized Controlled Dietary Modification Trial	48,835 postmenopausal women	50–79 years	Randomized controlled trial	8.1 years	reduction of total fat intake from $\geq$32%E to $<$20% E		The dietary intervention had no significant effects; HR were 0.97 (0.90–1.06) for incident CHD; 1.02 (0.90–1.15) stroke and 0.98 (0.92–1.05) CVD. Trends toward greater reductions in CHD risk were observed in those with lowest intakes of SFA or trans fat; HR were 0.82 (0.67–0.99) and 0.84 (0.69–1.02), respectively.
McGee et al. (1985)	The Honolulu Heart Program	8006 Japanese men residing in Hawaii		Prospective cohort	10 years	Dietary fat g/d using 24h dietary recall and % Energy as fat	systolic blood pressure, number of cigarettes per day, physical activity, BMI, and age	Fat and SFA as g/d were not associated but % of Energy as fat and SFA were inversely associated with stroke mortality.
Atkinson et al. (2011)	The Caerphilly cohort	2710 men	middle-aged	Prospective cohort	18 years	intakes of total fat, SFA, unsaturated fat, white fish and oily fish via FFQ	Smoking status, adult social class, marital status, alcohol intake, vitamin C intake, vegetable fibre intake, blood pressure, cholesterol, BMI, fasting glucose, diabetes and atrial fibrillation.	HR for risk of stroke in the highest quintile of unsaturated fat and oily fish intakes were 0.66 (0.41–1.05, P-trend $=0.13$) and 0.66 (0.41–1.05, P-trend $=0.09$), respectively.
Iso et al. (2001a)	The Nurse Health Study	79,839 women	34–59 years	Prospective cohort	14 years	Fish and omega-3 polyunsaturated fatty acid intake	Age, menopausal stats and postmenopausal hormone use, vigorous exercise, alcohol intake, use of aspirin, multivitamin use, history of hypertension, BMI, fruits and vegetable, SFA, trans fat, linoleic acid, animal protein and calcium intakes.	HR for total stroke and thrombotic infarction in the highest intake category of fish were 0.48 (0.21–1.06) and 0.49 (0.26–0.93) and those for n-3 fatty acids intake were 0.72 (0.53–0.99) and 0.67 (0.42–1.07), respectively.

He et al. (2003)	Health Professional Follow up Study	43,732	40–75 years	Prospective cohort	14 years	Intake of total fat, cholesterol, and specific types of fat.	BMI, physical activity, history of hypertension, smoking status, aspirin use, multivitamin use, and consumption of alcohol, potassium, fibre, vitamin E, fruit and vegetables, total energy intake, and hypercholesterolaemia	Comparing the highest fifth of intake with the lowest fifth, the RR of ischemic stroke was 0.9 for total fat, 1.20 for animal fat, 1.07 for vegetable fat, 1.16 for SFA, 0.91 for MUFA, 0.88 for PUFA, 0.87 for trans fat, and 1.02 for dietary cholesterol. None was significant
Kiage et al. (2014)	The REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort	17,107 men and women	$\geq$45 years	Prospective cohort	7 years	Trans fatty acids intake	Age, smoking status, race, region, alcohol use, education, waist circumference, level of physical activity, diabetes, ischemic heart disease, hypertension, baseline stroke, heart failure, kidney failure, statin use, regular aspirin use, total energy and energy-adjusted SFA, MUFA, PUFA, and protein.	HR for total and ischemic stroke by 1-SD (2-g/d) increase in trans fatty acids intake was 1.14 (1.02, 1.28) and 1.13 (1.00, 1.28) for men and 0.93 (0.79, 1.11) and 0.93 (0.77, 1.12).for women.
Gillman et al. (1997)	Framingham Heart Study	832 men	45–65 years	Prospective cohort	20 years	Intakes of total fat, SFA, MUFA, PUFA via 24-h DR.	Age, energy, cigarette smoking, glucose intolerance, BMI, blood pressure, blood cholesterol level, physical activity, and intake of vegetables and fruits.	For each 3% increment in total fat, 1% of SFA and MUFA intakes, the multivariable-adjusted RR of ischemic stroke were 0.84 (0.75–0.92), 0.90 (0.83–0.96) and 0.87 (0.81–0.94), respectively.
Sauvaget et al. (2004)	Adult Health Study (AHS)	3731 Japanese men and women	35–89 years	Prospective cohort	14 years	Intakes of total fat, animal fat, SFA, MUFA PUFA and cholesterol via 24-h DR.	Radiation dose, city of exposure, smoking and drinking status, BMI, and history of hypertension and diabetes, fruit and vegetable intake, lymphocyte count, blood cholesterol level, total energy intake and weight.	The risk reduction of cerebral infarction in the highest tertiles on intakes versus lowest were 62% (CI, 82%–18%) for animal fat and 63% (CI, 82%–22%) for cholesterol, respectively.
Seino et al. (1997)		1182 rural Japanese men and 1469 women	$\geq$40 years	Prospective cohort	15.5 years	Dietary lipid via FFQ	Stratified by age and sex, and adjusted for blood pressure- and atrial fibrillation	RR for cerebral infarction in the highest quintiles of total fat, SFA, MUFA and PUFA were 0.94, 0.68, 0.28 and 1.57, respectively. None of which were significant
He et al. (2002)	Health Professional Follow up Study	43,671	40–75 years	Prospective cohort	12 years	Intakes of fish and ώ3 PUFA via FFQ	Age, BMI, history of hypertension, smoking, alcohol consumption, use of aspirin, use of multivitamins, physical activity, potassium, magnesium, fruits, vegetables, total energy, total fat, SFA, trans fat, fish oil and hypercholesterolemia. No significant associations were found between fish or ώ3 PUFA intake and risk of hemorrhagic stroke.	HR of ischemic stroke was 0.57 (0.35–0.95).

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Table 2 (Continued)

Published year/ ref no	*Study/cohort name*	*Sample size/Study population*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/outcome*	*Adjusted variables*	*Results*
Yaemsiri et al. (2012)	The Women's Health Initiative Observational Study	87,025 postmenopausal women	50–79 years	Prospective cohort	7.6 years	Dietary trans fat via FFQ	Age, race, education, family income, years as a regular smoker, hormone replacement therapy use, total MET hours per wk, alcohol intake, history of coronary heart disease, history of atrial fibrillation, history of diabetes, aspirin use, use of antihypertensive medication, use of cholesterol-lowering medication, BMI, systolic blood pressure, total energy intake, dietary vitamin E, fruits and vegetable and fiber intakes.	HR for ischemic stroke in highest trans fat quintile was 1.39 (1.08–1.79, *P*-trend = 0.048). No significant associations were found between intakes of SFA, MUFA, or PUFA
Boden-Albala et al. (2009)	The Northern Manhattan Study (NOMAS)	3183 subjects	≥ 40 years	Prospective cohort	5.5 years	Intakes of total fat, SFA, MUFA and PUFA via FFQ.	Age, race/ethnicity, sex, education, BMI, hypertension, diabetes, coronary artery disease, moderate alcohol consumption, current smoking, previous smoking, physical activity	HR of ischemic stroke 1.6 (1.2–2.3) for total fat >65g/d. HR 1.7 (0.8–2.3) for highest intake quintile of SFA,
Iso et al. (2003)		4775 Japanese	40–69 years	Prospective cohort	14 years	Intakes of fat by single 24-h dietary recall	Age, sex, community, total energy intake BMI, history of hypertension, serum total cholesterol levels, serum glucose, smoking status, ethanol intake, and (for women) menopausal status and the time intervals of the baseline surveys.	HR of intraparenchmal hemorrhage in the highest total fat and SFA quartiles were 0.46 (0.20, 1.04, *P* = 0.05) and 0.30 (0.12, 0.71, *P* = 0.005), respectively.
Iso et al. (2001b)	Nurses' Health Study	85,764 women	34–59 years	Prospective cohort	14 years	Intakes of fat via FFQ	Age, smoking status, time interval, BMI, alcohol intake, menopausal status and postmenopausal hormone use, vigorous exercise, usual aspirin use, multivitamin use , vitamin E use, n3 fatty acid intake, calcium intake, and histories of hypertension, diabetes, and high cholesterol levels and total energy intake.	HR of intraparenchmal hemorrhage in the lowest SFA and trans fat intakes were 2.36 (1.10–5.09; *P* = 0.03) and 2.50 (1.35 to 4.65; P:=0.004), respectively
Yakoob et al. (2014)	The Health Professionals Follow-Up Study; the Nurses' Health Study	594 incident stroke cases and 594 controls of 51,529 men and 121,700 women		Case-control study	8.3 years	Plasma and erythrocyte fatty acids by gas-liquid chromatography	Matched by age, sex, race, and smoking and further adjusted for alcohol, family history of diabetes, parental history of MI, menopausal status in the NHS; postmenopausal hormone use in the NHS; consumption of fish, processed meats, unprocessed meats, fruit, vegetables, whole grains, coffee, sugar-sweetened beverages, glycemic load, dietary calcium, PUFA, and total energy; plasma total 16:0, 18:0 and .trans 18:1 and 18:2.	Pooled RR of total stroke were 0.85 (0.54–1.33) for plasma 15:0, 0.99 (0.67–1.49) for 17:0, 0.89 (0.55–1.45) for trans 16:1 *n*-7, and 1.05 (0.62–1.78) for 14:0. Results were similar for ischemic and hemorrhagic stroke subtypes.

Wiberg et al. (2006)		2313 middle-aged men	50 years	Prospective community based study.	32 years	Serum cholesterol ester fatty acids concentrations	Use of antihypertensive, antidiabetic, and lipid-lowering drugs, hypertension, diabetes, the metabolic syndrome, serum cholesterol, smoking, atrial fibrillation, cardiovascular disease, smoking and physical activity.	HR for brain infarction with higher palmitic (16:0), palmitoleic (16:1), and oleic acid (18:1) in cholesterol esters were 1.14 (01.01–1.29), 1.19 (1.05–1.34) and 1.23 (1.09–1.39), respectively. Whereas that of linoleic acid (18:2 *n*-6) was 0.81 (0.72–0.92).
Iso et al. (2002)		197 incident stroke and 197 control Japanese subjects	40–85 years	Nested case-control.	From 1984 and 1993 to end of 1998	Serum Linoleic Acid (LA) and other FA (SFA, MUFA and PUFA).	Matched by sex, age, community, year of serum storage, and fasting status and further adjusted for BMI, smoking status, ethanol intake, hypertension status, serum total cholesterol levels, and log-transformed triglyceride levels and serum glucose and for selected serum fatty acids.	The multivariate OR associated with a 1-SD increase in LA were 0.72 (0.59–0.89) for total stroke, 0.66 (0.49–0.88) for ischemic stroke, 0.63 (0.46–0.88) for lacunar infarction, and 0.81 (0.59–1.12) for hemorrhagic stroke. The respective odds ratios for 1-SD increase in SFA were 1.13 (1.05–1.65), 1.35 (1.01–1.79), 1.44 (1.03–2.01), and 1.21 (0.82–1.80).
Yaemsiri et al. (2013)	The Women's Health Initiative Observational Study cohort (WHI).	964 matched pairs of postmenopausal women	50–79 years	Nested case-control	Between 1993 and 2003.	Serum fatty acids	Matched by age, race, and length of follow-up and further adjusted for BMI smoking status, diabetes, aspirin use, blood pressure and blood cholesterol	The OR and 99.9% CI of ischemic, evident for lacunar but not cardiometabolic stroke, associated with a 1-SD increment in serum FA concentration were 1.38 (1.05–1.83) for linoelaidic acid, 1.27 (1.06–1.51) for palmitic acid, 1.20 (1.01–1.43) for oleic acid, 0.72 (0.59–0.87) for docosapentaenoic acid, 0.72 (0.59–0.87) for docosahexaenoic acid and 0.81 (0.67–0.98) for arachidonic acid.
Yamagishi et al. (2013b)	The Atherosclerosis Risk in Communities (ARIC) Study	3870 white US (1859 men and 2011 women)	45–64 years	Prospective study.	19.9–22.1 years	Plasma cholesterol ester (CE) and phospholipid (PL) fatty acids	Age and sex, smoking status, cigarette-years and alcohol intake	Age and sex-adjusted HR for ischemic stroke were 1.93 (1.23–3.04, *P* for trend = 0.01) for CE fraction, and 1.64 (1.05–2.57, *P* for trend = 0.03) for PL fraction of SFA. Similarly, those of palmitoleic acid (MUFA) were 1.86 (1.20–2.87, *P* for trend = 0.003) for CE fraction, and 1.52 (0.99–2.34, *P* for trend = 0.005) for PL fraction. Contrary were those for LA (*n*-6 PUFA), HR were 0.64 (0.43–0.97, *P* for trend = 0.13) for CE fraction and 0.69 (0.45–1.05, *P* for trend = 0.24) for PL fraction. No associations of ω-3 and ω-6 polyunsaturated fatty acids with ischemic stroke

(*Continued*)

Table 2 (Continued)

Published year/ ref no	*Study/cohort name*	*Sample size/Study population*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/outcome*	*Adjusted variables*	*Results*
Simon et al. (1995)	The Multiple Risk Factor Intervention Trial (MRFIT)	96 men with incident stroke and 96 control subjects	35–57 years	Nested case-control	6.9 years	Serum fatty acids	Matched by age, clinical center, treatment group, and date of randomization and further adjusted for plasma cholesterol level, diastolic blood pressure, and cigarette smoking	1 SD increase in ALA was associated with 37% decrease in the risk of stroke ($P < .05$).
Chowdhury et al. (2012)		794,000 subjects of 26 prospective cohort studies and 12 RCT published until September 2012.	Meta-analysis		Fish consumption and ώ 3 fatty acids.	The pooled RR for cerebrovascular disease in the highest versus lowest tertiles of dietary ώ 3 FA and serum ώ 3 FA concentrations were 0.1.04 (0.90–1.20) and 1.04 (0.89–1.08). In RCT, the RR for cerebrovascular disease in the ώ 3 FA supplement compared with the control group in primary prevention trials was 0.98 (0.89–1.08) and in secondary prevention trials was 1.17 (0.99–1.38).		
Wang et al. (2006)		347,747 subjects Primary CVD prevention reported in 1 RCT, in 25 prospective cohort studies, and for secondary CVD prevention reported in 14 RCT, in 1 prospective cohort study.	Systematic review		Fish or *n*-3 fatty acids intakes.	Trials: fish oil significantly reduced all-cause mortality, myocardial infarction, cardiac and sudden death, and stroke, with benefits of fish oil stronger in secondary than in primary prevention. Cohort studies: fish consumption was associated with lower rates of all-cause mortality and adverse cardiac outcomes. The effects on stroke were inconsistent. *n*-3 FAs from fish or fish-oil supplements, but not of-linolenic acid, reduced the rates of all-cause mortality, cardiac and sudden death, and possibly stroke.		

Table 3 Associations of dietary fat intake and serum fatty acids levels with risk of coronary heart disease

Published year/ ref no	*Study/cohort name*	*Sample size/study population*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/ outcome*	*Adjusted variables*	*Results*
Oomen et al. (2001)	The Zutphen elderly study	667 men	64–84 years	Prospective cohort	10 years	Trans fat intake via dietary survey	Age, intake of energy, BMI, smoking, alcohol intake, use of vitamin supplements, intake of SFA, MUFA, PUFA and cholesterol and for fibre.	Positive association; RR for a difference of 2% of E in trans fatty acid intake at baseline was 1.28 (95%CI 1.01–1.61) with a RR of 1.33 (95%CI 0.96–1.86) for fatal CHD. RR in highest tertile ($\geq$4.86%E) versus lowest tertile ($<$3.11%E) of trans fat intake was 2.00 (1.07–3.75; *P*-trend = 0.03).
Leosdottir et al. (2007)	The Malmö Diet and Cancer Study	28,098 (61% women)	Middle aged	Prospective cohort	8.4 years	Intakes of fat via FFQ	Age, smoking habits, alcohol consumption, socio-economic status, marital status, physical activity, BMI, fibre intake, blood pressure, total fat intake for the ratio between unsaturated- and saturated fats.	HRs of acute CHD in the highest quartile of intakes among men were 0.77 for total fat, 0.81 for SFA, 1.06 for MUFA and 1.16 for PUFA and among women were 0.99, 1.02, 0.87, and 1.20, respectively. No significant associations in multivariable model
Yamagishi et al. (2013a)	The Japan Public Health Center-based prospective Study	38,084 Japanese men and 43,847 women	45–74	Prospective cohort	11.1 years	Dietary SFAs via FFQ	Age; sex; energy intake; cohort; cigarette smoking status; alcohol intake; body mass index; sports at leisure time; walking and standing time; perceived mental stress; energy-adjusted dietary intakes of carbohydrate, protein, cholesterol, vegetables, fruit, and calcium.	SFAs intake was positively associated with myocardial Infarction; HR 1.39 (0.93–2.08), trend *P* = 0.046.
Yamagishi et al. (2010)	The Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC) Study	58,453 Japanese men and women	40–79 years	Prospective cohort	14.1 years	Intakes of SFA via FFQ	Age, sex, a history of hypertension and diabetes, smoking status, alcohol consumption, BMI, mental stress, walking, sports, educational level, and dietary intakes of total energy, cholesterol, *n*-3 and *n*-6 polyunsaturated fatty acids, vegetables, and fruits	RR for CHD and myocardial infarction mortality were 0.93 (0.65–1.35; *P*-trend = 0.86) and 0.85 (0.56–1.29; *P*-trend = 0.40), respectively
Laaksonen et al. (2005)	The Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study	1551 men	50–79 years	Prospective cohort	15 years	dietary linoleic and total polyunsaturated fatty acid (PUFA) intake via 4-d DR	Age, year of examination, smoking, alcohol intake, adult socioeconomic status, vigorous physical activity, plasma lipid-standardized αtocopherol levels, plasma ascorbic acid level, and dietary total energy, energy-adjusted SFA and fiber intakes, LDL-cholesterol levels, fasting concentrations of insulin and nonesterified fatty acids and BMI.	HR of CVD death were 0.16 (0.23–0.91, *P* = 0.03) for LA, 0.63 (0.33–1.21, *P* = 0.18) for ALA and 0.45 (0.23–0.90, *P* = 0.02) for dietary PUFA intake.

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Table 3 (Continued)

Published year/ ref no	*Study/cohort name*	*Sample size/study population*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/ outcome*	*Adjusted variables*	*Results*
Yamagishi et al. (2008a)	The Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC) Study	57,972 Japanese men and women	40–79 years	Prospective cohort	12.7 years	Intakes of fish and *n*-3 PUFA via FFQ	Age, sex, a history of hypertension and diabetes, smoking status, alcohol consumption, BMI, mental stress, walking, sports, educational level, and dietary intakes of total energy, cholesterol, and *n*-6 PUFA vegetables, and fruits	RR for CHD and myocardial infarction mortality were 0.86 (0.62–1.19; *P*-trend = 0.41) and 0.77 (0.53–1.10; *P*-trend = 0.22), respectively in highest versus lowest quintiles of fish intake, and were 0.95 (0.62–1.43; *P*-trend = 0.58) and 0.75 (0.47–1.19; *P*-trend = 0.14), respectively in highest versus lowest quintiles of *n*-3 PUFA intake.
Siri-Tarino et al. (2010)		347,747 subjects of 21 prospective cohort studies published until September 2009.		Meta-analysis	5–23 years	Saturated fat intake		The pooled relative risk estimates that compared extreme quantiles of saturated fat intake were 1.07 (95%CI: 0.96, 1.19; *P* = 0.22) for CHD, 0.81 (95%CI: 0.62, 1.05; *P* = 0.11) for stroke, and 1.00 (95% CI: 0.89, 1.11; *P* = 0.95) for CVD. None was significant
de Souza et al. (2015)		347,747 subjects of prospective cohort, case-control, nested case-control, or case-cohort studies published until May 2015.	Meta-analysis		SFA and trans fat intakes	With SFA, RR were 0.97 (0.84–1.12) for CVD mortality, 1.15 (0.97–1.36) CHD mortality, 1.06 (0.95–1.17) incident total CHD, 1.02 (0.90–1.15) incident ischemic stroke, and 0.95 (0.88–1.03) for type 2 diabetes. With total trans fat intake RR were (1.28, 1.09–1.50) for CHD mortality, and 1.21 (1.10–1.33) for total incident CHD but were 1.07 (0.88–1.28) for ischemic stroke and 1.10 (0.95–1.27) for type 2 diabetes	
Schwingshackl and Hoffmann (2014)		32 cohort studies (42 reports) of 841,211 subjects	A systematic review and meta-analysis Literature until June 2nd, 2014		MUFA (of both plant and animal origin), olive oil, oleic acid, and MUFA: SFA ratio	High MUFA was not associated CHD; a pooled RR of 0.96, (0.90–1.01, *P* = 0.13).		
de Oliveira Otto et al. (2013)	The Multi-Ethnic Study of Atherosclerosis (MESA)	2837 US adults	45–84 years	Prospective cohort	10 years	Dairy phospholipid fatty acids including 15:0, 14:0, and trans-16:1*n*7	Age, sex, race/ethnicity, field center, education, cigarette smoking, alcohol, physical activity, intakes of whole-fat dairy, processed and unprocessed meat, total energy, fiber, and fruits and vegetables, and mutually for each dairy fat biomarker (14:0, 15:0, and trans-16:1*n*7).	Each SD-unit of 15:0 was associated with 26% lower CHD risk; RR 0.74 (0.60–0.92).
Esrey et al. (1996))	The Lipid Research Clinics Prevalence Follow-Up Study (LRC)	4546 North American men and women	≥30 years	Prospective cohort	12 years	%E of total fat, SFA, MUFA, PUFA via 24-h DR.	Abstract. No data on confounder full text needed	Among 30–59 year olds RR of CHD mortality with increasing %E intake were 1.04 (1.01–1.08) for total fat, 1.11 (1.04–1.18) for SFA, 1.08 (1.01–1.16) for MUFA and 0.96 (0.94–0.99) for carbohydrate. None of the dietary components were significantly associated with CHD mortality among those aged 60–79 years.

McGee et al. (1984	The Honolulu Heart Program	8000 men of Japanese ancestry	≥30 years	Prospective cohort	10years	%E from macronutrients.	Age, systolic blood pressure, serum cholesterol, cigarettes smoked per day, and physical activity index, carbohydrates, vegetable protein, %E sFA, and %E PUFA	Men who developed CHD also had a higher mean intake of %E from protein, total fat, SFA and PUFA than men who remained free of coronary heart disease. The associations with %E SFA and %E PUFA lost significance in multivariate model.
Kushi et al. (1985)	The Honolulu Heart Program	1001 men of Japanese ancestry	≥30 years	Prospective cohort	20 years	Key and modified Hegsted dietary scores.	Abstract. No data on confounder full text needed	A higher Keys score (a high score indicates a high intake of SFA and cholesterol and a relatively low intake of PUFA) associated with an increased risk of CHD; RR = 1.60.
Shekelle et al. (1981)	The Western Electric study	1900 men	Middle-aged	Prospective cohort	20years	Serum cholesterol and SFA, PUFA intake via Key and modified Hegsted dietary scores.	Abstract. No data on confounder full text needed	Positive association between diet score and serum cholesterol concentration, and a positive association prospectively between mean base-line diet score and the 20-year risk of death from CHD
Ascherio et al. (1996)	Framingham Heart Study	43,757 men	40–75 years.	Prospective cohort	6 years	Intakes of total fat, SFA, cholesterol, LA, ALA and trans fat via FFQ.	Age; BMI; smoking habits; alcohol consumption; physical activity; history of hypertension or high blood cholesterol; family history of myocardial infarction before age 60; profession, fibre intake and for energy.	R for fatal CHD were 2.21 (1.38–3.54) and 1.78 (1.11–2.84) in the highest versus lowest quintile of SFA and trans fat intake. Adjustment for fiber intake attenuated the association greatly. RR for MI for a 1% increase in energy from ALA was 0.41 (0.21–0.80).
Hu et al. (1997)	Nurse Health Study	80,082 women	34–59 years	Prospective cohort	14 years	Intakes of total fat, SFA, MUFA, PUFA and trans via FFQ.	Age; time period; BMMI; cigarette smoking, menopausal status, parental history of myocardial infarction before 65 years of age; multivitamin use; vitamin E supplement use; alcohol consumption, history of hypertension; aspirin use, vigorous exercise, percentage of energy from protein; total energy intake and dietary cholesterol.	Compared with equivalent %E from carbohydrates, the RR for CHD were1.41 (0.97–1.41) for 5%E SFA, 1.93 (1.43–2.61) for 2%E trans fat, 0.81 (0.65–1.00) for 5%E MUFA, 0.62 (0.46–0.85) for 5%E PUFA and 1.02 (0.97–1.07) for 5%E total fat. Replacement of 5% E from saturated or 2% E from trans with equivalent %E from unsaturated fats was associated with 42% and 53% reduction in risk of CHD, respectively.
Hu et al. (1999)	Nurse Health Study	80,082 women	34–59 years	Prospective cohort	14 years	Intakes of short, medium and long chain SFA and PUFA/SFA ratio via FFQ.	Age; time period; BMMI; cigarette smoking, menopausal status, parental history of myocardial infarction before 65 years of age; multivitamin use; vitamin E supplement use; alcohol consumption,	Intakes of longer-chain SFA (12:0–18:0) but not short- to medium-chain SFA (4:0–10:0) were associated with the risk of CHD. The multivariate RR for a 1%E increase from stearic acid was 1.19 (95%

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Table 3 (Continued)

Published year/ ref no	*Study/cohort name*	*Sample size/study population*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/ outcome*	*Adjusted variables*	*Results*
							history of hypertension; aspirin use, vigorous exercise, percentage of energy from protein; total energy intake and dietary cholesterol.	CI: 1.02, 1.37). The highest versus lowest deciles of PUFA/SFA ratio had a RR of 0.58 (0.41, 0.83; P for trend <0.0001).
Howard et al. (2006)	the Women's Health Initiative Randomized Controlled Dietary Modification Trial	48,835 postmenopausal women	50–79 years	Randomized controlled trial	8.1 years	Reduction of total fat intake from $\geq$32%E to $<$20% E		The dietary intervention had no significant effects; HR were 0.97 (0.90–1.06) for incident CHD; 1.02 (0.90–1.15) stroke and 0.98 (0.92–1.05) CVD. Trends toward greater reductions in CHD risk were observed in those with lowest intakes of SFA or trans fat; HR were 0.82 (0.67–0.99) and 0.84 (0.69–1.02), respectively.
Kromhout et al. (1995)	16 cohorts in seven countries.	12,763 men	middle-aged	case-control study.	25 years	Dietary SFA and trans fat via a FFQ.	Abstract. No data on confounder full text needed	Strong positive associations between CHD death and intakes of the four major SFAs, lauric, myristic, palmitic, and stearic acid ($r>0.8$, $P<0.001$); the trans fatty acid elaidic acid ($r=0.78$, $P<0.001$); and dietary cholesterol ($r=0.55$, $P<0.05$).
Jakobsen et al. (2009)		344,696 persons	$\geq$35 years	pooled analysis of 11 American and European cohort studies	4–10 years	Intakes of SFA, MUFA PUFA and carbohydrates via FFQ.	% E for intakes of MUFA, PUFA, trans fat, protein, and age, baseline year, total energy, smoking,BMI, physical activity, history of hypertension, alcohol intake, intakes of fiber and cholesterol.	For a 5% E lower from SFA, the HR for higher % E from PUFA was 0.87 (0.77, 0.97) for incident CHD and 0.74 (0.61, 0.89) for coronary deaths. For a 5% E lower from SFA, the HR for higher % E from carbohydrate was 1.07 (1.01, 1.14) for incident CHD and 0.96 (0.82, 1.13) for coronary deaths. MUFA intake was not associated with CHD. No effect modification by sex or age was found.
Oh et al. (2005)	Nurse Health Study	78,778 women	35–59 years	Prospective cohort	20 years	Intakes of total fat, SFA, MUFA PUFA and trans fat via FFQ.	Age, BMI, smoking, alcohol, intake, parental history of myocardial infarction, history of hypertension, menopausal status and hormone use, aspirin use, multivitamin use, vitamin E supplement use, physical activity, and energy, protein, cholesterol intake and intakes of SFA, MUFA, PUFA, and trans fat; LA; marine n-3fatty acids; cereal fiber; and fruits and vegetables.	RR for the highest versus. the lowest quintile were 0.75 (0.60, 0.92; $P=0.004$) for LA and 1.33 (1.07, 1.66; $P=0.01$) for trans fat. Total fat, SFA and MUFA intakes were not associated with risk of CHD.

Pietinen et al. (1997)	Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ABTC)	21,930 smoking men	50–69 years	Prospective cohort	6.1 years	Intakes of SFA, MUFA, PUFA, trans fat, cholesterol, LA, ALA and oleic acid via a FFQ.	Age, treatment group, smoking, BMI, blood pressure, intakes of energy, alcohol, and fiber, education, and physical activity.	RR of coronary death were 1.39 (1.09–1.78) for trans fat and 1.30 (1.01–1.67) for ώ-3 fatty acids. No associations between intakes of SFA, MUFA, LA, ALA or dietary cholesterol and the risk of coronary deaths. For major coronary events (first non-fatal MI), similar bur weaker associations were found.
Ascherio et al. (1994)		239 MI patients and 282 controls	<76 years	Case-control study		Fish consumption and ώ 3 fatty acids	Matched for age, sex and resident town and adjusted for smoking, history of hypertension, BMI, alcohol intake, family history of ischemic heart disease, and physical activity, intake of saturated fat, monounsaturated fat, linoleic acid, and cholesterol.	They reported as RR, I think it should be OR for highest versus lowest quintile of trans fat = 2.44; (1.42, 4.19; for trend $P < .0001$) for total subjects, 2.17 (1.11, 4.24; for trend $P = .001$) for men and 3.65 (0.90, 14.70; for trend $P = .02$) for women.
Dolecek (1992)	the Multiple Risk Factor Intervention Trial (MRFIT)	12,866 men	middle-aged	An intervention group and usual care group	10.5 years	Dietary PUFA measured by multiple DR.	Abstract. No data on confounder full text needed	No significant associations with mortality were detected for linoleic acid (18:2*n*-6), the predominant dietary PUFA.
Iso et al. (2006)	The Japan Public Health Center based Study (JPHC)	41,578 Japanese men and women	40–59 years	Prospective cohort	10 years	Fish and *n*-3 PUFA intakes via a FFQ.	Age, sex; smoking status; alcohol intake, BMI; history of hypertension or diabetes, medication use for hypercholesterolemia, education level, sports at leisure time, quintiles of dietary intake of fruits, vegetables, SFA, MUFA, n6 PUFA, cholesterol, and total energy; and public health area.	HR in the highest (180 g/d) versus lowest (23 g/d) quintiles of fish intake were 0.63 (0.38–1.04) for total CHD, 0.44 (0.24–0.81) for definite MI, and 1.14 (0.36–3.63) for sudden cardiac death. Mainly nonfatal coronary events (HR = 0.43 [0.23–0.81]) but not for fatal coronary events (HR = 1.08 [0.42–2.76]). Similar association with *n*-3 FA intake; HR were 0.35 (0.18–0.66) for definite MI and 0.33 (0.17–0.63) for nonfatal coronary events.
Li et al. (2015)	The Nurse Health Study	84,628 women	30–55 years	Prospective Cohort	24–30 years	Intakes of SFA, MUFA PUFA and carbohydrates via multiple FFQ.	Energy intake, the energy contribution from protein, cholesterol intake, alcohol intake, smoking status, BMI, physical activity, use of vitamins and aspirin, family history of MI and diabetes, and presence of baseline hypercholesterolemia and hypertension, dietary intake of fruits and vegetables, the percentage of energy from all fat types simultaneously.	HR was 0.80 (0.73–0.88; *P*-trend <0.0001) for high PUFA. Intake. Replacing 5%E from SFA with equivalent %E from PUFAs, MUFA, or carbohydrates from whole grains was associated with a 25%, 15%, and 9% lower risk of CHD, respectively Replacing SFA with carbohydrates from refined starches/added sugars was not significantly associated with CHD risk ($p > 0.10$).

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Table 3 (Continued)

Published year/ ref no	*Study/cohort name*	*Sample size/study population*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/ outcome*	*Adjusted variables*	*Results*
Mozaffarian et al. (2010)		13,614 subjects of 54 RCT published until June 2009.		A meta-analysis		High intakes of PUFA as a replacement of SFA for at least 1 year.	The overall pooled risk reduction was 19%; RR = 0.81 (0.70–0.95, $P = 0.008$) which corresponds to 10% reduced CHD risk; RR = 0.90 (0.83–0.97) for each 5% E of increased PUFA	
Farvid et al. (2014)		310,602 individuals of 13 prospective cohort studies	>19 years	Systematic review and meta-analysis Literature until June, 2013		Intake of linoleic acid (LA)		A pooled RR of 0.85 (0.78–0.92, $P = 0.004$) for CHD events and 0.79 (0.71–0.89, $P = 0.001$) for CHD deaths.
Chowdhury et al. (2014)		32 observational studies (530,525 participants) of fatty acids from dietary intake; 17 observational studies (25,721 participants) of fatty acid biomarkers; and 27 randomized, controlled trials (103,052 participants) of fatty acid supplementation.					RR in highest versus lowest tertiles of intakes for CHD were1.02 (0.97–1.07) for SFA, 0.99 (0.89–1.09) for MUFA, 0.93 (0.84–1.02) for *n*-3 PUFA, 1.01 (0.96 –1.07) for *n*-6PUFA, and 1.16 (1.06–1.27) for trans fatty acids. For circulating fatty acids were 1.06 (0.86–1.30), 1.06 (0.97–1.17), 0.84 (0.63–1.11), 0.94 (0.84–1.06), and 1.05 (0.76–1.44), respectively. For Supplementations were 0.97 (0.69–1.36) for α-linolenic, 0.94 (0.86–1.03) for *n*-3 PUFA, and 0.89 (0.71–1.12) for *n*-6 PUFA.	
Sun et al. (2007b)	The Nurses' Health Study	166 cases and 327 controls from 32,826 participants	30–55 years	Case-control study.	6 years	Plasma and erythrocyte dairy fatty acids Trans fatty acids in plasma or erythrocyte	Matched for age at blood draw, smoking status, fasting status and time of blood draw, and adjusted further for BMI, postmenopausal status, postmenopausal hormone use, physical activity, alcohol intake, aspirin intake, parental history of MI before age 65y, history of hypertension, diabetes and hypercholesterolemia, and linoleic acid and total	RR in the highest versus lowest tertile of 15:0 concentrations in plasma was 2.36 (1.16–4.78; *P*-trend = 0.03).
Sun et al. (2007c)	The Nurses' Health Study	166 cases and 327 controls from 32,826 participants	30–55 years	Case-control study.	6 years	Plasma and erythrocyte fatty acids Trans fatty acids in plasma or erythrocyte	Matched for age at blood draw, smoking status, fasting status and time of blood draw, and adjusted further for BMI, postmenopausal status, postmenopausal hormone use, physical activity, alcohol intake, aspirin intake, parental history of MI before age 65y, history of hypertension, diabetes and hypercholesterolemia, and linoleic acid and total	RR in the highest versus lowest quartiles of erythrocyte trans fatty acids concentrations was 3.3 (1.50–7.20; *P*-trend = < 0.01).
Aro et al. (1995)		671 men with acute MI and 717 controls	≤70 years	case-control study		Adipose tissue FA, mainly trans isomer (C18:1)	Age, centre, smoking, and BMI, socioeconomic status	The risk of acute MI did not differ significantly across quartiles of adipose C18:1 trans FA: OR in the highest versus lowest quartile = 0.97 (0.56–1.67).

Wang et al. (2003)	The Atherosclerosis Risk in Communities (ARIC) Study	3591 participants	45–64 years	Prospective cohort	10.7 years	Plasma cholesterol ester (CE) and phospholipid (PL) fatty acid composition	Age, sex, smoking status, alcohol intake, sports index, special diet, dietary cholesterol, percentage of energy intake from fat	RR of CHD for the highest versus lowest quintile were 1.42 (*P*-trend: 0.026) for CE SFAs, 1.31 in CE and 1.44 in PL for dihomo-γ-linolenic acid (*P*-trend: 0.05 and 0.017, respectively), 0.59 in CE and 0.65 in PL for arachidonic acid (p: 0.016 and 0.024, respectively) No association with total MUFAs, total PUFAs, *n*-3 PUFAs, or *n*-6 PUFAs.
Wu et al. (2013)	The health professionals follow-up study	459 CHD cases and 879 matched controls	40–75 years	Case-control study	1993–1995–2008	Lipophilic index (LI) by summing the products between FA levels and melting point of each FA	Matched for age and smoking status, and further adjusted for alcohol intake, physical activity, family history of MI, history of hypertension, history of hypercholesterolemia, aspirin use, cholesterol-lowering medication use, antihypertensive medication use, alternative healthy eating index and BMI.	RR in highest versus lowest quintiles was 1.61 (1.03–2.53; *P*–trend = 0.04)
Harris et al. (2007)		25 prospective cohort and case control studies published from 1996 until 2005.	Meta-analysis		Tissue fatty acids composition	Lower LA content was associated with increased risk for non-fatal events. Tissue proportions of DHA (with or without EPA) were significantly reduced in the cases. Both LA and ALA tended ($P = 0.02$) to be depressed in cases In non-fatal endpoint studies, only LA was significantly associated with case status. While in In the fatal endpoint studies, reduced EPA + DHA and DHA alone were both significantly associated with case status.	
Mozaffarian and Rimm (2006)	Systematic review and meta-analysis published through April 2006				Fish intake	Modest consumption of fish (eg, 1–2 servings/wk), higher in the *n*-3 PUFA, and EPA and DHA, associated with reduced risk of coronary death by 36% (20%–50%; $P < .001$).		
Whelton et al. (2004)		19 observational studies (14 cohort and 5 case-control)	Meta-analysis of data published before May 2003		consumption of fish	RR of total and fatal CHD in fish consumers versus non consumers were 0.86 (0.81–0.92; $p < 0.005$) and 83 (0.76–0.90; $p < 0.005$), respectively		
He et al. (2004)		222,364 individuals of 11 studies	Meta-analysis of data published before May 2003	11.8 years	consumption of fish	Compared with nonconsumers, the pooled RR of CHD mortality were 0.89 (0.79–1.01) for fish intake 1–3 times per month, 0.85 (0.76–0.96) for once per week, 0.77 (0.66–0.89) for 2–4 times per week, and 0.62 (0.46–0.82) for 5 or more times per week.		

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Table 3 (Continued)

Published year/ ref no	*Study/cohort name*	*Sample size/study population*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/ outcome*	*Adjusted variables*	*Results*
Eshak et al. (2014)	The Japan Public Health Center-based prospective Study	72,012 Japanese men and women	45–74 years	Prospective cohort	15 years	Smoking status and seafood/ fish intakes via 2 FFQ 5 y apart.	Age; sex; public health center; alcohol intake, BMI; histories of hypertension and diabetes; medication use for hypercholesterolemia; sports during leisure time; and quintiles of dietary intake of fruits, vegetables, SFA, MUFA, *n*-6 PUFA, cholesterol, and total energy.	Compared with never smokers, HR in light and moderate smokers were 2.39 (1.60, 3.56) and 2.74 (1.90, 3.95) among low seafood/fish eaters; whereas among high seafood/fish eaters were 1.13 (0.64, 1.99) and 1.29 (0.95, 2.04), respectively. Compared with heavy smokers with a low seafood/fish intake, light smokers with a high seafood/fish intake had substantially reduced risk of CHD; HR = 0.57 (0.32, 0.98).
Chowdhury et al. (2012)	The Caerphilly cohort	347,747 subjects		Systematic review	18 years	Fish or *n*-3 fatty acids intakes.	Trials: fish oil significantly reduced all-cause mortality, myocardial infarction, cardiac and sudden death, and stroke, with benefits of fish oil stronger in secondary than in primary prevention. Primary CVD prevention reported in 1 RCT, in 25 prospective cohort studies, and for secondary CVD prevention reported in 14 RCT, in 1 prospective cohort study. Cohort studies: fish consumption was associated with lower rates of all-cause mortality and adverse cardiac outcomes. The effects on stroke were inconsistent. *n*-3 FAs from fish or fish-oil supplements, but not of-linolenic acid, reduced the rates of all-cause mortality, cardiac and sudden death, and possibly stroke.	

Table 4 Associations of dietary fat intake and serum fatty acids levels with risk of other and total cardiovascular disease

Published year/ ref no	*Study/cohort name*	*Sample size/study population*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/outcome*	*Adjusted variables*	*Results*
Tanasescu et al. (2004)	The Nurses' Health Study	5672 women with type 2 diabetes	30–55 years	Prospective cohort	1980–1998	Intakes of fat via FFQ	Age, smoking, postmenopausal hormone use, parental history of myocardial infarction, alcohol intake, moderate/ vigorous activities, BMI, total caloric intake, protein intake, fiber intake, multivitamin use, vitamin E supplement use, and medication use.	RR of CVD were 1.37 (1.12–1.68; $P=0.003$) for an increase of 200 mg cholesterol/1000 kcal and 1.29 (1.02–1.63; $P=0.04$) for 5% of energy intake from saturated fat
Leosdottir et al. (2007)	The Malmö Diet and Cancer Study	28,098 (61% women)	Middle aged	Prospective cohort	8.4 years	Intakes of fat via FFQ	Age, smoking habits, alcohol consumption, socio-economic status, marital status, physical activity, BMI, fibre intake, blood pressure, total fat intake for the ratio between unsaturated- and saturated fats.	No significant associations in multivariable model between total fat, SFA, MUFA and PUFA with CVD events.
Yamagishi et al. (2013a)	The Japan Public Health Center-based prospective Study	38,084 Japanese men and 43,847 women	45–74	Prospective cohort	11.1 years	Dietary SFAs via FFQ	Age; sex; energy intake; cohort; cigarette smoking status; alcohol intake; body mass index; sports at leisure time; walking and standing time; perceived mental stress; energy-adjusted dietary intakes of carbohydrate, protein, cholesterol, vegetables, fruit, and calcium.	HR for total CVD in the highest SFA intake was 0.82 (0.69–0.96; $P=0.01$).
Yamagishi et al. (2010)	the Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC) Study	58,453 Japanese men and women	40–79 years	Prospective cohort	14.1 years	Intakes of SFA via FFQ	Age, sex, a history of hypertension and diabetes, smoking status, alcohol consumption, BMI, mental stress, walking, sports, educational level, and dietary intakes of total energy, cholesterol, *n*-3 and *n*-6 PUFA, vegetables, and fruits	RR for heart failure, cardiac arrest and total CVD mortality were 0.99 (0.64–1.52; *P*-trend = 0.83), 0.50 (0.23–1.10; *P*-trend = 0.11) and 0.82 (0.69–0.97; *P*-trend = 0.05), respectively
Guasch-Ferré et al. (2014)	The PREvención con DIeta MEDiterránea (PREDIMED) study	7216 men and women	55–80 years	Prospective cohort	4.8 years	Olive oil consumption via FFQ	Age, sex, intervention group, BMI, smoking status, alcohol intake, educational level, leisure time physical activity, prevalence of diabetes, hypertension, and hypercholesterolemia, use of antihypertensive medication, use of statins. And Mediterranean diet adherence	RR in highest versus lowest tertiles of total olive intakes were 0.65 (0.47–0.89) for incident CVD and 0.52 (0.29–0.93) for total CVD mortality

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Table 4 (Continued)

Published year/ ref no	*Study/cohort name*	*Sample size/study population*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/outcome*	*Adjusted variables*	*Results*
Laaksonen et al. (2005)	The Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study	1551 men	50–79 years	Prospective cohort	15 years	Dietary linoleic and total polyunsaturated fatty acid (PUFA) intake via 4-d DR	Age, year of examination, smoking, alcohol intake, adult socioeconomic status, vigorous physical activity, plasma lipid-standardized αtocopherol levels, plasma ascorbic acid level, and dietary total energy, energy-adjusted SFA and fiber intakes, LDL-cholesterol levels, fasting concentrations of insulin and nonesterified fatty acids and BMI.	HR of CVD death were 0.16 (0.23–0.91, $P=0.03$) for LA, 0.63 (0.33–1.21, $P=0.18$) for ALA and 0.45 (0.23–0.90, $P=0.02$) for dietary PUFA intake.
Yamagishi et al. (2008a)	The Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC) Study	57,972 Japanese men and women	40–79 years	Prospective cohort	12.7 years	Intakes of fish and *n*-3 PUFA via FFQ	Age, sex, a history of hypertension and diabetes, smoking status, alcohol consumption, BMI, mental stress, walking, sports, educational level, and dietary intakes of total energy, cholesterol, and *n*-6 PUFA vegetables, and fruits	RR for heart failure, cardiac arrest and total CVD mortality were 0.76 (0.53–1.09; *P*-trend = 0.10), 0.73 (0.36–1.46; *P*-trend = 0.16) and 0.82 (0.71–0.95; *P*-trend = 0.007), respectively in highest versus lowest quintiles of fish intake, and were 0.58 (0.36–0.93; *P*-trend = 0.03) and 0.81 (0.67–0.98; *P*-trend = 0.01), respectively in highest versus lowest quintiles of *n*-3 PUFA intake.
Siri-Tarino et al. (2010)		347,747 subjects of 21 prospective cohort studies published until September 2009.		Meta-analysis	5–23 years	Saturated fat intake		The pooled relative risk estimates that compared extreme quantiles of saturated fat intake were 1.07 (95%CI: 0.96, 1.19; $P=0.22$) for CHD, 0.81 (95%CI: 0.62, 1.05; $P=0.11$) for stroke, and 1.00 (95%CI: 0.89, 1.11; $P=0.95$) for CVD. None was significant
de Souza et al. (2015)		347,747 subjects of prospective cohort, case-control, nested case-control, or case-cohort studies published until May 2015.		Meta-analysis		SFA and trans fat intakes	With SFA, RR were 0.97 (0.84–1.12) for CVD mortality, 1.15 (0.97–1.36) CHD mortality, 1.06 (0.95–1.17) incident total CHD, 1.02 (0.90–1.15) incident ischemic stroke, and 0.95 (0.88–1.03) for type 2 diabetes. With total trans fat intake RR were (1.28, 1.09–1.50) for CHD mortality, and 1.21 (1.10–1.33) for total incident CHD but were 1.07 (0.88–1.28) for ischemic stroke and 1.10 (0.95–1.27) for type 2 diabetes	
Schwingshackl and Hoffmann (2014)		32 cohort studies (42 reports) of 841,211 subjects		A systematic review and meta-analysis Literature until June 2nd, 2014		MUFA (of both plant and animal origin), olive oil, oleic acid, and MUFA:SFA ratio		A pooled RR of 0.88 (0.80–0.96, $P=0.004$) for CVD mortality and 0.91 (0.86–0.96, $P=0.001$) for CVD events.

Marik and Varon (2009)		39,044 patients of 11 Prospective, randomized, placebo-controlled clinical trials		Systematic review	15.5 years	Dietary supplements of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)	OR with *n*-3 fatty acids supplements were 0.87 (0.79–0.95, $P=0.002$) for CVD death, 0.87 (0.76–0.99, $P=0.04$) sudden cardiac and 0.92 (0.85–0.99, $P=0.02$) for nonfatal cardiovascular events.	
Woodward et al. (2011)	The Scottish Heart Health Extended Cohort Study	3944 participants	40–59 years	Prospective cohort	19.5 years	Adipose tissue fatty acids by gas chromatography.	Age, sex, total and HDL-cholesterol, systolic blood pressure, smoking, hypertensive medication use, diabetes, socio-economic status and family history.	RR for incident CVD in highest versus lowest quartiles were 1.29 (1.05–1.59; *P*-trend0.004) for adipose MUFA and 0.77 (0.63–0.94; *P*-trend–0.01) for adipose *n*-3 PUFA. No associations with adipose SFA or *n*-6 PUFA.
Warensjö et al. (2008)	The Uppsala Longitudinal Study of Adult Men (ULSAM)	1885 men	50 years	Prospective cohort	3.7 years	Dietary fat biomarkers	Total cholesterol, BMI, smoking, physical activity, and hypertension.	RR for CVD mortality with higher serum concentrations of myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1) and LA (18:2*n*-6) were 1.12 (1.02–1.23), 1.15 (1.04–1.26), 1.18 (1.07–1.30) and 0.85 (0.78–0.94), respetively
de Oliveira Otto et al. (2013)	The Multi-Ethnic Study of Atherosclerosis (MESA)	2837 US adults	45–84 years	Prospective cohort	10 years	Dairy phospholipid fatty acids including 15:0, 14:0, and trans-16:1*n*7	Age, sex, race/ethnicity, field center, education, cigarette smoking, alcohol, physical activity, intakes of whole-fat dairy, processed and unprocessed meat, total energy, fiber, and fruits and vegetables, and mutually for each dairy fat biomarker (14:0, 15:0, and trans-16:1*n*7).	Each SD-unit of 15:0 was associated with 19% lower CVD risk; RR 0.81 (0.68–0.98).
Howard et al. (2006)	The Women's Health Initiative Randomized Controlled Dietary Modification Trial	48,835 postmenopausal women	50–79 years	Randomized controlled trial	8.1 years	reduction of total fat intake from $\geq$32%E to <20% E		The dietary intervention had no significant effects; HR were 0.97 (0.90–1.06) for incident CHD; 1.02 (0.90–1.15) for stroke and 0.98 (0.92–1.05) for CVD.
Nettleton et al. (2008)	The Atherosclerosis Risk in Communities (ARIC) study	14,153 participants	45–64 years	Prospective cohort	13 years	Whole grain, Egg and dairy fat intakes via FFQ	Energy intake, age, sex, race/center, education level, physical activity level, smoking and drinking status, and prevalent disease status: cardiovascular disease, diabetes, and hypertension.	RR of heart failure with higher egg intake was 1.23 (1.08–1.41) and with high-fat dairy was 1.08 (1.01, 1.16)
Mozaffarian et al. (2005a)		4738 adults	$\geq$65 years	Prospective cohort	12 years	Intakes of baked/broiled fish, fried fish via FFQ	Age, gender, race, enrollment site, education, diabetes, BMI, prevalent CHD, prevalent stroke/transient ischemic attack, total caloric intake, intake of either fried fish or tuna/other fish, smoking, leisure-time physical activity, and intakes of SFA, fruits, vegetables, and alcohol.	RR of heart failure in the highest ($\geq$5 times/week) versus lowest (<once monthly) of tuna/other fish intake was 0.68 (0.45–1.03).

(*Continued*)

Table 4 (Continued)

Published year/ ref no	*Study/cohort name*	*Sample size/study population*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure measures/outcome*	*Adjusted variables*	*Results*
Belin et al. (2011)	The Women's Health Initiative study	84, 493 women	50–79 years	Prospective cohort	10 years	Intakes of baked/broiled fish, fried fish, and omega-3 fatty acid (eicosapentaenoic acid + docosahexaenoic acid, α-linolenic acid), and trans-fatty via FFQ	Age, ethnicity, education, physical activity, smoking, alcohol, diabetes, hypertension, atrial fibrillation, MI/ coronary artery bypass graft/ percutaneous transluminal coronary angioplasty, BMI, time-dependent MI, fiber, fruit/vegetable servings, fried fish servings, SFA intake (%), DHA + EPA (%), ALA (%), LA (%), fried food servings, and sodium intake (mg).	RR of heart failure in the highest (≥5 times/week) versus lowest (<once monthly) of Baked/broiled fish intake was 0.70 (0.51–0.95). No significant associations were found between EPA + DHA, ALA, or trans-fatty acid intake and incident HF.
Dijkstra et al. (2009)	The Rotterdam Study	5299 subjects	Mean age 68 years	Prospective cohort	11.4 years	Fish or its *n*-3 fatty acids, 0045PA plus DHA	Age, sex, total energy intake, smoking, BMI, education, and intake of alcohol, fat, saturated fat, trans-fat and meat.	RR of heart failure in the highest versus lowest quintiles of EPA plus DHA intake was 1.00 (0.73–1.36) in men and 0.75 (0.54–1.04) in women. For fish intakes ≥20 g/day, the RR was 0.96 (0.78–1.18) compared with no fish intake.
Levitan et al. (2009)	The Cohort of Swedish Men	39,367 men	45–79 years	Prospective cohort	7 years	Fatty fish and marine omega-3 fatty acids via FFQ	Age, BMI, physical activity, energy, alcohol, fibre, sodium, and red or processed meat consumption, education, family history of myocardial infarction at <60 years, cigarette smoking, marital status, self-reported history of hypertension, and high cholesterol.	RR of heart failure in the moderate (1 time/week) and the highest (≥3 times/week) versus lowest (none) of fatty fish intake was 0.76 (0.60–0.97) and 0.97 (0.61–1.55), respectively. RR in the third and highest versus lowest quintiles of *n*-3 fatty acids intake were 0.67 (0.50–0.90) and 1.00 (0.77–1.29), respectively.
Levitan et al. (2010)	The Swedish Mammography Cohort	36,234 women	48–83 years	Prospective cohort	9 years	Fatty fish and marine omega-3 fatty acids via FFQ	Age, education, BMI, physical activity, cigarette smoking, living alone, postmenopausal hormone use, total energy intake, alcohol intake, fiber intake, sodium intake, intake of red or processed meat, family history of myocardial infarction before 60 years, self-reported history of hypertension, self-reported history of high cholesterol.	RR of heart failure in the moderate (2 time/week) and the highest (≥3 times/week) versus lowest (none) of fatty fish intake was 0.70 (0.53–0.94) and 0.91 (0.0.59–1.40), respectively. RR in highest versus lowest quintiles of *n*-3 fatty acids intake was 0.75 (0.58–0.96).

Djoussé et al. (2012)	176,441 subjects from 7 prospective studies			Meta-analysis Literature until August 31, 2011		Fish and marine omega-3 fatty acids	A pooled RR of heart failure in the highest versus lowest category of fish intake was 0.85 (0.73–0.99, $P = 0.04$), corresponding value for marine *n*-3 fatty acids was 0.86 (0.74–1.00, $P = 0.05$).	
Yamagishi et al. (2008b)	The Atherosclerosis Risk in Communities (ARIC) Study	15,792 persons	45–64 years	Prospective cohort	13 years	Plasma fatty acid composition	Age, sex, BMI, SBP, antihypertensive medication use, plasma total and high-density lipoprotein (HDL)-cholesterols, prevalent diabetes, smoking status, alcohol intake, sports index, education level.	RR of heart failure was 2.71 (1.64–4.45) for myristic acid, 14:0. For women only, AA 0.38 (0.16–0.91) and DHA 0.16 (0.07–0.40) associated inversely with heart failure risk.
Wang et al. (2006)	The Caerphilly cohort	347,747 subjects Primary CVD prevention reported in 1 RCT, in 25 prospective cohort studies, and for secondary CVD prevention reported in 14 RCT, in 1 prospective cohort study.		Systematic review	18 years	Fish or *n*-3 fatty acids intakes.	Trials: fish oil significantly reduced all-cause mortality, myocardial infarction, cardiac and sudden death, and stroke, with benefits of fish oil stronger in secondary than in primary prevention. Cohort studies: fish consumption was associated with lower rates of all-cause mortality and adverse cardiac outcomes. The effects on stroke were inconsistent. *n*-3 FAs from fish or fish-oil supplements, but not of-linolenic acid, reduced the rates of all-cause mortality, cardiac and sudden death, and possibly stroke.	

For risk of ischemic stroke, some studies reported inverse associations with intakes of total fat (Gillman et al., 1997; Sauvaget et al., 2004; Seino et al., 1997), SFA (Tanasescu et al., 2004; Yamagishi et al., 2013a; Gillman et al., 1997; Seino et al., 1997), MUFA (Gillman et al., 1997), and PUFA (Iso et al., 2001a; He et al., 2002); others reported no association with intakes of total fat (Leosdottir et al., 2007; He et al., 2003), SFA (Leosdottir et al., 2007; He et al., 2003; Seino et al., 1997; Yaemsiri et al., 2012), MUFA (Leosdottir et al., 2007; He et al., 2002, 2003; Sauvaget et al., 2004), PUFA (Leosdottir et al., 2007; Yamagishi et al., 2008a; He et al., 2003; Gillman et al., 1997; Sauvaget et al., 2004; Yaemsiri et al., 2012), or trans fat (He et al., 2003), while some other studies reported positive associations with intakes of total fat (Boden-Albala et al., 2009), PUFA (Seino et al., 1997), and trans fat (Kiage et al., 2014; Yaemsiri et al., 2012).

For risk of hemorrhagic stroke (intraparenchymal hemorrhage), some studies reported inverse associations with intakes of total fat (Iso et al., 2003), SFA (Tanasescu et al., 2004; Yamagishi et al., 2013a; Iso et al., 2001b, 2003), and trans fat (Boden-Albala et al., 2009); others reported no association with intakes of total fat (He et al., 2003; Gillman et al., 1997; Iso et al., 2001b), SFA (He et al., 2003; Gillman et al., 1997), MUFA (He et al., 2003; Gillman et al., 1997; Iso et al., 2001b, 2003), PUFA (Yamagishi et al., 2008a; Iso et al., 2001a,b, 2003; He et al., 2003; Gillman et al., 1997), or trans fat (He et al., 2003), while no studies, so far, reported positive associations between intakes of dietary fat and risk of hemorrhagic stroke.

The associations of circulating biomarkers of fatty acids with risk of stroke were much easier to understand than those with dietary fatty acids intake. In one hand, the associations with hemorrhagic stroke were reported in only two studies and there were no associations with serum levels of fatty acids (Yakoob et al., 2014; Wiberg et al., 2006). On the other hand, risk of ischemic stroke associated positively with serum levels of SFA (Wiberg et al., 2006; Iso et al., 2002; Yaemsiri et al., 2013; Yamagishi et al., 2013b), MUFA (Wiberg et al., 2006; Yaemsiri et al., 2013; Yamagishi et al., 2013b), and trans fatty acids (Wiberg et al., 2006; Yaemsiri et al., 2013), but inversely associated with serum levels of *n*-3 and *n*-6 fatty acids, namely, with ALA (Simon et al., 1995), DPA and DHA (Yaemsiri et al., 2013), LA (Wiberg et al., 2006; Iso et al., 2002; Yaemsiri et al., 2013; Yamagishi et al., 2013b), and AA (Yaemsiri et al., 2013). High serum SFA, namely, stearic acid, was associated with increased risk of total stroke in one study (Simon et al., 1995); biomarkers for diary fat (C14:0, C15:0, C17:0, and trans 16:1*n*-7) were not associated with either ischemic or hemorrhagic stroke in one study (Yakoob et al., 2014), and *n*-3 and *n*-6 PUFA were not associated with risk of ischemic stroke in another study (Yamagishi et al., 2013b).

The mechanisms of associations between fat and risk of stroke seem to be affected by several factors which include the usual intake of fat and fatty acids in diet (very low intake versus usual or high intake) (Yamagishi et al., 2013a; Iso et al., 2001b, 2002, 2003), the method of calculating fat intake (gm/d versus percentage of total energy) (McGee et al., 1985), the prevalence of stroke and stroke subtypes in the population (McGee et al., 1985; Atkinson et al., 2011; Iso et al., 2001a,b, 2002, 2003; He et al., 2002, 2003; Kiage et al., 2014; Gillman et al., 1997; Sauvaget et al., 2004; Seino et al., 1997; Yaemsiri et al., 2012, 2013; Boden-Albala et al., 2009; Yakoob et al., 2014; Wiberg et al., 2006; Yamagishi et al., 2013b; Simon et al., 1995), the affected site of stroke (cortical or perforator's artery area) (Iso et al., 2001a,b, 2002; Yakoob et al., 2014; Wiberg et al., 2006; Yaemsiri et al., 2013; Yamagishi et al., 2013b), and by serum cholesterol levels (Leosdottir et al., 2007; Yamagishi et al., 2013a; McGee et al., 1985; Iso et al., 2001b, 2002, 2003; Simon et al., 1995; Atkins et al., 1993).

Dietary fat intakes and plasma fatty acids composition were also related to risk of other cardiovascular disease. SFA intake was not associated with mortality from heart failure or cardiac arrest (Tanasescu et al., 2004). Contrarily, *n*-3 PUFA supplementation was associated with decreased risk of sudden cardiac death (Marik and Varon, 2009); also, fish and *n*-3 PUFA intakes were inversely associated with risk of mortality from heart failure, but not with risk of mortality from cardiac arrest in another study (Yamagishi et al., 2008a). Incident heart failure was associated with intakes of egg and high fat diary, but not with fish intake (Nettleton et al., 2008); however, two studies have shown a decreased risk of incident heart failure with intakes of tuna and other boiled and backed fish, but not fried fish (Mozaffarian et al., 2005a; Belin et al., 2011). A high intake of fish and EPA plus DHA was associated with lower hear failure risk, especially in women (Dijkstra et al., 2009). Two Swedish studies showed that fatty fish rather than total fish intakes were associated with a decreased risk of heart failure in men (Levitan et al., 2009) and women (Levitan et al., 2010). In a meta-analysis, the pooled RR for heart failure in the highest versus lowest categories of fish and *n*-3 fatty acids intakes were 0.85 (0.73–0.99; *P*-trend = 0.04) and 0.86 (0.74–1.00; *P*-trend = 0.05), respectively (Djoussé et al., 2012). Higher plasma levels of SFA, especially myristic (14:0) acid, were associated positively with incident heart failure in men and women of the ARIC study, while higher AA (20:3, ω6) and long-chain *n*-3 PUFA, especially DHA (22:6,ω3), were associated inversely with heart failure in women but not in men (Yamagishi et al., 2008b).

Total Fat/SFA

Several studies confirmed that if total fat intakes exceeded 35% of total energy, it would be difficult to avoid high intakes of SFA. Since Keys' early findings of increased LDL-cholesterol concentrations with increasing total fat/SFA intake (Keys et al., 1957), many studies, based on the fact that high blood cholesterol is a strong risk factor of CVD (Prospective Studies Collaborations, 2007), have investigated the associations of total fat/SFA with risk of CVD (Tanasescu et al., 2004; Yamagishi et al., 2008a, 2013a; Esrey et al., 1996; McGee et al., 1984, 1985; Kushi et al., 1985; Shekelle et al., 1981; Ascherio et al., 1996; Hu et al., 1997, 1999; Howard et al., 2006; Kromhout et al., 1995; Jakobsen et al., 2009; He et al., 2003; Gillman et al., 1997; Seino et al., 1997; Iso et al., 2002, 2003; Yakoob et al., 2014; Wiberg et al., 2006; Yaemsiri et al., 2013; Yamagishi et al., 2013b), and regardless to the logic of beneficial impacts of reducing SFA intake on cardiovascular health, the evidence for a clear positive, independent association is lacking (Leosdottir et al., 2007; Siri-Tarino et al., 2010; de Souza et al., 2015; Hu et al., 1997; Howard et al., 2006; Oh et al., 2005; Pietinen

et al., 1997; Atkinson et al., 2011; He et al., 2003; Gillman et al., 1997; Seino et al., 1997; Yaemsiri et al., 2012; Iso et al., 2001b; Yakoob et al., 2014).

In men, there were no associations of total fat intakes with risk of CHD (Ascherio et al., 1996) or between total fat or specific fat subtypes and risk of stroke (Iso et al., 2002). While in women, two prospective studies (Hu et al., 1997; Oh et al., 2005) showed no association between intakes of total fat or SFA and risk of CHD; the first study (Oh et al., 2005) reported no association, while the second study (Hu et al., 1997) reported a positive association between SFA intake and risk of CHD, which appeared to be evident for only longer chain SFA (12–18 Carbon atoms), but not short to medium chain SFA (4–10 Carbon atoms) (Hu et al., 1999). As the strong positive association between total cholesterol and CHD was only observed among populations with high total cholesterol levels in the Seven Countries Study (Prospective Studies Collaborations, 2007), SFA-CHD association has been generally weak among Asian populations, given lower distribution of SFA consumption compared with Western populations. Only one study showed a positive association between SFA and CHD in Asia (Yamagishi et al., 2013a).

In some studies, total fat and SFA intakes were inversely associated with risk of ischemic (Yamagishi et al., 2010, 2013a; Gillman et al., 1997; Sauvaget et al., 2004; Seino et al., 1997) and hemorrhagic (Yamagishi et al., 2010, 2013a; Iso et al., 2001b, 2003) stroke, while other studies showed no association with either ischemic (Leosdottir et al., 2007; He et al., 2003; Yaemsiri et al., 2012) or hemorrhagic (He et al., 2003; Gillman et al., 1997; Iso et al., 2001b) stroke. Two meta-analyses of prospective cohort studies showed no significant associations of dietary total fat and SFA with an increased risk of CHD, stroke or total CVD (Siri-Tarino et al., 2010; de Souza et al., 2015). SFA intake was not associated with mortality from heart failure or cardiac arrest (Yamagishi et al., 2010), while plasma levels of SFA were positively associated with incident heart failure (Yamagishi et al., 2008b). In a randomized controlled trial of 48,835 postmenopausal women aged 50 to 79 years, a dietary intervention that reduced total fat intake and increased vegetables, fruit, and grain intakes did not reduce the risk of CHD, stroke, or total CVD in postmenopausal women (Howard et al., 2006).

MUFA

Intakes of MUFA and SFA are highly correlated in general; clinical trials with high MUFA diets showed no effect on blood pressure (Mutanen et al., 1992; Shah et al., 2007); however, a hypotensive effect was reported in one study (Ferrara et al., 2000). In a systematic review, a high MUFA diet showed favorable outcomes as regard metabolic syndrome and cardiovascular risk factors (Gillingham et al., 2011). In a meta-analysis of 60 randomized controlled trials, MUFA intake improved the metabolic profile with respect to total cholesterol, HDL-cholesterol, and triacylglycerol concentrations (Mensink et al., 2003). It is worth mentioning here that most of the favorable associations between MUFA and metabolic profile were evident when intakes of MUFA increased and SFA decreased.

Olive oil of Mediterranean diet as a surrogate of high MUFA intake was inversely associated with total CVD incidence and mortality (Guasch-Ferré et al., 2014). An inverse association was reported between MUFA intake and risk of CHD mortality in both men (Jakobsen et al., 2009) and women (Shekelle et al., 1981). Contrarily, one study reported a positive association between high MUFA intake and CHD mortality in men; however, the authors attributed that to a high correlation between MUFA and SFA in their analysis (Hegsted and Ausman, 1988). Some prospective studies could not find any associations between MUFA intake and risk of CHD (Oh et al., 2005) or risk of stroke (He et al., 2003). However, the analysis of other prospective cohort studies revealed an inverse association between dietary MUFA and CHD risk (RR = 0.81; 95%CI 0.68–0.93) (Gillingham et al., 2011). In a 14-year follow-up of women in the Nurses' Health Study, a 5% increase in energy intake from MUFA was associated with a relative risk of CHD of 0.81 (95%CI 0.65–1.00) (Hu et al., 1997). In some studies, MUFA intakes were inversely associated with risk of total (Schwingshackl and Hoffmann, 2014) and ischemic (Gillman et al., 1997) stroke, while other studies showed no association with either ischemic (Leosdottir et al., 2007; He et al., 2003; Sauvaget et al., 2004; Yaemsiri et al., 2012) or hemorrhagic (Iso et al., 2001a, b; He et al., 2003; Gillman et al., 1997) stroke. A systematic review of 32 cohort studies including 841,211 subjects showed a significant stroke risk reduction for the comparison of the top versus bottom thirds of MUFA intake (RR: 0.83, 95%CI 0.71, 0.97, $P = 0.02$) (Schwingshackl and Hoffmann, 2014).

n-3 PUFA

Fish or fish oil intake were widely used as a surrogate for *n*-3 PUFA intake in many epidemiological studies. Despite the growing evidence that dietary *n*-3 PUFA can reduce the risk of CHD, stroke, and heart failure, fish and n3 PUFA-CVD association has been considered generally nonlinear (Mozaffarian and Rimm, 2006); which explains why fish and n3 PUFA-CHD association was more evident in low-intake Western populations (Mozaffarian and Rimm, 2006; Whelton et al., 2004; He et al., 2004) than in high-intake populations like Japanese (Yamagishi et al., 2008a; Iso et al., 2006; Eshak et al., 2014). Most mortality studies (regardless in Western or Eastern populations) showed relatively weak or null associations between fish intake and risk of CHD (Yamagishi et al., 2008a; Mozaffarian and Rimm, 2006; Whelton et al., 2004; He et al., 2004). In Japanese populations, fish and *n*-3 fatty acids intakes showed nonsignificant inverse associations with CHD mortality (Yamagishi et al., 2008a) and a weak inverse association with risk of incident CHD, which was evident for nonfatal but not fatal CHD (Iso et al., 2006), and high fish intake attenuated the smoking-induced risk of nonfatal CHD (Eshak et al., 2014).

Inverse associations of fish and *n*-3 PUFA intakes with risk of stroke, especially thrombotic infarction, were reported among women of the Nurse's Health study (Iso et al., 2001a). In a meta-analysis of 26 prospective studies and 12 randomized controlled trials, fish intake and *n*-3 PUFA supplementation were associated with a modestly reduced risk of stroke (RR: 0.88, 95%CI 0.81–0.96

for ≥5 fish servings a week versus 1 serving a week) (Chowdhury et al., 2012). In another meta-analysis of 7 prospective studies, dietary intakes of fish marine *n*-3 fatty acids were associated with a decreased risk of heart failure; RR (95%CI) was 0.85 (0.73–0.99; $P=0.04$) comparing the highest to lowest categories of fish intake and 0.86 (0.74–1.00; $P=0.05$) for marine *n*-3 PUFA intake (Djoussé et al., 2012). Systematic reviews of prospective, randomized, placebo-controlled clinical trials confirmed the effect of *n*-3 fatty supplementation on risk of CVD (Marik and Varon, 2009; Wang et al., 2006); the multivariable OR (95%CI) were 0.87 (0.79–0.95, $P=0.002$) for cardiovascular deaths, 0.87 (0.76–0.99, $P=0.04$) for sudden cardiac death, and 0.92 (0.85–0.99, $P=0.02$) for nonfatal cardiovascular events (Marik and Varon, 2009). The effect of *n*-3 fatty acids has been proven to be independent from the effect of PUFA in the same diet (Mozaffarian et al., 2005b).

Several mechanisms for the beneficial effects of *n*-3 PUFA on cardiovascular health (Kinsella et al., 1990) have been postulated; reducing atherosclerosis via modulating endothelial function (von Schacky et al., 1999), reducing platelet aggregation (De Caterina et al., 2000) and inhibiting the production of thromboxane A2 (Harker et al., 1993), preventing arrhythmias (Nair et al., 1997), favorable lipid outcome and low triglycerides and increasing high HDL-cholesterol concentrations (Harris, 1989, 1997), reducing inflammatory biomarkers (James et al., 2000), and moderately decreasing blood pressure (Morris et al., 1993).

n-6 PUFA

Higher *n*-6 PUFA intake modified blood lipid concentrations (Djoussé et al., 2012; Keys et al., 1957; Mozaffarian et al., 2005b; Mensink and Katan, 1992). Omega-6 PUFA can suppress key mediators of the atherosclerotic process such as adhesion molecules, chemokines, and interleukins via anti-inflammatory properties (De Caterina et al., 2000). However, in human studies, higher plasma levels of *n*-6 PUFAs were not associated with any beneficial changes of serum lipid or plasma inflammatory markers levels (Becker et al., 1983; Nelson et al., 1997).

Serum and, to a lesser extent, dietary LA and PUFA were inversely associated with CVD mortality and total CVD events in the Kuopio Ischemic Heart Disease Risk Factor (KIHD) Study (Levitan et al., 2010), while in another study serum *n*-6 PUFA concentrations were not associated with risk of CVD (Yamagishi et al., 2008b).

In prospective cohort studies, the associations of *n*-6 PUFA intakes with risk of CHD were inconsistent; no associations ((Esrey et al., 1996; Pietinen et al., 1997; Harris et al., 2007)), nonsignificant inverse associations (McGee et al., 1984; Ascherio et al., 1996; Dolecek, 1992), and inverse associations (Marik and Varon, 2009; Kushi et al., 1985; Shekelle et al., 1981). However, in a meta-analysis of 25 case-control and prospective cohort studies, LA intake was inversely associated with CHD risk, whereas AA intake was unrelated to CHD risk; the effect size (95%CI) was −0.28 (−0.04 to −0.53; $P=0.02$) for LA and 0.03 (0.25 to −0.19; $P=0.81$) for AA (Harris et al., 2007).

An inverse association between serum levels of LA and risk of stroke, especially ischemic stroke, was reported in a nested case-control study of Japanese cohort, the Circulatory Risk in Communities Study (CIRCS) (Iso et al., 2002). On the other hand, in diet studies of total *n*-6 PUFA intake, neither risks of ischemic (He et al., 2003; Gillman et al., 1997; Iso et al., 2001b), hemorrhagic (He et al., 2003; Iso et al., 2001b, 2003), or stroke mortality (Sauvaget et al., 2004) were associated with *n*-6 PUFA intakes.

Higher serum levels of AA were associated with lower risk on incident heart failure in women, but not men of ARIC study (Yamagishi et al., 2008b).

Trans Fatty Acids

Among women of the Nurse Health study, intakes of trans fatty acids were correlated with high levels of plasma biomarkers of inflammation and endothelial dysfunction, such as C-reactive protein, interleukin-6, soluble tumor necrosis factor receptor 2, E-selection, and soluble cell adhesion molecules (Lopez-Garcia et al., 2005). In addition, intakes of trans fatty acids was not only associated with increased levels of LDL-cholesterol, but also with decreased levels of HDL-cholesterol (Mensink et al., 1992, 2003; Mozaffarian et al., 2005b). Similar effects were reported with trans fatty acids content in erythrocytes (Sun et al., 2007c).

Trans fatty acids intakes showed no associations with risk of total CVD in two studies (Leosdottir et al., 2007; Iso et al., 2001b). Contrarily in many literatures, the CHD associations with trans fatty acids intake (Oomen et al., 2001; Ascherio et al., 1994, 1996; Hu et al., 1997; Howard et al., 2006; Kromhout et al., 1995; Oh et al., 2005; Pietinen et al., 1997), plasma (Sun et al., 2007b,c), and adipose tissue (Aro et al., 1995) trans fatty acids biomarkers levels were evident. Trans fatty acids intake was positively associated with risk of total (Kiage et al., 2014) and ischemic (Kiage et al., 2014; Yaemsiri et al., 2012) stroke; however, no associations could be detected in 14-year prospective cohort study of male US healthcare professionals (He et al., 2003). However, trans fatty acids intake was inversely associated with risk of hemorrhagic stroke in one study (Iso et al., 2001b). The serum biomarkers levels of trans fatty acids (Shekelle et al., 1981; Hu et al., 1997), but not the serum levels of trans isomer of dairy fat, trans 16:1*n*-7 (Yakoob et al., 2014), were associated with risk of stroke. However, an increased dairy fat intake was associated with risk of incident heart failure in the ARIC study (Nettleton et al., 2008).

Substitution of Different Types of Fat

Substituting fatty acids for carbohydrates resulted in the reduction of LDL-cholesterol and HDL-cholesterol and increase of triglycerides. However, when MUFA or PUFA replaced SFA, total, LDL- and HDL-cholesterol concentrations have been shown to decrease (Jakobsen et al., 2009; Summers et al., 2002; Hodson et al., 2001). Moreover, substituting PUFA for SFA may have favorable outcomes on insulin sensitivity (Summers et al., 2002). It is worth mentioning here that, regarding their effects on lipids

and lipoproteins, the effect of SFA in diet is generally modulated by content and/or availability of PUFA, and the effects of substituting SFA with carbohydrates differ by the glycemic index and load of the carbohydrate. A review has shown that replacing SFAs with PUFAs was protective against CHD in some, but not all studies (Hu and Willett, 2002), while substituting total carbohydrate for SFA was associated with no or modest higher risk of CHD (Astrup et al., 2011). In a recent prospective study, replacing 5% of energy intake from SFA with equivalent energy intakes from whole grain carbohydrates, MUFA or PUFA, yielded a 9%, 15%, and 25% reductions in risk of CHD, respectively (Li et al., 2015). In a systematic review and meta-analysis of randomized controlled trials, each 5% of energy from SFA replaced by PUFA was associated with a 10% reduction in risk of CHD (Mozaffarian et al., 2010), which confirmed results from a pooled analysis of 11 cohort studies (Jakobsen et al., 2009). Only one study reported the effect of those substitutions on risk of stroke so far and showed that SFA intake was consistently inversely associated with stroke mortality in substitution models; significant or nonsignificant positive associations with stroke mortality were found when SFA was replaced by MUFA, PUFA, or carbohydrate (Yamagishi et al., 2010).

Dietary Pattern With Different Fat Content

Low fat, high carbohydrate diets versus high fat, low carbohydrate diets

The low prevalence of CHD in populations that consume very low fat diets in Asia (Cui et al., 2015) has led to the concept that low fat diets can protect against CHD. However, such populations are physically active and lean. Previously, low fat, high carbohydrate diets, compared with high fat diets, were associated with physically inactive, overweight, or obese populations with the development of atherogenic dyslipidemia (Krauss, 2001) which was characterized by high triacylglycerol and low HDL-cholesterol concentrations. In the Women's Health Initiative Dietary Modification Trial, the replacement of 7%–8% of fat intake with complex carbohydrates over 6 years did not modify the lipid profile (triglycerides, HDL-cholesterol, or lipoprotein subclasses) (Howard et al., 2010) and did not significantly reduce the risk of CHD, stroke, or CVD in postmenopausal women (Howard et al., 2006).

On the other hand, generally any fat that replaces carbohydrate in the diet raises HDL-cholesterol and decreases triacylglycerol concentrations. Moreover, in several long-term prospective studies that have examined the associations between diet and risk of CVD, rates of CHD did not substantially differ across populations that consumed approximately one third of energy from fat (Ascherio et al., 1996; Hu et al., 1997).

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Digital Data (mHealth and Social Media)

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Overview

Mobile technology use has seen a massive rise in recent years with mobile data usage expected to grow by more than 50% by 2020 (Statistica, 2016a). Technology has revolutionized the healthcare sector, and mobile health (mHealth) has become an increasingly popular mode of healthcare delivery. Advances in mobile devices coupled with high speed internet means phones are becoming more than communication devices and are acting more as hubs for controlling accessories, as well as portals that other devices can communicate through. In 2013 there were 15 million people who used app-enabled mHealth and wearable mobile-fitness hardware devices, and this number is forecast to rise to more than 95 million by 2018 (Statistica, 2016b).

This article will examine how mobile technology can potentially be used in cardiovascular research and medicine, with emphasis on how it can improve awareness and behavior management on the part of the patient, and allow health care providers to monitor health outcomes.

Social Media

Social media usage has been increasing steadily over the last decade, and this rise has increased exponentially with the increased popularity of internet-capable "smart" mobile devices. In 2015, there were approximately 2.15 billion social media users, and this number is estimated to rise to 2.95 billion by 2020 (Statistica, 2016c). **Table 1** shows the user base for the top 10 current social media platforms.

While penetration is greatest in North America (59% of the population have at least one social media account), usage in developing countries is expected to rise significantly in the future—usage in China and India alone is expected to increase by more than 250 million users by 2020 (Statistica, 2016c). This means that social media provides an ideal platform to target users in countries where the risk of cardiovascular diseases (CVDs) like stroke is on the rise.

While social media is not used as widely by public healthcare organizations as by other industries (such as music and television), they have considerable reach—the American Heart Association, for example, has nearly 900,000 people who "like" their Facebook page, who have access to any information posted by the organization on that page. Using platforms such as Facebook or Twitter to promote relevant healthcare messages provides the best value for money method of information dissemination available.

Awareness

While the prevalence of behavioral and other modifiable risk factors of CVD (O'Donnell et al., 2010) including stroke has reached epidemic proportions worldwide(Go et al., 2014; Feigin et al., 2016), there is evidence that modifying health behaviors is feasible, improves health outcomes, reduces healthcare costs (Spring et al., 2013), and can reduce an individual's risk by up to 90% (Goldstein et al., 2011; Rose, 1981; Feigin et al., 2016; O'Donnell et al., 2016).

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Table 1 Top 10 social media platforms by user numbers

Platform	*Active users (× million)*
Facebook	1590
WhatsApp	1000
Facebook Messenger	900
QQ	853
WeChat	697
QZone	640
Tumblr	555
Instagram	400
Twitter	320
Baidu Tieba	300

Source: http://www.statista.com/statistics/272014/global-social-networks-ranked-by-number-of-users/.

However, the level of public awareness of CVDs, such as stroke, its risk factors, and how to manage them is low, particularly in communities with low socioeconomic status, in ethnic minority groups and in rural areas (Alkadry et al., 2011; Ryan, 2010). In addition, current high-risk CVD prevention strategies do not target people with low to moderate risk of CVD, where the majority of strokes and heart attacks occurs (Dalton et al., 2013).

Using social media platforms to promote healthcare messages has been shown to improve health awareness (Lapointe et al., 2014). With the viral, peer to peer nature of social media, the dissemination of information can far outstrip the original audience of the message.

The most successful example of social media usage in recent times would be the "Ice bucket Challenge" for ALS. The statistics associated with this campaign highlight how effective social media can be in spreading awareness about a health condition (even if only temporarily).

- In two and a half months between June and September 2014, more than a million "ice bucket challenge" videos were uploaded to Facebook alone.
- The campaign raised $115 million for the ALS Association in 2014.
- Between July 29 and August 24 2014, donations to the ALS Association more than tripled the total donations received in 2013 ($70 million vs. $18.1 million).
- In August 2014, hits to the ALS Wikipedia page rose from the average of ~160,000 hits to nearly 3 million (McCarthy, 2014).

While this is an extreme example, the ALS campaign highlights what is possible with social media, and any awareness campaigns about cardiovascular conditions will need to incorporate at least a partial social media component in order to facilitate effective dissemination.

Interaction

Health professional–community interactions

In addition to improving awareness, social media can provide an effective method of interaction with other stakeholders (patients, other health professionals, community members) that would not be possible through other means. The peer to peer nature of social media means that health care providers can also seek input from end users to gain ideas about products or services that could be beneficial, or to gain feedback about existing interventions. Health researchers can use similar techniques to promote awareness about research trials, and also as a potential recruitment tool.

The Break Dengue project is an example of using social media to first promote a health project (using Facebook to gain 160,000 followers—nicknamed the DengueBreakers—who then raised awareness about the condition among their own networks), and leveraging this base to launch a prize contest where followers could submit ideas for prevention methods that could then be implemented based on feasibility. Using social media to build the profile for the project allowed the organization to reach a much wider audience to source ideas from (www.Breakdengue.org).

Community–community interactions

Another important aspect of social media interaction is that it lets like-minded people congregate and share ideas and views in a virtual setting.

Sites like PatientsLikeMe (https://www.patientslikeme.com/) allow people with health concerns or conditions to connect with others in similar situations. This provides users with an opportunity to share experiences about living with their conditions, provide support to and receive support from others with the similar illnesses, and compare symptoms and treatments.

For stroke survivors, who can often end up with long-term disabilities, getting supportive social interaction can significantly improve quality of life, and indeed health outcomes (Seeman, 1996). Where there are potential mobility issues which would make

normal face-to-face social interaction difficult, the availability and usage of forums could represent a valuable alternative. A number of major organizations such as the American Heart Organisation (http://supportnetwork.heart.org/stroke) and Stroke Association in the United Kingdom (https://www.stroke.org.uk/forum) have online forums set up to facilitate such interaction, and with higher levels of tech savviness, usage of social media as a tool for social interactions among people with specific health conditions is only likely to grow.

Health professional–health professional interactions

Lastly, social media provides a platform for health professionals to interact and collaborate on an unprecedented level. While "mainstream" social media sites are not ideal for professional collaboration, specialized platforms such as Sermo (http://www.sermo.com/) and Allnurses (www.allnurses.com) allow health professionals to interact with others to facilitate better health outcomes. Platforms such as this are particularly important for sharing important or relevant content (much quicker, and generally with more reach than traditional medical journals) as well as potentially being a portal for getting second opinions about challenging cases from a much larger pool of professionals than a physician or health professional would normally have access to in a short time frame.

Disadvantages of Social Media

As shown above, social media provides a huge opportunity to improve awareness about health conditions and interactions between interested stakeholders; but there are some significant drawbacks that must be taken into consideration.

The most important downside to social media is that the open, peer to peer nature that facilitates knowledge dissemination from health professionals is open to misinformation from third parties. The lack of any regulations about data validity or accuracy means that this incorrect information can also circulate rapidly. It is important as health providers to therefore have a sizeable social media presence wherever possible—this means that patients and general consumers are more likely to check and get health education and information from reputable sources.

Another important consideration is the legal and ethical issues surrounding any information provided through social media, and the privacy and security around health data. While patient-submitted information is usually fine from this perspective, health professionals and researchers need to be extremely careful around the responses they provide. Social media presence also has additional risks around information being stolen through compromised user accounts, data mining, employee's inadvertently positing confidential data online, etc. While these often do not have great health implications, there could be significant legal and commercial implications that need to be carefully considered.

mHealth Technology

While social media is a great tool for improving knowledge about health conditions, it is not an ideal medium for monitoring or managing patients or research participants. The growth of mHealth means that there are a rapidly growing number of tools health professionals, researchers, and the general population can use for these purposes. In broad terms, mHealth tech is split into two main categories, those that are aimed at individuals and those that are aimed at improving functionality within the health sector itself. However, there is often some crossover between the two, with data from end user applications informing healthcare and research organizations and vice versa. We will be using the Stroke Riskometer™ application as an example through this section.

The Stroke Riskometer™

The Stroke Riskometer application was developed by the National Institute for Stroke and Applied Neurosciences to address the lack of awareness around stroke and associated risk factors (Feigin et al., 2015). Users complete a questionnaire and are provided with their risk of stroke using a validated algorithm (Parmar et al., 2015), and identified risk factors. The PRO version of the app also provides management tips to improve user risk. Additionally, users can submit their data as part of the international RIBURST study (**Fig. 1**).

Individual Use

Given the risk factors associated with CVD, there are a lot of applications and technology aimed at generic health factors such as diet and exercise that decrease users' CVD risk despite not being explicitly marketed for this purpose. Activity trackers like Fitbit™ have huge userbases (recent filings indicate there are approximately 19 million registered users with over 9 million "active" users (Goode, 2015), and research indicates that usage of wearable devices such as these does show an increase in physical activity (Cadmus-Bertram et al., 2015; Fritz et al., 2014)). Similarly, diet trackers and calorie counters such as MyFitnessPal™ also show efficacy when used on a regular basis, with research suggesting users of this technology were able to maintain dietary changes better than those who did not use them (Wang et al., 2016). Similar generic applications are also available for blood pressure, smoking cessation, and other associated risk factors.

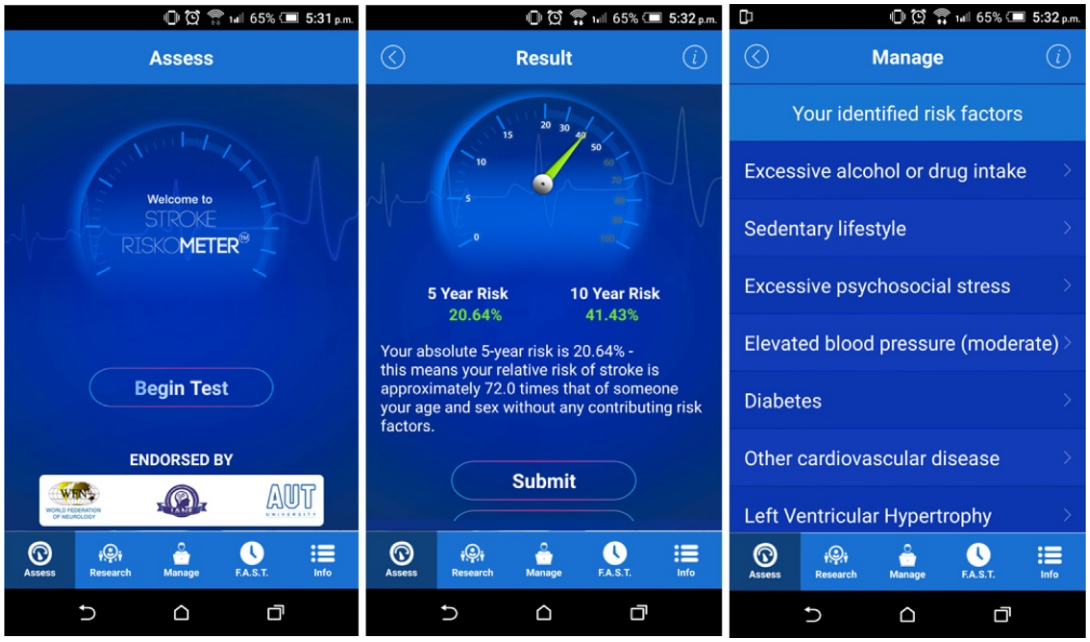

Fig. 1 Screenshots from the Stroke Riskometer™.

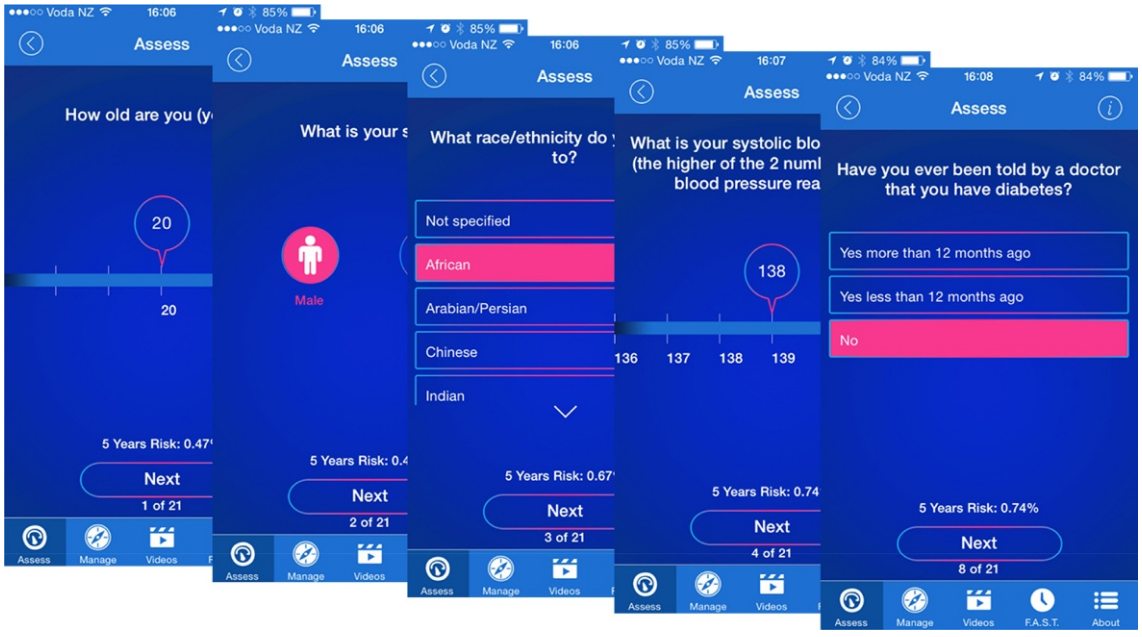

Fig. 2 Questions from the Stroke Riskometer™.

For CVD-specific mHealth applications or devices, the first priority for management needs to be personal risk awareness. While social media is a good medium for improving general awareness, mHealth needs to be more personalized, where the user gains an awareness of his/her own risk factors and identifies what he/she needs to be doing in order to minimize his/her chances of suffering from the relevant condition (Fig. 2).

In addition to personal awareness, ideally the application or device should provide users with information on what they can do to minimize or mitigate any identified risks. This is a potentially complicated issue to address, as ideally the aim should be to not only provide users with as much relevant information as possible, but also to prompt them to seek advice from their health professional when warranted. Fig. 3 shows some of the management advice provided in the Stroke Riskometer app.

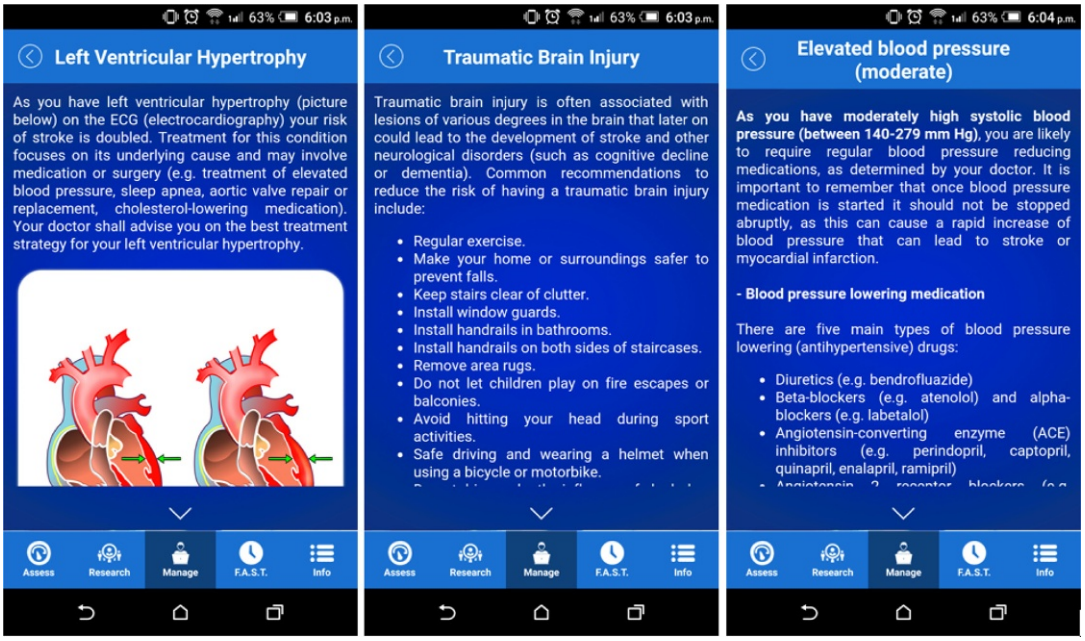

Fig. 3 Management advice from the Stroke Riskometer PRO.

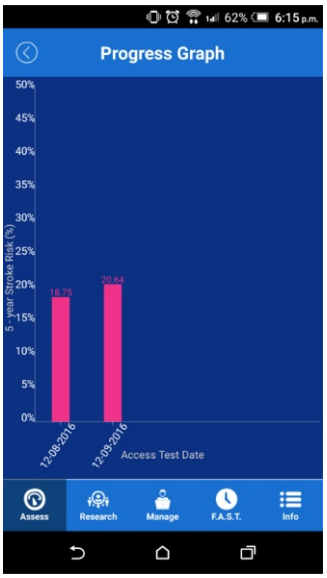

Fig. 4 Stroke Riskometer Progress graph.

Lastly, these applications should have a monitoring component so users can see if they are making progress, and track whether any changes in health behavior lead to changes in the risk of having an adverse event. Fig. 4 shows the Stroke Riskometer progress graph, which users can use to track changes in their risk of stroke.

Health Sector Use

For the health sector, the main emphasis for using mHealth technologies should be data driven. Mobile technology allows aggregation of data (from both individuals and wider population groups) at a significantly lower resource cost than current data collection methods. Collecting and analyzing the data allows the health sector to identify gaps in current health programs, assess the suitability and uptake of promotions being rolled out, and most importantly measure the outcomes of projects to determine whether they are working or not.

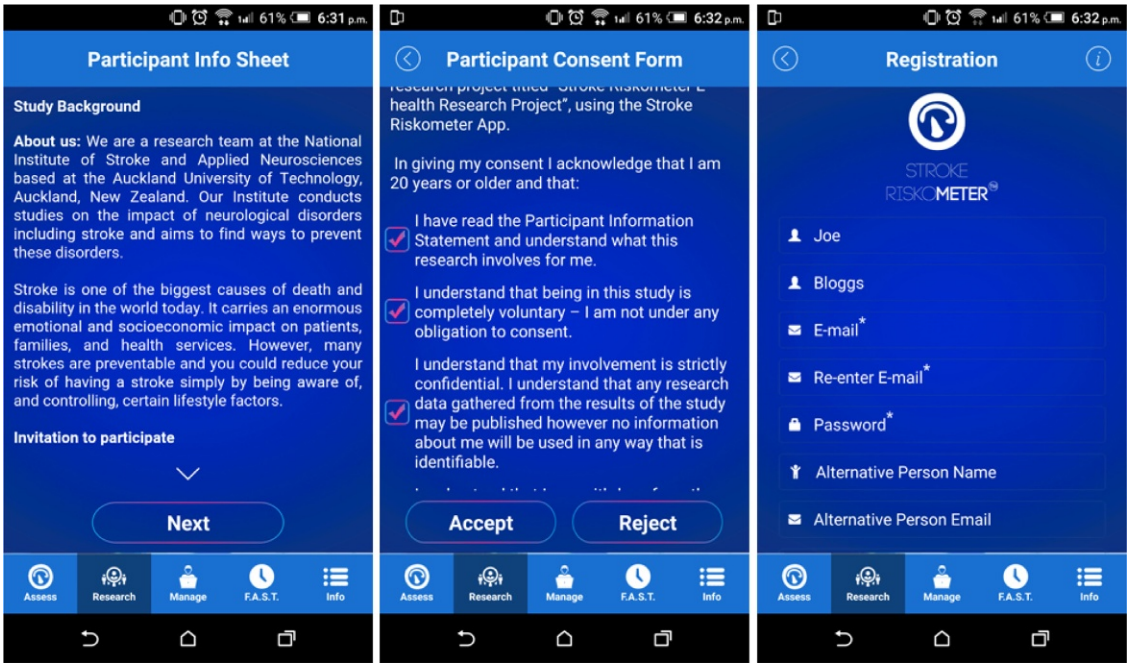

Fig. 5 Consenting process for Stroke Riskometer data collection.

Data collection and mining

As stated earlier, collecting the data from self-use applications and devices allows health professionals to acquire data from the population group without the traditional face-to-face interview method, which saves both time and costs (allocating and training interviewers, time taken for data entry, etc.). However, care needs to be taken to ensure that any intervention meets ethics criteria, which can be tricky particularly for international projects with different levels of ethical thresholds. This is especially important with respect to research data. Health professionals and researchers need to work closely with their commercial and legal teams and ensure any mHealth products or interventions meet all legislative requirements. Fig. 5 shows the consent process used by the Stroke Riskometer for data collection.

Personalized mHealth

On an individual level, mHealth technology can be used to track patients at high risk or those who have been diagnosed with health conditions to ensure that quick action can be taken if their conditions deteriorate. The level of sophistication for this technology is variable, ranging from relatively simple Bluetooth-enabled blood pressure monitors and weight scales that transmitted data when readings were outside preset limits (Logan et al., 2007; Scherr et al., 2006) through to more complicated products like personal monitors which record ECG, compare the readings with reference ECG readings which account for predetermined patient risk factors and clinical data, and send out stratified levels of alarm messages to the users health provider if arrhythmia is detected (Rubel et al., 2005).

Short message service

In developing countries, where internet coverage and usage is still suboptimal, mobile text messaging also provides an opportunity to reach people who may not have access to smart mobile devices or reliable internet connections. Short message service (SMS) messaging has been used extensively for behavior modification programs such as smoking cessation (Haug et al., 2009), diabetes management (Krishna and Boren, 2008), and weight loss (Patrick et al., 2009). SMS-based interventions generally indicate good acceptability and uptake, but demonstrate variable results with respect to changes in health outcomes. However, this method of healthcare provision is a viable option in situations where more personalized or individualized care is not feasible operationally or financially.

What Makes a Good mHealth Product?

With the rapid improvements in mHealth technology, there is a massive scope for development of applications, software, and other devices that could have great potential in informing people about the risks and risk factors associated with CVDs, and monitoring and managing the symptoms of those who suffer from these conditions.

However, for those in the health sector, this technology is often unfamiliar—what works from a commercial standpoint (which is important to get people to accept and use the technology) can often be at odds with what is required for a health or research

Table 2 Key mHealth development considerations

Cost	This is often the most important barrier, but there are more things to consider than simply initial development cost—any new technology should factor in running costs and potential further developments. For example, costs for server space for data storage, database monitoring and upkeep, development time for future changes, etc. can be overlooked during the initial development phase
Maintenance	It is important to have a strategy to make sure there are resources in place for monitoring. Any product should be easy to work with and maintain over the life of the project
Usability and accessibility	This is essential for successful implementation. The product should be straightforward and easy to use—the end user should be able to intuitively use the product with minimal external guidance
Performance	Attention should be paid to ensure that the technology should work fast with good reliability. If the product is slow (i.e., long load times or lag for data submission), users are less likely to continue to engage with and use it
Volume	The backend of any new products should be able to handle high transaction volumes, and quickly respond to requests looking through large amounts of data (this is important at the analysis stage)
Security and privacy	Precaution must be taken to ensure that there are minimal chances of the product being hacked. Ideally all collected data should be encrypted, and access to the data should be restricted and recorded
Compatibility	Any technology should be able to work in all foreseeable environments. Testing should be done to ensure products work across different operating systems, browsers, etc. as well as communicate seamlessly with any linked programs or databases
Reusability and extensibility	For "future proofing," any new technology should be designed so it can be reskinned, rescaled, and used for other projects to maximize cost-effectiveness and utility

perspective (getting enough accurate information to draw meaningful conclusions from). While it is important to work in conjunction with your technical and commercial partners to develop the best all-round application or device, **Table 2** highlights some of the key items that should always be taken into consideration.

Summary

Social media and mHealth have significantly changed the way health care and research can be administered. People have more access than ever to information about their health and what they can do to improve it. Similarly, health providers have access to more information about patient health and lifestyle than ever before. There is very little doubt that social media and mHealth can greatly improve awareness about CVD and risk factors. However, there needs to be more research to determine the actual effectiveness of individual interventions before wide-scale rollouts, as current data suggests the outcomes of mHealth and social media-based health projects have varying levels of success.

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Diseases of the Mitral Valve

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Abbreviations

AF	Atrial fibrillation
MR	Mitral regurgitation
MS	Mitral stenosis
MVA	Mitral valve area
MVP	Mitral valve prolapse
TEE	Transesophageal echocardiography
TTE	Transthoracic echocardiography

Overview

The mitral valve was so-named by Vesalius in the 16th century from comparison of the cusps to a bishop's mitre or hat. An in-depth understanding of the mitral valve's structure and function was clearly evident at that time in the drawings of Leonardo da Vinci.

Extending from a semirigid fibrous frame called the mitral valve annulus are two asymmetric saloon door-like leaflets termed anterior and posterior (although predominantly orientated superiorly and inferiorly). These leaflets are attached by chordae tendineae to papillary muscles which together ensure that the valve stays closed during cardiac contraction. The normal mitral valve area (MVA) is ~4–5 cm^2 and is the portal through which diastolic left ventricular filling occurs via the left atrium.

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Guidelines published in 2014 by the American College of Cardiology (ACC) in collaboration with the American Heart Association (AHA) are referenced in this text (Nishimura et al., 2017). These guidelines provide grading based on the strength of evidence supporting each clinical practice and will be referred to as follows:

Class I: Procedure or treatment is supported by sufficient evidence and is recommended.

Class IIa: There is conflicting evidence/divergence of opinion regarding the given procedure/treatment but recommendations are generally in favor of its usefulness/efficacy.

Class IIb: There is greater conflicting evidence over usefulness/efficacy of procedure and recommendations are less well established.

Class III: There is sufficient evidence that the procedure/treatment is not useful/effective and may be harmful in some cases.

Mitral Stenosis

Background

Mitral stenosis (MS) is narrowing of the mitral valve orifice that leads to impaired blood flow from the left atrium to ventricle during diastole. Rheumatic degeneration remains the predominant etiology of MS in the developing world (Carapetis et al., 2005). In contrast, degenerative mitral annular calcification is the predominant etiology of MS in industrialized countries. Rare functional causes of MS include infective endocarditis with large vegetations, and left atrial myxoma. Treatment strategies for MS are based upon patient symptoms, valve anatomy, hemodynamics including consequences for the left atrium and pulmonary circulation and right ventricle as well as concomitant and comorbid disease.

Staging of MS

Stage A patients are at risk of MS and include those with mild valve doming during diastole. Stage B (progressive MS) includes patients with mild or moderate MS where the MVA > 1.5 cm^2, and the morphology may involve more pronounced diastolic valve doming or commissural fusion. Stage C is defined as asymptomatic severe MS (MVA ≤ 1.5 cm^2), while Stage D involves patients with symptomatic severe MS.

Pathophysiology

Following an episode of acute rheumatic fever, patients may remain asymptomatic for decades prior to the development of subclinical rheumatic MS. Repeated episodes of rheumatic carditis eventually lead to fibrosis and thickening of the mitral valve leaflets. The classical rheumatic lesions are fusion of the commissures and subvalvular degeneration which together reduce mitral valve orifice area to cause MS. As the valve area progressively narrows, the diastolic mitral valve gradient rises, which leads to higher left atrial pressure. Chronically elevated left atrial pressure leads to inexorable left atrial enlargement, which can predispose patients to atrial fibrillation (AF) and thromboembolic events including stroke (Chiang et al., 1998, Lee et al., 2014). Elevations in left atrial pressure are transmitted backward to the pulmonary vasculature causing pulmonary hypertension resulting in shortness of breath, orthopnea, and paroxysmal nocturnal dyspnea. Untreated patients may report that these latter symptoms may improve at the same time they develop progressive lower limb edema which denotes the development of right heart failure that may ultimately lead to anascarca.

Signs and Symptoms

Presentation may occur as a consequence of gradual functional decline (e.g., fatigue or exertional dyspnea) or in some cases due to acute deterioration (e.g., acute pulmonary edema, AF, thromboembolism, rarely hemoptysis). In very rare cases, an enlarged left atrium may compress the recurrent laryngeal nerve causing hoarseness and Ortner's syndrome, which accounts for approximately 0.25–0.5% of all cases of recurrent laryngeal nerve palsy. Acute decompensations in previously stable MS patients may occur during exercise, infection, stress, thyrotoxicosis, AF, and pregnancy (which will be discussed later in this section) (Norrad and Salehian, 2011).

Examination findings of mitral stenosis include a malar flush, AF, and jugular venous distension. Characteristic auscultatory findings include a loud first heart sound and the absence of silence during diastole (with namely an opening-snap and a mid-diastolic rumble). The first heart sound may be reduced if the valve has limited mobility from calcification. A loud P_2 can be appreciated if pulmonary hypertension is present. With worsening stenosis and higher left atrial pressure, the opening snap may be heard earlier during diastole therefore prolonging the duration of the murmur. Factors that could reduce flow across the valve and mask the murmur of MS include right heart failure, severe tricuspid regurgitation, or concomitant aortic stenosis.

Diagnosis

Initial electrocardiography may reveal a patient in AF with left atrial enlargement, right ventricular hypertrophy, and right axis deviation due to associated pulmonary hypertension. Chest radiography can exhibit left atrial dilation, enlargement of the main pulmonary arteries, and pulmonary congestion.

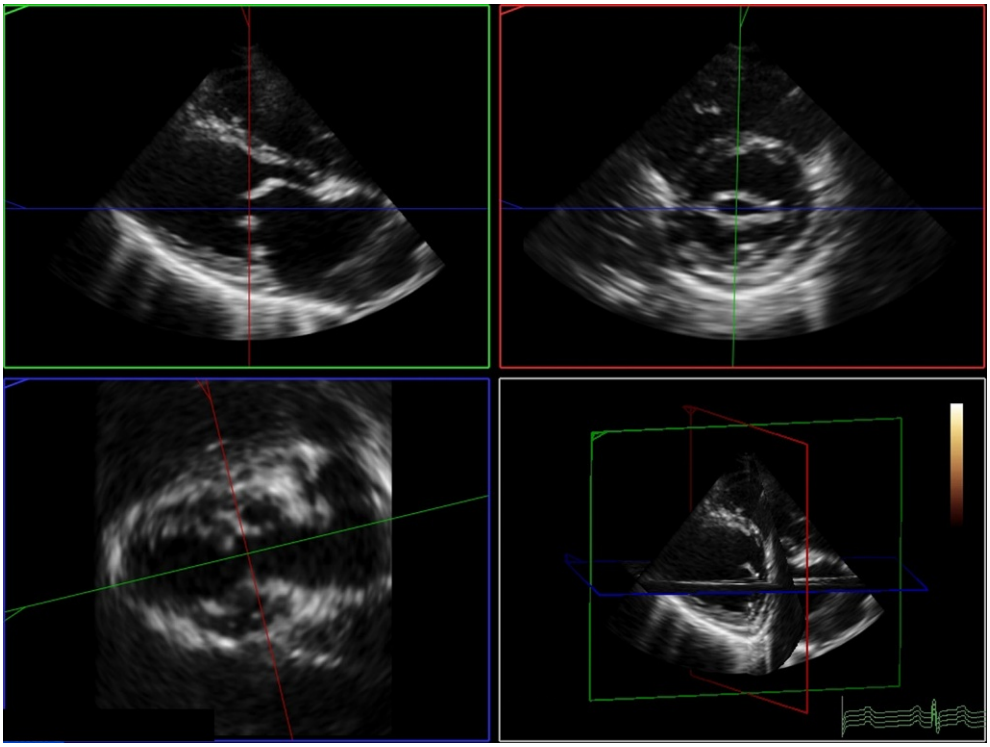

Fig. 1 Three-dimensional echocardiographic planimetry demonstrating how to obtain an accurate mitral valve orifice area in severe rheumatic mitral stenosis by measuring at the leaflet tips during maximal diastolic opening. Characteristic rheumatic anterior mitral valve leaflet doming is evident in the top left image while bicommissural fusion is evident in the top right image.

Transthoracic echocardiography (TTE) is recommended in all patients with suspected MS (Class I) (Nishimura et al., 2017). TTE can establish MS diagnosis and identify hemodynamic severity (mean pressure gradient, MVA, and pulmonary artery pressures). Due to the frequent occurrence of other valvular disease alongside MS, it is important to investigate coexisting valvular lesions, especially the tricuspid and aortic valves. TTE helps determine valve morphology, which is fundamental in developing treatment strategy and suitability for mitral commissurotomy (Class I) (Nishimura et al., 2017). Scoring systems to evaluate significant aspects of valve anatomy including valve mobility, calcification, thickening, deformity, and commissural areas have been developed (Baumgartner et al., 2009).

Transesophageal echocardiography (TEE) is indicated in patients being considered for percutaneous mitral balloon commissurotomy (PMBC) to look for left atrial thrombus and presence of mitral regurgitation (MR) (Class I) (Nishimura et al., 2017). Where there is an equivocal difference or discordance between resting Doppler echocardiography and the severity of symptoms/signs, exercise testing with Doppler or cardiac catheterization can identify the response of mean mitral gradient and pulmonary artery pressures (Class I) (Nishimura et al., 2017). While the transmitral pressure gradient is generally $\geq$10 mmHg in severe MS, this measurement is subject to variations in heart rate and forward flow. MVA is a more consistent parameter for defining MS. Valve area should be measured using planimetry and the pressure half time method (time taken for the peak transvalvular pressure gradient to fall by half, i.e., MVA = 220/ P × ½ t). Three-dimensional echocardiography may enhance evaluation of valve area and morphology (Fig. 1).

Medical Therapy

Anticoagulation with warfarin or heparin is indicated in patients with MS and concomitant AF, left atrial thrombus, or prior embolic events (Class I) (Nishimura et al., 2017). Anticoagulation may also be considered if there is significant left atrial dilation of 5–5.5 mm (Class IIb) (Nishimura et al., 2017). Tachycardia reduces diastolic filling time and may be poorly tolerated by patients with MS. Thus heart rate control with beta-blockers, calcium channel blockers, digoxin, or ivabradine can be beneficial in MS patients with AF and rapid ventricular response (Class IIa) (Nishimura et al., 2017). It is also beneficial in normal sinus rhythm patients who are symptomatic during exercise (Class IIb) (Nishimura et al., 2017).

Patients with a history of rheumatic heart disease are at risk of recurrence. Long-term antibiotic prophylaxis with penicillin is indicated in patients with a history of rheumatic fever, rheumatic carditis, or rheumatic valve disease. Duration of therapy varies widely depending on the extent of cardiac disease, patient age, and timing of most recent episode (Cilliers, 2006). Current guidelines do not recommend routine antibiotic prophylaxis against endocarditis for patients with MS (Wilson et al., 2007).

While medical therapy optimizes patient symptoms and risks for embolic events and recurrent rheumatic valve disease events, it does little to alter the natural progression of MS.

Surgery

There are three surgical options available for MS patients: PMBC, surgical mitral commissurotomy, and mitral valve replacement (MVR).

In 1984, Inoue (a cardiovascular surgeon from Japan) and his colleagues, first described PMBC, a method whereby a balloon catheter is used to fracture fused commisures and restore MVA (Inoue et al., 1984). This method has shown equal or better success rates, equivocal restenosis rates, and larger valve area with improved durability when compared to surgical commissurotomy, all the while being less invasive (Turi et al., 1991, Arora et al., 1993, Dean, 1994, Reyes et al., 1994). There are a number of contraindications that must be considered with PMBC; MVA > 1.5 cm^2, presence of a left atrial thrombus, moderate 2+ mitral leak, severe/bicommissural calcification, and concomitant coronary artery disease requiring bypass surgery. In the absence of contraindications, PMBC has a Class I indication in symptomatic patients with severe MS (MVA less than 1.5 cm^2, Stage D) (Nishimura et al., 2017). It has a Class IIa indication in asymptomatic patients with very severe MS (MVA less than 1 cm^2, Stage C) without contraindication (Nishimura et al., 2017). There are three Class IIb indications for PMBC outlined in the AHA/ACC Valvular Heart Disease Guidelines as follows; asymptomatic patients with severe MS (MVA < 1.5 cm^2, Stage C) and favorable valve morphology for PMBC with new onset AF; in symptomatic patients with MVA > 1.5 cm^2 but evidence of hemodynamically significant MS with a mean mitral valve gradient >15 mmHg or pulmonary artery wedge pressure >25 mmHg on exercise, in severely symptomatic patients (New York Heart Association (NYHA) Class III or IV) with severe MS (MVA < 1.5 cm^2, Stage D) with suboptimal valve morphology and who are poor surgical candidates. Major complications of PMBC include mortality 0.5%–4%, embolism 0.5%–5%, hemopericardium 0.5%–10%, and severe regurgitation in 2%–10% (Vahanian et al., 2012). PMBC can also play a role in the palliative care of patients with poor valve morphology who are not surgical candidates (Fawzy et al., 2005).

Closed surgical commissurotomy was first described by Harken and Bailey in 1948 (Harken et al., 1951). Following the advent of cardiopulmonary bypass, open mitral commissurotomy using a midline sternotomy or left thoracotomy replaced the closed approach in the 1960s and 1970s. However, the closed technique is still widely used in developing countries where rheumatic heart disease is most prevalent. Another surgical option is MVR with mechanical or biological prostheses. Both surgical commissurotomy and MVR have a Class I indication in severely symptomatic patients (NYHA Class III or IV) with Stage D MS who are not high risk surgical candidates and have either failed previous PMBC or have contraindications to PMBC (Nishimura et al., 2017). If a patient with Stage C or D MS is undergoing cardiac surgery for other reasons, mitral valve surgery has a Class I indication (Nishimura et al., 2017). Likewise, mitral valve surgery has a Class IIb indication in patients undergoing cardiac surgery who also have concomitant moderate MS (1.6–2 cm^2) (Nishimura et al., 2017). Lastly, mitral valve surgery with left atrial appendage removal has a Class IIb indication in Stage C or D MS patients who have recurrent embolic events while on anticoagulation (Nishimura et al., 2017). Given the less invasive and less costly approach of PMBC, it is important to consider all options before proceeding to more advanced surgical measures.

Pregnancy and MS

Pregnancy increases plasma volume by approximately 50% and heart rate by 20%–30%, resulting in a rising transmitral gradient that may trigger new onset of symptoms or exacerbation of preexisting symptoms (Norrad and Salehian, 2011). Most patients with MS will experience clinical deterioration during pregnancy (Hameed et al., 2001). Pulmonary edema, AF, and thromboembolic events are not uncommon complications. One study found that pregnant MS patients had a 67% incidence of cardiac events, while there was a 44% incidence of fetal complications such as premature birth, intrauterine growth restriction, or death (Silversides et al., 2003).

When MS is suspected, all patients should ideally undergo TTE before pregnancy in order to identify the best treatment plan prepregnancy and during gestation (Class I) (Nishimura et al., 2017). Those found to have severe valve stenosis of Stages C or D should be seen by a cardiologist with expertise in managing MS during pregnancy and all treatment options discussed (Class I) (Nishimura et al., 2017). Of note, mechanical valve prostheses require continued anticoagulation, which may pose maternal and fetal risks. However, bioprosthetic valves are thought to undergo accelerated degeneration during pregnancy, with 60%–80% requiring replacement within 10 years (Hung and Rahimtoola, 2003). It is recommended that a multidisciplinary team approach of anesthesiologists, obstetricians, surgeons, and cardiologists should be implemented to develop management plans in a tertiary care center (Class I) (Nishimura et al., 2017).

Pregnancy is a hypercoaguable state and patients with concurrent MS and AF should be prescribed warfarin in the second and third trimester (Class I) (Nishimura et al., 2017). Controversy still remains over the most appropriate anticoagulant for the first trimester. Beta-blockers (Class IIa) and diuretics (Class IIb) are reasonable therapies in patients free from contraindication (Nishimura et al., 2017). However, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers should be avoided due to teratogenic effects (Class III) (Nishimura et al., 2017).

Surgical intervention is recommended before pregnancy for symptomatic patients with MVA < 1.5 cm^2, Stage D (Class I) (Nishimura et al., 2017). While PMBC is the preferred method, surgical commissurotomy and MVR may also be used depending on institutional experience.

Severe MS patients categorized as Stage D during pregnancy and are experiencing NYHA III to IV heart failure symptoms despite medical therapy should be considered for PMBC provided valve morphology is favorable (Class IIa) (Nishimura et al., 2017). Stage D patients without suitable valve morphology may be considered for PMBC if NYHA IV refractory heart failure symptoms are ongoing. Surgery is reserved for severely symptomatic patients unsuited for PMBC, having failed all medical therapies and bed rest. The MS patient who does not have severe heart failure symptoms should not be considered for surgery (Class III) (Nishimura et al., 2017). One study concluded that, where possible, surgical intervention should be delayed until 12–14 weeks gestation to prevent radiation exposure during organogenesis (Hameed et al., 2009).

Mitral Regurgitation

Background

MR is the leakage of blood backward from the left ventricle to the left atrium during systole. MR can be divided into primary (degenerative) and secondary (functional) subtypes depending on the mechanism underlying MR. Primary MR includes all etiologies which affect the mitral valve apparatus such as myxomatous disease, infective endocarditis, radiation heart disease, or rheumatic heart disease. Myxomatous valve prolapse is the most common cause of MR in developed countries and will be discussed later in this chapter. By contrast, in secondary MR the mitral valve apparatus is structurally normal and MR results from distortion from surrounding structures such as left ventricular enlargement and/or annular dilation (**Fig. 2**).

Staging of MR

The stages of primary and secondary MR are assessed separately. In the rare cases where the two mechanisms occur together, one usually predominates and guides therapy. In treating the regurgitant "valve problem" of primary MR, correction is curative (Nishimura et al., 2017). However in secondary MR, where there is a "valve plus a pump problem," the left ventricular component drives the prognosis making the staging and treatment of secondary MR complicated and unclear in comparison to primary disease.

In primary MR, staging is divided into four categories, A through D. Stage A patients have mild valve prolapse and thickening with normal coaptation. There may or may not be a MR jet, if so it is typically small (<20% left atrial area on Doppler). Progressive Stage B MR is present when there is severe valve prolapse with normal coaptation, prior infective endocarditis or rheumatic valve changes with leaflet restriction with loss of coaptation (20%–40% left atrial area on Doppler). Stage C asymptomatic severe MR is seen with severe valve prolapse and flail leaflet, rheumatic changes with loss of coaptation, prior infective endocarditis, and/or thickening of leaflets due to radiation damage (>40% left atrial area on Doppler; regurgitant volume >60 mL). This Stage C classification is divided into C1 and C2, with better left ventricular ejection fractions (LVEF) of >60% and left ventricular end systolic dimension (LVESD) <40 mm in C1, with the alternate values accounting for C2. Patients with severe MR who are symptomatic are categorized as Stage D.

Patients with secondary MR are also divided into four categories from A to D. Stage A patients are at risk of MR and have cardiomyopathy or coronary artery disease but normal mitral valve anatomy. Progressive Stage B patients have regional wall

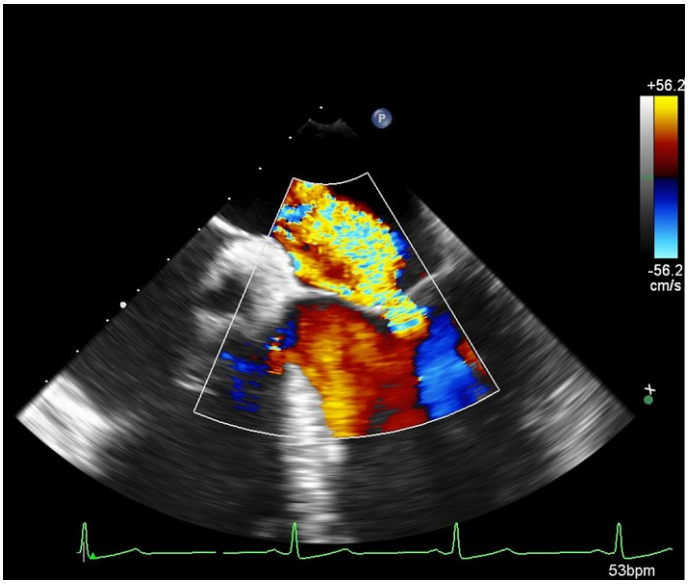

Fig. 2 Color Doppler two-dimensional echocardiographic image of a centrally directed jet of severe functional mitral regurgitation secondary to left ventricular enlargement.

abnormalities with some tethering of valve leaflets, annular dilation, and loss of central coaptation with effective regurgitant orifice (ERO) $<0.2\ cm^2$ and regurgitant volume <30 mL. Severe MR is defined by severe mitral leaflet tethering and severe loss of central coaptation (ERO $>0.2\ cm^2$ with a regurgitant volume >30 mL and characterizes Stage C (asymptomatic patients) and Stage D (symptomatic patients).

Pathophysiology

MR overloads the volume of the left ventricle and atrium with both stroke volume and added regurgitant volume. The way in which the ventricle copes with this added volume depends on the rapidity at which the regurgitation develops. In the acute setting, the ventricle is unable to remodel in size to compensate for higher volumes. Hence, acute MR is characterized by hyperdynamism and tachycardia to maintain stroke volume. Left atrial and pulmonary vasculature pressures may rise resulting in pulmonary congestion and edema. In contrast, with chronic MR the left ventricle dilates gradually over time in order to compensate for the volume load. However, this mechanism does eventually fail resulting in high left atrial and pulmonary venous pressures with eventual pulmonary hypertension late in the course of disease. Development of AF may precipitate the onset of symptoms in the presence of otherwise asymptomatic chronic MR.

Signs and Symptoms

Due to left ventricular remodeling, chronic MR can remain asymptomatic for decades. Later on, symptoms may include dyspnea, fatigue, orthopnea, paroxysmal nocturnal dyspnea, and palpitations produced by AF. However in the acute MR patient, symptoms develop rapidly due to lack of left ventricular compensation (e.g., postmyocardial infarction with papillary muscle rupture and resultant acute MR.

On auscultation, a soft S_1 with a blowing pansystolic murmur is best heard over the cardiac apex in the left lateral position at end expiration. The murmur can be heard radiating to the axilla. Later on, examination findings may include AF, a palpable heave at the left sternal edge, and/or a displaced apex beat.

Diagnosis

A simple electrocardiogram can reveal the presence of AF or left atrial enlargement (P mitrale waves). Chest radiography may show evidence of left atrial or ventricular enlargement. TTE is indicated for investigation of both primary and secondary MR in order to establish etiology, baseline left ventricular functioning, ERO, regurgitant volumes, and estimation of pulmonary vasculature pressures (Class I) (Nishimura et al., 2017).

In primary MR, cardiac magnetic resonance imaging can further evaluate the above parameters if TTE is inconclusive (Class I) (Nishimura et al., 2017). TEE can elaborate on diagnostic information if noninvasive investigations provide inadequate data (Class I) (Nishimura et al., 2017). An intraoperative TEE can help guide repair (Class I) (Nishimura et al., 2017). Where there is discrepancy between symptom severity at rest and upon exertion, exercise hemodynamics via stress echocardiography or cardiac catheterization is reasonable (Class IIa) (Nishimura et al., 2017).

Tests that have a Class I indication for secondary MR include nuclear or positron emission tomography, cardiac magnetic resonance imaging, cardiac CT angiography, coronary arteriography, or cardiac catheterization (Nishimura et al., 2017) to better assess the left ventricular substrate.

Medical Therapy

Acute MR should be ruled out in patients with myocardial infarction experiencing sudden hemodynamic compromise. Such patients require urgent mitral valve surgery. Until then, nitroprusside and nitroglycerin may help to reduce afterload and maintain forward cardiac output. In the hemodynamically unstable, intraaortic balloon pump placement should be considered.

In the normotensive, asymptomatic MR patient with chronic primary MR (Stage B or C1) and normal systolic LV function, vasodilator therapy is not indicated (Class III) (Nishimura et al., 2017). However, medical therapy for systolic dysfunction is reasonable in Stage D patients with ejection fractions less than 60% who are poor surgical candidates.

In patients with secondary MR classed as Stage B, C, or D with heart failure and poor ejection fractions, guideline-directed medical therapy (GDMT) for heart failure should be employed, utilizing medications such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers, and aldosterone antagonists. Cardiac resynchronization therapy should be considered in Stages B through D patients who meet criteria for device implantation.

Surgery

Primary

In the symptomatic patient, mitral valve surgery is recommended in Stage D MR with LVEF >30% (Class I) or <30% (Class IIb) (Nishimura et al., 2017). Transcatheter mitral valve repair can be considered in the severely symptomatic Stage D patient (NYHA III or IV) despite optimal GDMT with favorable anatomy and good life expectancy but severe comorbidities that contribute to surgical risk (Class IIb) (Nishimura et al., 2017).

In the asymptomatic Stage C2 patient with LVEF 30%–60% and LVESD > 40 mm, mitral valve surgery is recommended (Class I) (Nishimura et al., 2017). Mitral valve repair is reasonable in Stage C1 patients with LVEF > 60% and LVESD < 40 mm in whom there is a >95% chance of successful repair and <1% risk of mortality when performed under at a Heart Valve Center of Excellence (Class IIa) (Nishimura et al., 2017). In the asymptomatic Stage C1 patient with the aforementioned LVEF and LVESD values, with new onset AF or pulmonary hypertension (pulmonary artery systolic arterial pressure >50 mm Hg) mitral valve repair is also reasonable (Class IIa) (Nishimura et al., 2017). In general, mitral valve repair is preferable to replacement, the latter surgery used either after failed valve repair or if the anatomy is unsuitable for repair (Nishimura et al., 2017). Concomitant mitral valve surgery is indicated in patients undergoing other cardiac procedures with chronic severe (Class I) or chronic moderate (Class IIb) MR (Nishimura et al., 2017).

Secondary

Mitral valve surgery is reasonable in chronic severe Stage C or D patients who are undergoing Coronary Artery Bypass Grafting (CABG) or Aortic Valve Replacement (AVR) (Class IIa) (Nishimura et al., 2017). Mitral valve surgery may be considered in Stage B patients who are undergoing cardiac surgery for other indications (Class IIb) (Nishimura et al., 2017). In the severely symptomatic NYHA III or IV Stage D patient with persistent symptoms despite optimal GDMT, mitral valve surgery should be considered (Class IIb) (Nishimura et al., 2017).

Pregnancy and MR

Patients with MR generally tolerate pregnancy well in the absence of significant left ventricular dysfunction. While plasma volume rises by 50%, the systemic vascular resistance is reduced in pregnancy, therefore reducing the regurgitant volume across the mitral valve. However, patients with preexisting left ventricular dysfunction, pulmonary hypertension, or heart failure may experience exacerbation of MR symptoms. AHA/ACC guidelines recommend that all patients with suspected MR should undergo TTE before pregnancy (Class I) (Nishimura et al., 2017). All patients with severe MR (Stage C or D) should have prepregnancy counseling with a cardiologist with expertise in managing such patients (Class I) (Nishimura et al., 2017). These Stage C or D patients should be followed in a tertiary care center by a Heart Valve Team consisting of cardiologists, surgeons, anesthesiologists, and obstetricians (Class I) (Nishimura et al., 2017). As in patients with MS, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers should be avoided during gestation (Class III harm) (Nishimura et al., 2017).

In terms of surgical intervention, valve repair or replacement is recommended before pregnancy in Stage D patients (Class I) (Nishimura et al., 2017). Valve repair before pregnancy in the asymptomatic severe MR patient (Stage C) may be considered in patients with suitable valve morphology with whom detailed discussions regarding the operation and associated risks have taken place (Class IIb) (Nishimura et al., 2017). During pregnancy, valve operation is reasonable in the Stage D patient with intractable NYHA IV heart failure symptoms (Class IIa) (Nishimura et al., 2017). There is a Class III harm indication for such patients in the absence of heart failure symptoms (Nishimura et al., 2017).

Mitral Valve Prolapse

Background

Mitral valve prolapse (MVP) is the abnormal excessive motion of part or all of one or both mitral valve leaflets behind the mitral annulus into the left atrium during systole as seen in outflow tract views. It is the most commonly diagnosed valvular disorder and the most prevalent MR etiology requiring surgical intervention (Guy et al., 1980). MVP occurs in around 5% of the general population, with women exhibiting a higher incidence than their male counterparts (Devereux et al., 1986). However, this incidence was seen to be variable with age amongst females in the Framingham Study. MVP rates were found to be as high as 17% in females aged 20–29 years but only 1% in females aged in their 80s. (Savage et al., 1983).

Pathophysiology

MVP is caused by myxomatous degeneration with subsequent expansion and prolapse of the cusp. (Hanson et al., 1996) The chordae tendineae also experience elongation and may rupture with resultant loss of leaflet tethering (Jeresaty et al., 1985). The commonest flail type involves the middle posterior scallop (P2) (**Fig. 3**). These patients can deteriorate from an initially benevolent asymptomatic systolic click to a mid–late systolic murmur, and later to a pansystolic murmur with or without left heart dilation.

While often sporadic, there appears to be a genetic component to MVP with an autosomal dominant mode of inheritance seen in documented familial cases (Hayek et al., 2005). MVP is also seen among inherited connective tissue disorders such as Marfan's and Ehlers–Danlos syndrome.

Signs and Symptoms

Many patients with MVP may remain asymptomatic even in the presence of significant MR. However, a subset of patients may develop a constellation of symptoms including atypical chest pain, panic attacks, palpitations, dyspnea, exercise intolerance, and syncope. On auscultation, a midsystolic click with a late-systolic murmur can be best heard over the cardiac apex.

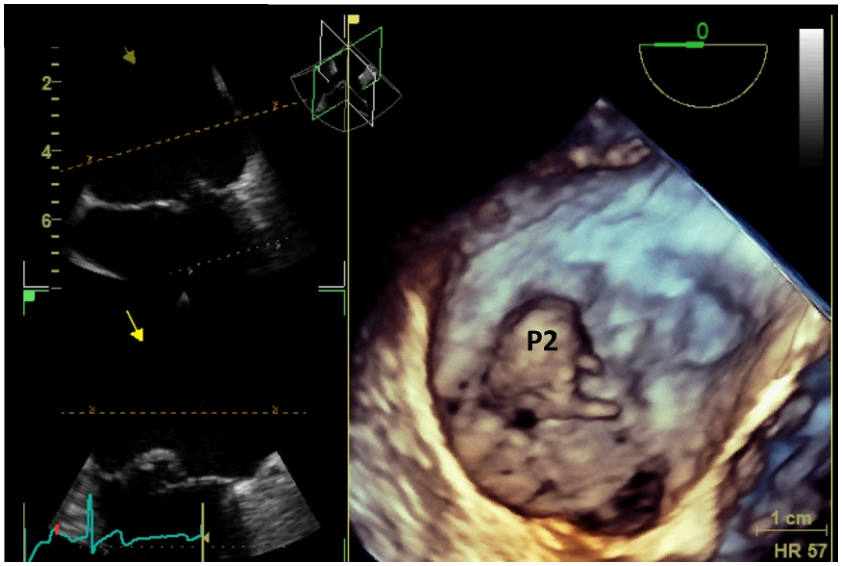

Fig. 3 Three-dimensional echocardiographic view of a flail the middle posterior segment (P2) of the posterior mitral valve leaflet as seen from the left atrium (the surgeon's view).

Diagnosis

TTE is used to investigate MVP (Class I) (Nishimura et al., 2017). Owing to the mitral valve's saddle shape, normal mitral valves may be detected as false positives. To minimize this, diagnosis is most reliable when assessed on parasternal long-axis or apical three-chamber view. Electrocardiography and chest radiographs are likely to be normal with the exception of severe MR.

Medical Therapy

In the asymptomatic patient, no specific treatment is necessary. Nor do they require antibiotic prophylaxis for endocarditis (Nishimura et al., 2017). Beta-blockers may relieve symptoms in certain patients but would not influence disease course (Shah, 2010). Patients with MVP but no MR should be reviewed every 3–5 years. Echocardiography should be performed in the patients with new onset symptoms or suspicious clinical examination. Those with severe MR should have yearly echocardiography performed (Nishimura et al., 2017).

Surgical Treatment

Surgery is indicated in the presence of any severe MR presuming low operative risk and a high likelihood of repair (Nishimura et al., 2017). In general, surgery for MVP is generally associated with low mortality and low reoperation rates (at 10 years, survival was 88.1% and freedom from reoperation was 91.1% in one study) (Glauber et al., 2015).

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Diuretic Therapy

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Abbreviations

ADHF	Acute decompensated heart failure
AHF	Acute heart failure
GFR	Glomerular filtration rate
HF	Heart failure

Introduction

Despite limited evidence, diuretic therapy has become a first-line therapy for the management of patients with acute heart failure (AHF). AHF is a life-threatening clinical syndrome that is characterized by an abrupt onset of symptoms, usually necessitating hospitalization. While this syndrome can occur de novo, it most frequently occurs in patients with a known diagnosis of HF in whom fluid has accumulated over days to weeks. In extreme cases, AHF can lead to cardiogenic shock, due to a low cardiac output state and a low systemic blood pressure, collectively preventing adequate end-organ perfusion. However, a consistent feature across the spectrum of AHF is fluid retention, which can manifest as ankle swelling, ascites, and/or pulmonary edema. Therapeutic strategies to relieve congestion can relieve symptoms and improve a patient's quality of life.

Before modern diuretics therapy was available, fluid retention was managed using rotating tourniquets to reduce cardiac preload, the insertion of Southey tubes subcutaneously to facilitate the drainage of fluid (Southey, 1877), recurrent venesection (Krishnakumar et al., 2007), and use of digoxin (Withering, 1785). In the 1920s, mercury-based therapies were noted for their diuretic effect. First noted by a medical student when these mercury-based agents were used for the treatment of syphilis, Merbaphen (novasurol) became a popular diuretic that could be administered as an intramuscular injection for the management of HF. It was not until the 1950s that more recognizable diuretics were introduced, first with the discovery of thiazide diuretics (Novello and Sprague, 1957) and later, in the 1960s, with loop diuretics (Robson et al., 1964). Such discoveries were considered revolutionary for the treatment of HF.

In the present era, the evidence supporting diuretic usage is lacking. While there is evidence to support their role in effectively relieving congestion and thereby attenuating brain natriuretic peptide levels (Palazzuoli et al., 2015), their effect on cardiovascular and all-cause mortality is largely derived from small under-powered randomized studies (Felker et al., 2009). Nevertheless, diuretic therapy within HF is considered a "class I" recommendation from international guidelines, meaning usage is generally considered beneficial (Yancy et al., 2016; Ponikowski et al., 2016). International guidelines suggest diuretics should be used for the relief of dyspnea and edema in patients with signs and symptoms of congestion, irrespective of left ventricular ejection fraction, with the

specified aim of achieving and maintaining a euvolemic state. They also recommend the use of the lowest possible dose of diuretic to achieve euvolemia to prevent hypotension, worsening renal function and neurohormonal activation. A lower dose of diuretic may enable up-titration of a disease modifying agents such as angiotensin converting enzyme inhibitors or sacubitril/valsartan, beta-blockers, and mineralocorticoid receptor antagonists (Ponikowski et al., 2016; Yancy et al., 2016). In this review, we discuss the evidence base for the use of these modern diuretics in the management of AHF.

Types of Diuretic

There are four pharmacological classes of diuretics used in HF (Fig. 1):

(a) Loop diuretics (furosemide, bumetanide, torsemide, ethacrynic acid)
(b) Thiazide diuretics (hydrochlorothiazide, bendroflumethiazide, Indapamide, or the "thiazide-like" metolazone)
(c) Directly acting potassium-sparing diuretics (amiloride and triamterene)
(d) Mineralocorticoid receptor antagonists (spironolactone, canrenoate, and eplerenone).

Loop Diuretics

Loop diuretics act on the ascending limb of the loop of Henle, blocking the reabsorption of up to 20%–30% of filtered sodium by inhibiting the sodium, potassium, and chloride co-transporter. This results in an intense, and usually short-lived, diuresis. Loop diuretics must be delivered to the lumen of the nephron and its actions are dependent on glomerular filtration being sufficiently preserved.

Loop diuretics can be administered orally, intravenously as a slow injection, or as an infusion. They have a rapid onset of action, working within minutes when given intravenously or within 30 min when given orally (Maxwell et al., 2002). They have a short duration of action, and consequently they may have to be given several times in a day to maintain the diuretic effect and to minimize rebound sodium reabsorption. However, gut edema may alter the rate of absorption of loop diuretics, but this effect is variable across the drug class. For instance, oral bumetanide achieves higher rates of absorption when compared with oral furosemide when administered to patients with gut edema (Sica, 2003). Thus, switching from oral furosemide to oral bumetanide can often lead to a diuresis that would not have been achieved with oral furosemide. Bumetanide is also more potent than furosemide, with 1 mg of oral bumetanide equivalent to approximately 40 mg of furosemide, and demonstrates higher bioavailability (approximately 70%) compared with oral furosemide (varying from 10% to 110%) in patients with marked fluid retention or gut edema. Furthermore, as furosemide is renally excreted, it can accumulate if administered to patients with renal dysfunction. Bumetanide and torasemide undergo hepatic elimination. However, torasemide has a longer half-life (3–4 h), meaning it should be administered less frequently than furosemide and bumetanide.

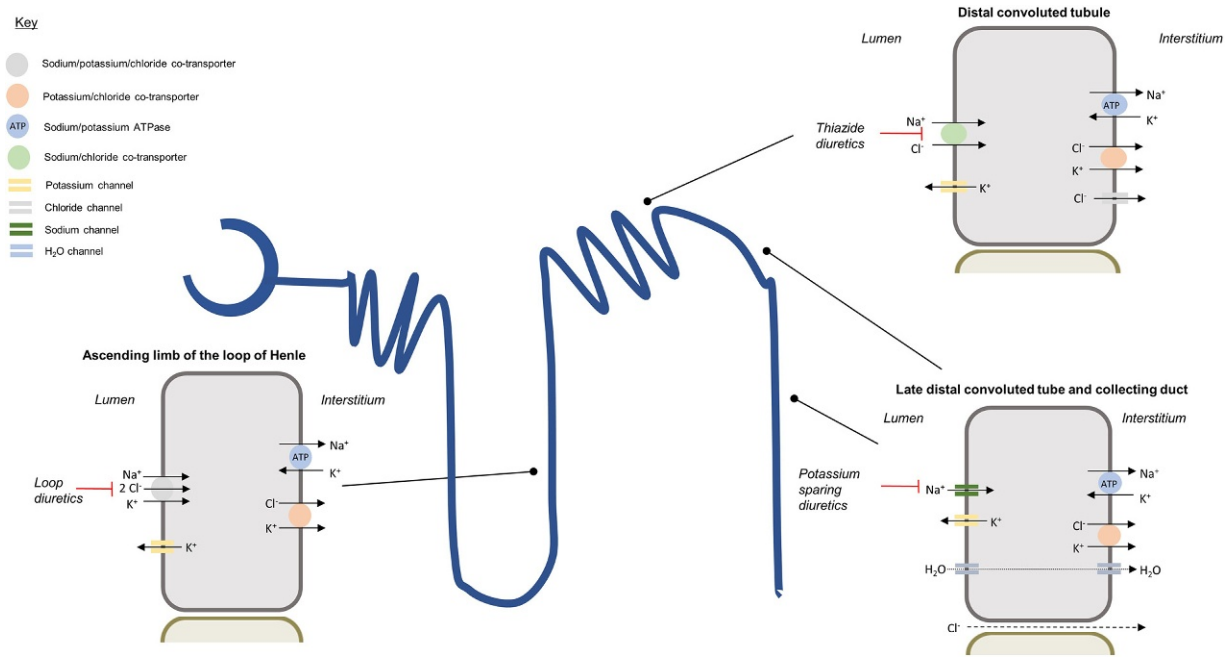

Fig. 1 Highlights mechanisms of actions of the different diuretics on the different areas of the nephron.

Table 1 Summary of common side effects of diuretics in acute decompensated heart failure

Hypokalaemia with loop and thiazides
Hyperkalemia with potassium sparing diuretics
Hyponatremia more frequent with thiazides than loop
Impaired glucose metabolism with loop and thiazides
Ototoxicity with high-dose loop
Acute gout with loop and thiazide
Activation of RAAS and sympathetic nervous system leading to progression of LV dysfunction

RAAS, renin-angiotensin-aldosterone system.

All loop diuretics may cause changes in systemic hemodynamics that are initially unrelated to the degree and extent of natriuresis that they induce. For instance, short-term administration of furosemide can lead to a rapid increase in venous capacitance and a decline in cardiac filling pressure, coincident with a rise in plasma renin activity. This effect predominates over any rise in systemic vascular resistance in patients with pulmonary edema or decompensated AHF (Brater et al., 1984a).

The side-effects of loop diuretics are summarized in **Table 1**, this includes: risk of ototoxicity (highest with ethacrynic acid); transient hearing loss, which may occur in patients receiving rapid intravenous bolus injection—so injection at a rate above 4 mg/min is not recommended; and permanent sensorineural hearing loss, which may occur at doses equivalent to furosemide 1000 mg per day (Rybak, 1985).

Thiazide and Thiazide-Like Diuretics

Thiazide diuretics such as bendroflumethiazide, hydrochlorothiazide, and indapamide act on the distal tubule by inhibiting sodium and chloride reabsorption. This results in a ~10%–15% reduction in sodium reabsorption. Compared with loop diuretics, while associated with a slower onset (1–2 h), they offer a more prolonged (12–24 h) and milder diuretic effect. The combination of a mild diuretic effect and long duration of action produces sodium excretion comparable to loop diuretics throughout 24 h (Vermeulen and Chadha, 1982). However, this also results in side effects such as nocturia, in addition to hypokalemia. Administered as a solo agent, thiazides are ineffective in individuals with a glomerular filtration rate below 30 mL/min. However, when thiazides, or indeed thiazide-like diuretics such as metolazone, are used in combination with loop diuretics, they facilitate sequential nephron blockade and are useful when managing refractory edema. Unlike thiazide diuretics, thiazide-like diuretics also act on the proximal tubule, where 60%–70% of sodium is reabsorbed and therefore can produce a profound diuresis.

Potassium Sparing Diuretics

Potassium sparing diuretics (such as amiloride) produce a mild diuretic effect by blocking the sodium/potassium exchange pump in the distal tubule. This exchanger is highly active in patients with HF who receive both a loop and thiazide diuretic. As potassium sparing diuretics tend to have a weak diuretic effect, they are mainly used in combination with thiazide or loop diuretics to prevent hypokalemia, as they are more effective than potassium replacement (Townsend et al., 1984; Ghosh et al., 1987; Kohvakka, 1988). However, usage is associated with a risk of hyperkalemia, particularly in patients with known renal dysfunction (Krishna et al., 1988).

Low-dose aldosterone (mineralocorticoid) receptor antagonists tend not to be used principally for diuresis in HF, but instead are administered for their potent antifibrotic properties to patients with chronic HF (RALES, 1996). However, in patients with fluid retention associated with right-sided HF, liver impairment, and ascites, very high circulating levels of aldosterone are detectable and high doses of aldosterone receptor antagonists (200–400 mg/day) can be useful in this setting for a diuretic effect.

There is increasing evidence, from multiple small studies, that high doses of spironolactone (>100 mg /day) can induce a potent natriuresis, sufficient to provide a negative sodium balance in patients resistant to loop diuretics (Chamsi-Pasha et al., 2014). The ATHENA-HF (Aldosterone Targeted Neurohormonal Combined With Natriuresis Therapy in HF) trial is a randomized, double-blind, placebo-controlled trial that aims to further evaluate the role of high-dose spironolactone (>100 mg/day) compared with placebo (or continued low-dose spironolactone use) on N-Terminal pro-Brain Natriuretic Peptide (NT-proBNP natriuretic peptide) levels for hospitalized AHF patients (Butler et al., 2016). The investigators of this study hypothesize that in patients with AHF, the early use of higher doses of spironolactone will produce a significant reduction in NT-proBNP, from randomization to 96 h, compared with standard care. This study should provide more insight into the safety and efficacy of higher doses of spironolactone in AHF and substantiate current clinical concerns relating to acute kidney injury and hyperkalemia.

Evidence Base for the Use of Diuretics in AHF

The evidence base supporting diuretic use in AHF is limited. A summary of contemporary trials evaluating diuretic use within acute decompensated HF (e.g., trials containing patients hospitalized with worsening symptoms or those with New York Heart Association II–III characterized by fluid retention) is detailed in **Table 2**.

Table 2 Summary of contemporary trials evaluating diuretic use within acute decompensated heart failure patients

Trials of diuretics for ADHF	*Sample size*	*Population studied*	*Trial design*	*Diuretic drug*	*Outcomes*
Placebo-controlled trials					
Kouroukli, 1976	30	ADHF	Cross-over	Bumetanide vs. furosemide vs. placebo	Effective water excretion with both loop diuretics
Haerer, 1990	60	NYHA II-III	Unblinded, controlled, nonrandomized	Piretanide vs. placebo	Improvement in all clinical and hemodynamic parameters in the absence significant side effects
Kleber, 1990	247	Mild CHF	Multicentre, double-blind, double-dummy, randomized	Oral HCT vs. ibopamine vs. HCT + ibopamine vs. placebo	Greater reduction in body weight with diuretics
Patterson, 1994	66	NYHA II-III	Randomized, parallel, double-blind, multicenter	Oral torasemide 5 mg vs. torasemide 10 mg vs. placebo	Greater reduction with Torasemide
Sherman, 1986	38	Mild-moderate CHF	Double blind, randomized, parallel	Piretanide vs. placebo	At 28 days, improved symptoms with piretanide
Thiazide vs. loop diuretic					
Stewart, 1965	11	ADHF, Resistant edema	Cross-over trial	Furosemide vs. bendrofluazide vs. Ethacrynic acid	Furosemide at doses of 200 mg leads to effective diuresis, similar to ethacrynic acid, and better than bendrofluazide
Crawford, 1988	47	CCF, primary care based	Unblinded	Frusemide-amiloride vs. cyclopenthiazide-potassium	Furosemide lead to better response, more free from PND and orthopnoea
Gonska, 1985	30	NYHA II-III	Open, controlled, randomized study	Piretanide vs. hydrochlorothiazide-triamterene	Significant Weight loss in both groups. Piretanide group were more recompensated (10 vs. 4)
Gabriel, 1981	18	Elderly with cardiac edema	Cross-over trial design	Bendrofluazide, frusemide and bumetanide	Bumetanide least change in plasma potassium compare to bendrofluazide
Comparison between loop diuretics					
Noe, 1999	240	NYHA II-III	Prospective study, randomized	Torasemide vs. furosemide	At 6 months, Torasemide group were heavier but had better quality of life at 1 month only
Stroupe, 2000	193	ADHF	Prospective, randomized, nonblind study	Torasemide vs furosemide	At 1 year Torasemide leads to lower readmission to hospitalization 18 vs. 34%; $P = 0.013$. Torasemide was more cost-effective
Murray, 2001	234	ADHF	Open-label trial	Oral torasemide vs. furosemide	Less readmission with torasemide 32% vs. 19%; $p < 0.01$ at 1 year and more improved dyspnea and fatigue scores
Muller, 2003	237	Primary care NYHA II-IV	Open randomized trial	Oral torasemide vs. furosemide	At 9 months, more clinical improvement and better quality of life in patients with Torasemide
Low dose vs. high dose loop					
Felker, 2011	308	ADHF	Prospective, 2 × 2 factorial design, double-blind, randomized trial	IV furosemide bolus low vs. high dose vs. continuous low vs. high dose	Over 72 h. No difference for symptoms or renal function between IV bolus vs. continuous infusion. Higher dose (2.5 × oral dose at admission) had better secondary outcomes such as relief of dyspnea, change in weight, fluid loss, with worsening renal function, but there was no worse 60-day outcome
Combined thiazide and loop diuretic					
Sigurd, 1975	18	ADHF	Randomized cross-over trial, single dose	Bumetanide and bendrofluazide	More diuresis with combined treatment
Channer, 1994	33	ADHF	Randomized	Iv furosemide and bendrofluazide or metolazone	Bendrofluazide and metolazone were equally effective in establishing a diuresis

Placebo-Controlled Trials of Diuretics

Placebo-controlled, randomized trials that have evaluated diuretic therapy in HF have been limited to a relatively small number of patients, ranging from 3 to 247 patients. These studies reported that diuretics significantly improve symptoms in HF (Kourouklis et al., 1976; Haerer et al., 1990; Kleber and Thyroff-Friesinger, 1990; Patterson et al., 1994; Sherman et al., 1986; Stewart and Edwards, 1965). However, none were powered to estimate the effect on mortality, but a meta-analysis of three short-term studies reporting mortality up to 12 months suggested a 75% mortality reduction [95% CI 16%–93% $P = 0.03$], but the main issue with this analysis was that the number of deaths was low—12 in the placebo group and 3 in the diuretic group—and the majority of the patients were unlikely to have met the contemporary definition of acute decompensated HF (Faris et al., 2002).

Thiazide Versus Loop Diuretics

Several small studies suggest that loop diuretics appear to be more effective at decongestion than thiazides alone in the management of HF (Crawford et al., 1988; Gonska and Kreuzer, 1985; Stewart and Edwards, 1965). Thiazide diuretics have a less harsh onset and longer duration of action, and for this reason they may be more acceptable to patients over loop diuretics (Funke Kupper et al., 1986). However, they are also more likely to result in hypokalemia and hyponatremia (Stewart and Edwards, 1965; Gabriel and Baylor, 1981; Vermeulen and Chadha, 1982).

Comparison Between Loop Diuretics

Comparisons between loop diuretics have principally evaluated torasemide and furosemide, with findings of these studies summarized in **Table 2**. Assessed using a randomized and unblinded study design, oral torasemide appears to offer a lower HF-related readmission rate compared to oral furosemide (Noe et al., 1999; Stroupe et al., 2000; Murray et al., 2001; Muller et al., 2003). It has been suggested that torasemide is also better tolerated by patients, compared with furosemide, with less urinary urgency (Muller et al., 2003). However, international guidelines provide no recommendations between specific loop diuretics.

Dose of Loop Diuretic and Bolus Versus Continuous Infusion of Loop Diuretic

The Diuretic Optimization Strategies Evaluation (DOSE) trial, assessed the initial diuretic strategies in patients with acute decompensated HF (Felker et al., 2011). DOSE used a 2×2 factorial design and randomized 308 acutely decompensated HF patients to receive intravenous furosemide, either as a twice-daily bolus or a continuous infusion, using either a low-dose (intravenous dose numerically equivalent to the patient's oral dose) or a high-dose (an intravenous dose 2.5 times higher than the patient's oral dose) regime. Dose adjustments were permitted following 48 h of intravenous therapy. At 72 h, there were no significant differences in either: (1) global assessment of symptoms or (2) change in serum creatinine; the studies predefined coprimary endpoints. Patients randomized to receive a high-dose intravenous furosemide regime reported an improvement in dyspnea (area under the curve for dyspnea 4478 ± 1550 in low dose vs. 4669 ± 1496 in high dose, $P = 0.04$), reduction in weight (-6.1 ± 9.5 lbs in low dose vs. -8.7 ± 8.5 lbs in high dose, $P = 0.01$), and greater net fluid loss (3575 ± 2635 mL in low dose vs. 4899 ± 3479, $P = 0.001$). However, the high-dose intravenous furosemide regime was also associated with a greater risk of serum creatinine measurements rising by >0.3 mg/dL within 72 h (23% vs. 14%, $P = 0.04$). There was no difference in length of initial hospital stay or in days alive and out of hospital at day 60. In addition, there were no statistically significant differences between continuous infusion or twice-daily bolus injection of furosemide in terms of the patients' global assessment of symptoms, mean change in serum creatinine, net fluid loss, change in weight, or length of hospital stay. This supported findings from a previous study (Allen et al., 2010).

While the DOSE trial did not achieve either coprimary endpoint, secondary outcome measures suggest that high-dose intravenous furosemide can enable rapid decongestion, with concomitant symptomatic relief, at the expense of an increased risk of renal dysfunction, with no medium-term clinically significant consequences.

Door to Diuretic Time and Prognosis

In a prospective registry based study assessing the time taken to give diuretics to patients presenting with acute decompensated heart failure, Matsue et al found that among 1291 patients, door to diuretic time of less than 60 minutes was associated with lower in-patient mortality (2.3 versus 6, P=0.002) and in a multivariate analysis, patients receving diuretics within 60 minutes had lower inpatient mortality (odds ratio of 0.39 95% confidence interval (0.20 to 0.76); p=0.006) (Matsue et al., 2017).

Diuretic Resistance

A frequent problem experienced when treating patients with advanced HF is diuretic resistance. Diuretic resistance is a term used when the diuretic response is either diminished or lost before the therapeutic goal can be reached. Diuretic resistance is common, which occurs in up to one-third of hospitalized patients (Ravnan et al., 2002), and is associated with a poor prognosis (Neuberg et al., 2002). There are several mechanisms that contribute toward diuretic resistance (see **Fig. 2**), including:

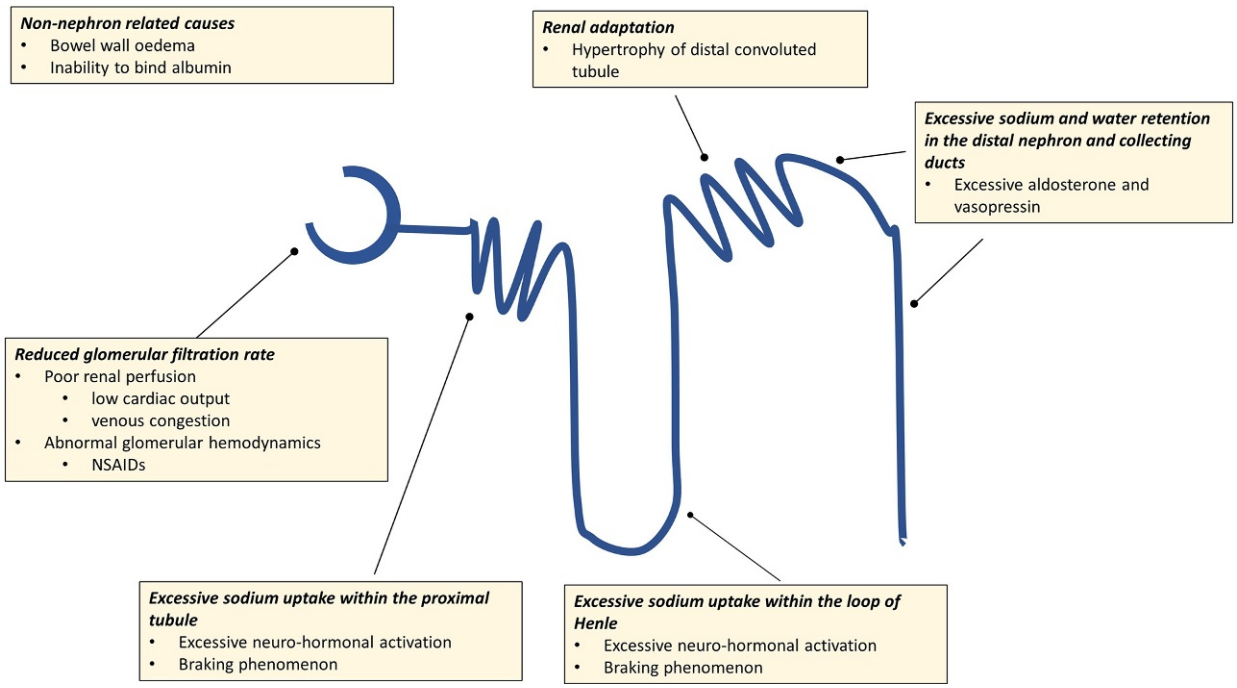

Fig. 2 Highlights the different mechanisms of diuretic resistance. Some are not related to the nephron and some involving different parts of the nephron.

- *Decreased bioavailability of diuretics*: In congested states, bowel wall edema may lead to reduced diuretic absorption. This effect is particularly noticeable with oral furosemide (Brater et al., 1984b; Vasko et al., 1985).
- *Reduced glomerular filtration rate* (*GFR*): A reduction in the GFR can occur due to poor renal perfusion, secondary to either low cardiac output or venous congestion. Additionally, chronic kidney disease or acute kidney injury can also prevent diuretics from entering the nephron's proximal tubule lumen. Both mechanisms reduce the delivery of diuretics and therefore any potentially beneficial diuretic effects cannot be harnessed (de Silva et al., 2006).
- *Excessive sodium uptake within the proximal tubule, loop of Henle, and the distal tubules* may occur secondary to the following mechanisms:
  - *E*xcessive neuro-hormonal activation (renin-angiotensin system and vasopressin) leading to excessive sodium and water retention throughout the proximal and distal tubules
  - The presence of the *Braking phenomenon*. This is a rebound of excessive sodium reabsorption that occurs when there is no diuretic detectable within lumen of either the proximal tubule or the loop of Henle. This typically occurs in the period between boluses of loop diuretics.
- *Renal adaptation*: The chronic use of diuretics may lead to hypertrophy of distal convoluted tubule, which may lead to more sodium and water resorption (Stanton and Kaissling, 1988; Kaissling and Stanton, 1988).
- *Drug interaction*: NSAIDS, aspirin (Bartoli et al., 1980), pioglitazone

Importantly, compliance with diuretic therapy needs to be addressed with the patient, their family, or their careers, prior to establishing a diagnosis of diuretic resistance.

Strategies for Decongestion in AHF Patients with Impaired Diuretic Responsiveness

Several strategies can be employed to aid the decongestion of patients with acute HF who manifest impaired diuretic responsiveness. These include diuretic and nondiuretic strategies.

Diuretic Strategies for Decongestion

1. Changing the route of administration from oral to intravenous (thus overcoming bioavailability issues);
2. Continuous infusion of loop diuretic rather than intermittent bolus injections (although this may only offer a minor improvement);
3. Using higher doses of intravenous loop diuretics to increase the diuretic dose reaching the tubules, particularly in the context of a low GFR;
4. Sequential nephron blockade by using a combination of diuretics (Kiyingi et al., 1990; Sigurd et al., 1975) such as metolazone or bendroflumethiazide in addition to a loop diuretic. This approach requires close monitoring, with attendant risks of marked electrolyte disturbance, hypotension, dehydration, and worsening renal function.

5. Restricting excessive dietary sodium and fluid intake may improve diuretic resistance, as less sodium and water are processed by the nephron. This strategy can be unpleasant for patients and should be limited to not less than 1.5 L of fluid intake daily.

Nondiuretic Strategies for Decongestion (Summarized in Table 3)

1. Renal dose dopamine.

   The exact mechanism through which renal dose dopamine (2–3 mcg/kg/min) increases renal blood flow remains debated, but is likely to involve a combination of increased cardiac output (via beta-1 receptors, traditionally associated with doses of 5–10 mcg/kg/min) and reduced renal and peripheral vasodilation (via peripheral dopaminergic receptors). (Bock and Gottlieb, 2010; Elkayam et al., 2008).

   The addition of dopamine to standard therapy for patients with AHF and a preserved systolic blood pressure have been evaluated in two reasonably sized randomized trials.

   - In the DAD-HF II trial (Triposkiadis et al., 2014), 161 patients with AHF with mean systolic blood pressures of 157 ± 28 mmHg were randomized to dopamine 5 mcg/kg/min with furosemide vs. furosemide alone. The trial was prematurely discontinued due to a high incidence of tachycardia and the lack of benefit within the low-dose dopamine arm, with respect to 60-day or 1-year mortality, hospitalization for HF, or overall change in dyspnea score.
   - In the ROSE-AHF trial (Chen et al., 2013b), patients with AHF and a median systolic blood pressure of 114 (104–127) mmHg received low-dose dopamine at 2 mcg/kg/min, but did not significantly increase their cumulative urine output or improve renal function, as measured by cystatin C levels at 72 h. In addition, there were no significant differences between secondary endpoints such as HF rehospitalization rate, death, or adverse events for 60 days. The incidence of tachycardia was higher in the dopamine group.

   Therefore, current data do not support the use of dopamine in nonhypotensive patients presenting with AHF. However, the role for low-dose dopamine in AHF with hypotension, in the absence of cardiogenic shock, merits further study. In our own clinical practice, we do use renal dose dopamine in congested patients with impaired diuretic responsiveness, who demonstrates a systolic blood pressure of 80–100 mmHg to aid decongestion.

2. *Vasopressin receptor antagonists* (*aquaretics*)

   Aquaretics promote the loss of electrolyte-free water by inhibiting vasopressin 2 receptors, which subsequently prevents recruitment of aquaporin 2 channels to the luminal cell membrane within the nephron's collecting duct. Furthermore, aquaretics use is not associated with many of the side effects seen with traditional diuretics, with no alteration in renal blood flow, glomerular filtration rate, changes in serum potassium, creatinine or osmolality, and no activation of either noradrenaline or plasma renin (Narayan and Mandal, 2012). Aquaretics are particularly useful in the context of euvolaemic or hypervolaemic hyponatraemia (sodium <135 mmol/L). The effectiveness of tolvaptan, a vasopressin 2 receptor antagonist, was assessed in the Efficacy of Vasopressin Antagonism in HF Outcome Study with Tolvaptan (EVEREST) trial. This involved over 4100 patients with AHF, randomized to tolvaptan or placebo. Tolvaptan had no effect on total mortality or HF hospitalization when compared to placebo (Konstam et al., 2007; Gheorghiade et al., 2007). However, tolvaptan did significantly improve hyponatremia in patients with a baseline serum sodium of less than 134 mmol/L and edema score at day 7. The effect on serum sodium was maintained throughout a maximum of 40 weeks of treatment.

3. *Adenosine antagonists*

   Adenosine antagonists can potentially increase glomerular filtration and enhance the diuretic effect of diuretic drugs. The effectiveness of rolofylline, a selective A1 adenosine receptor antagonist, was assessed in the PROTECT trial, in which 2033 patients hospitalized with AHF were randomized to rolofylline or placebo. Rolofylline did not have any beneficial effect and was associated with an increased risk of seizures (Massie et al., 2010).

4. *Hypertonic saline to facilitate effect of loop diuretics*

   Several randomized single-blind studies from a single group have demonstrated that a combination of a hypertonic saline infusion (150 mL of 3% sodium chloride) and 250 mg furosemide infusion is superior to a furosemide infusion alone, in terms of increasing diuresis and serum sodium levels, and in reducing the length of hospital stay. They also reported that there was lower mortality (12.9% vs. 23.8%; $P < 0.0001$) and readmission rate (18.5% vs. 34.2%; $P < 0.0001$) at 57 months of follow-up (Paterna et al., 2011). The mechanism of action is unclear, but expansion of intravascular volume, improved renal blood flow, and shift of fluid from the interstitium into the circulating volume (Tuttolomondo et al., 2011) have been postulated as potential mechanisms. This method of decongestion and diuresis requires replication in other centers and in double-blind studies.

5. *Other alternative strategies aiding diuresis*

Table 3 Trials involving nondiuretic strategies for patients with HF

UNLOAD trial (Costanzo et al., 2007)
ROSE-HF trial (Chen et al., 2013a)
CARRESS-HF trial (Bart et al., 2012)
EVEREST trial (Konstam et al., 2007)
PROTECT trial (Massie et al., 2010)

Several alternative agents may also be helpful in improving responsiveness to diuretics. These include glucocorticoids and levosimendan

- Glucocorticoids may promote diuresis and protect renal function in patients with AHF. Liu and colleagues reported the effects of prednisolone (1 mg/kg/day or 60 mg/day) given over 4 weeks (followed by a tapering-off regimen) in patients with diuretic resistant AHF with mean furosemide dose of 212 ± 25 mg/day in addition to a thiazide and spironolactone in an unblinded uncontrolled study of 13 patients (Liu et al., 2007). They reported an improved diuresis after a 3–4-day lag period, weight loss (9.4 ± 3 kg), and an improved renal function with a fall in serum creatinine (55 ± 49 umol/L) over a median of 20 (11–40) days. The proposed mechanism of action of glucocorticoids includes: (1) increased expression of natriuretic peptide receptor-A (NPR-A) in the kidney and the hypothalamus: a mechanism that appears reduced in patients with HF (Liu et al., 2011) and (2) increasing renal blood flow through dilatation of the renal vasculature via increased renal prostaglandin, nitric oxide, and dopamine production (Massari et al., 2012). Subsequently, an open-label randomized controlled study assessing the effects of prednisolone on outcomes compared to standard care was designed (Liu et al., 2014). This study was terminated prematurely due to poor recruitment as patients were reluctant to receive glucocorticoids. From the 102 patients with AHF who were randomized to receive glucocorticoids or standard treatment, the glucocorticoid arm demonstrated an improvement in renal function at 7 days (serum creatinine -0.14 vs. -0.02 mg/dL ($P < 0.05$)) and improved symptoms. Thirty-day survival was better in the group receiving glucocorticoids compared to standard care; however, the number of deaths was low (3 deaths in glucocorticoid vs. 10 deaths in standard treatment group, $P < 0.05$). Further research is needed to confirm whether glucocorticoids may be beneficial in this setting.
- Levosimendan, an inodilator and calcium sensitizer, was studied in patients presenting with AHF in the REVIVE studies. These showed that when levosimendan was given intravenously, with a loading dose and as 24-h infusion, to patients with AHF, there was improved renal function and improved responsiveness to diuretics leading to rapid relief of symptoms (Packer et al., 2013). However, side effects including an increased risk of arrhythmia and hypotension were also reported. In our clinical practice, levosimendan is given without a loading dose and instead given after 24–72 h at low doses, such as 0.05 mcg/kg/min (**Table 4**).

Table 4 Diuretic trials in progress

Trial	*Sample size*	*Inclusion criteria*	*Trial design*	*Intervention*	*Primary outcome*
Safety and efficacy of the combination of loop with thiazide-type diuretics in patients with decompensated HF (CLOROTIC Trial) Trullas et al (NCT01647932) Change in body and patient reported dyspnoea in 24 h	304	Admission for acute decompensated HF	Double-blind,	randomized, placebo-controlled trial	Loop + thiazide diuretic therapy vs. loop diuretic therapy
Loop diuretic dosage in patients with AHF and renal failure: conventional vs. carbohydrate antigen 125-guided therapy (IMPROVE-HF trial) Nunez et al (NCT02643147) Change in renal function at 24 and 72 h	170	Admission for acute decompensated HF and renal dysfunction	Double-blind,	randomized, placebo-controlled trial	CA 125 guided loop diuretic therapy vs. conventional loop diuretic therapy
Diamox/Aldactone to increase the urinary excretion of sodium: an investigational study in congestive HF (DIURESIS-CHF trial) (NCT01973335) 1. Spironolactone arm: incidence of hypo- (serum potassium <3.5 mmol/L) or hyperkalemia (serum potassium >5.0 mmol/L) [Time Frame: 72 h] 2. Acetazolamide arm: natriuresis 24 h	80	Admission for acute decompensated HF, renal dysfunction and serum sodium <136 mmol/L	Double-blind, factorial	assignment, randomized controlled trial	1. Upfront therapy with spironolactone and loop diuretic vs. loop diuretic 2. Azetolamide + low dose loop diuretic vs. high-dose loop diuretic
Prospective, randomized, parallel-group pilot study comparing IV furosemide to subcutaneous furosemide in acute decompensated HF patients Russell et al (NCT02579057) Urine output: The volume of urine produced in milliliters over the 6 h after drug delivery will be measured	40	History of at least 3 months treated HF (NYHA class II/III/IV), or recent hospitalization for HF; presenting to Heart Failure Bridge Clinic (HFBC) with decompensated HF symptoms	Prospective,	Randomized, Parallel-Group Pilot Study	Intravenous furosemide vs. Subcutaneous furosemide

Conclusions

While the evidence base supporting diuretic therapy in HF is not extensive, they remain a fundamental cornerstone to the management of patients with AHF for symptomatic relief and are endorsed by international guidelines. The initial management strategy likely involves administration of a loop diuretic, such as furosemide, and the evidence suggests that an initial "high dose" strategy either by twice daily bolus injection or continuous infusion is likely to be more successful than a slower, more "ramped" approach. In cases of diuretic resistance, the addition of a thiazide or thiazide-like diuretic may enhance diuresis, but this will require close monitoring of fluid balance and electrolytes. The addition of other strategies to improve the effectiveness of diuretic therapy also appears to have a limited evidence base, but may be useful in some populations.

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Driving and Syncope

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Introduction

Syncope is a common medical symptom, characterized by an abrupt and transient loss of consciousness and inability to maintain postural tone due to cerebral hypoperfusion, with rapid and spontaneous recovery (Sheldon et al., 2015).

Syncope is indicative of an underlying disease, and a common clinical condition seen by practitioners. In the general population, the estimated lifetime prevalence is approximately 20% (Chen et al., 2006). The incidence has predominantly a trimodal appearance with a peak at 20 and 60 years and at 80 years with the third peak occurring 5–7 years earlier in men (Ganzeboom et al., 2003; Ruwald et al., 2012). Among patients, there is almost a twofold increase in the incidence in patients 70–79 years old versus 60–69 years old (Soteriades et al., 2002). The incidence also is higher in patients with cardiovascular disease than without (Soteriades et al., 2002). In patients with syncope, approximately 3%–10% will have an episode while driving (Li et al., 2000; Maas et al., 2003; Sorajja et al., 2009).

Driving itself carries risk. Worldwide, approximately 1.3 million persons die annually from road crashes, which translates to about 3500 deaths per day. Another 20–50 million persons are estimated to be injured from road crashes. Traffic safety data from the United States compiled from 2014, reported some 6.1 million crashes, involving 32,675 deaths and 2.34 million injured persons. This rate equates to a person dying every 16 min due to a motor vehicle crash (Administration NHTS, 2014). While most accidents are attributed to human error, there remain a number of accidents, approximately 2%–11% that can occur from syncope with the potential for morbidity and mortality (Sorajja et al., 2009; Nume et al., 2016). In fact, patients with syncope have almost double the incidence rate of motor vehicle crashes (Nume et al., 2016). In addition, the amount of motor vehicle crashes related to sudden incapacitation is likely underreported, as many study analysis rely on patients who seek medical attention for their syncope. In one study, 29% of patients with syncope while driving reported an injury, but only 60% of these injured patients sought medical care for their injury (Sorajja et al., 2009).

Causes of Syncope

Previously the occurrence of sudden death while driving most commonly was attributed to coronary artery disease (Antecol and Roberts, 1990; Grattan and Jeffcoate, 1968; Halinen and Jaussi, 1994). More recent data show the etiologies for syncope while driving to be similar to the causes of syncope in the general population, with the most common cause being neurally mediated syncope (**Table 1**) (Sorajja et al., 2009; Blitzer et al., 2003). Tilt table testing in one small series confirmed a neurally mediated origin of syncope while driving (Li et al., 2000). This pathophysiology requires further evaluation, but the mechanism could have contribution from venous pooling, cutaneous vasodilatation from a warm car environment, and strong emotional stimulation while driving (Sorajja et al., 2009). One small study, evaluating patients with syncope with driving, found a vasodepressor response on head-up tilt table testing to be more common (Folino et al., 2012).

Among other identified etiologies, cardiac arrhythmias make up the next largest group (11.8%), with bradyarrhythmias being more common than ventricular arrhythmias, and supraventricular tachycardia (Sorajja et al., 2009). Interestingly, in the Triggers of Ventricular Arrhythmias (TOVA) study (Albert et al., 2007), the risk of ventricular tachyarrhythmias was not elevated during driving, but rather in the 30-minute period after driving. Of note, some patients have potentially more than one etiology, and many patients, approximately 20%–30%, continue to have an unknown cause for their syncope despite evaluation (Sorajja et al., 2009; Blitzer et al., 2003).

Syncope Recurrence

While the majority of patients have only one episode of syncope, approximately 12%–22% have recurrence (Soteriades et al., 2002; Sorajja et al., 2009). The recurrence of syncope mainly depends on correct diagnosis of the etiology and the efficacy of in-place

Table 1 Causes of syncope (Sorajja et al., 2009)

Cause of syncope	*Syncope while driving (n = 381)*	*Syncope not while driving (n = 3496)*	P *value*
Neurally mediated	142 (37.3)	1247 (35.7)	.54
Bradyarrhythmia	25 (6.6)	221 (6.3)	.86
SVT	8 (2.1)	71 (2.0)	.93
Ventricular tachyarrhythmia	20 (5.2)	130 (3.7)	.14
Structural heart disease	1 (0.3)	9 (0.3)	.99
Cerebrovascular disease	14 (3.7)	100 (2.9)	.37
CSH	12 (3.1)	100 (2.9)	.75
Orthostatic intolerance	18 (4.7)	223 (6.4)	.20
Others	87 (22.8)	1044 (29.9)	.004

Table 2 Estimated 12-month risk of syncope (Sumner et al., 2010)

Measure	*Categories*	N	*12-month syncope risk (%)*	*95% CI*	*Log rank statistic (df) (P-value)*
Number of spells in the previous year	0.1	55	21.9	8.8–33.2	33.4 (3)
	2.3	62	40.6	25.2–52.8	$P < .0001$
	4–6	49	49.4	30.3–63.3	
	>6	38	69.1	44.8–82.7	
Life time number of spells	<6	65	35.7	21.1–47.6	26.2 (3)
	6–9	39	31.4	47.2–10.9	$P < .0001$
	10–20	52	33.2	18.3–45.4	
	>20	48	71.1	51.6–82.8	
Frequency of spells prior to the previous year	≤0.15	49	49.6	30.6–63.5	12.1 (3)
	0.16–0.60	52	25.5	11.8–37.1	$P = .0071$
	0.61–1.65	51	41.6	23.8–55.2	
	>1.65	52	55.9	36.6–69.3	

Estimated 12-month survival rates with 95% CI and the log rank statistic.

treatment (Task Force for the Diagnosis and Management of Syncope et al., 2009). For patients with a cardiac cause or neurocardiogenic cause of their syncope, the risk of recurrence is increased compared to other etiologies (Soteriades et al., 2002; Sorajja et al., 2009). For vasovagal syncope, one predictor is the number of syncope events in the prior year. In patients with abnormal tilt table test with less than 2 syncopal episodes in the prior year, a 22% recurrence rate over 1 year was observed. In comparison, those patients with 2 or 3 syncopal episodes in the prior year had a 41% recurrence rate (**Table 2**) (Sumner et al., 2010). However, in a metaanalysis of vasovagal syncope, even among highly symptomatic patients, the spontaneous remission rate is high (Pournazari et al., in press). For patients presenting to an emergency department for evaluation of syncope, one meta-analysis showed recurrence to be almost linear over time increasing from 0.3% at 1 month to 22% at 24 months (Solbiati et al., 2015). For those patients with syncope while driving, the actuarial recurrence of syncope while driving is actually quite low, being 0.7% at 6 months (Sorajja et al., 2009). This recurrence rate overall is similar to those patients who have an index syncopal event not while driving (Sorajja et al., 2009). However, those patients with syncope not while driving tend to have more recurrences in follow-up (**Fig. 1**). In analysis of prospective data from the Prevention of Syncope Trial 1 and 2, the occurrence of syncope while driving was 0.48% and the estimated probability of syncope while driving was 0.62% per year; however it is unknown how much patients actually drove in this study (Tan et al., 2016).

In terms of prediction of morbidity within 7–30 days and mortality within 1 year of initial evaluation, a number of clinical tools are available to try and determine if patients require expedited work-up or not. Many of these risk scoring systems, such as OESIL, SFSR, Boston Syncope Rule, STEPS, and the Syncope risk score, most commonly include age and presence of an abnormal ECG (Colivicchi et al., 2003; Quinn et al., 2004; Grossman et al., 2007; Costantino et al., 2008; Sun et al., 2009). However, these studies are limited by sample size, inclusion of all syncope patients regardless of cause, and inconsistent definitions of syncope and its associated risk across these studies (Colivicchi et al., 2003; Quinn et al., 2004; Grossman et al., 2007; Costantino et al., 2008; Sun et al., 2009). As a general rule, these scoring systems should not replace clinical judgment, but can be considered as an adjunct in decision-making.

Rationale for Driving Privileges and Restrictions

In the United States and many developed countries, motor vehicle travel remains an important means of transportation. On a societal level, driving is a privilege, but for many persons, driving is a necessity due to demands such as from family and work. For a

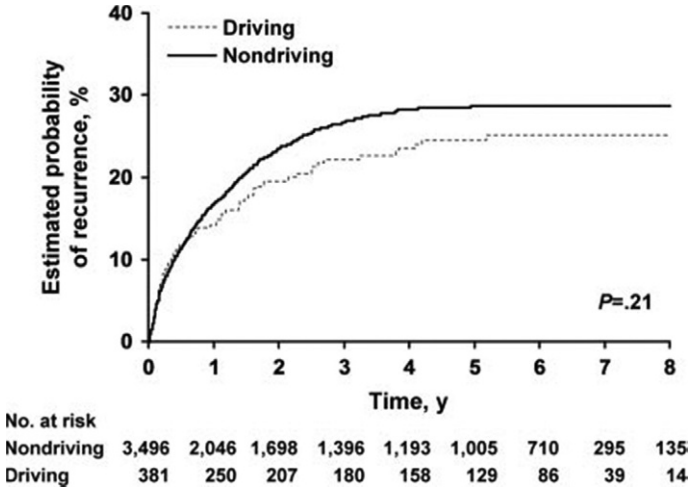


Fig. 1 Recurrence of syncope in syncope while driving and not while driving (Sorajja et al., 2009).

patient to be fit to drive, many factors come into consideration, which include knowledge of road laws, vision acuity, and alertness among others (Sorajja and Shen, 2010).

For the public interest, the main concern is whether a person can drive a vehicle safely enough to avoid causing harm to others. Restrictions on driving and driving guidelines have been developed to prevent or reduce motor vehicle accidents with the outcome being improved personal and public safety. However, accidents still occur and sudden driver incapacitation contributes to 1%–3% of all motor vehicle accidents, and 8%–10% are believed to be due to a heart condition (Petch, 1998; Epstein et al., 1996). Despite the incapacitation, only 2% or less of reported cases result in injury or death to other road users or bystanders (Ostrom and Eriksson, 1987; Parsons, 1986).

Determining Risk of Harm from Syncope While Driving

The determinant principal factors can be summarized in the risk of harm (RH) formula. This formula quantifies the amount of risk that can occur with sudden incapacitation, and then the level of risk can then be judged as acceptable or not. Society recognizes that certain driving groups, such as younger and elderly drivers, are allowed to drive despite their higher risk of potentially harming others (Simpson et al., 2004; Curtis and Epstein, 2009; Larsen et al., 1994). As such, policy should be fair to other higher risk groups, while considering the welfare of others where these higher risk individuals will drive. The threshold of acceptable risk may depend on the eye of the beholder. While a society may accept a limited number of higher risk drivers, the goal should not necessarily be to allow as many high-risk drivers as possible. Conversely, driving restrictions should not be prohibitive to certain higher risk drivers who number far fewer than the cohort of younger and elderly drivers.

The RH formula is derived from 4 components: (1) time spent driving or distance driven in a given time period (TD); (2) size of the vehicle driven (V); (3) risk of sudden cardiac incapacitation (SCI); and (4) the probability that incapacitation will cause a fatal or injury-producing accident (Ac). The formula can be expressed as:

$$\mathrm{RH} = \mathrm{TD} \times V \times \mathrm{SCI} \times \mathrm{Ac}$$

The time spent driving (TD) for noncommercial drivers is usually 4% daily (approximately 1 h) and less than 36,000 km per year or less than 720 h per year. For commercial drivers, the time spent driving is usually 25% daily (approximately 6 h) with longer distances and hours logged than the private driver definition (Simpson et al., 2004). Longer and shorter durations of driving can be accounted for with adjustment in this portion of the formula.

For the size of the vehicle (V), those with a weight over 3500 kg are attributed a value of 1, and this amount includes vehicles such as ambulances, semitrucks, and buses. Vehicles less than 3500 kg are assigned a value of 0.28. A list of representative vehicles and their weights are given in **Table 3**. Of note, motorcycles do not have a "V" assigned, but theoretically would be much lower than 0.28. Conversely, the weight of a bus can increase to 16,000 kg with a load of people and belongings, and the weight of a semi-trailer truck with a trailer can be up to 50,000 kg in the United States. With such a significant amount of mass, the "V" assigned should be theoretically different than a car weighing just over 3500 kg, but this difference is not accounted for in the formula.

The risk of sudden cardiac incapacitation varies among persons depending on their underlying conditions. Common conditions associated with syncope or sudden incapacitation are listed in **Table 4**. For commercial drivers, the risk of sudden cardiac incapacitation (SCI) is acceptable if their annual risk is 1% or less. This threshold is based on prior data from patients who are at least 3 months post-myocardial infarction and are able to complete seven METS on stress testing (Simpson et al., 2004).

Table 3 Types of Motor Vehicles and Weight (Sumner et al., 2010)

Moped/motorcycle	225–500 kg
Compact car	1350 kg
Midsize car	1600 kg
Large car	2000 kg
Compact truck/SUV	1600 kg
Midsize truck/SUV	1900 kg
Large truck/SUV	2500 kg
Ambulance	5500 kg
Semi-trailer truck	10,500 kg
Bus	12,500 kg

Table 4 Causes of syncope and risk of loss of consciousness

Bradycardia: 4%–5% annually (Ng Kam Chuen et al., 2014; Aste et al., 2016)
Brugada syndrome: 28%–40% prevalence (Sacher et al., 2006, 2012)
Carotid sinus syndrome: 7%–40% annually (Gaggioli et al., 1995; Claesson et al., 2007)
Hypertrophic cardiomyopathy: 2%–19% prevalence (Kofflard et al., 2003; Kim et al., 2016)
Long QT syndrome: 17%–34% prevalence, 5% annually (Moss et al., 1991; Goldenberg et al., 2008)
Orthostatic intolerance: 12%–31% prevalence (Atkins et al., 1991; Sarasin et al., 2002; Beckett et al., 1999)
Supraventricular tachycardia: 4%–14% prevalence (Page et al., 2016; Walfridsson and Walfridsson, 2005)
Vasovagal syncope: 40% prevalence, 22%–69% (Sumner et al., 2010; Tan et al., 2016)
Ventricular arrhythmias in primary prevention patient: 2% annually (Thijssen et al., 2011; Freedberg et al., 2001; Bardy et al., 2005)
Ventricular arrhythmias in a secondary prevention patient: 8% annually (Thijssen et al., 2011; Freedberg et al., 2001; Bansch et al., 1998; Kim et al., 2015)

The probability of a fatal or injury-producing accident (Ac) to other road users or bystanders is approximately 2% for all drivers (Ostrom and Eriksson, 1987; Parsons, 1986).

When these data are plugged into the RH formula for a commercial driver:

$$\begin{aligned} \text{RH} &= \text{TD} \times V \times \text{SCI} \times \text{Ac} \\ \text{RH} &= 0.25 \times 1 \times 0.01 \times 0.02 \\ \text{RH} &= 0.00005 \end{aligned}$$

This RH equates to a 1 in 20,000 chance of harm from a commercial driver. As a result, for a noncommercial driver, the SCI resulting in the equivalent RH can be calculated after adjusting for time spent driving (TD) and the vehicle size (V):

$$\begin{aligned} \text{RH} &= \text{TD} \times V \times \text{SCI} \times \text{Ac} \\ 0.00005 &= 0.04 \times 0.28 \times \text{SCI} \times 0.02 \\ \text{SCI} &= 0.22 \end{aligned}$$

While a 22% chance of SCI may be acceptable for most noncommercial drivers, if the time spent driving increases or the size of vehicle increases, this SCI also increases and may no longer be acceptable. Conversely, for commercial drivers, if the time spent driving decreases or the vehicle size decreases, the RH may become acceptable.

Based on this RH formula, many untreated conditions, such as bradycardia or supraventricular tachycardia that result in syncope, would disqualify both commercial and noncommercial driver patients from driving. However, appropriate treatment, such as with a pacemaker or ablation, would reduce these patients' risk of SCI to less than 1% and allow for driving to be resumed (Brunner et al., 2004). Some conditions have a higher rate of recurrence and SCI, which may not allow for patients to drive on a temporary or even permanent basis. While the annual risk of SCI may be less than the 22% threshold, this fact does not preclude the presence of higher risk periods of time where the risk of SCI is excessive. For instance, as the time from a ventricular tachyarrhythmia event increases, the risk of incapacitating ventricular arrhythmias decreases. At some point, the RH may be acceptable for noncommercial drivers to resume driving, but in commercial drivers the acceptable risk threshold may never be reached.

Of note, ventricular arrhythmias should not be taken as an equivalent for syncope. For patients with an ICD who receive an appropriate shock for ventricular tachyarrhythmias, only 31% experience syncope or near syncope during their event (Thijssen et al., 2011; Freedberg et al., 2001). The degree and severity of symptoms including loss of consciousness depend on the rate and duration of the ventricular arrhythmia as well as a patient's underlying cardiac dysfunction (Zipes et al., 2006). For primary prevention patients, using SCD-HeFT data, ICD discharge occurred in 7.5% of patients annually including appropriate and inappropriate shocks. Patients with appropriate shocks comprised 5.1% of the overall cohort (Bardy et al., 2005). So for primary prevention patients, we would expect an annual rate of syncope of 1.6% (or 0.31×0.051). In addition, the presence of an ICD only addresses the risk of SCD, and appropriate ICD therapy is frequently too late to prevent loss of consciousness even when programmed to deliver an immediate defibrillation (Task Force for the Diagnosis and Management of Syncope et al., 2009; Olshansky et al., 2008;

Bansch et al., 1998). For secondary prevention patients, the risk of recurrent ventricular is approximately 25% annually (Fig. 2) (Bansch et al., 1998; Kim et al., 2015). Thus for these patients, we would expect an annual rate of syncope of 7.8% (or 0.31×0.25).

It should be emphasized that the RH formula is one method for determining RH from driving, but it should not replace clinical judgment. As clinicians, patients with the same condition may have very different prognoses depending on comorbidities, therapy, and compliance with therapy. For most conditions, there is incomplete data regarding recurrence of syncope or sudden cardiac incapacitation with and without optimal therapy. Intuitively, patients with syncope are more likely to have a motor vehicle crash, and the data bears that out (Nume et al., 2016). Certain patients are more likely to have a motor vehicle crash from syncope; these include an approximate twofold increased risk for men compared to women, and those patients aged 18–35 years (Nume et al., 2016).

In addition, the RH formula does not supercede local driving regulations. The authors strongly recommend that physicians, who care for syncopal patients, know the pertinent driving laws and restrictions, particularly in regard to the duty of the patient or the physician to report a patient's inability to drive a motor vehicle.

In our large retrospective cohort, the patients with syncope while driving had a more frequent occurrence of injury (28.6% vs. 23.7%) than those without episodes while driving, but there was no significant increase in injuries requiring hospital care (Sorajja et al., 2009). This finding may be related to neurocardiogenic syncope being the most frequent cause, and the presence of a prodrome being a protective factor. As a group, there is no increase in mortality for syncope while driving patients compared to age- and sex-matched controls (Fig. 3). Prior studies have suggested sudden natural death while driving poses no

		Event rate		Risk of SCI	
Event	ICD indication	Observed 6-mo	Observed 12-mo	Based on 6-mo event rate	Based on 12-mo event rate
Shock since the first therapy	ATP as the first therapy	9.9%	15.6%	0.058	0.048
	Shock as the first therapy	30.0%	39.3%	0.158	0.122
	Primary prevention	13.6%	19.4%	0.079	0.060
	Secondary prevention	21.7%	30.3%	0.120	0.094
	Primary prevention, ATP as the first therapy	6.8%	11.3%	0.041	0.035
	Secondary prevention, APT as the first therapy	16.1%	23.2%	0.092	0.072
	Primary prevention, shock as the first therapy	26.8%	35.3%	0.144	0.109
	Secondary prevention, shock as the first therapy	33.4%	45.0%	0.172	0.140
First shock since implant	All	4.9%	8.2%	0.003	0.025
	Primary prevention	3.5%	6.2%	0.021	0.019
	Secondary prevention	8.8%	13.7%	0.052	0.042
Second shock since the first shock	All	24.8%	34.3%	0.135	0.106
	Primary prevention	21.7%	30.7%	0.120	0.095
	Secondary prevention	29.2%	40.1%	0.155	0.124
Third shock since the second shock	All	40.5%	54.0%	0.200	0.167
	Primary prevention	41.2%	53.7%	0.203	0.166
	Secondary prevention	41.2%	56.7%	0.203	0.176

ICD = implantable cardioverter-defibrillator; SCI = sudden cardiac incapacitation.

Fig. 2 Risk of sudden cardiac incapacitation based on event rates of ICD shocks (Kim et al., 2015).

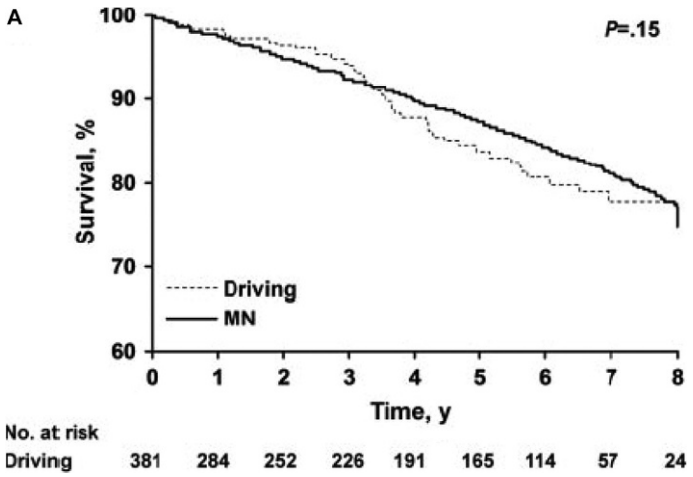
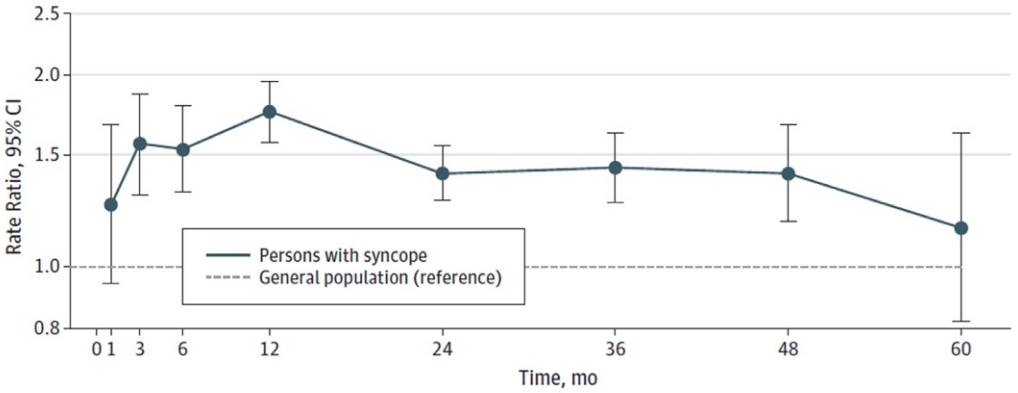


Fig. 3 Survival of patients with syncope while driving compared to the general population (Sorajja et al., 2009).



Months	0-1	1-3	3-6	6-12	12-24	24-36	36-48	48-60
Events, No.	47	114	158	317	391	252	130	33
PY	3277.9	6350.5	8948.2	15739.4	24603.5	16179.7	8963.8	2816.4
RR	1.25	1.56	1.53	1.75	1.40	1.43	1.40	1.15
95% CI	0.94-1.67	1.30-1.87	1.31-1.79	1.57-1.96	1.27-1.55	1.26-1.62	1.18-1.67	0.82-1.62
P Value	.13	<.001	<.001	<.001	<.001	<.001	<.001	.41

Fig. 4 Time elapsed from syncope and risk of motor vehicle crashes (Nume et al., 2016).

threat to other road users (Christian, 1988), but the presence of underlying cardiac disease, including ventricular tachycardia and myocardial infarction, carries a decreased survival rate similar to what has been shown in other studies (Sorajja et al., 2009; Kapoor, 1990). While some studies show the risk of recurrence to decrease from the index evaluation, one study found the risk of motor vehicle crash to remain elevated through 5 years of follow-up (**Fig. 4**) (Sorajja et al., 2009; Nume et al., 2016; Thijssen et al., 2011).

Ethical Considerations of Driving Restrictions

In the United States, private or noncommercial automobile driving is regulated by individual states which may or may not require reporting of a syncopal event. The laws for driving restrictions are a form of ethical judgments that attempt to balance the rights of the individual and the good of society. The two notions do not necessarily coincide, as an individual is allowed to act in whatever manner they choose as long as that action does not infringe upon the rights of others. Actual harm does not have to occur, as the potential for harm is the real threshold. For instance, drunk driving does not have to result in an accident, but the potential for harm is great enough that driving incapacitated due to alcohol is illegal. For medical providers caring for patients with syncope, the question distills to what is the risk of harm.

As a society, we accept a certain level of risk by letting higher risk drivers continue to drive. For teenagers aged 16–19 years in the United States, 2270 teenagers were killed by motor vehicle crashes and another 221,313 teenagers were injured in 2014 (Prevention CfDCa,https://www.cdc.gov/motorvehiclesafety/older_adult_drivers/). Considering this cohort comprises approximately 7% (22.4 million people) of the US population which was 320 million in 2014 (Bureau USC,https://www.census.gov/popclock/), the RH by teenage drivers is 0.01, which is significantly higher than what is established as acceptable (RH = 0.00005) for commercial drivers.

Similar for elderly drivers, those older than 65 years, in the United States, 50 older adults were killed and 214,000 were injured in motor vehicle accidents in 2012 (Prevention CfDCa,https://www.cdc.gov/motorvehiclesafety/older_adult_drivers/). Older adults make up 13% of the US population, which was 315 million in 2012, resulting in a cohort of 41 million (Bureau USC,https://www.census.gov/popclock/). With these numbers, the RH is 0.005, which is also quite a bit higher than the acceptable risk for commercial drivers.

Put another way, the risk for older adults and teenagers driving is 100–200 times higher than what has been deemed acceptable for commercial drivers. There are also aggravating and mitigating factors too. For instance, younger drivers tend to be more frequently intoxicated, wear seat beats less often, and drive at excessive speed (Halinen and Jaussi, 1994; Simons-Morton et al., 2005; Voas et al., 2012), but with more driving experience, we as a society expect their driving to improve. Elderly drivers, on the other hand, are cognizant of their limitations and tend to drive shorter distances and less so at night (Prevention CfDCa,https://www.cdc.gov/motorvehiclesafety/older_adult_drivers/).

Of note, many patients will not stop driving after being recommended to do so, regardless of how short or long the driving restriction is. Due to occupational and social demands on the patient, the compliance with driving restrictions is simply poor. The driving restriction given by most physicians is at least 6 months (Curtis et al., 1995; Akiyama et al., 2001). In the Antiarrhythmics versus Implantable Defibrillators (AVID) trial, the most common driving proscription was 6 to 11 months. However, 57% of

patients resumed driving within 3 months, and 78% within 6 months did so mainly because being unable to drive represented a "severe hardship" for 59% of the patients (Akiyama et al., 2001). In another study, 79% of patients remembered their physician-recommended driving restriction of 3 to 6 months, but 100% had continued to drive (Maas et al., 2003).

There are benefits to advising a driving restriction, however. Whether patients have or have not been shocked by their ICD, patients may continue to drive, but they impose self-restrictions such as driving shorter distances and only "if necessary"(Conti et al., 1997). In a large Canadian study of patients deemed unfit to drive, those patients who were warned by their physicians about driving had a 45% reduction in the annual rate of crashes (Redelmeier et al., 2012).

The probable need of some sort of driving restriction and the lack of compliance with it poses a legal and ethical morass. One key aspect is the principle of patient–physician confidentiality. Under almost any circumstance, a physician has an obligation to maintain confidentiality for a patient's medical condition. However, if a patient's condition poses a significant risk to others, then this information should not be withheld from the appropriate recipients or authorities. In this situation, the ethical principles of beneficence (doing good for society) outweigh the principle of confidentiality.

Driving Restriction Recommendations By Different Societies

The driving restrictions from American Heart Association, American College of Cardiology, and the Heart Rhythm Society are summarized in **Table 5** (Shen et al., 2017). The European Society of Cardiology (Task Force for the Diagnosis and Management of Syncope et al., 2009) has a separate set of recommendations, summarized in **Table 6**. While these recommendations do not cover all scenarios, they cover the far majority of them, and they still require physician discretion in applying them to patients.

There are several pertinent differences between the different societal recommendations. The main difference is the lack of recommendations for commercial driving in the AHA/ACC/HRS Guidelines. While commercial drivers' licenses are issued by each state and Washington D.C., these drivers are federally regulated in the United States by the Department of Transportation and Federal Motor Carrier Safety Administration who develop and issue the standards that must be met for a person to be a commercial driver. Other differences in the AHA/ACC/HRS include the separation of treatment by pharmacologic means and invasive procedures for a number of conditions such as supraventricular tachycardia, depressed ejection fraction with and without structural heart disease, and ventricular tachycardias (Task Force for the Diagnosis and Management of Syncope et al., 2009; Shen et al., 2017). Of note, whether the episode of syncope occurs while driving or not should not necessarily result in different treatment or driving restrictions (Sorajja et al., 2009; Sorajja and Shen, 2010).

Table 5 Suggested driving restrictions after an episode of syncope per AHA/ACC/HRS (Shen et al., 2017)

Condition	*Symptom-free waiting time*
OH	1 month
VVS, no faints prior year	No restriction
VVS 1–6 faints per year	1 month
VVS >6 faints per year	Not fit to drive until symptoms resolved
Situational syncope other than cough syncope	1 month
Cough syncope, untreated	Not fit to drive
Cough syncope, treated with cough suppression	1 month
Carotid sinus syncope, untreated	Not fit to drive
Carotid sinus syncope, treated with permanent pacemaker	1 week
Syncope due to nonreflex bradycardia, untreated	Not fit to drive
Syncope due to nonreflex bradycardia, treated with permanent pacemaker	1 week
Syncope due to SVT, untreated	Not fit to drive
Syncope due to SVT, pharmacologically suppressed	1 month
Syncope due to SVT, treated with ablation	1 week
Syncope with LVEF <35% and a presumed arrhythmic etiology without an ICD	Not fit to drive
Syncope with LVEF <35% and presumed arrhythmic etiology with an ICD	3 month
Syncope presumed due to VT/VF, structural heart disease and LVEF ≥35%, untreated	Not fit to drive
Syncope presumed due to VT/VF, structural heart disease and LVEF ≥35%, treated with an ICD and guideline directed drug therapy	3 months
Syncope presumed due to VT with a genetic cause, untreated	Not fit to drive
Syncope presumed due to VT with a genetic cause, treated with an ICD or guideline directed drug therapy	3 months
Syncope presumed due to a nonstructural heart disease VT such as RVOT or LVOT, untreated	Not fit to drive
Syncope presumed due to a nonstructural heart disease VT such as RVOT or LVOT, treated successfully with ablation or suppressed pharmacologically	3 months
Syncope of undetermined etiology	1 months

Table 6 Suggested driving restrictions after an episode of syncope per ESC (Task Force for the Diagnosis and Management of Syncope et al., 2009)

Diagnosis	*Group 1 (private drivers)*	*Group 2 (professional drivers)*
Cardiac arrhythmias		
Medical treatment	After successful treatment is established	After successful treatment is established
Pacemaker implant	After 1 week	After appropriate function is established
Successful catheter ablation	After successful treatment is established	After long-term success is confirmed
ICD implant	In general low risk, restriction according current recommendations	Permanent restriction
Reflex syncope		
Single/mild	No restrictions	No restriction unless it occurred during high risk activity
Recurrent and severe	After symptoms are controlled	Permanent restriction unless effective treatment has been established
Unexplained syncope	No restrictions unless absence of prodrome occurrence during driving, or presence of severe structural heart disease	After diagnosis and appropriate therapy is established

Summary

Syncope is a common condition that is implicated with motor vehicle crashes. Most patients have a similar prognosis to the general population, unless they have a cardiac etiology for their syncope. However, neurocardiogenic syncope remains the most common cause. For patients, the RH from syncope while driving can be estimated, but clinical judgment should still be used. While a driving restriction may be beneficial in terms of allowance for therapy to become effective, the majority of patients will resume driving before the end of the recommended restriction. As such, all physicians, who care for patients with syncope, should be familiar with local driving regulations.

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Echocardiography in Heart Failure

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Introduction

Heart failure (HF) is a prevalent disease affecting an estimated 5.1 million Americans and carries significant mortality and morbidity (Yancy et al., 2013). Echocardiography is an integral tool for the evaluation and treatment of patients with varying stages of HF or those with symptoms suggestive of HF. As an imaging modality, echocardiography is widely available, does not involve radiation, and provides real-time information with high temporal resolution. In HF, echocardiographic techniques allow for noninvasive assessment of cardiac structure, myocardial function, and hemodynamics for the purposes of initial diagnosis and subsequent clinical management. Early establishment and characterization of HF, whether systolic or diastolic, leads to earlier initiation of therapies that can improve clinical outcomes.

Diagnosis and Initial Evaluation of HF

Heart failure is often suspected in patients who present with nonspecific symptoms such as dyspnea, fatigue, or edema. Although careful physical examination may reveal signs of cardiac dysfunction, these findings may be subtle and insufficient for assessment of volume status or cardiac output. Transthoracic echocardiography with Doppler provides a noninvasive assessment of hemodynamics to establish a diagnosis of HF and characterize its severity. Echocardiography also allows for delineation of cardiac anatomy to help determine the etiology of HF, which is most commonly due to left ventricular (LV) myocardial dysfunction but may also arise from valvular abnormalities, right ventricular (RV) myocardial dysfunction, or pericardial pathology. A wide spectrum of cardiomyopathies due to genetic or acquired factors lead to adverse remodeling of the ventricular myocardium. Subsequent impairment of ventricular contractility or relaxation, which can both be assessed by echocardiography, results in the clinical syndrome of HF.

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Hemodynamics and Ventricular Function

Ascertainment of hemodynamic status is paramount in the acute management of heart failure and determination of prognosis. Unlike invasive catheterization, echocardiography allows for serial monitoring of hemodynamic status outside of the intensive care unit. Hemodynamic assessment by echocardiography encompasses measurement of circulatory flow (i.e., cardiac output) as well as evaluation of filling pressures in the cardiac chambers. Global LV systolic function can be described by several echocardiographic parameters including estimation of cardiac output. Diastolic function, assessed noninvasively by echocardiography, provides diagnostic and prognostic information and is also a key component in characterization of overall myocardial performance. In addition, evaluation of right ventricular function and hemodynamics plays an important and increasingly recognized role in the management of all forms of heart failure.

Ejection Fraction

Perhaps the most prevalent metric of LV systolic function is ejection fraction (LVEF), due to its prognostic value and therapeutic implications in heart failure patients (Quiñones et al., 2000; Cintron et al., 1993). LVEF is related to the proportion of the end-diastolic volume that constitutes the stroke volume (volume of blood ejected from the LV per heartbeat). Quantitation of LVEF by two-dimensional echocardiography (2DE) can be performed in multiple different ways. The recommended method is the biplane method of discs (modified Simpson rule), which involves deriving end-diastolic and end-systolic volumes by tracing the LV cavity in the apical 4-chamber and 2-chamber views, as shown in **Fig. 1**. Volumes are calculated by the summation of the volumes of parallel discs along the long-axis of the LV, each of equal thickness and variable diameter, defined by the traced endocardial borders. Alternatively, official recommendations state that LVEF may be calculated by three-dimensional echocardiography (3DE), which avoids geometric assumptions and the potential pitfall of foreshortening the LV apex (**Fig. 2** and **Video 1**). 3DE is more accurate and reproducible than 2DE for measuring LV volumes and calculating LVEF (Zhang et al., 2013; FASE et al., 2012). Progressive improvements in digital measurement algorithms and ease of use have led to increasing adoption of 3DE.

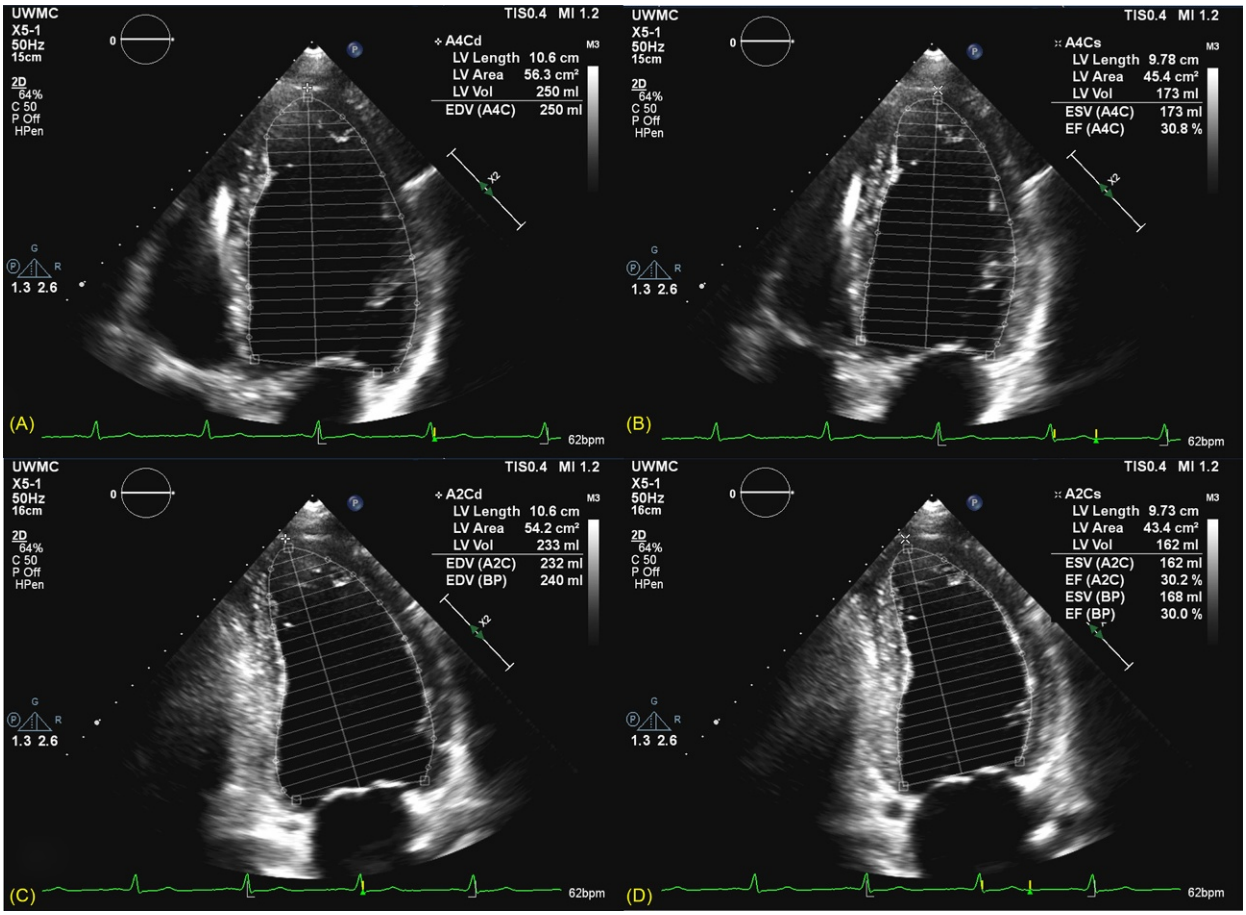

Fig. 1 LVEF by biplane method of discs in a patient with dilated cardiomyopathy. Volumes are derived from endocardial tracing of the LV cavity from the apical 4-chamber and apical 2-chamber views at end-diastole (A and C) and at end-systole (B and D). The difference between the end-diastolic and end-systolic volume yields the stroke volume, which is divided by the end-diastolic volume to yield LVEF. In this patient, the LVEF is moderately–severely reduced at 30%.

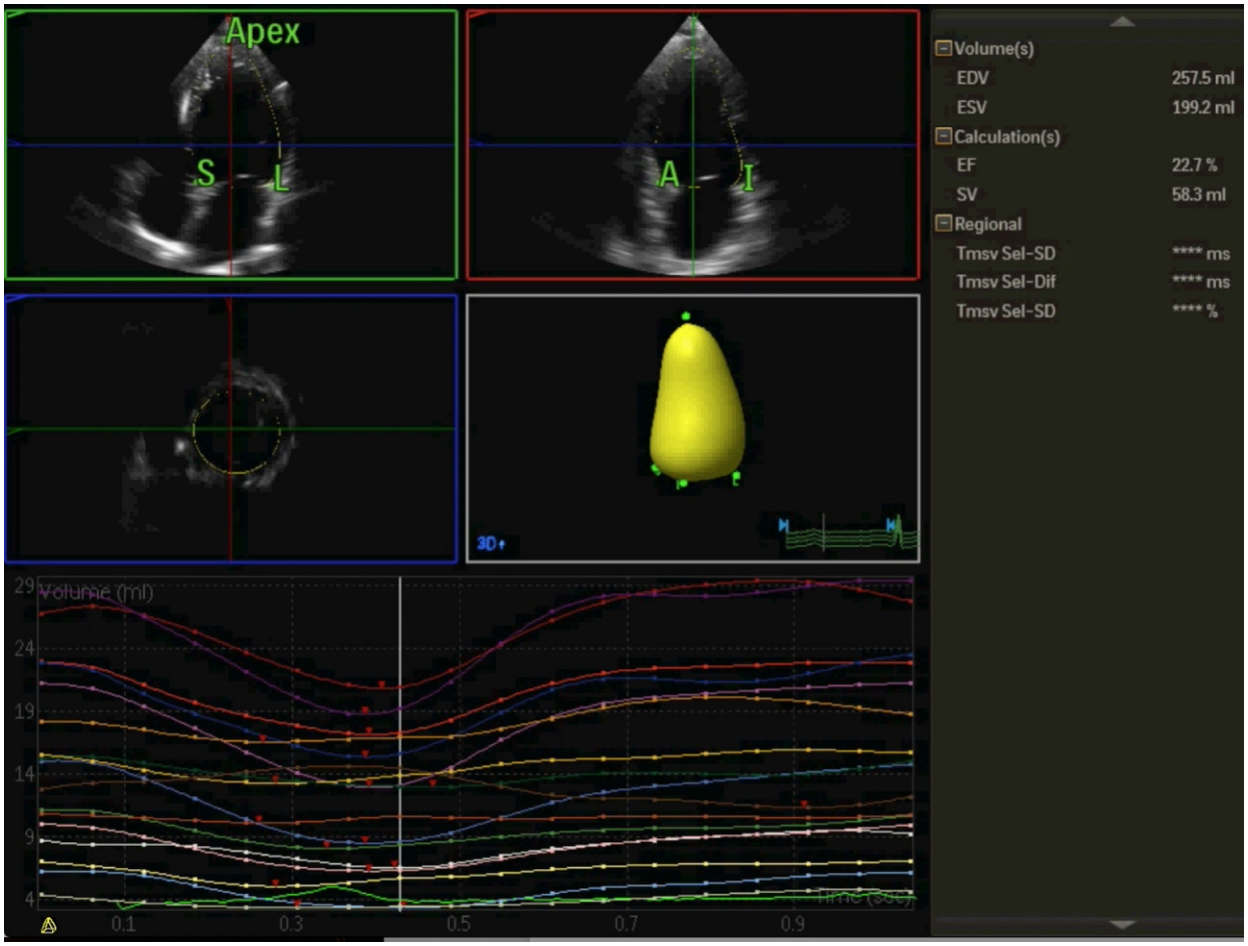

Fig. 2 LVEF by 3D echocardiography (3DE) in a patient with dilated cardiomyopathy. Endocardial tracing of full volume data acquired by 3DE generates a volume-rendered representation of the LV cavity throughout the cardiac cycle. The bottom panel shows volumetric changes over time due to segmental wall motion, divided per the 17-segment model. (See also **Video 1**.).

Other Measures of Systolic Function

Stroke volume may be derived from the change in LV volume from end-diastole to end-systole, using the biplane method of discs. Stroke volume can also be obtained by pulsed wave Doppler at the left ventricular outflow tract (LVOT), as shown in **Fig. 3**. Utilizing this method, stroke volume is calculated as the product of the cross-sectional area of the LVOT and the flow velocity at the LVOT over the duration of flow, or velocity-time integral (VTI). Stroke volume multiplied by heart rate results in cardiac output. Measurement of cardiac output by echocardiography can identify diminished cardiac function, even in the setting of normal LVEF, volume depletion, right ventricular dysfunction, or left ventricular hypertrophy.

Although ejection fraction and cardiac output by echocardiography are valuable and widely used indices of LV systolic function, they are influenced by changes in preload and afterload. The Doppler-derived dP/dt contractility index is a parameter that may be independent of heart rate and less dependent of loading conditions. Impaired contractility causes LV pressure to rise less rapidly during isovolumic contraction. This rate of change in the LV pressure is reflected by the slope of the mitral regurgitation jet by continuous wave Doppler. Systolic dysfunction prolongs not only the isovolumic contraction time but also the isovolumic relaxation time, in addition to shortening the ejection time of the left ventricle. The myocardial performance (or Tei) index can be calculated from these intervals, obtained by spectral Doppler, and represents a measure of combined systolic and diastolic function (Tei et al., 1995). Both the myocardial performance index and dP/dt derived by Doppler have prognostic value in patients with heart failure (Kolias et al., 2000; Dujardin et al., 1998; Arnlöv et al., 2005; Mikkelsen et al., 2006).

Diastolic Function

Heart failure with reduced ejection fraction generally involves impairment of both systolic and diastolic function. There has also been increasing recognition of an entity of heart failure with preserved ejection function (HFpEF) resulting from isolated diastolic

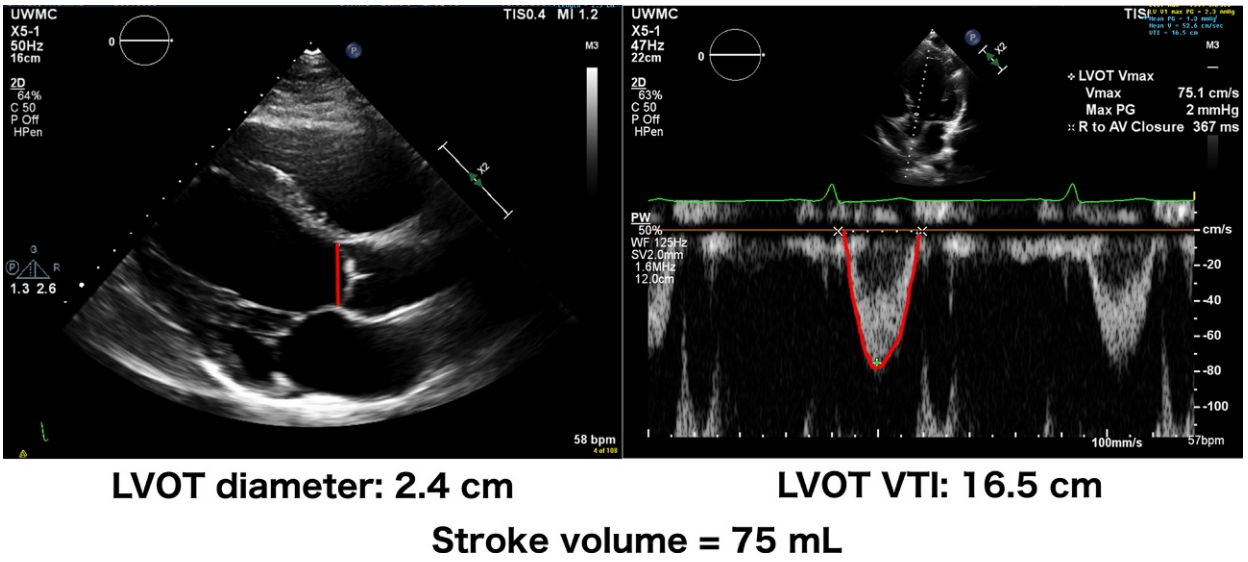

Fig. 3 Stroke volume by LV outflow tract (LVOT) Doppler. Cross-sectional area of the LVOT can be calculated from the LVOT diameter measured from the parasternal long-axis view (left image). Velocity–time integral (VTI) is obtained from pulsed-wave Doppler across the LVOT in the apical 5-chamber view (right image). Using the continuity equation, the LVOT cross-sectional area multiplied by the VTI yields stroke volume.

dysfunction. As evaluated by the integration of various echocardiography parameters, diastolic dysfunction has been shown to be associated with the development of heart failure and worse overall prognosis (Kane et al., 2011; Aljaroudi et al., 2012).

Left ventricular filling can be assessed by the spectral Doppler pattern of mitral inflow velocity, which represents the pressure gradient between the left atrium (LA) and left ventricle during diastole (**Fig. 4A**). Early transmitral flow (E wave) corresponds to active LV relaxation and is influenced by the relative compliances of the LA and LV. Late transmitral flow (A wave) is generated by atrial contraction and affected by the compliance of the receiving ventricular chamber. Impaired LV relaxation leads to a diminished E wave relative to the A wave. Furthermore, the deceleration time of the E wave is prolonged due to the longer time for equilibration of LA and LV pressures. With severe diastolic dysfunction (i.e., restrictive filling), high left atrial pressure causes early diastolic filling to occur rapidly via predominantly passive flow, resulting in a steep E wave with a short deceleration time and a small A wave. Persistence of restrictive filling pattern portends a worse prognosis in patients with heart failure (Pinamonti et al., 1997).

Diastolic function can also be inferred from left atrial size and filling. Chronically elevated left atrial pressure resulting from diastolic dysfunction causes left atrial enlargement (**Fig. 4C**). Pulmonary vein flow pattern on Doppler reflects left atrial filling, which normally occurs predominantly during ventricular systole. During early diastole, the left atrium serves as a conduit that simultaneously fills the left ventricle and receives blood from the pulmonary veins. As diastolic dysfunction progresses, LA pressure increases and the systolic component (S wave) of pulmonary vein flow into the left atrium diminishes as compared to the diastolic component (D wave), as shown in **Fig. 4D**. Atrial contraction during late diastole causes some retrograde flow into the pulmonary veins (Ar wave), which is more pronounced in diastolic dysfunction. Furthermore, prolongation of the Ar wave duration as compared to the antegrade mitral A wave has been shown to be an indicator of elevated LV filling pressure and worsened clinical outcome in heart failure (Dini et al., 2000).

Tissue Doppler of the mitral annular velocity provides yet another method of evaluating diastolic function. Left ventricular contraction causes excursion of the mitral annulus longitudinally toward the LV apex. During myocardial relaxation in diastole, the mitral annulus returns to its initial position, the velocity of which can be assessed by tissue Doppler sampling at the medial and lateral annulus (**Fig. 4B**). The early diastolic wave (e') diminishes to less than the late diastolic wave (a') as diastolic dysfunction progresses. Combined with the mitral inflow Doppler, mitral annular tissue Doppler during early diastolic filling can be used to estimate LV filling pressures. The ratio of E/e' has been shown to correlate well with invasive measurements, such that $E/e' > 15$ is associated with elevated LV filling pressures and $E/e' < 8$ with normal filling pressures (Ommen et al., 2000). Caveats to the measurement of e' velocities include mitral annular calcification, abnormal septal motion from conduction delay, lateral wall motion abnormalities, and mitral valve prostheses.

Right Ventricular Function and Hemodynamics

Determining right ventricular function and hemodynamics plays a central role in not only cardiomyopathies involving primary right heart dysfunction or pulmonary hypertension but also in heart failure due to left heart disease. In patients with left heart failure, right ventricular dysfunction is associated with high mortality and morbidity (Meyer et al., 2010; Di Salvo et al., 1995). Compared to that of the LV, right ventricular performance is more difficult to quantify by 2D echocardiography due to the complex

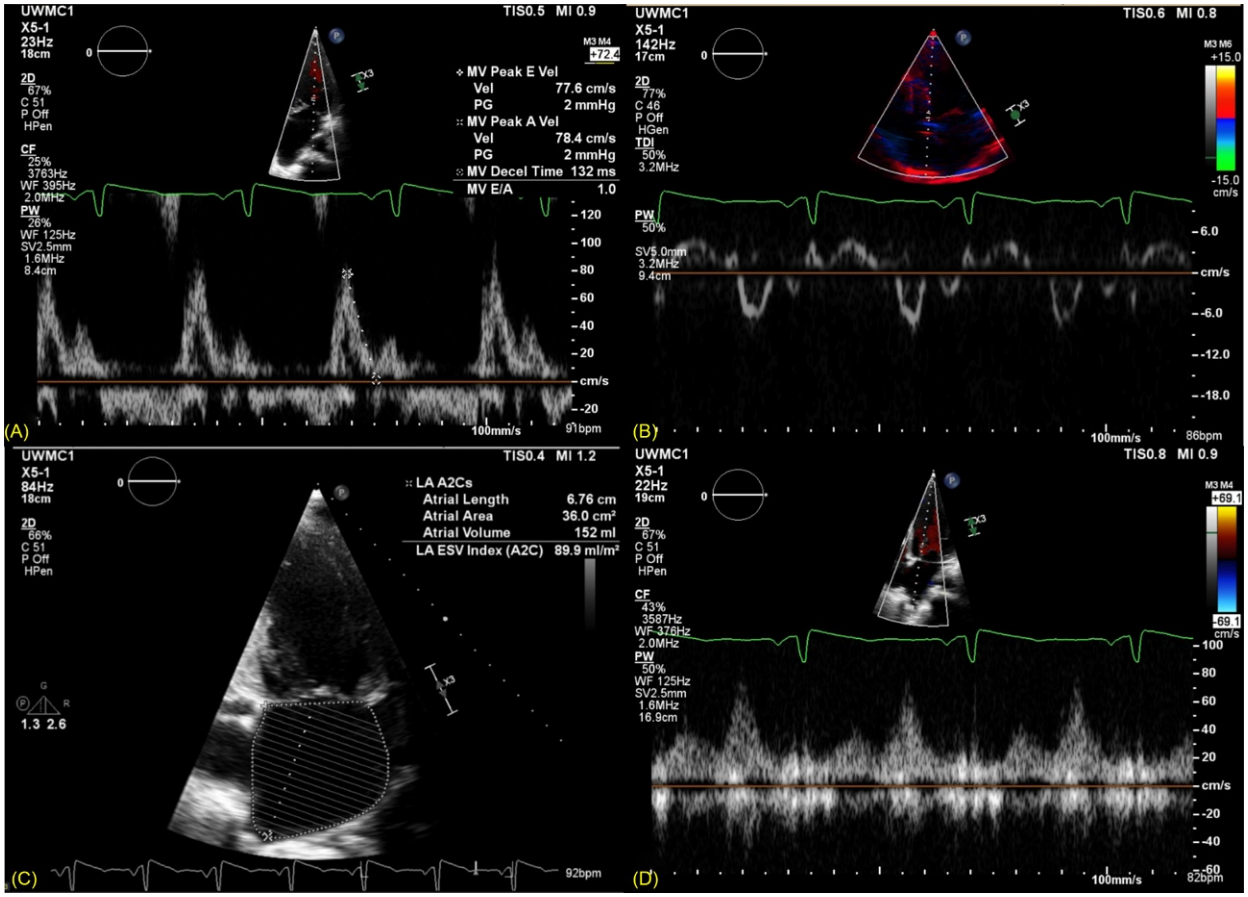

Fig. 4 Diastolic function in a patient with systolic heart failure. A, Mitral inflow shows steep deceleration of the E wave and a relatively diminutive A wave. B, Tissue Doppler of the medial annular velocity shows a reduced e′ and an E/e′ ratio of 13. C, Left atrial volume is severely increased. D, Pulmonary vein flow shows a greater diastolic than systolic component. Taken together, this patient likely has abnormal diastolic relaxation and elevated left atrial pressure.

anatomy of the right ventricle (RV) invalidating geometric assumptions for volume calculations. Given that RV contraction occurs primarily in a longitudinal fashion from base to apex, its systolic function can be gauged by the linear motion of the lateral tricuspid annulus by M-mode echocardiography in a metric known as the tricuspid annular plane systolic excursion (TAPSE), demonstrated in **Fig. 5A**. Similarly, the peak systolic velocity of the tricuspid annulus can be measured by tissue Doppler (s′), as shown in **Fig. 5B**. Despite its one-dimensional measurement and dependency on the angulation of Doppler interrogation, TAPSE has been shown to correlate well with radionuclide-derived RV ejection fraction (RVEF) (Rudski et al., 2010). Another method of assessing RV systolic function is fractional area change (FAC), which is calculated as the difference between the end-diastolic and end-systolic areas of the RV cavity by planimetry from the apical 4-chamber view (**Fig. 5C** and **D**). FAC has been shown to correlate with RVEF by cardiac MRI and is of prognostic value after myocardial infarction (Anavekar et al., 2007). The myocardial performance index used for assessing LV function can also be applied to the RV and is known as the RV index of myocardial performance (RIMP), which is independent of ventricular geometry. Although technical challenges preclude its widespread use, RVEF calculation by 3D echocardiography has been shown to be more reproducible and accurate than 2D methods (Jenkins et al., 2007; Gopal et al., 2007).

Right heart hemodynamics can be characterized by echocardiography in a multitude of ways. Right atrial (RA) pressure is approximated by the size of the inferior vena cava (IVC), which empties into the right atrium, and its change with respiration (**Fig. 6A**). Increased pressure in the right atrium causes dilation of the IVC and decreased inspiratory collapse. The Doppler pattern of hepatic vein flow is also affected, such that there is a predominance of the diastolic wave during which atrial emptying occurs. Right ventricular afterload can be estimated by the peak tricuspid regurgitation velocity by Doppler, which reflects the gradient between the RV and RA during systole (**Fig. 6B**). In the absence of pulmonic stenosis, RV systolic pressure is equivalent to pulmonary artery (PA) systolic pressure. Other Doppler methods can be employed to estimate pulmonary pressures, especially in the absence of sufficient tricuspid regurgitation. End-diastolic pulmonary regurgitation velocity combined with RA pressure estimates PA diastolic pressure (**Fig. 6D**). Similarly, mean PA pressure can be derived from the peak pulmonary regurgitation velocity. Pulmonary hypertension due to backward transmission of elevated LV filling pressures in heart failure can lead to remodeling in the pulmonary vasculature and RV dysfunction. Elevated pulmonary vascular resistance causes early peaking and

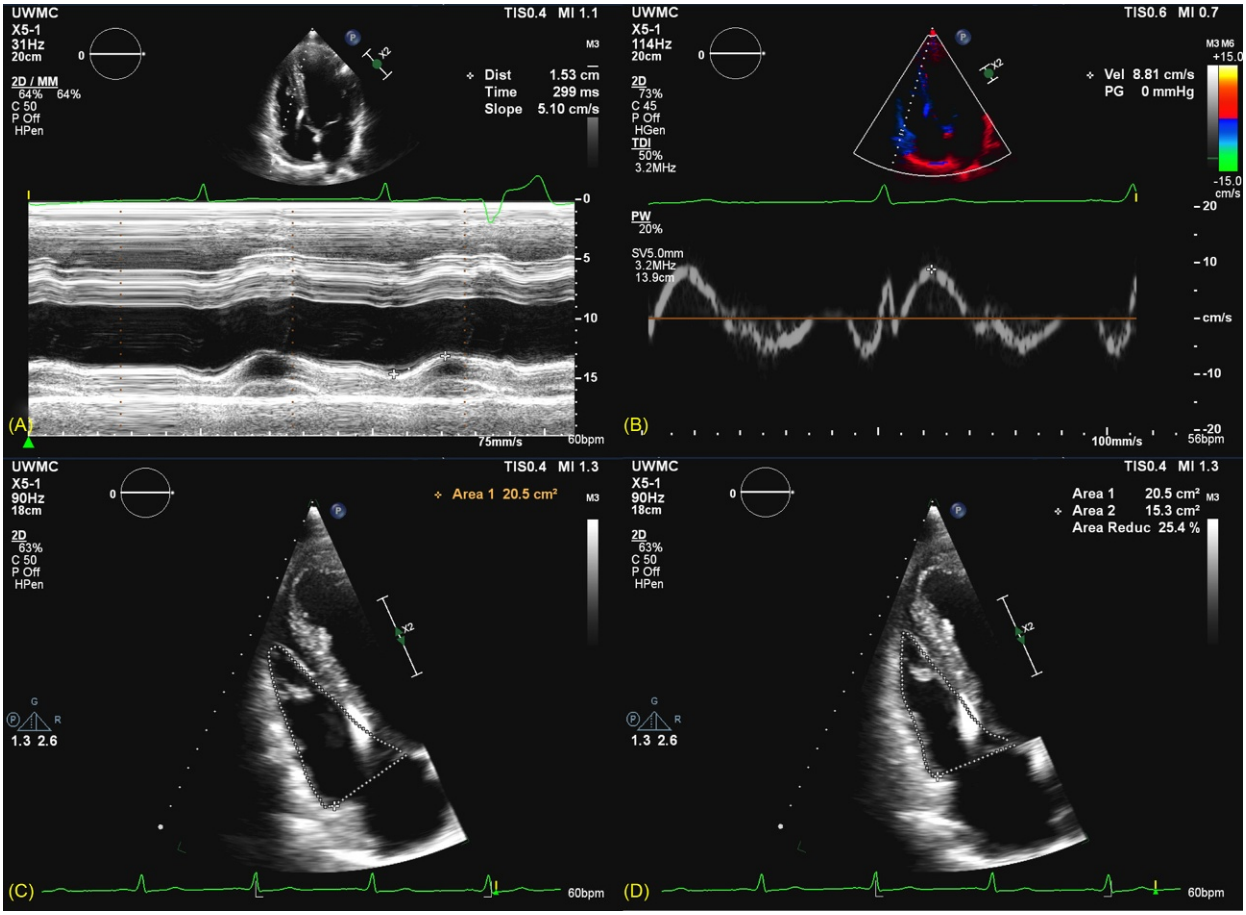

Fig. 5 Right ventricular function in a patient with dilated cardiomyopathy. A, Tricuspid annular plane systolic excursion (TAPSE) is mildly reduced at 1.5 cm. B, Tissue Doppler of the peak systolic tricuspid annular velocity (s′) is also mildly reduced at 8.8 cm/s. Fractional area change (FAC) is calculated from the traced area of the RV cavity from the RV focused view at end-diastole (C) and end-systole (D). In this case, the FAC of 25% is also decreased.

mid-systolic deceleration of flow in the RV outflow tract (RVOT), resulting in a short acceleration time and mid-systolic notching in the Doppler waveform (Fig. 6C). Furthermore, the ratio of the peak tricuspid regurgitation velocity (an indicator of PA pressure) to the RVOT velocity-time integral (an indicator of flow) provides a surrogate marker for pulmonary vascular resistance (Abbas et al., 2003). In patients with heart failure, worse clinical outcomes are associated with pulmonary hypertension and RV dysfunction (Ghio et al., 2013). Estimation of PA pressure and RA pressure by echocardiography thus serves an important role in the hemodynamic assessment of patients with heart failure and can obviate the need for invasive right heart catheterization.

Strain Imaging

Strain imaging assesses systolic and diastolic function of the ventricle by detecting myocardial deformation, which results from lengthening or shortening of myocardial fibrils. Strain and strain rate are expressed in terms of magnitude along a directional vector, which allows for deconstruction of myocardial mechanics into longitudinal, circumferential, and radial components. Using tissue Doppler techniques, strain rate is derived from the difference in tissue velocities at various sample volumes in the myocardium and the distance between the samples. Integrating tissue Doppler strain rate over time yields strain. Due to the one-dimensionality and angle dependency of Doppler interrogation, strain imaging by this technique is limited in accuracy and reliably assesses only longitudinal deformation.

Speckle tracking utilizes the interference pattern of backscattered signals from small structures within the myocardium, termed "speckles," to track myocardial displacement over time. Thus, strain is directly measured in a two-dimensional fashion such that circumferential and radial strain can be assessed, in addition to longitudinal strain. Global longitudinal strain (GLS), the averaged longitudinal strain across the entire myocardium from the apical window, serves as an indicator of myocardial function with several important clinical applications, particularly the detection of systolic dysfunction in the setting of normal LVEF. Because longitudinal strain is affected by endocardial myocardial dysfunction, GLS may detect subclinical myocardial disease prior to transmural involvement, such as in hypertensive or diabetic heart disease (Marwick, 2006; Saito et al., 2016; Mor-Avi et al., 2011). In coronary artery disease, subendocardial ischemia is associated with abnormal segmental longitudinal strain prior to the

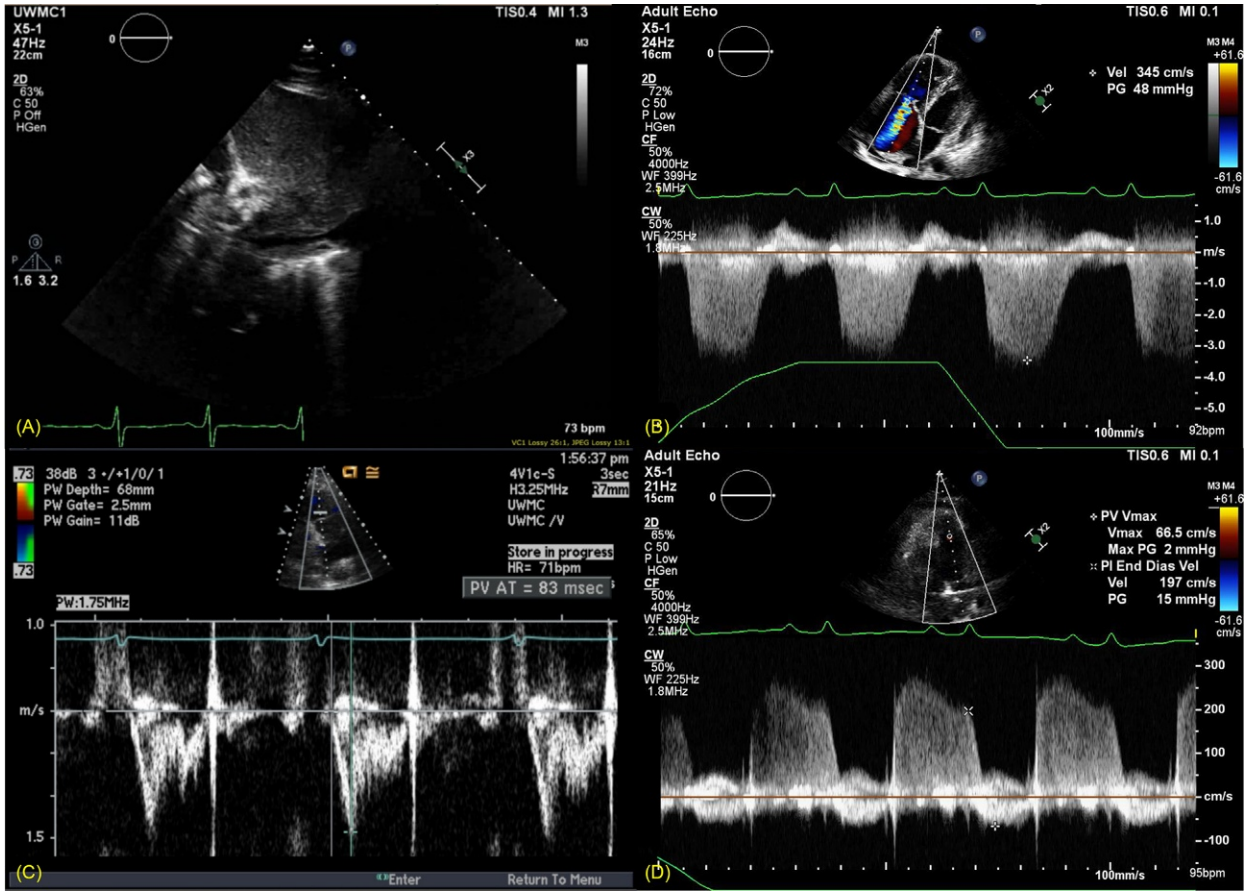

Fig. 6 Right heart hemodynamics in severe pulmonary arterial hypertension. (A) Inspiratory collapse of the IVC of $\geq$50% suggests normal right atrial pressure. (B) Systolic pressure gradient across the tricuspid valve, calculated from peak tricuspid regurgitation velocity by Doppler and estimated right atrial pressure, indicates an elevated PA systolic pressure of 48 mmHg. (C) Doppler profile of the RVOT shows a short acceleration time and mid-systolic notching, suggestive of elevated pulmonary pressures and pulmonary vascular resistance. (D) End-diastolic velocity of the pulmonary regurgitation jet indicates an elevated PA diastolic pressure of 15 mmHg above RA pressure.

development of visually evident hypokinesis (Montgomery et al., 2012). Strain imaging also allows for early detection of chemotherapy-induced cardiotoxicity in oncology patients (Sawaya et al., 2012; Negishi et al., 2014; Thavendiranathan et al., 2014). Reduction in GLS in hypertrophic cardiomyopathy can differentiate it from physiologic hypertrophy in athletes and serves as a predictor of adverse events (Reant et al., 2015). Regardless of the etiology, restrictive cardiomyopathy is invariably associated with reduced GLS. However, in cardiac amyloidosis, there is a characteristic pattern of reduced strain at the base with sparing of the apex (Phelan et al., 2012), as shown in **Fig. 7** and **Videos 2–4**. In patients with systolic heart failure, global longitudinal strain predicts the occurrence of major adverse cardiac events (Nahum et al., 2010).

Myocardial Structure and Morphology

Left Ventricular Chamber Size

In patients with systolic heart failure, impaired myocardial contractility causes the LV cavity to progressively dilate as an adaptive measure to increasing end-diastolic volume. Evidence of LV remodeling can be seen by echocardiography via estimation of its chamber size, which is commonly reported as a linear dimension from the parasternal long-axis view, biplane-derived volume from the apical window, or a 3D volume. End-diastolic dimension, 2D end-diastolic volume, and 2D end-systolic volumes have all been shown to have prognostic value (Lee et al., 1993; Solomon et al., 2005). Dilation of the other cardiac chambers typically accompanies that of the left ventricle. Remodeling due to heart failure also results in transformation of the LV cavity into a more spherical, rather than ellipsoid, shape (Konstam et al., 2011). Sphericity index, derived from the ratio of the lengths of the LV major and minor axes, has been shown to correspond to exercise capacity in patients with systolic heart failure (Tischler et al., 1993).

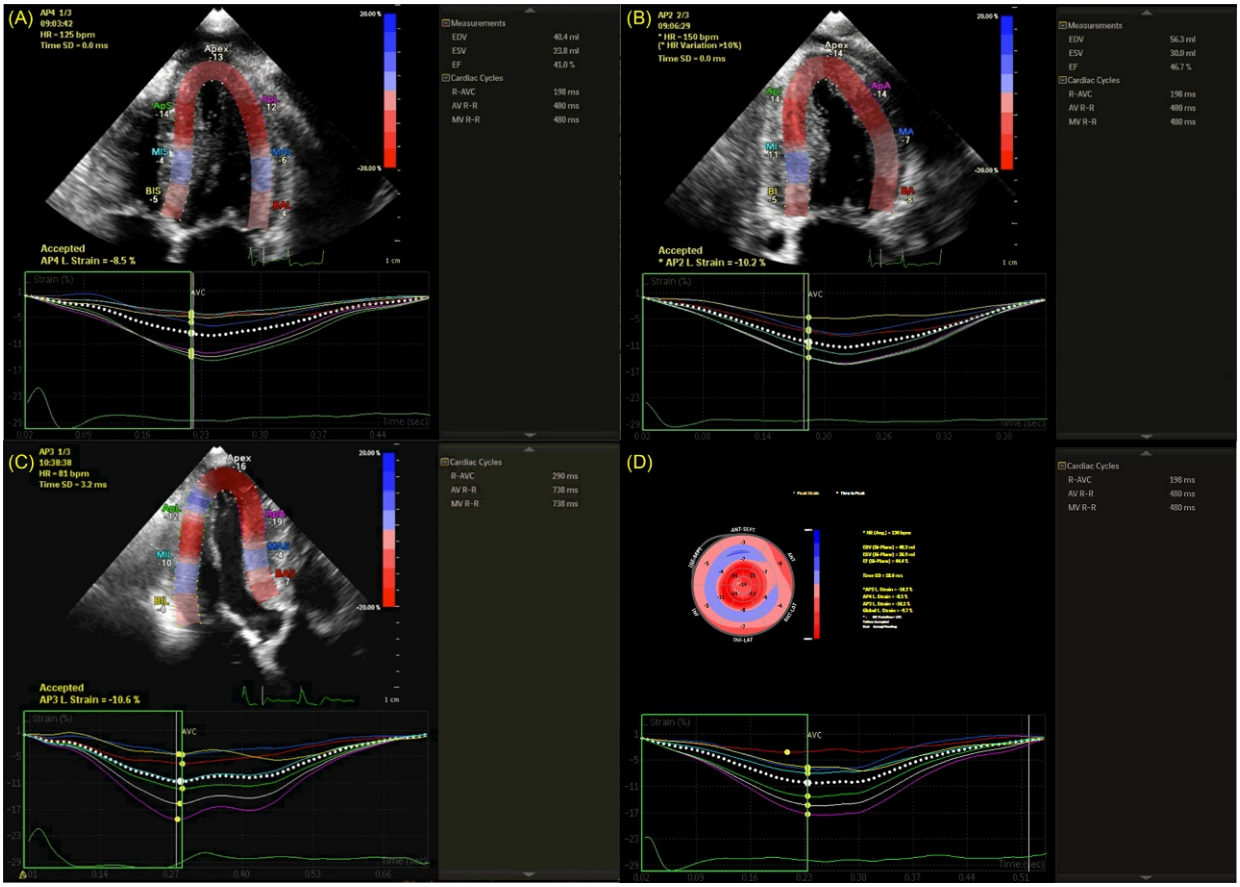

Fig. 7 Speckle tracking longitudinal strain in cardiac amyloidosis. Regional longitudinal strain is obtained by speckle tracking echocardiography from the apical 4-chamber (A), 2-chamber (B), and 3-chamber (C) views. Polar map, or bull's eye plot, of the peak systolic strain (D) shows a pattern of apical sparing characteristic of amyloidosis. Global longitudinal strain was reduced at −9.7%. (See also **Videos 2–4**).

Left Ventricular Geometry and Mass

The pathologic response to myocardial injury and hemodynamic changes associated with heart failure is a combination of hypertrophy and elongation of cardiomyocytes. This maladaptive response, in addition to cardiomyocyte necrosis and fibrosis in the extracellular matrix, results in macroscopic changes in the LV geometry. Not only does the ventricular cavity dilate but LV wall thickness may diminish. Left ventricular wall thickness with respect to chamber size is reflected by the echocardiographic measure of relative wall thickness (RWT). Distinct patterns of ventricular geometry caused by remodeling have been described, based on relative wall thickness and LV mass (LVM). The pathologic classifications are termed concentric remodeling (normal LVM with increased RWT), eccentric hypertrophy (increased LVM with normal RWT), and concentric hypertrophy (increased LVM and RWT). Compared to normal LV geometry, each of these patterns is associated with worse cardiovascular outcomes, particularly concentric hypertrophy (Verma et al., 2008).

The remodeling process in heart failure typically results in an increase in the LV mass, which can be estimated by echocardiography. Using linear measurements of posterior wall thickness, septal wall thickness, and the end-diastolic dimension, the LV mass may be calculated. LV mass can also be derived from the difference between the areas bound by the epicardium and the endocardium, using either the area-length method or truncated ellipsoid method by 2DE. Direct volumetric measurements by 3DE may yield more accurate estimation of LV mass, as compared to 2D or linear methods, but has yet to gain widespread use (Mor-Avi et al., 2004; Chuang et al., 2000). Increased LV mass, indexed to body surface area, has been associated with worse cardiovascular outcomes and also higher mortality in patients with heart failure (Quiñones et al., 2000; Armstrong et al., 2012; Verma et al., 2008).

Regional Remodeling and Fibrosis

In addition to altering the global LV geometry, remodeling due to heart failure can also cause regional changes in the myocardial architecture, particularly in ischemic cardiomyopathy. Ischemic myocardial injury due to obstructive coronary artery disease

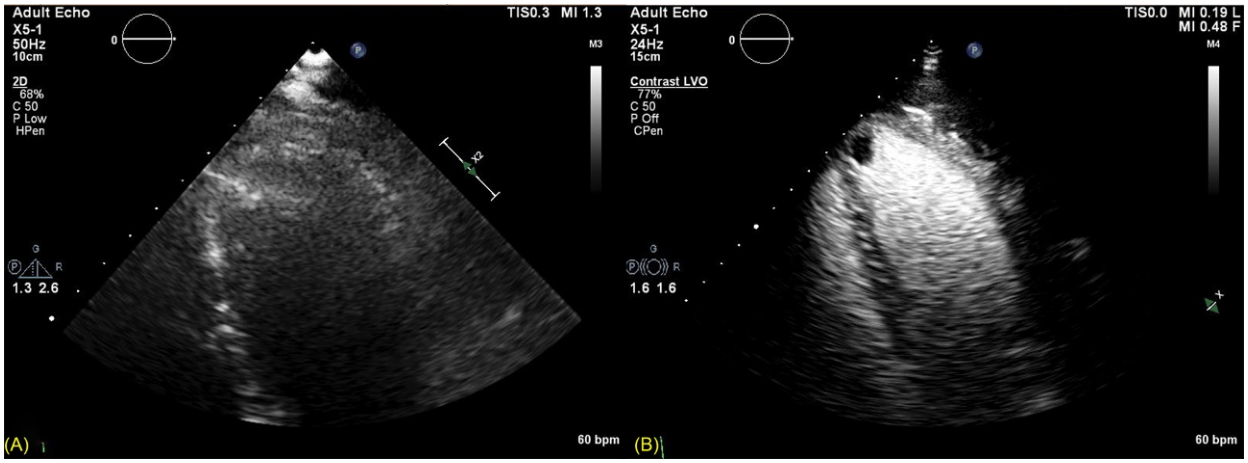

Fig. 8 Left ventricular thrombus in a patient with apical akinesis. (A) Poor endocardial definition without contrast enhancement limits the detection of mural thrombus formation. (B) Opacification of the LV cavity with contrast greatly improves endocardial definition and reveals a filling defect at the apex likely representing a thrombus. (See also **Video 8**.).

diminishes wall thickening in the affected territory during physiologic stress, which can be induced by exercise or dobutamine infusion. Regional wall motion abnormalities can be qualitatively visualized by 2D echocardiography and semiquantitated by strain imaging. As myocardial infarction develops, regional wall motion abnormalities become apparent at rest. Dobutamine stress echocardiography can identify hibernating myocardium with contractile reserve that may recover in function following coronary revascularization and lead to improved survival (Afridi et al., 1995, 1998). In areas of transmural infarction, replacement fibrosis causes the myocardial wall to become akinetic and appear thinned and bright by echocardiography over time (**Videos 5–7**). Nonischemic etiologies of cardiomyopathy can also cause fibrotic changes resulting in a similar echocardiographic appearance, though usually in a noncoronary distribution. Aneurysms can occur in areas of scarred myocardium and have a propensity for LV thrombus formation, which carries a high risk of systemic embolization (van Dantzig et al., 1996). Presence of an LV thrombus can be detected by echocardiography, particularly with the aid of contrast for chamber opacification (**Fig. 8** and **Video 8**).

Functional Mitral Regurgitation

Left ventricular remodeling associated with heart failure leads to mitral regurgitation (MR) via the alteration of the opposing tethering and closing forces involved in mitral valve function. Spherical LV dilatation causes apical and lateral displacement of the papillary muscles leading to leaflet tethering as well as flattening of the saddle-shaped mitral annulus (Ray, 2010). Focal scarring due to ischemic disease may also cause papillary muscle displacement. Annular dilatation further perpetuates MR, as does impairment of the force of mitral valve closure by LV systolic dysfunction.

Echocardiography plays an important role in both elucidating the mechanism of and quantifying the severity of MR. Visualization of the entire mitral valve apparatus and the left ventricle allows for assessing whether MR is due to primary leaflet abnormality or functional in nature. Conventional echocardiographic methods for quantifying severity are more difficult in functional MR due to the noncircular regurgitant orifice area as well as the dynamic nature of MR, which is highly dependent on loading conditions and timing in the cardiac cycle (Ray, 2010). Furthermore, functional MR is often eccentric or comprised of multiple regurgitant jets. 3D echocardiography allows for direct planimetry of the vena contracta (**Fig. 9**) without geometric assumptions and has been well validated (Thavendiranathan et al., 2013).

Thorough evaluation of functional MR by echocardiography is essential due to its significant prognostic implications. Functional MR has been associated with increased mortality and heart failure hospitalizations in patients with both ischemic and nonischemic cardiomyopathy, independent of LV systolic function or severity of heart failure (Trichon et al., 2003; Rossi et al., 2011; Bursi et al., 2010). Therapies for functional mitral regurgitation with the most clinical benefit have been those directed at reversal of ventricular remodeling, such as guideline-directed medical therapy of heart failure, coronary revascularization, and cardiac resynchronization therapy in certain situations. Surgical valve repair or replacement has not been demonstrated to improve survival in patients with severe, functional MR (Calafiore et al., 2013; Wu et al., 2005). However, mitral valve surgery may have a limited role in patients undergoing concomitant coronary artery bypass or other cardiac surgery (Mihaljevic et al., 2007; Chan et al., 2012). Transcatheter mitral valve repair, such as with the MitraClip device, has emerged as a feasible therapeutic option for severe functional MR, particularly in patients at high operative risk (Feldman et al., 2011; Mauri et al., 2013). Transesophageal echocardiography, often with 3D, is crucial for patient selection and procedural guidance of Mitraclip placement.

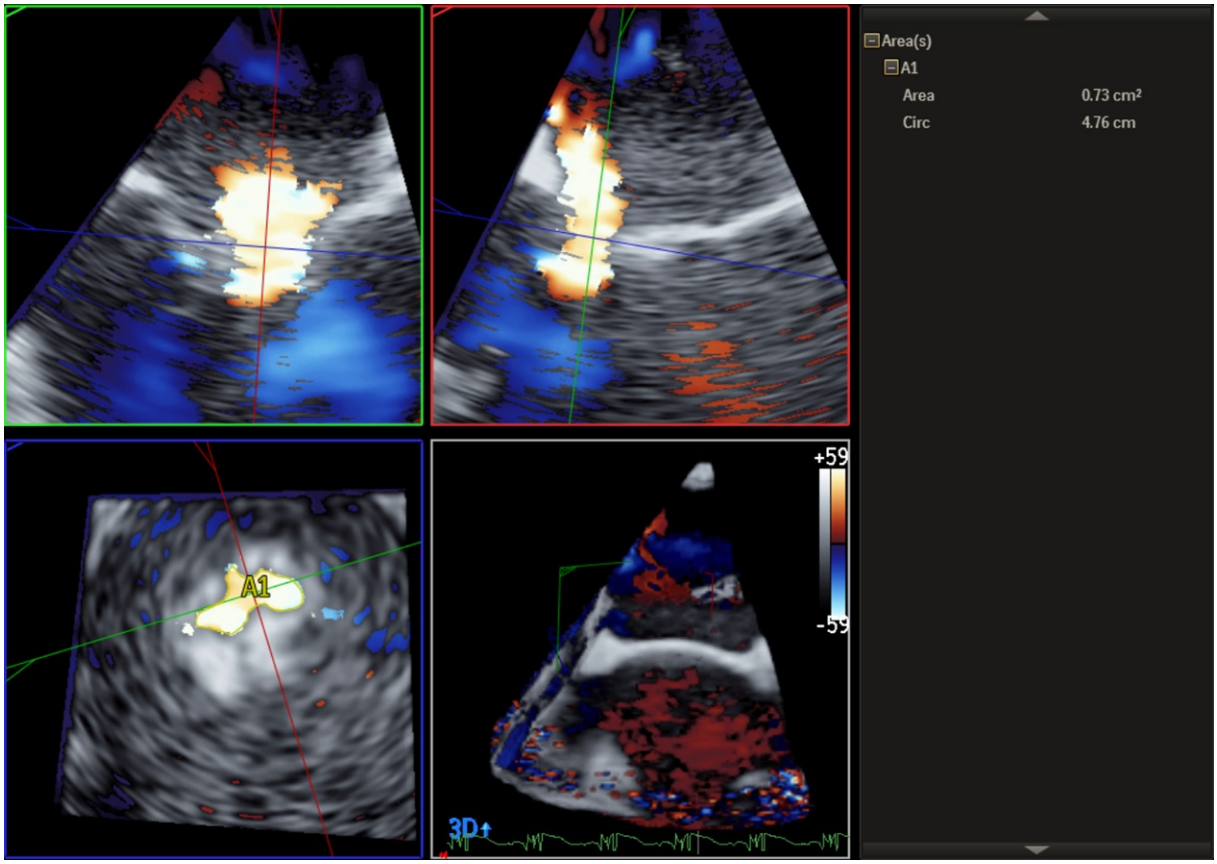

Fig. 9 3D echocardiography in severe functional mitral regurgitation. Multiplanar views of the mitral regurgitant jet by 3D color Doppler showing a noncircular vena contracta area, which measures 0.73 cm^2 by direct planimetry (*bottom left*), consistent with severe MR.

Guidance of HF Therapy

Medical Therapy

Echocardiography serves an important role in not only diagnosis and prognostication of heart failure but also in guidance of HF therapy. Evaluation of hemodynamics, ventricular function, and cardiac structure by echocardiography is essential in the management of acutely decompensated heart failure, particularly when the precipitant of clinical decompensation is unclear (American College of Cardiology Foundation Appropriate Use Criteria Task Force et al., 2011). Furthermore, evaluation of filling pressures and volume status by echocardiography is valuable in guiding diuresis in the acute setting. Medical therapies for chronic heart failure also often depend upon echocardiographic assessment of LV ejection fraction. Medications that mediate neurohormonal blockade such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), angiotensin receptor neprilysin inhibitors, beta-blockers, and aldosterone antagonists have demonstrated clinical benefit in terms of reduction in mortality only in heart failure with reduced LVEF (Anon, 1999; Packer et al., 2001; The Consensus Trial Study Group, 1987; SOLVD Investigators, 1991; Pfeffer et al., 1992, 2003; Cohn et al., 2001; Granger et al., 2003; Pitt et al., 1999, 2003; McMurray et al., 2014). Beta-blocker therapy has been associated with evidence of reverse remodeling, in terms of increase in LVEF and reduction in LV mass, end-diastolic volume, and end-systolic volumes (Lowes et al., 1999; Groenning et al., 2000). Several large echocardiographic studies have also shown similar reverse remodeling with ACE inhibitor and ARB therapy (St John Sutton et al., 1994; Solomon et al., 2005; Wong et al., 2002). Aldosterone antagonist therapy may also influence LV remodeling, though the evidence is less robust (Cicoira et al., 2002; Vizzardi et al., 2010). Monitoring clinical response with echocardiography allows for optimization of these neurohormonal and other adjunctive medical therapies in the longitudinal care of systolic HF patients.

Device-Based Therapy

In addition to its impact on medical therapy of heart failure, echocardiography also helps in identifying HF patients who would benefit from an implantable cardioverter–defibrillator (ICD) placement or cardiac resynchronization therapy (CRT). Current guidelines for ICD placement for primary prevention of sudden cardiac death from ventricular arrhythmia are based on LV systolic

function by echocardiography (Epstein et al., 2008). Mortality benefit of primary prevention ICD therapy has been shown for patients with an LVEF ≤35% despite optimal medical therapy and at more than 40 days following myocardial infarction (Moss et al., 2002; Bardy et al., 2005; Hohnloser et al., 2004). The benefit of prophylactic ICD implantation may be less in those with systolic HF due to nonischemic cardiomyopathy (Kober et al., 2016). Following ICD implantation, echocardiography allows for evaluation of potential late complications, including defibrillator lead infection or severe tricuspid regurgitation.

Mechanical dyssynchrony in patients with systolic HF causes inefficient ventricular contraction and further impairs systolic function. Cardiac resynchronization therapy utilizes biventricular pacing to reestablish intraventricular and interventricular synchrony. Echocardiographic evidence of LV reverse remodeling, reduction of functional MR, and improvement in ventricular performance have been shown to follow resynchronization therapy (Sutton et al., 2006; St John Sutton et al., 2015). Current guidelines recommend CRT for symptomatic patients with an LVEF ≤35% and a widened QRS duration, particularly in a left bundle branch block (LBBB) pattern, due to the mortality benefit in several clinical trials (Bristow et al., 2004; Moss et al., 2009; Cleland et al., 2005; Tracy et al., 2012). Despite these criteria, hemodynamic response to CRT is not achieved in all patients. Doppler echocardiographic parameters of dyssynchrony, such as septal-lateral wall delay (intraventricular delay) and the difference between LV and RV preejection intervals (interventricular delay), have yielded inconsistent results in predicting CRT response (Chung et al., 2008). However, speckle tracking measures, especially anteroseptal–posterior wall delay by radial strain, have demonstrated correlation with clinical and LVEF response to CRT (Gorcsan et al., 2010; Tanaka et al., 2010). LV lead positioning with the guidance of radial strain imaging has also been shown to optimize CRT response (Saba et al., 2013; Khan et al., 2012). Three-dimensional speckle tracking techniques may offer further insight into the relationship between mechanical dyssynchrony and CRT response. Although intraventricular synchrony has a greater hemodynamic impact than atrioventricular (AV) synchrony (Auricchio et al., 1999), optimization of the AV delay may be performed by echocardiographic assessment of diastolic filling patterns (Gorcsan et al., 2008).

Advanced Heart Failure Therapy

Several heart failure risk prediction models for determining the rate of adverse outcomes are based on LVEF derived from echocardiography in addition to clinical features and lab findings (Levy et al., 2006; Pocock et al., 2013). High-risk patients with end-stage heart failure despite optimal medical therapy and CRT, if indicated, often warrant consideration for advanced heart failure therapies (AHFT), such as a left ventricular assist device (LVAD) or orthotopic heart transplantation. Echocardiography serves an important role in AHFT screening and managing patients who have undergone LVAD implantation or cardiac transplantation.

Mechanical Circulatory Support

Mechanical circulatory support (MCS) devices consist of short-term, often percutaneously placed, support devices and long-term, durable ventricular assist devices (VADs) for either the left or the right ventricle. Percutaneous MCS devices offer temporary ventricular support as a bridge to recovery, transplantation, or durable VAD. Furthermore, temporary MCS devices provide periprocedural ventricular support for high-risk, complex percutaneous coronary interventions or arrhythmia ablations (Dangas et al., 2014). Durable MCS devices serve as either a bridge to transplantation or destination therapy for patients who are not transplant candidates.

Echocardiography plays a central role in patient selection and in monitoring of MCS device function. Criteria for LVAD implantation include the presence of severely depressed LV ejection fraction and LV cavity dilatation. Significant aortic regurgitation can render an LVAD ineffective and is a contraindication for the Impella, a percutaneous transaortic device used for temporary support. Careful assessment of RV function with echocardiography is also essential for the determination of whether mechanical RV support is necessary, given the prevalence of RV failure in the LVAD population (Kirklin et al., 2014; Kormos et al., 2010). Echocardiography guides positioning of temporary MCS devices for initial placement (e.g., transseptal puncture for the percutaneously-placed TandemHeart) and subsequent management, since these devices, particularly the Impella, tend to migrate (**Fig. 10** and **Video 1**).

In terms of routine surveillance of durable LVADs, echocardiography is utilized to monitor for subclinical LVAD dysfunction and to assess response to mechanical support. Current practice guidelines recommend obtaining surveillance echocardiograms at predetermined intervals in LVAD patients (Stainback et al., 2015). Furthermore, echocardiography can diagnose the cause of symptoms or device alarms that may result from complications such as inflow or outflow cannula obstruction (due to thrombosis or kinking), pump thrombosis, RV failure, and tamponade. These complications are associated with hemodynamic changes that cause overfilling or underfilling of the LV. Overfilling manifests as LV dilatation with bowing of the interventricular septum toward the RV and can result from LVAD pump failure (most commonly, pump thrombosis) or cannula obstruction, which can be evaluated by pulsed-wave Doppler interrogation of cannula flow (**Fig. 11B** and **C**). Underfilling from conditions of decreased preload (RV failure, tamponade, sepsis, or volume depletion) manifests as a smaller LV cavity and may cause suction of endocardial structures into the inflow cannula, resulting in reduced cardiac output and potentially ventricular arrhythmias.

Another application of echocardiography in LVAD management is in optimization of continuous-flow LVAD rotor speed for the patient's hemodynamics. The frequency and degree of aortic valve opening is a key measure for these speed-optimization studies, as it reflects the relative contributions to the cardiac output by the LVAD versus the contractile function of the ventricle. The frequency,

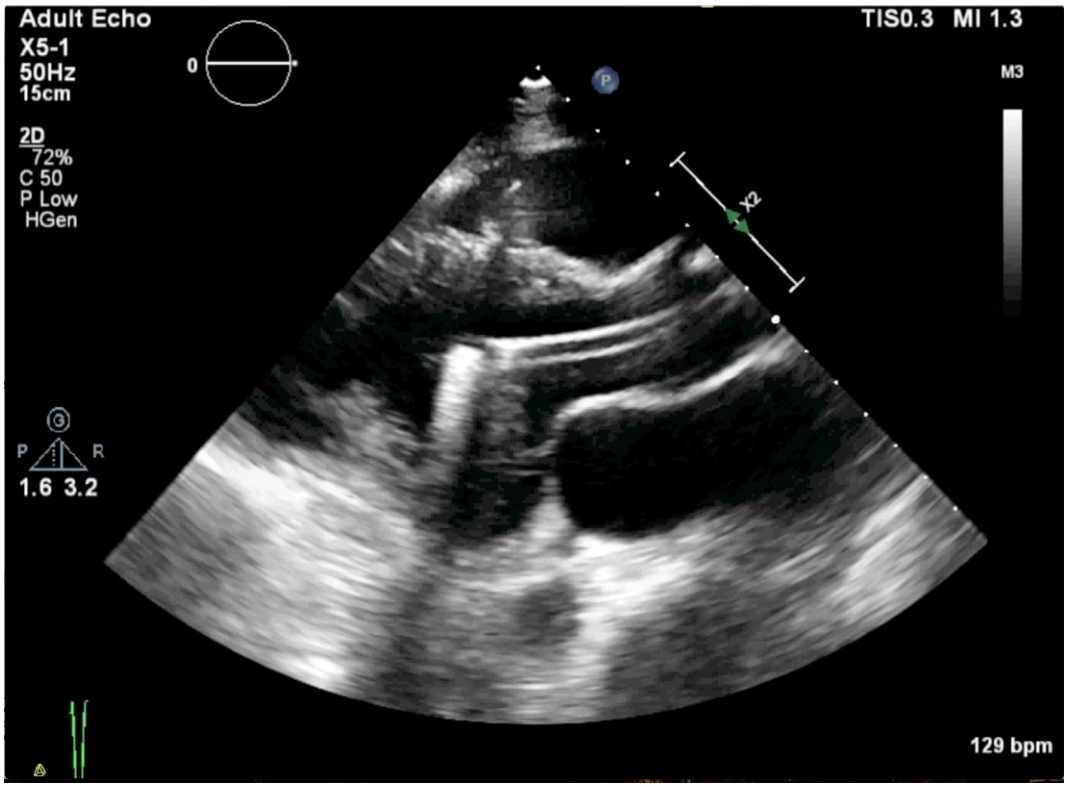

Fig. 10 Cannula positioning of an Impella percutaneous ventricular assist device. Parasternal long-axis view showing an Impella device positioned across the aortic valve with the inflow cannula in the LV cavity. The device should be angulated toward the LV apex with the inlet area located approximately 4–4.5 cm from the aortic valve. Transthoracic echocardiography provides real-time imaging for positioning the Impella device. (See also **Video 1**.).

duration, and extent of aortic valve opening may be assessed by M-mode echocardiography (**Fig. 11A**). Determination of the optimal LVAD speed is based on aortic valve opening as well as the degree of LV filling. High-speed settings result in LV underfilling and cessation of aortic valve opening, and the converse holds true for low speeds. In addition to hemodynamic optimization, echocardiographic imaging at varying LVAD speeds is also used in ramp studies to evaluate for pump thrombosis when the expected changes in terms of reduced LV filling and decreased aortic valve opening do not occur at increasing speeds. During weaning studies when LVAD speed is gradually decreased, improvement in LVEF, increased aortic valve opening, and lack of significant LV dilatation serve as indicators of myocardial recovery on which candidacy for LVAD explant is based.

Transplant Cardiology

In patients with advanced heart failure who are considered for heart transplantation, echocardiography is instrumental in the pretransplantation process. Candidacy for transplantation is based on numerous clinical factors, in addition to echocardiographic findings of severe left ventricular dysfunction. Pulmonary hypertension secondary to chronically elevated LV filling pressures can lead to irreversible pulmonary vascular remodeling and has been associated with increased mortality following cardiac transplantation, primarily due to right heart failure (Costard-Jäckle and Fowler, 1992). As a result, severe pulmonary hypertension unresponsive to vasodilator therapy may preclude transplantation. Although pulmonary pressures are typically evaluated by catheterization for transplant candidacy, echocardiography allows for noninvasive monitoring of right heart hemodynamics (**Fig. 6**). Another component of the pretransplantation process is evaluation of donor heart function, which involves echocardiographic assessment of biventricular function and screening for valvular or congenital abnormalities (Zaroff, 2004). Neurohormonally mediated LV systolic dysfunction, as well as regional wall motion abnormalities in the absence of coronary artery disease, is common in potential donors and may preclude use for transplantation (Dujardin et al., 2001).

Following cardiac transplantation, echocardiography continues to be an important tool for evaluation of graft function. In the early perioperative period, LV systolic dysfunction can be an indication of primary graft failure due to prolonged ischemic time, reperfusion injury, hyperacute rejection, or suboptimal donor heart. RV dilatation and dysfunction are common during this period due to the high afterload from residual pulmonary hypertension in the recipient (Bhatia et al., 1987; Hosenpud et al., 1987). Postsurgical pericardial effusions are associated with undersized donor hearts and raise the concern for infection (due to immunosuppression) or rejection (Hauptman et al., 1994). Tamponade physiology resulting from large effusions or focal loculations may be readily assessed by echocardiography.

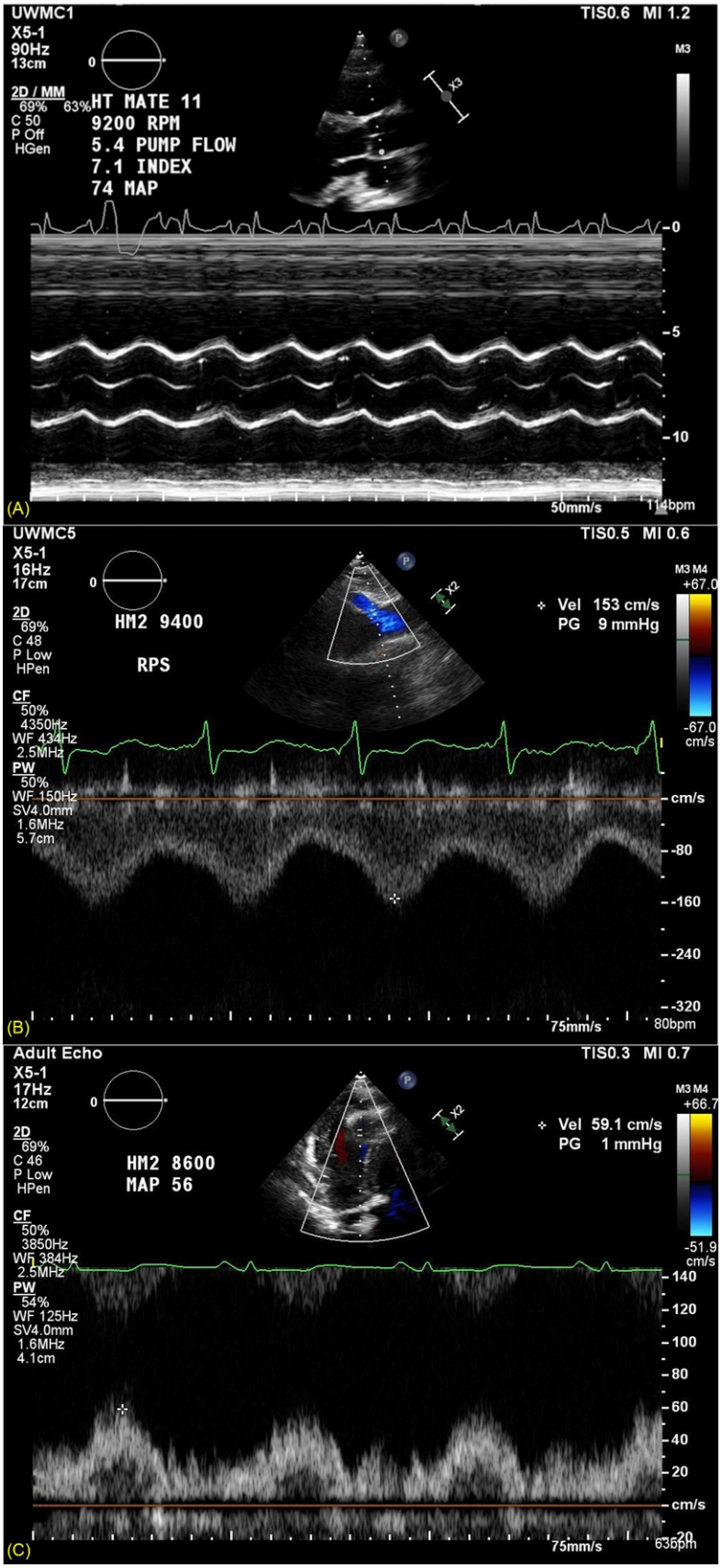

Fig. 11 Assessment of LVAD function in a Heartmate II continuous-flow device. (A) M-mode across the aortic valve shows intermittent, partial opening of the aortic valve. (B) Pulsed-wave Doppler interrogation of the outflow cannula showing laminar flow into the ascending aorta with normal velocities (<2 m/s). (C) Pulsed-wave Doppler interrogation of the inflow cannula showing laminar flow from the LV apex with normal velocities.

Current practice guidelines recommend routine surveillance for acute rejection, cellular or antibody-mediated, by endomyocardial biopsy in at least the first year posttransplantation (Costanzo et al., 2010). However, in patients presenting with symptoms concerning for acute rejection, echocardiography can assess graft function more rapidly and lead to earlier initiation of therapy. In acute rejection, systolic dysfunction presents later than diastolic dysfunction, which is not specific to rejection. There may be a role for strain imaging in the screening for acute rejection noninvasively (Kato et al., 2010). Late complications, occurring several years after transplant, are most commonly related to cardiac allograft vasculopathy (CAV), which is characterized intimal hyperplasia of the coronary arteries. CAV may be subclinical or present as heart failure, myocardial infarction, or sudden cardiac death. Screening for CAV by coronary angiography can be challenging due to the diffuse and concentric involvement. Intravascular ultrasound (IVUS) is more sensitive for detecting CAV and predicts adverse cardiac outcomes but still requires invasive catheterization (Tuzcu et al., 2005; Kobashigawa et al., 2005). Dobutamine stress echocardiography correlates well with the gold standard of IVUS in terms of detection of CAV and carries similar prognostic value (Spes et al., 1999). Furthermore, dobutamine stress echocardiography appears to have higher diagnostic accuracy than nuclear myocardial perfusion imaging for CAV, with the exception of quantification of myocardial blood flow with positron emission tomography (PET) (Bacal et al., 2004; Wu et al., 2010; Mc Ardle et al., 2014).

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Echocardiography: Assessment of Valve Structure and Function

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Abbreviations

2D	Two dimensional
3D	Three dimensional
AR	Aortic regurgitation
AS	aortic stenosis
LA	Left atrium
LV	Left ventricle
LVOT	Left ventricular outflow tract
MR	Mitral regurgitation
MS	Mitral stenosis
PISA	Proximal isovelocity area
PR	Pulmonic regurgitation
PS	Pulmonic stenosis
RA	Right atrium
RV	Right ventricle
RVOT	Right ventricular outflow tract
TEE	Transesophageal echocardiogram
TR	Tricuspid regurgitation
TS	Tricuspid stenosis
TTE	Transthoracic echocardiogram
VC	Vena contracta

Introduction

Valvular heart disease is a significant cause of morbidity and mortality around the world (Nkomo et al., 2006). Echocardiography serves as the workhorse imaging modality in the diagnosis of valvular heart disease and in many cases, remains the gold standard. Advances in echocardiography (both due to widespread availability and technological improvements) have helped improve our understanding of valve structure and function and simplified the diagnosis of valve disease. Several major society guidelines have

been published which comprehensively (often using multiple modalities) address the identification and quantification of valve disease (Nishimura et al., 2014 Jul; Zoghbi et al., 2017; Baumgartner et al., 2009; Lancellotti et al., 2010a,b). In this article, we have outlined the role of echocardiography in understanding valve structure and function and reviewed various ultrasound principles and echocardiographic techniques in the diagnosis of valve disease encountered in adults.

Ultrasound Principles in the Assessment of Valve Structure and Function

Knowledge of the principles of ultrasonography is crucial to image heart valves and interpret valvular pathology. Otherwise, significant errors in the diagnosis and grading of severity of valve disease may occur.

Two-Dimensional Echocardiography

Two-dimensional B mode (2D) transthoracic echocardiography (TTE) is the first step in the assessment of valve structure and function. Visualization of valves depends on ultrasound penetration as well as careful probe manipulation in standard views to obtain orthogonal 2D images of three-dimensional valvular and subvalvular structures. Such imaging is influenced by the ultrasound principles underlying 2D image formation.

Fundamental versus harmonic imaging

Tissue harmonic imaging (THI) has greatly improved endocardial border delineation (improved lateral resolution) and reduced artifacts (such as near field clutter) which are common with fundamental imaging in TTE (Thomas and Rubin, 1998). Many echocardiographic laboratories now image exclusively with THI. It is important to note, however, that THI imaging leads to reduced axial resolution which makes cardiac structures appear thicker than when fundamental imaging is used (van Wijk and Thijssen, 2002). Although leading edge to leading edge measurement technique can resolve this to some extent in the assessment of chamber dimensions, there is limited data for such adjustment in measurement of valve thickness. With application of THI, many laboratories report an increase in diagnosis of increased valve thickness and sclerosis. For example, the mitral valve leaflets appear thicker with THI compared to fundamental imaging (**Fig. 1**) (Gorgulu et al., 2005). This can lead to false diagnosis of mitral valve pathology and can also alter therapeutic decisions. In the case of mitral stenosis, valvular and subvalvular thickness play an important role in the choice of percutaneous versus surgical therapies (Prior et al., 2000). In otherwise eligible patients, THI imaging may lead to a falsely high valve score precluding the option of balloon valvuloplasty. In such cases, fundamental imaging should be used judiciously to improve accuracy of diagnosis. Transesophageal echocardiography (TEE), by elimination of ultrasound tissue attenuation inherent to TTE, improves diagnosis of valve pathology by virtue of the ability to image at both fundamental and higher frequencies than TTE (see what follows). Despite these limitations, THI has improved our overall ability to diagnose valve disease simply by better visualization of the valve.

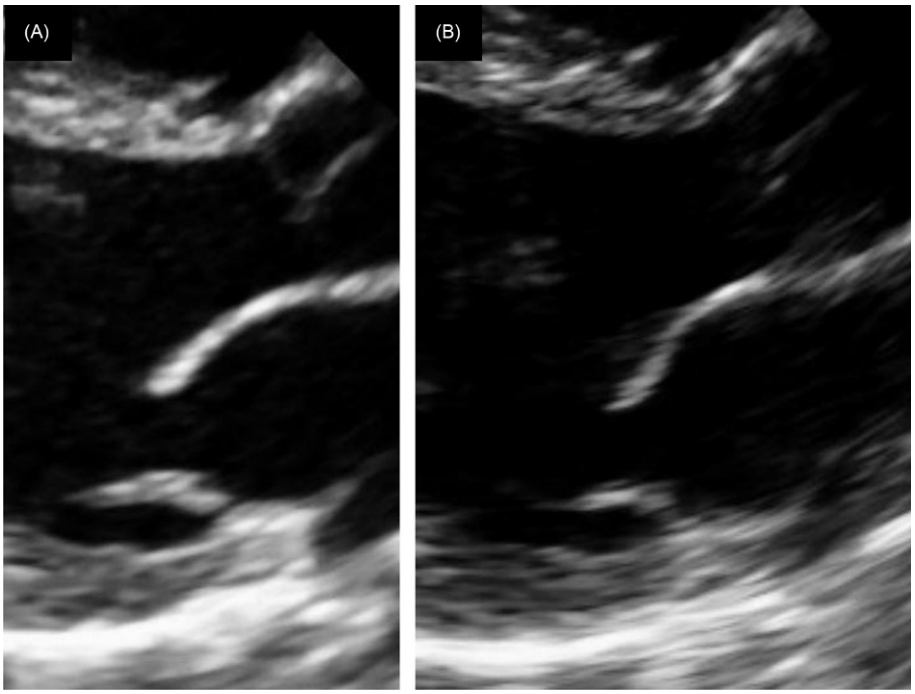


Fig. 1 Effect of harmonic imaging on assessment of valve structure. The anterior mitral valve leaflet appears thicker with harmonic imaging (transmit energy 1.5 MHz/receiving frequency 3 MHz, panel A) compared to fundamental imaging (transmit/receive at 3 MHz, panel B).

High frequency imaging

The higher the frequency of ultrasound, the better the axial resolution at the cost of reduced penetration. In patients with reasonable ultrasound penetration, high frequency TTE (3–4 MHz) imaging improves resolution of valve structures than lower frequency imaging. Though higher frequency, phased array TTE transducers are available (up to 12 MHz), diagnostic quality imaging is not feasible in many adult patients.

Depth of imaging and focal zone

Because valves are often far field in standard echocardiographic views, every attempt should be made to improve both spatial and temporal resolution. Reducing depth, matching sector size to the area of interest and/or zoomed-in views of the valve in question improve resolution and signal-to-noise ratios. Reducing sector size is the single most important step in increasing frame rate which in turn improves temporal resolution.

Doppler Echocardiography

Both color and spectral Doppler play a crucial role in the assessment of valvular function. Whereas color Doppler is essential to detect the presence of regurgitation, spectral Doppler is essential to quantify the extent of both regurgitation and stenosis.

Spectral Doppler

Pulse wave (PW) Doppler and continuous wave (CW) Doppler are critical modalities in quantifying flow across valves. Briefly, these techniques are based on the Doppler principle: the frequency of a waveform emitted by a moving object changes relative to the position of the observer. This change is called Doppler frequency shift and is summarized by the Doppler equation:

$$\mathbf{f_d = 2f_t V(\cos \theta)/c}$$

where f_d = Doppler shift frequency, f_t = transmit ultrasound frequency, v = velocity of blood flow, c = speed of ultrasound in medium (blood in case of echocardiography), and θ = angle of flow relative to ultrasound beam (angle of insonation).

When frequency shift information is available, velocity of blood flow is computed instantaneously on contemporary Doppler systems. A major limitation is the assumption that cosine angle is zero (=1). As the angle of insonation increases (cosine >0 degrees), the velocity is underestimated. Because blood flow in normal and abnormal valve function states varies three dimensionally, angle correction by estimating the angle of insonation is often inaccurate and should be avoided.

In PW Doppler, discrete pulses of ultrasound are transmitted and received by a single transducer element with specific time windows to listen to the reflected signal. By placing the sample volume at a location of interest, Doppler frequency shifts at that site are detected and processed to resolve velocity. Because the pulses are not simultaneously transmitted and received, this modality is limited by the sampling rate, that is, pulse repetition frequency (PRF). If the velocity of flow at the area of the Doppler sample volume is high, the higher frequencies shift and a higher pulse repletion frequency is needed to accurately detect such shift. The Nyquist limit refers to the maximum velocity that can be accurately quantified within the given sample volume and is determined by the formula:

$$\mathbf{Nyquist\ \ limit = PRF/2}$$

The longer the distance of the sample volume from the transducer, the lower the Nyquist limit because longer time is taken for the pulse to be transmitted and reduced. When the velocities exceed the Nyquist limit, aliasing occurs. In contrast, CW Doppler is continuously emitted from a dedicated transducer element while a separate element simultaneously and continuously receives returning signals (Fig. 2). While this mode is not constrained by the limitations posed by the Nyquist limit, the location of the signal cannot be specified. Thus, PW Doppler is used to localize flow disturbance and quantify lower velocities (usually less than 2 m/s), while CW Doppler resolves higher velocities without the ability to localize.

Assessment of valve function using spectral Doppler echocardiography is based on the assessment of flow and pressure gradients via the application of physical principles of fluid mechanics. The following pitfalls should be recognized while using spectral Doppler techniques in valve disease.

Measurement of pressure gradients

The Bernoulli equation is used to estimate the pressure gradient between two chambers separated by an orifice. A simplified form of the Bernoulli equation assesses convective acceleration while ignoring flow acceleration (which is almost nonexistent at flows being interrogated in clinical echocardiography) and viscous fraction (which is minimal for blood flow across even the most stenotic valves):

$$\mathbf{P_1 - P_2 = 1/2\rho\left(V_2^2 - V_1^2\right)}$$

where P_1 and P_2 are pressures proximal and distal to the orifice, V_1 and V_2 are velocities proximal and distal to the orifice, and ρ is the density of blood.

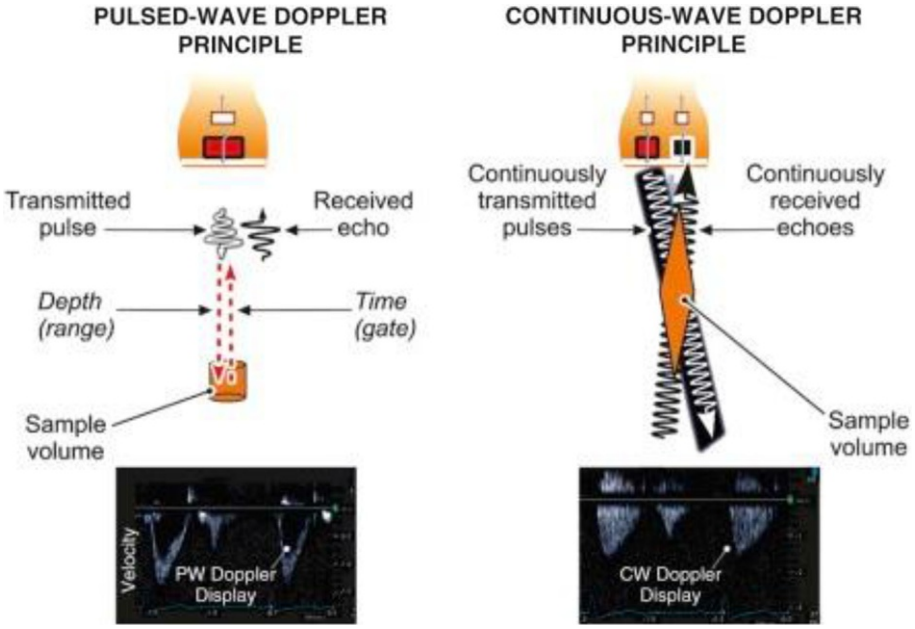


Fig. 2 Difference between pulse wave and continuous wave Doppler. Reproduced with permission from *Braunwald's Heart Disease*, 10th edn, 2015.

Because the velocity proximal to a stenotic orifice in the heart is often quite low compared to that distal to the orifice, they are often ignored. After solving for 1.06×10^3 kg/m^3 as ρ at room temperature, a very simple equation is derived:

$$\mathbf{P1 - P2 = 4(V_2)^2}$$

This simplified formula is sufficient in most cases but can overestimate the pressure gradient in cases where V_1 is not insignificant. An example is a case of aortic stenosis (AS) with increased left ventricular outflow tract (LVOT) velocity due to increased flow or LVOT obstruction. In these cases, V_1 should be included in the equation when the velocity is over 1.5 m/s (Baumgartner et al., 2009). In addition, pressure gradient is underestimated if the ultrasound beam is not aligned parallel with flow through the orifice which leads to underestimation of velocity (higher cosine angle of insonation, especially above 20 degrees). Of note, CW Doppler is employed to resolve the maximal velocity which is of interest in estimation of pressure gradients. When location of flow at a particular area is of interest in calculating volumes, PW Doppler is used.

Derivation of volume from Doppler echocardiography

Volume assessment is a two-step process. First, flow rate is calculated by integrating the velocities resolved throughout the relevant phase of the cardiac cycle at the site of interest (using PW Doppler) into a velocity time integral (VTI). Second, flow rate (VTI) is multiplied by the cross-sectional area (CSA) of the region of interest to calculate the magnitude of flow (volume):

$$\mathbf{Volume = VTI \times CSA}.$$

Because the diameter (D) of a given structure is easily measured by 2D echocardiography while CSA is not, the region of interest is assumed to be a circle and the CSA is derived by the formula:

$$\mathbf{CSA = \pi \times (D/2)^2}$$

This calculation can be applied to assess effective orifice area (EOA) of stenotic valves by the application of the continuity equation. The continuity equation is based on the principle of conservation of mass which states that flow in one region of the heart should be equal to flow in another region. When applied to stenotic valves, flow proximal to the stenosis is equal to flow distal to the stenosis provided there is no regurgitation or shunt. For example,

$$\mathbf{LVOT_{VTI} \times LVOT_{CSA} = Aortic\ Valve_{VTI} \times Aortic\ Valve_{EOA}}[11]$$

There are three significant pitfalls in this process. One, if the ultrasound beam is not aligned parallel with flow (angle of insonation >0 degrees), velocities are underestimated which in turn will underestimate flow and volume. Second, in estimating the flow proximal to the stenosis, the sample volume should be placed carefully at the area of laminar flow. For example, while calculating LVOT flow, the sample volume should be placed about 5–10 mm away from the aortic valve to avoid contamination of the PW Doppler signal by turbulence that occurs closer to a stenotic aortic valve. If flow turbulence is captured, LVOT velocity and thus flow is overestimated. Similarly, the modal velocity (densest portion) and not the outer edges of the PW Doppler signal should be traced. Third, CSA calculation depends both on accurate calculation of diameter and the region of interest confirming to the assumption of circular geometry. If the diameter is calculated incorrectly (such as in patients with poor ultrasound windows), the magnitude of the

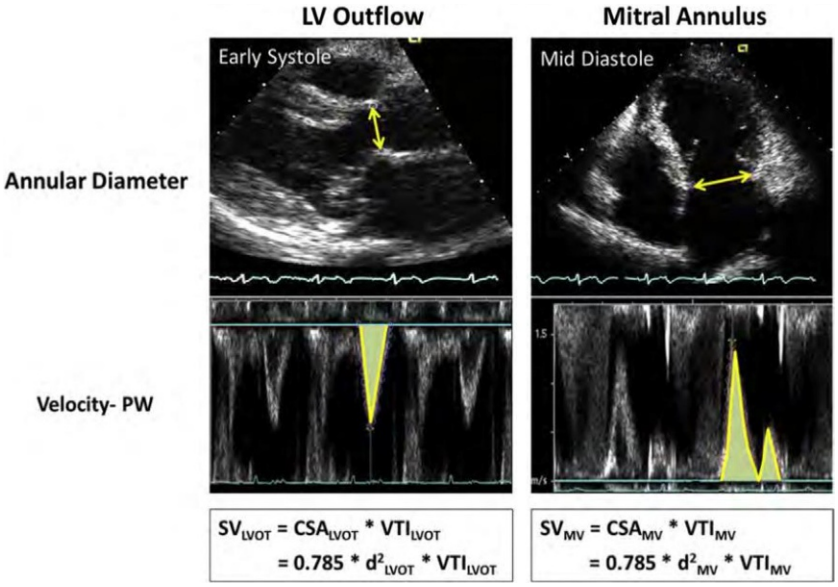


Fig. 3 Application of the principle of Doppler volumetric assessment in mitral and aortic regurgitation. The difference between the stroke volume across the regurgitant valve and the normal valve represents regurgitant volume. Reproduced with permission from Zoghbi et al. (2017). *Journal of the American Society of Echocardiography*, **30** (4), 303–371.

error is squared. Similarly, CSA is underestimated if the anatomical structure (such as the LVOT, a crucial step in the derivation of aortic valve area in AS) is noncircular which leads to underestimation of EOA.

Spectral Doppler evaluation can also be used in quantification of regurgitant volumes by calculating the difference in flow across the regurgitant valve and stroke volume (Rokey et al., 1986). This is termed the Doppler volumetric method. For example, regurgitant volume of mitral regurgitation (MR) can be calculated as the difference between total transmitral stroke volume (VTI of transmitral flow at annulus × CSA of mitral annulus) and the stroke volume across the LVOT. Because several calculations are required and due to the multiple assumptions inherent to this calculation (that both mitral annulus and LVOT are circular), errors are common (Fig. 3).

An important method in estimating regurgitant volumes is the proximal isovelocity surface area (PISA method) which requires a combination of color and CW Doppler.

Color Doppler

Qualitative assessment by color Doppler is the most common echo parameter used for detection and assessment of severity of valvular regurgitation. Color flow Doppler imaging produces a color-coded map of Doppler frequency shifts detected using PW ultrasound. By convention, flow toward the transducer is coded red and flow away is coded blue. Because multiple areas within a color sector need to be interrogated for flow information, fewer pulses are used per scan line and the frequency shift between each area of measurement is color encoded and compared using a technique called autocorrelation. The transducer also switches rapidly between color Doppler and B mode imaging to produce a combined image (which is an illusion because scanning in these modalities does not occur simultaneously). Techniques such as a variance mapping (which compares the degree of difference between different velocities to the mean velocity in a given sample volume and encodes these differences with green providing a multitude of shades) are used to better depict turbulence within the regurgitant flow.

A well-defined regurgitant color Doppler jet consists of three parts—proximal flow convergence (representing the area where blood flow accelerates as it converges through the regurgitant orifice), vena contracta (VC, narrowest portion of the regurgitant jet which is just distal to the regurgitant orifice and is in essence the effective regurgitant orifice area, EROA), and the jet area (Fig. 4). The most important error in the assessment of color Doppler in valve regurgitation is overreliance on jet area and length. First, color Doppler does not measure actual regurgitant flow but rather provides an estimate of the change in velocities due to the regurgitation. A wall hugging eccentric jet may appear smaller because it loses momentum sooner and cannot entrain a larger pool of blood flow velocities compared with a central jet arising from similar regurgitant orifice area. Even indexing jet area to chamber size can also be misleading in the presence of severe chamber dilation.

Pitfalls in color Doppler imaging (Zoghbi et al., 2017; Thomas et al., 1990)

a. *Frame rate*: When frame rates are low, a coherent color Doppler jet cannot be realized and valve regurgitation can be missed or underestimated. Principles to improve frame rate in color Doppler include reducing depth and decreasing size of both color Doppler and 2D sectors.
b. Color Doppler settings (Fig. 5).

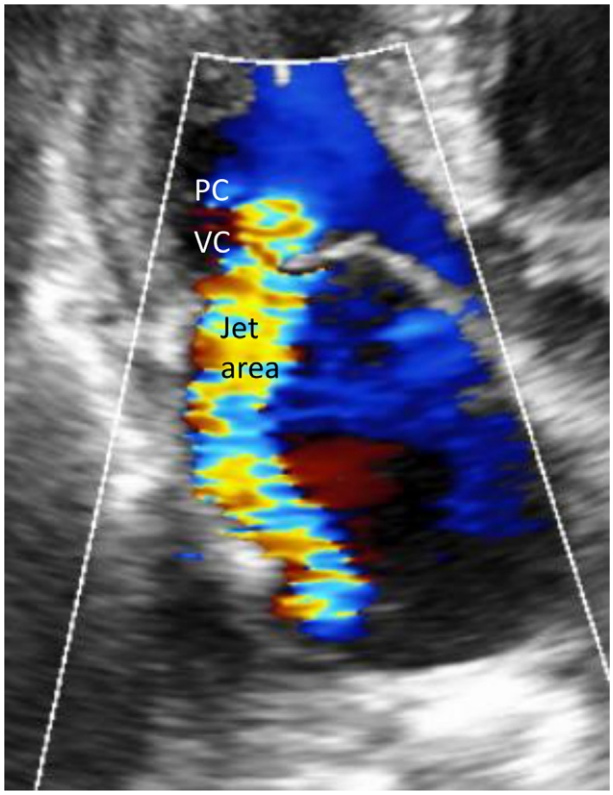


Fig. 4 The three areas of a regurgitant jet by color Doppler. *PC*, proximal flow convergence; *VC*, vena contracta.

Gain: Excess gain can overestimate color jet size by recruiting random noise while too low gain leads to underestimation. Gain should be high enough to avoid Doppler signal within the tissues.

Nyquist limit: As a special application of PW ultrasound, velocities resolved by color Doppler are subject to the Nyquist limit. Low Nyquist limit settings lead to overestimation by merging lower velocity blood flow in the chamber into the regurgitant jet. A Nyquist limit of at least 50–60 cm/s is recommended. Similarly, a high Nyquist limit may underestimate regurgitation by failing to detect lower velocity flow with the regurgitant jet.

High frequency imaging: While high-frequency imaging improves diagnosis of valve structure, it can make color Doppler jets appear larger due to increased detection of lower velocities. This effect needs to be considered especially in TEE imaging because many commercially available probes offer a range of imaging frequencies between 3.5 and 8 MHz.

c. *Temporality*: Color jet area is instantaneous while regurgitant volume is not. This principle is key in avoiding overestimation of severity of regurgitation. Assessment of regurgitation severity by color Doppler is based on jet area/dimension compared to the chamber into which the jet empties. Jet area/size is calculated at a maximum instantaneous time, while regurgitant volume depends on both flow rate and duration of flow. This is significant in cases of mitral valve prolapse where a mid to late systolic MR jet may appear significant by color Doppler but due to the shorter duration of regurgitation, the regurgitant volume is less impressive.

d. *Hemodynamics*: Color Doppler depiction of regurgitant jet depends on jet momentum which depends on both orifice area and jet velocity. Jet velocity in turn depends on the driving pressure across the regurgitant valve. Thus, elevation of LV systolic pressures (due to systolic hypertension) can make mitral regurgitant jet appear large and correction of blood pressure may reduce the jet area. Similarly, the color jet of MR may appear small when LV systolic pressures are low (such as with sedation in a dehydrated patient or intraoperatively). In acute severe regurgitation, the jet may appear laminar due to the lower driving pressure across the regurgitant orifice. This underscores the importance of integrating patient hemodynamics into color Doppler assessment.

e. *Image plane*: Deviation from standard imaging planes may be needed to clearly define the regurgitant jet. In eccentric jets, standard echocardiographic views to evaluate valve structure may not capture the full extent of Color jet area. In addition, the same view may not capture the flow convergence and jet area simultaneously. In these cases, individual components should be defined in separate optimized views.

Color Doppler can be used to quantitate regurgitant flow by assessment of proximal flow convergence and VC width.

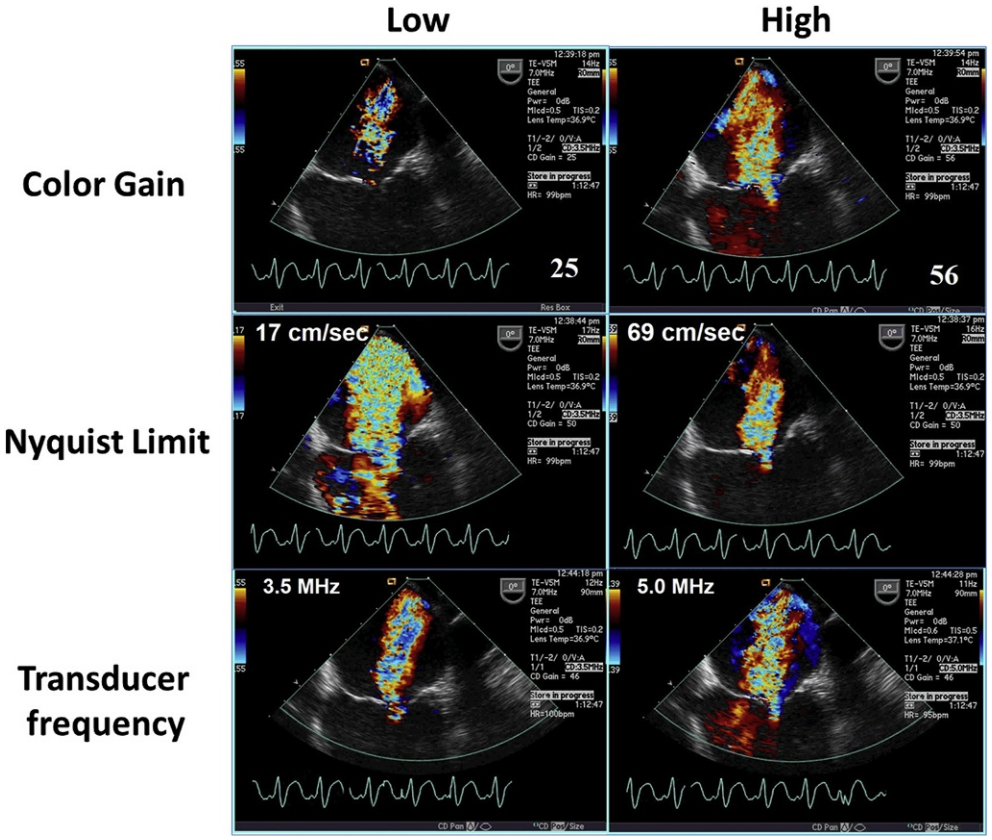


Fig. 5 Effect of gain, transducer frequency, and Nyquist limit on the color Doppler appearance of mitral regurgitation. Reproduced with permission from Zoghbi et al. (2017). *Journal of the American Society of Echocardiography*, **30** (4), 303–371.

Vena contracta width

Measurement of VC dimension provides a semiquantitative measure of severity of regurgitant lesion (Baumgartner et al., 1991). By virtue of being the narrowest portion of the regurgitant jet, VC represents the EROA.

Pitfalls in measurement of vena contracta

a. *Shape and form*: 2D VC measurement provides a single measure of a three-dimensional EOA. Depending on the plane chosen, a single measurement may overestimate or underestimate true VC size. This is especially true when the VC is an ellipse (such as a case of secondary MR) rather than a circle. 3D assessment of VC provides accurate assessment of VC area but is limited by the poor temporal and spatial resolution of current generation 3D systems and not well validated.
b. *Measurement unit*: Because VC width quantified is in millimeters, even a small error in measurement can result in a misleading assessment of severity of valvular regurgitation. Axial resolution of ultrasound is better than its lateral resolution, therefore VC is best measured in the parasternal views in both mitral and aortic regurgitation (AR).
c. *Severity of regurgitation*: VC is best at identifying severe regurgitant lesions but lacks sufficient accuracy to distinguish less severe cases. Values in intermediate range require verification by another method of quantification.

Proximal isovelocity surface area

The proximal isovelocity surface area (PISA) method represents an application of the continuity principle and utilizes both color and spectral Doppler (Schwammenthal et al., 1994). As blood converges toward a regurgitant orifice, it accelerates and forms concentric hemispherical shells whose boundaries contain blood cells of similar velocity (isovelocity surfaces). As the blood gets closer to the orifice, the velocity increases and aliasing occurs and is depicted by a distinct red blue interface at the boundary of the shell. By adjusting the color Doppler baseline in the direction of the regurgitant flow (reducing the Nyquist limit) and turning off variance mapping, this interface is accentuated and radius of the shell can be measured. Assuming the shell is hemispheric, the surface area can be calculated ($2\pi r^2$) and finally the flow rate can be estimated by multiplying the surface area with the aliasing velocity (**Fig. 6**). Based on the continuity principle, the flow rate at isovelocity surface is similar to the flow rate through the regurgitant orifice (Flow rate at isovelocity shell = EROA × regurgitant jet velocity). By measuring the maximum velocity of the regurgitant jet by CW Doppler, the

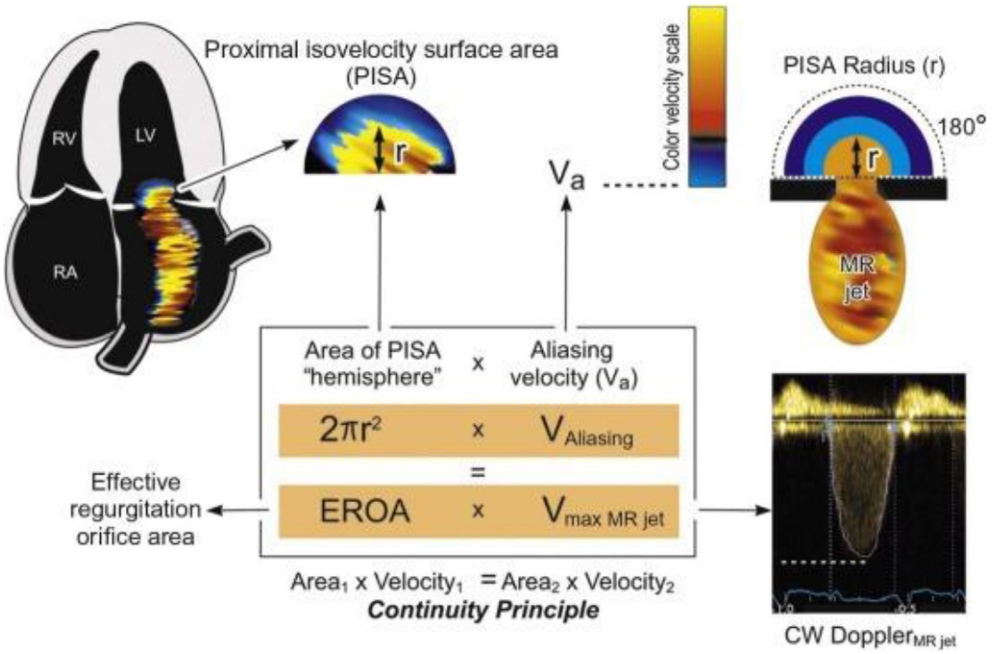


Fig. 6 Estimation of regurgitant volume and effective regurgitant orifice area (EROA) using the PISA method. This example shows the measurement of the PISA radius and the timing of the selection of the color frame for measurement (*solid yellow arrow*), corresponding to the maximal jet velocity by CWD (*dashed arrow*). *Reg*, regurgitation; *PKV*, peak velocity of regurgitant flow by CWD; *VTI*, velocity time integral of the regurgitant jet by CWD Reproduced with permission from *Braunwald's Heart Disease*, 10th edn, 2015.

EROA can be calculated. Regurgitant volume can then be derived by multiplying the EROA by VTI of the regurgitant jet (obtained by tracing CW velocity curve). Despite being technically challenging, accurate estimation of regurgitant volume and EROA is possible in many cases where the proximal flow convergence zone is clearly defined. In patients with multiple jets, volumes and EROA can be calculated for each individual jet and combined to provide an assessment of severity.

Pitfalls in the estimation of regurgitation by PISA

a. *Flow convergence*: It is difficult to obtain a clear flow convergence zone in all cases. Off-axis views are sometimes required. In some cases, valve thickening and calcification preclude accurate depiction of flow convergence. An additional pitfall is the challenge of identification of the orifice which is a critical component of estimation of radius.
b. *PISA shape, plane, and orifice geometry*: EROA estimation of the PISA method is based on three geometric assumptions which can introduce error into calculations. The first assumption is that the isovelocity shell is hemispheric which is not always true. Some shells appear flat and some assume a tear drop shape. The flow convergence zone may be constrained by a ventricular wall (especially in eccentric jets). Though angle correction can be used, this introduces another degree of complexity into an already complicated equation (Pu et al., 1995). It introduces a significant source of error. The second assumption is that the orifice lies in a flat plane which does not hold good in all cases, for example, the plane may be conical in secondary MR with leaflet tethering. The final assumption is that of a circular orifice geometry, the most noTable exception is that of an elliptical orifice in secondary MR.
c. *Timing and duration of measurements*: EROA by the PISA method is an instantaneous measure of an average EROA through a phase of the cardiac cycle. Thus, EROA can be overestimated in regurgitant jets limited to a small part of the cardiac cycle (late systolic MR due to mitral valve prolapse) or underestimated in jets with a dynamic variation of EROA (such as the bimodal secondary MR) (Topilsky et al., 2012). In addition, the measurement of PISA radius and the MR velocity should be timed to the same point of the cardiac cycle.
d. *Locating the regurgitant orifice*: The PISA radius is drawn from the location of the regurgitant orifice to the point of first Color Doppler aliasing. The location of the regurgitant orifice can sometimes be challenging to define. In practice, one of three methods are useful: (i) Location of VC can be used to draw the radius because it represents location of EROA; (ii) often, a horizontal Color Doppler flow occurs at the EROA can be a marker; and finally (iii) turning off the Color Doppler flow is possible in some PACS software (EchoPAC, GE) which allows for visualization of the anatomical orifice area.

In conclusion, Doppler echocardiographic quantification is useful in many cases of valvular regurgitation. In some cases, however, significant discrepancy can occur and multiple methods should be used to obtain the best possible estimate of severity. Accurate flow quantification and chamber volume assessment by cardiovascular magnetic resonance imaging (CMR) is helpful in such cases (Zoghbi et al., 2017).

Three-dimensional echocardiography

Current generation three-dimensional (3D) echocardiography is based on processing data obtained from a matrix-array transducer which simultaneously emits and receives ultrasound in multiple planes. Improvements in processing technologies allow for real-time rendering of 3D images as well as acquisition of full volume data sets that are suïTable for postprocessing (Lang et al., 2012). The ability to generate 3D images has helped improve our understanding of valve structure as well as facilitated easier identification of abnormalities such as mitral valve prolapse or flail leaflets. During postprocessing, multiplanar reconstruction can be performed to obtain in-plane measurements. This has especially been useful in cases such as rheumatic mitral stenosis. Integration of color Doppler to 3D echo has helped clarify several pitfalls and assumptions in standard 2D measurements such as PISA and VC. By virtue of the large amount of data that is generated and processed, currently available 3D suffers from poor spatial and temporal resolution compared with 2D echocardiography.

Currently, 3D echocardiography is superior to 2D in the following aspects of valvular heart disease: (a) accurate estimation of chamber size and volumes by eliminating the assumptions on which 2D volumetry is based as well as avoiding apical foreshortening (Mor-Avi et al., 2008); (b) assessment of 3D valve anatomy in some cases such as mitral valve disease (e.g., a surgeon's view with identification of leaflet segments in a single view), and (c) guiding catheter-based interventional procedures such as mitral clip implantation. The most important pitfall of 3D echocardiography relevant to valve disease is the failure to understand that a poor 2D image results in a worse 3D image. Time should be spent optimizing 2D TTE images first. In cases of poor ultrasound penetration, a 3D dataset rarely helps. With better resolution and less interference, 3D imaging is an invaluable resource in TEE imaging (Lang et al., 2012).

Stress echocardiography

Stress echocardiography plays an important role in three subsets of patients with valve disease: (a) to assess the hemodynamic effects of asymptomatic severe valve disease, (b) when resting echocardiography results are incongruent with exertional symptoms, and (c) when low flow states blur the distinction between true stenosis and failure of valve opening due to low flow itself (pseudo stenosis). Exercise testing with or without concomitant echocardiography is a valuable tool in an objective assessment of functional capacity in patients with apparently asymptomatic severe valve disease (Lancellotti et al., 2005). When noninvasive testing is performed to assess the impact of mechanical stress on myocardial and valve function, exercise is preferred over pharmacological stress because physical activity is more physiological. Exercise modalities that enable assessment at peak heart rate (semisupine exercise and bicycle ergometry) are preferred over those where assessment is performed post peak-stress (treadmill exercise). This is particularly important in some patients with seemingly moderate mitral stenosis at rest who have significant increases in mitral valve gradients and RV systolic pressure with exercise. When a low flow state (such as reduced LV systolic function) exists, increasing flow with a pharmacological agent such as dobutamine allows for distinction between true stenosis and pseudo-stenosis. This is applied commonly in AS in the setting of a low stroke volume (Takeda et al., 1999; Nishimura et al., 2002).

Contrast echocardiography

By utilizing the reflective properties of gas filled microspheres, endocardial border definition is improved by utilization of commercially available ultrasound opacification agents (Mulvagh et al., 2008). This allows accurate assessment of LV volumes and estimation of LV systolic function (Thomson et al., 2001). These agents also help enhance Doppler signals and can be used to better delineate the Doppler velocity trace across a valve. This has been mostly studied in the assessment of RV systolic pressure using the jet of TR and in enhancing the signal of AS (Himelman et al., 1989; Nakatani et al., 1992). Because dispersion of ultrasound increases artifact and causes blurring around CW Doppler signals, care should be taken to avoid measuring artifact which can result in overestimation of velocities.

Speckle tracking echocardiography

A wealth of information now exists that assessment of LV mechanics provides incrementally useful information in assessment of the myocardial effects of left-sided valve disease (Yingchoncharoen et al., 2012; Magne et al., 2012; Lancellotti et al., 2010c). In patients with asymptomatic severe valve disease with normal cardiac function, assessment of LV longitudinal strain by speckle tracking echocardiography can help diagnose early stages of myocardial dysfunction. This can be particularly useful in serial longitudinal follow-up. However, widespread clinical application of this technique in valve disease is in its infancy and requires validation in larger studies. The lack of intervendor compatibility also limits its use in routine clinical practice especially in multivendor labs where a patient may receive serial follow-up studies on different machines.

Transesophageal echocardiography

Transesophageal echocardiography (TEE) is a valuable tool in the assessment of valve anatomy because of the close proximity of the TEE probe to the heart, unhindered by structures such as adipose tissue, ribs, and lungs, and its ability to employ high-frequency imaging that enables better resolution (Hahn et al., 2013). However, a carefully performed TTE outperforms a TEE in some situations:

a. Interrogation of Velocities Across the Aortic Valve. Except for the deep transgastric apical view (which can be difficult to obtain in nonintubated patients), a spectral Doppler beam cannot be aligned parallel to transaortic flow during TEE.

b. Evaluation of Anterior Structures – Anterior structures such as the pulmonic valve are nearfield in TTE and far field in TEE. Similarly, though TEE enables an en face evaluation of the tricuspid valve, reverberation artifact from the aortic root can sometimes effect imaging of the tricuspid valve in the esophageal views.

Integrated approach to echocardiographic evaluation of valves

Echocardiographic evaluation often starts with a review of the clinical history and an assessment of overall health status of the patient. Evaluation performed on supine patient in a hospital bed may preclude accurate valve assessment by reducing available ultrasound windows and preventing parallel alignment of Doppler beam with the valve of interest. In such cases, a follow-up study after the patient recovers enough to be examined thoroughly is sometimes required. Heart rate and blood pressure recorded at the time of the study are essential in estimating the hemodynamic effects of valve disease. For example, the heart rate at which the mitral stenosis gradient is recorded should be part of all reports because the mean gradient across the valve is heavily dependent on flow (which, in turn, is affected by heart rate). Similarly, the blood pressure at the time of the exam can influence the assessment of severity of left-sided regurgitant valve lesions, especially MR.

Echocardiographic evaluation often starts with the assessment of overall chamber size and volumes and then 2D evaluation of valve structure. Multiple views should be used to assess valve structure. En face views of the aortic valve and mitral valve opening are easily obtained by TTE, while similar views are difficult to obtain for the tricuspid valve. When a valve abnormality is found, attention should be paid to the structure and function of the affected cardiac chamber. For example, left atrial size in mitral stenosis and MR, LV dimensions, volume, and systolic function in AR and MR and RV dimensions and function and IVC size in tricuspid regurgitation (TR) help assess the hemodynamic burden of valve disease. Color Doppler is used to detect valvular regurgitation and also to detect flow acceleration across stenotic valve. In stenotic valves, the presence of flow acceleration allows one to cross check the accuracy of CW Doppler measurement across stenotic valves. For example, flow acceleration during diastole across a mitral valve with calcified annulus should alert one to assess mitral valve gradients despite what appears to be adequate leaflet tip motion.

Spectral Doppler images are then assessed to evaluate gradients and effective orifice areas across stenosis and regurgitant volumes and effective regurgitant areas. Some commercially available PACS systems (EchoPAC, GE) allow the interpreter to manipulate raw image data to change gain and alter color Doppler scale (which is crucial in PISA assessment for regurgitant valves). Attention should be paid to the intercept angle between the ultrasound beam and the flow being interrogated. Discrepancies in degree of severity of valve disease (especially AS) are often related to his error. If such a discrepancy is detected, additional images should be obtained to correct the technical error or use additional nonstandard views (such as CW Doppler evaluation of AS from the right parasternal window). Similarly, assessment of flow in structures effected by regurgitant flow (PW Doppler of pulmonic veins in MR, descending aorta in AR, hepatic veins in TR) provides valuable information of its hemodynamic significance. In selected cases, 3D imaging is performed to better assess valve structure though poor ultrasound penetration may prevent diagnostic quality 3D TTE imaging.

TEE is often used in cases where TTE does not provide definitive information or in cases where surgical repair or transcatheter therapies are planned. Despite the higher resolution of TEE images, TTE is superior for assessment of gradients across stenotic valves (especially AS). Additionally, TEE is useful in surgical planning (such as mitral valve repair for primary MR) or assessing suitability for transcatheter valve therapies (mitral valve anatomy for MitraClip).

Aortic Valve

Anatomy of Aortic Valve

The aortic valve complex consists of valve leaflets, the aortic root, and a virtual annular ring formed by basal attachment of leaflets to the left ventricle (LV) (Piazza et al., 2008). A normal aortic valve has three leaflets or cusps which are designated by their relationship to the origin of the coronary arteries—right, left, and noncoronary. The area enclosed by each leaflet and the corresponding outpouching of the aortic root form the respective sinuses of Valsalva. The noncoronary leaflet adjoins the interatrial septum and the commissure of the right and noncoronary cusp lies over the membranous intraventricular septum and the cardiac conduction system; the commissure of the noncoronary and left coronary cusps point toward the mitral valve; and the commissure of the right and left cusps point toward the pulmonary leaflets. The leaflets are anatomically divided into free margins, body or "belly" of the leaflet, and the basal attachments. The leaflet margins are attached to the aortic root with their commissures forming the highest points of attachment at the sinotubular junction. The leaflet margins are mostly uniform except for a thickened node (nodule of Arantius) which facilitates optimal coaptation. During systole, the leaflet body and the margins fall backward onto the aortic root to allow free flow of blood from the ventricle and the aorta. During diastole, these leaflets bulge forward and coapt to prevent regurgitation.

The basal portions of the leaflets are semicircular; they attach all the way from the aortic root, cross the anatomic ventriculo-aortic junction, and attach to the LV myocardium at their lowest point of attachment (hinges). An imaginary line joining the hinges at the level of the ventricular myocardium forms the annulus. The aortic annulus is thus a virtual planar representation of a complex

three-dimensional structure and actually lies in the LV. The leaflets, their basal attachments, and the virtual annular ring thus form the shape of a coronet.

Echocardiographic Assessment of Aortic Valve Structure

Two-dimensional TTE is often sufficient in the identification of normal aortic leaflet anatomy. In a standard parasternal long-axis view, the right and noncoronary cusps are visualized (though a slight tilt to the left depicts right and left cusps), while the parasternal short axis provides an en face view. In the parasternal short-axis view at the level of the aortic valve, the noncoronary cusp is adjacent to the interatrial septum while the right cusp is adjacent to the RVOT (**Fig. 7**). In those with poor parasternal windows, a subcostal short axis en face view can be frequently obtained. The apical views are best suited for Doppler evaluation though care should be taken to align the ultrasound beam as co-axial with blood flow as possible. The corresponding views by TEE are: (a) mid-high esophageal 45–60 degrees short-axis en face view and (b) mid-esophageal 120 degrees long-axis view (corresponds to the parasternal long-axis TTE view). Doppler evaluation by TEE requires deep transgastric apical views to ensure coaxial alignment of the probe.

Aortic Stenosis

Echocardiography can be considered the de facto standard for the evaluation of AS in modern clinical practice. Direct measurement of transvalvular gradients by cardiac catheterization is rarely required (Baumgartner et al., 2009).

Assessment of valve structure in aortic stenosis

The most common causes of AS are calcific degenerative stenosis, bicuspid aortic valve disease, and rheumatic valve disease and echocardiography often helps delineate the etiology. When evaluating a case of suspected AS, the initial anatomic evaluation should include identification of the number of leaflets. The parasternal short-axis view at the level of the aortic valve is most helpful to identify the number of leaflets as well as assess leaflet mobility and degree of calcification. Bicuspid aortic valve disease is congenitally acquired and commonly results in fusion of the right and left coronary cusps (**Fig. 8**) (Siu and Silversides, 2010). In systole, an elliptical fish mouth-shaped systolic orifice is identified with two commissures. A raphe is often present on the larger of the bicuspid valve leaflets and can mimic a tri-leaflet valve. In the parasternal long-axis views, systolic doming and an asymmetric closure suggest a bicuspid aortic valve. The parasternal short-axis view also helps in the differentiation of calcific AS and rheumatic AS. In rheumatic AS, thickening and calcification are most prominent along the margins of the cusps. This is accompanied by commissural fusion which results in a triangular systolic orifice with blunted corners. In contrast, calcific AS of a tricuspid valve is prominent in the body of each cusp. Because the commissures are free and open, this results in a stellate orifice. The degree of calcification and restricted leaflet motion can also be assessed in this view. Degree of calcification is an important predictor of outcomes and should be described quantitatively as part of echocardiographic evaluation (Rosenhek et al., 2000). Unlike mitral stenosis where planimetry is useful for the assessment of valve area, it is less helpful in the evaluation of AS (Okura et al., 1997). Though it appears to correlate well with AVA by continuity equation in small studies, there are three major sources of error in measuring AVA by planimetry: (a) valve calcification and resultant reverberations may limit identification of the valve orifice, (b) planimetry should be performed at the area of the cusp tips; if measured more proximally, the valve area is overestimated, and (c) the effective orifice area (EOA) does not correspond with the location of geometric orifice area (GOA) (Gilon et al., 2002). The EOA is often distal to the GOA (aortic aspect) and smaller. Three-dimensional geometry of the stenotic valve plays an important role

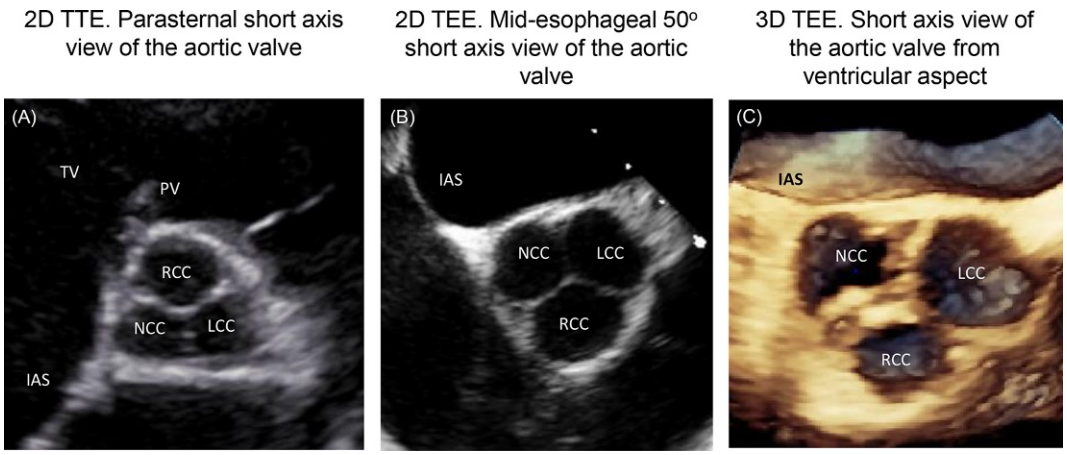


Fig. 7 Identification of aortic valve cusps by 2D TTE (panel A), 2D TEE (panel B), and 3D TEE (panel C). *IAS*, interatrial septum; *RCC*, right coronary cusp; *NCC*, noncoronary cusp; *LCC*, left coronary cusp; *PV*, pulmonic valve; *TV*, tricuspid valve.

with a smaller EOA for a flatter valve than a long tapering valve with the same GOA (Gilon et al., 2002). In addition, the primary predictor of outcome is EOA rather than GOA, because it better represents the hemodynamic burden on the LV. Finally, an old-fashioned axiom that has far outlived its usefulness is that "if the valve opens, it is not severely stenotic." Visual 2D estimation of valve severity is an insensitive method to assess severity and has high interobserver variability (Quader et al., 2015). This axiom should be modified for current day practice in the following manner "If the transaortic gradients are high but valve looks relatively disease free, look for subvalvular pathology (such as a subaortic membrane or LVOT obstruction)."

In the era of transcatheter aortic valve replacement, TEE (which is rarely useful in cases of AS except when the valve is not well-visualized by TTE) plays an important role in preoperative and intraoperative guidance of trans-valve replacement. Annular and LVOT dimensions can be accurately assessed using 3-D TEE and these correlate well with CT measurements (Altiok et al., 2011).

Assessment of valve function in aortic stenosis

After valve anatomy is established, the degree of aortic valve stenosis is assessed using Doppler ultrasound. The most important hemodynamic parameters are aortic valve peak velocity (AV Vmax), mean transaortic gradient, aortic valve area (AVA) by continuity equation, and dimensionless index (or Doppler velocity index) (Fig. 9).

Peak velocity and gradients across the aortic valve

The peak velocity and gradients across the aortic valve are measured using CW Doppler (Baumgartner et al., 2009). The five important principles in the accurate assessment of peak velocity and mean transaortic gradient are:

a. *Doppler beam alignment*. It is crucial to align Doppler beam in the direction of blood flow across the stenotic aortic valve. This is the most important source of error. In the standard echocardiographic views (apical five-chamber and three-chamber views), the jet flow may not be aligned well with CW Doppler beam despite appropriate patient positioning. This is especially true in elderly patients with aortic ectasia where the aorta arises at an angle with the LVOT. It is important to interrogate the aortic valve using multiple acoustic windows such as the right parasternal or suprasternal windows and using a nonimaging, dedicated CW

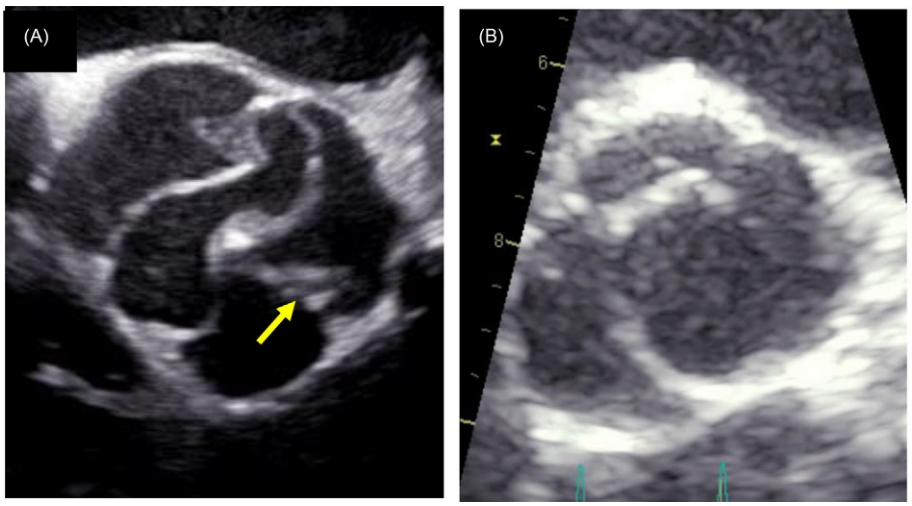
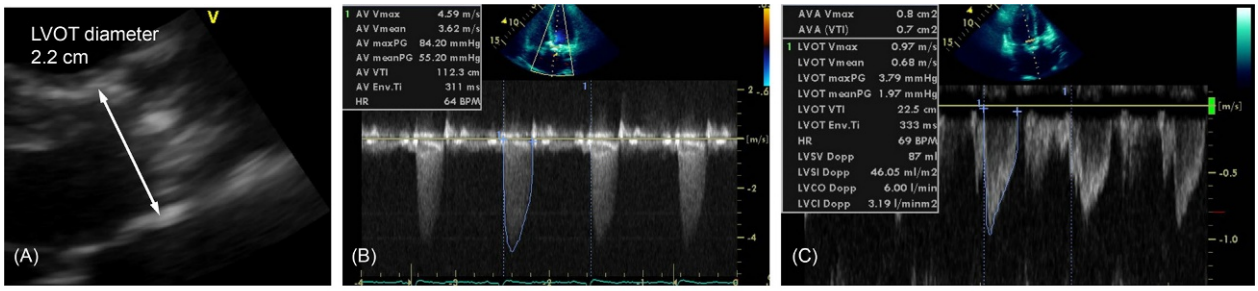


Fig. 8 Echocardiographic appearance of congenitally bicuspid (panel A) and unicuspid aortic valves (panel B). Yellow arrow denotes raphe.



Doppler Velocity Index = $AV_{VTI}/LVOT_{VTI}$= 22.5/112.3 = 0.20

Fig. 9 Doppler assessment of severe aortic valve stenosis. (Panel A) Thickened and calcified aortic valve in parasternal long-axis view by TTE. (Panel B) Significantly increased transvalvular velocity and gradient. (Panel C) Determination of valve area by continuity equation by measurement of LVOT velocity and flow.

Doppler transducer (PEDOF probe). This probe has the advantage of allowing better transducer positioning and angulation as well as a better signal-to-noise ratio.

b. *Avoid cosine error*. The intercept angle between blood flow and the ultrasound beam should be kept within 20 degrees. Because the three-dimensional direction of the jet is unpredictable, angle correction which is commonly used in vascular ultrasound should not be used.
c. *Avoid measuring Doppler artifact*. Due to transit time effects, CW Doppler curves often demonstrate Doppler blur toward the tip of the velocity curve. Care should be used in recording only the dense portions of the outer edge in measuring peak velocity and mean trans-aortic gradient.
d. *Avoid recording other sources of increased flow*. Velocity curves from two other cardiac pathologies can be mistaken for trans-aortic flow. The jet of MR (especially eccentric jets) can be distinguished by its onset soon after mitral valve closure (during isovolumetric contraction time) as well as the shape of the signal (rounded compared with the peaked appearance of severe AS). In patients with dynamic outflow obstruction (such as hypertrophic cardiomyopathy), the velocity curve is late peaking with a concave upward curve during the early part of systole (dagger-shaped).
e. *Average measurements*. Conditions such as atrial fibrillation and frequent premature atrial and ventricular contractions can alter flow across the aortic valve due to changes in stroke volume resulting from variable LV filling times. In atrial fibrillation, avoid cardiac cycles with very small filling times. In the presence of ectopy, avoid post-extrasystolic beats. In general, three or more cardiac cycles are averaged in sinus rhythm, while at least five consecutive beats should be averaged in atrial fibrillation.

In addition to quantification of gradients, a marker of severity that can be derived from CW Doppler signal is the time to peak velocity. A delay in time to peak velocity (analogous to the late peaking murmur by auscultation) is positively correlated with severity of AS (Kamimura et al., 2016).

Aortic valve area by continuity equation

Calculation of AVA by applying the Continuity equation is the standard method of assessment of severity of AS (Kamimura et al., 2016; Zoghbi et al., 1986; Baumgartner et al., 1990). It is based on the concept that flow across the LVOT tract is equal to flow across the aortic valve. While jet velocity and mean transaortic gradient depend on flow, the valve area is relatively independent of flow rate except in cases of low-flow low-gradient AS with the degree of valve opening is impaired by the low-flow itself. Some sources of error are:

a. Measurement of LVOT Diameter. The most significant error that can be introduced into the measurement of aortic valve area by continuity equation is in the assessment of the LVOT diameter. The LVOT diameter is measured in the parasternal long-axis zoomed-in view, within a centimeter of the valve orifice (corresponding to where the laminar LVOT flow velocity signal is obtained in the apical views) and parallel to the aortic valve plane. In some cases, averaging for several beats is appropriate. Despite careful measurement of the LVOT diameter, a significant error in the assessment of LVOT area results from the presumption that the LVOT is circular in shape. 3D echo studies reveal that the shape of the LVOT is highly variable and is often oval (Altiok et al., 2011). This results in underestimation of the area of the LVOT which eventually leads to underestimation of aortic valve area (falsely severe).
b. Assessment of LVOT VTI. The LVOT velocity is recorded using PW Doppler from an apical five-chamber or three-chamber view. Sample volume is usually placed about 5–10 mm proximal to the aortic valve to obtain a smooth laminar velocity curve with a narrow band of velocities (modal) throughout systole. The VTI is measured by tracing the dense modal velocity throughout systole while avoiding the blur at the peak of velocity curve.
c. Pressure Recovery. A source of discrepancy between Doppler-derived AVA by continuity equation and that AVA derived from cardiac catheterization is the phenomenon of pressure recovery (Oh et al., 1988). In general, peak velocity and gradients obtained by CW Doppler are often higher than that measured by cardiac catheterization. CW Doppler resolves highest velocity across the stenotic valve (at the region of VC where velocity is highest and pressure is lower due to conversion of kinetic energy to potential energy) while a double lumen catheter placed during cardiac catheterization often measures the difference in gradient between the LV and a point distal to the VC in the aorta (where potential energy is converted back to kinetic energy and pressure has recovered). The impact of this is usually small but can be significant when the ascending aorta is small (Baumgartner et al., 1999).

Dimensionless Doppler velocity index

Because calculation of the LVOT area introduces a significant error in the measurement of the aortic valve area, the dimensionless Doppler Velocity Index (DVI) which is independent of the LVOT area has simplified the assessment of severity of AS (Niederberger et al., 1996):

$$\mathbf{DVI} = \mathbf{LVOT}_{\mathbf{Vmax\ or\ VTI}} / \mathbf{AV}_{\mathbf{Vmax\ or\ VTI}})$$

Although peak velocity or VTI can be used to calculate DVI, we prefer VTI because small errors in calculating peak velocity (e.g., measuring Doppler blur) are amplified when Vmax is used instead of VTI. A DVI of less than 0.25 correlates well with severe AS. In addition to being a useful diagnostic marker, a low dimensionless index has prognostic significance (Otto et al., 1986).

Table 1 shows the echo parameters for grading severity of aortic stenosis.

In routine practice, cases which do not meet all criteria for a given severity of AS are often encountered. Some of the reasons for noncongruence between gradients and AVA have already been discussed earlier (Rusinaru et al., 2015) and a stepwise approach to

resolution is depicted in Fig. 10. A final important factor is that the guideline criteria for severity are inherently inconsistent with a mean AV gradient below 40 mmHg in a significant number of patients with an AVA <1 cm^2, by both Doppler echocardiography and cardiac catheterization (Michelena et al., 2013; Minners et al., 2008). An AVA cutoff of 0.8 cm^2 has been proposed for severe AS because that area correlates better with a mean gradient >40 mmHg. Indexing for body surface can be helpful but can overestimate severity in larger patients. Reduced flow obviously reduces gradients across the aortic valve and two different low-flow, low-gradient (LF-LG) states are well characterized—LF-LG AS with reduced LV ejection fraction and LF-LG AS with preserved LV ejection fraction (reduced LV stroke volume index <35 mL/m^2) (Minners et al., 2010). A detailed discussion is beyond the scope of this article but detection of true severity often requires additional testing such as dobutamine echocardiography and multimodality imaging (Pibarot and Dumesnil, 2012).

In conclusion, an important consideration in incorporating echo parameters in clinical practice is to correlate the echo findings with the clinical status of the patient. There is no echocardiographic holy grail that precludes such careful patient individualization (Clavel et al., 2017).

Effect on cardiac chambers in aortic stenosis

The LV bears the hemodynamic burden of AS. Severe AS causes concentric LV hypertrophy due to pressure overload. The thickened walls with a small ventricular cavity lead to diastolic dysfunction which can cause symptoms by itself. In addition, it leads to a low stroke volume which can affect the assessment of valve stenosis (LF-LG AS with preserved ejection fraction). Presence of severe LV hypertrophy can be used as a marker to guide clinical decision making especially in a patient with asymptomatic severe stenosis. This is supported by the observation that despite reversal of LV hypertrophy with valve replacement, maladaptive changes persist at an ultrastructural level (Michelena et al., 2014).

Table 1 Echocardiographic grading of aortic stenosis

	Aortic sclerosis	*Mild*	*Moderate*	*Severe*
Aortic jet velocity (m/s)	<2.5 m/s	2.6–2.9	3.0–4.0	>4.0
Mean gradient (mmHg)		<20	20–40	>40
AVA (cm^2)		>1.5	1.0–1.5	<1.0
Indexed AVA (cm^2/m^2)		>0.85	0.60–0.85	<0.6
Dimensionless index (peak velocity or VTI)		>0.50	0.25–0.50	<0.25

AVA, aortic valve area; *VTI*, velocity time integral.

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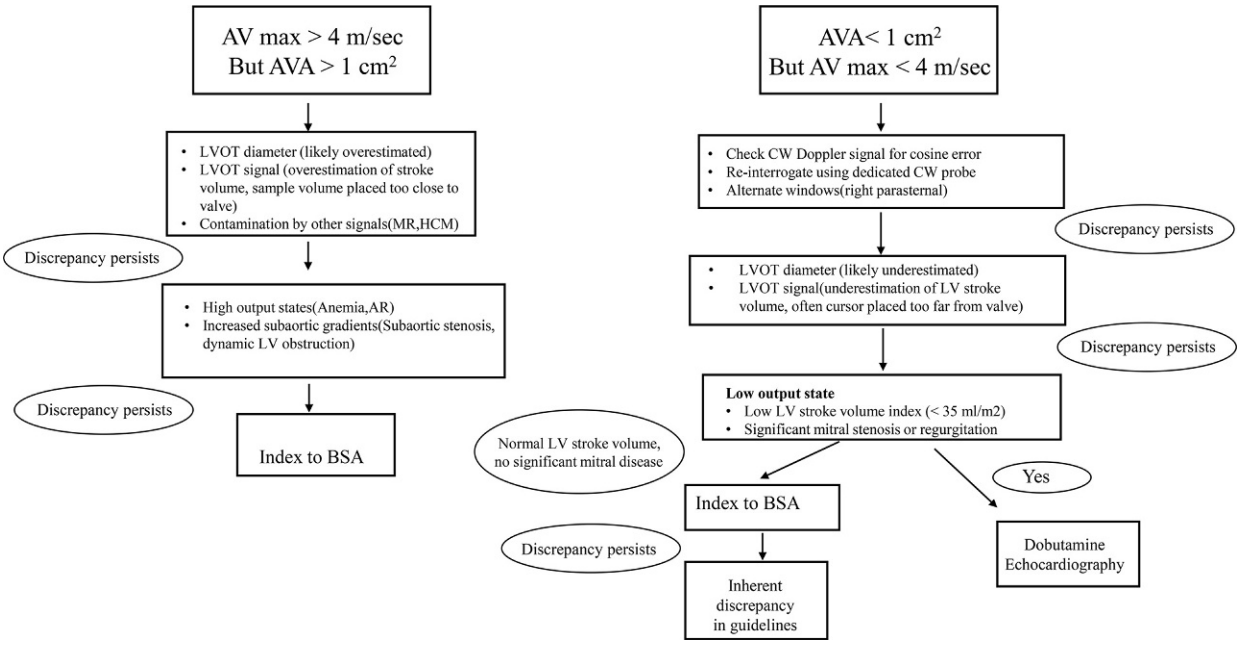


Fig. 10 Approach to resolving discrepancy between Doppler valve area and trans-aortic velocities in suspected severe aortic stenosis.

Stress echocardiography in aortic stenosis

Stress echocardiography is useful in the clinical management of AS. Exercise testing is an accepted measure for the assessment of functional status in a patient with asymptomatic severe AS. This is often performed without the use of echocardiography, though limited data suggests that the increase in mean pressure gradient with exercise predicts outcome. Dobutamine stress echocardiography is invaluable in the assessment of LF-LG AS. In patients with LF-LG severe AS (AVA less than 1 cm^2 with a peak velocity less than 4 m/s and mean gradient less than 40 mmHg), graded infusion of dobutamine can be used to distinguish between true severe stenosis versus pseudo severe stenosis (reduced valve opening due to low-flow itself) (Takeda et al., 1999; Nishimura et al., 2002). In addition, the absence of contractile reserve (failure to increase stroke volume by more than 20%) is a predictor of poor outcomes though its presence should not preclude valve replacement (Minners et al., 2010). In the era of transcatheter valve replacement, patients who are previously considered to be too high risk for surgical aortic valve replacement can now benefit from a less invasive option.

Effect of concomitant valve disease in aortic stenosis

Mixed aortic valve disease

The estimation of AS severity can be affected when more than moderate AR is present. AR leads to high transaortic flow with resultant higher than expected AV maximum and mean gradient for the calculated AVA (**Fig. 11**). These high transaortic gradients (irrespective of the dominant valve lesion) have independent prognostic value and should be used to drive decision making in addition to symptom status (Yarbrough et al., 2012). In general, a combination of moderate AS and moderate AR can be considered to represent severe mixed aortic valve disease.

Effect of concomitant mitral stenosis

Mitral stenosis can co-exist in patients with rheumatic as well as calcific AS. Due to impaired LV filling, mitral stenosis causes low cardiac output. This can lead to a LF-LG AS state despite normal LV function and lead to underestimation of severity of AS.

Effect of concomitant mitral regurgitation

In patients with AS, both primary (concomitant mitral valve disease) and secondary MR (due to LV pressure overload) can occur. Transesophageal echocardiography should be performed before surgical or transcatheter therapies in patients with suspected primary MR to establish etiology and severity of mitral valve disease. Secondary MR due to LV pressure overload often improves after correction of AS while more than moderate primary MR likely needs to be addressed along with the aortic valve intervention. In the presence of severe MR (both primary and secondary), a LF-LG AS can occur due to reduced LV forward stroke volume.

Aortic Regurgitation

Assessment of valve structure in aortic regurgitation

During echocardiographic assessment of AR, all portions of the aortic valve complex should be evaluated. The number of cusps should be carefully evaluated by identification of the commissures. Dilation of the aortic root and the ascending aorta should be assessed. The mechanism of AR can be classified based on etiology (primary or secondary) to chronicity (acute or chronic). A useful classification method for the mechanism of AR is based on leaflet motion and is an adaptation of the Carpentier classification for mitral valve disease (Zoghbi et al., 2017) (**Fig. 12**). Type I is associated with normal leaflet motion and is subdivided into: type IA due to dilatation of the ascending aorta and sinuses of Valsalva; type IB due to dilatation of the sinuses and the sinotubular junction alone; type IC due to annular dilatation and type ID due to cusp perforation or fenestration of the leaflets. Type II is associated with excessive leaflet motion from leaflet prolapse. Type III is associated with restrictive leaflet motion from causes such as degenerative calcification or rheumatic heart disease. In cases of new onset or acute AR, presence of oscillating echodensities such as vegetations should be carefully investigated which can cause acute severe regurgitation.

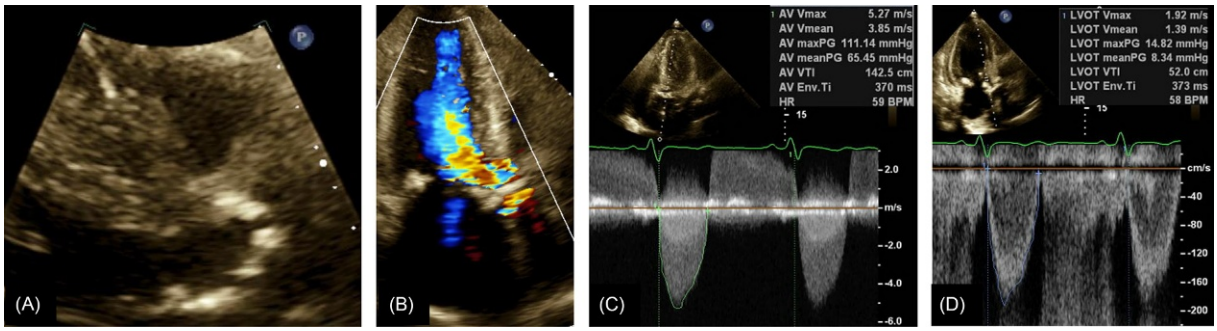


Fig. 11 Mixed aortic valve disease. In this example, TTE shows thickened aortic valve and restricted valve opening (panel A, mid systole) with significant regurgitant jet occupying the entire LVOT (panel B). The transvalvular gradient was significantly increased due to stenosis (panel C) and increased stroke volume (panel D). The mean gradient across the severely diseased aortic valve thus reflects the combined effect of stenosis and regurgitation.

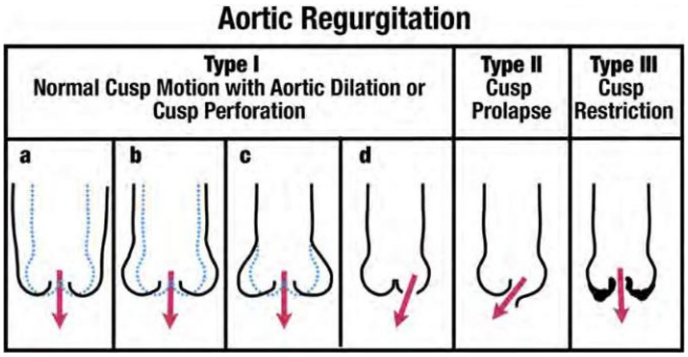


Fig. 12 Depiction of mechanisms of aortic regurgitation using the Carpentier classification. Type Ia shows sinotubular junction enlargement and dilatation of the ascending aorta. Type Ib shows dilatation of the sinuses of Valsalva and sinotubular junction. Type Ic shows dilatation of the ventriculo-arterial junction (annulus). Type Id shows aortic cusp perforation. Reproduced with permission from Zoghbi et al. (2017) *Journal of the American Society of Echocardiography*, **30** (4), 303–371.

*Assessment of valve function in aortic regurgitation (*Table 2*)*

Color Doppler imaging

Color-flow Doppler imaging forms the basis of the detection of AR by echocardiography. The three components of the jet of regurgitation with (flow convergence, VC, and jet area) are best visualized in the TTE parasternal long-axis view. The origin of the jet is sometimes best visualized in the parasternal short-axis view. The width of the jet in the LV outflow tract is a key component of the assessment of AR. The length of the jet is an unreliable parameter for severity because it is dependent on the difference of pressure between the aorta and the LV. In a centrally directed jet, the ratio of the width of the AR jet to the LVOT diameter provides a semiquantitative measure of the severity of regurgitation. The ratio is obtained in the parasternal long-axis view and the ratio more than 65% indicates severe AR, while less than 25% indicates mild AR (Egbe et al., 2016) (**Fig. 13**). Apical views should not be used for calculation of jet width because the regurgitation jets often appear wider due to poor lateral resolution of ultrasound. The ratio can be misleading in eccentric jets or in patients with multiple jets.

Vena contracta width

Width of the VC can be measured in a carefully optimized zoomed-in, parasternal long-axis view (at a Nyquist limit of 50–60 cm/s). VC >6 mm indicates severe AR, while <3 mm indicates mild AR (Perry et al., 1987). An advantage of the VC of method is that it can be useful even in eccentric jets.

PISA method

The PISA method can be used to calculate regurgitant volume (RVol) and EROA can be accomplished, typically in zoomed-in, parasternal, or apical long-axis view (Tribouilloy et al., 2000a). The radius of the proximal isovelocity shell is measured after defining the shell in a standard fashion (zoomed in view, baseline shift of the Nyquist limit in the direction of the flow). By calculating the radius of the shell and measuring AR peak velocity and VTI by CW Doppler, the EROA and regurgitant volume can be measured. Severe AR is defined by EROA ≥ 0.3 cm^2 and RVol >60 mm. Unlike MR, PISA quantification of AR is more challenging because flow convergence is often masked by aortic valve thickening and calcification.

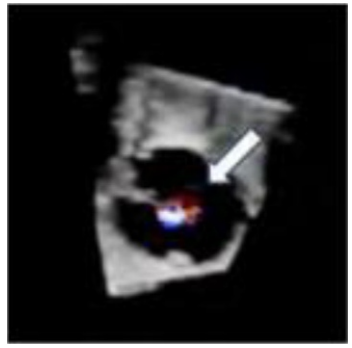
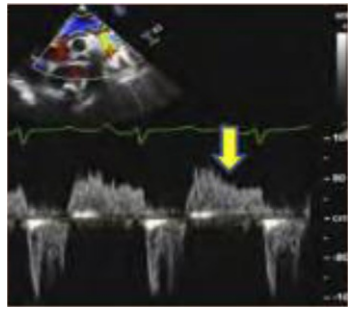
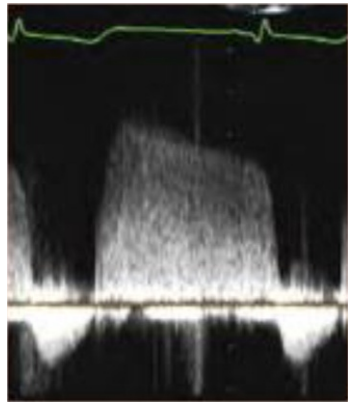
Doppler volumetry

Doppler volumetric assessment is occasionally used in measurement of regurgitant volume. Using PW Doppler, the stroke volume at the LVOT and the stroke volume at the mitral valve annulus are measured. RVol is the difference between these volumes. This method is laborious and the area calculation is based on the assumption that both the LVOT and the mitral annulus are circular structures. It is not valid in the presence of more than mild MR.

Density of the Doppler signal

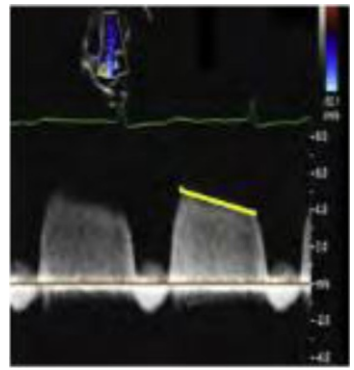
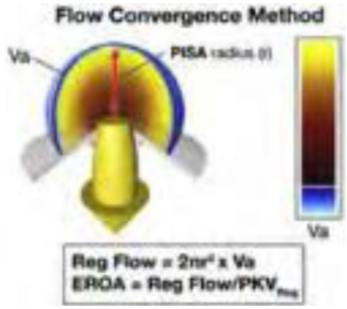
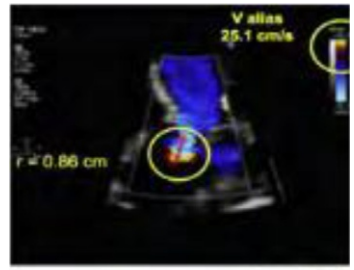
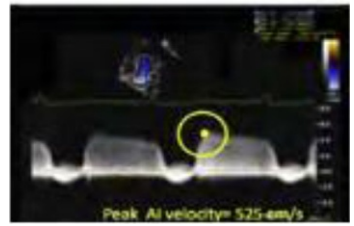
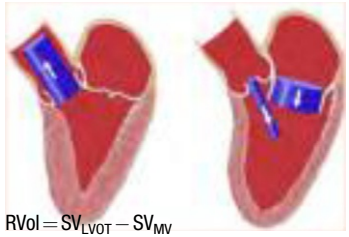
The density of the CW Doppler signal of AR compared with forward aortic flow is sometimes used as a qualitative measure of severity. A dense jet indicates significant regurgitation. A caveat is that CW Doppler interrogation should be in plane with the regurgitant jet or even a severe, eccentric jet can produce a faint or incomplete Doppler signal. Pressure halftime (PHT) of the AR signal provides an indication of the equalization of pressures between the aorta and the LV and thus AR severity. PHT is defined as the time interval in milliseconds between the peak AR gradient in early diastole to the time point where the gradient is half the maximum. PHT <200 ms suggests severe AR (especially in acute AR), whereas PHT >500 ms suggests mild AR. PHT is less useful in chronic severe AR, where compensatory LV remodeling can produce PHT between 200 and 500 ms. By virtue of its dependence on the aorto-ventricular gradient, PHT is unreliable in the presence of severe LV systolic or diastolic dysfunction due to another cause (in these cases, even mild AR may produce short PHT) or during treatment of systemic hypertension (high diastolic pressure can produce short PHT and treatment of hypertension lengthens PHT) (Tribouilloy et al., 1998).

Table 2 Echocardiographic assessment of aortic regurgitation

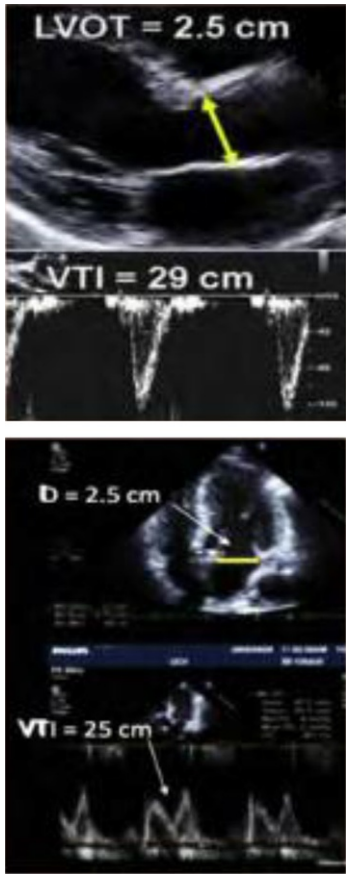
Modality	*Optimization*	*Example*	*Advantages*	*Pitfalls*
Color flow Doppler 3D: 3D VC	• Color flow sector should be narrow • Align orthogonal cropping planes along the axis of the jet • Choose a middiastolic cycle • Noncoaxial jets or aliased flow may appear "laminar" but still represent regurgitant flow		• Multiple jets of differing directions may be measured	• Dynamic jets may be over- or underestimated
Pulsed wave Doppler: Flow reversal in proximal descending aorta	• Align insonation beam with the flow in the proximal descending or abdominal aorta		• Simple supportive sign of severe AR • More specific sign if seen in abdominal aorta • Can be obtained with both TTE and TEE	• Depends on compliance of the aorta; less reliable in older patients • Brief velocity reversal is normal • Can be present in arteriovenous fistula in upper extremity, ruptured sinus of Valsalva • May not be holodiastolic in acute AR
CWD Density of regurgitant jet	• Align insonation beam with the flow • Adjust overall gain		• Simple • Density is proportional to the number of red blood cells reflecting the signal • Faint or incomplete jet is compatible with mild or trace AR	• Qualitative • Perfectly central jets may appear denser than eccentric jets of higher severity • Overlap between moderate and severe AR

(*Continued*)

Table 2 (Continued)

Modality	*Optimization*	*Example*	*Advantages*	*Pitfalls*
Jet deceleration rate (pressure half-time)	• Align insonation beam with the flow • Usually best from apical windows • In eccentric jets, may be best from parasternal window, helped by color Doppler		• Simple • Specific sign of pressure relation between aorta and LV • If long, excludes severe AR	• Qualitative • Poor alignment of Doppler beam may result in lower pressure half-time • Affected by changes that modify LV-aorta pressure gradient (if short, implies significant AR or high LV filling pressure)
Quantitative Doppler: EROA, regurgitation volume, and fraction				
	• Align insonation beam with the flow • Lower the color Doppler baseline in the direction of the jet • Look for the hemispheric shape to guide the best lower Nyquist limit • CWD of regurgitant jet for peak velocity and VTI	 	• Rapid quantitative assessment of lesion severity (EROA) and volume overload (RVol)	• Feasibility is limited by aortic valve calcifications • Not valid for multiple jets, less accurate in eccentric jets • Limited experience • Small errors in radius measurement can lead to substantial errors in EROA due to squaring of error.
SV method  RVol = $SV_{LVOT} - SV_{MV}$	• LVOT diameter measured at the annulus in systole and pulsed Doppler from apical views at same site • Mitral annulus measured at middiastole; pulsed Doppler at the annulus level in diastole • Total LV SV can also be measured by the difference between LV end-diastolic volume and end-systolic volume		• Quantitative, valid with multiple jets and eccentric jets. Provides both lesion severity (EROA, RF) and volume overload (RVol) • Verily results using LV end-diastolic volume and LV end-systolic volume	• Difficulties measuring mitral annulus diameter, particularly with annular calcification • Not valid for combined MR and AR, unless pulmonic site is used

- LV volumes are best measured by 3D. Contrast may be needed to better trace endocardial borders. If 3D not feasible, use 2D method of disks



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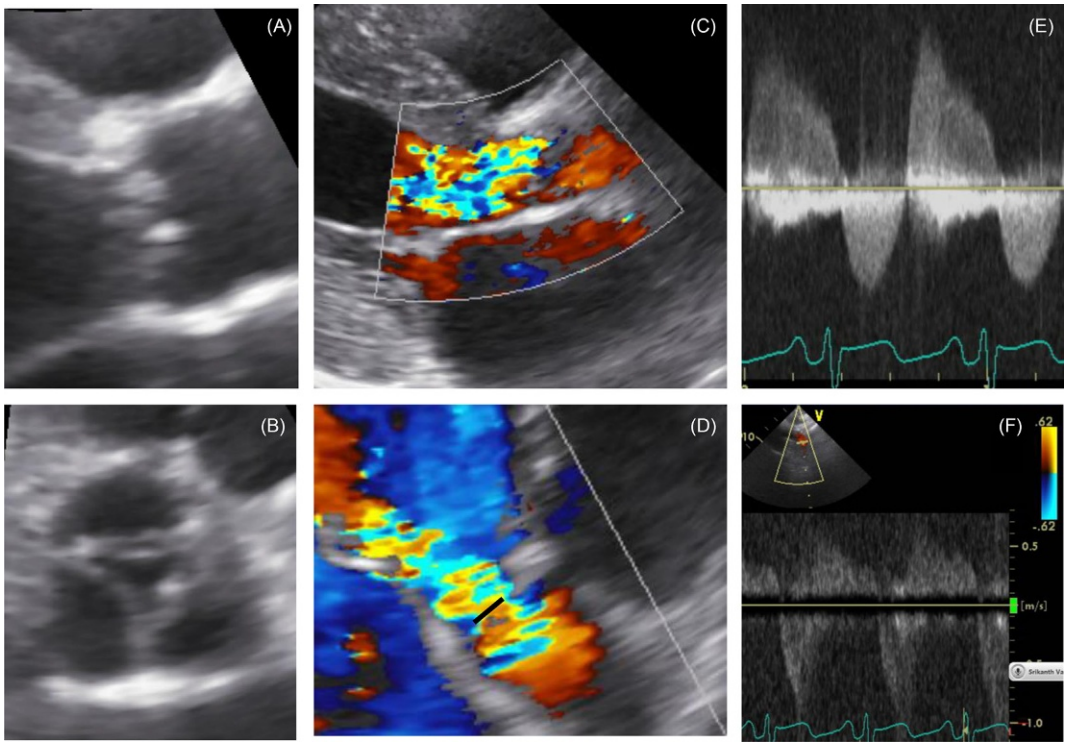

Fig. 13 Echocardiographic evaluation of a patient with severe aortic regurgitation. 2D TTE shows thickened leaflets with cusp eversion causing a significant coaptation defect during diastole (panels A and B). Color Doppler imaging shows the AR jet occupying most of the LVOT (panel C) with a vena contracta width of 9 mm (panel D). The CW Doppler profile of AR is as dense as forward flow profile (panel E). Holodiastolic flow reversal was present in proximal descending aorta (panel F).

Holodiastolic flow reversal in descending aorta

A useful sign of severe AR is the presence of holodiastolic flow reversal in the descending aorta. This is best accomplished by interrogating the proximal descending aorta from the suprasternal window and the abdominal aorta from subcostal views by PW Doppler. Presence of holodiastolic flow reversal in the abdominal aorta is specific for severe AR, while its presence in the proximal descending aorta indicates that the AR is at least moderate. Two useful semiquantitative measures that can be derived from the PW Doppler signals are: (a) an end-diastolic velocity more than 20 cm/s is often present in severe AR (Griffin et al., 1991); (b) a rough assessment of regurgitant fraction by the ratio of the VTI of reversed flow to forward flow.

Conditions that produce holodiastolic flow reversal in the absence of severe AR include a calcified noncompliant aorta in the elderly, presence of arteriovenous fistula in the upper extremity in patients with kidney disease, and rarely the presence of a patent ductus arteriosus or aortic dissection. Flow reversal may only occupy a small part of diastole despite severe AR in acute severe AR and with low heart rates.

Table 3 summarizes the echo parameters for grading severity of AR. Quantification of AR using these parameters has been validated prospectively and has good diagnostic and prognostic validity (Tribouilloy et al., 1991).

Effect on cardiac chambers in aortic regurgitation

Severe AR produces LV volume overload and ultimately reduced LV systolic function. Assessment of LV volume and function is thus crucial especially in the follow-up of AR. Left ventricular ejection fraction <55% or an LV end-systolic dimension >55 mm are predictors of outcomes and are useful in surgical decision making (Nishimura et al., 2014 Jul). Presence of normal LV volume and function almost rule out chronic severe AR. 3-D echocardiography allows more accurate assessment of LV volumes by eliminating the assumptions inherent to 2-D assessment and avoiding apical foreshortening. An additional supportive sign of severe AR (particularly acute) is its effect on the mitral valve. Due to the rapid rise of pressure in the LV during diastole in severe AR, the mitral valve closes prematurely. Presence of premature mitral valve closure provides a very valuable supportive sign of severe AR and surgical correction is recommended. By virtue of its excellent temporal resolution, M mode echocardiography is a valuable tool in detecting premature mitral valve closure.

Other modalities in aortic regurgitation

3-D echocardiography especially during TEE can be useful in the assessment of valve anatomy and measurement of annular dimensions. Integration of color flow with 3-D imaging can provide 3-D assessment of VC assessment. Exercise echocardiography can be useful in the assessment of functional status and LV function in patients with asymptomatic chronic severe AR and can be used to predict LV function recovery after valve replacement (Detaint et al., 2008).

Table 3 Echocardiographic grading of severity of chronic aortic regurgitation

Structural parameters	*Mild*	*Moderate*	*Severe*
Aortic leaflets	Normal or abnormal	Normal or abnormal	Abnormal/flail, or wide coaptation defect
LV size	Normal	Normal or dilated	Usually dilated
Qualitative Doppler			
Jet width in LVOT, color flow	Small in central jets	Intermediate	Large in central jets; variable in eccentric jets
Flow convergence, color flow	None or very small	Intermediate	Large
Jet density, CW	Incomplete or faint	Dense	Dense
Jet deceleration rate, CW (PHT, ms)	Incomplete or faint slow, >500	Medium, 500–200	Steep, <200
Diastolic flow reversal in descending aorta, PW	Brief, early diastolic reversal	Intermediate	Prominent holodiastolic reversal
Semiquantitative parameters			
VCW (cm)	<0.3	0.3–0.6	>0.6
Jet width/LVOT width, central jets (%)	<25	25–45 46–64	≥65
Jet CSA/LVOT CSA, central jets (%)	<5	5–20 21–59	≥60
Quantitative parameters			
RVol (mL/beat)	<30	30–44 45–59	≥60
RF (%)	<30	30–39 40–49	≥50
EROA (cm^2)	<0.10	0.10–0.19 0.20–0.29	≥0.30

LV, left ventricle; *CW*, continuous wave Doppler; *PW*, pulse wave Doppler; *PHT*, pressure half time. *LVOT*, left ventricular outflow tract; *VCW*, vena contracta width EROA denotes effective regurgitant orifice area. *RVol*, regurgitant volume; *RF*, regurgitant fraction.
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Effect of other valve disease in aortic regurgitation

As described earlier, the presence of mixed AS and AR can affect the assessment of either valve lesion. The presence of MR affects the Doppler volumetric assessment of AR. Co-existing MR makes Doppler volumetric assessment of RVol unreliable and subjects the LV to the excess hemodynamic burden.

Mitral Valve

Anatomy of Mitral Valve

The mitral valve complex consists of two mitral leaflets, the mitral annulus and a subvalvular (chordae tendineae, and papillary muscles) apparatus interacting with the LV (Wahi et al., 2000; Ho, 2002). The mitral leaflets are commonly called anterior and posterior, though aortic and mural are probably more appropriate terms based on their anatomic orientation (Fig. 14). The anterior or aortic leaflet is trapezoid shaped and is larger and thicker than the posterior or mural leaflet. It is anchored to the fibrous portion of the mitral annulus (intravalvular fibrosa) and is adjacent to commissure between the left and noncoronary cusps of the aortic valve. The posterior leaflet is crescent shaped and smaller but occupies a longer circumferential area of the mitral annulus. The posterior leaflet often has indentations which demarcate it into three scallops (lateral P1, central P2, and medial P3). Though these indentations are not well developed in the aortic leaflet, the corresponding sections are labeled A1, A2, and A3. The two commissures of the mitral valve are anterolateral (A1–P1) and posteromedial (P3–A3). The normal mitral valve orifice area is 4–6 cm^2.

The mitral annulus is a dynamic, D-shaped fibromuscular structure which three-dimensionally resembles a nonplanar saddle. The straight edge of the D is formed by the anterior leaflet and is fibrous, while the circular portion is formed by the posterior leaflet and is muscular. This explains why the posterior annulus enlarges preferentially in cases of annular dilation. The normal annular orifice area is anywhere between 6 and 10 cm^2 in healthy adults. In secondary MR, flattening of the mitral annulus and absence of dynamic motion in addition to annular dilation contribute to valvular dysfunction.

The valve leaflets are anchored to the LV via fibrous structures called chordae tendineae which originate from papillary muscles (a few tertiary chordae arise directly from the ventricular myocardium). The chordae are fan-like and are inserted into the ventricular aspects of the leaflets. Primary or marginal chordae attached to the leaflet tips, while secondary or basal chordae attached to the body of the leaflets. A pair of thick secondary chordae called the strut chordae are commonly seen inserted into the anterior leaflet and are often preserved during mitral valve replacement. A normal mitral valve complex consists of two papillary muscles, anterolateral and posteromedial. The anterolateral papillary muscle has a single head and receives dual blood supply from the obtuse marginal branch of the left circumflex and the diagonal branch of the left anterior descending artery. The posteromedial papillary muscle commonly has two heads and is supplied either by the right coronary artery or the circumflex coronary artery (thus

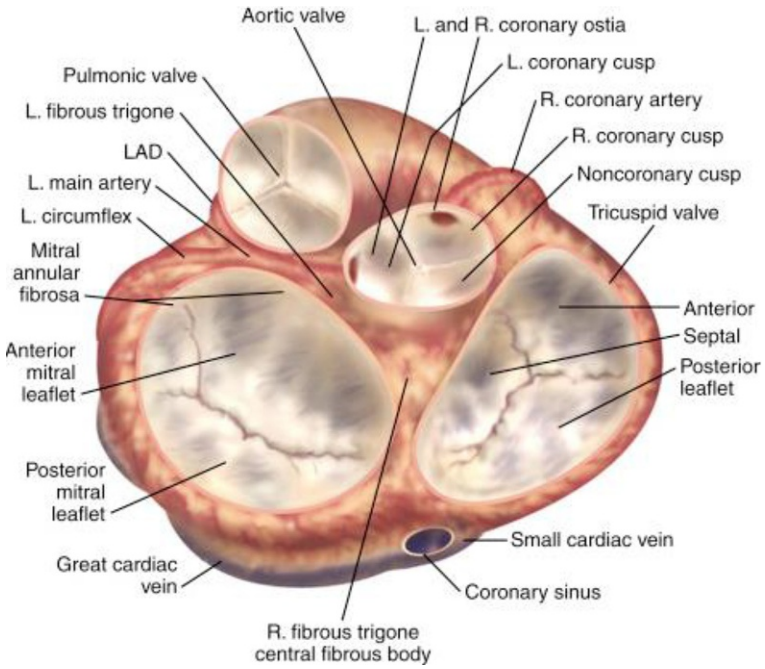


Fig. 14 Anatomical relationship of the mitral valve segments to surrounding structures. Reproduced with permission from Otto, C. M. et al. (2013). *Textbook of Clinical Echocardiography*, 5th edn, 2013.

making it more prone to ischemia). Contraction of the papillary muscles maintains the relationship between the LV and the mitral valve annulus and promotes normal coaptation. In patients with cardiomyopathy, remodeling of the ventricle disrupts this balance and produces malcoaptation.

Echocardiographic Assessment of Mitral Valve Structure (Fig. 15)

The mitral valve complex is evaluated in various orthogonal views by TTE. Leaflet segments can be identified in standard TTE views (marker to the right of the screen): A2-P2 in parasternal long-axis view; A2/A3 and P1/P2 in the apical four-chamber view, P1-A2-P3 in the apical two-chamber (commissural view); and finally an en face view in the parasternal short-axis view. In the parasternal short axis, A1-P1 are lateral most while A3-P3 form medial segments. Chordae and papillary muscles are assessed in parasternal long-axis or apical views (especially the apical two-chamber view). Apical views allow co-axial Doppler interrogation of the mitral valve. The papillary muscles can be identified in the parasternal short axis at approximately 4 o'clock (anterolateral) and 8 o'clock (posteromedial) positions.

Leaflet segment can be precisely identified by TEE examination from standard mid esophageal (ME) position by manipulating the probe at various angles and degrees of flexion: (A) ME 0 degrees—A2 and P2; at this angle, slight withdrawal—A1–P1 and slight advancement—A3–P3; (B) ME 45–60 degrees—commissural view, P1–A2–P3; (C) ME 90 degrees—two-chamber view—A2,3–P3; and (D) ME 120 degrees—long-axis view—A2–P2. An en face view of the valve is obtained in a transgastric 0 degree short-axis view of the mitral valve, this requires anteflexion of the probe. 3D TEE can help identify segments and pathology in a single frame and image can be manipulated to view the valve from either the left atrial (surgeons view) or the LV perspective.

Mitral Stenosis

Assessment of valve structure in mitral stenosis

Even in industrialized nations, the most common cause of mitral stenosis (MS) is rheumatic heart disease (RHD) (McCarthy et al., 2010). Rheumatic MS is characterized by commissural fusion, chordal fusion, and shortening, leaflet thickening (mostly at the tips) and calcification; this combination which finally lead to restriction of leaflet motion and stenosis (**Fig. 16A**). Common 2-D echocardiographic signs include a "hockey-stick appearance" of the mitral valve in the parasternal long-axis view due to restriction of leaflet motion and commissural fusion in the parasternal short-axis view resulting in a "fishmouth" appearance.

The second common cause is degenerative or calcific mitral stenosis which results from mitral annular calcification and its extension into the leaflets (**Fig. 16B**). Restriction of leaflet motion occurs at the bases (relatively free leaflet tips) and commissural fusion is absent. Rarer causes include radiation (**Fig. 16C**), carcinoid disease, and drug-induced valvulopathy.

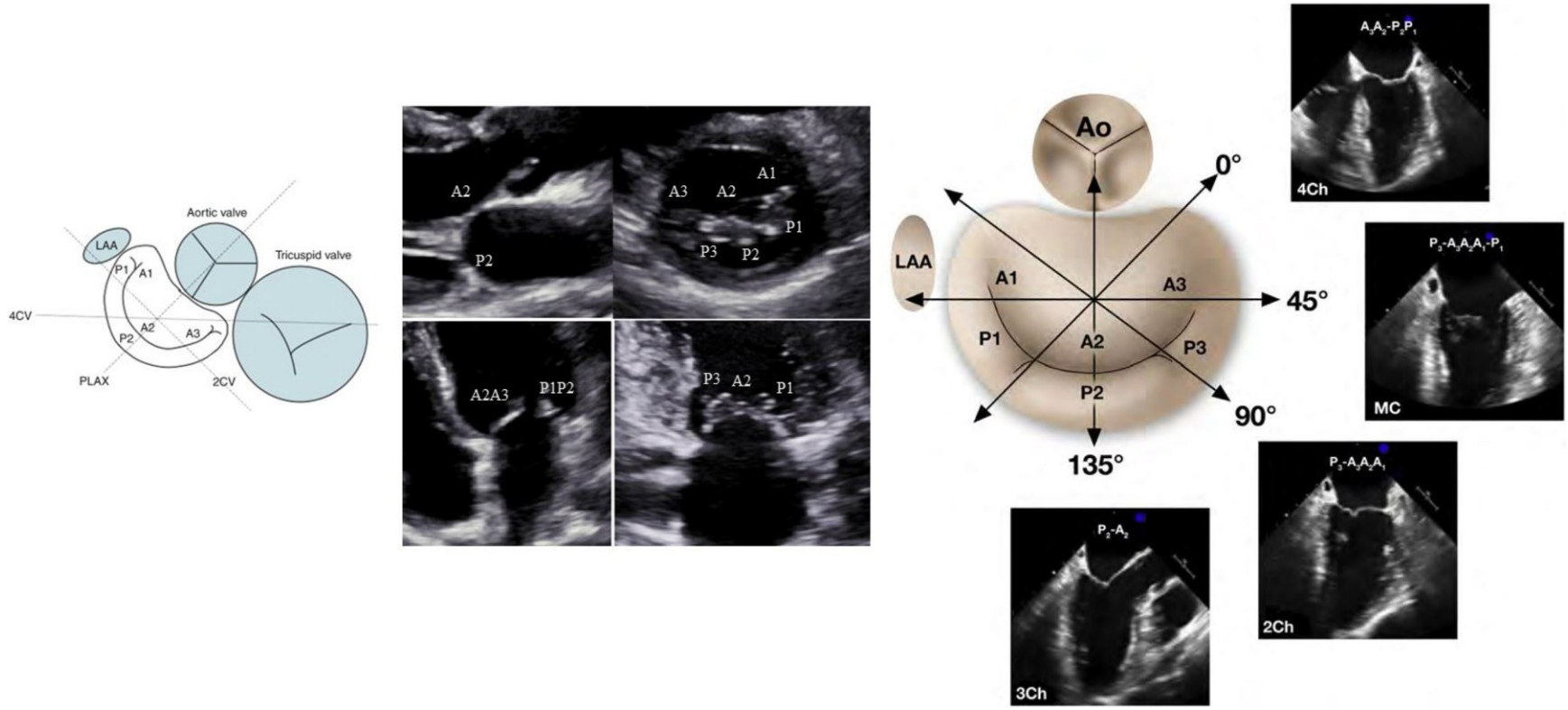


Fig. 15 Identification of mitral valve leaflet segments by 2D TTE and 2D TEE. Adapted with permission from Hahn, R. T. et al. (2013). *Journal of the American Society of Echocardiography*, **26** (9), 921–964 and Hutchinson, S. J. *Principles of Echocardiography*.

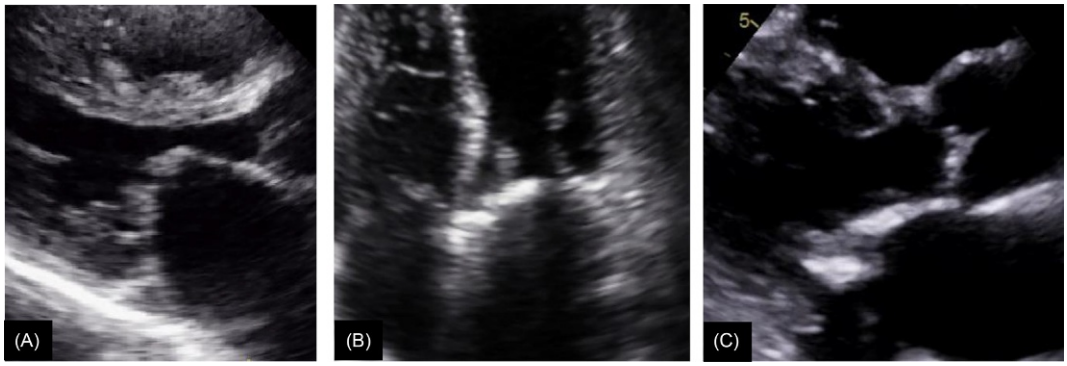


Fig. 16 Mitral valve structure in rheumatic mitral stenosis (leaflet tip restriction is the hallmark with relatively free bases) (panel A, mid diastole), calcific mitral stenosis in a patient with end stage renal disease (basal leaflet calcification with relatively free tips) (panel B) and mixed (radiation induced and mitral annular calcification) mitral stenosis (calcified anterior leaflet which was affected by mediastinal radiation to treat lymphoma) (panel C).

Evaluation of valve structure and MS is crucial both to establish diagnosis, define severity, as well as guide therapy. Mitral valve planimetry is the gold standard for the assessment of mitral valve area (MVA). It is obtained by direct tracing of the mitral orifice on the parasternal short-axis view at the tips of leaflets. Measurement distal or proximal to the leaflet tips can lead to under- or overestimation of the valve area. Planimetry is more accurate in patients with noncalcified leaflets and is best measured in middiastole (Baumgartner et al., 2009). Averaging of several measurements is recommended especially in patients with concomitant atrial fibrillation. It is important to note that planimetry was validated in patients with rheumatic MS and MVA by planimetry of leaflet tips may underestimate severity in MS due to causes such as calcific MS which predominantly affects the bases. 3-D guided measurement of mitral valve orifice area can improve the accuracy of planimetry measurement (Iung et al., 2003; Faletra et al., 1996; Sebag et al., 2005).

Percutaneous balloon mitral valvuloplasty (BMV) is a very effective method of alleviating symptoms severe MS. This method works by splitting of the commissures and thus suited for rheumatic MS (can cause acute severe MR if used in calcific MS with free leaflet tips and open commissures). It should be avoided in patients with extremely calcified leaflets and chordae. Several scores are available to assess suitability for BMV. These scores depend on thorough assessment of leaflet and chordal anatomy and of these, the Wilkins score is often used (Zamorano et al., 2004). Here, TEE is often useful in patients with poor TTE windows. Presence of moderate or more severe MR makes BMV a less desirable option.

Assessment of valve function in mitral stenosis

Doppler assessment of MS severity depends on assessment of valve gradients and estimation of MVA.

Peak velocity and gradients across the mitral valve

The mean gradient across the stenotic mitral valve is derived by tracing the transmitral diastolic velocity curve obtained by CW Doppler (Wilkins et al., 1988). This is best assessed in apical views to allow for parallel alignment of the ultrasound beam with the flow. The transmitral gradient is dependent on heart rate and flow (co-existing high flow states or MR) in all patients with MS and cycle length in those in atrial fibrillation. In patients with AF, at least five measurements should be averaged and extremes of cycle lengths should be excluded. Transmitral gradient of more than 10 mmHg (at a heart rate of about 70 bpm) suggests severe stenosis. Peak velocity is not used to assess severity.

Mitral valve area by pressure halftime

Mitral valve area (MVA) can be derived using the empirical formula:

$$\mathbf{MVA = 220/PHT}$$

where the pressure halftime (PHT) is defined as the time interval in milliseconds between the peak mitral gradient in early diastole in the time point where the gradient is half the maximum (Nishimura et al., 1994). Similar to measurement of mitral gradient, pressure halftime is best measured using CW Doppler and should be averaged in cases of atrial fibrillation. In cases of bimodal pattern (ski slope), the deceleration slope in mid-diastole should be traced (Thomas and Weyman, 1987) (**Fig. 17**). Despite its ease of performance, PHT depends on left atrial compliance and LV lusitropy and compliance (Gonzalez et al., 1987). In patients with LV diastolic function such as the elderly, PHT may be unreliable. It is best avoided in calcific MS where PHT has not been validated and often overestimates MVA (Karp et al., 1989a).

Other methods to assess mitral valve area

The MVA can be calculated similar to the estimation of aortic valve area using continuity equation and often correlates well with other methods (**Fig. 18**) (Akram et al., 2009). This is a useful additional parameter to assess MVA. MVA is calculated by dividing flow across the LVOT by VTI of the mitral flow at leaflet tips:

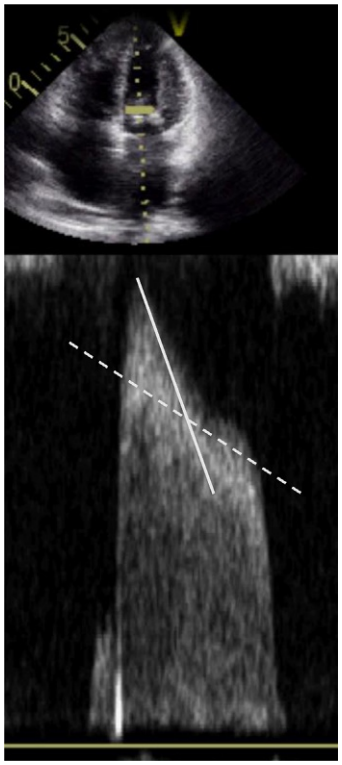


Fig. 17 Estimation of pressure half time (PHT) in a patient with a bimodal transmitral flow pattern. The dashed line represents the appropriate method for estimation of mitral valve area by PHT. The mitral valve area is overestimated by tracing PHT using the initial slope (*solid line*).

$$\mathrm{MVA} = \mathrm{LVOT}_{\mathrm{VTI}} \times \mathrm{LVOT}_{\mathrm{CSA}}/\mathrm{MV}_{\mathrm{VTI}}).$$

The proximal isovelocity surface area method can also be used but it is technically demanding and requires multiple measurements and angle correction because the orifice is quite obviously not flat.

Effect on cardiac chambers

The predominant effect of MS is on the left atrium (LA), pulmonary venous pressures, and thus the right ventricle (RV). With worsening MS, the LA size and pressure increase with two significant consequences: (a) increased LA remodeling and fibrosis which can result in atrial fibrillation. This is often poorly tolerated by patients with MS whose cardiac output (preload) is dependent on atrial contraction and (b) increased LA pressure is transmitted to the pulmonary circulation resulting in pulmonary venous hypertension and resultant RV dysfunction. The presence of RV dilation and dysfunction as well as concomitant TR should thus be carefully assessed during echocardiography. Tricuspid valve repair should be considered in patients with a dilated annulus (>40 mm or >21 mm/m^2) in patients being considered for mitral valve surgery (Nishimura et al., 2014).

The LV has traditionally been considered protected from the hemodynamics of MS. However, abnormalities in LV longitudinal and circumferential strain can be detected in many patients with severe MS. (Bonow et al., 2006) These abnormalities resolve soon after balloon mitral valvuloplasty, suggesting that abnormal loading conditions cause LV dysfunction rather than an intrinsic myocardial abnormality.

Echo parameters used to diagnose severe MS are depicted in **Table 4**.

Stress echocardiography in mitral stenosis

Exercise echocardiography is very useful in the assessment of the symptomatic patient with less than severe MS on resting echocardiogram. This is not an uncommon clinical scenario and reflects how a given MVA causes less than severe obstruction to flow at resting conditions but severe obstruction at higher flow rates. Measurement of transmitral gradient and pulmonary artery systolic pressure at peak exercise is necessary for accurate assessment of the hemodynamic significance of stenosis. Due to its ability to obtain gradients at peak exercise, semisupine exercise echocardiography is preferred to upright treadmill stress echocardiography. An elevation of RV systolic pressure (>60 mmHg) and transmitral gradients (often >15 mm) suggests exercise limiting MS. (Nakatani et al., 1988) Dobutamine stress echocardiography is rarely used because increase in flow created by dobutamine is not physiological.

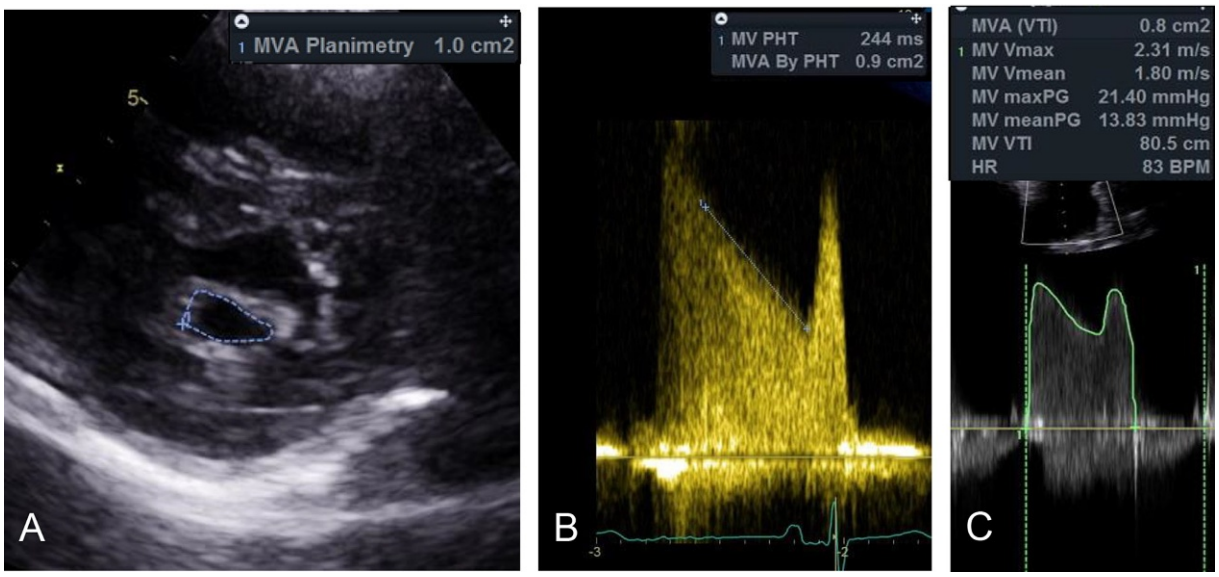


Fig. 18 Mitral valve area calculation in a patient with severe rheumatic mitral stenosis. There is good agreement between all three methods used: planimetry (panel A), pressure halftime (panel B), and continuity equation (panel C).

Table 4 Echocardiographic criteria for severe mitral stenosis

	Severe mitral stenosis
Specific findings	
Valve area (cm^2)	<1.5
Supportive findings	
Mean gradient (mmHg)	>5–10
Pulmonary artery pressure (mmHg)	>30 (2009 ASE guidelines [Ref 4])
	>50 (2014 ACC guidelines [Ref 2])

Effect of concomitant valve disease in mitral stenosis

Mixed mitral valve disease

Similar to mixed aortic valve disease, the presence of MR affects both the assessment and treatment of mitral stenosis. The increased flow from MR increases transmitral flow and gradients. Though PHT has traditionally been considered independent of MR, available data is conflicting and this method is best avoided. Presence of moderate MS and moderate MR can be labeled severe mixed mitral valve disease. Presence of MR also effects calculation of MVA by continuity equation.

Effect of concomitant aortic stenosis

As noted earlier, severity of AR may be underestimated due to the reduced stroke volume from MS.

Effect of concomitant aortic regurgitation

The PHT method for calculation of MVA is not valid in cases of severe AR (which effects LV compliance).

Mitral Regurgitation

Assessment of valve structure in mitral regurgitation

Echocardiography is useful in defining the mechanism of MR which can broadly be divided into two categories: Primary MR due to an intrinsic leaflet abnormality and secondary MR due to left heart remodeling in the presence of a normal and intact mitral valve apparatus (McCarthy et al., 2010). Within primary MR, the Carpentier classification based on leaflet motion provides a method of classification and helps planning of surgical repair (Fig. 19). Leaflet motion is normal in type I (e.g., leaflet perforation and annular dilation due to secondary MR), excessive in type II (e.g., degenerative mitral valve disease such as mitral valve prolapse or presence of flail leaflet), and restrictive in type III, which can be further divided into type IIIa where leaflet motion is restricted during both

diastole and systole (e.g., rheumatic valve disease, radiation-induced valve disease) and type IIIb where leaflet motion is restricted during systole alone (e.g., leaflet tethering due to LV dilatation in secondary MR) (Zoghbi et al., 2017).

The most common cause of primary MR is myxomatous degenerative valve disease such as mitral valve prolapse (MVP). In MVP, leaflets are thick and redundant and the coaptation point moves beyond the mitral annulus into the LA during systole (see online **Videos 1** and **2**). Echocardiography is useful in detecting MVP and distinguishing between two major causes—Barlow's disease and fibroelastic deficiency. In Barlow's disease, the mitral valve leaflets are diffusely thickened and redundant while redundancy and prolapse are limited to few leaflet segments in fibroelastic deficiency. The diagnosis of MVP is usually made in the parasternal long-axis view where the coaptation point of the mitral valve is displaced at least 5 mm posterior to the mitral annular plane. A key pitfall in the 2-D echo diagnosis of MVP is diagnosing it based purely on apical four-chamber or two-chamber views. In these views, the mitral valve leaflets are imaged along the low points of the saddle-shaped mitral valve annulus; this makes the leaflets appear to be displaced into the left atrium even in normal subjects (Sengupta et al., 2014). However, in patients with significant MR and poor parasternal windows, apical views can provide visual assessment of thickening of leaflets and leaflet motion though not being diagnostic of MVP except in severe cases.

MR due to MVP is usually limited to the mid-to-late systole. When chordal rupture appears, the prolapsed leaflet becomes flail (see online **Video 3**). Flail leaflet is defined by the presence of mobile leaflet edge with its tip pointing into the LA. In these cases, MR is usually severe and is holosystolic. Identification of a flail leaflet is crucial because its presence usually indicates significant MR and predicts adverse outcomes if left unrepaired. In secondary MR, distortion of the mitral valve apparatus by left heart remodeling (both LA and LV) occurs in the presence of usually normal leaflets. In the presence of adverse LV remodeling, leaflet mild coaptation occurs either due to regional or global LV systolic dysfunction. A common cause of secondary MR due to regional LV systolic dysfunction is hypokinesis of the inferior wall after a myocardial infarction. This results in tethering of the posterior leaflet and increased distance of the posterior papillary muscle from the intravalvular fibrosa. The direction of the MR jet in these cases is usually posterior. In contrast, global LV dysfunction results in several changes. The mitral annulus is frequently dilated, becomes flat-shaped, and is nondynamic (Zoghbi et al., 2017). Due to LV dilation, there is increased distance between the papillary muscles. This combination results in symmetric tethering of both leaflets resulting in apical displacement and malcoaptation. Because the posterior portion of the mitral annulus is muscular, it dilates more than the fibrous anterior annulus. The MR jet is frequently central.

In the era of trans-catheter mitral valve therapies, it is important to understand a few caveats in the distinction between primary and secondary MR. Mild leaflet thickening can occur in secondary MR as a reaction to the abnormal tethering forces on the displaced mitral leaflets (Levine et al., 1988). Chordal rupture and a flail leaflet can occur in patients with secondary MR. Similarly, LV dysfunction due to either ischemic or nonischemic cardiomyopathy can develop in a patient with known primary MR and leaflet anatomy may reflect characteristics of both types. In this condition, LV chamber size and function may reflect the underlying

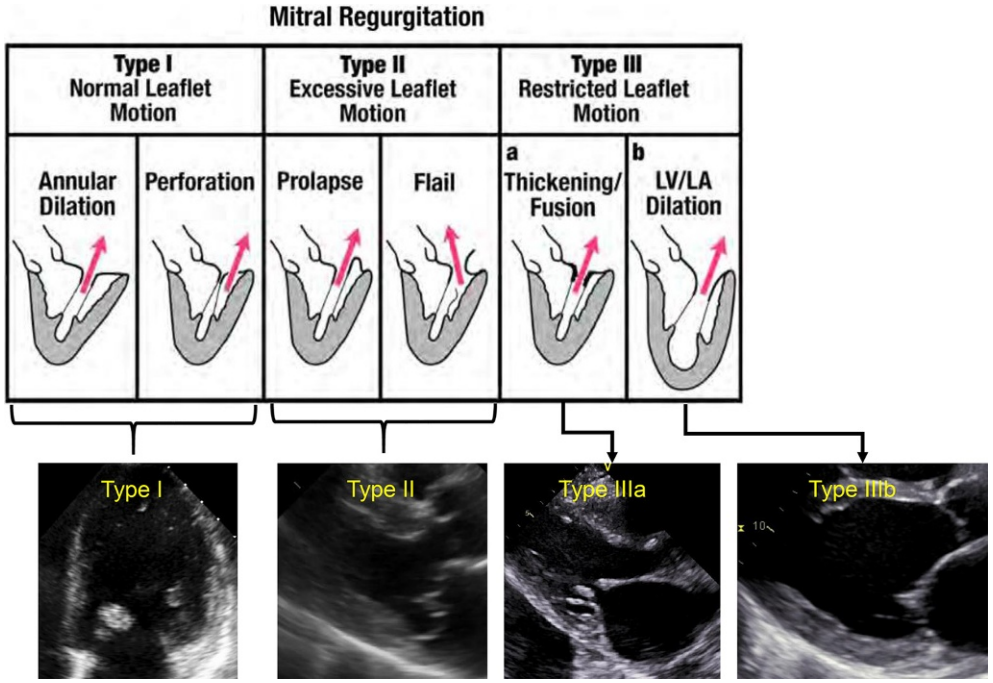


Fig. 19 Mechanism of mitral regurgitation according to Carpentier classification and examples of their representative TTE appearance. Endocarditis exemplifies type I; mitral valve prolapse illustrates type II; rheumatic mitral regurgitation with leaflet tip restriction and malcoaptation represents type IIIa; and leaflet malcoaptation due to LV enlargement is an example of type IIIb. Adapted with permission from Zoghbi et al. (2017). *Journal of the American Society of Echocardiography*, **30** (4), 303–371.

cardiomyopathy rather than the hemodynamic burden of MR alone. These types of mixed mitral valve disease (by etiology) should be clearly defined to enable accurate diagnosis, guide effective therapies, and avoid regulatory scrutiny (e.g., the Mitraclip device is currently approved only for primary MR in the United States and etiology should be clearly defined prior to this intervention).

Based on chronicity, MR can either present acutely or chronically. Echocardiography plays a crucial role in the timely identification and management of acute MR which often results in hemodynamic compromise. 2-D echo helps identify the pathology such as the flail leaflet due to a ruptured cord, leaflet destruction due to endocarditis, or ruptured papillary muscle due to acute myocardial infarction. Due to its sudden onset, the left atrial pressure increases rapidly and the stroke volume is reduced because of the lack of compensatory LV remodeling. Due to this low LV- LA gradient, color Doppler imaging can be unimpressive and may display little turbulence. TEE is often helpful in precise identification of valve pathology.

*Assessment of valve function in mitral regurgitation (*Table 5*)*

Color Doppler imaging

Color Doppler imaging is the primary modality for the detection of MR. Trivial MR is a common finding in normal subjects. When an MR jet is identified, effort should be made to display all three components of the jet—flow convergence, VC, and jet area. This may require slightly off axis views especially in cases of eccentric regurgitation and sometimes multiple views to define each component. Though the jet area and length are often used clinically for grading MR severity (Dal-Bianco et al., 2009), they can be very misleading.] An eccentric jet (such as in the presence of a flail leaflet) may appear small as part of the jet is constrained by the left atrial wall and loses kinetic energy quickly which prevents it from recruiting surrounding blood. Volume and loading conditions such as hypertension also affect jet area and explain the discrepancy when echocardiography is performed at different times (Zoghbi et al., 2017). Though the jet area would be similar in different echocardiographic views when the ERO is central, it can appear markedly different in the presence of elliptical or slit-like orifice. For example, in secondary MR where origin of the jet is slit-like along the region of malcoaptation, imaging in the commissural view (apical two-chamber view) produces a large jet area even in the presence of a small EROA. VC width provides a semiquantitative method of assessment of severity of MR. (Chaliki et al., 1998)VC $\geq$7 mm is consistent with severe MR, while VC $<$3 mm usually denotes mild MR. Additional methods of quantification should be used in case of intermediate values of VC (3–6 mm) due to substantial overlap between MR grades. Multiplanar reconstruction using 3-D echocardiography may provide more accurate assessment of VC with and allows direct calculation of the VC area (which corresponds to EROA) (see online **Video 4**). A 3D-derived VC area $>$0.41 cm^2 can differentiate between moderate and severe MR. (Tribouilloy et al., 1992) Due to the challenges posed by the lower spatial and temporal resolution of 3D echo and the lack of outcome data, this method is not widely used.

Spectral Doppler

The flow convergence or PISA method is the most reliable echocardiographic method for the determination of regurgitant volume (RVol) and EROA. The proximal isovelocity shell is optimized in a standard fashion (apical zoomed-in view of the mitral leaflet, lowering the Nyquist limit in the direction of the flow to define the boundary of the shell) and the radius calculated. Using the maximal velocity and VTI of the CW jet of MR, RVol and EROA can be estimated (**Fig. 20**). The pitfalls in PISA assessment have been described earlier (Section 2.2.2.3.1). In part, they are related to the assumption that the shell is hemispheric and the orifice is circular. The shell may not be well delineated in eccentric jets with the flow convergence are can be constrained by the LV wall. Similarly, severity of MR can be underestimated in patients with noncircular EROA geometry (such as secondary MR). Finally, both the PISA method and VC are performed on single frame images and can be misleading when the MR is limited to part of systole (such as MVP).

A simplified PISA method

By shifting the aliasing velocity to 40 cm/s and assuming an MR velocity of 5 m/s, the PISA equation can be simplified to:

$$\mathbf{EROA} = \mathbf{r^2/2} \ldots .. [\mathbf{80}]$$

This method may not hold good in patients with extremes of blood pressures or in severe LV systolic dysfunction.

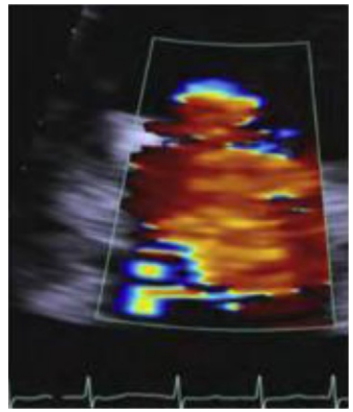
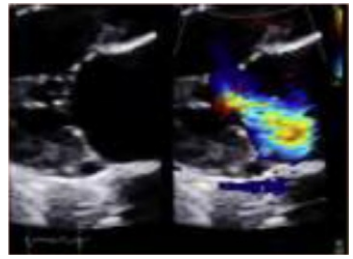
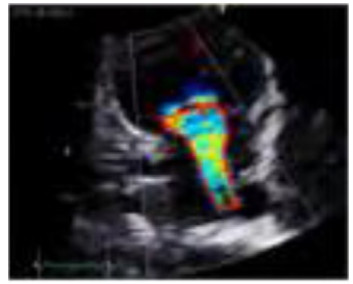
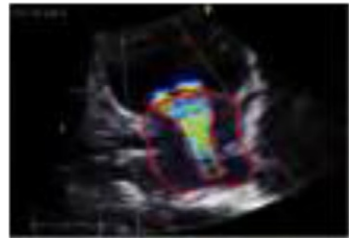
Doppler volumetry

Doppler volumetric assessment can be used to calculate the RVol by estimating the difference between the stroke volume at the LVOT and the mitral valve annulus. PW Doppler is used to calculate the VTI of flow in the LVOT and at the level of the mitral annulus. The calculation of the area of the LVOT and the mitral annulus assumes that both of these structures are circular in shape. When this assumption is not true, this introduces significant error in calculation. In addition, the calculation is often laborious and is uncommonly used in clinical practice.

Supportive Doppler signs

There are some supportive signs for severity of MR on PW and CW Doppler assessment of mitral valve. The most important supportive PW Doppler sign of the severity of MR is PW evaluation of pulmonary venous flow. Presence of systolic flow reversal correlates well with an EROA $>$0.3 cm^2 and thus is often present in severe MR. (Pu et al., 2001) Flow reversal in two or more pulmonary veins (which is better demonstrated by TEE) is more specific because a less than severe eccentric jet can selectively enter a single pulmonary vein and display reversal. Reduced or blunted systolic velocity is less useful because coexisting LV diastolic or systolic dysfunction cause increased LA pressure and reduce systolic flow from the pulmonic veins (S wave $<$D wave).

Table 5 Echocardiographic evaluation of mitral regurgitation

Modality	*Optimization*	*Example*	*Advantages*	*Pitfalls*
Color flow Doppler 2D: Proximal flow convergence	• Align direction of flow with insonation beam to avoid distortion of hemisphere from noncoaxial imaging • Zoomed view • Variance off • Change baseline of Nyquist limit in the direction of the jet • Adjust lower Nyquist limit to obtain the most hemispheric flow convergence (typically 30–40 cm/s) • Measure the radius from the point of color aliasing to the VC		• Rapid qualitative assessment • Absence of proximal flow convergence usually a sign of mild MR	• Multiple jets • Eccentric jets • Constrained jet (LV wall) • Nonhemispheric shape, particularly functional MR • Overestimation when MR not holosystolic
VCW	• Parasternal long-axis view • Zoomed view • Imaging plane for optimal VC • Best measured when proximal flow convergence, VC, and MR jet aligned in same plane		• Surrogate for regurgitant orifice size • Independent of flow rate and driving pressure for a fixed orifice • Can be applied in eccentric jets • Less dependent on technical factors • Good at separating mild ($<$0.3 cm) from severe MR ($\geq$0.7 cm)	• Problematic in the presence of multiple jets • Convergence zone needs to be visualized for adequate measurement • Overestimation when MR not holosystolic
Jet area or jet area/LA area ratio	• Apical view • Zoom view • Measure largest jet alone or as ratio to LA area in same view	 	• Easy to measure	• Shown to be imprecise in multiple studies, particularly in eccentric, wall-impinging jets • Dependent on hemodynamic (especially LV systolic pressure) and technical variables • Overestimation when MR not holosystolic

(*Continued*)

Table 5 (Continued)

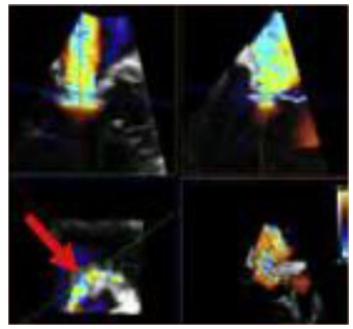
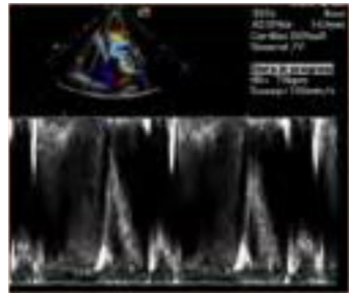
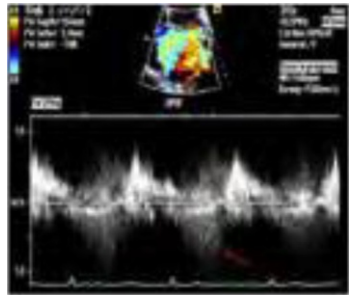
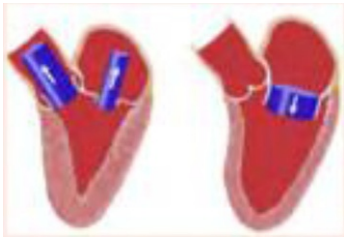
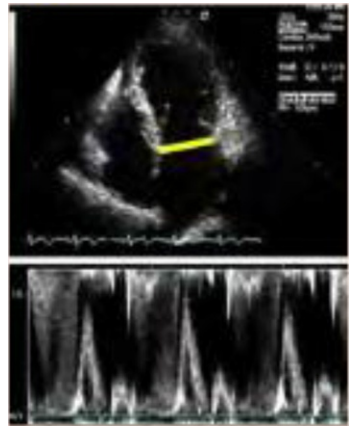
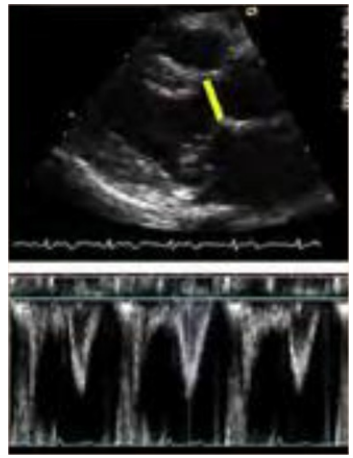
Modality	*Optimization*	*Example*	*Advantages*	*Pitfalls*
Color flow Doppler 3D: 3D VCA	● Color flow sector should be as narrow as possible to improve volume rates and line density ● Align orthogonal cropping planes along the axis of the jet ● Planimeter the high velocity aliased signal of VC, avoiding low velocity (dark color) signals		● Multiple jets of differing directions may be measured ● Can identify severe functional MR in some cases where PISA underestimates EROA	● Subject to color Doppler blooming ● Limited temporal and spatial resolution ● Overestimation when MR not holosystolic ● Multiple jets may be in different planes, must be analyzed separately and then added ● Cumbersome, often requires offline analysis
Pulsed wave Doppler Mitral inflow velocity	Align insonation beam with the flow across the MV at leaflet tips in apical four- chamber view		● E velocity $\geq$1.2 m/s a simple supportive sign of severe MR (volume load) ● Dominant A-wave inflow pattern virtually excludes severe MR ● Can be obtained with both TTE and TEE	● Depends on LV relaxation and filling pressures ● High E velocity not specific for severe MR in secondary MR, atrial fibrillation, and mitral inflow stenosis
Pulmonary vein flow pattern	Use small sample volume (3–5 mm) placed 1 cm into pulmonary vein		● Systolic flow reversal in more than one pulmonary vein is specific for severe MR ● Normal pulmonary vein pattern suggests low LA pressure and hence nonsevere MR	● Eccentric MR of mild or moderate severity directed into a pulmonary vein alters flow pattern ● Systolic blunting is not specific for significant MR (common in secondary MR and present in elevated LA pressure, atrial fibrillation) ● Not valid for combined MR and AR, unless pulmonic site is used

Table 5 (Continued)

Modality	*Optimization*	*Example*	*Advantages*	*Pitfalls*
SV method  $RVol = SV_{MV} - SV_{LVO}$	• LVOT diameter measured at the annulus in systole and pulsed Doppler from apical views at same site • Mitral annulus measured at middiastole; pulsed Doppler at the annulus level in diastole • Total LV SV can be measured by pulsed Doppler technique at mitral annulus or by the difference between LV end-diastolic volume and end-systolic volume • LV volumes are best measured by 3D. Contrast may be needed to better trace endocardial borders. If 3D not feasible, use 2D method of disks	Mitral annulus  LVOT 	• Quantitative, valid with multiple jets and eccentric jets • Provides both lesion severity (EROA, RF) and volume overload (RVol) • Validated against CMR in isolated MR	• Cumbersome, needs training; small errors in each different measurement can combine to magnify error in final results • Pulsed Doppler method (mitral SV) and LV volume method may give different results

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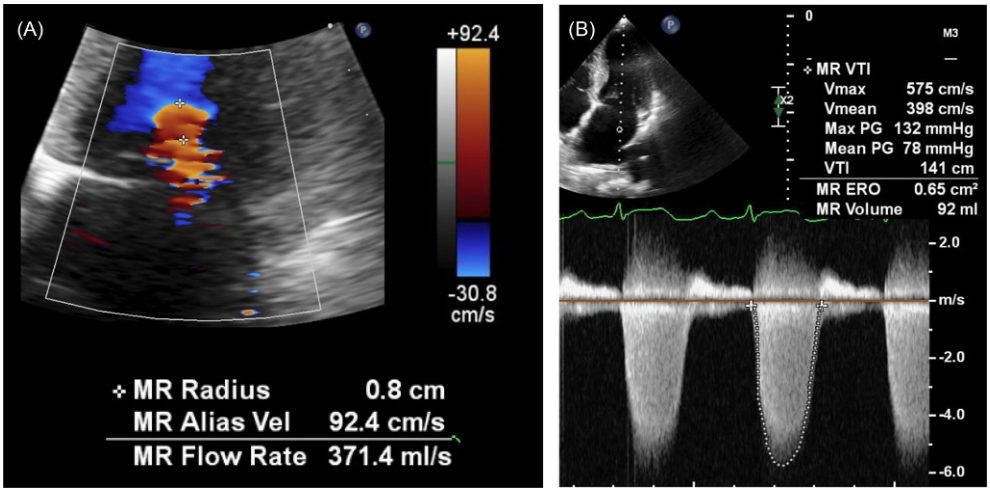

Fig. 20 Assessment of regurgitant volume and EROA in a patient with severe primary MR.

In patients with severe primary MR, the E velocity of transmitral flow pattern is usually high due to increased diastolic flow. In fact, severe MR can be almost excluded in the presence of an A wave-dominant impaired relaxation pattern. This may not be reliable in several conditions such as young age (where the mitral flow is E-wave dominant), secondary MR (where increased E velocity may be due to elevated LA pressure), and mitral annular calcification or MS (where flow is accelerated due to obstruction).

A simple nongeometric method relies on the principle that degree of regurgitant flow is reflected by the ratio of MV_{VTI} and $LVOT_{VTI}$ (Pu et al., 1999). This ratio is less than 1 in normal individuals because the mitral valve area is greater than the LVOT area. A ratio of >1.3 supports severe MR. This finding is however dependent on LV function and should only be used in conjunction with other parameters.

The shape and density of the CW Doppler MR jet can provide qualitative insight into the severity of MR. Compared to forward flow, significant MR causes echodense MR jet signal; lesser degrees of MR produce fainter signals. Similarly, the jet appears triangular and truncated in cases of severe MR with elevated LA pressure due to early peaking of MR jet signal with rapid decrease due to elevated LA pressure.

Echo parameters for grading severity of MR are depicted in **Table 6**. Echo variables for quantification of MR have been tested prospectively and have good prognostic validity (Tribouilloy et al., 1994).

A significant area of confusion in recent times has been the differing indices of severity for primary and secondary MR. (Nishimura et al., 2014 Jul) This was based on several studies showing that an EROA >0.2 cm^2 was associated with worse prognosis in secondary MR. This led to its inclusion as a cut-off for severity for severe secondary MR in the 2014 ACC/AHA valve guidelines (Nishimura et al., 2014 Jul). However, there is no data that valve interventions at that cut-off improve outcomes. In addition, patients with secondary MR are at a high risk of perioperative morbidity and mortality by virtue of their LV disease and valve surgery does not cure the underlying problem. This has led to a recent focused update which now aligns markers of severity equally between primary and secondary MR. (Enriquez-Sarano et al., 2005).

Effect on cardiac chambers in mitral regurgitation

The primary hemodynamic effect of MR is borne by the LV; hence, accurate assessment of LV volume and function is crucial in the assessment of severity. An LV end-systolic diameter >40 mm (>22 mm/m2 when indexed to BSA) and/or LV ejection fraction less than 60% justify surgical intervention. 3-D echocardiography provides a better assessment of LV volumes. LV dimensions and volumes should be indexed to body surface area especially in patients with smaller body surface areas to avoid underestimation of the hemodynamic burden. LA volume provides insight into the severity of MR especially in patients in sinus rhythm and who have no history of hypertension. Assessment of RV size and function is crucial in assessing the impact of elevated pulmonary venous pressures due to MR. The RV systolic pressure should be estimated by CW Doppler interrogation of the TR jet. Measurement of the tricuspid annulus and detection of the severity of TR is useful especially in patients undergoing mitral valve surgery so concomitant tricuspid valve repair can be planned (Nishimura et al., 2017).

Other modalities in mitral regurgitation

TEE, especially 3-D TEE places a very important role in the assessment of mitral valve anatomy especially in patients being considered for mitral valve repair. Accurate assessment of segments involved in prolapse or flail as well as accurate measurement of mitral annular size help surgical planning. Exercise echocardiography plays an important role in evaluation. In addition to assessment of functional capacity in patients with asymptomatic severe MR, response of LV function and the magnitude of increase in PA pressure with exercise provide useful diagnostic and prognostic information (Dreyfus et al., 2005; Leung et al., 1997).

Table 6 Echocardiographic grading of severity of mitral regurgitation

	MR severity[a]			
	Mild	*Moderate*		*Severe*
Structural				
MV morphology	**None or mild leaflet abnormality** (e.g., mild thickening, calcifications or prolapse, mild tenting)	Moderate leaflet abnormality or moderate tenting		**Severe valve lesions** (primary: flail leaflet, ruptured papillary muscle, severe retraction, large perforation; secondary: severe tenting, poor leaflet coaptation)
LV and LA size[b]	Usually normal	Normal or mild dilated		Dilated[c]
Qualitative Doppler				
Color flow jet area[d]	**Small, central, narrow, often brief**	Variable		Large central jet (>50% of LA) or eccentric wall-impinging jet of variable size
Flow convergence[e]	**Not visible, transient or small**	Intermediate in size and duration		**Large throughout systole**
CWD jet	Faint/partial/parabolic	Dense but partial or parabolic		Holosystolic/dense/**triangular**
Semiquantitative				
VCW (cm)	<0.3	Intermediate		≥0.7 (>0.8 for biplane)[f]
Pulmonary vein flow[g]	**Systolic dominance** (may be blunted in LV dysfunction or AF)	Normal or systolic blunting[g]		Minimal to no systolic flow/**systolic flow reversal**
Mitral inflow[a]	**A-wave dominant**	Variable		E-wave dominant (>1.2 m/s)
Quantitative[h,i]				
EROA, 2D PISA (cm^2)	<0.20	0.20–0.29	0.30–0.39	≥0.40 (may be lower in secondary MR with elliptical ROA)
RVol (mL)	<30	30–44	45–59[h]	≥60 (may be lower in low flow conditions)
RF (%)	<30	30–39	40–49	≥50

ROA, Regurgitant orifice area.

Bolded qualitative and semiquantitative signs are considered specific for their MR grade.

Most valid in patients >50 years old and is influenced by other causes of elevated LA pressure.

[a]All parameters have limitations, and an integrated approach must be used that weighs the strength of each echocardiographic measurement. All signs and measures should be interpreted in an individualized manner that accounts for body size, sex, and all other patient characteristics.

[b]This pertains mostly to patients with primary MR.

[c]LV and LA can be within the "normal" range for patients with acute severe MR or with chronic severe MR who have small body size, particularly women, or with small LV size preceding the occurrence of MR.

[d]With Nyquist limit 50–70 cm/s.

[e]Small flow convergence is usually <0.3 cm, and large is ≥1 cm at a Nyquist limit of 30–40 cm/s.

[f]For average between apical two- and four-chamber views.

[g]Influenced by many other factors (LV diastolic function, atrial fibrillation, LA pressure).

[h]Discrepancies among EROA, RF, and RVol may arise in the setting of low or high flow states.

[i]Quantitative parameters can help subclassify the moderate regurgitation group.

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Effect of other valvular disease in mitral regurgitation

The presence of mixed mitral valve disease (MS and MR) complicates the assessment of either stenosis or regurgitation. In general, the guiding principle remains that moderate stenosis and moderate regurgitation signify severe mixed valve disease. The mitral valve diastolic gradient is a useful parameter because it signifies the combined hemodynamic flow burden of both lesions.

Effect of aortic valve disease

In patients with AS, both primary (concomitant mitral valve disease) and secondary MR (due to LV pressure overload) can occur. TEE should be performed before surgical or transcatheter therapies in patients with suspected primary MR to establish etiology and severity of mitral valve disease. Secondary MR due to LV pressure overload often improves after correction of AS while more than moderate primary MR likely needs to be addressed along with the aortic valve intervention. In the presence of severe MR, a LF-LG AS can occur due to reduced LV forward stroke volume. The presence of more than mild AR complicates the measurement of regurgitant volumes by the Doppler volumetric method. In addition, the combined hemodynamic burden of coexisting MR and AR can lead to LV dilation and dysfunction disproportionate to the severity of each lesion.

Patients with significant MR should be screened for coexisting tricuspid valve disease. In patients with mitral valve prolapse, tricuspid valve prolapse can also occur. Due to RV dilation from severe MR, secondary TR often occurs. They should be carefully assessed prior to mitral valve surgery because uncorrected significant TR causes worse postoperative outcomes.

Tricuspid Valve

Anatomy of Tricuspid Valve

The normal tricuspid valve has an area of 4–6 cm^2 oriented nearly vertical (highest anteriorly) (Lancellotti et al., 2003). The three cusps are labeled anterior (antero-superior or infundibular), posterior (marginal) and septal. The anterior cusp is the largest while the septal is the smallest. The annulus is nonplanar and fibromuscular (fibrous portion corresponding to the septal leaflet). Thus, in cases of annular dilation, the septal leaflet is often unaffected and dilation occurs in the AP plane. The tricuspid subvalvular apparatus consists of three muscles–anterior, posterior, and septal papillary muscles and their true chordae tendinae. The anatomy and chordal distribution of papillary muscles is varied. The anterior papillary muscle is the largest and supplies chordae to the anterior only or the anterior and septal leaflet. The posterior PM is often bifid or trifid and provides chordae to the posterior and septal leaflets. The septal PM is the smallest and provides chordae to the septal and anterior leaflets. In addition, the septal leaflet is also supported by chordae that arise from the ventricular septum.

Echocardiographic Assessment of Tricuspid Valve Structure (Fig. 21)

By 2D TTE, the tricuspid valve is viewed from the parasternal RV inflow view, parasternal short-axis view at aortic valve level, apical four-chamber, and subcostal views. Identification of individual tricuspid valve leaflets in the 2-D views is controversial. 3D analysis as well as pathological examination of valves have demonstrated how leaflet identification by 2D TTE can be erroneous. In the 2-D parasternal long-axis (right ventricular inflow) view, the anterior leaflet is consistently visualized as the more proximal leaflet arising from the anterior free wall. The leaflet furthest from the probe is usually the septal leaflet, though the posterior leaflet can sometimes occupy this position. Identification of septal leaflet is facilitated by probe rotation to the interventricular septum into view. In the parasternal short-axis view at the level of the aortic valve, the posterior leaflet lies adjacent to the RV free wall and either the septal or anterior leaflet is adjacent to the aortic root. In the apical four-chamber view, the septal leaflet is consistently visualized adjacent to the septum, and the anterior leaflet is usually visualized along the RV free wall (occasionally the posterior leaflet is seen). The subcostal view usually displays the anterior and septal leaflets.

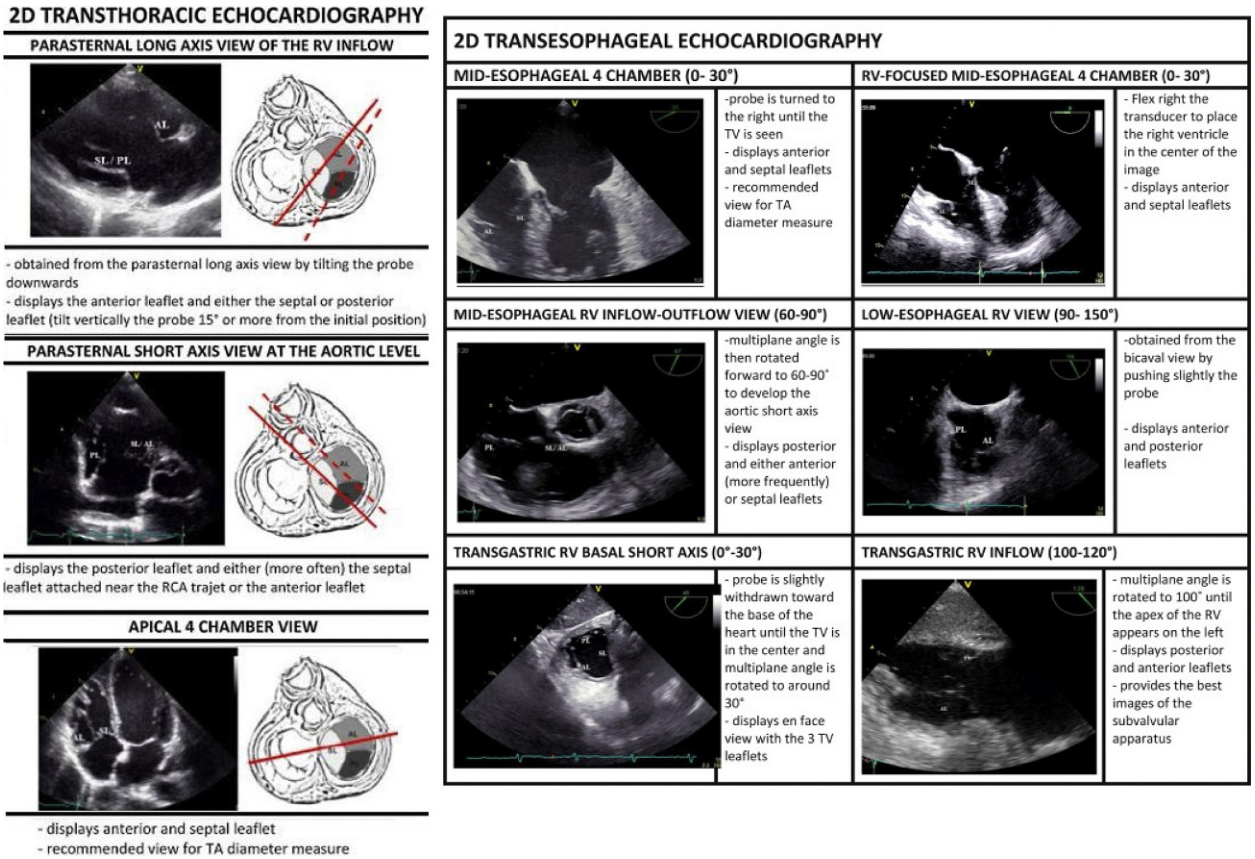

Fig. 21 Tricuspid valve leaflet identification using 2D TTE and 2D TEE. Adapted with permission from Huttin, O et al. (2016). *Archives of Cardiovascular Diseases*, **109** (1), 67–80.

3-D imaging of the tricuspid valve enables both en face visualization of the three leaflets as well as accurate identification of each leaflet in a corresponding 2 D view. The tricuspid valve annulus is slightly apically displaced compared with the mitral valve annulus.

By virtue of its location, the tricuspid valve is anterior and thus far field from the TEE transducer. The tricuspid valve is best visualized in ME 0 degrees (anterior and septal leaflets) and by clockwise rotation from ME level at 30 and 60 degrees; mid-high esophageal level 45 degrees, and clockwise rotation from the transgastric 0 and 90 degree views. TR jet velocity is best measured in a view in which the ultrasound beam and the regurgitant jet are most parallel, often between 30 and 90 degrees in the ME level.

Tricuspid Stenosis

TS is the least common valvular stenosis and is commonly caused by rheumatic heart disease. Other causes include carcinoid syndrome, mechanical obstruction due to tumors and endocarditis, and rarely pacemaker lead induced adhesions (**Fig. 22**, see online **Video 5**).

Assessment of valve structure in tricuspid stenosis

Anatomical assessment of the tricuspid valve is best accomplished through the parasternal RV inflow view as well as an apically focused four-chamber view. The presence of valve thickening and calcification, reduced leaflet opening and RA size can be assessed. An en face view of the valve is best obtained using 3D echo.

Assessment of valve function in tricuspid stenosis

CW Doppler evaluation of the valve provides assessment of trans-tricuspid gradient (Rogers and Bolling, 2009). Unlike the mitral valve, tricuspid flow is affected by respiration, thus Doppler flow measurements are best made at end expiration or should be averaged. As in MS, measurement should be averaged for at least five cardiac cycles in the presence of atrial fibrillation. The mean pressure gradient >5 mmHg has been considered to indicate hemodynamically significant TS. Similar to MS, PHT can be useful. Despite not being well validated, PHT $\geq$ 190 ms corresponds to a valve area $\leq$1 cm^2 (**Table 7**) (Fawzy et al., 1989; Pearlman, 1991; Yousof et al., 1985).

Effect on cardiac chambers in tricuspid stenosis

The right atrium bears the brunt of the hemodynamic consequences of TS. Severely enlarged right atrium and a dilated inferior vena cava are supportive criteria to suggest hemodynamically significant TS.

Stress echocardiography

Owing to its rarity, the role of stress echocardiography in tricuspid stenosis is unknown.

Effect of concomitant valve disease in tricuspid stenosis

The presence of TS should be meticulously investigated in all patients with rheumatic MS. Significant TS reduces RV cardiac output and this could theoretically underestimate severity of MS.

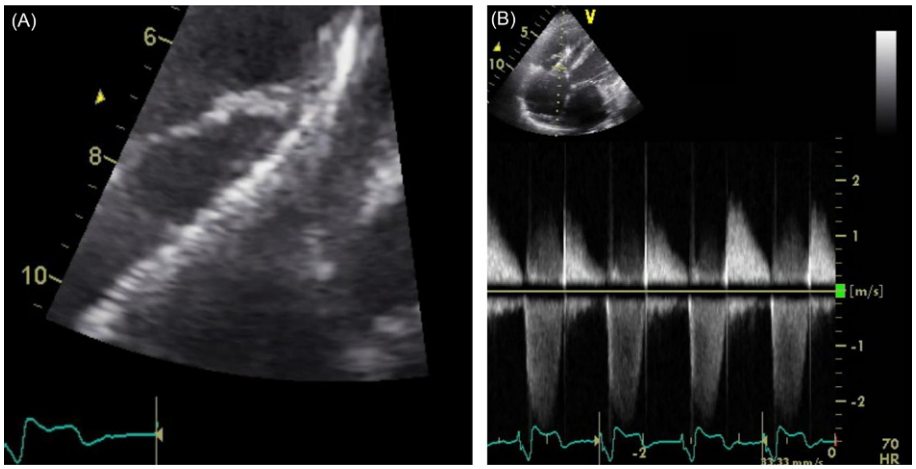

Fig. 22 Severe tricuspid stenosis due to defibrillator lead adhesions. Mean gradient across the valve was 8 mmHg.

Table 7 Echocardiographic findings suggestive of severe tricuspid stenosis

Specific findings	
Mean pressure gradient	>5 mmHg
Inflow time-velocity integral	>60 cm
Pressure halftime	>190 ms
Valve area by continuity equation	<1 cm^2
Supportive findings	
Enlarged right atrium, > moderate	
Dilated inferior vena cava	

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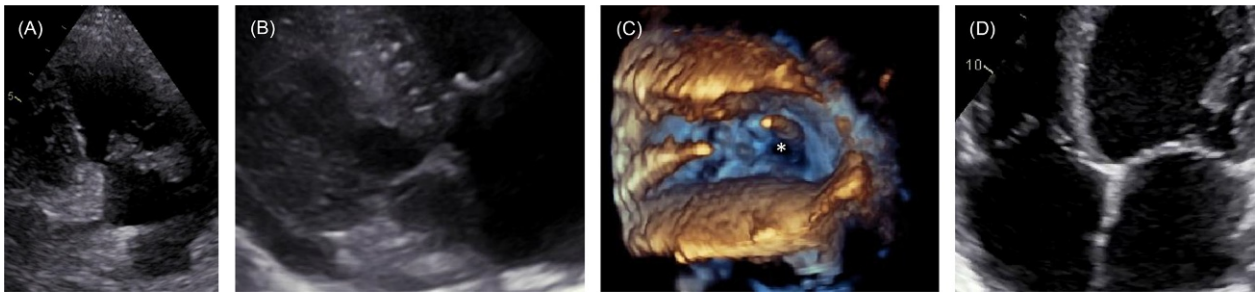

Fig. 23 Tricuspid valve appearance in significant TR due to endocarditis (panel A), carcinoid syndrome (panel B), pacemaker lead with restricted posterior leaflet causing malcoaptation (panel C), and secondary TR resulting from leaflet malcoaptation in dilated cardiomyopathy (panel D, mid systole).

Tricuspid Regurgitation

Though long ignored and considered a "forgotten valve disease," the prognostic importance of both primary and secondary TR is being increasingly recognized (Karp et al., 1989b; Topilsky et al., 2014).

Assessment of valve structure in tricuspid regurgitation

The tricuspid valve is best visualized in the standard and RV focused apical four-chamber view; parasternal right ventricular inflow view; parasternal short-axis view at the aortic level, and subcostal four-chamber views. Mechanism of TR can be divided into primary (rheumatic valve disease, degenerative valve disease, endocarditis, trauma and rarer causes such as congenital heart disease, carcinoid or drug induced valvulopathy) and secondary (RV and RA remodeling). (**Fig. 23**) Leaflets and chordae are thickened and retracted in rheumatic TR and leaflet motion is restricted. Tricuspid valve prolapse and flail can co-exist in patients with MVP. Carcinoid involvement of the tricuspid valve often results in thickening and immobility of the entire length of the leaflet with restricted motion and wide open TR (see online **Videos 6** and **7**). Secondary or functional TR is the result of tricuspid annulus dilatation, with structurally normal leaflets that are tethered and tented. These features are best demonstrated in apical four-chamber view. The normal tricuspid diastolic annulus diameter is 28 ± 5 mm in apical four-chamber view and a diameter >35 mm is considered abnormal.

Assessment of valve function in tricuspid regurgitation

Color flow Doppler

Trivial TR can be seen during echocardiographic exam in 65%–75% of normal individuals and facilitates the measurement of RV systolic pressure. Color flow imaging is the first step in TR diagnosis and provides an estimate of TR severity (Nath et al., 2004). TR can be graded based on the jet area: <5 cm^2 (mild), 5–10 cm^2 (moderate), and >10 cm^2 (severe). As with other regurgitant lesions, color jet area is influenced by hemodynamic (RA pressure, volume) and technical factors (gain) and tends to underestimate the severity of eccentric jets. VC width is a semiquantitative method for TR severity estimation. A VC width ≥ 7 mm is quite sensitive and specific for severe TR (Gonzalez-Vilchez et al., 1994). Similar to MR, 3D assessment provides an accurate assessment of VC area. (Tribouilloy et al., 2000b) Unlike MR however, there is no clear cut off between mild and moderate TR.

Spectral Doppler

EROA and RVol can be assessed by the PISA method but is seldom used in clinical practice. When a clear isovelocity shell is defined, the PISA radius, aliasing velocity and Vmax and VTI of CW TR jet can be used to calculate EROA and RVol. An EROA ≥ 40 mm^2 and

RVol ≥45 mL characterize severe TR. The contrast in RVol between MR and TR despite similar EROA reflects the smaller difference in RV-RA (smaller TR velocity) compared with LV–LA pressures.

The shape and density of CW Doppler TR jet signal provides a means for qualitative assessment of TR severity. The denser the envelope compared to the forward flow, the more severe the TR. In severe TR (especially acute) rapid equalization of RA and RV pressure causes a triangular truncated envelope (V cut off sign) (**Fig. 24**). While RV systolic pressure can be measured in lesser grades of TR, caution should be exercised in patients with wide open TR where the velocity of the TR jet is low due to the small pressure difference between the RA and RV. An E wave >1 m/s by PW Doppler at leaflet tips is an indirect marker of severe TR by providing an estimate of early transtricuspid flow.

The most important PW Doppler indicator of TR severity is systolic flow reversal in the hepatic veins. In the subcostal view, the right superior hepatic vein (1–2 cm proximal to the junction with the inferior vena cava) is interrogated. In severe TR, flow is directed toward the right atrium and reflected back into the hepatic veins; this causes systolic flow reversal and provides a specific marker of severe TR (Egbe et al., 2016). Caution should be exercised in interpreting hepatic vein flows in patients with atrial fibrillation or flutter. Echo parameters for grading severity of TR are depicted in **Table 8**.

Effect on cardiac chambers in tricuspid regurgitation

Severe chronic TR is associated with RA, RV, and IVC dilation which are important supportive signs. In fact, normal right heart volumes and IVC dimension rule out significant chronic TR.

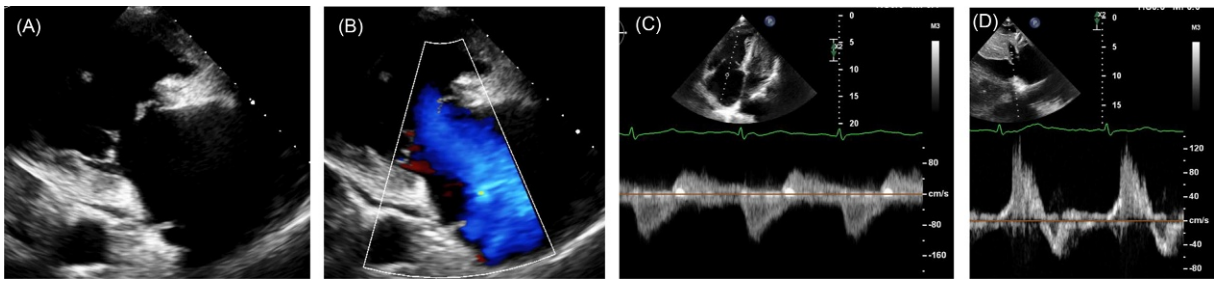

Fig. 24 Echocardiographic evaluation in a patient with severe, wide open TR due to a flail anterior leaflet (panel A). Due to the very high RA pressure, normal RV pressure and large EROA, rapid equalization of pressures between RA and RV occurs and is depicted by laminar color Doppler flow (panel B) and a low velocity CW Doppler TR signal (panel C). Systolic flow reversal is present in the hepatic veins (panel D).

Table 8 Grading the severity of chronic tricuspid regurgitation by echocardiography

Parameters	*Mild*	*Moderate*	*Severe*
Structural			
TV morphology	Normal or mildly abnormal leaflets	Moderately abnormal leaflets	Severe valve lesions (e.g., flail leaflet, severe retraction, large perforation)
RV and RA size	Normal	Mild dilation	Usually dilated
Inferior vena cava diameter	Normal <2 cm	Normal or mildly dilated 2.1–2.5 cm	Dilated >2.5 cm
Qualitative Doppler color			
Flow jet area	Small, narrow, central	Moderate central	central jet or eccentric wall impinging jet of variable size
Flow convergence zone	Not visible—transient or small	Intermediate in size and duration	Large throughout systole
CWD jet	Faint/partial/parabolic	Dense, parabolic, or triangular	Dense, often triangular
Semiquantitative			
Color flow jet area (cm^2)	Not defined	Not defined	>10
VCW	<0.3	0.3–0.69	≥0.7
PISA radius	≤0.5	0.6–0.9	>0.9
Hepatic vein flow	Systolic dominance	Systolic blunting	Systolic flow reversal
Tricuspid inflow	A-wave dominant	Variable	E-wave >1.0 m/s
Quantitative			
EROA	<0.20	0.20–0.39	≥0.40
R Vol	<30	30–44k	≥45

TV, tricuspid valve; *RV*, right ventricle; *RA*, right atrium; *CWD*, continuous wave Doppler; *VCW*, vena contracta width; *PISA*, proximal isovelocity surface area; *EROA*, effective regurgitant orifice area; *R Vol*, regurgitant volume.

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Other modalities in tricuspid regurgitation

The role of exercise echocardiography in TR is unclear. 3D imaging allows to accurate identification of leaflets and associated pathology.

Effect of concomitant valve disease in tricuspid regurgitation

Presence of significant primary TR complicates the assessment of RV function from left-sided valve disease (such as MS and MR).

Pulmonic Valve

Anatomy of Pulmonic Valve

Anatomically, the pulmonic valve is located anterior, superior, and to the left of the aortic valve. Normal pulmonic valve has three cusps—right, left, and anterior.

Echocardiographic Assessment of Pulmonic Valve Structure (Fig. 25)

Pulmonic valve is best visualized in the parasternal short-axis view the aortic level (**Fig. 25A**). In the parasternal long-axis view, tilting the probe cranially (sometimes moving up an intercostal space) also provides an excellent view of the pulmonic valve and the pulmonary artery including its bifurcation (**Fig. 25B**). Color Doppler and spectral Doppler interrogation of the valve can be accomplished. In some patients with poor transthoracic echo windows, subcostal views can sometimes be useful. 3-D echocardiography is useful in cases with good quality 2-D imaging and is useful in defining valvular anatomy. By virtue of its anterior location, the pulmonic valve is best seen by TTE. By TEE, the best views to visualize the pulmonic valve are aortic short-axis view with an (between 40 and 60 degrees) and mid-to-high esophageal long-axis view (between 110 and 130 degrees).

Pulmonic Stenosis

Assessment of valve structure in pulmonic stenosis

Valvular PS results from thickening and the fusion of the leaflets and can be either congenital or acquired. Congenital PS is by far the most common cause. The leaflets are often thickened and dysplastic and can be bicuspid. Thickened leaflets with systolic bowing can be seen (**Fig. 26**, see online **Video 8**). Post stenotic pulmonary artery dilatation can sometimes be visualized.

Assessment of valve function in pulmonic stenosis

Increased flow velocity and gradient across the stenotic valve can be assessed by CW Doppler (Chen et al., 2013). Unlike left-sided valve stenosis, measurement of gradients forms the predominant method to assess severity of stenosis. Severe stenosis is defined by a peak jet velocity more than 4 m/s and a corresponding peak gradient >64 mmHg (**Table 9**). Compared to other valve stenosis, peak gradients have been traditionally used to define severity due to good correlation with catheterization-derived gradients (Lima et al., 1983; Silvilairat et al., 2005). Pulmonic valve area can be calculated using the continuity equation:

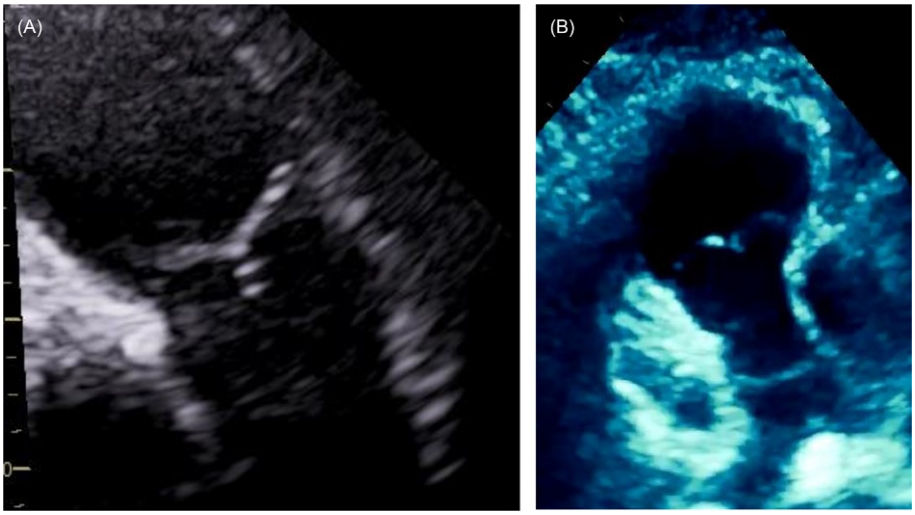

Fig. 25 2D TTE imaging of pulmonic valve. Zoomed-in parasternal short-axis view at aortic level (panel A). A cranial tilt to the probe in the parasternal long-axis position provides a dedicated view of the RVOT, pulmonic valve, and proximal pulmonary artery (panel B).

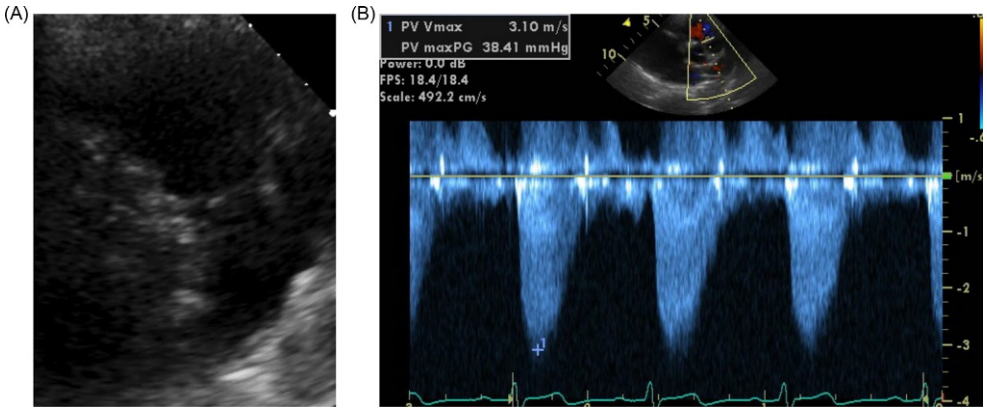

Fig. 26 Echocardiographic evaluation of a patient with pulmonic stenosis. Panel A shows the doming appearance of pulmonic valve (see online **Video 8**); CW Doppler evaluation in panel B quantifies gradient across the stenotic valve (mild stenosis).

Table 9 Echocardiographic grading of pulmonary stenosis

	Mild	*Moderate*	*Severe*
Peak velocity (m/s)	<3	3–4	>4
Peak gradient (mm Hg)	<36	36–64	>64

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$$\mathrm{PVA} = \mathrm{CSA_{RVOT}}^{*}\ \mathrm{VTI_{RVOT}})/\ \mathrm{VTI_{PV}}$$

However, due to the difficulty in imaging the RVOT and the inability to obtain coaxial Doppler interrogation, this is rarely used in clinical practice. Color Doppler mapping and PW Doppler can be used to rule out other levels of obstruction at the sub- or supravalvular level.

Effect on cardiac chambers in pulmonic stenosis

Severe PS causes RV pressure overload and right ventricular hypertrophy. RV free wall thickness >5 mm (measured a few centimeters below the tricuspid annulus in the subcostal view) is used to define increased RV wall thickness. 3-D echocardiography and CMR provide better assessment of RV volume and function than 2D echo.

Other modalities in pulmonic stenosis

As noted earlier, TEE is used in patients with poor transthoracic window or in planning and guiding transcatheter pulmonic valve therapies. When possible, 3D echo provides en face views of the valve. The role of stress echocardiography is limited to functional capacity assessment and largely unstudied.

Effect of concomitant valve disease in pulmonic stenosis

In cases of congenital PS, careful evaluation is required to rule out co-existing abnormalities. When hemodynamically significant PS is present, it complicates the evaluation of other valve structures on the right heart. Similar to other valve diseases, severity of mixed pulmonic stenosis and regurgitation can be tough to assess.

Pulmonic Regurgitation

A majority of normal individuals have mild, functional, PR which are small, central jets by color Doppler.

Assessment of valve anatomy in pulmonic regurgitation

Valve pathology such as a flail leaflet and right heart size and function should be assessed. TTE is the preferred modality.

Assessment of valve function in pulmonic regurgitation

Unlike other valvular regurgitation, the quantification of degree of PR is not supported by robust data (Table 10).

Color Doppler imaging is useful in evaluating the spatial location of the jet (central, eccentric) and assessing its severity (Williams et al., 2002). A ratio of the jet diameter to the width of the RVOT just below the valve provides a semiquantitative measure

of severity (severe PR >65% of the RVOT) (Fig. 27, see online Video 9). Unlike other valves, VC width is often estimated as a ratio of the annular diameter and a cutoff >0.5 suggests severe PR (Williams et al., 2002). A PHT <100 ms is a good indicator of significant PR but is unreliable in the setting of high RV end-diastolic pressures (Renella et al., 2010). Though EROA by PISA can be calculated, this approach has not been well validated. By CW Doppler, signal density may provide a qualitative measurement of the regurgitation while a steep deceleration of the Doppler spectrum or an early termination of diastolic regurgitant flow might be a sign of severe PR. A PR index (ratio of PR time to total diastolic time traced using CW Doppler) <0.77 provides a quantitative

Table 10 Echocardiographic and Doppler parameters useful in grading severity of pulmonic regurgitation

Parameter	*Mild*	*Moderate*	*Serve*
Pulmonic valve	Normal	Normal or abnormal	Abnormal and may not be visible
RV size	Normal	Normal or dilated	Dilated
Jet size, color Doppler	Thin (usually <10 mm in length) with a narrow origin	Intermediate	Broad origin; variable depth of penetration
Ratio of PR jet width/pulmonary annulus			>0.7
Jet density and contour (CW)	Soft	dense	Dense; early termination of diastolic flow
Deceleration time of the PR spectral Doppler signal			Short, <260 ms
Pressure half-time of PR jet			<100 ms
PR index		<0.77	<0.77
Diastolic flow reversal in the main or branch PAs (PW)			Prominent
Pulmonic systolic flow (VTI) compared to systemic flow (LVOT VTI) by PW	Slightly increased	Intermediate	Greatly increased
RF	<20%	20%-40%	>40%

RV, right ventricle; *CW*, continuous wave Doppler; *PW*, pulse wave Doppler; *PR*, pulmonic regurgitation; *LVOT*, left ventricular outflow tract; *EROA*, effective regurgitant orifice area; *RF*, regurgitant fraction.

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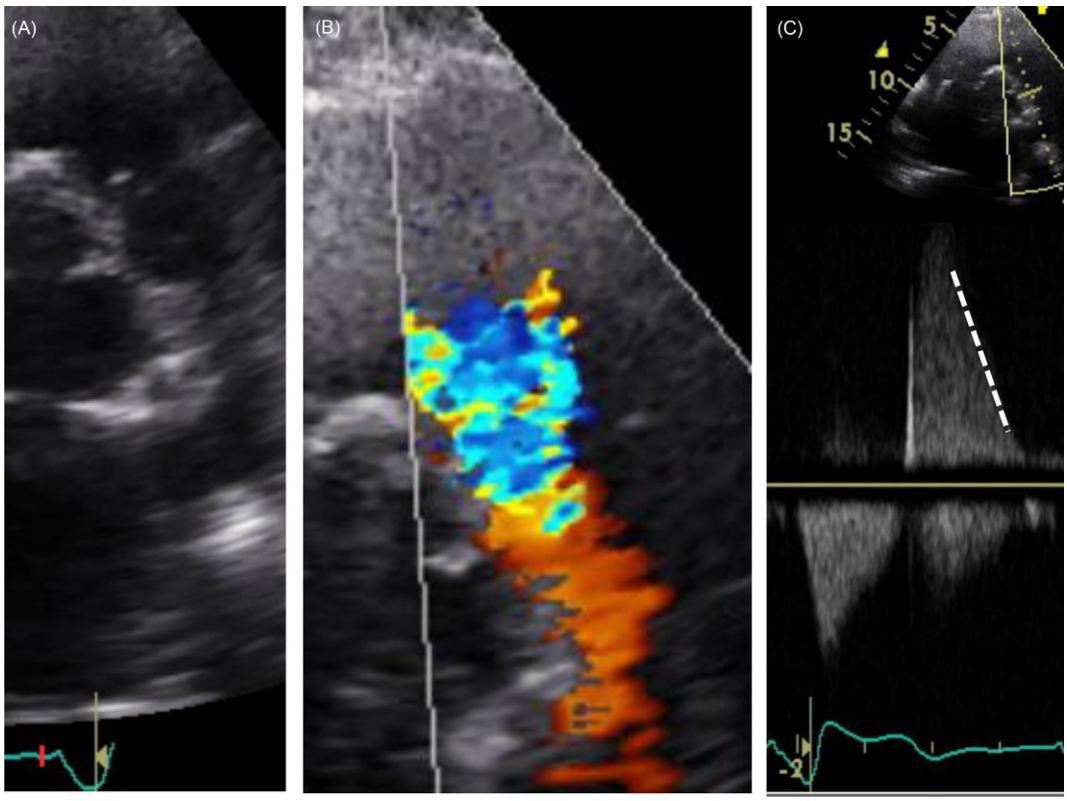

Fig. 27 Echocardiographic evaluation of a patient with severe pulmonic regurgitation due to flail leaflet (panel A, Video 9). Color Doppler shows a significant jet of PR occupying the entire RVOT (panel B). CW Doppler shows dense PR jet with a short pressure half time (*dashed line*, PHT = 60 ms).

measure of this phenomenon and suggests severe PR (Silversides et al., 2003). Analogous to AR, holodiastolic flow reversal in the branch pulmonary arteries is a useful parameter that suggests severe PR.

Effect on cardiac chambers in pulmonic regurgitation

The dominant hemodynamic effect of PR is on the RV. While commonly ignored in the past, RV size and function provide valuable prognostic information and guide decision making. The presence of a dilated RV is not specific for severe PR especially in patients without concomitant pulmonary hypertension or in complex congenital heart disease. Serial follow-up studies are often more valuable in assessing worsening hemodynamic burden of PR.

Other modalities in pulmonic regurgitation

3D echo (and CMR) are superior in the assessment of RV size and function which play an important role in timing of surgical intervention.

Effect of concomitant valve disease in pulmonic regurgitation

Presence of significant PR complicates assessment of the effect of other valve lesions on RV size and function.

Conclusions

This article provides an overview of the importance of echocardiography in the evaluation of valve structure and function. Despite advances in 3D technology and rapid growth of alternative modalities of imaging such as CMR, 2D, and Doppler echocardiography provide a rapid, easily available, and cost effective method to evaluate and manage valve disease.

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Economics of Heart Failure

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Economic Aspects of Heart Failure

The syndrome of heart failure (HF) is one of the most prevalent cardiovascular conditions worldwide, with the number of cases rising as the population progressively ages in many parts of the globe (Khatibzadeh et al., 2013). Overall, it is estimated that 26 million people across the globe are affected at any given time (Ambrosy et al., 2014). In the United States alone, HF is estimated to affect 5.7 million people aged 20 years or more with an incidence approaching 10 cases per 1000 per annum in those aged 65 years or more (Mozaffarian et al., 2016). From a health-care service perspective, in North America and Europe combined, HF contributes to >1 million hospitalizations per annum (Ambrosy et al., 2014). Reports from Asia (Shimokawa et al., 2015), Australia (Chan et al., 2016), and even Africa (Keates et al., 2017) indicate an equivalent or at least emerging (with respect to sub-Saharan Africa) burden of disease associated with the syndrome. Consequently, HF-related health-care expenditure (direct costs) accounts for ~2% of the total health-care budget in the US, with equivalent or even higher proportional expenditure in many other high-income countries around the world (Cook et al., 2014).

Iterative guidelines (Ponikowski et al., 2016) for the gold-standard diagnosis and treatment of acute and chronic forms of HF reflect an expanding armory of therapeutic options with the potential to substantially increase HF-related health-care costs, from the introduction of new biomarkers to detect the syndrome at an earlier clinical stage, to device-based therapies to treat more advanced clinical cases. Isolated HF is rare (<10% of cases). In the majority of the cases, beyond the common antecedents of hypertension and coronary artery disease, HF often occurs in the setting of multimorbidity; concurrent management of these comorbidities adding to the plethora of health-care costs already associated with the syndrome (Stewart et al., 2016). As such, HF commonly copresents with other high-cost conditions such as respiratory disease, renal dysfunction, diabetes, and atrial fibrillation (AF) that both complicate its management and prolong hospitalization (Wiley et al., 2017).

Key Components of Heart Failure Costs

Given the diversity of health-care systems (with different cost-dynamics imposed by a variety of funding models) around the world, there is no definitive source of health-care costs associated with HF. Therefore, in this chapter, health-care expenditure relating to HF is expressed in US dollars and the lowest cost price is listed (unless specified otherwise). Specific costs were obtained from the Healthcare Bluebook (2017).

Direct Costs

The direct costs of HF include those activities specifically arising from a patient's diagnosis, medications, device-based and surgical treatment, HF management, and, at the end-of-life, palliative care. A primary hospital admission for HF (where it is listed as the principal diagnosis) is usually the most expensive direct cost associated with the syndrome, with initial high-level care, investigations of cardiac function (such as transthoracic echo and brain-type natriuretic peptide (NP) levels) and high cost pharmacological (e.g., IV inotropic support) and device-based therapies (e.g., insertion of a cardiac pacemaker with the capacity for internal defibrillation) now becoming increasingly common. Chronic pharmacological treatment, requiring ongoing specialist HF surveillance in the community, also contributes to the direct cost of managing the syndrome. It is estimated that costs directly associated with HF in the US will have increased from $21 billion in 2012 to $53 billion by 2030 (Heidenreich et al., 2013). However, as already noted, estimates of total HF-related expenditure from a whole health service and societal perspective vary widely from country to country, with US-based estimates, at least from an absolute cost perspective, being substantially higher than many other countries (Nicholson et al., 2016).

Initial screening and diagnosis

Due to the relatively low cost (>$550), ease, lack of contraindications, and critical details obtained, echocardiography (typically using the least expensive option of a transthoracic approach as opposed to the more expensive transesophageal approach (>$594)) is generally the preferred modality for diagnosing underlying cardiomyopathy and associated cardiac dysfunction, whether it be in the form of HF with reduced (HFrEF) or preserved ejection fraction (HFpEF). Accordingly, echocardiography represents the cornerstone for the diagnosis (and ongoing management) of HF (Ponikowski et al., 2016; Yancy et al., 2013).

As per contemporary expert guidelines (Ponikowski et al., 2016; Yancy et al., 2013), initial HF investigations may involve determining the plasma concentration of NPs (>$89), especially in the cases where echocardiography is not immediately available. While relatively inexpensive, NP testing cannot be used to definitively establish a diagnosis of HF, and is only recommended to rule out HF. This is also the case for a 12-lead electrocardiogram (ECG) (>$41), which cannot be used to definitively diagnose HF due to the low specificity of the test. However, the 12-lead ECG is relatively inexpensive, often available in situations in which an echocardiogram is not, and can be used to determine which patients require echocardiography. Regardless of the combination of diagnostic tests applied, they require trained technicians to perform the test(s), and expert cardiology review to diagnose the presence of HF and, if confirmed, the extent, nature, and underlying cause of associated cardiac dysfunction, further adding to the costs involved. A range of common diagnostic tests (most notably a chest x-ray (>$68)) is also typically undertaken during the diagnostic phase of management. If, after echocardiography, the diagnosis or causality of HF requires further elucidation, additional imaging options of varied costs include cardiac magnetic resonance (>$1,154), positron emission tomography (>$2,809), coronary angiogram (>$8,911), and coronary computed tomographic angiography (>$918).

Pharmacological treatment

Initial pharmacological treatment of the most common form of HF, HFrEF secondary to ischemic cardiomyopathy, generally entails a combination of angiotensin converting enzyme inhibitors (>$9 per 30 day supply) and beta-blockers (>$7 per 30 day supply), with mineralocorticoid receptor antagonists (>$9 per 30 day supply), ivabradine (>$209 per 30 day supply), digoxin (>$21 per 30 day supply), hydralazine and isosorbide dinitrate (>$97 per 30 day supply), and/or loop-diuretics (>$6 per 30 day supply) considered on an individual basis. The replacement of angiotensin converting enzyme inhibitors with angiotensin receptor neprilysin inhibitors (>$215 per 30 day supply) may also be indicated (Ponikowski et al., 2016). The total pharmacological treatment cost per patient per annum is estimated to be between $750 and $1626 (Voigt et al., 2014). While the evidence-based management of HFpEF (an increasingly more common form of HF within the aging populations of high-income countries) is less clear, the cost (per annum) of treating such patients is likely to be equivalent given the typical presentation with hypertension and/or AF. For example, in combination with typical signs and symptoms of congestion associated with the syndrome, active treatment is mandated (Ponikowski et al., 2016; Yancy et al., 2013).

Device-based therapies

There are currently two nonsurgical device based therapies routinely recommended for the treatment of HFrEF: implantable cardioverter-defibrillators (ICDs) (>$64,560) and cardiac resynchronization therapy (CRT) (Ponikowski et al., 2016; Yancy et al., 2013). While US costing for CRT is not made widely available, a Belgian assessment has placed the price at >$23,000 for the device alone (Van Brabandt et al., 2010). Costs for these devices are extremely high, and both require ongoing monitoring and maintenance by cardiologists and highly trained technicians once implanted, with the prospect of replacement in younger individuals with prolonged survival. There is a broad variability in both device cost, and the cost of remotely monitoring devices within and between countries. Analysis of cardiac implantable device usage within the Asia-Pacific region revealed that the cost of remote monitoring can vary from as low as an additional 5% of the device cost (in Japan), to as high as an additional 20% in expenditure (in China, Hong Kong, and South East Asia) (Lau and Zhang, 2013).

Surgical procedures

Many surgical procedures can be used to treat HF depending on the underlying causes and consequences of the syndrome. Current guidelines recommend the application of coronary artery bypass grafting (CABG) (>$51,072), percutaneous coronary intervention

(PCI) (>$21,694), surgical aortic valve replacement (>$60,609), transcatheter aortic valve implantation (TAVI) (>$78,415), and mitral valve surgery (>$62,364) on a case-by-case basis (Ponikowski et al., 2016; Yancy et al., 2013). More rarely, heart transplantation may be considered (>$196,774). The insertion of an intraaortic balloon pump (IABP) (>$181,340) or a left ventricular assist device (LVAD) (>$187,535) is most commonly used as bridging strategies until patients can undergo a more permanent procedure. However, LVADs are increasingly being used as a permanent solution for patients not eligible for heart transplantation (Kilic, 2015). It is worthwhile to note that these costs represent only the surgical procedure, and not the cost of hospitalization pre and postprocedure monitoring, and specialist management and treatment in the longer-term. These costs are substantive.

HF-specific management: Hospital and community-based care

As noted, hospitalization (particularly in the form of recurrent, unplanned admissions denoting ongoing clinical instability in a subset of high cost patients) represents a substantive component of the overall economic burden of HF, as demonstrated in **Fig. 1** using data collected from a recently completed, large, multicenter trial of HF management in Australia where the primary outcome was health-care costs during 12 months follow-up (Scuffham et al., 2017). Typically, those admitted with acute HF require management (noting a substantive burden imposed by those who receive Emergency Department management without subsequent admission) in high-cost facilities. Moreover, the short- and longer-term rate of recurrent hospitalization is extremely high; in the United States, as in many parts of the world, all-cause readmission is as high as 20%–25% within 30-days of an HF admission (Jencks et al., 2009; Wiley et al., 2017). A recent estimate has placed the median hospital cost for HF hospitalization in the US at $23,051 (Bress et al., 2016) and hospitalization costs are projected to rise 154% between 2012 and 2030 (Heidenreich et al., 2013). While the majority of people admitted to hospital for HF for the first time are discharged home, 21% are discharged to a long-term care facility/residential care. Significantly, the proportion of those requiring such long-term care increases with age and each subsequent HF-related admission, denoting the progressive and debilitating nature of the syndrome in affected individuals (Chan et al., 2016).

Community-based care (often specialist in nature) is critical to the ongoing management of HF, particularly for those recently admitted with acute HF and/or treated with specialist pharmacotherapy requiring routine monitoring (e.g., electrolyte levels and renal function) or devices (e.g., routine pacemaker and ICD checks to ensure optimal function). This component of health-care includes primary care physician visits (>$176 per 15 min visit), medical specialist (>$505 per visit), allied health outpatient appointments (>$195 per visit), and ongoing surveillance costs (Voigt et al., 2014). For high-risk hospitalized patients, post-discharge multidisciplinary management (typically HF nurse-led) is also recommended to reduce the risk of rehospitalization and to prolong survival. The cost of applying these multifaceted programs will vary according to the level of specialist outpatient, community/home visits, remote monitoring (from structured telephone support and remote monitoring techniques), and referrals to other services (from pharmacological reviews to exercise programs) applied. In the recently completed WHICH? II Trial undertaken in the subsidized health-care system of Australia, the median cost of applying an intensive form of HF management was $285 per month per patient (Scuffham et al., 2017).

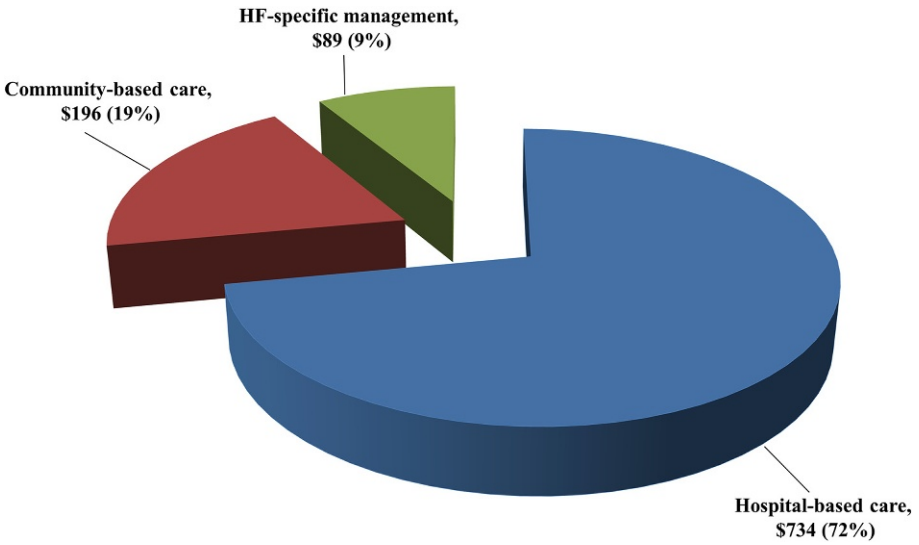

Fig. 1 Breakdown of HF costs (per person, per month). Data obtained from Scuffham, P. A., Ball, J., Horowitz, J. D., et al. (2017). Standard vs. intensified management of heart failure to reduce healthcare costs: results of a multicentre, randomized controlled trial. *European Heart Journal*. https://doi.org/10.1093/eurheartj/ehx259 [Epub ahead of print].

Palliative care

Within an aging HF patient population in whom survival rates have improved (Mamas et al., 2017), the need for palliative care is becoming increasingly more common. It is suggested that patients with advanced and intractable forms of HF now represent the second most common reason for hospice admission (after cancer patients) (Heidenreich et al., 2013). However, compared to patients with the common forms of malignancy, their end-of-life trajectory is quite unclear and unpredictable, broadly differing between patients. Perhaps due to the difficulty in predicting the life expectancy of patients with HF, they are more likely to be either discharged alive or to remain in hospice for more than 6 months (Heidenreich et al., 2013). Accordingly, costs accumulated by HF patients during the last 6 months of life are high, and continue to rise (Unroe et al., 2011), with recent estimates placing this figure at an average of $23,606 (Nicholson et al., 2016).

Indirect Costs

The indirect costs associated with HF (particularly in the setting of multimorbidity) are often difficult to define. However, they can be broken down into two broad categories: microeconomic versus macroeconomic costs (as detailed below). As described by Stewart and colleagues (2002), it is critical to consider the indirect cost of HF through its contribution to hospital admissions where the syndrome is listed as a secondary diagnosis. Reflective of the complicated nature of HF (as a syndrome that complicates more clearly defined clinical conditions), the ratio of primary versus secondary HF admissions is often 1:1.5–2.0. Consequently, when considered in economic analyses, these admissions almost double estimated HF-related expenditure (Chan et al., 2016).

Microeconomic costs

Microeconomic costs can be defined as out-of-pocket expenses associated with HF treatment and management. These costs typically infer negative, downstream economic consequences such as the loss of monetary savings or the sale of a house to cover health-care expenses (Braunwald and Bonow, 2012). Microeconomic costs may vary depending on the health-care system and are influenced by the age and broader socioeconomic profile of the affected individual, the cost and comprehensiveness of health insurance, transport to health-care appointments, pharmacological costs, and potential loss of employment/income due to illness.

Macroeconomic costs

Macroeconomic costs relate to the loss of worker productivity and economic growth when individuals with HF and their carers reduce work hours or drop out of the workforce entirely as a result of illness (Braunwald and Bonow, 2012). Macroeconomic costs are difficult to quantify. However, whole population data from Sweden suggest that the wider societal impact of HF is larger than the most common forms of cancer for both men and women in respect to total life-years and quality-adjusted life-years lost (Stewart et al., 2010).

The Cost Dynamics of HF: A Case Study

As outlined above, management and treatment of HF can be associated with significant direct and indirect costs. **Fig. 2** details the sequence of events relating to a patient's diagnosis of HF and the associated costs. The key economic aspects of their case are outlined below.

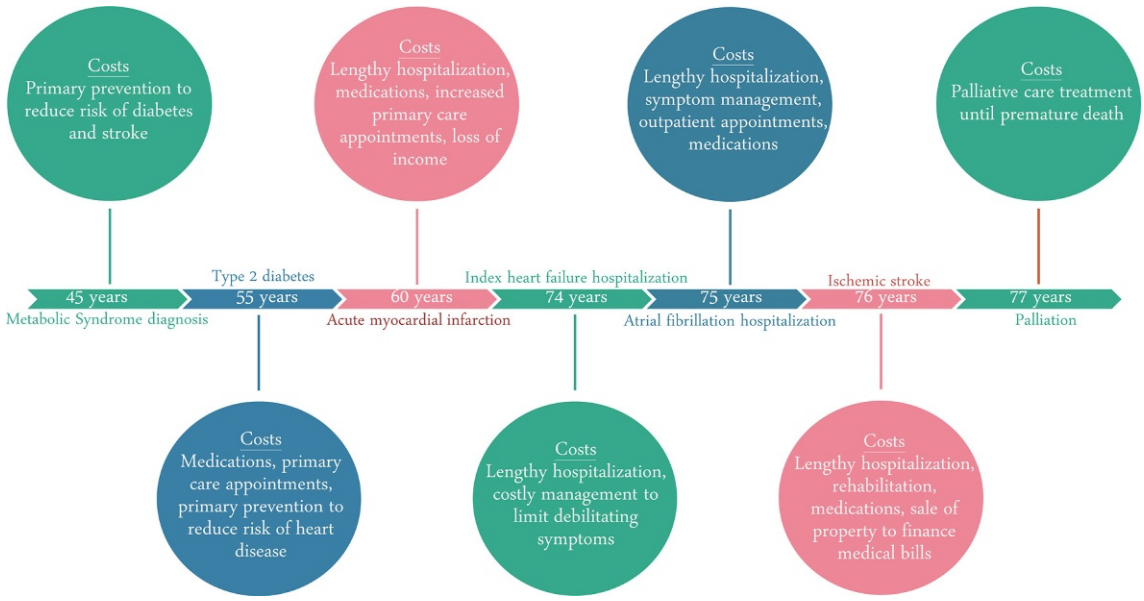

Fig. 2 Sequence of events relating to the patient's diagnosis of heart failure.

Primary and Secondary Care

The patient had regular visits with his primary care personnel, who were essential for early detection of disease and management of symptoms to prevent (or at least delay) subsequent HF-related morbidity and mortality that would be costly for an individual to societal perspective. His primary care personnel were involved in both pharmacological and nonpharmacological management of his symptoms. Secondary care commenced when the patient's primary care physician referred him immediately for an acute hospital admission with specialist cardiology treatment and management.

Treatment and Management

The patient's diagnosis of HFrEF (characterized by progressive cardiac dysfunction and multimorbidity—including type 2 diabetes and renal disease) required costly inpatient and outpatient management. Due to the typical natural history of the syndrome, this management was aimed at providing clinical stability, rather than a cure, in order to reduce costly hospitalizations and prolong his life (Ponikowski et al., 2016).

Palliative Care

As the patient's HF symptoms worsened, his ability to perform daily self-care activities declined markedly and, despite the support of his wife, he was placed into a nursing home for long-term, high-level care. His subsequent advanced-stage HF was treated with palliative management until he died at the age of 77 years, 3 years following his de novo acute HF presentation.

The Impact of HF and Multimorbidity

As shown in Fig. 2, those affected by HF have health issues that are both complicated and not confined to the syndrome. As is often the case in HF (Stewart et al., 2016), in the presented case study, HF contributed to subsequent hospitalizations for the management of chronic AF and an ischemic stroke, these conditions contributed to his clinical instability and decline. The 12 most common comorbidities in HF are hypertension, coronary artery disease, anemia, arrhythmias (predominantly AF), cognitive dysfunction, depression, diabetes, musculoskeletal disorders, renal dysfunction, respiratory disease, sleep disorders, and thyroid disease (Stewart et al., 2016). The close link between multimorbidity and incremental health-care costs in patients affected by HF is now well established (Glynn et al., 2011; Picco et al., 2016) with a recent study finding that multimorbid HF patients have an increased risk of 30-day all cause readmission upon discharge compared to those with lone HF (Wiley et al., 2017). This economic effect is well demonstrated in the recently reported WHICH? II Trial of HF management (Scuffham et al., 2017). Figs. 3 and 4 show a breakdown of length of stay and hospitalization costs accumulated over a 12-month follow-up of the WHICH? II Trial cohort. These data demonstrate that of $7,489,876 total health-care expenditure due to hospitalization during 12-month follow-up, only $1,944,461 (26%) was HF-related, meaning the residual portion of expenditure ($5,545,415 or 74% was attributable to HF-related multimorbidity).

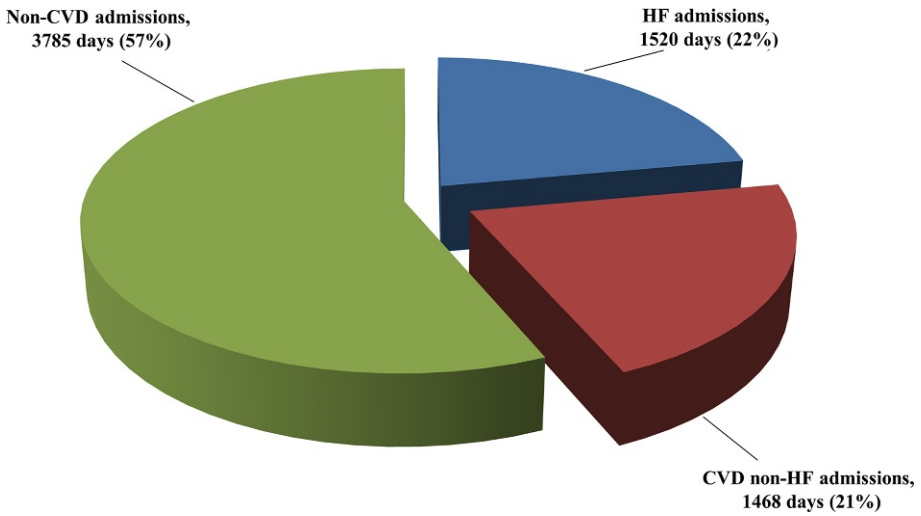

Fig. 3 Breakdown of 12-month hospitalization length for 391 HF patients. Data obtained from Scuffham, P. A., Ball, J., Horowitz, J. D., et al. (2017). Standard vs. intensified management of heart failure to reduce healthcare costs: results of a multicentre, randomized controlled trial. *European Heart Journal*. https://doi.org/10.1093/eurheartj/ehx259 [Epub ahead of print].

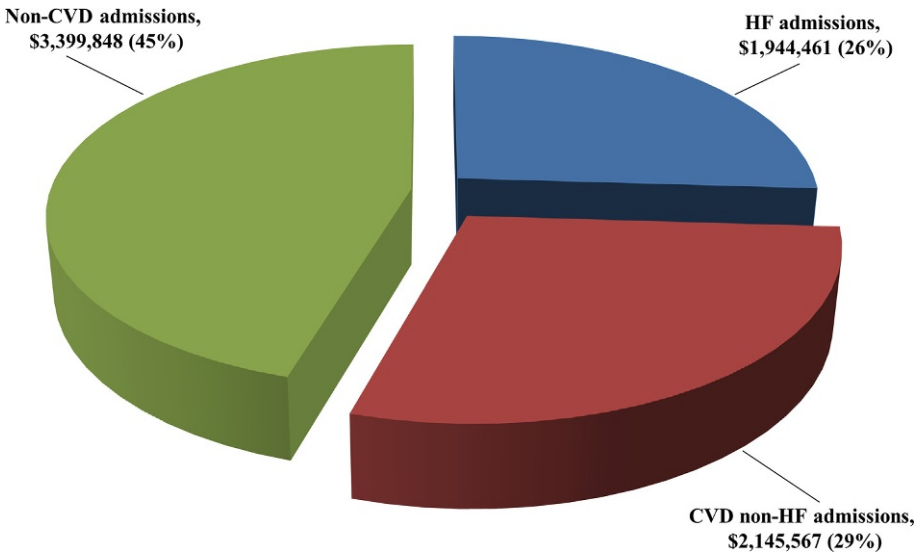

Fig. 4 Breakdown of 12-month hospitalization costs for 391 HF patients. Data obtained from Scuffham, P. A., Ball, J., Horowitz, J. D., et al. (2017). Standard vs. intensified management of heart failure to reduce healthcare costs: results of a multicentre, randomized controlled trial. *European Heart Journal*. https://doi.org/10.1093/eurheartj/ehx259 [Epub ahead of print].

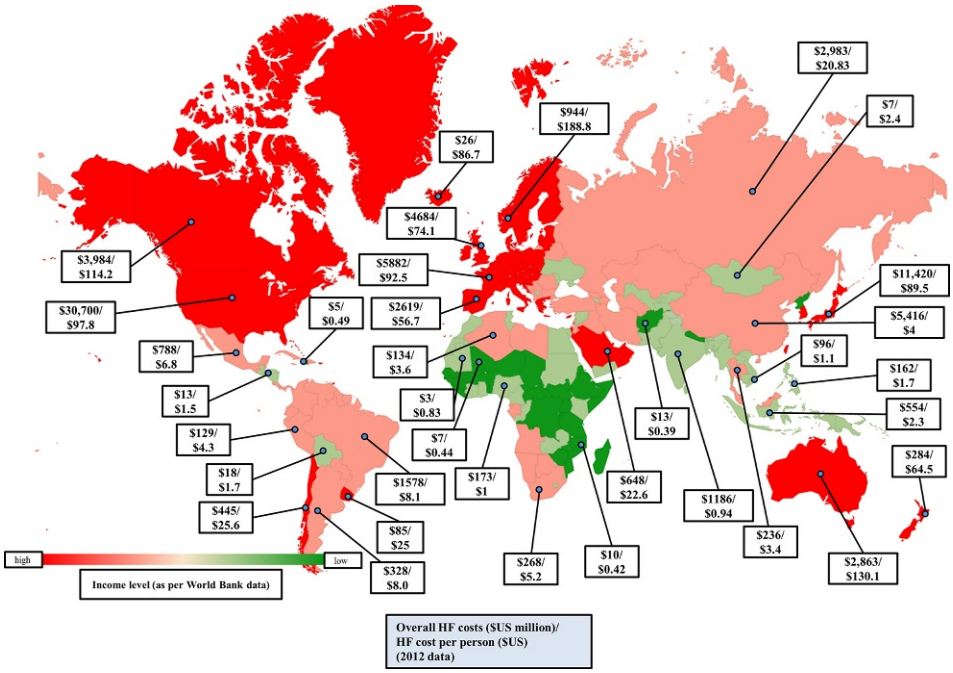

Fig. 5 Global comparison of HF costs. Data obtained from Cook, C., Cole, G., Asaria, P., Jabbour, R. & Francis, D. P. (2014). The annual global economic burden of heart failure. *International Journal of Cardiology* 3, 368–376. The World Bank (2017). GNI per capita, Atlas method (current US$). http://data.worldbank.org/indicator/NY.GNP.PCAP.CD (accessed 1 May 2017); Population Reference Bureau. (2012). 2012 world population data sheet. [online] Washington: PRB, pp. 6–9. http://www.prb.org/Publications/Datasheets/2012/world-population-data-sheet.aspx (accessed 0505.17).

Global Economic Burden of Heart Failure

As noted previously, it is now estimated that HF affects more than 26 million people globally, with figures on the rise as many populations progressively age (Ambrosy et al., 2014; Bui et al., 2011). Increasing HF prevalence places a varied burden on the global economy (as shown in Fig. 5). However, the economics of HF is difficult to calculate and compare between countries due to the complexity of the syndrome, variability in definitions, and the differing characteristics of health-care and insurance systems

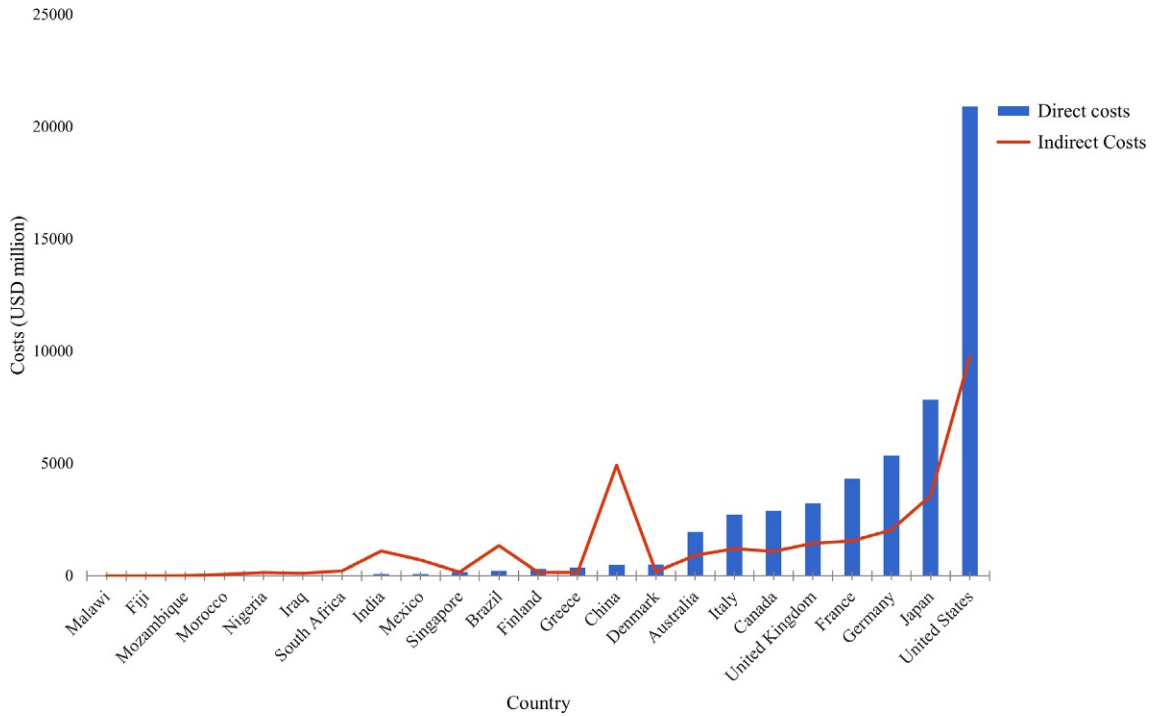

Fig. 6 Direct and indirect HF costs per country. Data obtained from Cook, C., Cole, G., Asaria, P., Jabbour, R. & Francis, D. P. (2014). The annual global economic burden of heart failure. *International Journal of Cardiology* 3, 368–376.

(Voigt et al., 2014). Despite these caveats, Cook and colleagues (2014) estimated that in 2012, the annual economic cost of HF was $108 billion globally (comprising $65 direct and $43 billion indirect expenditure). The cost dynamics of HF-related expenditure varied greatly between high-income and low- and middle-income countries (LMICs), the former spending a larger proportion of direct costs, while the pattern was reversed in LMICs (Fig. 6).

A Low- and Middle-Income Country Perspective

When compared to high-income countries, the cost of HF in LMICs differs for a variety of reasons. For example, the average age of HF patients in sub-Saharan Africa is markedly lower than many other regions of the world. This places a burden on the economy through loss of income and productivity, and through needing to care for those who may normally be caregivers themselves. For example, direct HF costs are substantially lower in Nigeria (approximately 50% of the total cost of HF) as compared to high-income countries (Ogah et al., 2014). The mean cost of care per HF patient per annum in Nigeria is approximately $2000: while this is far lower than the approximate cost of $20,000 in the US, this represents a far higher proportion of the gross national income (GNI) per capita, at 72% and 36%, respectively (Voigt et al., 2014; The World Bank, 2017). However, the approximate mean cost of care per annum (for patients with HFrEF) in Turkey was $608, representing only 6% of their per capita GNI (Aras et al., 2016). In China, the per annum HF cost was $4204, representing 53% of their per capita GNI (Huang et al., 2017), demonstrating a large variation in the relative economic burden of HF among LMICs.

Cost Pressures in Heart Failure

As the prevalence of HF is predicted to increase in the foreseeable future, so too will the economic burden. An aging population will contribute to a greater proportion of men and women at higher risk of HF (Cook et al., 2014). The US-based data has indicated that HF prevalence will increase by 46% during the period 2012–2030 (Heidenreich et al., 2013). Concurrently, improved health-care and expanded treatment options are contributing to increased longevity following an initial diagnosis of HF, which in turn increases its economic impact. As highlighted previously, while this is occurring globally, there may be a greater and more adverse economic impact of HF in LMICs. Additionally, more people are being diagnosed with HF than ever before. This reflects both increased awareness and an expansion of the syndrome definition, recent European guidelines defining HFpEF and HF with midrange ejection fraction (HFmrEF) in addition to the more traditionally recognized HFrEF (Ponikowski et al., 2016). As surveillance efforts and definitions have broadened, so too have HF risk factors. Globally, there are currently historically high levels of obesity and

physical inactivity. As noted, there is also an alarming trend in the growing burden of multimorbidity, contributing to increased rates of hospitalization, and more complicated treatment patterns (Stewart et al., 2016).

Cost Evaluation in Heart Failure

It is worth noting that much of the early (and cheapest) treatment modalities in HF were (and still remain) untested from a robust scientific and subsequent health economic perspective, loop-diuretics being the most obvious example. However, with an evolving armory of more expensive pharmacological agents and devices, in particular, there is increasing need to evaluate the cost of different diagnostic and treatment modalities in HF, from an individual to societal perspective. Either in the form of cost-effectiveness estimates (e.g., quality adjusted life-years gains per health-care expense) or cost–benefit analyses (e.g., potential cost-savings relative to an old treatment), most new therapeutic modalities in HF are formally assessed before being subject to subsidized funding by governments or health insurance agencies. Rarely is one therapeutic option [one recent example being the superiority of home-based versus clinic-based management of HF in Australia (Maru et al., 2015)] proven to be "dominant" over another in terms of both health benefits and cost-savings. For example, in the UK, the National Institute for Health and Care Excellence established a cost-effective threshold (relative to optimal pharmacotherapy) of up to £30,000 per quality adjusted life-year gained to support a recommendation of implanting a device with cardiac resynchronization and internal defibrillator capacities in a subset of patients with HF (National Institute for Health and Excellence, 2014). Increasingly, in the setting of limited financial resources and an expanding patient population with HF, thresholds for subsidizing therapeutic strategies (when individuals cannot pay directly) will undoubtedly become more stringent.

Reducing the Economic Burden of Heart Failure

Having provided an overview of the nature of the economic burden of HF, how then do we reduce its adverse impact? Around half of all US HF-related hospitalizations (the greatest component of HF expenditure) are potentially preventable (Braustein et al., 2003). Recent Australian data placed the median cost per month of hospital-based care for patients with HF at $734, compared to only $196 per patient per month for community-based care, and $89 per patient per month for HF-specific management (Scuffham et al., 2017). Any strategy that reduces hospital stay by even a modest amount, therefore, is likely to have a favorable economic profile.

Accordingly, reducing readmission rates in those patients with clinical instability could lead to a substantial lowering of HF-related expenditure. While some factors leading to high readmission rates are largely beyond the scope of the direct health-care system (such as severity of illness and socioeconomic factors) potentially modifiable factors include:

- *Increased communication between health-care providers*: As highlighted previously, multimorbidity and HF is common, being the primary cause of hospitalization for those with HFpEF (Stewart et al., 2016). As such, it is necessary for HF patients to see a variety of specialists in addition to their primary care physician. Increasing communication levels between these practitioners may lead to a clearer understanding of the patient's health-care plan to all involved (practitioners, patient, and other carers), which may, in turn, lead to greater adherence and reduced likelihood of rehospitalization. This is especially apparent in patients in long-term care facilities, who often have a large amount of personnel, with a broad range of skill- and knowledge-levels involved in their care (Heckman et al., 2016).
- *Providing clear and high-quality discharge instructions to patients*: Along the same lines, ensuring that this information is then provided to patients in a manner which is clear, easily understood, and remembered, is important. For all but the sickest HF patients, the majority of time is spent at home, and if the patient does not understand or cannot remember their health-care instructions, they are at increased risk of not following them.
- *Implementing early outpatient appointments*: Beginning HF-related outpatient appointments soon after discharge may lead to a reduction in hospital readmission rates (Soundarraj et al., 2017). A recent study revealed that follow-up appointments were only organized for 50% of patients discharged home post HF-related hospitalization, and that appointments were less likely to have been organized for patients aged over 65 years, and for patients with HFpEF (vs HFrEF) (Goyal et al., 2016).
- *Reducing initial hospital stay*: A fine balance needs to be found between ensuring a hospitalization stay is not unnecessarily long and ensuring that a patient is not discharged prematurely, increasing the likelihood of recurrent instability and readmission or death.
- *Specialist management*: Recent American research has suggested that intensive care unit (ICU) admission, while significantly more expensive than general medicine stays, was not significantly associated with differences in 30-day mortality for HF. As such, being more selective with those patients admitted to ICU may lead to a reduction in hospitalization costs without compromising the care of the patient (Valley et al., 2017).

Specialist HF management programs, particularly in the form of outreach models of care have the potential to markedly reduce rehospitalization and prolong survival (Maru et al., 2015), although implementing more intensive management may not necessarily deliver cost-savings (Scuffham et al., 2017).

The cost of new device-based therapies and pharmaceutical treatments for HF continue to exert an increasing economic burden. As previously discussed, while necessary, both of these treatment techniques can be quite expensive. There is a variety of potential cost saving mechanisms associated with HF-related devices. Improving the battery life of devices has been highlighted as of potential benefit—recent studies have shown that longer device battery life could save 60.4 million Swedish Krona (USD6.83 million) over a 6-year period in Sweden, and reduced device-related costs by 29%–34% over 15 years in Europe (Gadler et al., 2016; Boriani et al., 2013). Additionally, reducing the variability of cardiac device costs between and within countries may reduce costs. The price of cardiac devices is often established via confidential agreements between hospitals and/or insurance companies, and device companies—a method which may work to inflate prices. Similarly, there is broad variability in pharmaceutical pricing.

Summary

The economic footprint of HF, from the individual to societal level in nearly all part of the globe is substantial. In the US alone it is estimated that costs directly associated with the management of HF will rise from a current level of >$21 billion per annum to >$53 billion per annum by 2030 (Heidenreich et al., 2013). Indirect costs attributable to HF are likely to double these figures. Hospital costs and an increasing armory of expensive pharmacological and device-based therapies in an increasing number of older patients with complex clinical needs will be instrumental in driving the future cost burden imposed by the syndrome. Beyond preventing progression to HF, key targets to limit its economic impact include reducing the number of preventable hospital admissions (often recurrent in the same individual) and reducing the inflationary costs of new therapeutic strategies.

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Relevant Websites

Gapminder tools—www.gapminder.org.
Healthcare Bluebook—www.healthcarebluebook.com.
National Institute of Health Care Excellence—www.nice.org.uk.

Electrocardiographic Monitoring Strategies (Holter, Implantable Loop Recorder, in Between)

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Introduction

The understanding and application of the electrocardiogram (ECG) has evolved immensely since 1912, when Einthoven first described his equilateral triangle to the Chelsea Clinical Society in London, and coined the term "EKG" in his landmark paper published in the Lancet (Barold, 2003). Advancements in the ECG continued in the early 1900s when Emanuel Goldberger created the augmented leads, which led to the modern-day 12-lead ECG (Burch, 1978). The ability to monitor patients remotely is largely attributed to the work of Norman Holter, who developed the first "Holter Monitor" in the 1940s. It allowed for a single ECG to be recorded for several hours, giving physicians the ability to monitor their patients ECG outside of the hospital setting (Roberts and Silver, 1983; Holter, 1961; Holter and Generelli, 1949). The original Holter monitor was a 75-pound backpack that utilized analog electronics and large batteries (Holter, 1961). Although it lacked practicality in its design, the Holter monitor revolutionized our ability to record and analyze ambulatory ECG data of patients not in hospital (Roberts and Silver, 1983) (**Fig. 1**).

Since the original Holter monitor, substantial technological advancements provide the ability to monitor patients continuously from days to years, and as a result, our diagnostic capabilities have dramatically improved. Clinical trials examining the role of implantable cardiac monitors (ICMs) have validated their role in patients with unexplained syncope. Furthermore, they have shown increased utility in patients with vasovagal syncope and postmyocardial infarction arrhythmia detection. The era of smartphones has brought with it the potential for cardiac-monitoring technology integrated into our daily activities. This can offer real-time feedback to patients and couple ECG monitoring to other physiological parameters (blood pressure, oxygen saturation, etc.).

This article will focus on summarizing the different modalities of electrocardiographic monitoring used in the context of common cardiac scenarios.

Clinical Scenarios Necessitating Cardiac Monitoring

Cardiac monitoring aids clinicians in obtaining a rhythm profile and helps to establish a symptom–rhythm correlation. Various monitoring modalities are utilized in patients with palpitations, syncope, postmyocardial infarction arrhythmia detection, atrial fibrillation management, and detection of atrial fibrillation in patients with cryptogenic stroke. With the continued advances in monitoring technology, the scope of clinical application continues to expand.

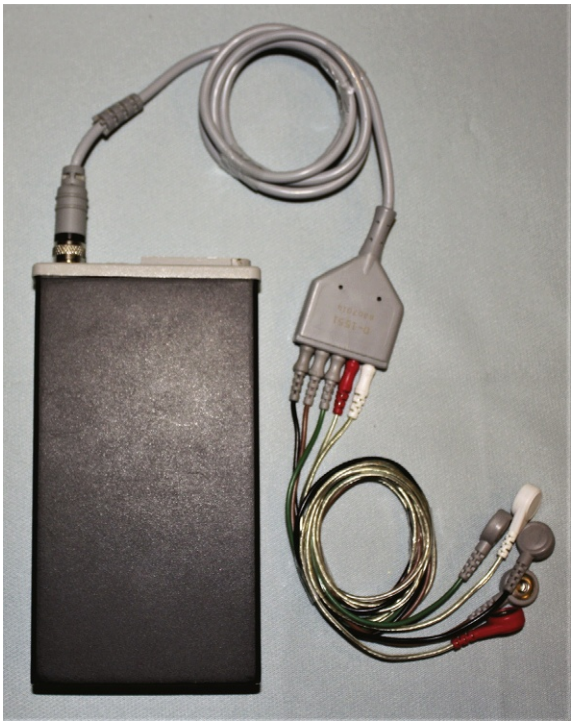

Fig. 1 Holter monitor device. The patient wears the device using a shoulder strap or belt loop, attached to 3–5 skin electrodes for continuous monitoring. An event button (not shown) at the *top* of the housing of the device is pressed in the event of symptoms to mark the recording.

Palpitations

Palpitations represent one of the most commonly encountered symptoms by general practitioners and emergency department (ED) physicians, and frequently prompt referral to a cardiologist (Zimetbaum and Goldman, 2010). Palpitations can be completely benign or abnormal depending on the clinical scenario; therefore, a careful history needs to be elicited (Zimetbaum and Goldman, 2010; Weber and Kapoor, 1996). In patients with palpitations that remain unexplained after initial evaluation with routine ECG, cardiac monitoring is often considered. Monitoring can help establish symptom–rhythm correlation and detect asymptomatic arrhythmias that are inferred to be relevant, which can help to establish a diagnosis. Typically, the interval between palpitations can be lengthy and therefore, the type of continuous monitoring strategy chosen needs to be carefully considered (Zimetbaum and Goldman, 2010). Multiple studies have compared the diagnostic yield of the different monitoring devices, an important consideration for choosing the appropriate device based on symptom frequency.

Syncope

Syncope is a common presentation encountered by primary care and ED physicians. It accounts for 1%–3% of ED visits and 0.6%–1% of all hospitalizations (Olde Nordkamp et al., 2009). It is estimated that the lifetime cumulative incidence of syncope in the general population is between 35% and 39%, with the majority of patients having their first syncopal episode before the age of 30 (Sud et al., 2009). Despite its prevalence, the management of syncope remains a challenge for many physicians. Obstacles in the diagnosis of syncope include the high spontaneous remission rates as well as the episodic and unpredictable frequency of events. Syncope falls under the broad definition of "transient loss of consciousness" and therefore the differential diagnosis for patients presenting with syncope can be extensive in some cases (Olde Nordkamp et al., 2009; Anon, 2016). The differential can include a number of life-threatening causes, which often leads to physicians feeling compelled to complete an extensive diagnostic work-up. Monitoring is often considered when the syncope likely attributed to a cardiac etiology. However, despite a full diagnostic evaluation, the etiology of the syncope is not found in approximately one-third of patients. Therefore, monitoring strategies are utilized to help record or exclude an arrhythmia as a contributing cause of syncope (Zimetbaum and Goldman, 2010; Subbiah et al., 2013). Extended monitoring is typically necessary to record the ECG during a spontaneous episode of syncope, but asymptomatic arrhythmias may have compelling inferential use (Krahn et al., 2004).

Atrial Fibrillation

With an increasingly aging population, the prevalence of atrial fibrillation (AF) continues to rise (Colilla et al., 2013). It is estimated that by 2030, 12.1 million individuals will be living with AF in the United States (Colilla et al., 2013). Many of the adverse events

associated with AF can be correlated with episode duration, frequency, and overall arrhythmia burden. Therefore, there is a great deal of interest in AF monitoring to help guide anticoagulation therapy, rate control, rhythm control efficacy, and burden in patients with heart failure (Botto et al., 2009). Multiple studies have examined the role and utility of different monitoring strategies, and there is evidence that longer duration ECG monitoring enhances arrhythmia detection rates (Boriani et al., 2014; Andrade and Krahn, 2013; Brachmann et al., 2016; Korompoki et al., 2016; Sposato et al., 2015).

Postmyocardial Infarction Arrhythmia

Individuals who survive an acute myocardial infarction (AMI) that are left with left ventricular dysfunction (ejection fraction $\leq$40%) are at high risk of sudden death due to cardiac arrhythmias (Solomon et al., 2005; Huikuri et al., 2001). There is evidence to support that prophylactic implantation of an implantable cardioverter-defibrillator (ICD) late after an AMI reduces all cause mortality (Hohnloser et al., 2004). This benefit is lessened when the ICD is implanted early after an AMI. In addition, use of left ventricular function alone selects high-risk individuals for ICD implantation, but has a modest effect on population sudden death risk, because the majority of sudden deaths occur in apparently low-risk patients (Adabag et al., 2010; Myerburg et al., 1992) Prolonged ECG monitoring is an area of interest to identify improved noninvasive risk stratifiers that may help identify which patients post AMI may find the most benefit from ICD implantation. The Cardiac Arrhythmias and Risk Stratification after AMI (CARISMA) trial was the first large-scale prospective trial that utilized a prolonged ECG monitoring strategy to help identify invasive and noninvasive risk markers that may predict the occurrence of arrhythmias in patients post AMI (Huikuri et al., 2009). Overall, evidence to support prolonged ECG monitoring in these patients is limited by low positive predictive value, and more studies will be necessary to make definitive recommendations.

Atrial Fibrillation and Embolic Stroke of Undetermined Source

As many as 25% of patients presenting with an ischemic stroke have no probable cause identified after a thorough evaluation, which includes an ECG, echocardiogram, in-patient telemetry, magnetic resonance imaging (MRI), or computed tomographic (CT) imaging of the brain. These patients are ultimately diagnosed as having a cryptogenic stroke or more recently termed as an embolic stroke of undetermined source (ESUS) (Sanna et al., 2014; Saver, 2016; Seet et al., 2011). Some of these strokes are attributed to embolism of venous thrombi through a patent foramen ovale (PFO) (Meier et al., 2013; Calvert et al., 2011). Several randomized clinical trials have demonstrated that closure of PFO failed to yield significant benefit (Carroll et al., 2013), suggesting that ischemic strokes through paradoxical embolism of venous thrombi are very rare. More recently, studies have focused on better understanding the extent to which AF contributes to ESUS. AF increases risk of thromboembolic strike as much as fivefold (Boriani et al., 2014). In the evaluation of patients with ischemic strokes, the incidence of either persistent or paroxysmal AF predating the stroke is approximately 15% (Joundi et al., 2016; Liao et al., 2007). Following their stroke, an additional 8% and 5% of patients presenting with sinus rhythm are identified as having AF through inpatient cardiac telemetry or 24-h monitoring, respectively (Brachmann et al., 2016). Clinical trials using longer duration monitoring strategies have shown an even greater yield in the identification of AF in this patient population and will be discussed further.

Electrocardiographic Monitoring Modalities

Short-Term Recording

Inpatient telemetry

Classically, hospital electrocardiographic monitoring allowed for basic heart rate and rhythm monitoring. Advancements in technology now allow for evaluation of more complex rhythm disturbances, including myocardial ischemia, extent and nature of bradycardia, ectopy, ventricular arrhythmia burden/morphology, and QT interval prolongation. The challenge lies in which patients warrant inpatient monitoring. Logistical and economic constraints limit the number of patients that can be monitored at any given time. In the case of syncope, inpatient ECG monitoring is recommended for patients deemed to be at high risk for an arrhythmic cause, such as those with known structural heart disease (Subbiah et al., 2008; Simpson et al., 1999). The European Society of Cardiology (ESC) and Canadian Cardiovascular Society (CCS) have outlined characteristics, which necessitate urgent hospitalization (**Table 1**). Despite these recommendations, the overall yield for 72-h inpatient monitoring is only 16%; nonetheless, in select patients, this modality of monitoring is appropriate and justified for risk stratification.

Holter monitoring

Holter monitoring is the most commonly utilized diagnostic tool for ambulatory monitoring and consists of a portable recording device attached to cutaneous electrodes on the chest wall (Roberts and Silver, 1983; Galli et al., 2016). Holter monitors can provide 2, 3, or 12 electrodes and typically provide recordings for 24–48 h; however, newer monitors now have the capability of offering up to 2 weeks of monitoring (Galli et al., 2016). The data is stored in the device using digital media, analyzed with software by a technologist, and subsequently edited and reported by the physician.

Table 1 ESC and CCC guidelines for inpatient telemetry

Canadian cardiovascular society	*European society of cardiology*
● Major risk factors (should have urgent cardiac assessment) ○ Abnormal ECG (any bradyarrhythmia, tachyarrhythmia, or conduction disease; new ischemia or old infarct) ○ History of cardiac disease (ischemic, arrhythmic, obstructive, valvular) ○ Hypotension (systolic BP $<$90 mm Hg) ○ Heart failure (either past or present) ● Minor risk factors (could consider urgent cardiac assessment) ○ Age $>$60 ○ Dyspnea ○ Anemia (hematocrit $<$0.30) ○ Hypertension ○ Cerebrovascular disease ○ Family history of SCD ○ Syncope while supine, during exercise or with no prodrome	● Severe structural or coronary artery disease (CHF, low LVEF, or previous MI) ● Clinical or ECG features of arrhythmic syncope: ○ Exertional or supine syncope ○ Palpitations at time of syncope ○ Family history SCD ○ Nonsustained VT ○ Bifascicular block or other intraventricular conduction abnormalities (QRS $\geq$120 ms) ○ Inadequate sinus bradycardia ($<$50 bpm) or sinoatrial block without negative chronotropic medication or physical training ○ Preexcited QRS complex ○ Prolonged or shortened QT interval ○ Brugada pattern ○ Negative T waves right precordial leads, epsilon waves, and ventricular late potentials (suggestive of ARVC)

Abbreviations: *BP*, blood pressure; *SCD*, sudden cardiac death; *CHF*, congestive heart failure; *LVEF*, left ventricular ejection fraction; *MI*, myocardial infarction; *VT*, ventricular tachycardia; *ARVC*, arrhythmogenic right ventricular cardiomyopathy.

Table adapted from Sheldon, R. S., Morillo, C. A., Krahn, A. D., O'Neill, B., Thiruganasambandamoorthy, V., Parkash, R., et al. (2011). Society position statement standardized approaches to the investigation of syncope: Canadian cardiovascular society position paper. *Canadian Journal of Cardiology* **27**(2), 246–253 and Moya, A., Sutton, R., Ammirati, F., Blanc, J. J., Brignole, M., Dahm, J. B., et al. (2009). Guidelines for the diagnosis and management of syncope (version 2009). *European Heart Journal* **30**(21), 2631–2671.



Fig. 2 Evidence for arrhythmic etiology for syncope on Holter monitor. Rhythm strip demonstrating a greater than three second pause in a patient with documented syncope while wearing a 24-h Holter monitor.

The advantages of Holter monitoring lie in its widespread accessibility, low cost, and easy application. Most importantly, it offers continuous electrocardiographic monitoring and requires minimal patient participation for data acquisition. Patients are asked to maintain a diary of symptoms and activity during the monitoring period to help establish a symptom–rhythm correlation. This form of monitoring is most beneficial in clinical scenarios where there is a suspected arrhythmic cause of the syncope and the symptoms occur frequently (Fig. 2). In reality, the majority of patients with syncope experience weeks, months, or years between episodes, which limits the diagnostic yield of Holter monitoring. This has been demonstrated in several large studies, in which only 4% of patients had symptoms during the monitoring period. In studies where patients were not carefully selected, the yield of detecting an arrhythmic cause of syncope was as low as 1%. In patients with palpitations, Holter monitors are frequently used despite evidence that the diagnostic yield is only 35% unless symptoms occur with daily frequency. In one study comparing Holter monitors to event recorders (see below) in patients with uninvestigated palpitations, a symptom–rhythm correlation was obtained in 67% of patients with event monitors compared with 35% of patients with Holter monitoring (Kinlay et al., 1996). It was also demonstrated that event monitors proved to be a more cost-effective approach in these patients.

While they are frequently utilized, Holter monitors have a number of notable limitations. The physical size of these monitors creates a significant burden for patients. This ultimately results in limiting their ability to maneuver throughout their daily activities, which may precipitate their symptoms. The brief duration of monitoring, as discussed above, results in low diagnostic yield in patients experiencing infrequent symptoms. The lack of real-time data transmission requires close interaction between the patient and ordering physician. Noncompliance in maintaining a log of symptoms and using event markers can further limit the diagnostic value.

Prolonged Recording

External cardiac event recorders

Event recorders are newer-generation devices, which allow for prolonged monitoring. These devices can be divided into two categories: loop recorders and event recorders. The loop recorders can further be divided into external loop recorders (ELRs) and implantable loop recorders (ILRs).

Event recorders, sometimes referred to as nonlooping recorders, are a form of noncontinuous ambulatory ECG monitors that can be used for 14–30 days (Fogel et al., 1997; Sciaraffia et al., 2014). The event recorder is a portable handheld or wrist-based device that is activated when symptoms occur (**Fig. 3**). Patients activate the device, which will then record an ECG rhythm strip. The duration of ECG recording depends on the device model, but averages 1–2 min. This event then must be directly transmitted to the receiving center, which will convert the information into a single lead rhythm strip (Wu et al., 2003). Nonlooping event recorders are suitable devices for investigating symptoms that are sustained long enough to record the rhythm with the application of a recorder (Saarel et al., 2004).

The major advantage to these devices is that they are small and easily worn by patients and avoid the skin irritation associated with the fixed recording electrodes that are required for loop recorders. However, these devices present with a few disadvantages, including the required technical skills to transmit the data to the central monitoring station and the coherency of the patient to activate the device during times of symptoms. Additionally, the mode of recording does not allow rhythm to be captured during the onset of symptoms and therefore, brief arrhythmias may be missed before the device is activated (Bhandari et al., 1992). These limitations often result in low diagnostic yield, especially during episodes that result in disabling symptoms that prevent activation of the device (Kus et al., 1995). Patients are now using smartphone-based applications to record either heart rate or an actual ECG during symptoms, leading to patients bringing their own recordings to medical attention. This enabling technology clearly has merit for improving access to monitoring, but requires context to prevent medical information overload.

Loop-recorders

ELRs are attached to the patient through 1–3 chest electrodes or a single-patch electrode, which are only removed for bathing and showering. The transmitter of most ELRs is the size of a pager or small mobile phone and can be worn on a belt or within nine meters of the patient to enable transmission (**Fig. 4**) (Sciaraffia et al., 2014). Rhythm recording is either initiated by patient activation or automatically triggered by inputted programmable criteria. The recorder will register the ECG data for a few seconds to minutes before and after the recorder has been activated (according to preprogrammed durations). The memory "loop" or buffer allows the patient to answer both "what just happened?" as well as "what is happening?" Given that loop recorders can be activated when symptoms are present, it allows for accurate symptom–rhythm correlation and ultimately can help exclude or diagnose a causative role for rhythm disturbances as the etiology of patients' syncope or palpitations (**Fig. 5**) (Farwell et al., 2004; Lombardi



Fig. 3 Cardiac event recorder device. Four electrodes are present on the back of the device to permit single lead rhythm capture. A record button (*top left*) is pressed at the onset of symptoms, and the recorded event is transmitted to a base station over an analog phone line.



Fig. 4 ELR device. The record button is pressed in the event of symptoms to store a predetermined time (up to 9 min) of rhythm data prior to the onset of symptoms along with the ensuing minute. The phone receiver is also placed over this button to transmit data over an analog phone line.

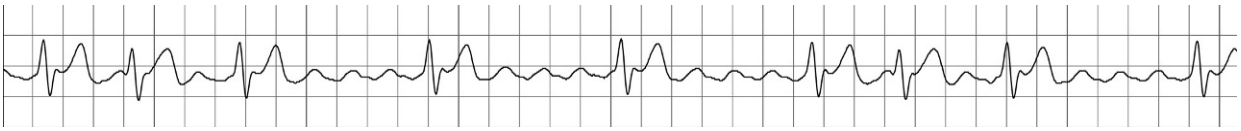

Fig. 5 ELR tracing during symptoms of palpitations. Sample tracing of atrial flutter in a patient experiencing palpitations.

et al., 2005). These devices can theoretically be used indefinitely as long as weekly battery changes are performed. However, because of practical limitations, they are typically worn for several weeks to a month.

ILRs, or more recently known as ICMs, have become the primary investigative tool in patients with recurrent unexplained syncope who have had negative initial investigations (Farwell et al., 2004, 2006). Several manufacturers make devices, with a widening scope of capabilities and form factors. They are increasingly smaller devices (Medtronic's Reveal LINQ, one-third the size of an AAA battery) that are designed for subcutaneous implantation in the chest wall using a minimally invasive technique (**Fig. 6**) (Krahn et al., 1999). The evolution and development of smaller form factors in ICMs has allowed for the creation of these less invasive devices (Krahn et al., 2014). The projected battery life of most ICMs is in the order of 36 months.

Prior to implantation, external ECG mapping is typically used to help determine the site of insertion that will provide optimal R-wave amplitude (Krahn et al., 1997). These leadless devices record a single-lead ECG signal through two electrodes within the device. The typical position of the ICM usually lies in the left parasternal region, with an adequate signal usually obtained with implantation anywhere in the left hemithorax (Krahn et al., 1998). The device is anchored to the muscular plane to avoid mechanical instability, displacement, or migration, all of which may create artifact and reduce R-wave amplitude. In patients concerned with the aesthetic impact of an anterior chest surgical scar, there is the option for anterior axillary implantation, which recently has been shown to be as effective as the standard approach in achieving adequate R- and P-wave amplitudes (Miracapillo et al., 2010, 2016). Thus far, implantation of ICMs has been shown to be safe and associated with very low infection rates, with emerging data to support feasibility of in-office implantation with semisterile precautions (Rogers et al., 2017).

Similar to other external event recorders, ICMs have been developed to correlate symptoms with recorded cardiac rhythms. They are especially useful in noncompliant patients as there are no external parts or batteries to manage. At the time of implantation, the patient and their family members are instructed on how to activate the device (Sciaraffia et al., 2014). Once symptoms occur, a handheld activator is used to record the episode. The data can be transmitted over a standard phone line or via transtelephonic monitoring. Remote monitoring can allow for prompt intervention and therapy if a relevant arrhythmia is detected. Most devices have the capability to store 40–60 min of a single lead ECG. Therefore, if arrhythmias are very frequent, they may be missed, because either the memory is full or the device is programmed to stop storing new data, or old episodes are overwritten by the new data (Hoefman et al., 2007). Therefore, timely data transmission is essential, especially for patients living in remote areas where access to specialized clinics is both difficult and costly. Remote monitoring has the ability to virtually eliminate concern regarding memory capacity, since any recorded events are transmitted daily. This has however led to information overload at the receiving end, where many monitored patients generate immense workloads for device clinics.



Fig. 6 ICM. Examples of two different ICMs from Medtronic, (A) Reveal LINQ and (B) Reveal XT. The two devices are placed next to an AAA battery to provide size perspective.

ICMs have been shown to be effective in patients with infrequent palpitations. In patients without severe heart disease and infrequent ($\leq$1 episode/month), sustained ($>$1 min) palpitations, conventional testing with a 24-h Holter monitor led to diagnosis (with symptom–rhythm correlation) in only 21% of patients versus 73% in patients tested with an ICM (Galli et al., 2016).

To date large-scale studies of ICMs have focused on their utility in patients with recurrent syncope with an uncertain diagnosis. The ESC has given a Class I recommendation for the use of ICMs in the early phase of evaluation of patients with recurrent syncope (Task Force for the Diagnosis and Management of Syncope et al., 2009). Three studies from the International Study on Syncope of Uncertain Etiology (ISSUE) investigators examined different groups of patients with unexplained syncope after they had undergone initial conventional testing (Moya et al., 2001; Brignole et al., 2001; Menozzi et al., 2002). The first of the studies examined 111 patients with unexplained syncope who had undergone tilt table testing. These patients received an ICM irrespective of their tilt table result. In 34% of these patients, syncope recurred irrespective of their tilt table response, the most common rhythm during the episodes were marked bradycardia or asystole (Moya et al., 2001). The second study examined patients with underlying bundle branch block and negative electrophysiological testing. Syncope recurred in 22 of the 52 patients in the study wherein the most common rhythm finding was again marked bradycardia, mainly attributed to complete AV block (Brignole et al., 2001). The third study included 35 patients with syncope, overt heart disease, and negative electrophysiological testing (Menozzi et al., 2002). Of these patients, 54% (19/35) had recurrent symptoms, with bradycardia in four, supraventricular tachyarrhythmias in five, and ventricular tachycardia in one. This third study by the ISSUE investigators was the first to demonstrate an ongoing risk for cardiac arrhythmias despite negative electrophysiological testing in this patient population, and further strengthened the evidence for the use of ICMs in this patient cohort.

The follow-up ISSUE-2 was a nonrandomized, multicenter observational study that investigated the efficacy of therapies based on ICM diagnosis in patients with recurrent suspected neurocardiogenic syncope (Brignole et al., 2006). This demonstrated that diagnostic information obtained from early ICM implantation allowed for safe and effective therapy in patients with neurocardiogenic syncope. Based on these promising results, the ISSUE-3 study was initiated, which examined 511 patients over the age of 40 with recurrent vasovagal syncope, who were all implanted with an ICM (Brignole et al., 2012). In the 17% with asystolic recurrence, dual chamber pacemaker therapy was superior to those whose pacemaker was turned off in the overall prevention of recurrent syncope. This study further strengthened the evidence that prolonged monitoring with an ICM is useful in patients with recurrent vasovagal syncope who would be considered for a permanent pacemaker if marked bradycardia was detected (the minority).

The PICTURE study (Place of reveal In the Care pathway and Treatment of patients with Unexplained Recurrent Syncope) was a large prospective, multicenter study that evaluated the usage and diagnostic effectiveness of ICMs for the everyday evaluation of patients with unexplained syncope (Edvardsson et al., 2011). Overall, the results demonstrated that implantation with an ICM resulted in high diagnostic yield (78%) and should be considered early on in the workup of patients with unexplained syncope to reduce the overall number of tests required to lead to a diagnosis. Specific rhythms diagnosed were not reported by the investigators, but historically bradycardia outweighs tachycardia in most case series.

To date, there have been only a few randomized trials that directly compared ICMs with conventional testing strategy for syncope. The first trial was the Randomized Assessment of Syncope Trial, which clearly demonstrated the superiority of prolonged monitoring strategy with ICM over conventional testing in the diagnosis of syncope (55% vs. 19%) (Krahn et al., 2001, 2003). This was followed by the Eastbourne Syncope Assessment Study (EaSyAS), which examined 201 patients with recurrent unexplained syncope (Farwell et al., 2004). The population was randomized to receive the ICM (Reveal plus, Medtronic) compared to conventional testing. The results demonstrated that after 17 months of follow-up, 43% of patients with an ICM versus 6% with conventional testing received an electrocardiographic diagnosis for their syncope (Farwell et al., 2006). As a result, they had more rapid access to tailored therapy, which led to a longer time to a second syncopal episode. ICM was also shown to be a more cost-effective strategy compared to conventional testing. The FRESH study from France examined 78 patients with recurrent or severe syncope who were randomized to ICM or conventional testing (Podoleanu et al., 2014). This population consisted of low-risk patients with syncope, which did not include any subject with cardiac disease. After 14 months of follow-up, the results clearly favored ICMs over conventional testing in establishing a diagnosis of syncope. Finally, the most recent trial EaSyAS II followed 246 patients for 20 months after randomization into different arms: conventional strategy versus syncope clinic, versus ICM and syncope clinic versus an ICM alone. Finally, the most recent EaSyAS II trial followed 246 patients for 20 months after they were randomized to one of four arms: 1) conventional management, 2) syncope clinic alone, 3) ICM alone or 4) ICM and syncope clinic. Patients randomized to the ICM arm had an overall significantly shorter time to diagnosis, compared to conventional management (Sulke et al., 2016).

There is growing evidence for the utility of ICMs for the detection of AF, and not surprisingly, several studies have demonstrated that longer monitoring strategies improve detection rates (Giada et al., 2012). It has been estimated that serial 7-day Holter monitors or event recorders may miss up to 30% of AF episodes (Kirchhof et al., 2007). Guidelines have not yet adopted ICMs as the gold standard for AF detection in clinical trials evaluating the efficacy of interventions such as ablation or cardioversion. Prolonged rhythm monitoring with ICMs in assessing AF burden is currently being examined in large clinical trials to assess the role of oral anticoagulation in subclinical AF.

The hypothesis that loop recorders improve the detection of AF in patients with ESUS has been examined by a number of large-scale studies (Brachmann et al., 2016; Saver, 2016; Yong et al., 2016). The Cryptogenic Stroke and Underlying AF (CRYSTAL AF) trial examined 441 patients over the age of 40 with a diagnosis of cryptogenic stroke or transient ischemic attack (TIA) of undetermined cause and randomized them to receive an ILR or conventional ECG testing (Sanna et al., 2014; Thijs et al., 2016). The study reported the detection of AF to be 8.9% at 6 months, 12.4% at 1 year, and 30% at 3 years with ICM monitoring, compared to 1.4%, 2.0%, and 3.0% at 6 months, 1 year, and 3 years, respectively, for the control group (Brachmann et al., 2016; Thijs et al., 2016). The 30-day Cardiac Event Monitor Belt for Recording AF after a Cerebral Ischemic Event (EMBRACE) trial examined a total of 572 patients, who were randomized to receive an ELR or 24-h Holter monitor (Gladstone et al., 2014). This patient population included those over the age of 55 without known AF and a diagnosis of ESUS or TIA of undetermined cause. The results showed that testing with an ELR was superior to Holter monitoring in detecting AF in this population. AF that lasted 30 sec or longer was detected in 16.1% of patients with an ELR, versus 3.2% in the patients with Holter monitoring after 30 days. A meta-analysis examining 50 studies and over 10,000 patients with recent stroke found that 23.7% of the patients were diagnosed with AF after their hospital admission (Sposato et al., 2015; Joundi et al., 2016). Currently, there is no clear consensus on the type of extended monitoring that should be utilized in patients with a diagnosis of cryptogenic stroke to detect low-burden or silent AF. Overall, these studies provide convincing evidence that a prolonged monitoring strategy with either an ELR or ILR is a reasonable approach in the work-up of patients with cryptogenic stroke and negative findings on conventional testing.

The aforementioned experiment clearly demonstrates the role and superiority of extended monitoring in the detection of subclinical AF (SCAF) in patients with ESUS. The Asymptomatic Atrial Fibrillation and Stroke Evaluation in Pacemaker Patients and the Atrial Fibrillation Reduction Atrial Pacing Trial (ASSERT) demonstrated that SCAF was associated with a 2.5-fold increased risk of stroke or systemic embolism (Healey et al., 2012). Despite the evidence of SCAF in patients with thromboembolic strokes, the causal and temporal relationships between SCAF and embolism are not entirely clear, since timing of AF did not correspond to subsequent stroke or TIA, and most stroke patients did not have recent AF. SCAF may be associated with embolic stroke without direct causality.

The TRENDS study evaluated the relationship between long-term atrial tachycardia (AT)/AF burden and thromboembolism in patients that had stroke risk factors and were scheduled to receive a pacemaker or ICD for a class I/II indication (Glotzer et al., 2009; Ziegler et al., 2010). A subgroup analysis of this study was conducted, which examined 40 patients who suffered a stroke, systemic embolism, or a TIA. It was demonstrated that of the 20 subjects with AF prior to the embolic event, only 30% had AF within the 1 month prior to the embolic event and the timing for the most recent AF episode was an average of 166 ± 189 days prior to the embolic event (Daoud et al., 2011). The ASSERT study demonstrated that only 15% of patients with a SCAF-associated embolic event had evidence of SCAF, which was >6 min in duration 1 month prior to the event (Healey et al., 2012). More importantly, most of the SCAF occurring before these embolic events were less than 48 h, which has previously been felt to be the minimum duration necessary for thrombus formation in the left atrial appendage (Healey et al., 2012; Weigner et al., 1997). Patients in the ASSERT trial had an absolute stroke risk of 1.7% per year, which is lower than previously published reports. The initial results of the ASSERT trial were followed up by a posthoc analysis, which focused on the duration of device-detected subclinical AF and the occurrence of stroke (Van Gelder et al., 2017). In this substudy, patients with SCAF ≤6 min were excluded from the study. The 2455 patients that were included in the post-hoc analysis were stratified based on the longest single episode of SCAF. They defined four categories of SCAF: (1) no SCAF, (2) SCAF with a duration of >6 min–6 h, (3) SCAF between >6 h–24 h, and (4) SCAF >24 h. The

post-hoc analysis revealed that SCAF lasting >6–24 h occurred in 7% of patients and episodes lasting >24 h occurred in 11% of patients. Importantly, they reported a significantly higher absolute risk (3.1%) of ischemic stroke or systemic embolism compared to patients with no SCAF. This absolute risk is similar to patients with clinical AF and higher than that reported in the primary ASSERT study. In patients with SCAF <24 h, their risk of stroke or systemic embolism was not significantly different from those that did not have SCAF. Overall, the more robust analysis of the data from the ASSERT study highlights the importance of SCAF duration on overall stroke risk, a topic that remains controversial. Collectively, these results highlight the importance for careful interpretation of SCAF that is identified during prolonged ECG monitoring. Currently, there is no consensus regarding the role of anticoagulation in patients with SCAF. There is a need for randomized trials to help better understand the temporal relationship of SCAF and embolic events, several of which are underway.

Mobile cardiac outpatient telemetry

Real-time continuous attended cardiac monitoring has emerged over the past decade with the aim of overcoming some of the limitations of Holter monitors and ELRs. These systems are commonly referred to as mobile cardiac outpatient telemetry (MCOT); these systems were developed to overcome some of the limitations associated with Holter and ELR monitoring systems (Joshi et al., 2005). MCOT systems are similar in size to the ELR and consist of three chest electrode leads attached to a small sensor, which is similar to a pager clipped onto a belt. For patients not able to tolerate adhesive electrodes, there is the option for a chest belt with three built-in nonadhesive electrodes. A key distinguishing characteristic of the MCOT is the associated monitoring device, which is in constant communication with the sensor and records the cardiac rhythm independent of any patient participation. While in the home, the monitor transmits events and pertinent rhythm strips transtelephonically to a service center that is staffed with monitoring specialists (Olson et al., 2007). While outside of their home, patients carry their monitor and the events are transmitted through cellular signals. In the event no signal is available, most MCOT systems are equipped to store 24 h of ECG data, which can then be transmitted to the monitoring center once a signal has been restored. Patients are also able to record symptoms and activity directly into the monitoring device. Monitoring specialists provide detailed reports to the ordering physicians on a daily basis, which include heart rate trends, representative ECG events of predetermined cardiac rhythms, and urgent reports based on the ordering physicians' criteria.

Thus far, there have been very few RCTs comparing the MCOT system to other forms of external monitoring devices. In one RCT, 266 patients with symptoms of syncope, presyncope, or severe palpitations with a negative 24-h Holter monitor were randomized to an MCOT device or ELR for up to a period of 30 days (Rothman et al., 2007). A diagnosis was made in 88% of patients with an MCOT compared to 75% in those with an ELR. In a subgroup analysis examining those patients with presyncope or syncope, a diagnosis was made in 89% versus 69% for MCOT and ELR devices, respectively. Overall, MCOT devices were also found to be superior in identifying clinically significant arrhythmias (41% vs. 15% in MCOT vs. ELR devices, respectively). In one study investigating the diagnostic yield of MCOT systems in patients with a diagnosis of cryptogenic stroke, a monitoring period of 30 days was not sufficient to identify occult AF in a significant proportion of these patients who were later found to have AF.

Patch ECG monitors

Holter monitors have a number of limitations, including their bulk, which creates a significant inconvenience for patients. This often leads to patient noncompliance or results in limited maneuverability, which lowers diagnostic yield (Diemberger et al., 2015). While there are now 2-week Holter monitors available, the desire to create long-term monitoring devices with ease of use for patients has led to the development of patch ECG Monitors (PEMs) (**Fig. 7**) (Lobodzinski and Laks, 2012). PEMs are wearable ECG



Fig. 7 Patch ECG monitor device. Examples of two different patch ECG monitors, (A) Zio XT cardiac monitor from iRhythm technologies, (B) CardioSTAT cardiac monitor. These devices are water-resistant, wire-free, single-use devices that allow for prolonged (up to 7 days) ECG monitoring. Each patch has a centrally placed button that can be activated by the patient to mark their symptoms.

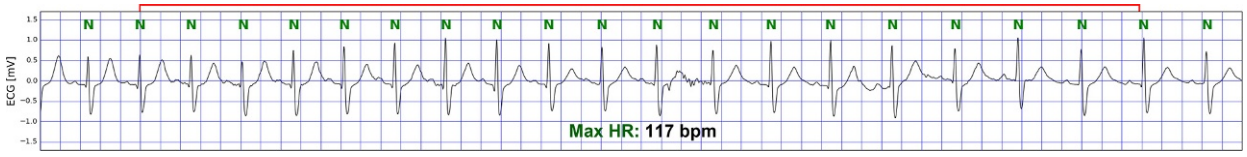

Fig. 8 Example tracing from PEM. Sample tracing of sinus rhythm from a patient wearing a CardioSTAT cardiac monitor.

monitors capable of continuously recording ECG data for up to 14 days (Barrett et al., 2014; Cheung et al., 2014). PEMs can record data without visible electrodes or lead wires and fall into two main categories, those that function as recording devices only and those that can record and transmit. The Zio Patch (iRhythm technologies) is a single-channel, single-use, waterproof PEM device that is capable of continuous recording for up to 14 days (Fig. 8). The patch is placed in the left pectoral region and does not require any patient activation. There is a single button on the device that allows the patient to mark a symptomatic episode. At the end of the recording period, the patient simply mails the device in a prepaid envelope to the monitoring center, and within 3 days, a finalized report is provided to the ordering physician. In a direct comparison to 24-h Holter monitors, 146 patients that were referred for cardiac arrhythmia evaluation underwent simultaneous ambulatory ECG monitoring with a Holter monitor worn for 24 h and the Zio Patch worn for a goal of 14 days (Barrett et al., 2014; Cheung et al., 2014). The mean wear-time in days for the Holter monitor and PEM was 1.0 (range, 0.9–1.0) and 11.1 (range, 0.9–14.0), respectively. The primary outcome was the detection of arrhythmias during the wear-time of each device. In this study, the PEM detected 96 arrhythmias compared to 61 events with the Holter monitor. Of these recorded events, both the Holter monitor and PEM detected 60 of the same events. The results of this study demonstrated a clear increased diagnostic yield with the PEM and strong patient preference for the PEM over the Holter monitor, with 81% preferring the PEM. Clinician satisfaction was also higher, with 90% feeling a definitive diagnosis was made with the PEM versus 64% with the Holter monitor. Multiple manufacturers are emerging in this space. It is clear that PEMs will change the face of ambulatory ECG monitoring.

Future Directions

With recent advances in ICM design and the presence of many different manufacturers, it is clear that long-term electrocardiographic monitoring will continue to be area of growth and development in the field of cardiology. The focus will be directed toward the design of products that are minimally invasive, utilize modern technology, and integrate seamlessly into the daily lives of patients. Increasingly, there is a great deal interest and research dedicated to smartphone ECG monitoring.

Smartphone monitoring

It is estimated that by 2020, there will be 6.1 billion smartphone users, approximately 70% of the world's population. With this expansion in smartphone usage, there have been a large number of medical applications and ancillary products being designed to utilize this technology. These applications provide a unique opportunity to implement monitoring strategies that integrate seamlessly into the daily lives of patients (Konecny and Saxon, 2016). Thus far, there is little data on the effectiveness of mobile health technology in detecting arrhythmias. The Smartphone Pediatric ElectrocARdiogram (SPEAR) trial examined pediatric patients with documented paroxysmal arrhythmia using the AliveCor® heart monitor (AHM) and found the device was able to generate tracings of diagnostic and therapeutic qualities in children with diagnoses of AF and SVT (Nguyen et al., 2015). Patient satisfaction was very high, with 98% indicating ease in obtaining tracings and 93% reporting ease in transmitting the recorded tracings. This study was followed by the iTransmit study, which enrolled 60 patients and directly compared the AHM to TTM. Patients were then asked to record their rhythms using both monitors simultaneously whenever they had symptoms, at least once a week (Tarakji et al., 2015). The AHM had 100% sensitivity and 97% specificity for the detection of AF and atrial flutter, and the vast majority of patients (92%) preferred using the AHM for detection of their AF as opposed to the TTM.

Smartphone-based applications that have been designed to analyze the pulsatile signals by illuminating the fingertip using the smartphone camera have been shown to be successful in distinguishing AF from sinus rhythm (McManus et al., 2013). The first study using this technology conducted by McManus and colleagues utilized an iPhone 4S camera to analyze 76 patients with AF scheduled to undergo elective cardioversion (McManus et al., 2016). Pre- and post-ECGs were recorded along with pulse signal recordings using the iPhone 4S camera. The novel algorithm used in this study, which was previously validated, demonstrated 100% and 96.05% accuracy in identifying AF and normal sinus rhythm, respectively. Subsequent follow-up study using the same PULSE-SMART technology demonstrated the successful discrimination in pulse recordings during AF, sinus rhythm, premature atrial contractions, and premature ventricular contractions (McManus et al., 2016). While the technology and software behind the pulse-sensing algorithm provides the promising possibility for ECG monitoring in every person with a smartphone, a number of important issues still need to be considered, including the management of previously healthy patients who are identified with AF using this technology. Should they be further investigated? Is there a need for anticoagulation in those with patients identified as having significant risk of stroke based on the $CHADS_2$ scoring system? More data will be needed to properly address these questions.

Table 2 Comparison of ambulatory electrocardiographic monitoring devices

Device	*Advantages*	*Limitations*	*Indications*
Holter monitor	• Low cost • Continuous monitoring • Record and then analyze data (not real time)	• Short duration of monitoring and low diagnostic yield • Size of device limits daily activities, which may precipitate symptoms	• Patient with very frequent symptoms ($\geq$1 week)
Event recorder	• Low cost • Easily portable • Record and then analyze data (not real time)	• Poor patient compliance with device application • Technical experience required to transmit data • Onset of arrhythmia is not captured • Patient coherence needed during arrhythmia to activate device • Short-lasting arrhythmias may not be recorded	• Compliant patients with intersymptom interval $\leq$4 weeks
ELR	• Prospective and retrospective recording capabilities • Ability to record asymptomatic arrhythmias based on inputted programmable criteria • Record and then analyze data (not real time)	• Poor patient compliance to wearing device • Must be removed for bathing, swimming • May lose electrode contact during sweating/physical activity • Poor electrocardiographic recordings • Continuous device maintenance with weekly battery replacement	• Compliant patients with intersymptom interval $\leq$4 weeks • Syncope and presyncope where preceding/onset of symptoms or arrhythmia is important
ICM (or ILR)	• Prolonged monitoring without any external electrodes or other parts to manage • Highest diagnostic yield of all monitoring devices • Record and then analyze data (not real time) • Use of remote monitoring to access stored data, extends memory capacity dramatically, and allows next day notification of events	• Invasive implantation with risk of local complications • High cost	• Early-phase evaluation of patients with recurrent syncope and absence of high-risk features • Growing role in atrial fibrillation monitoring for burden, symptom–rhythm correlation, potential role in influencing antithrombotic therapy decisions (being assessed in clinical trials)
Mobile outpatient cardiac telemetry	• Continuous monitoring • Real-time data transmission • Patient activation to report symptoms	• High cost • Not widely available • Poor patient compliance to wearing device • Continuous device maintenance	• Compliant patients with intersymptom interval $\leq$4 weeks

To date, there has not been a randomized controlled trial comparing smartphone ECG monitoring to conventional monitoring strategies. The iHEART study is a single-center, prospective randomized controlled trial currently underway (NCT02731326). The trial will randomize 300 participants 1:1 to receive iHEART intervention, which included a smartphone equipped with an AliveCor® Mobile ECG and accompanying Kardia application or standard cardiac care for 6 months. The investigative arm will receive behavior-altering motivational text messages throughout the study period. The standard arm will receive guideline-based care at the discretion of their clinical provider based on ACC/AHA/HRS guidelines for management of patients with AF. This study has the potential to provide robust data on rhythm recording quality, diagnostic yield, and ultimately on the potential of smartphone ECG monitoring in the ambulatory setting.

Conclusion

Electrocardiographic monitoring has evolved dramatically since the development of the Holter monitor. Devices with progressively longer recording capabilities have significantly enhanced the ability of clinicians to obtain silent or symptomatic physiological data. With the development of ICMs, we have an enhanced ability to diagnose intermittent arrhythmias in a number of different clinical settings. **Table 2** summarizes the comparative advantages, limitations and indications for the different modes of ambulatory electrocardiographic monitoring. The continued development of novel, less invasive, and user-friendlier devices will undoubtedly advance the field of electrocardiographic monitoring even further. Ongoing clinical trials will be needed to determine the suitability of these devices in helping clinicians investigate and diagnosis a variety of clinical conditions.

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Electrophysiology Approaches for Ventricular Tachycardia

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Introduction

Ventricular tachycardia (VT) and fibrillation (VF) are the most common cause of cardiac arrest and sudden death. Hence, the presence of ventricular arrhythmia warrants careful assessment to determine risk for arrhythmic death and its prevention. In the absence of structural or genetic abnormalities, these arrhythmias are termed idiopathic. While idiopathic VF is a rare life-threatening condition, idiopathic monomorphic VT usually originates from a single focus or may be due to interfascicular reentry. Idiopathic VT is usually considered benign and infrequently associated with sudden death. Occasionally, ventricular arrhythmias such as frequent Premature ventricular contractions (PVCs), repetitive VT, or slow incessant VT can initiate systolic LV dysfunction or worsen preexisting heart failure.

Types of VT

Nonsustained VT refers to episodes that last less than 30 s. Episodes of VT that persist for longer than 30 s cause syncope or cardiac arrest or require an intervention such as cardioversion or overdrive pacing for termination is commonly designated *sustained VT*. VT at a rate less than a 100 bpm is usually due to an *accelerated idioventricular rhythm* but occasionally it may be due to a slow reentrant tachycardia involving a scar.

Monomorphic VT has the same QRS configuration from beat to beat, indicating a stable VT origin from a focus or structural substrate. The ECG morphology suggests the ventricular origin (Josephson and Callan, 2005). A left bundle branch block-like configuration, defined by a dominant S wave in lead V1, suggests that the arrhythmia originates in the right ventricle or interventricular septum. A right bundle branch block-like configuration defined by a dominant R wave in V1 typically originates from the left ventricle (**Fig. 1**). VT that originates from the base of the heart usually has an inferiorly directed frontal plane axis with positive QRS in the inferior limbs leads and prominent R waves in the apical precordial leads (V5 and V6). Inferior wall origin is suggested by superiorly directed frontal plane axis with predominantly negative QRS complexes in the inferior limb leads (**Fig. 1**).

Polymorphic VT has a continually changing QRS axis indicating a varying ventricular activation sequence. A fixed anatomic substrate is not required. Acute myocardial ischemia, genetic arrhythmia syndromes, or electrolyte disturbances are the causes. Polymorphic VT associated with QT prolongation often has a characteristic waxing and waning QRS amplitude referred to as "torsade de pointes (TdP) (Roden, 2008)."

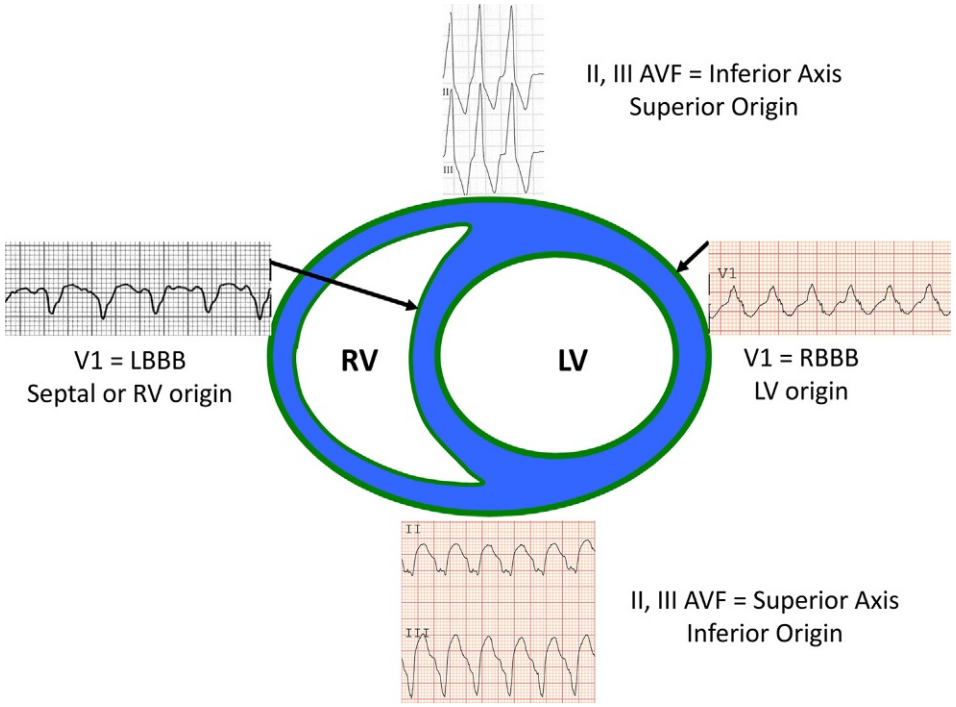

Fig. 1 Morphology and frontal plane axis of a monomorphic suggests site of origin (see text for details). Reproduced from John, R.M. and Stevenson, W.G. (2014). Ventricular arrhythmias. In: Kasper, D.L., Fauci, A.S., Hauser, S.L., Longo, D.L., Jameson, J.L. and Loscalzo, J. (eds.) Harrison's principles of internal medicine (19th edn.). New York: McGraw Hill. Chapter 277.

Diagnosis

Because ventricular arrhythmias originate below the His bundle, the QRS duration is typically wider than 120 ms. However, VT that originates from the Purkinje fibers or the high septum can be relatively narrow and mimic supraventricular arrhythmias. The main differential diagnoses of wide complex tachycardia include VT, supraventricular tachycardia (SVT) with aberration, or preexcited tachycardias. SVTs with preexisting wide QRS, arrhythmias associated with drug toxicity, and pacemaker-mediated tachycardia may also present with wide complex tachycardia. Clinically, the most common cause of a wide complex tachycardia is VT, accounting for 80% of cases (Miller and Das, 2009). A history of structural heart disease increases the probability of VT with a positive predictive value of >95% (Alzand and Crijns, 2011). Patients with VT tend to be older. Age below 35 years and recurrent events over >3 years support SVT with aberration. Hemodynamic stability is not a good marker for VT diagnosis as VT can present with stable hemodynamics.

Several ECG criteria have been published to distinguish monomorphic VT from SVT with aberration. In general, SVT with aberration tend to follow typical bundle branch block of fascicular block patterns, whereas VT that originates outside of the bundle branches or Purkinje system tend to have atypical patterns. Scar-related reentrant VT exit into ventricular myocytes. Hence, initial ventricular activation is usually slower compared with terminal activation. Lead aVR yields a negative QRS during SVT as the ventricular activation wavefront is conducted away from this lead. The presence of an initial R in aVR is thus supportive of VT (Alzand and Crijns, 2011; Vereckei et al., 2008). In addition, the direction of ventricular activation from supraventricular stimulation gives rise to an R wave in at least one or several precordial leads, therefore the absence of RS complex in the precordial leads indicates VT (**Fig. 2**) (Vereckei et al., 2008). None of these criteria are perfect. Because a definitive diagnosis is mandatory for assessing risk and guiding therapy, an electrophysiologic study maybe required if the diagnosis remains in doubt. ECG artifacts can mimic VT, particularly polymorphic VT, and can lead to inappropriate treatments if not recognized.

Management of Ventricular Arrhythmias

VT frequently cause symptoms, but some are recognized on routine evaluation or present with progressive heart failure symptoms. The common presentation is with palpitations, syncope, or cardiac arrest. Initial management of an unstable arrhythmia should follow established advanced cardiac life support guidelines. History and physical examination should focus on identifying heart disease. Potential aggravating factors should be sought and addressed, including electrolyte imbalances, stimulants such as caffeine and amphetamine analogues, and other drugs. The electrocardiogram often provides the first indication of underlying heart disease or a genetic arrhythmia syndrome (see below). Coronary artery disease is the initial major consideration in adults. Ventricular

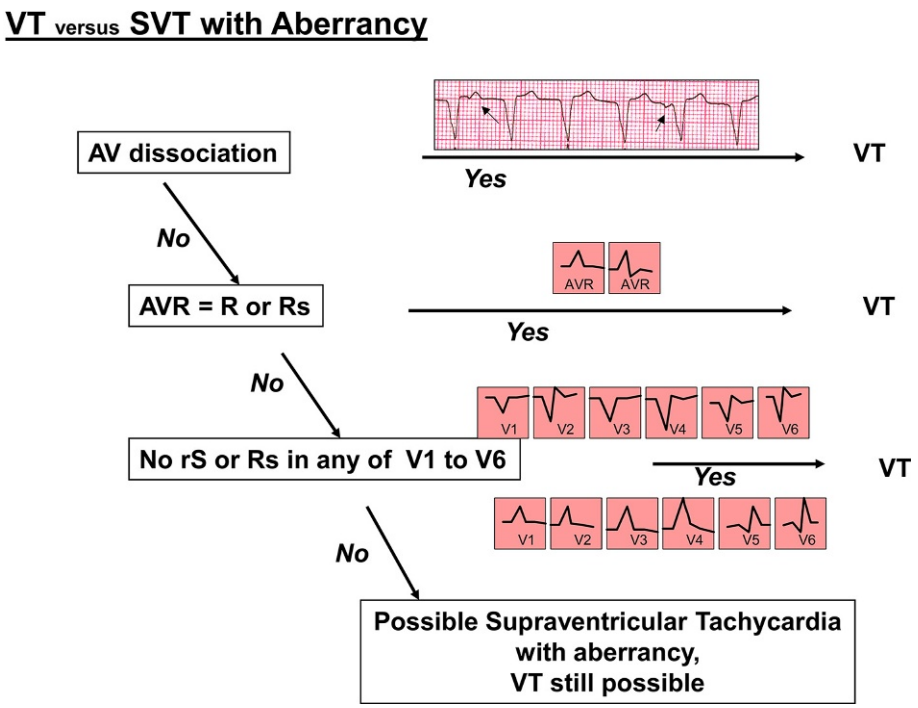

Fig. 2 Algorithm to differentiate ventricular tachycardia (VT) from supraventricular tachycardia (SVT) with aberration. Reproduced from John, R.M. and Stevenson, W.G. (2014). Ventricular arrhythmias. In: Kasper, D.L., Fauci, A.S., Hauser, S.L., Longo, D.L., Jameson, J.L. and Loscalzo, J. (eds.) Harrison's principles of internal medicine (19th edn.). New York: McGraw Hill. Chapter 277.

imaging, usually with echocardiography, is warranted to detect myopathies and assess ventricular function. Cardiac MR imaging and ^{18}F-fluoro-deoxy-glucose PET imaging with CT can be helpful in assessing the presence of scar, infiltration, and inflammation (Blankstein and Waller, 2016; MEMBERS WRITING COMMITTEE and MEMBERS ACCF TASK FORCE, 2010). Long-term management strategies for VT management are determined primarily by the nature of the underlying heart disease and the risk for sudden arrhythmic death. The specifics of the arrhythmia can often direct one to specific evaluation and management.

Premature Ventricular Contractions (PVCs) and Nonsustained VT

PVCs are common, and are observed in up to 27% of healthy individuals during exercise testing (Morshedi-Meibodi et al., 2004). Their prevalence increases with age and with the presence and severity of heart disease. Exercise-induced and recovery-phase PVCs or nonsustained VT (NSVT) are associated with increased mortality, likely because they are markers for underlying heart disease. In the absence of structural heart disease or an underlying genetic arrhythmia syndrome, prognosis is excellent. Specific therapy is rarely required; beta-blockers may be used for control of symptoms.

Very frequent PVCs, typically in excess of 20% of total heartbeats per 24 h, or incessant repetitive monomorphic VT (Figs. 3 and 4) can depress LV ejection fraction in a manner similar to tachycardia-induced cardiomyopathy from incessant SVT or rapid atrial fibrillation (Cha et al., 2012). However, accurate measurement of ventricular function can prove difficult due to frequent arrhythmia. In addition, an underlying cardiomyopathy may be present causing the PVCs. Reassessment of ventricular function after suppression of the arrhythmia with drugs or ablation is often required to clarify the initiating event.

Sustained Monomorphic VT

When structural heart disease is present, monomorphic VT is usually due to scar-related reentry and tends to be recurrent. Although myocardial ischemia can act as a trigger for such VT, revascularization therapies alone are unlikely to prevent recurrences. More than 40% of patients presenting with sustained monomorphic VT will have recurrent arrhythmia within 2 years and there is a risk of sudden death (Kuck et al., 2010). An implantable cardioverter defibrillator (ICD) should therefore be considered in such patients. ICDs terminate, but do not prevent VT episodes. Recurrent VT is common and is associated with increased mortality and hospitalizations and reduced quality of life if VT elicits ICD shocks (Aliot et al., 2009; Poole et al., 2008). Antiarrhythmic drugs or catheter ablation are used to reduce the frequency of recurrences and to control incessant VT. It is important to achieve adequate control of VT before implantation of an ICD to prevent recurrent ICD shocks. In experienced centers, ablation for VT should be considered early in such situations (see what follows) (Aliot et al., 2009).

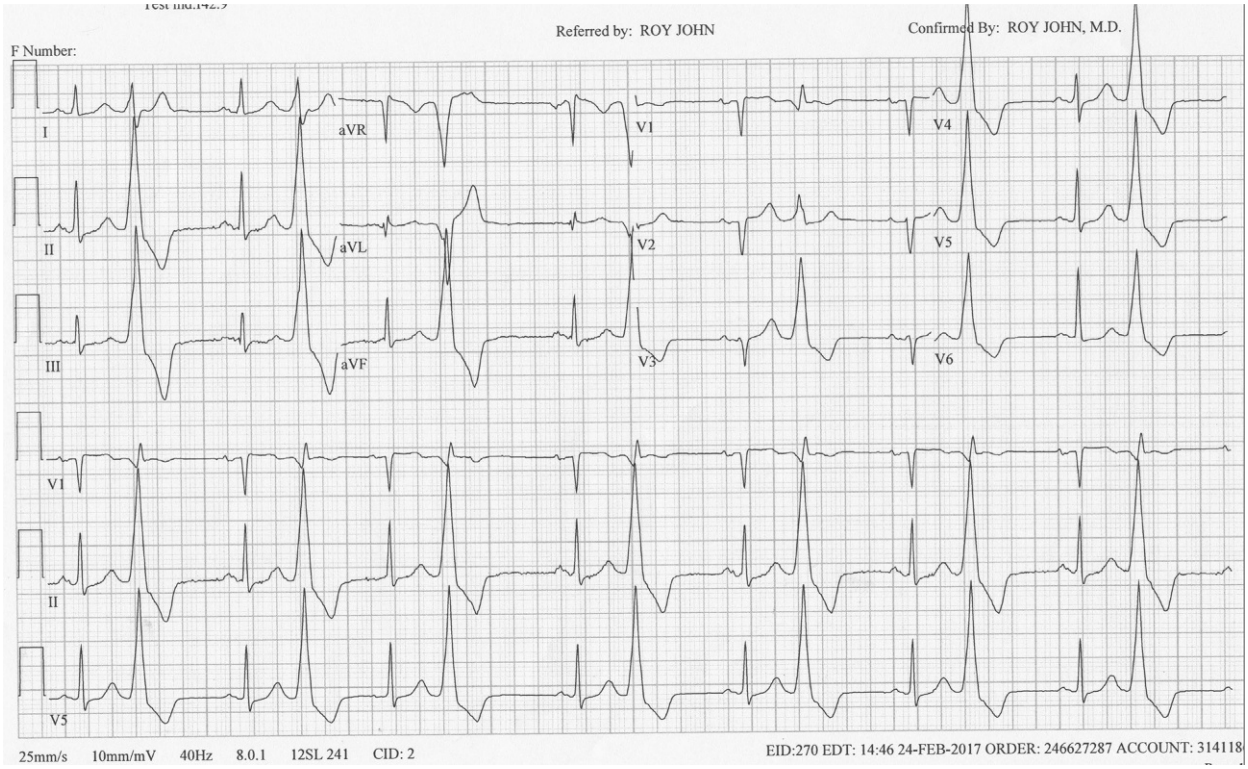

Fig. 3 Frequent MM PVC of LV outflow tract origin.

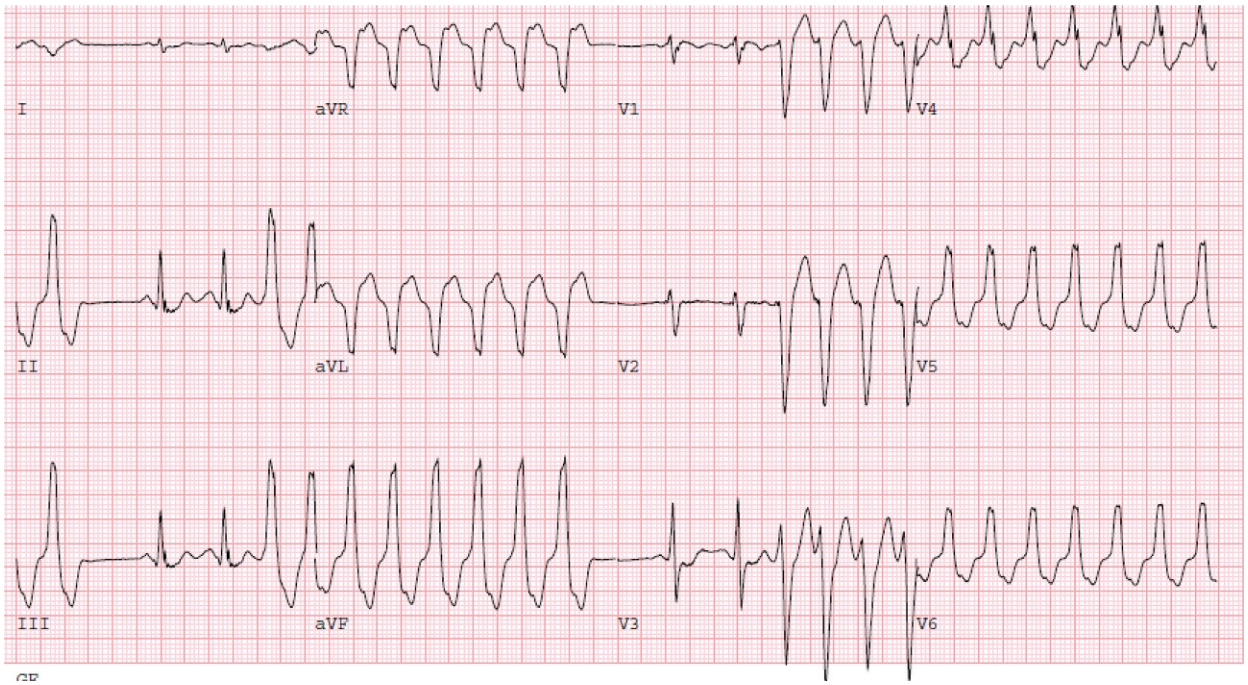

Fig. 4 A 50-year-old male with exercise-induced palpitation. During an ETT, single monomorphic PVCs were present. During recovery phase after 8 min of exercise, he developed repetitive monomorphic VT with morphology similar to PVCs at rest. The VT has a left bundle branch morphology with rightward inferior axis. Because of continued symptoms despite beta-blockers, he underwent ablation of a focus in the anterior septal aspect of the right ventricular outflow tract with suppression of the arrhythmia and no recurrence.

Idiopathic VTs are those that occur in the absence of structural heart disease. Most commonly, these are monomorphic and originate from the right ventricular outflow tract, although several other sites including the left ventricular outflow tract, papillary muscles, and perivalvular areas can give rise to these VTs. The diagnosis requires exclusion of underlying heart disease, and diseases such as ARVC and sarcoidosis that can cause VTs with similar characteristics to idiopathic VTs. Distinguishing idiopathic VT from VT with structural heart disease is of critical importance as the latter often warrants an ICD. Detection of ventricular scar on cardiac imaging can be helpful (MEMBERS WRITING COMMITTEE and MEMBERS ACCF TASK FORCE, 2010). Idiopathic monomorphic VT may be present with frequent PVCs that occur in clusters or as repetitive monomorphic VT usually during exercise (**Fig. 4**). Fascicular reentrant VT can similarly cause monomorphic VT that may respond to verapamil. Although idiopathic VTs occasionally cause syncope, sudden death is rare (Shimizu, 2009). Beta-blockers, calcium channel blockers, or catheter ablation (see what follows) are often effective in controlling symptoms.

Polymorphic VT and Resuscitated VF

Polymorphic VT generally indicates the presence of significant heart disease due to acute myocardial ischemia, cardiomyopathies, or a genetic arrhythmia syndrome (see what follows). Drugs causing QT prolongation and metabolic derangements are other causes (**Fig. 5**) (Roden, 2008). Unless a clear precipitant can be identified and corrected, an ICD is often warranted (Epstein et al., 2008). Polymorphic VT or VF complicating the first 48 h of acute myocardial infarction is associated with greater in-hospital mortality, but not greater long-term mortality for patients who survive to hospital discharge, and an ICD is not warranted (Mehta et al., 2009). Patients who are found in VF and resuscitated may have had sustained monomorphic VT or polymorphic VT as the initial arrhythmia, requiring a broader evaluation for specific diagnoses.

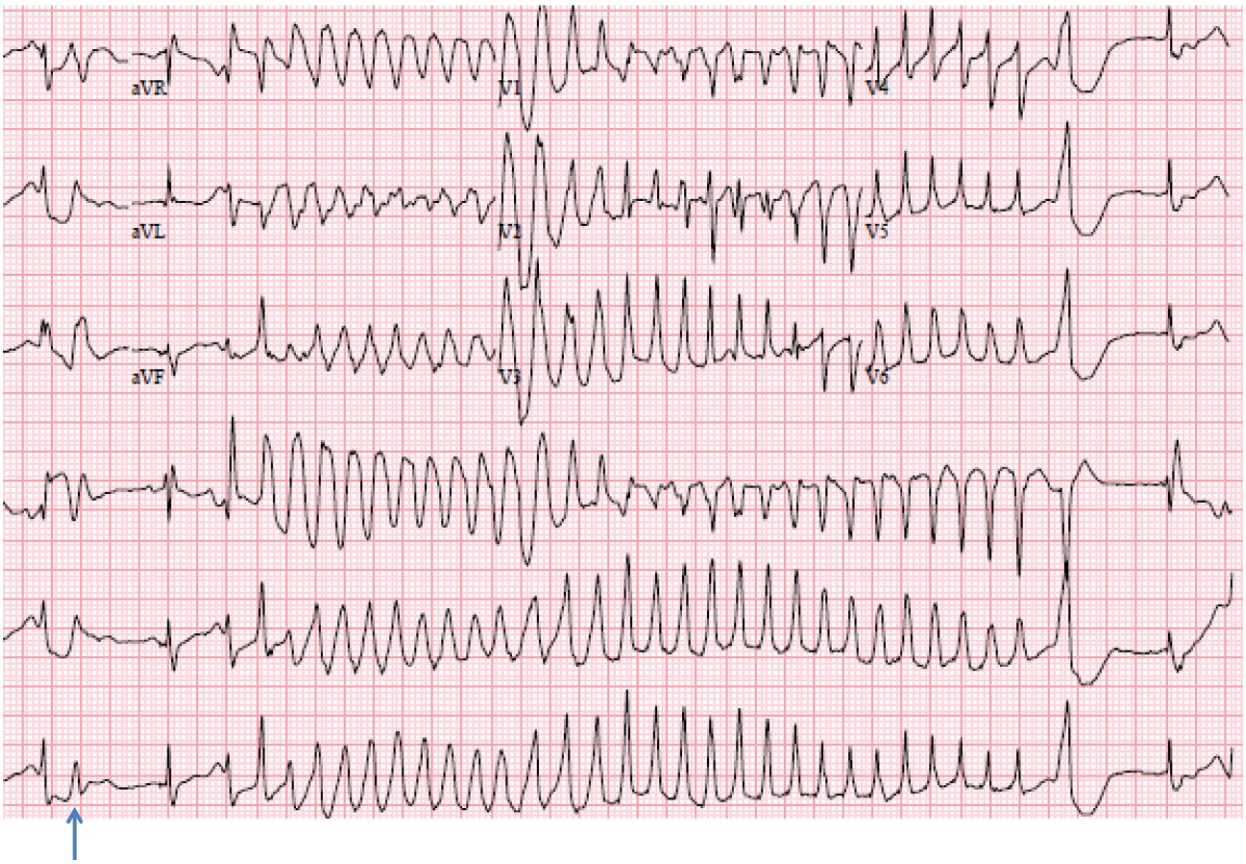

Fig. 5 A 40-year-old male developed recurrent VT following abdominal surgery. He was being treated with levofloxacin and haloperidol and had a resting ECG that showed QT prolongation at baseline. The 12-lead ECG trace in figure shows a closely coupled PVC (*arrow*) with a compensatory pause followed by a sinus beat with longer QT and PVC arising from the T wave that initiates polymorphic VT. This initiation with long short short coupling is typical of torsade de pointes VT associated with QT prolongation. In this particular case, the use of QT prolonging drugs (levafloxacin and haloperidol) on an underlying long-QT syndrome provoked the arrhythmia.

Structural Heart Disease Associated With VT

Coronary Artery Disease

Ischemic cardiomyopathy with infarct scar is the most common substrate for VT. Infarct-related monomorphic VT typically arises from regions of poorly coupled surviving myofibers within scar tissue that characteristically demonstrates slowed electrical conduction. These arrhythmia substrates related to ischemic cardiomyopathy are commonly located subendocardially but can also occur intramurally and subepicardially. A single area of infarct scar can give rise to multiple morphologies of VT (Aliot et al., 2009). Progressive fibrosis and ventricular remodeling can give rise to onset of VT late after a myocardial infarction. A small proportion (approximately 10%) of monomorphic VT in ischemic cardiomyopathy is due to automaticity or reentry involving the Purkinje system (Lopera et al., 2004).

Nonischemic Cardiomyopathy

Nonischemic cardiomyopathy (NICM) is a heterogeneous group of diseases including idiopathic cardiomyopathy, genetic LV cardiomyopathies, arrhythmogenic RV cardiomyopathy (ARVC), inflammatory disease (sarcoid heart disease, myocarditis, Chagas disease), and hypertrophic cardiomyopathy (HCM). Key differences in the VT substrate of NICM compared to ischemic cardiomyopathy include: (i) generally smaller scars with multiple reentrant VTs despite small scar; (ii) predilection for anatomical location around the valve annuli or septum; (iii) less frequent transmural scars; (iv) more frequent intramural scars; (v) extensive epicardial scarring that may occur in the presence of normal endocardium or extend beyond the region of endocardial scarring (Piers et al., 2013; Desjardins et al., 2013). Because the replacement scar of NICM is smaller and diffusely distributed especially during its early stages, patients may present with polymorphic VT or VF. As the disease progresses, scarring becomes more confluent. Sustained monomorphic VT is due to scar-mediated reentry in over 80% of patients with the remainder being focal in origin or due to bundle branch reentry.

Inherited cardiomyopathies

Genetic, familial cardiomyopathies account for 30%–40% of cases originally diagnosed as idiopathic or nonischemic dilated cardiomyopathy (Jacoby and McKenna, 2012). Some are associated with skeletal muscular dystrophy. Autosomal dominant, recessive, X-linked, and mitochondrial inheritance patterns are recognized. Mutations in genes coding for structural proteins of the nuclear lamina (lamins A and C) and the SCN5A gene are particularly associated with conduction system disease and ventricular arrhythmias in association with a cardiomyopathy (Van Tintelen et al., 2007). One study suggested that defects in the giant molecule titin maybe one of the most common causes of inherited dilated cardiomyopathy (Herman et al., 2012).

HCM is the most common genetic cardiovascular disorder occurring in 1 in 500 individuals and is a prominent cause of sudden death before the age of 35 years (Gersh et al., 2011). Most cases are due to mutations in sarcomeric proteins, but once the diagnosis is made, genotyping has a limited role in assessing sudden death risk. Sudden death can be due to polymorphic VT/VF. Risk factors include young age, nonsustained VT, failure of blood pressure to increase during exercise, recent (within 6 months) syncope, ventricular wall thickness >3 cm, and possibly the severity of LV outflow obstruction (Christiaans et al., 2011). Cardiac MRI demonstrating scar has been suggested as a high-risk profile (Chan et al., 2014). An ICD is recommended for high-risk subjects, but the specific risk profile warranting an ICD continues to be debated. Surgical myectomy is effective for relief of symptoms from outflow obstruction and has been associated with a sudden death rate of less than 1% per year (Gersh et al., 2011). The reported annual rate of sustained VT or sudden death after transcoronary ethanol septal ablation has been reported to range between 1% and 5% (Gersh et al., 2011).

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a group of genetic disorders characterized by VT arising in regions of fibrosis and/or fatty replacement of ventricular myocardium (Corrado et al., 2017). Most are due to mutations in genes involved in cell to cell adhesion proteins in desmosomes, such as plakoglobin and plakophilin. Approximately 50% has a familial transmission with an autosomal dominant inheritance. Left ventricular involvement occurs in approximately a third of cases and can predominate. The sinus rhythm ECG displays inverted T-waves in the anterior precordial leads in over 75% of patients. Sudden death and ventricular arrhythmias are often the initial presentation and frequently provoked by exercise (Corrado et al., 2017). Right ventricular monomorphic VT may be the initial manifestation of ARVC and care must be taken to differentiate it from idiopathic RV outflow VT. If a diagnosis of ARVC is confirmed, an ICD is usually warranted. Autosomal recessive forms result in cardiocutaneous syndromes such as Naxos disease and Carvajal syndromes associated with plantar and palmar hyperkeratosis.

Genetic Arrhythmia Syndromes

An increasing number of single gene mutations are being defined that cause arrhythmias and sudden death by disrupting cardiac ion channel function (channelopathies) or causing cardiomyopathy. Most are uncommon, or rare, but the first point of medical contact is often the primary care physician when an otherwise healthy individual develops arrhythmia symptoms or is seen after the sudden death of a relative. The electrocardiogram is an important screening tool, but abnormalities can be subtle and can vary from day to day in some disorders (Juntilla et al., 2012; Narayanan and Chugh, 2015). Family screening is further complicated by substantial

variability in penetrance, such that the same mutation can cause frequent arrhythmias in one individual and be asymptomatic in another. Genotyping is playing an increasing role in confirming the type of disorder, identifying other affected family members, and in some, but not all disorders, helping to assess risk of sudden death.

Abnormalities of Repolarization and the QT Interval

The *congenital long-QT syndrome* (LQTS) is due to mutations in genes encoding cardiac ion channels or their supporting proteins. The corrected QT (QTc) interval is typically prolonged to greater than 440 ms in men and 460 ms in women. Syncope or cardiac arrest due to TdP VT is the presenting symptom (**Fig. 5**). Depending on the mutation, other clinical features can include bradycardia, dysmorphic features, and deafness. Several different forms of congenital LQTS have been identified, but three groups of mutations that lead to LQT-1, LQT-2, or LQT-3 syndromes account for 90% of cases (Cerrone and Priori, 2011; Ackerman et al., 2011). The most common form, LQT-1, is due to a loss-of-function mutation in the gene encoding the alpha subunit for the slow inward rectifier potassium channel (KvLQT1). Symptoms typically occur during exertion, notably swimming. LQT2 is due to a loss-of-function mutation of the hERG gene that reduces rapid component of the delayed rectifier potassium current I_{Kr}. Arrhythmias are often triggered by surprise, such as a sudden loud noise. LQT3 is due to a mutation that increases the cardiac sodium current. Sudden death during sleep is a notable feature. Most patients with LQT-1 and LQT-2 respond to beta-blocker therapy especially of symptoms are provoked by adrenergic stimulation (Goldenberg et al., 2012). Markers of increased risk include longer QT intervals (particularly >0.5 s), female gender, and a history of syncope or cardiac arrest. Recurrent syncope despite beta-blocker therapy or high-risk profiles merit consideration of an ICD (Epstein et al., 2008). Left cardiac sympathetic denervation is effective when beta-blockers are not tolerated or when arrhythmias occur despite adequate beta blockade (Schwartz et al., 2004). Avoidance of QT prolonging drugs is critical for all patients with the LQTS including those who are genotype positive, but have normal QT intervals.

Acquired LQTS is common due to electrolyte disturbances and drugs blocking the I_{Kr} currents mediated via the KCNH2 potassium channel, the primary mechanism of action of drugs such as sotalol and dofetilide. QT prolongation with associated risk factors of hypokalemia, hypomagnesemia, bradycardia, older age, and female sex increases the risk for TdP VT. Common nonantiarrhythmic drugs include haloperidol, macrolide, and quinolone antibiotics, but a number of drugs have been implicated. Mutations affecting LQTS genes may be present in 5% of patients with drug-induced TdP and gene polymorphisms that contribute to the risk is present in another 5%–10% (Yang et al., 2002).

The *short QT syndrome* is very rare compared to the LQTS. The QTc is shorter than 0.36, and usually less than 0.3 s. The genetic abnormality causes a gain of function of the potassium channel (I_{Kr}) or reduced inward depolarizing currents. The abnormality is associated with atrial fibrillation, polymorphic VT, and sudden death (Patel et al., 2010).

The *Brugada syndrome* is diagnosed by characteristic electrocardiographic appearance with ST segment elevation in at least two of leads V1–V3 with a typical coved morphology (referred to as Type I Brugada ECG) either spontaneously or induced by administration of a sodium channel blocking drug, combined with syncope, resuscitated cardiac arrest, or a family history of the disorder (Priori et al., 2013). Syncope and sudden death are due to polymorphic VT with a predilection during sleep or acute illnesses associated with fever. There is male predilection. Gene mutations are identified in fewer than 30% of patients, and most have involved the cardiac sodium channel gene SCN5A with an autosomal dominant inheritance pattern. Abnormal conduction and fibrosis in the RV outflow tract and overlap with ARVC have been described, suggesting that the syndrome may have multiple causes (Priori et al., 2013). An ICD is indicated for individuals who have had unexplained syncope or been resuscitated from cardiac arrest. Quinidine has been shown to be effective for the prevention of recurrent ventricular arrhythmias.

Catecholaminergic Polymorphic VT

Catecholaminergic polymorphic VT (CPVT) is due to mutations in the ryanodine receptor or the sarcoplasmic calcium binding protein calsequestrin 2. These mutations result in abnormal calcium handling, and polymorphic ventricular arrhythmias that can resemble those of digitalis toxicity, for example, bi-directional VT. Patients typically present with syncope or cardiac arrest during exertion or emotional upset (Priori et al., 2013). ICD, restriction of physical activity, and beta-adrenergic blocker are the mainstays of therapy. The antiarrhythmic drugs flecainide and propafenone are effective for arrhythmia suppression (van der Werf et al., 2011). Left cardiac sympathectomy can be helpful in refractory cases (Wilde et al., 2008).

Drug Therapy for Ventricular Arrhythmias

Drugs have an important role in reducing the frequency of recurrent symptomatic arrhythmias. Beta-adrenergic blockers are the most commonly used drugs because many arrhythmias are provoked by sympathetic stimulation, and they have a favorable safety profile. However, they have limited efficacy for suppressing reentrant arrhythmias associated with structural heart disease.

Membrane active antiarrhythmic drugs that block cardiac ion channels have little role in preventing sudden death in patients with structural heart disease, but are useful for reducing symptomatic arrhythmias. The sodium channel blocking drugs such as flecainide and propafenone are occasionally considered for idiopathic VTs, but these agents should be avoided in patients with heart disease. They have negative inotropic effects, and flecainide increased mortality when given to survivors of myocardial infarction (Zipes et al., 2006). Quinidine is better tolerated hemodynamically due to its effect on action potential duration and its vasodilatory

properties that tend to offset any negative inotropy from sodium channel blockade. However, all these drugs have significant proarrhythmic effects such that use of sodium channel blockers in patients with structural heart disease is largely limited to those with an ICD. Drugs that predominantly block potassium channels (sotalol, dofetilide) prolong repolarization and are better tolerated in heart failure. However, these drugs can cause TdP VT, and the risk is likely increased in heart failure due to electrophysiologic changes that accompany myocardial hypertrophy, impaired excretion of many drugs, and diuretic-induced hypokalemia. Careful monitoring for excessive QT prolongation during initiation is required.

Both oral amiodarone and sotalol reduce ventricular and atrial arrhythmias that can lead to ICD shocks (Connolly et al., 2006). Amiodarone is most effective for control of ventricular arrhythmias but extra-cardiac toxicities (prominently thyroid, lung, liver, and neurologic) prevent long-term use in more than 20% of patients. In a recent trial in patients with LVEF $\leq$35%, amiodarone had no effect on mortality in NYHA class II heart failure, but was associated with increased mortality in class III heart failure (Bardy et al., 2005). Nevertheless, amiodarone is a reasonable consideration for patients who have had spontaneous sustained VT or VF, but who have a contraindication to or refuse to have an ICD (Zipes et al., 2006). In patients who are refractory of combination of drugs, addition of ranolazine, a slow sodium channel blocker, has shown promise in case series and small studies (Bunch et al., 2011).

Implantable Cardioverter Defibrillators

ICDs are highly effective for termination of VF or tachycardia and have been shown to improve mortality in cardiac arrest survivors and in patients at risk for sudden death due to structural heart diseases (Epstein et al., 2008). ICDs are recommended only if there is also expectation for survival of at least a year with acceptable functional capacity. The exception is in cases of patients with end-stage heart disease who are awaiting cardiac transplantation outside the hospital, or who have left bundle branch block QRS prolongation such that they are likely to have improvement in ventricular function with cardiac resynchronization therapy from a biventricular ICD.

Despite their high efficacy for termination of ventricular arrhythmias, there is considerable morbidity associated with their use. ICD shocks are painful, associated with increased mortality, mostly from deteriorating heart failure and may result in post traumatic stress disorder. With improved programming parameters and early detection of lead malfunction, the annual rate of inappropriate or unnecessary shocks due to sinus tachycardia, atrial fibrillation, nonsustained VT, or lead malfunction has dropped to approximately 2.5% (Moss et al., 2012). Dual-chamber ICDs are better than single-chamber devices in reducing inappropriate shocks (Kolb et al., 2014). ICD implantation has a 3% risk of complications, including pneumothorax, perforation, bleeding, and heart failure decompensation, but the procedure-related mortality is less than 1% (Lee et al., 2010). ICD infections require device and lead removal, with a 0.25% procedural mortality in experienced centers. Patients receiving ICDs require careful follow-up to detect arrhythmias that are predictive of heart failure deterioration and monitoring for device malfunction, and assessment of battery life. Remote-monitoring systems that allow automated and patient-triggered transmissions of ICD recordings have simplified follow-up, and enhanced early detection of arrhythmias and device malfunction. A recent meta-analysis of three randomized trials suggests reduced mortality with remote monitoring of ICD patients (Hindricks et al., 2017).

Catheter Ablation for VT

Catheter ablation is a reasonable first-line therapy for many symptomatic idiopathic VTs (Aliot et al., 2009). Most idiopathic VTs originate from a focus in the right ventricular outflow tract and ablation in this location has 80%–90% success rate for prevention of recurrence. Success rates are less well defined, but likely lower for those arising in less common locations such as the left ventricular outflow tract, aortic root, along the AV valve annuli, and from the papillary muscles. Failure of ablation is often due to inability to induce the arrhythmia for precise localization, or VT origin in a location that is inaccessible or in close proximity to a coronary artery that precludes safe ablation.

In patients with structural heart disease, catheter ablation of VT is used to reduce the frequency of symptomatic VT that triggers ICD therapies, or to control VT storms that are life-threatening (see what follows) (Aliot et al., 2009). For arrhythmias that hemodynamically stable, mapping and ablation is performed during induced VT in an attempt to terminate VT. For hemodynamically unstable VTs, the likely arrhythmia substrate is sought by identifying areas of scar and abnormal conduction. These regions are indicated by low voltage and abnormal electrograms during stable sinus or paced rhythm. In this "substrate mapping" approach, regions within the scar likely to contain the reentry circuits can then be targeted for ablation without inducing VT. Substrate mapping can also be combined with limited mapping during VT for more focal targeting of the reentry circuit. Hemodynamic support with intraaortic counterpulsation or temporary ventricular assist devices has also been used to improve the ability to map during VT.

Catheter ablation reports are largely limited to experienced centers. Procedural complications occur in approximately 3% of patients with idiopathic VT, and 6% of patients with structural heart disease, including tamponade, stroke, heart block, and, most frequently, vascular access complications (Aliot et al., 2009). Procedural mortality is rare in idiopathic VT, but ranges from 1% to 3% for patients with structural heart disease, usually due to uncontrollable ventricular arrhythmias when the procedure fails. Risks

are greater with percutaneous epicardial access and ablation, which has a 5% risk of major complications, including coronary artery injury and pericardial bleeding, that is usually self-limited, but deaths have been reported.

When antiarrhythmic drug therapy and catheter ablation fails, or is not an option, surgical cryoablation, often combined with aneurysmectomy, can be effective therapy for recurrent VT due to prior myocardial infarction and has also been used successfully in a few patients with nonischemic heart disease (Anter et al., 2011). Few centers now maintain the expertise for this therapy. Transcoronary ethanol ablation has also been used, in a small number of patients who have failed catheter ablation and drugs, with modest success (Tokuda et al., 2011).

Management of VT Storm

An electrical or ventricular arrhythmia storm is increasingly being recognized as a distinct syndrome because of specific management issues and prognostic consequences that differ from a single episode of VT. An electrical storm refers to three or more episodes of VT or VF or ICD shocks within a period of 24 h and is associated with a high mortality (Sesselberg et al., 2007). In the long-term follow-up of AVID study participants who received a secondary prevention ICD, mortality was 5.6-fold higher compared to patients who had ventricular arrhythmia unrelated to a storm (Exner et al., 2001). Scar-related VT accounts for over 80% of VT storms (Nayyar et al., 2013). Acutely administration of beta-blockers, amiodarone, and sedation combined with general anesthesia is required to suppress sympathetic tone. Reversible causes such as ischemic and electrolyte disturbance should be sought and corrected. In the event of hemodynamic deterioration, ventricular support with assist devices, or extracorporeal membrane oxygenation might be required. Catheter ablation can be life saving and effective for suppression of a storm in 70% of patients (Nayyar et al., 2013). Failure of catheter ablation is associated with a high mortality. Left cardiac sympathetic denervation and thoracic epidural anesthesia have been reported with variable success for suppressing VT storms (Bourke et al., 2010). Patients with an electrical storm should be considered for transfer to a high volume, tertiary center for expert management.

Summary

The significance of ventricular arrhythmias relate to symptoms, associated structural or genetic heart disease and the risk of sudden cardiac death. The primary focus in assessment is the determination of the risk of arrhythmic death and its prevention. In the absence of a risk of sudden death, management is primarily aimed at symptom relief. The less common role of ventricular arrhythmias as a primary initiator of ventricular dysfunction is increasingly being recognized, but prospective clinical trials are just getting under way to determine if suppression of arrhythmia will improve outcome. Although management strategies for ventricular arrhythmias have been defined for several forms of heart disease, prevention of VT-related sudden death in the rare genetic arrhythmia syndromes remain a challenge. Data from multicenter registries and rapidly accumulating genetic database will continue to provide important insights to guide managements of these uncommon but important causes of sudden arrhythmic death.

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Emergency Medicine Approach and Management of Traumatic Injuries: An Overview

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Introduction

A multidisciplinary team made up of emergency physicians, surgeons, nurses, and ancillary staff is optimal to promptly and efficiently manage severely traumatized patients throughout the continuum of care. However, as this level of resources may not be available, it is critical that a formal process of planning for all stages of care occur to best understand and utilize the resources available. This planning should lead to a structure where each member of the care team will understand their well-defined role in the management of the trauma patient.

Preparation for the Patient

Prior to arrival at the hospital, emergency medical service (EMS) providers will typically inform the receiving emergency department (ED) regarding the arrival of a significant trauma patient. While there are expectations for ACS level one trauma centers regarding specific resource requirements on selected patients, trauma centers often use a combination of physiological and mechanistic criteria in deriving their formal activation process (Markovchick and Moore, 2007). Criteria based on mechanism alone, such as pedestrian struck, motor vehicle collision with intrusion, or prolonged extrication, have mixed results and relatively poor specificity, but when used in combination with anatomical or physiological criteria, the accuracy increases (Kohn et al., 2004). Generally it is felt that some reasonable level of over triage is preferred to under triage in assembling a bedside trauma response.

Evaluation of the Patient in the Emergency Department

The ED care of the trauma patient begins with an assessment of potentially life-threatening injuries. Although every patient should have a complete and thorough evaluation, the rapidity, extent, and amount of testing are related to the mechanism, initial findings, chief complaints, age, and co-morbidities of the patient. In the primary survey, a rapid assessment of the airway, breathing, and circulation is completed. In principle, as major injuries are identified, they are treated before moving to the next part of the primary survey. In reality, however, there are often multiple concurrent interventions. The ability to perform simultaneous interventions, however, often depends on both the number and makeup of the team. What is critical is that there is a team leader who is prioritizing interventions through a systematic, clear, and consistent approach. Specific injuries that must be immediately addressed include the loss of airway integrity, inability to oxygenate or ventilate, tension pneumo- or hemo- thorax, massive external hemorrhage, and cardiac tamponade. Geriatric trauma patients present an additional challenge. Patients >60 years of age have higher mortality and complication rates in comparison with younger patients with similar injuries. Special awareness must be given to this patient population (Campbell-Furtick et al., 2016; Kuhne et al., 2005).

Airway Management

Inadequate oxygenation or ventilation can be life-threatening but is often easily reversible. The airway assessment should include patency and ability to protect the airway both at the time of evaluation and in the near future. General indications for intubation include inability to protect the airway, inability to successfully oxygenate or ventilate, airway obstruction, and Glasgow Coma score <8. Oral intubation using rapid sequence intubation (RSI) is generally the optimal approach in the ED (Mayglothling et al., 2012). While the best induction agent to use is a source of ongoing discussion, common medications currently used include propofol, ketamine, and etomidate. Succinylcholine remains the most common agent for paralysis (Brown et al., 2015). All patients undergoing RSI should have a backup plan in place in the event of a failed intubation. While rare, in cases of severe maxillofacial trauma or direct tracheal injury, cricothyrotomy may be the initial step.

Breathing

This step focuses on rapid identification of chest injuries that may impair ventilation or oxygenation. As a general rule, all significantly traumatized patients should initially be placed on supplemental oxygen while the primary survey is occurring. The examination consists of visualization for signs of splinting, disordered or paradoxical breathing or obvious deformity followed by palpation for tenderness, subcutaneous air, evidence of fractures, and finally auscultation. While clinical decision rules exist to decrease the utilization of chest radiography in the trauma patient, significantly traumatized patients, unstable patients, or patients with evidence of chest trauma should undergo radiography (Rodriguez et al., 2013). In stable patients, a chest radiograph is essential to help aid in the diagnosis of suspected chest trauma, but should not delay a needle decompression or chest tube in the unstable patient. While a supine radiograph is the initial choice at the bedside, the use of bedside ultrasound have been repeatedly shown to has higher sensitivity than plain X-ray for a pneumothorax (Alrajhi et al., 2012).

Circulation

Initial management of circulatory status begins with immediate control of any large volume of external hemorrhage. Bleeding is often controlled by compression and elevation alone. Next, initial steps include rapid assessment of pressure, pulse, and perfusion. This includes, in addition to vital signs, an evaluation of mental status, skin tone and temperature, and respiratory rate. Clinically, differences in compensation occur for different types of injuries, ages, and blunted physiological responses in the elderly. Additionally, comorbidities and medications might alter or conceal shock. On the other hand, patients who have evidence of hypotension in the prehospital setting or ED, generally defined by a systolic pressure <90mmHg, have been shown to have a greater likelihood of severe injury and mortality, and this finding must be taken extremely seriously.

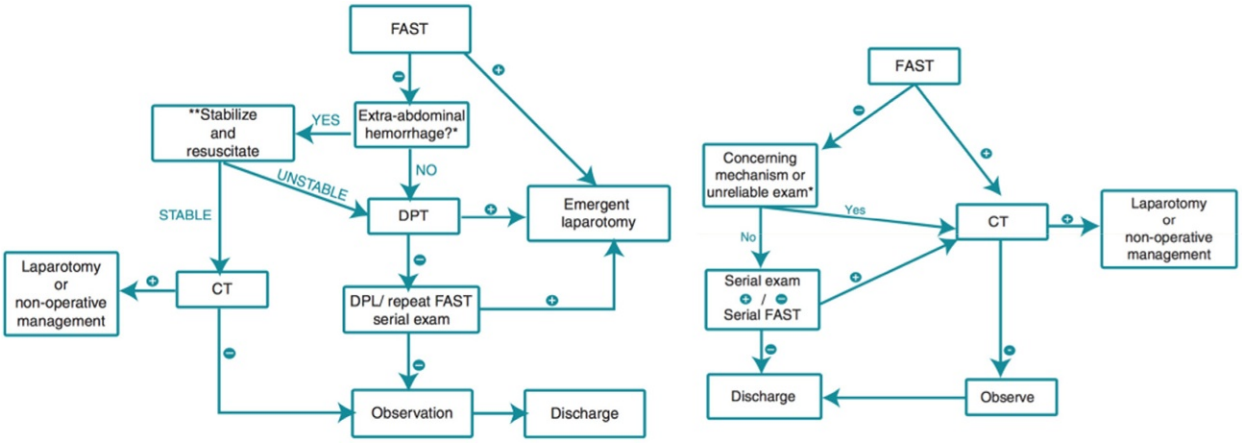
Concern for internal bleeding should lead to the establishment of intravenous access. All significantly traumatized patients should have two 14 or 16 gauge IVs placed, preferably in the antecubital fossa. If there is an inability to obtain access peripherally, it may be obtained via ultrasound guidance or central or intraosseous catheter.

The choice of fluids for resuscitation in the ED includes crystalloid and blood products (Perel et al., 2013). The goals of resuscitation are to rapidly diagnosing bleeding and critical injuries, prevent an uncompensated anaerobic state and rapidly attain definitive care for the injuries. Traditional teaching has been to initiate crystalloid resuscitation in a 20 mL/kg or 2 L bolus followed by blood products if persistent hypotension. However, there has been an ongoing trend toward less crystalloid fluid and greater use of blood products. This is consistent with an approach known as damage control resuscitation that includes avoidance of hypothermia and acidosis, permissive hypotension, hemostatic resuscitation with balanced blood products, damage control surgery, and eventual definitive care of the injury (Driessen et al., 2016). Concepts that are more germane to ED resuscitation include permissive hypotension (or limited resuscitation) and hemostatic resuscitation with balanced blood products. Permissive hypotension may be indicated in the patient who has not had definitive management or control of their bleeding. Rather than aggressive fluid or blood resuscitation to normotension, treatment goals in the nonhead-injured patient are systolic blood pressures around 90mmHg, although some studies have aimed lower. While animal trials clearly showed benefit to this approach, human studies have been more limited and less definitive (Mapstone et al., 2003). Although one large randomized controlled trial has shown this to be an effective and improved approach, some methodological concerns with the paper and other studies inability to find real benefit from the approach (although no worse outcomes) keep this from becoming a clear standard (Bickell et al., 1994; Dutton et al., 2002). Massive transfusion protocols that provide hemostatic resuscitation with balanced products may ameliorate some of coagulopathies associated with trauma, provide an improved method of resuscitation and ultimately lead to better patient outcomes. When massive transfusions are used, they should generally be adopted by a formal hospital policy agreed upon with department of surgery, blood bank, and the emergency department (Borgman et al., 2007; Hallet et al., 2013; Holcomb et al., 2011, 2015).

A focused abdominal sonography in trauma (FAST) examination should be performed on patients as an adjunct to the assessment of circulation. A FAST is a four view, directed ultrasound (US) examination to identify fluid within the abdomen and pericardial sac. The presence or absence of free intra-abdominal, intrathoracic, or intrapelvic hemorrhage diagnosed by ultrasound may direct the immediate management of trauma patients. Pericardial effusions or tamponade can be readily identified. The major limitation of US is that it shows fluid in the abdomen, but does not identify the degree of bleeding.

A FAST is primarily used in blunt trauma, however, a finding of free intraperitoneal fluid in penetrating trauma has an extremely high positive predictive value for an operative lesion, and should prompt strong consideration of an urgent laparotomy or

laparoscopy. A negative FAST, on the other hand, cannot rule out an intra-abdominal injury (Quinn and Sinert, 2011). An algorithm in the succeeding text shows one common way that ultrasound is incorporated into early clinical decision-making.



FAST for stable and unstable abdominal penetrating trauma patients. From Legome and Shockley, *Trauma a comprehensive emergency medicine approach.*2011 Chapter 13. Cambridge University Press.

Disability

The next step in the evaluation is an assessment of their neurological status. A rapid and focused examination should be performed with specific concentration on the Glasgow Coma score (see **Table 1**), pupillary examination and gross motor function.

While the initial score is important in planning treatment decisions and prioritizations, serial examinations are critical in following the course of the patient. Since many traumatized patients may have injuries or intoxicants that may make scoring difficult, emphasis on the motor score, with or without the pupillary examination has been found to have similar sensitivity in diagnosing intracranial injury (Brown et al., 2014; Chou et al., 2017; Kupas et al., 2016). The motor evaluation generally involves making sure the patient has no obvious focal weakness or evidence of spinal cord injury

Secondary Survey

After assessment and securing of the airway, breathing, and circulation, a complete head to toe physical exam should be performed.

Traumatic Head Injuries

Head trauma includes the full spectrum of injury, from superficial contusions, abrasions, and lacerations of the soft tissues, to injuries deep within the skull. The vast majority of patients who present to the ED with head trauma have blunt or closed head injury. Most injured patients fall into a category of mild traumatic brain injury category, with a GCS of 13–15 (CDC, 2016). Approximately 5%–10% of patients with mild traumatic brain injury will have an abnormal head CT scan, but only 0.5%–1.0% will require acute neurosurgical intervention for intracranial injury (Bazarian et al., 2003; Jagoda et al., 2009).

There are five distinct injuries frequently described: epidural hematomas, subdural hematoma, subarachnoid hemorrhages, diffuse axonal injury, and intraparenchymal hemorrhage. They may be seen in isolation or as a combination of injuries. An epidural hematoma is the result of an arterial injury, most commonly the middle meningeal artery secondary to direct force. Classically, it is thought that individuals might have a rapid deterioration following a "lucid interval," although this is neither specific nor highly sensitive. On CT imaging, a hyperdense lens-shaped or lenticular lesion is seen adjacent to the cranium. Frequently, emergent neurosurgical evacuation of the hematoma is indicated. Rarely, in exigent circumstance in underresourced areas, a burr hole in the emergency department may be performed. A subdural hematoma occurs when the bridging veins become disrupted. This typically

Table 1 Glasgow coma scoring

EYE	*Motor*	*Verbal*
4 = to spontaneous	6 = follows commands	5 = oriented
3 = to voice	5 = purposeful	4 = confused
2 = to pain	4 = withdrawal	3 = inappropriate
1 = none	3 = flexion	2 = incomprehensible
	2 = extension	1 = none
	1 = none	

causes a slow progressive bleed. Nevertheless, these types of bleeds can still cause severe neurological deterioration and often may have greater parenchymal injury and require neurosurgical intervention. On CT imaging, this hematoma is seen as a crescent-shaped hyperdense lesion. A subarachnoid hemorrhage (SAH) consists of bleeding in the subarachnoid space and can occur either in isolation or in combination with other intracranial bleeds. Intraparenchymal hemorrhages are caused by the disruption of the small intraparenchymal vessels secondary to shearing forces and management will vary based on size and location. Any type of intracranial bleed that causes increased intracranial pressure (ICP) can lead to cerebral herniation. Physical exam signs of herniation, include fixation and or dilation of one or both pupils, low GCS, and posturing (**Figs 1–4**).

Head-injured patients can exhibit significant agitation and combativeness, which can be dangerous and increase ICP. Additionally, they often have taken intoxicants that may obscure or worsen the injury (Easter et al., 2013). Clinical judgment must be used in



Fig. 1 Subarachnoid hemorrhage.



Fig. 2 Subdural hemorrhage.



Fig. 3 Epidural hemorrhage.



Fig. 4 Intraparenchymal hemorrhage.

deciding whether to chemically restrain these patients, especially prior to CT, or perform intubation and airway management due to a high concern for intracranial injury. One must also consider other reversible non traumatic causes of agitation, e.g., hypoglycemia, hypoxemia, hypotension, hypothermia, toxicology, and infection.

Despite many attempts, no specific medical treatments have been shown to improve outcome in severe brain injury. Although the full benefits are unclear, one very reasonable option is to follow the precepts of the National Brain Trauma Foundation (BrainTrauma.org), which appears to show benefit in mortality and morbidity of severely injured patients (Gupta et al., 2016). Much of the benefit, however, may be due to inpatient management including appropriate invasive monitoring and surgery. Much of the ED options are level 2 or 3, centered on the discussion of what remains unproved, e.g., hypothermia and hyperosmolar

Table 2 CT clinical decision rules

	Canadian CT head rule	*New Orleans criteria*
Inclusion criteria	Age >16 years GCS 13–15 with LOC or amnesia Head injury within 24 h	Age >3 years GCS 15 with LOC/PTA Head injury within 24 h
Risk factors identified	GCS <15 at 2 h post injury Suspected skull fracture ≥2 episodes of vomiting Age ≥65	Headache Vomiting Age >60 Persistent amnesia Visible trauma above the clavicles seizure
Exclusion criteria	Age <16 years Blood thinners Seizure after injury	
Sensitivity for injuries requiring neurosurgical intervention	100%	100%

therapy, or what to avoid, e.g., prolonged hyperventilation. In the ED, appropriate steps for the patient may include avoiding hypotension and hypoxia and consideration of antiepileptic drugs to avoid early post traumatic seizure and include basic maneuvers to decrease ICP such as raising the head of the bed to 30 degrees, providing adequate sedation and analgesia and treating fever. Early neurosurgical consultation is important to assist with decision making for surgical decompression, fluid drainage or intracranial monitoring.

One of the most common types of injuries to manage is the patient with a mild traumatic brain injury and a GCS of 15. Several clinical decision instruments have been created to help the clinician (Bouida et al., 2013; Haydel et al., 2000; Servadei et al., 2001; Stiell et al., 2001). A comparison of the two decision rules can be seen in **Table 2**. Performing advanced imaging on all patients in order to diagnose the few critical injuries is expensive and time-consuming and may cause eventual harm from the delayed effects of radiation. The authors recommend familiarity with a validated CT head injury rule or the American College of Emergency Physician head injury guidelines when caring for patients with a GCS of 13–15 with the loss of consciousness or posttraumatic amnesia.

Approximately one-third of adults older than 65 years of age will experience a fall each year (Hausdorff et al., 2001). Frequently, these individuals are on anticoagulant or antiplatelet agents. No well-validated decision rules comprehensively target patients with mild traumatic brain injury who are on these medications. The authors recommend that if resources are available, ED patients on an anticoagulant or antiplatelet agent, excluding aspirin, with more than minimal traumatic head injury should receive a CT scan of the brain to evaluate for an intracranial injury (Nishijima et al., 2012; Reddy et al., 2014; van den Brand et al., 2017). Trauma centers should create some protocol by which these individuals with evidence of intracranial injury have reversal of their agent rapidly to prevent further intracranial bleeding.

Neck Injuries

Penetrating and blunt trauma to the neck can cause a variety of injuries to vascular, respiratory, gastrointestinal, and neurological structures. The emergency physician must carefully assess for respiratory distress, stridor, bleeding, subcutaneous emphysema, expanding hematoma, or tracheal shift. Hard signs of significant vascular injury include a pulsatile hematoma, hemorrhagic shock, carotid bruit, absent pulse, or focal neurological deficit and generally are indications for early operative intervention. Early rapid sequence intubation should be strongly considered in someone with any acute airway concern or respiratory distress. In evaluating penetrating trauma, the neck has been divided into three anatomical zones not only to help clinicians both identify structures in each region, but also to help guide management. Zone one initiates at the clavicles and extends to the cricoid cartilage and includes all vital structures in this region. Injury to this area has been found to have the highest rate of mortality secondary to the difficulty in gaining access to these structures during surgical exploration (Kendall et al., 1998). Zone two is identified by the cricoid cartilage to the angle of the mandible. The platysma muscle covers most vital structures, and if intact, local wound care is all that is needed for patient management. Penetrating injuries deep to the platysma in zone two used to be routinely explored in the OR; however, currently, a stable patient may undergo CT imaging first, which may obviate further surgical intervention. Bronchoscopy and endoscopy are occasional needed if there is a high clinical suspicion for an aerodigestive injury. Zone three begins at the angle of the mandible and extends to the base of the skull. Vascular injuries are usually visualized clinically or by CT imaging, aerodigestive and neurological injuries can be more difficult to diagnose as CT imaging may appear normal.

Musculoskeletal Disorders of the Spine

The spine is a complex bony structure providing stability and protection. Injuries to the spine range from minor soft tissue contusions without structural abnormality to bony fractures, severed ligaments, and nerve damage. In a cooperative patient immobilized in a cervical collar, it should be loosened momentarily to evaluate for midline tenderness. With assistance, the patient

should be logrolled and the back should be examined for bruising, penetrating trauma, bony deformities, or bony step-offs. In an effort to maintain sensitivity for serious injury and reduce unnecessary imaging, two decision rules, NEXUS low-risk criteria (NLC) and the Canadian cervical spine rule (CCR) have been developed and should be a part of every emergency of physician's practice (Hoffman et al., 2000). Imaging the spine with CT is becoming the preferred modality to evaluate for bony pathology after blunt trauma. The benefits of improved sensitivity and specificity and better resolution should be balanced by greater radiation exposure and very small but real risk of radiation induced head and neck cancers.

Chest Trauma

Rapid evaluation and management is critical for the patient with significant chest trauma. The most severe and early life-threatening chest injury is blunt aortic injury, which is typically associated with frontal or nearside high-speed motor vehicle crashes with the lack of restraints and significant intrusion (Demetriades et al., 2008; LoCicero and Mattox, 1989). Other immediate life-threatening injuries of the chest include upper airway obstruction, tension pneumothorax, large hemothorax, flail chest, open chest wound, and cardiac tamponade. A tension pneumothorax occurs when air in the pleural space pushes the heart and mediastinal structures to the opposite side, compressing the contralateral lung and impeding venous return to the heart. Clinically, there will be absent breath sounds on the affected side and deviation of the trachea away from the side of injury. The treatment is immediate decompression of the affected side using a large bore, 14–16 gauge needle. Based on recent literature, the fourth or fifth intercostal space in the mid or anterior axillary line is probably the most effective area to insert the catheter. In order to ensure success, the needle and catheter must be appropriately long enough to penetrate into the pleural space (Akoglu et al., 2013; Inaba et al., 2015; Laan et al., 2016). With a successful decompression, one should hear a rush of air. While a needle thoracostomy will relieve a tension pneumothorax, a chest tube must still be placed afterward to evacuate air from the pleural space. Mechanisms of tension or pneumothorax without tension include laceration of the pleura or lung by a fractured rib, alveolar rupture from the compression of the chest against a closed glottis, or penetrating wound in the thorax. As noted earlier, a bedside ultrasound has very high accuracy for diagnosing pneumothorax (Soldati et al., 2008). The management of a significant pneumothorax includes tube thoracostomy placed to water seal. A small pneumothorax or one seen on CT alone may sometimes be managed expectantly. A hemothorax is the accumulation of blood in the pleural space. Bleeding can be from lacerations of the lung parenchyma or injuries from the intercostal vessels. The amount and rapidity of bleeding that occurs determines the management of such an injury. With a large hemothorax, patients will likely have diminished breath sounds and are often hypoxic and hemodynamically unstable. The treatment for a hemothorax includes evacuating the blood from the chest via a chest tube. In large or ongoing hemothoraxes, repletion of blood to restore intravascular volume may be required. This can be done using blood transfusions, but if available, an autotransfuser can be used to collect blood from the chest injury, filter it, and then reinfuse it back to the patient (Rhee et al., 2015). General indications for thoracotomy include an initial output of more than 1500 cc of blood from the chest tube or continued bleeding of greater than 200 cc/h over 4 h with hemodynamic instability; however, the decision may be altered by other injuries or co-morbidities and must ultimately be made by the surgeon who will be operating on the patient.

A flail chest occurs when there are three or more adjacent ribs fractured in more than one location. There is usually an associated pulmonary contusion. Physiologically, when a patient inspires, the flail portion may cave in. On expiration, the positive pleural pressure will cause the segment to flail out, causing a paradoxical motion of the chest and irregular respirations. This leads to significant pain during breathing and may result in both hypoxia and tachypnea. Management includes appropriate analgesia the including consideration of intercostal nerve blocks. In severe cases, intubation and mechanical ventilation may be required.

Traumatic cardiac tamponade is caused by the accumulation of blood in the pericardial sac from blunt or penetrating trauma. This accumulation of blood leads to decreased filling of the heart during diastole and decreased cardiac output. The classic signs and symptoms include muffled heart sounds, hypotension, and distended neck veins. Rarely are all three findings present (Stolz et al., 2017). The most sensitive test for pericardial effusion is by evaluating for pericardial blood using bedside ultrasound. Immediate treatment is fluid loading with a plan for needle decompression, a pericardial window or an emergent thoracotomy depending on both etiology and stability.

One of the most challenging situations for an emergency physician is the decision to perform a ED resuscitative thoracotomy for a trauma patient who presents in cardiac arrest or periarrest. In the appropriate setting, this procedure can be lifesaving by releasing of pericardial tamponade, controlling cardiac and great vessel wounds and cross clamping the aorta to limit hemorrhage and improve central perfusion. There are few scenarios where an ED thoracotomy for blunt thoracic injury is indicated. Time to loss of vital signs, surgery capabilities, and comorbidities all play a role in the decision. Patients with penetrating thoracic trauma, however, should receive a resuscitative thoracotomy if they had documented the loss of vital signs within 5–10 min of ED arrival, lose vital signs in the ED, or are hemodynamically unstable and found to have pericardial tamponade on ultrasound. In a study published in 2004, it was found that resuscitative thoracotomy to be futile in blunt trauma with prehospital CPR longer than 5 min and in patients with penetrating trauma with prehospital CPR duration greater than 15 min (Capote et al., 2013; Dennis et al., 2016; Powell et al., 2004; Slessor and Hunter, 2015).

Other potential serious but not immediately life-threatening injuries to the chest include fractures to the ribs and sternum. Fractures occur at the site of direct blows or at their posterior weak point as a result of compressive forces. Thoracic cage injuries usually result in significant pleuritic pain, localized tenderness and splinting, crepitus, ecchymosis, atelectasis, and increased risk for pneumonia. Multiple rib fractures interfere directly with the mechanics of ventilation. Fracture fragments may penetrate the pleura and lungs and result in pneumothorax and hemothorax. Adequate pain control should be provided, and patients should be instructed to perform incentive spirometry or take regular deep breaths throughout the day.

Sternal fractures are not particularly dangerous per se, but should be taken seriously and a search for underlying cardiac and pulmonary trauma should be undertaken. Generally, an isolated nondisplaced sternal fracture without associated findings requires analgesia alone (Sadaba et al., 2000).

Abdominal Trauma

Abdominal trauma, both blunt and penetrating, is a common cause of traumatic morbidity and mortality. Concerning findings for a patient with abdominal trauma include pain or tenderness and ecchymoses and abrasions on the abdominal wall. Increasing abdominal distention may be a marker of ongoing intraperitoneal bleeding. Physical examination is limited in its ability to identify the presence or absence of intraabdominal injury. Further diagnostic testing if frequently indicated despite minimal clinical findings in the patient with multiple or distracting injuries or limited examination due to comorbidities, age, or mental status.

The management of a patient with intra-abdominal trauma can be divided into the stable and unstable patient. The unstable patient should generally proceed immediately to the operating room without further diagnostic testing if any of the following are present: evisceration, gross blood per rectum, hematemesis, diaphragmatic injury, peritonitis, and hemodynamic instability with evidence of intraperitoneal injury on bedside ultrasound FAST examination or diagnostic peritoneal lavage (Stengel et al., 2001, 2005). For stable patient, there is further discrimination based on etiology. In the stable blunt trauma patient, diagnostic imaging is the mainstay of evaluation. FAST is recommended for all blunt trauma patients with a significant mechanism and is a may be a useful, if not well proved, screening tool (Stengel et al., 2001; Dammers et al., 2017). The FAST technique includes obtaining images of four standard views: right upper quadrant (Morrison pouch), subxiphoid, left upper quadrant, and suprapubic (pouch of Douglas) (**Figs 5** and **6**).

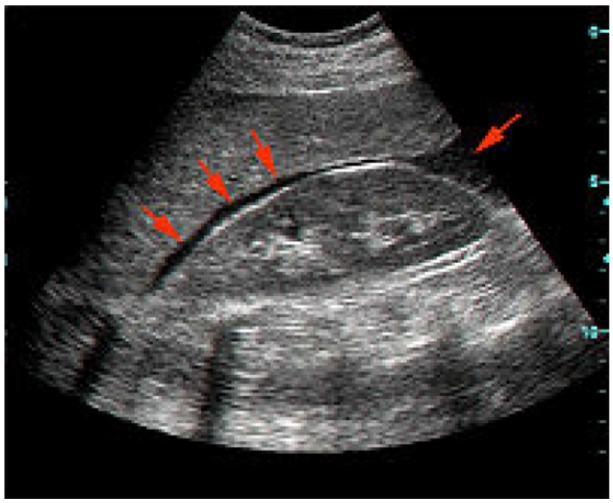


Fig. 5 Fluid in Morrisons pouch.

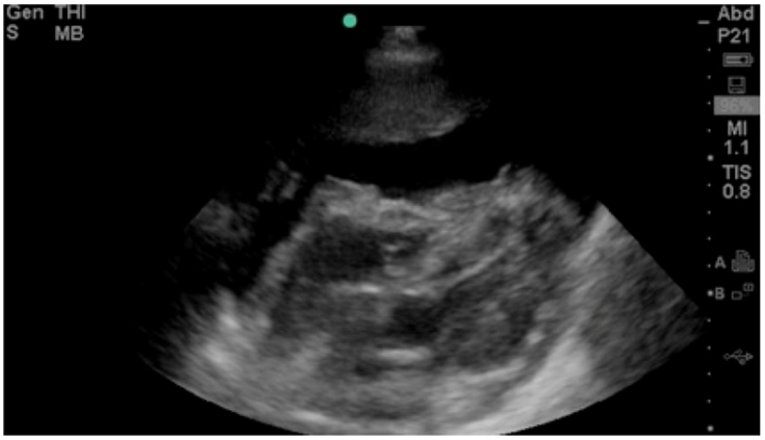


Fig. 6 Pericardial effusion.

Abdominal and pelvic CT is the standard diagnostic imaging modality for a blunt stable patient with concern for intraabdominal injury. CT provides excellent detail of solid organ and retroperitoneal injuries. Its performance in diagnosing hollow viscus injuries continues to improve. Because of the accuracy and detail of CT images, nonoperative management of solid organ injury is commonly and safely performed.

The initial treatment of patients with blunt abdominal trauma and finding of intr-abdominal injury includes fluid resuscitation, blood and product transfusion, and surgical consultation for possible operative repair. Indications for operative intervention include hemodynamic instability, diaphragmatic injury, and hollow viscus injury. Although high-grade spleen and liver injuries once routinely underwent surgery, the use of angiography with embolization has become more common (Schimmer et al., 2016).

In penetrating abdominal trauma, the most common mechanism is from knives and guns. In either case, hollow viscous injuries are more common than solid organ. Stab wounds, as opposed to gunshot wounds, tend to follow the track of the wound and have more predictability. In addition to wounds involving the abdominal cavity, injuries to the thoracic cavity must also be considered in patients with thoracoabdominal stab wounds or any gunshot wound. A FAST examination can be used in penetrating trauma as a diagnostic tool; specificity and positive predictive values are in the 90th percentile range for therapeutic laparotomy if ultrasound reveals free fluid. However, if the US is negative, further observation or testing is required (Quinn and Sinert, 2011).

Treatment options for penetrating abdominal trauma include observation, serial examinations, CT scanning, local wound exploration, laparoscopy, laparotomy, or combination of the aforementioned. Specific benefits and risks are dependent on multiple factors including comorbidities and associated injuries, mental status, and local resources.

Conclusion

The care of the severely injured trauma patient is complex. A multidisciplinary team made up of individuals familiar with the care of trauma patients is essential to promptly evaluate and treat these patients. Trauma patients may have suffered significant injuries despite normal vital signs and an initially benign physical examination. The appropriate use of imaging studies can promptly diagnose injuries and guide management and disposition. The placement of patients where they can receive definitive care, whether in the operating room, the ICU, or another facility, is a high priority in trauma care.

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Environmental Air Pollution: An Emerging Risk Factor for Stroke

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Introduction

Environmental medicine is a new field of practice and research (Reis and Roman, 2007; Calderón-Gareidueñas et al., 2016) dedicated to a worldwide, comprehensive and translational study of the effects of the environment on humans.

Environmental medicine uses four approaches in the study of environmental factors affecting people: by agents (such as chemicals), by milieu (such as water, air), by population (in children, in elderly), and by pathology (Calderón-Gareidueñas et al., 2016).

The World Health Organization defines air pollution as the "contamination of the indoor or outdoor environment by any chemical, physical, or biological agent that modifies the natural characteristics of the atmosphere" (Calderón-Gareidueñas et al., 2016).

Atmospheric pollution is a major problem of environmental health with an increasing concern for populations (Brunekreef and Holgate, 2002). It is well known that exposure to ambient air pollution increases mortality and morbidity, and shortens life expectancy (Feigin et al., 2016a; Murray and Lopez, 2013; Feigin et al., 2015). Moreover, cardiovascular disease, stroke, and cancer are the leading causes of death worldwide (Lozano et al., 2012) while their association with environmental air pollution increases (Henrotin et al., 2010; Mostafic et al., 2012; Danaei et al., 2005).

Previous studies observed a tight temporal association between exposure to air pollutants, stroke onset, and death induced by stroke (Feigin et al., 2016a; Shah et al., 2015). Results in the literature indicate controversies due to several factors as well as heterogeneous mechanisms, causes and stroke subtypes, various vascular risk factors, stroke triggers (Feigin et al., 2016a; Guiraud et al., 2010), and numerous air pollutants.

The present review focuses on the relationship between air pollution and stroke onset.

Toxicity of Air Pollutants

With the Industrial Revolution starting in the late 18th century, regional air pollution increased dramatically. The combustion of fossil fuels (coal, coke, gasoline, and diesel) needed to power industries, transportation, and housing, became responsible for the release of several contaminants into the atmosphere. However, the mix of air pollutants changed in the 20th century: oil and diesel replaced coal, and became the major energy sources used in expanding cities in Western countries. By the 1960s and 1970s, two major types of pollution were recognized: the London-type smog, linked to fossil fuel combustion and emissions of particulate matter (PM), and the Los Angeles-type smog, or photochemical oxidant pollution, the main compounds of which are ozone and secondary aerosols (sulfates and nitric oxides) (Calderón-Gareidueñas et al., 2016; Zhang and Samet, 2015).

Air pollution is a complex mixture of gases and particles whose composition varies geographically and temporally. Population-weighted annual average concentrations of particle mass with aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) and ozone are the two indicators used to quantify exposure to air pollution. $PM_{2.5}$ is the most consistent and robust predictor of mortality in studies of long-term exposure (Platt et al., 2014; Suissa et al., 2013).

Nowadays, only some atmospheric air pollutants are monitored, depending on their health effects and the regulations in place in different countries; these include ozone, sulfur dioxide, carbon monoxide, nitrogen dioxide, lead, and PM (**Fig. 1**). However, other air contaminants should also be measured because they include highly neurotoxic chemicals, such as volatile organic compounds, benzene, formaldehyde, tri- and tetrachloroethylene, toluene and polycyclic aromatic hydrocarbons, and metals such as lead, manganese, iron, mercury, arsenic, cadmium, and cobalt (Calderón-Gareidueñas et al., 2016; Kyle et al., 2001).

Airborne PM varies in its physical and chemical composition, source and particle size, and includes PM_{10} (particles >2.5 μm but <10 μm), $PM_{2.5}$ (fine particles >100 nm but <2.5 μm), and ultrafine PM (UFPM < 100 nm) and their components, the key pollutants found in European cities (Calderón-Gareidueñas et al., 2016).

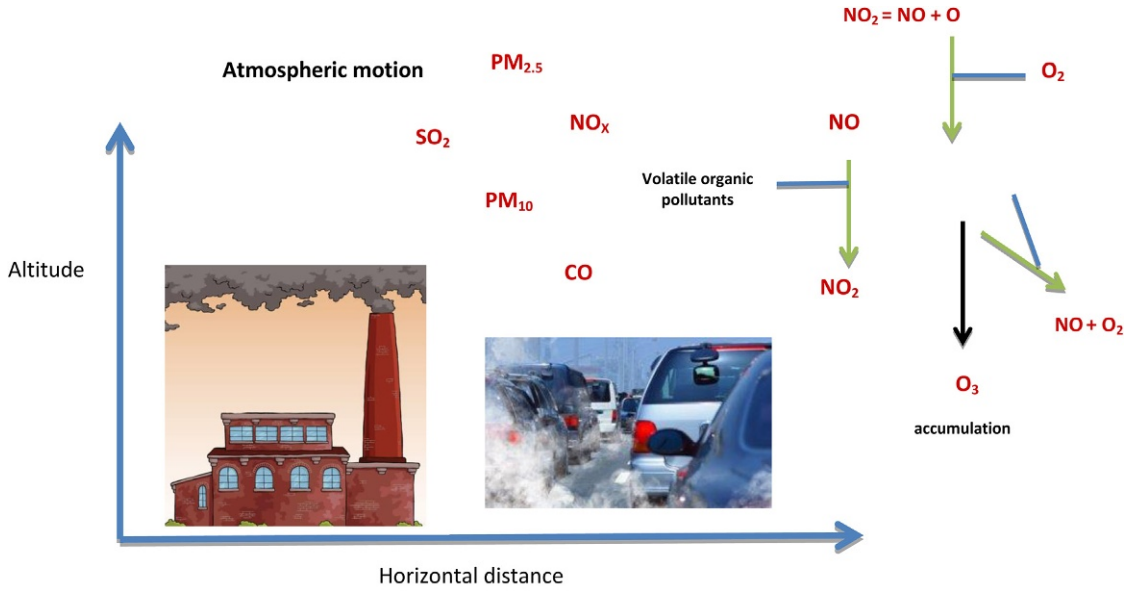

Fig. 1 The most toxic of atmospheric pollutants.

The neurological effects associated with sustained exposures to concentrations of outdoor air pollutants above the current international air quality standards are now an important issue for the millions of people living in megacities around the world. Moreover, the increasing worldwide warming is modifying all these conditions (Feigin et al., 2016a; Murray and Lopez, 2013; Feigin et al., 2015; Lozano et al., 2012).

Air Pollutants and Stroke Onset

Consequences have been clearly described first in terms of cardiovascular mortality and morbidity (Stafoggia et al., 2014; Samet et al., 2000; Katsouyanni et al., 1997; Pönkä and Virtanen, 1996; Hong et al., 2002; Maheswaran and Elliott, 2003; Kan et al., 2003).

With time, the association was confirmed mainly from long-term population-based studies. One of the first to evaluate stroke subtypes was conducted in the population-based stroke registry of Dijon, France, between March 1994 and December 2004 (Henrotin et al., 2007). The register recorded all first-ever strokes among residents of the medium-size town of Dijon (150 000 inhabitants), using standard stroke diagnostic criteria. Pollutant concentrations as sulfur dioxide (SO_2), carbon monoxide (CO), nitrogen oxides (NO_2), ozone (O_3), and PM_{10} were measured by three meteorological stations every hour.

A bi-directional case-crossover design was used to examine the association between air pollutant and stroke onset. The conditional logistic regression model included the meteorological parameters (temperature, relative humidity), influenza epidemics and holidays. The authors collected 493 large artery infarcts, 397 small artery infarcts, 530 cardioembolic infarcts, 67 indeterminate infarcts, 371 transient ischemic attacks, and 220 hemorrhagic strokes. For single-pollutant and for a 10 $\mu g/m^3$ increase of O_3 exposure, a positive associations was observed only in men, over 40 years of age, between ischemic stroke occurrence and O_3 levels with a 1-day lag. No significant association was observed for hemorrhagic stroke. In two-pollutant models, the effects of O_3 remained significant after each of the other pollutants was included in the model, in particular for PM_{10}. A significant association was observed for ischemic strokes of large arteries and for transient ischemic attacks (**Table 1**).

Moreover, the authors found an exposure-response relation between O_3 exposure and ischemic stroke. An increase in association in men with several cardiovascular risk factors (smoker, diabetes, dyslipidemia, and hypertension) was also observed (**Fig. 2**).

This work was associated with a second one adding stroke and myocardial ischemia (Henrotin et al., 2010). This work (Henrotin et al., 2010) evaluated the effects of short-term exposure to O_3 on ischemic heart and cerebrovascular disease, downtown the city of Dijon (150 000 inhabitants) for a long period from 2001 to 2007.

Table 1 Odds ratios describing the relationship between ischemic stroke subtypes (TOAST classification) and pollutant concentrations from 1-day lag, Dijon stroke registry (France), (Henrotin et al., 2007) from 1994 to 2004

	Ischemic stroke: large arteries (n = 262)[†]		*Ischemic stroke: lacunar (n = 193)*[‡]		*Ischemic stroke: cardioembolic (n = 208)*[§]		*Ischemic stroke: transient ischemic attacks (n = 172)*[¶]	
Pollutant	*ORa (95% CI)*	p *Value*	*ORa (95% CI)*	p *Value*	*ORa (95% CI)*	p *Value*	*ORa (95% CI)*	p *Value*
SO_2	1.088(0.785 to 1.508)	0.61	0.831 (0.605 to 1.412)	0.25	0.974 (0.707 to 1.344)	0.87	0.966 (0.659 to 1.416)	0.86
NO_x	0.988 (0.863 to 1.130)	0.20	0.896 (0.771 to 1.042)	0.15	1.028 (0.888 to 1.190)	0.70	0.860 (0.742 to 1.022)	0.09
O_3	1.140 (1.013 to 1.288)	0.03	1.077 (0.931 to 1.245)	0.31	1.113 (0.970 to 1.278)	0.12	1.206 (1.038 to 1.401)	0.01
CO	0.998 (0.994 to 1.003)	0.64	0.997 (0.992 to 1.001)	0.24	0.999 (0.995 to 1.004)	0.91	0.999 (0.995 to 1.004)	0.91
PM_{10}*	0.938 (0.769 to 1.144)	0.52	0.881 (0.698 to 1.111)	0.28	1.101 (0.895 to 1.354)	0.36	1.095 (0.833 to 1.432)	0.50

ORa: adjusted odds ratio for minimum temperature (1-day lag), maximum relative humidity (1-day lag), holidays (0-day lag), influenza epidemics (0-day lag), and for an increase of 10 μg/m^3 of pollutant.

*PM_{10}: data from 1999 to 2004:

[†]$n = 145$;

[‡]$n = 93$;

[§]$n = 92$;

[¶]$n = 80$

Daily levels of urban O_3 pollution, the incidence of first-ever, recurrent, fatal and nonfatal ischemic cerebrovascular events (ICVE) and myocardial infarction (MI), were correlated using a case-crossover design. The authors analyzed 1574 ICVE and 913 MI that occurred in Dijon, France from 2001 to 2007. SO_2, NO_2, Co, and PM_{10} were used to create bi-pollutant models. Using the adjusted OR, the effects of O_3 exposure were calculated for every 10 μg/m^3 increase in pollutants in multivariate logistic models adjusted for temperature, humidity, flu outbreaks and holidays.

The authors found a significant association between exposure to O_3 and recurrent ICVE with a 3-day lag (OR = 1.115; 95% CI 1.027 to 1.209). The direction and magnitude of the association between exposure to O_3 and recurrent MI were similar but not statistically significant. For incident events, the authors detected only a nonsignificant association for ICVE with a 2-day lag (OR = 1.041; 95% CI 0.996 to 1.089). In the subgroup analysis for ICVE, the authors observed an increased association with cardiovascular risk factors (OR = 1.523; 95% CI 1.149 to 2.018). For MI, the authors found an association with O_3 when hypercholesterolemia was present (OR = 1.111; 95% CI 1.020 to 1.211) and the association became stronger with the number of cardiovascular risk factors. The authors found a marked dose-response relationship.

The authors concluded that recurrent ICVE and MI could be triggered by short-term exposure to even low levels of O_3, especially among subjects with severe vascular risk factors, defining the population at risk, information useful for practitioners.

Recent metaanalyses suggest a tight temporal association between exposure to air pollution and admission to hospital for stroke or death induced by stroke (Shah et al., 2015).

Recent and worldwide data: three recent metaanalyses explored long-term exposure to air pollution and stroke events and found suggestive evidence of an association between long-term exposure to fine particles and stroke incidence. Stafoggia et al. (2014) found suggestive evidence of an association between $PM_{2,5}$ exposure and stroke incidence in European countries, although the main estimate did not reach statistical significance. A 5-μg/m^3 increase in annual $PM_{2,5}$ exposure was associated with 19% increased risk of incident stroke [hazard ratio (HR) = 1.19, 95% CI: 0.88 to 1.62]. Similar findings were obtained for PM_{10}. The results were robust to adjustment for an extensive list of cardiovascular risk factors and noise coexposure. The association with $PM_{2,5}$ was apparent in subgroups, among those ≥60 years of age (HR = 1.40, 95% CI: 1.05 to 1.87), among never-smokers (HR = 1.74, 95% CI: 1.06 to 2.88), and among participants with $PM_{2,5}$ exposure <25 μg/m^3 (HR = 1.33, 95% CI: 1.01 to 1.77).

Scheers et al. (2015) published a metaanalysis including 20 studies from three different continents. The authors found a pooled HR for each 10-μg/m^3 increment in PM_{10} at 1.061 (95% CI: 1.018 to 1.105) and 1.080 (95% CI: 0.992 to 1.177) for overall stroke events and stroke mortality, respectively. A stratified analysis by continent revealed that the association between stroke and long-term PM_{10} exposure was positive in North America (HR = 1.062 [95% CI: 1.015 to 1.110] and Europe (HR = 1.057 [95% CI: 0.973 to 1148]), but studies in Asia (HR = 1.010 [95% CI: 0.885 to 1.153]) showed a high degree of heterogeneity. In regard to exposure to $PM_{2,5}$ (Europe and North America combined), the HR for a 5-μg/m^3 increment was 1.064 (95% CI: 1.021 to 1.109) and 1.125 (95% CI: 1.007 to 1.256) for stroke events and mortality, respectively.

Environmental air pollution emerges as a leading risk factor for stroke worldwide (Feigin et al., 2016a).

Feigin et al. (2016a) used data from the Global Burden of Disease Study 2013 to estimate the population-attributable fraction (PAF) of stroke-related disability-adjusted life-years (DALYs) associated with potentially modifiable environmental, occupational, behavioral, physiological, and metabolic risk factors in different age and sex groups worldwide and in high-income countries and low-income and middle-income countries, from 1990 to 2013.

They evaluated attributable DALYs for 17 risk factors (air pollution and environmental, dietary, physical activity, tobacco smoke, and physiological) and six clusters of risk factors by use of three inputs: risk factor exposure, relative risks and the theoretical minimum risk exposure level. For most risk factors, they synthesized data for exposure with a Bayesian metaregression method or spatial-temporal Gaussian process regression.

Globally, 29.2% (95% UI 28.2–29.6) of the burden of stroke was attributed to air pollution.

In consequence, air pollution has emerged as a significant contributor to global stroke burden, especially in low-income and middle-income countries, and therefore reducing exposure to air pollution should be one of the main priorities to reduce stroke burden in these countries (Feigin et al., 2016a).

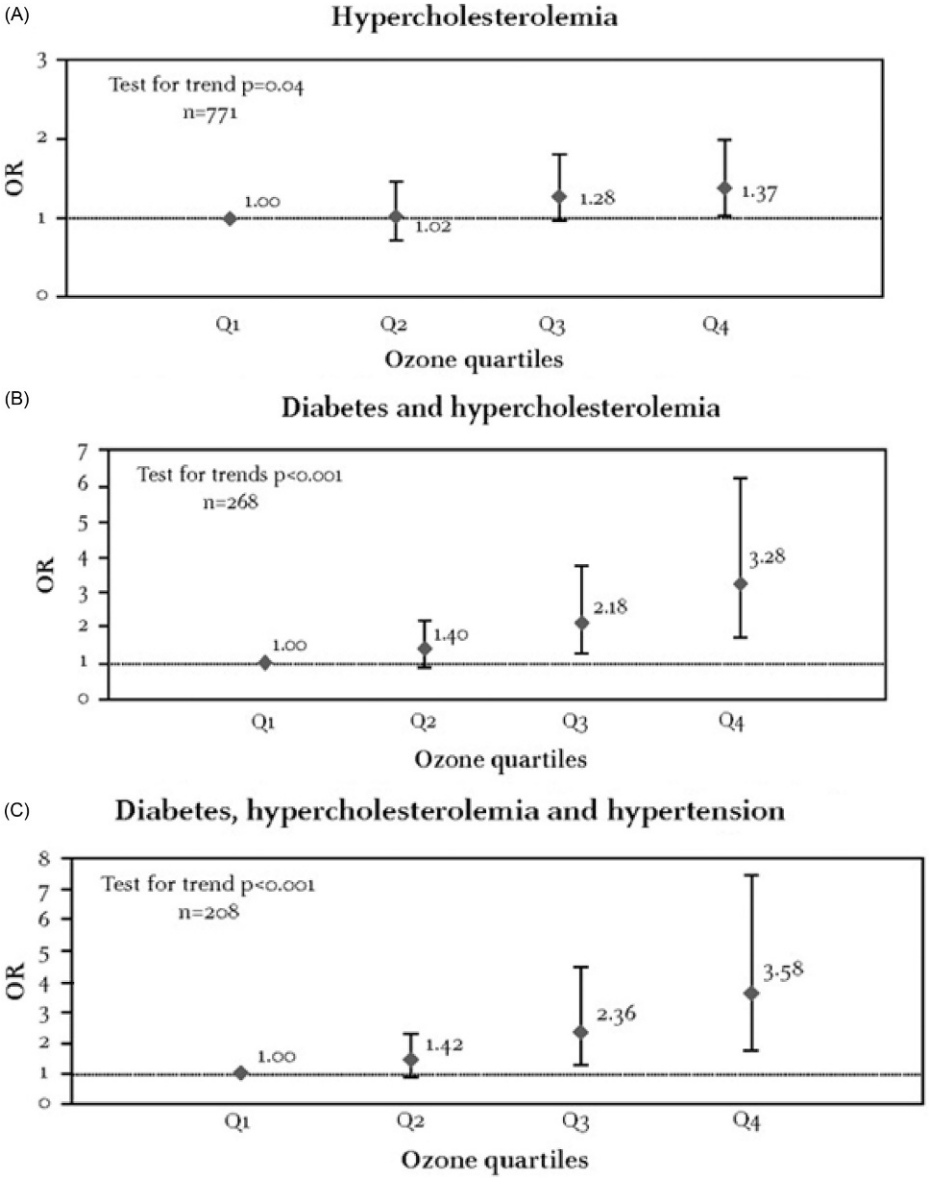

Fig. 2 Dose-response relationship (with 1-day lag) between O_3 quartiles and ischemic events according to the number of vascular risk factors (Henrotin et al., 2007).

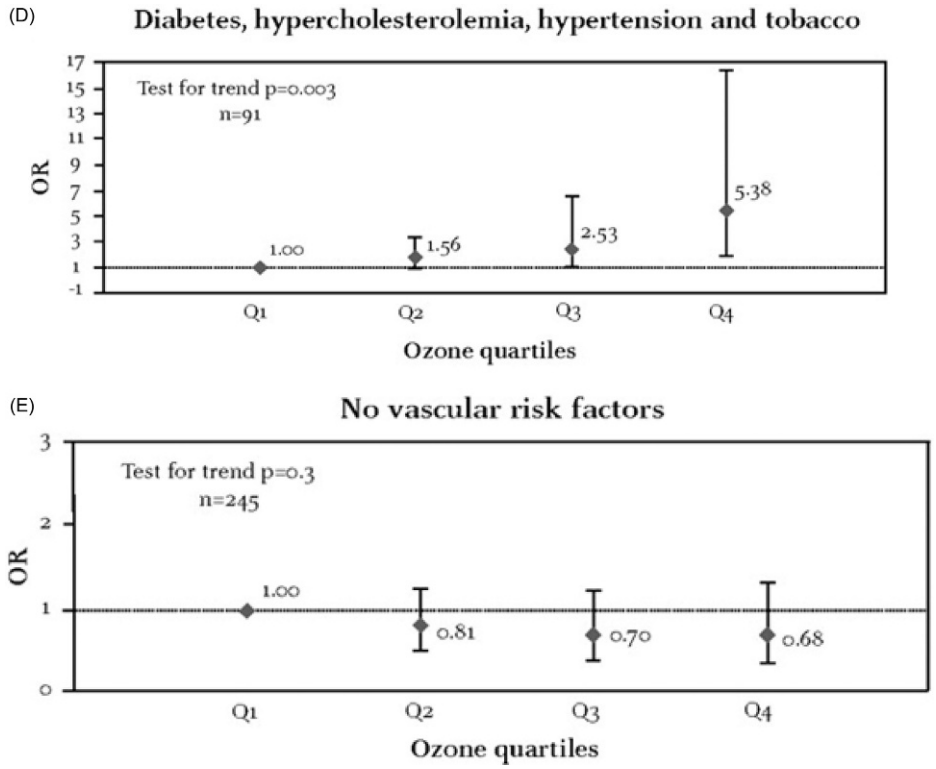

Fig. 2, Cont'd

Mechanisms of Stroke Induced by Air Pollutants

The mechanisms by which air pollution has multiple systemic effects in human, in particularly on the cardiovascular system and the brain, are not fully elucidated.

The penetration routes of the air pollutants into the body are questioned (Peters et al., 2006). Although pulmonary inhalation is the obvious exposure route, the nose-to-brain way has become of great interest. Translocation to the brain of very small particles via the olfactory epithelium in the nose has been demonstrated through experimental rodent studies with engineered nanoparticles (carbon nanoparticle, copper oxide nanoparticles, silver nanoparticles) and metals (e.g., radiolabeled isotope Mn, manganese oxide and chloride) (Heusinkveld et al., 2016; Brenneman et al., 2000).

Evidence of brain inflammatory processes and increase of biomarkers of inflammation come from laboratory studies in the brain of mice (Calderón-Gareidueñas et al., 2016; Campbell et al., 2005; Gerlofs-Nijland et al., 2010) and rats (Gerlofs-Nijland et al., 2010; Patchin et al., 2016). Levels of pro-inflammatory cytokines (IL-1a, TNF-a) and immune-related transcription factor NF-kB were increased in the brain tissue of mice exposed to small particles (Particulate Matter) compared with control animals. Using a nose-only exposure in rats, diesel engine exhaust and silver nanoparticles were able to induce inflammation in different brain regions (Patchin et al., 2016) or microglial activation (Patchin et al., 2016).

Inhalation of ozone has led to the lung-brain axis hypothesis: experimental data showed that cerebral microglia remains activated 24 h after an ozone exposure in rats, suggesting an on-going signal from lung to brain. Circulating cytokine-independent signals present in serum, that elevate the brain pro-inflammatory background, would be involved. Do these signals also act on the vascular endothelium? The release of vasoactive factors into the systemic circulation after pollutant inhalation (notably ozone), has been assessed in mice; these factors are capable of impairing vaso-relaxation to ACh via a CD36-dependent signaling mechanism. The presence of circulating factors appears to be independent of CD36 and inflammatory responses (Robertson et al., 2013). PM pulmonary inhalation also provokes a release locally and into the systemic circulation of bioactive species, pro-oxidative (i.e., reactive oxygen species) or inflammatory (i.e., cytokines such as IL-6 and TNF), and endothelins (vasoactive hormones) (Franchini and Mannuci, 2011).

Other pathophysiological hypotheses have been suggested to explain the increased risk of cardiovascular diseases including effects on: blood coagulation, endothelial dysfunction, systemic inflammatory responses, acute arterial vasoconstriction (Wellenius et al., 2013; Newman et al., 2015), or a propensity for arrhythmia (Link et al., 2013; Newby et al., 2015).

More recent works demonstrate that increase of air pollutant concentrations are significantly associated with methylation on tissue factors, interferon-gamma and interleukin 6 hypermethylation, toll-like receptor 2 hypomethylation and intercellular adhesion molecule 1.

A possible consequence is that changes in methylation may affect some genes (Bind et al., 2016). DNA methylation may be a consequence of air pollution.

Secondly, DNA methylation may be modulated by temperature and relative humidity. Therefore, temperature and relative humidity may also interact with air pollution and produce stronger adverse events (Bind et al., 2016).

Third, air pollution is associated with a shortening of telomere length. Telomere can be considered as the cell memory of exposure to oxidative stress and inflammation, and environmental air pollution is an important determinant of telomere length; these facts might provide new ways to understand the relationship between air pollution and cerebral vascular disease and will help to promote new preventive strategies (Haycock et al., 2014).

Conclusion

Ambient air pollution contributes substantially to the global burden of disease. (Calderón-Gareidueñas et al., 2016; Feigin et al., 2016a). This reflects both demographic and epidemiologic trends and increasing levels of air pollution in low- and middle-income countries, portending increasing burdens absent major reductions in level of ambient $PM_{2.5}$ (Feigin et al., 2016a). The challenges for future reductions in the burden of disease attributable to air pollution are considerable. Exposure to ambient air pollution and its associated burden of disease can potentially be lowered for entire populations via policy action at the national and subnational levels without the need to modify individual behaviors (Feigin et al., 2016a; Feigin et al., 2016b).

For the first time, Feigin et al. (2016a) showed that air pollution has emerged as one of the leading contributors to stroke burden worldwide, accounting for almost 30% of stroke-related disability-adjusted life-years. Moreover, global warming is modifying the impact of air pollution on stroke. Together, these considerations provide a global imperative for action to control and reduce air pollution (Feigin et al., 2016b).

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Environmental Risk Factors for Stroke and Cardiovascular Disease

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Introduction

The environmental neurology approach to diseases is systematic, holistic, and translational. This approach exists practically as long as the field of medicine itself; indeed, Hippocrates (460-370 BC) emphasized environmental factors in his "*Treaty of Water, Air, and Places*" (Hippocrates et al., 2003; Le Moal and Reis, 2011). To define the environment, we adopt words of Albert Einstein "*The environment is everything that isn't me.*" To this notion, we introduce the concept of environmental risk factors. A systematic approach should explore the multiple dimensions of these factors: their nature, sources, carriers, spatiotemporal characteristics, interactions (multicausality), and finally, health impacts (protective/harmless/noxious) (Reis and Roman, 2007).

In the case of stroke, for example, environmental risk factors can be classified in three categories depending on the nature of the risk (or agent), the milieu conveying the agent (e.g., air, water, soil, food), and the affected population (with a special interest for vulnerable population like children, pregnant women, and seniors). Environmental risk factors also have a geographical or spatial dimension (e.g., altitude, climate). In the previous chapter, we investigated the association of air pollution and stroke, as a well-recognized example of the role of the milieu. Here, we will study environmental factors from another point of view: their nature, be it physical, chemical, or biological. The impact of sociopsychological factors like stress and human interaction is considered elsewhere in this book.

Stroke, as a leading cause of mortality and morbidity around the world, is a major issue in the public health field. Neuroepidemiological studies currently underway are exploring all possible causes and risk factors for stroke. Among them, "new" environmental risk factors are being studied, taking into account the famous Hill's criteria (Hill, 1965). Nowadays, "biologically plausible" mechanisms have gained in importance and should be investigated extensively (Causality Meeting, 2016). In this chapter, we focus on some of the major targets and mechanisms underlying the risk factors involved in stroke: the heart, the vascular endothelium (container), and the blood (content).

Physical Environmental Risk Factors for Stroke

Environmental and Occupational Noise

The concern of the biomedical community and regulatory bodies about the cardiovascular impact of noise developed slowly in the 1980s. The health effects on adults of exposure to transport noise (road, air, and rail) were demonstrated first for hypertension and ischemic heart disease (Babisch, 2006). Addressing the impact of noise in stroke is quite recent; the first relevant publications appeared in 2010. Now, several years later, there is an agreement to consider that traffic noise is the second most important environmental risk factor for stroke in urban societies, after air pollution (Münzel et al., 2017). Comparative burden of disease studies have shown that environmental noise is ranked second in terms of disability-adjusted life years lost (DALYs) in Europe (Stansfeld, 2015). Several recent reviews have addressed the epidemiological evidences (Belojević and Paunović, 2016; Basner et al., 2015; Münzel et al., 2014) and the various potential mechanisms involved (Stokholm et al., 2013).

The impact of occupational noise exposure is controversial. No association has been shown between occupational noise exposure and stroke; risk has largely been attributed to lifestyle (Stokholm et al., 2013). However, in 2015 the Swedish Council

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on Health Technology Assessment stated that people who are exposed to noise at work "*more often develop stroke over time than people who are not subjected to noise at work*" (Swedish Council on Health Technology Assessment, 2015).

The sources of environmental noise include trains, road traffic, and aircraft. The impact of noise depends on the nature of the source (rail < road < air) (Münzel et al., 2014), its time of occurrence (daytime versus night), its physical characteristics (e.g., intensity, chronic versus acute), and the exposed population (vulnerability related to age).

Epidemiological studies about the relation between noise and stroke are sometimes contradictory. Long-term air pollution increases stroke risk, regardless of concomitant noise pollution (Hoffmann et al., 2015). Moreover, some epidemiological studies have found no associations; for instance, a study in a Danish cohort of individuals aged 50–64 and another in the Swiss national cohort (Huss et al., 2010) found no association between aircraft noise and stroke mortality (cited in Floud et al., 2013). However, in a Danish cohort of 57,053 people, residential road traffic noise exposure was associated with a higher risk (HR) for ischemic stroke: 14% HR per 10 dB higher exposure to noise for all participants and 27% HR per 10 dB higher exposure to noise for participants above 64.5 years. Stroke risk increased in a dose-dependent manner at exposure levels of 60 dB among the oldest participants (Sørensen et al., 2011). A cross-sectional study from six European countries used data from the Hypertension and Environmental Noise near Airports study including 4712 participants with 276 stroke cases. Members of the study population lived near the major airports in London, Amsterdam, Stockholm, Milan, Berlin, and Athens. An association between nighttime average aircraft noise and heart disease/stroke was found (after adjustment for sociodemographic confounders) for participants who had lived in the same place for ≥20 years (odds ratio: 1.25 per 10 dB). The association between 24-h average road traffic noise level and heart disease/stroke was also significant although the association was not adjusted for air pollution (Floud et al., 2013). In a population of 3.6 million people living near Heathrow airport, a study based on hospital admissions and mortality showed a significant excess risk of stroke and CVD. The noise intensity was investigated by grouping daytime aircraft noise and road noise into six categories from ≤51 to >63 dB in increments of 3 dB and nighttime aircraft noise into 5 dB categories (≤50, >50–55, and >55 dB). In multiple adjustment models, the relative risk (RR) for stroke was 1.24 for daytime aircraft noise (>63 dB v ≤51 dB) and 1.29 for nighttime noise (>55 dB v ≤50 dB). The RR of mortality was similar to those for hospital admissions at the higher noise levels. Thus the RR is the highest with high daytime noise levels and nighttime aircraft noise (Hansell et al., 2013). An 2009 U.S. study, recruited 6 million seniors (>65 years) living near one of the 89 largest airports to evaluate the association between airport-related noise and the risk of hospital admission for CVD and heart failure. Despite the study's methodological limitations, a statistically significant association between exposure to aircraft noise and risk of hospitalization for CVDs was shown (Correia et al., 2013).

One explanation of these contradictory results is related to the difficulty to deal with two major confounders, "indoor noise" produced by the neighborhood in a residential area (Tenaillon et al., 2015) and environmental (ambiant) air pollution, as this pollution shares many common sources with environmental noise (Tétreault et al., 2013).

The pathological effects of noise involve many functions: sleep disruption, disautonomic perturbations (e.g., increases of blood pressure and heart rate), stress reactions (release of stress hormone), and increase of oxidative stress, which in turn may result in vascular endothelial dysfunction and arterial hypertension (Münzel et al., 2014).

Altitude, Air Planes, and Erythropoietin

Altitude is a geophysiological condition which impacts all living organisms. Physical and climatological characteristics, decrease of available oxygen, lower atmospheric pressure and lower temperature, more frequent winds, and a larger exposure to solar radiation are determining Life. These conditions vary according to the altitude, low (<2000 m), moderate (2000–2999 m), high (3000–4999 m), and extreme (>5000 m) and also to the latitude.

Adaptation to high-altitude challenges the brain. As hypoxemia can be deleterious for cellular adaptation and survival, the regulatory homeostasis favors all mechanisms related to convective oxygen transport (cardiovascular, respiratory, and hematopoietic functions) that will counteract a possible cellular hypoxia (Petousi and Robbins, 2014). The first adaptative mechanism is hyperventilation which has the quickest effect. An increase in cerebral blood flow up to 31% of values measured at sea level can occur in few days (3–7) of a subject's transition to high altitudes. The return to sea-level base values follows, depending on the degree of the hypoxic stimulus and on the cerebrovascular sensitivity to hypoxia and carbon dioxide (Bor-Seng-Shu et al., 2012). The heart function is modified typically with tachycardia and no change in stroke volume but a slight temporary blood pressure increase. After a few days of adaptation, cardiac output returns to normal (Naeije, 2010). Supply of oxygen is favored by an adaptative release at the tissue level and an increase of the red blood cells (RBC) production. The direct effect of production is an increase of the hematocrit and the blood viscosity. Other factors contribute also to increased coagulability: namely, increases in platelets (mean platelet volume), erythrocyte aggregability, and plasma viscosity (Bor-Seng-Shu et al., 2012; Alper et al., 2009). Protection of the cellular metabolism involves hypoxia-inducible factor(s) (HIFs) (capable of inducing protective metabolites and proteins) (Kumar and Choi, 2015) as well as chemical agents such as nitric oxide (NO), carbon monoxide (CO), eicosanoid products, oxygen-derived free radicals, endothelins, K^+, H^+, and adenosine (Bor-Seng-Shu et al., 2012), and an control of key enzymes (Na-, K-ATPase). Oxygen-derived free radicals and H_2O_2, NO, and oxidized glutathione are the signaling messengers that make Na-, K-ATPase systems "oxygen-sensitive" (Bogdanova et al., 2016).

The number of exposed people varies; thus, more than 140 million people around the globe reside at high altitude (≥3000 m) in Africa (Ethiopian Highlands; Kilimanjaro), Asia (Himalaya Mountains and Tibet), and the American continents (Rocky Mountains and Andes Mountain Range) (Ronen et al., 2014). Two types of population must be distinguished, the native

mountain-born and the sea-level-born people who need to adapt. In fact, altitude stress and its neuro-cardiovascular consequences differ markedly depending on the cerebrovascular regulation and on the capability to adapt.

Impairment of the cerebral autoregulation is the major risk for travelers who rapidly reach altitudes above 2500 m. They may manifest several syndromes: altitude headache (HAH), acute mountain sickness (AMS), high-altitude cerebral oedema (HACO/HACE), and high-altitude pulmonary oedema (HAPO/HAPE) (Wilson et al., 2009; Yanamandra et al., 2014). Although rarely reported, acute stroke is possible during short-term visits to high-altitude regions (Chan et al., 2012). Thus, stroke or stroke-like episodes have been described in association with high altitude for the first time in 1895 (Szawarski et al., 2012). Differential diagnosis must consider cerebral venous thrombosis (Chan et al., 2012) and HACO, which can mimic stroke and provoke deficits; those resolve with the resolution of cerebral oedema (Yanamandra et al., 2014). In case of stroke, an asymptomatic patent foramen ovale should be excluded (Szawarski et al., 2012; Murdoch, 2015).

High-altitude impact during long-term stays (e.g., several months) has been investigated notably among soldiers stationed in the Himalaya Mountains. The relative risk for stroke above altitudes of 3000 m is significantly increased (Jha et al., 2002) by a factor as high as 10-fold (Niaz and Nayyar, 2003); extreme altitudes (over 5000 m) are associated with a 30-fold risk of spontaneous vascular thrombosis (Anand et al., 2001). Polycythaemia is clearly a major risk factor.

The effect of altitude on native populations is a different issue. Several publications (USA, Kashmir, South America, India) have shown that the incident stroke is lower in these populations compared to populations living at sea-level and lower altitudes (Mahajan et al., 2004), to the contrary of a Saudi Arabian study (al Tahan et al., 1998). A study in Cuzco (Peru), located at 3380 m above sea level, showed a crude prevalence ratio of 6.47 per 1000 (cohort of 3246 individuals). The prevalence risk was related to age, polycythemia, high alcohol consumption, residence's area (down-town versus suburban), blood pressure, and increased hematocrit. These two last parameters may vary following altitude (Jaillard et al., 1995). In the large Swiss National Cohort, a clear dose–response relationship between lifetime altitude exposure and mortality was demonstrated: mortality from coronary heart disease and stroke decreased with increases of altitude (22% per 1000 m and 12% per 1000 m, respectively). Switzerland has specific characteristics: altitude of residence between 200 and 2000 m and minimal changes in geographic latitude. However, this study has several limitations related to the methodology and the impossibility of collecting data on individual risk factors (e.g., atherosclerosis). The conclusion, that being born at higher altitude (although the range is not clear) has a protective effect, needs a clear confirmation. Possible physiological explanations could be found: solar radiation and Vit D, less air pollution, genetics, etc. (Faeh et al., 2009). In any case, these studies point to acclimatization, which differs from adaptation, as it is a long-term population-based process, involving both genetic and gene-environmental mechanisms (Petousi and Robbins, 2014). All populations living at high altitude do not show the same acclimatization. For example, at similar high altitudes, Tibetans have significantly lower hemoglobin concentrations than their Andean or Han Chinese immigrants counterparts (Petousi and Robbins, 2014); they also suffer less from chronic mountain sickness (CMS). Stroke risks should likewise be different.

The stroke's risk related to altitude during airplane travel (potentially associated with hypobaric and hypoxic conditions) is low even in patients with symptomatic carotid occlusion (Reynolds et al., 2014). Recently, the incidental risk has been reevaluated; it is now estimated to be 1 stroke in 35,000 flights (Álvarez-Velasco et al., 2016), or less than one in a million passengers (Humaidan et al., 2016). There is no difference between short-haul (<2 h) and long-haul flights (>2 h) for healthy individuals (Reynolds et al., 2014).

The role of blood erythropoietin (Epo) in the adaptation to high-altitude hypoxic exposure in humans led to its use as medicine and doping in competitive sports (Robinson et al., 2006). Epo causes a rise in red cell mass accompanied by elevated hemoglobin concentrations and hematocrit. Its side effects have been documented (Pope et al., 2014).

Climatic and Meteorological Variables: Seasonality and Temperature

In the context of an increasing interest in the health effects of climate change, climate and weather characteristics are now scrutinized as possible risk factors for stroke.

When considering the admission to a hospital or the mortality, the incidence of ischemic stroke seems to be cyclic during the year. Seasonality has been observed worldwide (Chen et al., 2013a) although some studies have reported no seasonality; for instance, a study in Taiwan (Lee et al., 2008). Furthermore, the peak season differs for different countries. In Japan (Takashima stroke register), an excess of ischemic stroke fatality occurs in spring followed by winter (after adjusting for age, gender); the rates are more when compared to during summer (Turin et al., 2009). In the United States, an epidemiological study conducted in the VA hospitals showed that the peak occurrence for ischemic stroke was mid-May, with the lowest occurrence in early December. The authors emphasized that, "Neither the region (i.e., climate) nor the race of the patient substantially modified the seasonal trend" (Oberg et al., 2000). In Melbourne, Australia, ischemic stroke occurrence was significantly higher during spring than summer (incidence rate ratio (IRR) 1.14) (Mao et al., 2015). Peak season was in winter in Hong Kong (Goggins et al., 2012) and in August in Eastern Turkey, with the lowest occurrence in spring (Anlar et al., 2002). Diverse explanations for these patterns have been proposed, including lunar patterns (Mao et al., 2015), rainfalls related to the El Niño Southern Pacific Oscillation (ENSO) (Kintoki Mbala et al., 2016), and in the case of global mortality, relations to air pollution, and meteorological variables (Qian et al., 2010).

The question addressed in a 2010 paper, "What is it with weather and stroke?" (McArthur et al., 2010), remains crucial. Most evidences suggest that cold and hot apparent temperatures (which consider both air temperature and humidity) are associated with stroke mortality, as shown by the eight Large Chinese Cities Study (Chen et al., 2013a). The results show a U-shaped relationship,

with increased risk at extreme high or low temperatures. The potential effect of cold temperature might last more than 2 weeks in contrast to the more immediate effect of hot temperature. However, the burden of stroke mortality is different. In the six large Chinese cities (2007–2013) the stroke burden was caused by cold temperature and was higher in males and seniors (Yang et al., 2016). The same patterns are known for mortality by all causes (Gasparrini et al., 2015; Medina-Ramón et al., 2006). Several subpopulations are particularly susceptible to temperature extremes, for example, patients with atrial fibrillation (Medina-Ramón et al., 2006). The relationship between heat waves and stroke has been addressed by several articles. Heat waves have a strong adverse effect on stroke mortality with an excess mortality of 6%–52% (Chen et al., 2015). A multicity case-only analysis of 50 U.S. cities showed that extremely high-temperature days posed a higher relative stroke mortality risk compared to pneumonia and CVD (Medina-Ramón et al., 2006). Heat wave (with a 2-day lag) is significantly associated with the risk of hospitalization for stroke (odds ratio [OR] 1.173) with more significance for men and over 80 years, in a study conducted in Pennsylvania (Ha et al., 2014). A Chinese study pointed to a clear spatial pattern of stroke mortality during heat waves; the risk was higher in rural areas (Luhe district) compared to the urban area of Nanjing, probably because of a higher vulnerability of the populations; this vulnerability seems to be linked to the socioeconomic level, the air condition availability, the population's age, and the medical facilities' access (Chen et al., 2015). Obviously, further works are needed to assess the real impact of weather (characterized by temperature, humidity, sunshine duration, etc.) on stroke subtypes and pathogenesis (hemorrhagic/stroke, lacunar/atherothrombotic/cardioembolic).

Biological explanations involve complex interactions between endothelium function, vasomotricity, blood pressure, brain demand for oxygen, and change of the quality of blood components (thrombogenic factors) and blood properties (viscosity). For example, cold induces vasoconstriction while heat promotes dilation (Chen et al., 2013a).

Geomagnetic Storms Can Trigger Stroke: A New Environmental Risk Factor

The term "geomagnetic activity" concerns natural variations in the geomagnetic field (Feigin et al., 2014). A geomagnetic storm is a temporary disturbance of the Earth's magnetosphere caused by a solar wind shock wave and/or cloud of magnetic field which interacts with the Earth's magnetic field; these are prominent during the solar maximum phase of the solar cycle. The sun has a solar cycle which averages 11 years in length; at the end of each cycle, the polar magnetic field of the sun reverses. During these cycles, there is an increased solar activity, which generally has a peak in the middle of the solar cycle. During the solar maximun, the increase of sunspots and the solar storms produce the emission of large quantities of electromagnetic and particle radiation. Theses events are known to disrupt technology such as power grids, magnetic compasses, damage satellite microchips and disturb radio and radar transmissions. The solar minimum refers to a relatively low solar activity. This takes place 5–6 years after the solar maximum.

Levels of geomagnetic activity are commonly measured by the Ap Index. Of all locales, Auckland (Feigin et al., 2014) had the most data collected during the solar maximum years, and even had high levels of geomagnetic activity including during 2003, a solar minimum year. Oxfordshire and Melbourne (Feigin et al., 2014) had data collected during solar minimum years only. However, the solar minimum that Oxfordshire data was collected for had high levels of geomagnetic activity. Melbourne (Feigin et al., 2014) data were collected during times of very low geomagnetic activity. The remaining Northern hemisphere cities had more data collected during solar minimum years: Dijon (8 out of the 11 years) and Northern Sweden (12 of the 20 years). Perth had data collected equally over solar maxima and solar minima. It is important to note that data collection for the four cities overlapped during very low levels of geomagnetic activity (1996–1997).

Overall, geomagnetic storms (Ap Index 60+) were associated with a 19% increase in the risk of stroke occurrence (95% CI, 11%–27%). The triggering effect of geomagnetic storms was most evident across the combined group of all strokes in those aged <65 years, increasing stroke risk by $>50\%$: moderate geomagnetic storms (60–99 Ap Index) were associated with a 27% (95% CI, 8%–48%) increased risk of stroke occurrence, strong geomagnetic storms (100–149 Ap Index) with a 52% (95% CI, 19%–92%) increased risk, and severe/extreme geomagnetic storms (Ap Index 150+) with a 52% (95% CI, 19%–94%) increased risk (test for trend, $P<2\times10-16$) (Feigin et al., 2014). It will be necessary to evaluate the impact of geomagnetic storm on air pollutants, as some researchers have suggested that they may act together on the brain and cardiovascular system (Stoupel, 2016).

Ionizing Radiations

Ionizing radiation has been assessed as a risk factor for cardiovascular diseases and sometimes for stroke in different exposure's conditions: military usage, occupational settings (miners), medical treatment (radiotherapy), and rarely in usual environmental exposures. Epidemiological studies have confirmed the radiation-related risks of noncancer outcomes. The stroke risk is related to the conditions of the exposure and the dose of the irradiation.

High doses of radiation for specific solid tumors and/or blood cancers (lymphoma and leukemia), involving specific targets (brain, neck, heart) are associated with an excess of deaths (heart disease or stroke) and an increased risk of stroke (Shimizu et al., 2010). The same risk has been shown among atomic bomb survivors in the Japanese survivors cohort. Radiation exposure has been associated with some CVDs, hypertension (Ozasa et al., 2017), and the risk of atherosclerotic disorders (Ozasa et al., 2016).

The cardiovascular effect of lower-radiation doses has recently been assessed. A systematic review and meta-analysis dedicated to moderate- and low-level whole-body ionizing radiation exposure found an excess population risks (mortality) and for all circulatory diseases. The risk ranged from 2.5%/Sv for France to 8.5%/Sv for Russia (Little et al., 2012). Between 1950 and 2003,

the Life Span Study registered about 9600 deaths related to stroke and 8400 related to heart failure in the Japanese cohort of atomic bomb survivors (86,611 members). For stroke, the estimated excess relative risk (ERR) per gray was 9% on the basis of a linear dose–response model. Individual radiation doses have been estimated and ranged from 0 to >3 Gy (86% received <0.2 Gy). The authors concluded that radiation may increase the rates of stroke and heart disease at moderate dose levels (mainly 0.5–2 Gy) although the results below 0.5 Gy were not statistically significant (Shimizu et al., 2010).

Two cohorts of miners bring precise data about occupational irradiation and notably radon. The Wismut cohort uranium mining company in Eastern Germany included 58,987 men from 1946 to 1989; 5141 deaths from heart diseases, and 1742 deaths from CVDs were observed. There was no evidence for mortality from CVDs when the ERR per unit of cumulative exposure to radon in working level months was analyzed (Kreuzer et al., 2010). Canadian authors came to the same conclusion in the Newfoundland fluorspar miners' cohort (2070 miners followed from 1950 to 2001) (Villeneuve et al., 2007). However, there are some data and clues that point to a relation between radiation and premature development of atherosclerosis (Villeneuve and Morrison, 1997; Xu and Cao, 2014; Simonetti et al., 2014).

Chemical Environmental Risk Factors for Stroke

The millennium years (1995–2005) were accompanied by a huge change of the dominant paradigm; environmental risk factors for CVDs had become a new field of research. The purpose was to better analyze and understand the behind-the-scene agents, notably the effects of various metals, chemicals, and obviously air pollution (Navas-Acien et al., 2005). These chemical compounds have been investigated as they are conveyed by multiple milieus, food, air, and water and related to multiple environmental sources and occupational exposures.

Arsenic, a common metal acting as poison and carcinogen, is nowadays considered as a risk factor for CDVs. In a systematic review of the epidemiologic evidence, in general populations, Navas-Acien et al. (2005) showed a significant increase of the RR for stroke which ranged from 0.69 to 1.53. In an occupational population the RR for stroke mortality ranged from 0.30 to 1.33. However, the best evidence has come from Taiwan; arsenic levels in drinking water were found to be associated with CVDs, in particular with stroke, despite methodological limitations. The RR between the highest and the lowest arsenic exposure's categories ranges from 1.19 to 2.69 for stroke. This is consistent with a role for high arsenic exposure in atherosclerosis. This review and a later one (Moon et al., 2012) pointed to the inconclusiveness of the chronic low-dose exposure to arsenic. Risk assessment, based on epidemiological studies (cohort, case-control cross-sectional, and ecological studies) from the United States, Taiwan, Bangladesh, and China, proposes a no-observed-adverse-effect level of 100 μg/L for arsenic in water, equivalent to an iAs dose of 0.009 mg/kg-day (Tsuji et al., 2014).

Cadmium is the second metal studied as risk factor for CVDs. In their 2013 review of cadmium studies, the team of Navas-Acien gave a pooled RR for stroke at 1.18 although the number of studies with stroke outcome was small (Tellez-Plaza et al., 2013a). The mechanisms of cadmium toxicity (data from animals and experimental) involve atherosclerosis (initiation and progression), increased blood pressure, and kidney damage (Tellez-Plaza et al., 2013b).

The third metal considered as a risk factor is lead. With a comparable methodology as that used for arsenic, Navas-Acien et al. (2007) have identified a positive association of lead exposure with the cardiovascular outcomes (included stroke) and obviously hypertension in general and occupational populations. Stroke mortality was higher among workers with the highest number of employment's years, under lead-exposure conditions (Navas-Acien et al., 2007).

For antimony, barium, chromium, nickel, tungsten, uranium, and vanadium, the scarcity of studies and data is insufficient to establish a role (Nigra et al., 2016).

Some organic compounds should also be considered as potential risk factors for stroke, even if reviews or meta-analyses are lacking. The use of pesticides increased significantly the risk of stroke mortality (RR = 1,91) among aerial applicator pilots versus flight instructors (Cantor and Silberman, 1999). Persistent organic pollutants (POPs) such as chlorinated organic compounds (polychlorinated biphenyls, (PCBs), dioxins, and chlorinated pesticides) were shown to increase the CVDs' risk; dioxin exposure is associated with higher risk and mortality for CVD (Chang et al., 2011; Humblet et al., 2008). The RR for stroke was 1.17 among patients discharged from the hospital after stroke (in New York State) and living near a POPs' source (e.g., waste sites) (Shcherbatykh et al., 2005). A hospitalization population-based study from the same team (Sergeev and Carpenter, 2011) confirmed this risk: the RR for stroke with hypertension was 13.4% higher in populations residing in zip codes containing or abutting POPs' environmental sources (RR = 1.134). The Prospective Investigation of Vasculature in Uppsala Seniors (PIVUS) studied background exposure to POPs. Plasma concentrations of 21 POPs (16 PCB congeners 3 organochlorine (OC) pesticides, 1 brominated diphenyl ether (BDE), and octachlorodibenzo-*p*-dioxin) were measured. During the 5-year of follow-up, 35 participants (among the cohort of 898, aged at baseline over 70) developed hospital-treated stroke. After adjusting for known stroke risk factors, the PIVUS showed that POPs' plasma concentrations significantly predicted the future risk of stroke (Lee et al., 2012).

Biological Environmental Risk Factors for Stroke

Infections and stroke

Although knowledge about the relation between infections and stroke goes back to the 19th century, with the discovery of the major infections' role in stroke (i.e., bacterial meningitis, sepsis, endocarditis), there has been an increasing interest in this topic over the last 25 years (Lindsberg and Grau, 2003). Besides the "classical" tropical infections, pediatric and adult strokes related to "minor" infections gain a greater attention.

In the tropics, the common central nervous system infections associated with stroke are cerebral malaria, Chagas' disease (which is an independent risk factor for stroke in South America), and neurocysticercosis (causing transient ischemic attacks as well as ischemic strokes). Other pathogens can be involved: bacteria (tuberculosis, syphilis, and brucellosis), viruses (hemorrhagic fevers due to arenavirus and flavivirus, Dengue fever, Japanese encephalitis, Nipah virus), HIV (in young Africans), fungi, and parasites. For example, gnathostomiasis is a cause of subarachnoid hemorrhage in south-east Asia (Sanchetee, 2009; Moghtaderi and Alavi-Naini, 2012). There have been several recent reports of clinical stroke cases linked to emerging viruses, such as Ebola and coronaviruses, including severe acute respiratory syndrome and Middle East respiratory syndrome (Bang et al., 2015). The reported frequency of cerebral infarction cases of neurocysticercosis has ranged from 2% to 12%. Extensive reviews are not available and epidemiological data are lacking. One estimation has been proposed but without a proper methodological support; the assertion is that "*Several diseases that are endemic in the tropics, can be responsible for up to 10% of the cases of strokes in adults.*" (Carod-Artal, 2007).

Although the first cases' reports of arterial ischemic stroke (AIS)/acute infantile hemiplegia related to infections issued in the seventies (Roden et al., 1975; Janaki et al., 1975) and though child infections are common, the interest in infectious pediatric strokes had developed recently, probably due to the AIS scarcity (2,4/100,000 US Children per year) (Fullerton et al., 2015). The Vascular Effects of Infection in Pediatric Stroke (VIPS) case control study demonstrated that childhood infections, including varicella zoster virus (VZV), are associated with an increased risk of AIS. Thirty-nine percent of the cases had a parental report of infection in the prior 4 weeks and the adjusted odds ratio (OR) between infection and childhood AIS was 6.5 (95% CI 3.3–13, for infection in the prior week). Based on serological evidences, the VIPS study also showed that recent herpesvirus infections (VZV, Herpes Simplex Virus HSV1, HSV2, EBV, CMV) are associated with an increased risk of AIS among children (29 days to 18 years old). Acute HSV infection (serologies suggestive of *primary* HSV infection) was present in 40% of AIS cases. Neither subjects with past infection nor reactivated cases with latent infection serological evidence were found to be at risk (Elkind et al., 2016). Although the biologic plausibility of a cerebral arteritis induced by VZV infection appears high, the plausibility of the HSV infection's role needs further investigation (Grose, 2016).

The relationship between herpesviruses and stroke has become an important issue because herpesviruses infect a large population: more than 95% of the world's population is infected by the VZV (Nagel and Gilden, 2014). Zoster can be complicated by a VZV vasculopathy (Nagel and Gilden, 2015). A recent systematic review and meta-analysis (Marra et al., 2017) pooled nine studies, published between 2009 and 2016, and found that the relative risk for stroke after a VZV infection was the highest at one postinfection month (RR of 1.78) and decreased progressively to 1.20 after one year. The RR was higher for a herpes zoster ophthalmic episode (RR: 2.05) and remain elevated for one year. The questioning of Borbinha et al. "*Should We Pay More Attention to Varicella Zoster Infection?*" should be probably, answered positively as more than 50% of strokes in young adults are cryptogenic (Borbinha et al., 2016).

Another recent review (Guiraud et al., 2010) has examined the stroke risk related to a large panel of infections in 11 case-controlled and 1 self-controlled case–series studies. The risk of stroke doubled or tripled within the first week after the infection. Thereafter, it decreased slowly, remaining still significant after 3 months. This increased risk exists regardless of the infection site (OR 2.4 for respiratory and OR 1.6 for urinary tract infection) or the agent type (OR 4.4 for bacterial and OR 2.6 for viral infection) when ORs are calculated during the first week.

Since then, new data have been issued. A meta-analysis analyzed the risk of cerebrovascular disease in the presence of IgG for *Chlamydia* pneumonia, showing that *C. pneumoniae* is strongly associated with stroke among patients with large artery atherosclerosis. However, the results (association or not) depended on the serological methods and infection's biomarkers (Chen et al., 2013b). The limits of meta-analyses are illustrated by the opposite results obtained in the case of *Helicobacter pylori*; one study showed that *H. pylori* infection contributes to the risk of ischemic stroke (Wang et al., 2012), the following study refuted it (Yu et al., 2014).

The mechanisms underlying the effects of infection on the cerebral vascular system are complex; there are several interactions and targets are numerous. The vessels can be damaged by the germs (vasculitis-angiitis), or the arterial vessel walls may develop atherosclerotic vascular lesions with inflammatory and prothrombotic alterations leading to thrombosis; embolic mechanisms can be related to infectious heart failure or directly to plasmatic circulating parasites (microembols). The outcomes are ischemic strokes (cerebral infarction) and/or hemorrhages (intracranial, subarachnoid) (Lindsberg and Grau, 2003; Sanchetee, 2009). The mechanisms of immune reactions involved in atherogenesis and the pathogenesis of stroke have also been investigated. An association of increased serum immune complexes (ICs) with stroke incidence was demonstrated. These ICs are the immune response to an acute infection preceding the stroke. *C. pneumoniae* or *Cytomegalovirus* could trigger such an immune response (Tarnacka et al., 2002).

Venomous bites and stings

"*Snake and spider bites, as well as scorpion stings envenoming are neglected diseases affecting millions of people all over the world*". This introductory sentence of Oscar H Del Brutto's article (Del Brutto, 2013) summarizes well the important burden of these issues despite the scarcity of published case reports and the absence of epidemiological studies about the neurological morbidity and mortality (including stroke) of venom exposure. Venoms are concentrates of complex mixtures of toxins targeting the central nervous system, the neuromuscular transmission, the cardiovascular system, and/or the coagulation cascade. Depending on the toxin, intracranial hemorrhage (Indian red scorpion) or ischemic strokes (snake) may occur (Del Brutto and Del Brutto, 2012).

Although the common occurrence of hymenopteran (wasp, bee) stings, primary neurologic manifestations including stroke, are rare. Around a dozen of cases have been published (Kulhari et al., 2016; Rajendiran et al., 2012; Bilir et al., 2013; Schiffman et al., 2004). No pathogenic hypotheses have been proposed.

Conclusions

Besides individual risk factors (nutrition, physical activity, behavior), we have shown that new and well-recognized, environmental risk factors are also related to stroke. Epidemiological investigations are needed to assess their importance. That is the question raised by Hankey in his 2006 publication's title: "*Potential New Risk Factors for Ischemic Stroke: What is Their Potential?*" (Hankey, 2006) although the impacts of air pollution was ignored at that moment. Nowadays, air pollution has become a major "new" environmental risk factor (Feigin et al., 2016). Should we expect the emergence of other new potent environmental risk factors? The environmental health approach is modifying our understanding of the causes of stroke (Boehme et al., 2017). Consequently the classical recommendations and strategies for prevention (Goldstein et al., 2011) have to be improved. Based on these new issues, physicians should better inform their communities and the policy makers about their respective roles and responsibilities. Together, we should act to reduce the impact of amenable environmental risk factors related to human activities, such as air pollution, noise, and chemical exposures.

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Epidemiology of Atherosclerotic Cardiovascular Disease

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Abbreviations

ASCVD	Atherosclerotic cardiovascular disease
BMI	Body mass index
CVD	Cardiovascular disease
DALYs	Disability-adjusted life years
HICs	High-income countries
IHD	Ischemic heart disease
LICs	Low-income countries
LLMICs	Low-, lower-middle-, and upper-middle-income countries
LMICs	Lower-middle-income countries
MI	Myocardial infarction
NCDs	Noncommunicable diseases
PAD	Peripheral arterial/artery disease
PVD	Peripheral vascular disease
TIA	Transient ischemic attack
UMICs	Upper-middle-income countries
WHO	World Health Organization
YLL	Years of life lost

Atherosclerotic Cardiovascular Diseases

Atherosclerosis, which is characterized by inflammation and dysfunction of the lining of blood vessels and the buildup of cholesterol, lipids, and cellular debris, can result in the formation of a plaque, which leads to the obstruction of blood flow and diminished oxygen supply to target organs (Anderson, 1999). Although atherosclerosis is widespread, the degree of it varies throughout the body with it more prevalent in certain areas. It is most common in the coronary arteries, cerebral arteries, and arteries supplying the lower limbs, in that order, with the resulting cardiovascular disease (CVD) caused by atherosclerosis differing depending on where it occurs (Davies and Woolf, 1993).

Atherosclerosis of the coronary arteries results in reduced coronary blood flow due to partial or complete occlusion, leading to insufficient oxygen reaching the heart muscle, particularly in the case of rupture or plaque formation (Arbustini et al., 1999). This results in ischemic heart disease (IHD), most commonly angina or myocardial ischemia through heart tissue necrosis. Atherosclerosis of the cerebral arteries can lead to cerebrovascular disease through a diminution of blood supply to the cerebral tissue. Momentary ischemia can result in transient ischemic attack (TIA), whereas more prolonged ischemia results in necrosis of the cerebral tissue due to occlusion of a cerebral artery (cerebral infarction), commonly referred to as ischemic stroke. Hemorrhagic

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strokes (cerebral hemorrhage), resulting from the rupture of an intracranial artery, are most commonly caused by cerebral aneurysms with evidence that the prevalence of these are also associated with atherosclerosis (Rinkel et al., 1998). Narrowing and progressive occlusion of the arteries supplying the limbs, through atherosclerosis, results in peripheral vascular disease (PVD), also referred to as peripheral arterial (or artery) disease (PAD). This is most commonly found in the lower limbs but on occasions does occur in the proximal arteries of the upper limbs (Welling et al., 1981).

Mortality

The most obvious burden from atherosclerosis comes from the deaths that result from it. ASCVDs make up the majority of all CVDs, which are the most common cause of death globally. Total CVDs accounted for around 18 million deaths in 2015, the equivalent of 32% of all deaths (31% for men and 34% for women). In the decade preceding 2015, there was an increase of around 10% in the number of people dying from CVD globally. Much of this change has occurred due to an increasing population and progressive aging (Nowbar et al., 2014), demonstrated by the decrease in age-standardized mortality rates (ASMRs) over the same period of time, from 384 deaths from CVD per 100,000 individuals in men in 2005 to 334/100,000 in 2015. Over the same period of time, ASMRs in women decreased from 298/100,000 to 233/100,000 (GBD, 2016).

Age standardization controls for changes in the age structure of the population and accounts for increasing longevity, which is important as ASCVDs are more common at older ages. This is reflected in the much lower global ASMRs in women (115.0/100,000) than men (172.8/100,000) for ischemic heart disease (IHD), despite smaller differences in the absolute number of deaths (men = 4.9 million IHD deaths and women = 4.0 million), as age standardization adjusts for the greater longevity in women. These 8.9 million global IHD deaths in 2015 make IHD the single leading cause of death worldwide, accounting for 16% of total deaths in both sexes. IHD also accounts for 52% of CVD deaths in men and 47% of CVD deaths in women (Wang et al., 2016). Stroke is the second most common single cause of death globally, accounting for more than 3 million deaths in both sexes in 2015, 35% of total CVD deaths. Of these stroke deaths, those from hemorrhagic stroke were slightly more common, making up 56% of all stroke deaths. This is largely due to greater mortality from hemorrhagic stroke in men, with ischemic and hemorrhagic stroke accounting for roughly equal number of deaths in women. In comparison with stroke and IHD, there are comparatively low numbers of deaths from PVD, making up less than 0.5% of CVD deaths in both sexes (GBD, 2016).

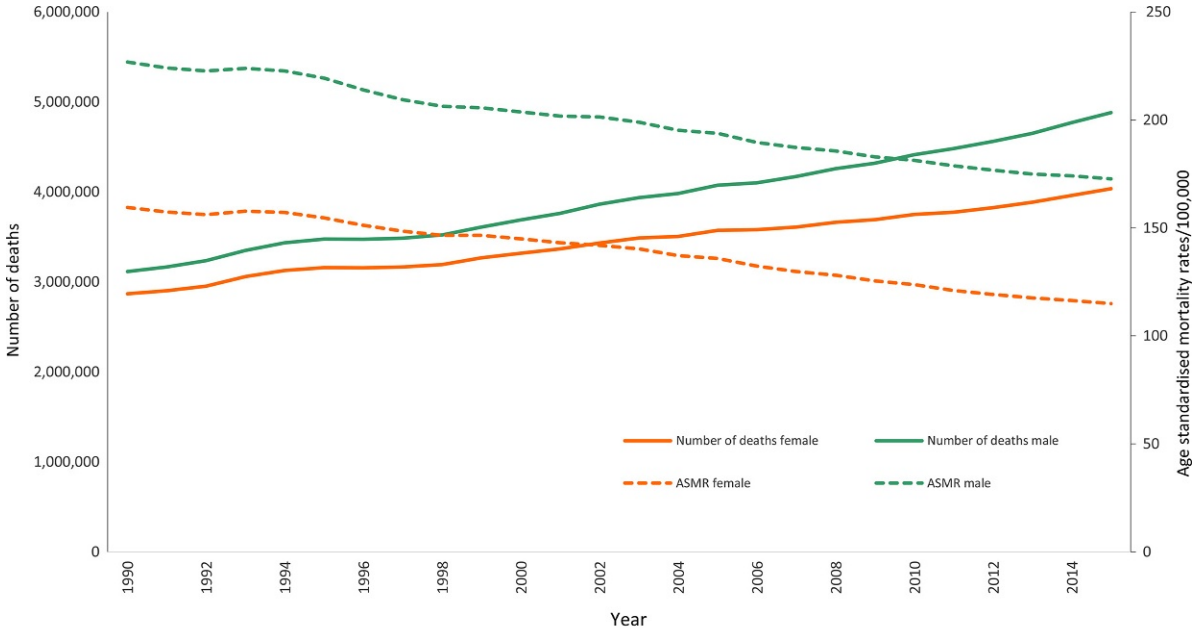
Although there has been little change in the number of deaths and ASMRs from PVD, the number of individuals dying from both IHD and stroke increased in the decade between 2005 and 2015. This has led to these two ASCVDs becoming the two most common causes of death globally, a result of changes in the causes of death in the last 30 years. In 1990, stroke was ranked the fifth most common cause of global deaths, rising to third in 2005 where it was behind lower respiratory disease and IHD; it is now second behind IHD, which has been consistently ranked the leading cause of death since 2005. In 1990, IHD was the fourth leading cause behind diarrheal disease, neonatal preterm birth complications, and lower respiratory infections (Wang et al., 2016). Population aging is one cause of this change, with shifts in population age structures estimated to account for more than 23% of increased mortality due to IHD and stroke. Although this population aging has led to increasing mortality for most causes, its relative contribution differs by disease. With a range in its influence from less than 4% for interpersonal violence and diarrheal diseases to 30% for Alzheimer's disease and other dementias (Wang et al., 2016) (**Fig. 1**).

Premature Mortality

To some people, deaths at older ages from diseases such as ASCVDs, along with other noncommunicable disease (NCDs), are inevitable as prevention and treatment of infectious disease and other causes improve. Deaths at younger ages may therefore be a greater demonstration of its burden, as these are deaths that many consider preventable. The number of CVD deaths in those under the age of 70 years, otherwise defined as premature mortality (GBD, 2013 Mortality and Causes of Death Collaborators, 2015, p. 13; Wang et al., 2016), has risen slightly from 2005, with 4.2 million men and 2.3 million women dying prematurely from CVD in 2015. Premature mortality from other causes has declined over the same period of time meaning that the percentage of premature deaths that arise due to CVD has risen, with CVD now accounting for 20% of all premature deaths in women and 23% in men (GBD, 2016). The World Health Organization (WHO) has declared that these current rates of premature deaths are unacceptable, as cost-effective interventions to prevent CVD are available (WHO, 2014a).

Around 2.2 million IHD deaths occur in men under the age of 70, equivalent to around 45% of all IHD deaths in this population. This compares to just under 1 million premature deaths in women, equivalent to less than 25% of all IHD deaths in females (Wang et al., 2016). These sex differences are also true for stroke, for which although it accounts for a greater percentage of the total premature deaths in women (38%) than men (33%), 43% of all stroke deaths in men occur in individuals aged under 70 years, compared with 29% of all stroke deaths in women. Of these premature stroke deaths, 70% are due to hemorrhagic stroke in both sexes. However, the number of premature deaths for both type of stroke has increased slightly in men since 2005 and decreased slightly in women over the same period. As with total deaths, PVD mortality is very rare, with one quarter of all PVD deaths in men occurring in those under 70 years of age, compared with around 10% in women (Wang et al., 2016).

Years of life lost (YLL) is a metric that takes into account the age at which deaths occur, by relating age at death to a standard life expectancy, thereby giving greater weight to deaths at younger ages.



Source: GBD, 2016. Burden of Disease Study 2015 Results. Institute for Health Metrics and Evaluation (IHME), Seattle, United States. http://ghdx.healthdata.org/gbd-results-tool

Fig. 1 Global trends in age-standardized mortality rates and number of deaths from ischemic heart disease, by sex, 1990–2015.

CVD accounts for 321 million years of life lost, just under 20% of all YLLs. IHD is the greatest individual cause of YLL globally with estimates that 99 million years of life were lost on account of IHD in men and 57 million lost in women. Increases in YLLs due to IHD were found for both sexes, with a 14% increase in YLL from IHD in men since 2005 and a 5% increase in women. This saw IHD move up from the fourth greatest cause of YLL in 1990, when it was ranked behind preterm birth, diarrheal diseases, and lower respiratory infections (GBD, 2013 Mortality and Causes of Death Collaborators, 2015, p. 13; Wang et al., 2016). In comparison, there is a slight decrease in overall YLLs from stroke, as a result of an 8% decrease in YLL due to stroke in women, contrasting a 5% increase among men. This pattern is the same for both types of stroke, ischemic and hemorrhagic. As with mortality figures, however, YLL due to hemorrhagic stroke is greater than for ischemic stroke. Although the largest increases in YLL over the last decade for ASCVDs come from PVD (a 28% increase in men and 30% increase in women), the absolute burden from PVD remains small, accounting for less than 0.2% of all YLLs due to CVD (GBD, 2013 Mortality and Causes of Death Collaborators, 2015, p. 13; Wang et al., 2016).

Morbidity

The burden of ASCVDs does not just come from the deaths caused by them. There are a number of people surviving with these diseases who are still affected by them. Disability-adjusted life years (DALYs) are a health gap measure that extends the concept of potential years of life lost due to premature death, to include equivalent years of "healthy" life lost by virtue of being in states of poor health or disability (Kassebaum et al., 2016). Globally, 347 million DALYs are lost due to CVD, with increases in these in men but not women in the last decade. IHD was the leading single cause of DALYs in 2015. However, despite an 11% increase in global DALYs due to IHD, from 147.8 million in 2005 to 164.0 million (men = 103.2 million and women = 60.8 million) in 2015, there was a 14.2% decrease in age-standardized DALYs over the same period of time (GBD, 2016; Kassebaum et al., 2016).

Although DALYs from stroke, the second leading single cause of DALYs, have shown little change since 2005 for all individuals, this conceals differences between the sexes, with a 6% increase in men and a 7% decrease in women. Hemorrhagic stroke accounts for more than three-fifths of all stroke DALYs in all ages (62%) and 72% for those aged under 70 years. Although DALYs due to PVD remain a relatively small burden, they have shown a greater risk than the other ASCVDs, with a 31% increase in the last decade and little change in age-standardized rates, compared with decreases in standardized rates for IHD and stroke (GBD, 2016; Kassebaum et al., 2016).

Prevalence

These increases in DALYs have occurred, in part, due to the rising global prevalence of CVD. The prevalence of a disease describes the number of people in a population who are currently living with the disease or, in the case of some ASCVDs, such as myocardial

infarction (MI) and stroke, the number of people living today who have ever suffered (and survived) an event. There have been increases of around 25% in the number of people living with CVD between 2005 and 2015, aligning with slight decreases in age-standardized rates, which demonstrate the impact of the growing population size and increasing longevity. Age-standardized prevalence rates in IHD decreased by around 3.4% in the 10 years leading up to 2015, but the number of people living with IHD increased from 87.5 million people in 2005 to 110.6 million in 2015 (64.4 million men and 46.1 million women) (Vos et al., 2016). Of this IHD prevalence, 65% comes from angina, 20% from heart failure, and 14% due to MI. IHD has shown the largest decreases in age-standardized prevalence rates of the three ASCVDs described here. Stroke has demonstrated 4% decreases in age-standardized rates, despite 21% more people living with the disease since 2005. PVD is the only ASCVD to show increases in age-standardized rates, albeit low ones, and accounts for around 40% of all prevalent CVD cases in men and women (GBD, 2016).

Changes in prevalence arise due to changes in incidence (the number of new cases that develop within a population over a specified period of time) and case fatality (the proportion of people dying from the disease over the course of it). Forty-five percent of total CVD incidence occurs due to IHD, close to 24 million individuals, with around half of these occurring in those under 70 years of age. PVD makes up 31% of all CVD incident cases in women and 26% in men with around 14.9 million incident cases of PVD in 2015. There were just under 9 million incident cases of stroke occurring in 2015. As with prevalence, PVD was the only ASCVD to demonstrate increases in age-standardized rates (GBD, 2016).

Global Inequalities

These global figures demonstrate the overall burden of ASCVDs, but they hide enormous inequalities that exist throughout the world, continents, and countries. Around 80% of CVD deaths occur in those countries classified as low-, lower-middle-, and upper-middle-income (LLMICs), often referred to as "developing countries." Although the greatest number of deaths from ASCVDs is found in upper-middle-income (UMI) countries, in which 90% of premature CVD occur deaths, absolute numbers do not account for differences in population size. ASMRs are greatest in lower-middle-income (LMI) countries for men and low-income (LI) countries for women. LI countries have also shown the smallest decreases in ASMRs and the largest relative increases in absolute number of deaths since 2005 (GBD, 2016).

High-income countries (HICs), also referred to as developed countries, have the lowest IHD ASMRs for both sexes, with ASMRs among men in HICs (87.9/100,000) lower than those found for women in all developing countries. Around 22% of global IHD deaths occur in HICs with this disparity greater for premature deaths, 88% of which occur in developing countries. There is also great variation between individual counties in the probability of dying from IHD, despite it being the most common single cause of death globally, with this ranging from 0.8% for women in Japan to more than 24% for men in Belarus (GBD, 2013 Mortality and Causes of Death Collaborators, 2015; Wang et al., 2016). Similar inequalities are found for stroke, with 87% of stroke deaths in men and 82% in women occurring in developing countries, along with 90% of all premature stroke deaths. There have, however, been decreases in stroke ASMRs in all country types, with these ASMRs lowest in HI countries for both ischemic and hemorrhagic stroke. PVD is the only ASCVD for which a greater number of deaths are found in developed than developing countries (61% of global deaths for men and 67% for women). However, HICs are also the only ones to show decreases in ASMRs in PVD, with all other countries demonstrating an increase. Rates and numbers of deaths of PVD remain low in comparison with the other disease types (GBD, 2016).

Similar inequalities are found for DALYs and YLLs from ASCVD. There have been small decreases in the number of DALYs and YLLs from IHD in the last 10 years in HI countries but increases in all developing country classifications, with the largest relative increases found in LICs. Similarly, decreases in DALYs and YLLs in HICs for stroke are compared with increases in LMICs and LICs. For both diseases, however, DALYs and YLLs are lowest in LICs (GBD, 2016).

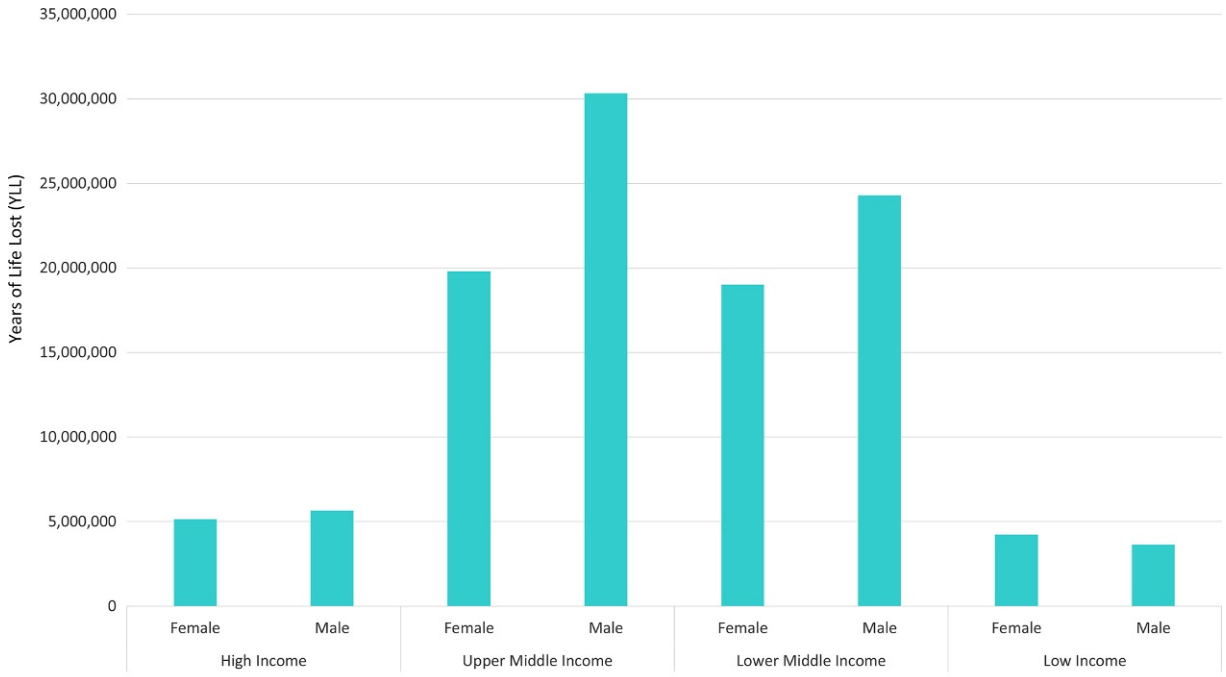
Although the burden of these ASCVDs is greatest in developing countries, the relative impact of them can differ due to the burden of other diseases. IHD and stroke are the top and second largest cause of YLLs globally, and this holds true for HICs and UMICs; however, IHD is only the fifth greatest cause of YLL and stroke the eighth in LMICs, with neither featuring in the top ten largest causes of YLL in LICs. Within developing countries, IHD and stroke feature in the top ten causes in all countries and regions except in sub-Saharan Africa in which neither feature (Wang et al., 2016) (**Fig. 2**).

Causes of ASCVDs

These global inequalities demonstrate inequalities between countries and regions in the treatment and the risk factors that cause atherosclerosis and its associated CVDs, with these causes often categorized as modifiable and nonmodifiable.

Nonmodifiable Risk Factors

Nonmodifiable risk factors include sex, age, and family history. As with all noncommunicable diseases (NCDs), ASCVDs take time to develop and are therefore more prevalent at older ages, with clinical manifestations seen mainly in the elderly. Although ASCVDs are very rare in those aged under 30 years, rising to become the main cause of death and disability in older age, the process of atherosclerosis that leads to these diseases actually begins in childhood with evidence of atherosclerosis found in teenagers (Berenson et al., 1992; Rose, 1982). The process of atherosclerosis develops in the different arteries at different ages, and it has



Source: GBD, 2016. Burden of Disease Study 2015 Results. Institute for Health Metrics and Evaluation (IHME), Seattle, United States. http://ghdx.healthdata.org/gbd-results-tool

Fig. 2 Years of life lost (YLL) to stroke, by sex and World Bank country income group.

been reported to be present in the aorta of almost every child, regardless of age, sex, or environment, from the age of 10 years (Strong et al., 1992). Studies in western countries have found advanced plaque to be common by the third decade and then to be found with increasing frequency in adult life, with clinical features and associated disease usually beginning after the fifth decade of life (Insull, 2009).

As demonstrated earlier in the article, when considering mortality and mortality from ASCVD, the burden of ASCVDs is greater for men, once greater longevity in women is controlled for. This is understood to be due to both behavioral and physiological factors. The behavioral risk factors that lead to atherosclerosis are commonly more prevalent in men, but it is also thought that the female sex hormones have a protective effect in the development of ASCVDs in women, with this protection lost after menopause (Rosano and Panina, 1998).

Having close relatives who have suffered ASCVDs has been found to be a strong predictor for developing these conditions (Holdt and Teupser, 2015). Diseases such as ASCVDs arise due to a large number of genes, with evidence that the risk of atherosclerosis is modulated through interaction between genetic factors and the environment (Holdt and Teupser, 2015). The major medical risk factors also arise in part due to complex genetic determination, and this complexity can detract from the use of genetic predisposition in targeting population and individual level interventions. This can limit the use of genetic prediction in clinical practice (Holdt and Teupser, 2015; Perk et al., 2012).

Modifiable Risk Factors

The WHO identifies eight key modifiable risk factors linked to ASCVDs. These include behavioral risk factors, alcohol use, tobacco use, diet, and physical inactivity, and intermediate (medical/physiological) risk factors, high blood pressure, high adiposity, high cholesterol, and high blood glucose (WHO, 2005, 2009). It is estimated that together, these risk factors account for the majority of deaths from these diseases (WHO, 2009) with evidence that exposure to these risk factors at all ages is linked with the development of ASCVDs over the life course (Labarthe et al., 2009; O'Flaherty et al., 2015).

Of these intermediate risk factors, hyperlipidemia (Scandinavian Simvastatin Survival Study Group, 1994; Shepherd et al., 1995) and hypertension both play an important role in the development of ASCVDs by causing damage to the lining of the blood vessels, which can result in the formation of atheromatous plaques (Munro and Cotran, 1988; Susic, 1997). Diabetes mellitus is closely linked to atherosclerosis, leading to dysfunction of the blood vessels and abnormalities of blood coagulation (Steiner, 1996). Adipose tissue, often measured in individuals through proxies such as body mass index (BMI) and waist circumference, is metabolically active, releasing a number of mediators that not only affect both the other ASCVD medical risk factors but also influence inflammation and thrombotic pathways, leading to endothelial dysfunction and atherosclerosis (O'Flaherty et al., 2015; Van Gaal et al., 2006). Due to the close interaction of all these conditions, many now consider the clustering of a number of these

risk factors within individuals as metabolic syndrome (Reaven, 2004; Townsend and Scriven, 2014). Also referred to as syndrome X, this is linked to increased risk of all ASCVDs (Gami et al., 2007).

These medical risk factors are closely associated to the behavioral risk factors, which lead to their development. Cigarette smoking is considered the major behavioral risk factor for ASCVDs at an individual level, with smoking damaging the blood vessel wall lining and causing an increased tendency for blood to clot (Ambrose and Barua, 2004). Diet is currently the greatest determinant of ASCVDs at a population level, due to the low population prevalence of smoking in comparison with unhealthy dietary intake. Dietary saturated fats are the main determinants of blood lipid levels, particularly total cholesterol (Clarke et al., 1997), while salt consumption is considered to be the single most important factor determining blood pressure levels (O'Flaherty et al., 2015), with diet also very closely linked to blood glucose levels and adiposity (Townsend and Scriven, 2014). Conversely, diets that are higher in healthy foods such as fiber, healthy fatty acids (e.g., omega-3), vitamins, potassium, and other minerals have been seen to have a protective effect (Brahmkshatriya et al., 2006; Connor and Connor, 1989; Harris et al., 1983; Nestel et al., 2002). Physical activity can lead to improvements in these medical risk factors (Kelley et al., 2005) with sedentary behavior, which is not only the absence of physical activity but also the engagement in activities that involve little movement and low energy expenditure (Reilly et al., 2008), seen as an independent risk factor to activity (Katzmarzyk et al., 2009; O'Flaherty et al., 2015). The WHO recommends that adults take at least 150 min of moderate-intensity aerobic physical activity throughout the week or at least 75 min of vigorous-intensity aerobic activity. Children and young people aged 5–17 years old should accumulate at least 60 min of moderate-to-vigorous-intensity physical activity daily (WHO, 2010a). The guidance on alcohol intake is less clear, with some types of alcohol thought to have a protective effect at low levels of consumption (Bell et al., 2017; Brahmkshatriya et al., 2006). Excess alcohol intake, however, has an impact on hyperlipidemia, liver disease, diabetes, and hypertension (Da Luz and Coimbra, 2004), leading to concerns over recommending the intake of even low levels of alcohol (Brahmkshatriya et al., 2006; Thun et al., 1997).

A Combined Approach to Reduce the Population Burden of ASCVDs

In order to prevent the development and impact of ASCVDs, the WHO recommends comprehensive prevention strategies that blend together two types of approaches: public health interventions aimed at reducing population-level risk factor levels and medical interventions targeted specifically at high-risk individuals, with the importance of risk factor and disease surveillance and monitoring also emphasized.

They recommend adopting a life-course approach to ensure prevention of risk factor exposure from early life, alongside interventions for adults at older ages, who are more likely to suffer ASCVDs (WHO, 2013). This advice reflects the success of countries that have achieved major declines in cardiovascular deaths, through reduced incidence and improved case fatality of ASCVD events (Smolina et al., 2012), as a result of population-wide primary prevention and individual health-care interventions (WHO, 2011a). The multinational monitoring of trends and determinants of cardiovascular disease (MONICA) project, coordinated by the WHO, attributed 75% of the decline in IHD mortality in men and 66% in women, to reduced incidence, with the resulting improvements attributed to improved survival after an event (Kuulasmaa et al., 2000; Tunstall-Pedoe et al., 1999, 2000), demonstrating the importance of this coordinated approach. Similarly, around one-third of the decline in stroke mortality was estimated to be due to reduced incidence, with the remaining two-thirds a result of improved case fatality (Thorvaldsen et al., 1995, 1997).

These findings support the view that population-wide primary prevention and individual health-care approaches go hand in hand in reducing the population burden of ASCVDs with the WHO integrating both into the voluntary global targets they have set for NCDs (WHO, 2013). In these targets, they include a 25% relative reduction in the risk of premature mortality from CVDs, reductions or containment in the prevalence of raised blood pressure, and a halting in the rise of diabetes and obesity. They also include targets for improved health treatment equality, with at least 50% of eligible people receiving drug therapy and counseling (including glycemic control) to prevent heart attacks and strokes, along with an 80% availability of affordable basic drug therapy (WHO, 2013, 2014b). Behavioral risk factor goals are also included, recommending 10% relative reductions in the harmful use of alcohol and the prevalence of insufficient physical activity and 30% relative reductions in the mean population intake of salt and the prevalence of current tobacco use in adults (WHO, 2013).

In order to help countries achieve these targets, the WHO recommends a package of interventions termed "best buys" that they say should be implemented to produce accelerated results in terms of lives saved, diseases prevented, and heavy costs avoided, by addressing the diseases and their key underlying risk factors. These best buy interventions were chosen with consideration to four key criteria: (i) health impact, (ii) cost-effectiveness, (iii) cost of implementation, and (iv) feasibility of scale-up, with a particular focus on resource constrained settings (Bloom et al., 2011; WHO, 2011b). These best buy interventions include those for both the treatment and prevention of ASCVDs, reflecting the combined approach they recommend.

Treatment

A major challenge in tackling ASCVDs, particularly in LMICs, is the hidden burden of disease (Gakidou et al., 2011). This comes from the number of people in the population who are suffering from ASCVDs but either who remain undiagnosed or who are diagnosed late (WHO, 2013, 2014a). These late diagnoses are more likely to result in expensive, more invasive intervention and death or disability (Deshpande et al., 2008). This arises due to a focus on treatment for ASCVDs, along with all NCDs, currently residing in hospital-centered acute care. This is particularly the case in many developing countries in which ASCVD patients

generally present themselves in hospitals at a point of acute events or long-term complications (WHO, 2013, 2014a). Treating late-stage disease requires expensive high-technology interventions. Early detection of disease is therefore recommended so that primary prevention strategies can be targeted to those at high risk of ASCVD (WHO, 2010b) with this approach feasible even in low-resource settings (WHO, 2010b, 2013, 2014a), with the WHO recommending that this improved treatment should be integrated into health systems alongside a focus on prevention (WHO, 2013, 2014a).

Within the best buys, the WHO promotes the treatment of high-risk individuals of IHD and stroke with a multidrug approach, recommending that a regimen of aspirin, statin, and blood-pressure-lowering agents could significantly reduce vascular events in people at high cardiovascular risk (WHO, 2013, 2014a). They also recommend the provision of aspirin to people following a heart attack and the use of drug therapy for blood sugar control for diabetes mellitus (Bloom et al., 2011; WHO, 2011b, 2013, 2014a).

As well as having a health impact, estimates suggest that these interventions will have an economic impact, with these health improvements leading to a restored or continued ability of individuals to lead flourishing lives and to participate actively in the workplace. A 10% reduction in the mortality rate due to IHD and stroke is predicted to reduce economic losses in developing countries by an estimated US$ 377 billion over the period 2011–25, approximately three times the yearly cost of scaling up "best buy" interventions for CVD (Bloom et al., 2011).

Prevention

Alongside these treatment recommendations, the WHO best buys include a number of interventions to encourage prevention through the targeting of behavioral risk factors. These include a raise of taxes on tobacco and alcohol, enforcing bans on the advertising of both, and bans on smoking in public places. They also recommend the provision of information on the harms of smoking on tobacco packaging, the mass-media campaigns on physical activity and diet, and the reformulation and labeling of foods with a focus on reducing salt and trans fat intake (Bloom et al., 2011; WHO, 2011b, 2013, 2014a). Although not included in the best buys, the WHO recommends a number of other cost-effective and low-cost population-wide interventions focusing on these behavioral risk factors, such as nicotine dependence treatment; restrictions on marketing of foods and beverages high in salt, fats, and sugar, especially to children; and food taxes and subsidies to promote healthy diets (WHO, 2011a).

Both the United Nations and the WHO recognize that decisions made outside of the health sector have a major bearing on factors that influence ASCVD-related risk, in particular to these behavioral determinants. They recommend, therefore, that prevention gains may be achieved by influencing public policies outside of health sectors, in domains such as trade, food and pharmaceutical production, agriculture, urban development, pricing, advertising, information and communication technology, and taxation policies (WHO, 2011a, 2013). Such a multisectoral approach recognizes that the determinants of ASCVDs, as with all NCDs, lie beyond these behavioral risk factors onto the factors that determine them. These include the social, or wider, determinants of health that come from economic transition, rapid urbanization, and 21st-century lifestyles (WHO, 2011a, 2013). As the responsibility for much of these issues lies outside of the health sector, multisectoral approaches with a public or population health perspective should be developed for effective prevention on a population level (O'Flaherty et al., 2015) (**Fig. 3**).

Multisectoral Action

Multisectoral action recognizes that combating the burden of ASCVDs requires firm commitments of resources and capabilities from a number of government sectors along with civil society, development agencies, private sector, and academia (Mamudu et al., 2011; WHO, 2011a, 2013). This health in all policies approach describes the integration of health focus in policies from all government sectors, allowing government and international organizations to provide leadership, establish necessary frameworks, create infrastructure, and create health policies. Within these approaches, other sectors can play key roles. For example, academia can contribute expertise; NGOs and civil society can lead in advocacy and support implementation, while the private sector can offer business skills, networks, and funds (Bloom et al., 2011; Mamudu et al., 2011; WHO, 2011a, 2013). Collaboration among these partners will allow societies to capitalize on individual strengths and realize benefits beyond the reach of any single entity to ensure that communities have the necessary resources to manage the growing burden of NCDs (Bloom et al., 2011; Mamudu et al., 2011; WHO, 2011a, 2013).

Such approaches also recognize the importance of all settings in which individuals live their lives to be a part of promoting a healthy lifestyle that may aid in the prevention of ASCVDs. There is strong evidence that healthy nutrition and physical environments in schools, workplaces, and communities; nutrition information and counseling in health care; and designing the built environment to promote physical activity can be effective (WHO, 2011a). However, these settings, while being considered part of the health environment, need help in recognizing and developing their place within it. Integration in multisectoral action, therefore, can aid in the implementation of health promotion approaches within them.

Surveillance and Monitoring

The WHO recommends the development of surveillance and monitoring programs, to support treatment and prevention efforts. Most countries have little usable mortality data and weak surveillance systems, but accurate data are vital for problem definition, to understand the distribution of the burden from ASCVDs, to target interventions, to predict future trends, and to provide impetus for action (WHO, 2011a, 2013).

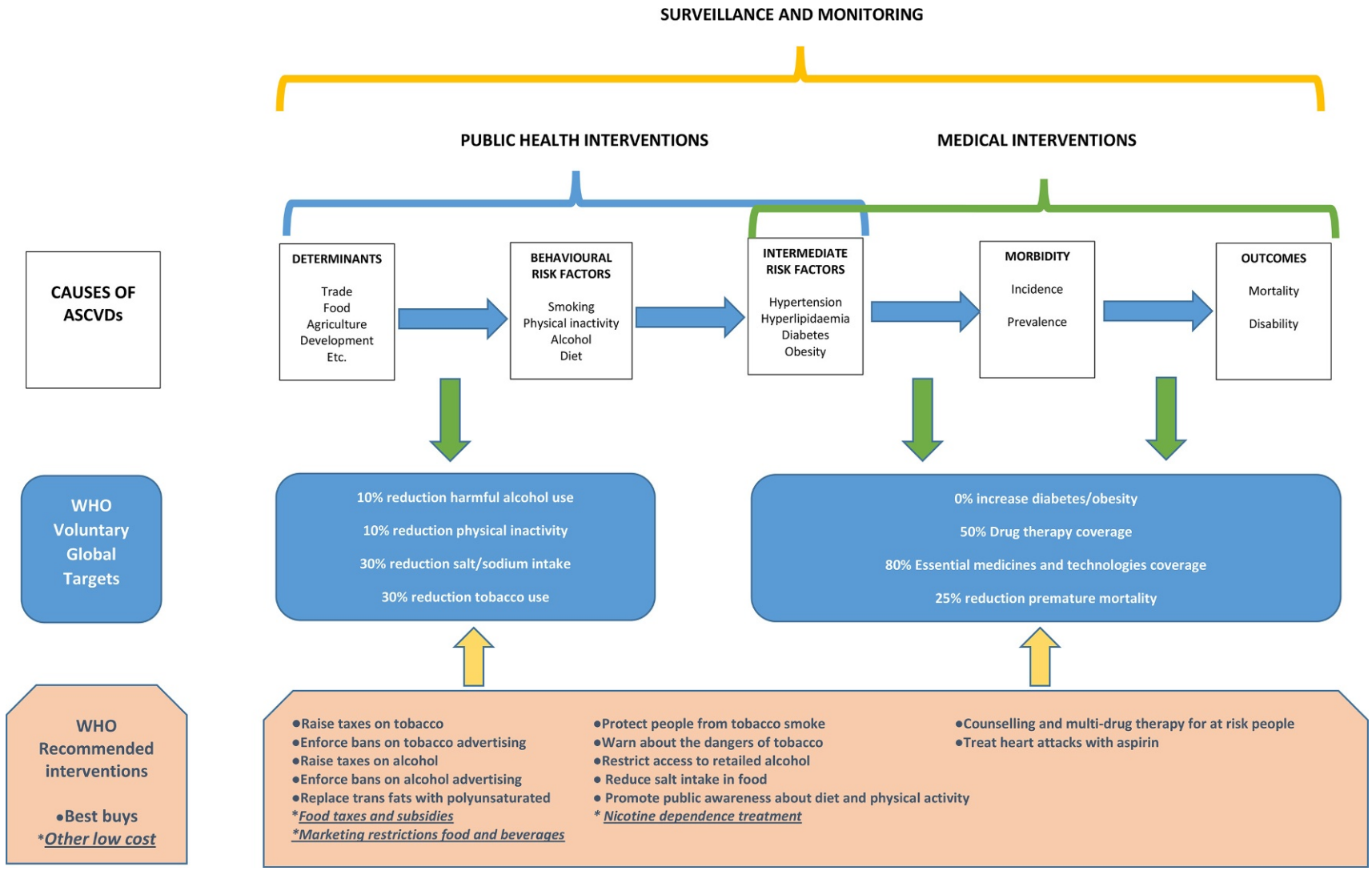


Fig. 3 A combined approach for the prevention of atherosclerotic cardiovascular disease.

Because of this, the WHO identifies improvements in country-level surveillance and monitoring as a priority in programs to prevent and treat ASCVDs, suggesting simple sustainable systems can be implemented in low-resource settings and still produce valuable data. The monitoring of ASCVDs is seen to be essential to the development of policies and program to prevent them, with three key areas of surveillance identified by the WHO:

1. Exposure to determinants and risk factors
2. Outcomes in the form of morbidity and mortality
3. Assessment of health-system capacity and response to prevent ASCVDs (WHO, 2011a, 2013, 2014a)

Although the WHO recommends that strengthening surveillance is a priority at the national and global levels, they acknowledge that this requires long-term funding to be effective, identifying surveillance of behavioral and metabolic risk factors in low-resource settings as the top priority (WHO, 2011a, 2013, 2014a).

Discussion

Atherosclerotic CVDs are the largest cause of death and disability in the world placing a greater burden on developing countries. A combination of public health and medical intervention is recommended to prevent and treat ASCVDs, in order to reduce both their incidence and case fatality. Early detection and intervention must be achieved alongside population-level prevention strategies that target the wider determinants of the ASCVD risk factors. This requires the collaboration of a number of sectors, such that responsibility in changing the environments in which individuals live our lives does not fall solely on health sectors and services. A coordinated approach to surveillance and monitoring will enable interventions to be targeted and evaluated and for health-system capacity to be improved.

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Epigenomics

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Introduction

Cardiovascular diseases (CVD) are a general term for conditions affecting the heart and blood vessels. CVD are the main cause of death and disability in the western world and a growing problem also in developing countries. The incidence of CVD has rapidly increased in several countries, and since human genetic code does not evolve much in a few decades through classical mutations, epigenetic effects are likely to be involved. Genome-wide association studies (GWAS) have been performed in different CVD conditions in order to identify genetic variants associated with disease probability. For example, the CARDIoGRAMplusC4D Consortium studied 63,746 coronary artery disease (CAD) cases and compared them to healthy population (130,681 cases), identifying multiple genetic risk locations and associated single nucleotide polymorphisms (SNPs) related to CAD (Doney et al., 2012). However, this study also observed that these variations explain only 10.6% of the heritability of CVD. It is becoming clear that the variations in the gene sequence alone are not enough to explain all differences, but factors affecting the readability of this sequence are equally important. Thus, epigenetic studies are needed to characterize the state of DNA methylation, histone modifications and non-coding RNA (ncRNA) expression, to generate more complete picture of the variability lying behind gene expression. This effort is now on the move, and at least one publication studying the chromatin organization behind the risk alleles and SNP variants, utilizing the data generated by consortiums such as CARDIoGRAM and METASTROKE, is already out (Haitjema et al., 2017). This article aims to offer the basic information about different epigenetic modifications and modifiers, as well as to introduce the current methodology for epigenetic studies.

Epigenetics

In eukaryotes, DNA is wrapped around a histone protein core thus allowing the tight packaging of chromatin in the nucleus. The nucleosome core consists of two of each of the histones H2A, H2B, H3 and H4 and approximately 147 bp of DNA that is wrapped around the histone octamer (Hayes and Hansen, 2001). The cores are connected by around 80 bp DNA stretch and histones H1 and H5 act as a linker to enable further chromatin compaction. The N-terminal tails of histones H3 and H4 extend from the core and their amino acid residues are subjected to a wide range of posttranslational modifications that affect chromatin structure and gene expression. Epigenetics as a term is used to refer to gene regulation mechanisms that are not affecting the DNA sequence itself but act 'above' (greek '*epi-*') it. This epigenetic information can also be heritable and involves modifications of DNA and the proteins attached to it. The most common epigenetic modifications include DNA methylation and posttranslational modifications of histones. In addition, RNA-mediated mechanisms contribute to the epigenetic regulation. These classes of epigenetic regulation are introduced in **Table 1**. Epigenetic regulation, the epigenome, helps to explain processes such as the maintenance of cellular identity in multicellular organisms and the ability to adapt to rapid environmental changes, since the epigenetic marks can be written, removed and rewritten in the genome by distinct enzymes (e.g., histone acetylases and histone deacetylases). The study of epigenome is referred to as epigenomics and it aims to identify the epigenetic factors and understand their function. The field has moved forward fast during the last decade together with the development of global epigenomics analysis tools such as different deep sequencing methods.

DNA Methylation

The cytosine residues on DNA can be covalently modified by addition of a methyl group. The methylation of DNA is catalyzed by DNA methyltransferase enzymes (DNMTs). In mammalian cells, there are three DNMTs: DNMT3a and DNMT3b act as de novo

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Table 1 Epigenetic modifications

Epigenetic class	*Modification*	*Function; effect for chromatin activity*	*References*
DNA methylation	Methylation of cytosine residue of DNA (5mC)	Silencing	(Li and Zhang, 2014)
	Hydroxymethylation of cytosine residue of DNA (5hmC)	Intermediate step for removal of 5mC to "erase" DNA methylation	(Kriukienė et al., 2012)
Histone modification	Acetylation	Mostly associates with active chromatin region	(Wu and Grunstein, 2000)
	Methylation	Both active and repressed chromatin state, depending on the modified residue and the level of methylation (mono-, di-, tri-)	(Barski et al., 2007)
	Phosphorylation	Transcriptional activation	(Rossetto et al., 2012)
	Ubiquitinylation	Transcriptional activation	(Zhang, 2003)
	Sumoylation	Transcriptional repression	(Cubeñas-Potts and Matunis, 2013)
RNA	miRNA	Posttranscriptional gene silencing; however, some studies suggest activation of genes	(Almeida et al., 2011)
	siRNA	Posttranscriptional gene silencing; transcriptional regulation both activating and repressing chromatin	(Turner and Morris, 2010)
	piRNA	Silencing of genome (transposons)	(Huang et al., 2013)
	lncRNA	Genomic imprinting; also fine-tuning of gene expression	(Kaikkonen et al., 2011)

DNA methyltransferases, establishing the DNA methylation patterns during development, while DNMT1 functions in the maintenance of the marks during DNA replication (Turek-Plewa and Jagodzinski, 2005). The biological function of the fourth DNA methyltransferase, DNMT2, is still uncertain but it has been shown to methylate transfer RNAs (tRNA) (Goll et al., 2006). Interestingly, DNMT3a and DNMT3b have also been linked to the demethylation pathway in a Ca^{2+}-ion dependent manner, and might therefore play a dual role in the regulation of DNA methylation and demethylation (Chen et al., 2013; van der Wijst et al., 2015). DNA methylation can also be reversed by an active process whereby 10–11 translocation (TET) enzymes hydroxylate the methyl groups (Tahiliani et al., 2009).

DNA methylation is typically considered a mark of transcriptionally repressed genes or genomic areas. Methyl-binding domain (MBD) family of proteins recognizes and binds methylated DNA and mediates the transcriptional state of the chromatin (Du et al., 2015). These proteins can act in conveying the multilayered epigenetic signal of DNA methylation and histone modifications. DNA methylation is considered to be a fairly stable silencing modification which plays a role in processes such as genomic imprinting and transposon silencing.

Several studies have been done to elucidate the role of DNA methylation in cardiovascular diseases (Laukkanen et al., 1999; Aavik et al., 2015; Turunen et al., 2009). Sharma et al. found 72 hypermethylated differentially methylated regions (DMR) from blood in CAD patients. 12 of these regions were further analyzed by bisulfite sequencing and 6 regions were found to be associated with CAD (Sharma et al., 2014). Mice with global reduction in DNA methylation have been shown to develop fatty streaks in aorta (Chen et al., 2001). Furthermore, knockout $ApoE^{-/-}$ mice show alterations in global DNA methylation in peripheral blood mononuclear cells and aorta even before typical vascular lesion formation occurred (Lund et al., 2004). In humans, a correlation between increased DNA methylation and lesion severity has been observed (Valencia-Morales et al., 2015). Recent genome-wide association study (GWAS) described associations between blood lipid levels and DNA methylation (Hedman et al., 2017).

Histone Modifications

Another well-known factor in the epigenome is modification of the histones. Histones consist of approximately 130 amino acid residues and have highly positive charge, which enables their strong association with negatively charged DNA. Histone "tails", 10–30 amino acid N-terminal domains protruding from the nucleosome, are subjected to posttranslational modifications and also act as platforms for nucleosome binding proteins (Luger et al., 1997; McGinty et al., 2014). Outside of the four core histones there are "linker" histones, H1 and H5, which interact with linker DNA that joins the nucleosomal structures, further condensing the chromatin (Bednar et al., 1998; Fan and Roberts, 2006). Nucleosome complex is modified by several different methods, including posttranslational modification of histones by histone modifying enzymes, replacement of the common histone proteins with special variants, and by altering the nucleosome position and composition by ATP-dependent chromatin modeling enzymes (Swygert and Peterson, 2014; Talbert and Henikoff, 2010; Hargreaves and Crabtree, 2011).

Currently several different histone modifications have been characterized: acetylation, methylation, phosphorylation, ubiquitinylation, sumoylation, ADP ribosylation, deimination, proline isomerization, citrullination, formylation, succinylation, butyrylation, propionylation and crotonylation (Tessarz and Kouzarides, 2014; Kouzarides, 2007). These may take place on over

60 different histone residues, and the methylation can occur in two or three different layers (mono- or di-, and mono-, di- or trimethylation) for arginine and lysine residues, respectively, thus creating a wide and complex landscape of regulation. This has been sometimes described as the histone code, where all the histone modifications and their interactions produce a code for the enzymes that are able to recognize and bind the specific modifications, and then recruit other proteins such as transcription factors (TFs) to modulate gene activity. Whether the histones form a unified 'code' is still unclear, but some patterns for histone modification occurrence have been observed (Wang et al., 2008). However, it is clear that some specific histone modifications are typically associated with either repressed or activated states of gene expression on specified genetic areas. These include histone H3 lysine K4 trimethylation and H3K27 acetylation on active promoters, H3K4 monomethylation on active enhancers, and H3K27 trimethylation and H3K9 dimethylation on repressed promoters (Heintzman et al., 2007; Stewart et al., 2005; Tie et al., 2009). Promoter-associated acetylation of lysines neutralizes the charge of lysine, weakening the bond between nucleosomal chromatin and histones, thus making the DNA more accessible for the transcriptional machinery (Zentner and Henikoff, 2013). Also, the interplay between different modifications has been detected, for example, phosphorylation of H3 serine S10 seems to be inhibitory for the methylation of H3K9 but instead promotes the acetylation of H3K14 (Rea et al., 2000). Extensive reviews are available for a more detailed description of histone modifications and modifying enzymes (Kouzarides, 2007; Lawrence et al., 2016; DesJarlais and Tummino, 2016).

Histones are modified by a variety of enzymes. The so-called *writers* are enzymes that establish modifications, such as histone lysine methyltransferases (KMTs) or histone acetyl transferases (HATs) that add the methyl or acetyl group, respectively, to their target histone residues. *Eraser enzymes* are able to remove these marks, histone lysine demethylases (KDMs) remove the methylation, and histone deacetylases (HDACs) the acetylation of the histones. Histone acetylation is usually associated with active genes and therefore the transcriptional coactivators, such as p300/CBP or SRC-1, often contain HAT activity (Ogryzko et al., 1996; Spencer et al., 1997). Correspondingly, the global transcriptional repressor complexes often associate with HDACs, for example, the complex of NCoR, mSin3, and RPD3 (Heinzel et al., 1997). KMTs and KDMs act more specifically, sometimes even mediating the methylation or demethylation of only one specific residue, such as is the case with KMT SUV39H1, which only methylates H3K9 (Rea et al., 2000). The same histone residue can be modified by different enzymes, such as in the case of H3K4 methylation where Jarid1B (also known as KDM5B) seems to demethylate di- and trimethylated H3K4, whereas LSD1 (also known as KDM1A) instead modifies the mono- and dimethylated forms of the same residue (Seward et al., 2007; Shi et al., 2004). *Reader enzymes* are transcription factors and enzymes that can bind DNA only when certain histone modifications are set on place. For example, HP1 proteins require the methylation of H3K9 by the associated KMT SUV39H1 for the generation of a favorable chromatin structure that it then binds and regulates transcriptional activity of the loci (Lachner et al., 2001). Review by Swygert and Peterson describes enzymes taking part in chromatin and histone remodeling in more detail (Swygert and Peterson, 2014).

Histone modifications and changes in their function have relevance for CVD as well. HDACs seem to have both pro- and antiangiogenic properties: HDAC inhibitors decrease the VEGF-induced migration and branching of human umbilical cord endothelial cells (HUVEC) (Deroanne et al., 2002). Opposing results were shown in the study by Jin et al., where HDAC inhibition in HUVECs resulted in enhanced sprout formation and upregulation of acetylated H3 levels (Jin et al., 2011). In vivo, HDAC3 expression in $ApoE^{-/-}$ mice aortas is increased, and *Hdac3* knock-down mice show more severe atherosclerotic phenotype (Zampetaki et al., 2010). HDAC9 gene region 7p21.1 has been identified as a strong risk locus for CAD and large artery stroke, a subtype of ischemic stroke (Dichgans et al., 2014). Azghandi et al. showed, that $Hdac9^{-/-}ApoE^{-/-}$ mice have less severe and fewer plaques when compared to $Hdac9^{+/+}ApoE^{-/-}$. Furthermore, healthy human subjects with HDAC9 risk allele rs2107595 showed higher mRNA expression of HDAC9 in their peripheral blood mononuclear cells. However, expression of the same risk allele did not have an effect on plaque morphology in human carotid artery plaques (Azghandi et al., 2015). In the study by Yang et al., increased expression of SUV39H was observed in mice after myocardial infarction. SUV39H is a methyltransferase responsible for H3K9Me3, a transcription repressive mark leading to formation of heterochromatin. Transgenic mice lacking SUV39H were noticed to have better survival when compared with wild-type animals (Yang et al., 2017).

RNA Transcripts

Even though only less than 3% of the genome encodes protein sequences, over 85% of the DNA is still transcribed to RNA (Hangauer et al., 2013). These RNA transcripts that are not translated into protein are called non-coding RNAs (ncRNAs) and include some functional RNAs, such as ribosomal RNAs (rRNAs) and tRNAs, that have been already well characterized with functions in protein synthesis. Recently the substantial role of other RNA transcripts has emerged with increasing number of publications reporting new functions for these 'junk RNAs'. The novel action of ncRNAs is in the regulation of gene expression, turning them into epigenetic actors. Different ncRNAs regulate gene expression at multiple levels: transcriptional, posttranscriptional, and translational levels. The ncRNAs are usually classified based on their size, long ncRNAs (lncRNAs) with the length of over 200 nt and small ncRNAs with the length less than that. Especially small RNAs are then categorized into subclasses, such as the best-studied examples of miRNAs, siRNAs, and piwi-interacting RNAs (piRNAs).

The best-known example of ncRNAs in gene regulation is the miRNAs that act in posttranscriptional gene silencing (PTGS). miRNAs target the 3′ UTR of mRNAs with complimentary sequence and upon binding to mRNA, can induce PTGS by inhibition of translation or resulting in degradation of the mRNA (Kim, 2005). Endogenous siRNAs expressed as long double-stranded RNAs from the genome, or exogenously administered siRNAs in research or therapeutic applications act in the same PTGS pathway.

Some ncRNAs play a role in establishing epigenetic modifications in the chromatin. For example, originally found from the centromeric regions, siRNAs were observed to form a nuclear RITS complex and thereby induce histone H3 lysine K9 methylation and chromatin condensation (Volep et al., 2002). In a study in *Schizosaccharomyces pombe*, an lncRNA termed BORDERLINE, which is further processed into siRNAs by Dicer, prevented histone H3 lysine K9 methylation outside the pericentromeric region (Keller et al., 2013). This ncRNA acts as a boundary for the HP1 protein that binds the H3K9 methylation and promotes the spreading of heterochromatin. The small RNAs generated from this lncRNA are not bound by Ago1 unlike the centromeric siRNAs, and they act in a locus-dependent manner independent of their sequence.

X-inactive specific transcript (XIST) is the best-known example of an lncRNA regulating epigenetic silencing. The X chromosome contains more genetic information than the gene-poor male Y chromosome and in female cells, one X chromosome must be silenced in order to prevent possible toxic effects of "double-dose" of gene expression. The inactive X chromosome transcribes an lncRNA XIST that is retained in the nucleus and localizes to the chromatin where it was produced. It also recruits chromatin modifying enzyme complexes such as PRC2 that increases methylation of H3K27 residues (da Rocha et al., 2014). The inactivation of the X chromosome also requires other ncRNAs such as TSIX, an antisense transcript for XIST, that represses XIST expression by a mechanism involving H3K36me3 on the XIST promoter (Ohhata et al., 2015). The interplay between the various ncRNAs and chromatin modifying enzymes in the regulation of X chromosome inactivation has not yet been fully resolved.

Recent study has shown that miRNAs are capable of binding to double-stranded DNA (Paugh et al., 2016). Some miRNAs have already been shown to have non-canonical functions and target promoters of genes, regulating their expression at the transcriptional level. In some studies, this has been associated with changes in epigenetic modifications, such as increase in H3K9me2 (Younger and Corey, 2011). piRNAs have been shown to mediate epigenetic modifications as well. Similarly to miRNAs, piRNAs were localized to their complementary sequences and recruit Piwi, HP1, and SUV-family KMTs that increase H3K9 methylation and reduce Pol II activity on the target sites (Huang et al., 2013). In this genome-wide study, Piwi and piRNA were shown to colocalize on both euchromatic and heterochromatic regions, preferably at pericentromeric and subtelomeric loci.

The biogenesis and action of ncRNAs have been studied extensively during the recent years. These findings are comprehensively described in many good reviews (Khorkova et al., 2015; Mattick and Makunin, 2006; Roberts, 2014). In addition, the association of ncRNAs with different diseases, including CVD, has been studied and reviewed (e.g., Almeida et al., 2011; Greco et al., 2015; Uchida and Dimmeler, 2015).

Tools for Studying Epigenetics

The tools for studying epigenetic phenomena have developed during the recent years toward more global analysis of epigenetic status of the cells and tissues. The first studies of, for example, DNA methylation were focused on specific genes of interest and/or total amount of DNA methylation in the cell, but with first microarray techniques and more recently the next-generation sequencing methods, the interest is moving towards mapping the whole genome at single residue level. Genomic high-throughput assays provide large amounts of data and the interpretation of the data may be challenging. Methods for epigenomics analysis are described briefly here and summarized in **Fig. 1**.

The basic tools for analysis of DNA methylation in the cell are digestion assays and bisulfite sequencing of DNA. In digestion assays, methylation-sensitive and -insensitive restriction enzymes are used to digest genomic DNA (Hatada et al., 1991). Methylation-sensitive endonucleases can only cleave unmethylated DNA and leave methylated DNA targets undigested. The digested DNA can be analyzed by sequencing or microarray, and that way the sites of methylation are mapped. The limitation of this method is that it only characterizes the sequences near the targets of chosen restriction enzymes, and is usually used to characterize only global levels of DNA methylation instead of identifying methylated DNA at the single residue level. Resolution is improved by using bisulfite sequencing method. In strand-specific bisulfite sequencing described first in 1992, bisulfite treatment is used to convert cytosines to uracil, whereas methylated cytosine residues remain unaffected (Frommer et al., 1992). The resulting DNA can be analyzed using various methods, often by sequencing after PCR of the regions of interest. The analysis can also be done using MALDI-TOF mass spectrometry or microarrays.

DNA methylation can also be studied using affinity-enrichment based methods. Methyl-binding domain (MBD) of MeCP2-protein has been bound to, for example, column resin to create affinity columns against methylated DNA (Zou et al., 2007). Fragmented DNA samples can be loaded to the columns and methylated DNA fractions eluted out for analysis with genome-wide techniques (such as MBDCap-seq/MethylCap-seq). Immunoprecipitation of methylated DNA (MeDIP) utilizes antibodies recognizing the methylated cytosine (5mC) in DNA (Mohn et al., 2009). DNA sample is fragmented and antibody used for immunoprecipitating those fragments that contain 5mC. This method is also used for the analysis of hydroxylated methylcytosine (5hmC). Purified fragments are analyzed by PCR, sequencing or microarray. When using these methods, the analysis resolution is restricted to the DNA fragment level of often 100–300 bp instead of identifying single-base modifications. However, depending on the research question, this can often be sufficient.

For histone modifications, chromatin immunoprecipitation (ChIP) is a widely used technique to identify local posttranslational modifications of the histone tails and to monitor changes in the modifications in response to different stimuli. The protocol was first described in 1984 when it was used for identifying RNA pol II occupancy on E. coli genes (Gilmour and Lis, 1984). Shortly, antibodies against specific histone modifications (such as trimethylation of histone H3 lysine K27) are used for immunoprecipitation and pulldown of the chromatin regions that have these modifications. ChIP can also be used to analyze the binding of

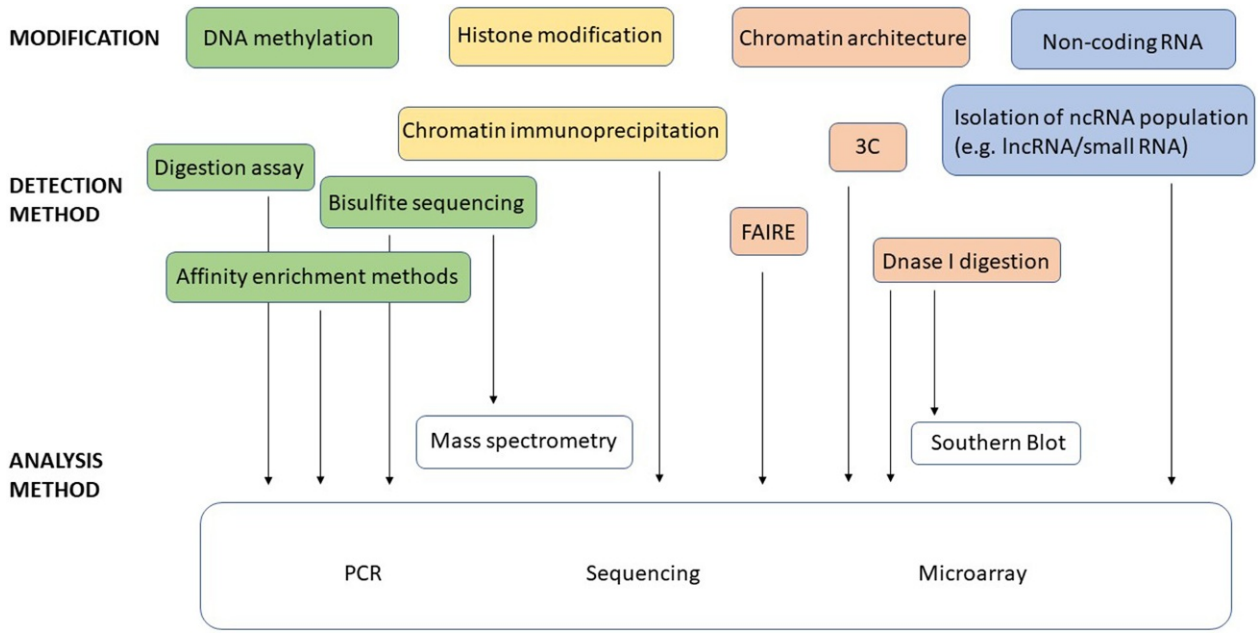

Fig. 1 Tools for studying epigenomics. Epigenetic modification (DNA methylation, histone modifications, chromatin architecture and non-coding RNA) can be studied using versatile detection methods. These methods are specific to each modification, such as antibody-based affinity methods in detection of DNA methylation or histone modification. Nevertheless, the final outcome of these methods is often analyzed with common analysis technologies: PCR, sequencing and microarray.

transcription factors and enzymes to the chromatin, using a specific antibody against the factor of interest. Usually, using either PCR to detect specific loci or deep sequencing for a more global view, the associated chromatin regions can be identified. ChIP experiments can also be analyzed using microarray (ChIP-chip), but the resolution of data is weaker compared to sequencing. The ChIP approach has been used, for example, for the identification of histone mark signatures of different chromatin elements, such as promoters or enhancers (Heintzman et al., 2007). Furthermore, during the last decade, the basic ChIP method has been developed to more specifically answer different research questions and to utilize more recent analysis technologies, so diverse ChIP methods have been described in the literature. Such are the μChIP-seq for low, micro-scale sample amount analysis, and the modern single-molecule real-time sequencing (SMRT; "third generation sequencing") of ChIP-samples (Dahl and Collas, 2008; Wu et al., 2016).

Epigenetic landscape can also be studied by chromatin accessibility methods. These studies can identify the active regions of chromatin, for example by using DNAse I—digestion of DNA. In the DNase-seq, DNase I—treatment digests the DNA that is not protected by nucleosome structure, so the regions that are sensitive to DNase are associated with active genes (Song and Crawford, 2010). Sequencing results in a more precise characterization, but also microarray or Southern Blot analysis can be used if single-base pair resolution is not necessary. Another method for the detection of open chromatin is the formaldehyde-assisted isolation of regulatory elements (FAIRE) that also result in the isolation of open, nucleosome-depleted chromatin regions (Giresi et al., 2007). In FAIRE, DNA and histones and other proteins associated with it are cross-linked with formaldehyde, sonicated to fragment the DNA, and DNA isolated with phenol-chloroform extraction. Only DNA that is not bound by nucleosomes and associated proteins remains in the aqueous phase in the extraction, thus resulting in the isolation of the open and active regions of the genome. The isolated fragments can be analyzed again with different methods, such as PCR, microarray, and sequencing. Chromatin architecture can also be studied using chromatin conformation capture (3C) technique that identifies the chromatin regions that are physically associated together, such as promoters with enhancers. 3C is often analyzed by PCR, but nowadays also by deep sequencing of the interactions globally (Hi-C) (van Berkum et al., 2010).

The RNA component of epigenetics is nowadays most often analyzed on a genome-wide scale using next-generation sequencing methods (Fernandez-Moya and Ehses, 2017). Deep sequencing can be performed for different populations of RNA to characterize the changes in both ncRNA and mRNA populations. mRNAs can be sequenced by isolating them based on their poly-A tails, still resulting in sequencing of some polyadenylated lncRNAs as well. Small RNAs and miRNAs can be sequenced separately based on their smaller length by using size exclusion. The role and function of miRNAs and other ncRNAs are mainly studied by cell line models, where it is easy to interfere with normal signaling using either overexpression or inhibition of the ncRNA of interest. Mostly, this is done using mimic or inhibitor oligonucleotides, respectively, that are transfected into the cells. Besides global sequencing methods, the expression of RNAs can be analyzed with qPCR. Modern techniques can detect low copy number transcripts and their expression differences, even on a single-cell level (Ziegenhain et al., 2017).

The methods described here only briefly scratch the surface of the numerous applications of these techniques. Detailed reviews are available that describe the progress of the technology and the use of these methods with different starting materials, such as when the starting cell number is fairly limited (Yong et al., 2016; Callinan and Feinberg, 2006; Khyzha et al., 2017).

Summary

Epidemiological studies have shown correlation between environment and epigenetics that can also be inherited to next generations. An example is from the Dutch Famine (1944–45), where families have been followed for decades after they were exposed to severe famine during the wartime. These studies have found that the region of insulin-like growth factor II (IGF2) gene was less methylated in individuals who were prenatally exposed to famine, compared with their other siblings (Heijmans et al., 2008). The DNA methylation status of the gene persisted for the lifetime of these individuals, showing the far-reaching consequences of environmental exposure.

With advances in technology, the single-base resolution mapping of epigenetic status (both DNA methylation and histone modifications) has become reality even at a single-cell level. Determining the genomic sequence may not be enough but the landscape needs more detailed characterization in the form of epigenetic studies. These studies will be necessary in the era of personalized medicine, to treat the individual patients with right drugs. Detailed characterization of epigenetic status in CVD, cancer and other diseases increases our understanding of the mechanisms of those diseases and will facilitate the development of epigenetic drugs in the future.

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Exercise and Cardiac Rehabilitation in Heart Failure

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Introduction

The cardinal symptom of heart failure is exercise intolerance secondary to fatigue and dyspnea. Exercise intolerance leads to significant reductions in patients' quality of life and functional level, estimated by the New York Heart Association (NYHA) classification. The aim of exercise training is to increase patients' exercise capacity and improve their functional level, quality of life, frequency of admission, and mortality.

Cardiac rehabilitation is a prescribed program of exercise training that has evolved into a multidisciplinary approach that may also include patient education, disease management, self-care counseling, and behavioral change coaching. Cardiac rehabilitation is defined by the Centers of Medicare and Medicaid Services (CMS) as a "program that furnishes physician-prescribed exercise, cardiac risk factor modification, psychosocial assessment, and outcomes assessment" (CMS, 2009).

Historical Perspective

Prior to the 2000s, the efficacy and safety of exercise training in heart failure were unestablished. Decades earlier, studies of exercise training first began in the care of patients with acute myocardial infarction (MI).

A patient suffering an MI in the first half of the 20th century would have been prescribed weeks of strict bed rest during which moving, let alone exercise, was verboten. After being established in a landmark paper by (Herrick et al., 1912), bed rest remained standard for decades until the early 1950s, when Levine and Lown (1952) began allowing patients to sit in armchairs and noticed shortened recovery times. With some controversy, physicians began to incorporate exercise earlier, and noted not just reduction in clots and bed sores but also decreased angina, increased exercise capacity, and decreased mortality.

Exercise training in heart failure has followed a similar arc from controversy to acceptance though decades after its adoption in MI. In the past, patients with heart failure were prescribed bed rest in an attempt to minimize symptoms and over concern that exercise would worsen left ventricular dysfunction (McDonald et al., 1972). Skepticism around the safety of exercise in heart failure persisted for logical reasons. In comparison to coronary heart disease patients, patients with heart failure are generally older, frailer, have more comorbidities, and are more prone to arrhythmia and fluid overload. In its 2005 guidelines on heart failure, the American College of Cardiology and American Heart Association (ACC/AHA) stopped short of providing a recommendation citing a lack of studies on long-term effects (Hunt et al., 2005).

In 2007, the National Institute of Health helped address this uncertainty by funding HF-ACTION, a large trial studying the efficacy and safety of exercise training in stable systolic heart failure. Based largely on results of the trial, the 2013 ACC/AHA guidelines provided a I–A recommendation for exercise training and a IIa–B recommendation for cardiac rehabilitation in stable patients with heart failure. CMS followed in 2014 with a National Coverage Decision approving cardiac rehabilitation in patients with stable, chronic heart failure defined as patients with left ventricular ejection fraction of 35% or less and NYHA class II–IV symptoms despite being on optimal heart failure therapy for at least six weeks, and who have not had recent ($\leq$6 weeks) or planned ($\leq$6 months) major cardiovascular hospitalizations or procedures (CMS, 2014).

Despite the approval of cardiac rehabilitation in stable heart failure, physician referrals and patient adherence to cardiac rehabilitation remain low. Also, patients with heart failure with preserved ejection fraction (HFpEF) and patients who have had admissions within six weeks remain ineligible. Cardiac rehabilitation has the potential to confer additional benefits in survival and

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quality of life in heart failure patients. To achieve its full potential, additional work is needed to improve referral and adherence rates and to study populations which lack robust evidence of benefit and safety.

Physiologic Basis for Exercise Training in Heart Failure

Measures of Exercise Capacity

Heart failure is characterized by exercise intolerance due to fatigue and dyspnea. In physiologic terms, this intolerance is due to reduced exercise capacity, which is often estimated by screening tools like the NYHA classification or by tests at submaximal effort such as the 6-min walk test. A more accurate estimate of exercise capacity is peak oxygen consumption, also known as maximal aerobic capacity (peak VO_2), which is the amount of oxygen consumed by the body at maximal effort. It can be measured during cardiopulmonary exercise testing (CPX), a standard first step after referral to cardiac rehabilitation. It is defined by the Fick equation as the product of cardiac output and the arteriovenous oxygen difference at peak exercise:

$$\text{peak } VO_2 = (HR^{*}SV)^{*}[C(a-v)O_2]$$

HR = heart rate; SV = stroke volume; C(a − v) is the difference in arterial and venous oxygen concentration. Peak VO_2 is often reported in mL/kg/min to allow for comparisons between people of different weight.

Another measure estimating exercise capacity is the ventilatory threshold (VT), which provides a better measure of exercise capacity at submaximal workloads. VT is based on the work of Wasserman and is thought to reflect the anaerobic threshold, or the point at which oxygen demand by muscle exceeds oxygen delivery (Wasserman, 1987). At low and moderate rates of exercise, expired gas (VE) rises linearly with VO_2. At a threshold work load, called VT, VE begins rising faster than VO_2 (see **Fig. 1B**). This divergence is thought to be due to the release of CO_2 from the conversion of lactic acid to lactate after the beginning of anaerobic respiration. Some dispute the accuracy and physiologic basis of VT as a marker for lactic acidosis and anaerobic respiration (Myers and Ashley, 1997; Hopker et al., 2011); however, given the increase in respiratory rate and dyspnea at VT, it remains a useful and conveniently measured surrogate for maximal sustained submaximal work rate. Other physiologic measures recorded during cardiopulmonary testing can be found in **Table 1**.

Reduced Exercise Tolerance in Heart Failure

In heart failure with reduced ejection (HFrEF), cardiac output is reduced by a lower stroke volume and lower heart rate reserve. Since VO_2 is the product of cardiac output and arteriovenous oxygen difference, it might be inferred that a decrease in stroke volume is the main driver behind reduced exercise capacity in HFrEF. Oddly, studies demonstrate a poor correlation between measures of resting ventricular function and exercise capacity suggesting other pathologies contribute (Franciosa et al., 1981). Studies comparing patients with heart failure to age and activity-matched controls show further defects in ventilation, skeletal muscle, endothelial function, and neurohormonal regulation (see **Table 2**) (Downing and Balady, 2011).

In heart failure with preserved ejection fraction (HFpEF), the left ventricle is not able to increase compliance to accommodate the greater diastolic filling rate needed during exercise. Dyspnea occurs due to a rise in left ventricular diastolic pressure and pulmonary capillary wedge pressure.

Physiologic Effects of Exercise Training

Peak VO_2 and VT have been shown to rise with exercise training in both healthy individuals and in patients with heart failure.

Interestingly, most studies have not shown increases in LVEF after exercise training. Much of the gain in exercise capacity is thought to be due to improvements in peripheral fitness. Studies have shown increases in mitochondrial density and type 1 oxidative fibers, and a decrease in sympathetic tone (see **Table 3**) (Downing and Balady, 2011). Cardiac adaptations, however, such as improvement in diastolic function have been documented.

Support for Cardiac Rehabilitation in Heart Failure

Prior to HF-ACTION, small trials had shown mixed data for the benefit of cardiac rehabilitation in heart failure, though meta-analyses supported a benefit in exercise capacity and quality of life and a decrease in hospitalizations. HF-ACTION was designed to definitively measure the safety and efficacy of exercise training in stable patients with systolic heart failure (LVEF <35%). Patients had to be medically optimized on ACEI/ARB and beta blocker. The study enrolled 2331 patients, NYHA Class II–IV (63% class II, 36% class III), of whom 40% had implantable defibrillators and 18% had biventricular pacing. The composite primary endpoint was all-cause mortality or hospitalization. Patients were randomized to either 36 sessions of supervised, moderate-intensity training (60%–70% of heart rate reserve) followed by home-based training or usual care. Subjects in the control arm were recommended to exercise 30 min per day at moderate intensity per ACC/AHA recommendations at that time. Halfway through the period, subjects in the experimental arm were given a treadmill or stationary bike and advised to exercise at moderate intensity for 40 min five times

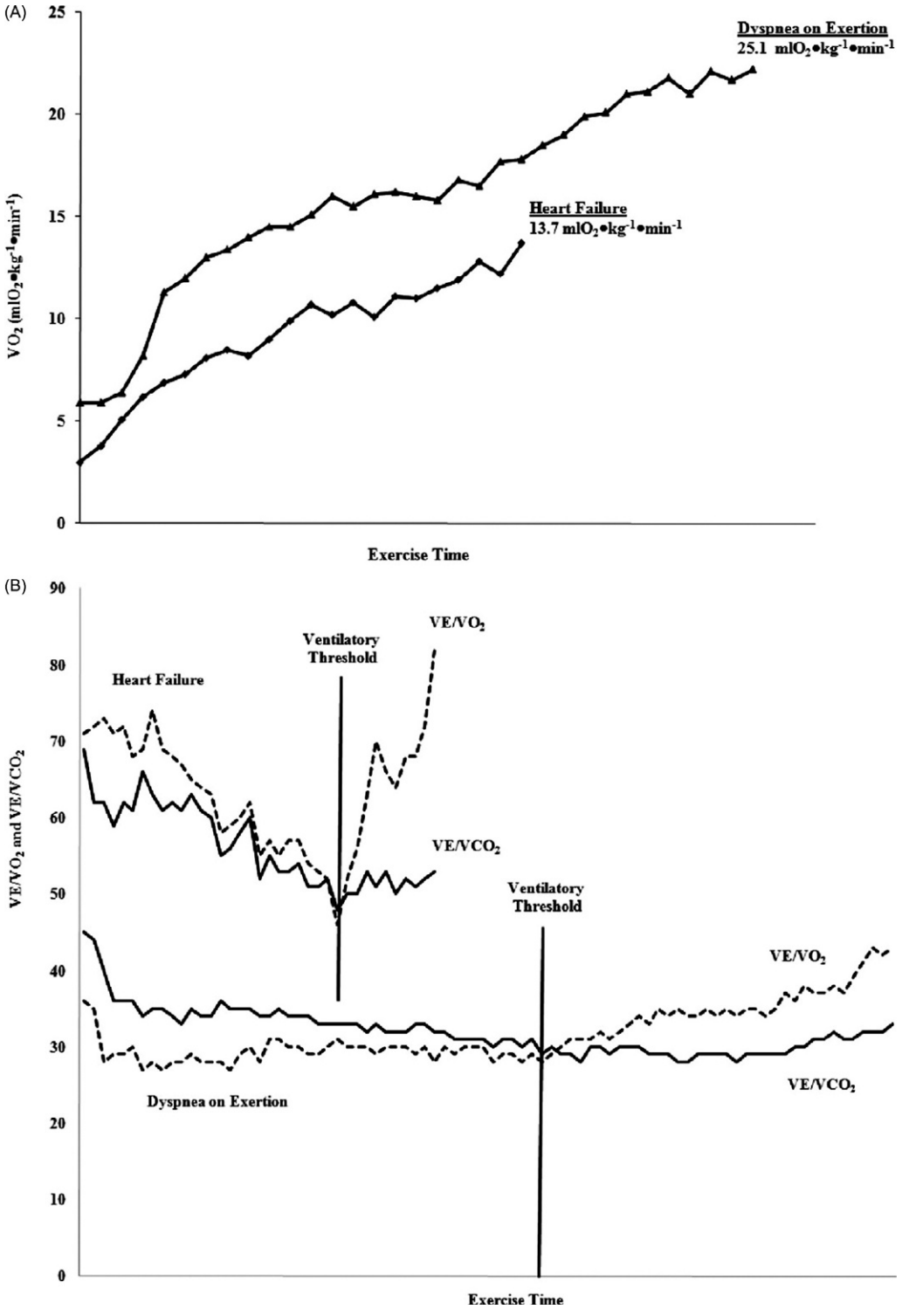

Fig. 1 (A) Peak VO_2, a measure of aerobic capacity, is reduced in heart failure. (B) determination of ventilatory threshold during cardiopulmonary exercise testing at point of increase in slope of VE/VO_2.

weekly. After a median follow-up of 30 months, the study found a nonsignificant 7% reduction in the primary combined endpoint ($P=0.13$). After adjusting for prespecified predictors of clinical outcome (baseline exercise duration, LVEF, Beck Depression Inventory II, history of atrial fibrillation, or flutter), investigators found a significant 11% reduction in the primary combined endpoint ($P=0.03$) (see Fig. 2). The secondary endpoint of cardiovascular mortality or heart failure hospitalization was also significantly reduced after adjustment (0.85, $P=0.03$). There was no significant difference in mortality (0.96, $P=0.7$). The study also established the safety of exercise training as adverse events were similar between the two groups.

Table 1 Comparison of physiologic parameters of heart failure and dyspnea on exertion

Reason for test	*Heart failure*	*Dyspnea on exertion*
Baseline characteristics	35-year-old female	35-year-old female
	Normal body weight	Normal body weight
Relevant past medical history	3-year history of idiopathic heart failure	2-year history of unexplained dyspnea on exertion
	Resting left ventricular ejection fraction 15%	Normal cardiac and pulmonary function
Aerobic capacity	Peak $\dot{V}_{O_2}$: 13.7 mL O_2 kg^{-1} min^{-1}	Peak $\dot{V}_{O_2}$: 25.1 mL O_2 kg^{-1} min^{-1}
	45% of predicted for age/sex	83% of predicted for age/sex
Peak RER	1.14	1.18
Submaximal exercise tolerance	$\dot{V}_{O_2}$ at VT: 8.5 mL O_2 kg^{-1} min^{-1}	$\dot{V}_{O_2}$ at VT: 14.0 mL O_2 kg^{-1} min^{-1}
Ventilatory efficiency	Abnormally elevated $\dot{V}E/CO_2$ slope	Normal $\dot{V}E/CO_2$ slope
Additional pulmonary function testing	No change in PEF after exercise compared with baseline	30% reduction in PEF after exercise compared with baseline
Breathing reserve	$(1 - \dot{V}E/MVV) > 20\%$ and within normal limits	$(1 - \dot{V}E/MVV) < 20\%$ and abnormally reduced
Electrocardiography	Occasional ventricular ectopy	No abnormalities detected
	Maximal heart rate = 122 bpm (66% of predicted) on a β-blocker	Maximal heart rate = 176 bpm (95% of predicted)
Hemodynamics	Blood pressure at rest: 110/60 mmHg	Blood pressure at rest: 118/72 mmHg
	Blood pressure at maximal exercise: 140/68 mmHg	Blood pressure at maximal exercise: 180/66 mmHg
Subjective symptoms	Maximal perceived exertion: 16/20	Maximal perceived exertion: 16/20
	Maximal dyspnea: 3/4	Maximal dyspnea: 4/4
	Reason for stopping: dyspnea	Reason for stopping: dyspnea
Interpretation	Severely diminished aerobic capacity and abnormal ventilatory efficiency: consistent with moderate to severe heart failure pathophysiology	Mildly diminished aerobic capacity; changes in pulmonary function after exercise and abnormally reduced breathing reserve are consistent with exercise-induced bronchospasm

PEF indicates peak expiratory flow rate; *bpm*, beats per minute.

Table 2 Pathophysiological mechanisms of exercise intolerance in heart failure (Downing and Balady, 2011)

Cardiac
Systolic and/or diastolic dysfunction
Reduced stroke volume
Elevated filling pressures
Secondary pulmonary hypertension and RV dysfunction
Mitral regurgitation
Reduced chronotropic reserve
Ventilatory system
Exaggerated minute ventilation relative to CO_2 production
Pulmonary hypertension and resulting pulmonary vascular damage and fibrosis
Ventilation/perfusion mismatch
Alveolar edema
Skeletal muscle
Reduced muscle mass
Reduced Type IIa fibers relative to Type IIb fibers
Reduced enzymes for oxidative metabolism and generation of ATP
Delayed resynthesis of high-energy compounds
Endothelial function
Reduced nitric oxide
Increased reactive oxygen species
Reduced vasodilatory response to shear stress
Neurohumoral system
Increased sympathetic activity
Low vagal activity
Increased levels of pro-inflammatory cytokines

Table 3 Beneficial effects of exercise in chronic heart failure (Downing and Balady, 2011)

Aerobic training
Increased exercise capacity
Lower heart rate response to submaximal exercise
Improved diastolic function
Improved endothelial function
Increased skeletal muscle oxidative capacity
Enhanced vagal tone and lower sympathetic tone
Reduced inflammatory cytokines
Lower all-cause mortality or hospitalization
Improved quality of life
Resistance training
Increased muscle strength and endurance

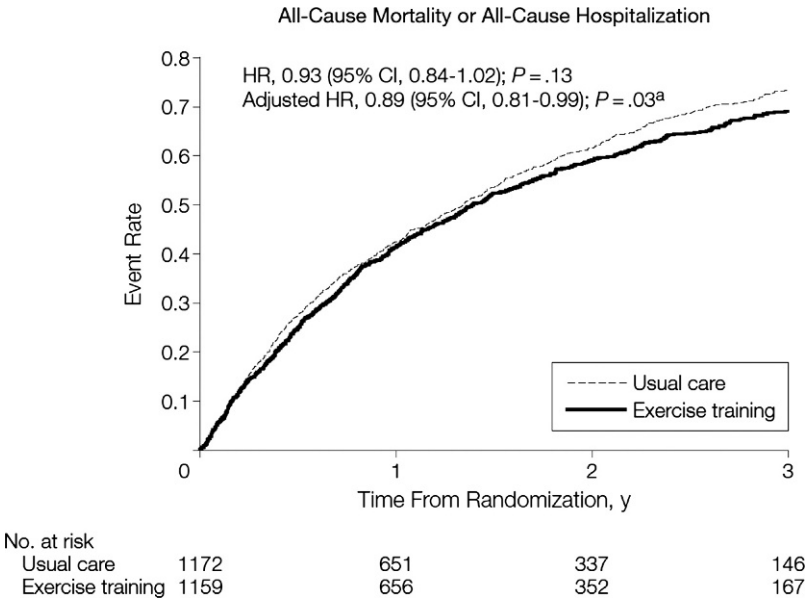

Fig. 2 Reduction in all cause mortality or hospitalization over the study period after adjustment for predictors.

The study was seen as disappointing to some for requiring statistical adjustment to achieve a significant result and for achieving a lower than expected effect size. The median improvement in peak VO_2 in the experimental group was 4%, much lower than the 10%–15% expected increase seen in previous studies. The results may have been affected by low adherence in the experimental group, as only 30% of patients in the experimental arm exercised at or above the target number of minutes per week. A subsequent analysis by Keteyian et al. (2011) investigated the relationship between volume of exercise and events and found that patients who achieved the prescribed exercise target had a 30% reduction in mortality and hospitalizations.

The importance of adherence was further demonstrated by a trial in Italy of 123 patients with stable heart failure randomized to a 10-year supervised exercise training program or control group. The trial was able to achieve 88% adherence to the exercise training program in the experimental group, which showed an increase in peak VO_2 of 14.7% compared to −2.5% for the control group ($P<0.01$) at 12 months (see **Fig. 3**). At 10 years, the experimental group had significantly fewer readmissions (0.64, $P<0.001$) and cardiac deaths (0.68, $P<0.001$) (Belardinelli et al., 2012). Unfortunately, a ten-year supervised exercise program is likely unfeasible, and it is unclear if these results could be replicated in an unsupervised program.

A 2014 Cochrane review of 33 trials of exercise-based rehabilitation in heart failure found no effect of exercise training on one-year mortality (0.93, 95% CI: 0.69–1.27) but a trend toward a reduction in mortality in trials with more than one year of follow-up (0.88, 95% CI: 0.75–1.02). Exercise training reduced overall hospitalization (0.75, 95% CI: 0.62–0.92) and heart failure-specific hospitalization (0.61, 95% CI: 0.46–0.80) and showed improved quality of life on the Minnesota Living with Heart Failure questionnaire (−5.8 points, 95% CI: −9.2 to −2.4). These benefits were independent of patients' age, gender, and severity of heart failure. The review highlights the need for further studies on cardiac rehabilitation in heart failure with preserved ejection fraction.

The safety and efficacy of cardiac rehabilitation has also been evaluated in specific populations. Rehab-VAD was a small trial ($n=26$) of patients with left ventricular assist devices that showed significant improvements in treadmill time, six-minute walk test,

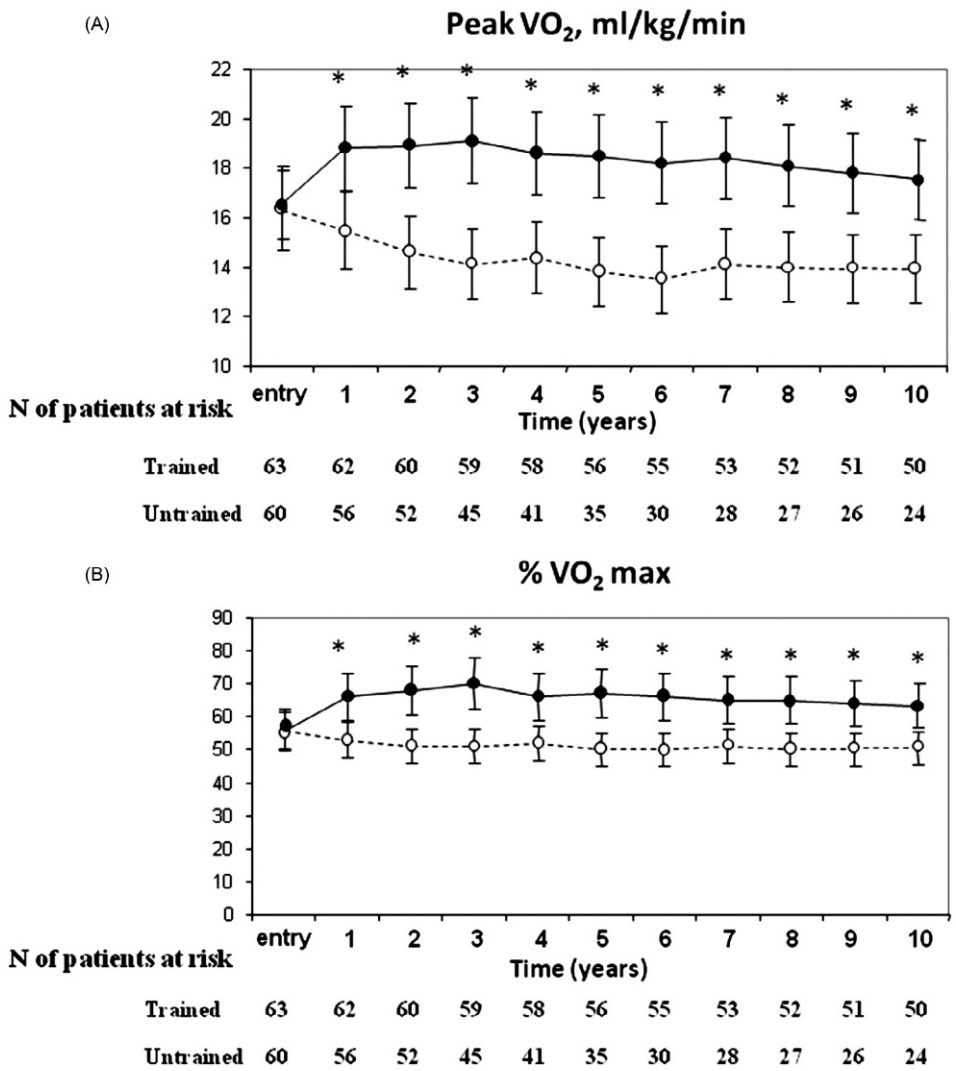

Fig. 3 Effect on aerobic capacity (peak VO_2) of a ten-year supervised exercise training program (Belardinelli et al., 2012).

and leg strength without significant improvement in peak VO_2 (Kerrigan et al., 2014). Sub-analysis of HF-ACTION by Luo et al. (2017) showed similar benefits from exercise for patients with atrial fibrillation (AF) as those in sinus rhythm even though patients with AF were older and had worse outcomes. REHAB-HF is currently in process and will evaluate the efficacy of cardiac rehab for 360 patients with acute decompensated heart failure over age 60 (Reeves et al., 2017).

Cardiac Rehabilitation in Practice

Cardiac rehabilitation, as developed in the treatment of acute MI, traditionally consists of three phases defined by setting and time. Phase I is inpatient rehabilitation occurring in the hospital after an acute event. It plays a smaller role today due to decreases in hospital admission lengths. Phase II occurs after discharge or upon referral. In the case of heart failure in the United States, it must begin at least six weeks removed from an acute cardiovascular hospitalization. It is commonly composed of 36 sessions over 3–4 months. Phase III refers to indefinite training occurring after the termination of Phase II and is not often covered by insurance in the United States.

The individual structure of cardiac rehabilitation programs can vary widely along a number of attributes (see **Table 4**). The optimal approach for different types of patients with heart failure has yet to be elucidated. Generally, resistance and inspiratory training is added as a complement to aerobic training, but respiratory training may be useful as a standalone therapy or a bridge for patients who are too frail or have a disability preventing use of an exercise machine. Aerobic exercise training is designed to increase exercise capacity; resistance training increases muscle strength and bone mass, and respiratory training can increase exercise capacity and ventilatory efficiency. Resistance training should be dynamic (isotonic) rather than static (isometric), which can increase blood pressure and lower stroke volume (Labate and Guazzi, 2015).

Table 4 Attributes of cardiac rehabilitation

Approach	*Examples*
Modality	Endurance aerobics
	Resistance training
	Respiratory training
Intensity level	Light
	Moderate
	High
Method	Continuous
	Interval
Application	Systemic muscle
	Regional muscle
	Respiratory muscle
Mode	Bicycle
	Treadmill
Setting	Hospital
	Center
	Home

Adapted from Labate, V. and Guazzi, M. (2015) Past, present, and future rehabilitation practice patterns for patients with heart failure. *Heart Failure Clinics* **11** (1), 105–115.

Several studies of high-intensity interval training (HIIT) have shown larger gains in peak VO_2 over moderate continuous training (MCT) (Wisloff et al., 2007). However, the SMARTEX trial, which randomized 261 patients with stable systolic heart failure to HIIT (90%–95% peak HR), MCT (60%–70% peak HR), or regular exercise found no significant difference between the improvement in aerobic capacity of HIIT and MCT. Additionally, 80% of MCT patients exercised above their target while 51% of HIIT patients exercised below their target, calling into question the general feasibility of HIIT in a heart failure population (Ellingsen et al., 2017).

Several studies have compared center and home-based therapies. A 2015 Cochrane Review on home-based versus center-based cardiac rehabilitation supports home-based cardiac rehabilitation as equally effective as center-based rehabilitation in short-term studies. The authors did not see a consistent difference in costs between center and home-based cardiac rehabilitation and recommend patient's needs and preferences drive decision (Taylor et al, 2015). The European Association on Cardiovascular Prevention and Rehabilitation has recommended center-based cardiac rehabilitation for patients with recent clinical stabilization or multiple comorbidities (Vanhees et al., 2012).

Challenges Facing Cardiac Rehabilitation

Increasing Utilization

Referral rates in the United States, which were at 13.3% of heart failure patients prior to Medicare approval, remain low (Golwala et al., 2015). One factor is certainly lack of cardiac rehabilitation services in certain geographic areas and a lack of capacity for all eligible patients in areas it exists (McNeely et al., 2016; Pack et al., 2014). Low provider referral rates in areas that do offer cardiac rehabilitation also play a role and vary largely at the hospital level, with bottom quartile hospitals referring less than 20% of eligible patients and top quartile hospitals referring over 80% (Aragam et al., 2015). Strategies shown to increase referral rates include the adoption of automatic referral systems in standard order sets and staff liaisons who help with the enrollment process (Mueller et al., 2009). Physicians must still play a role, however, as direct, in-person recommendations and discussion of potential barriers are a powerful predictor of cardiac rehabilitation participation.

Improving Adherence

A large challenge for cardiac rehabilitation programs is poor adherence and participation. HF-ACTION reported just 30% of patients achieved target exercise levels, a factor many cited as cause for the lower than expected increase in peak VO_2 of the experimental group. Many strategies can be used to improve adherence. Home-based cardiac rehabilitation has been shown to be as effective as center-based rehabilitation and can be more feasible for certain patients. Telehealth and mobile care plans have also been used to increase patient engagement and can assist in behavioral change. Consistent communication and reinforcement from all members of the rehabilitation team including case managers, liaisons, physical therapists, exercise physiologists, and physician can help drive participation. In addition, psychosocial and barrier assessment remains crucial as lower participation is found in low socioeconomic groups and depressive symptoms are found in nearly 30% of patients with heart failure (Kato et al., 2012).

Expanding Populations

Since 2014, Medicare only covers cardiac rehabilitation for patients with stable HFrEF on optimal therapy. Currently, the REHAB-HF trial is evaluating cardiac rehabilitation after acute exacerbation in elderly patients and including patients with HFpEF. More trials need to be performed to establish the long-term benefits of cardiac rehabilitation in patients with HFpEF, who comprise just over 50% of the population with heart failure. After CMS updated eligibility for cardiac rehabilitation in 2014, the percentage of patients with heart failure eligible for cardiac rehabilitation rose from 14% to 41%. This added just 27% of the heart failure population and left 59% ineligible mostly due to EF >35%. In analysis by Kelly et al. (2016), both HFpEF and HFrEF groups had one year mortality greater than 30% and readmission rates >65% suggesting an equal need for interventions that may reduce admissions and mortality. Given the lack of success of pharmaceutical trials in HFpEF, the potential for benefit from cardiac rehabilitation is intriguing and deserves greater study.

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Exercise, Physical Activity, and Cardiovascular Disease

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Introduction: Physical Activity, Exercise, and Heart Disease

For six decades, researchers have explored the cardiovascular benefits of exercise and physical activity, and documented consistent protective and therapeutic benefits for being physically active. This article reviews some of this evidence, with a focus on cardiovascular disease (CVD), defined here particularly as coronary heart disease (CHD).

Exercise is defined as planned and structured large muscle activity with the aim of improving maintaining physical fitness, performance, and health (Boden et al., 2013). It is a subset of "physical activity" which is more broadly defined as any movement involving large skeletal muscles leading to energy expenditure (Caspersen et al., 1985; Boden, 2013). Physical activity includes all large muscle movement throughout the day, at work, in travel to or from places, at home and in recreation or leisure time.

We review the primary prevention evidence for physical activity in reducing the risks of developing CVD among populations initially free of disease. We consider the new evidence on sedentary behavior (prolonged sitting time) and CVD. Then we consider the roles of physical activity and exercise for people with heart disease, and for common forms of heart disease: people with atrial fibrillation (AF) and patients with heart failure (HF), as exemplars of common cardiac conditions related to, or treated by physical activity and exercise. (Note that strictly this is tertiary prevention (as secondary prevention is screening for disease), but the term secondary prevention is widely used in cardiovascular health to mean patient-related prevention, so that this terminology is used here.)

The evidence for primary prevention comes from population-based epidemiological studies; the evidence for exercise and physical activity and potential benefits for people with cardiac disease comes from epidemiological, clinical, and basic science research. Hence, the perspectives in this chapter are multidisciplinary, emanating from both population and clinical sciences.

The Cardiovascular Health Benefits of Physical Activity: A Primary Prevention Approach

This section reviews the evidence for physical activity in the primary prevention of CVD, and is based on the epidemiological and population health research evidence accumulated over several decades. Primary prevention implies that in physical activity is beneficial in reducing the risk of developing CVD in populations of healthy individuals who are free of CVD. Much of this research emanates from epidemiological studies, starting in the 1950s with the observation that London bus drivers had higher CVD event rates than bus conductors, with the latter group being more active throughout the working day (Morris et al., 1953). Many studies followed, with consistent evidence provided for occupational and leisure time physical activity. Up until the 1990s, physical activity for health was usually defined as vigorous intensity "aerobic exercise," at least three times per week for at least 20 min each time (Kravitz et al., 2011). By 1990, there was a meta-analysis (Berlin and Colditz, 1990) and a systematic review (Powell et al., 1987) that clearly identified the risks of physical inactivity; on average, those who were least active were 1.9 times as likely to develop heart disease compared to the most active in the population.

A decade later, the US Surgeon General's report on the health benefits of physical activity was released (USSG, 1996) that redefined the minimal quantum of physical activity required to realize most cardiovascular and health benefits. This was a minimum recommendation of 30 min of at least moderate-intensity physical activity on most days of the week, a guideline that emphasized a new and more achievable level of physical activity for population health (USSG, 1996). The rationale for this was that most mortality risk and cardiovascular risk occurs in those who were completely inactive, and that maximum population benefit occurs if these inactive individuals start doing some activity, ideally half an hour a day (Powell et al., 2011). Benefit continued to accrue up to an hour a day of moderate-vigorous physical activity (MVPA), but beyond an hour a day, little additional benefit was noted (Arem et al., 2015). These benefits focused on all causes of mortality, but included risks of cardiovascular deaths, and risks of incident CVD events.

These cardiovascular benefits are now well established. Li and Siegrist (2012) conducted a meta-analysis of 21 epidemiological cohort studies, and compared to low active males, the men who met the recommended levels of activity had a 24% reduction in

CVD events; the same analysis showed a 27% risk reduction for women. These recent studies mostly focused on leisure time physical activity, with the role of occupational activity less clear (Li et al., 2013). The dose-response relationship was further verified by a 2011 meta-analysis of 33 cohorts (Sattelmair et al., 2011) which showed greater risk reduction for 300 min/week (an hour a day) compared to the minimum recommended level of 150 min/week of MVPA. The cardioprotective relationship was greater for women than men. Earlier meta-analyses had reached similar conclusions (Nocon et al., 2008; Oguma and Shinoda-Tagawa, 2004; Gebel et al., 2015), with even regular walking providing clear cardioprotection (Oguma and Shinoda-Tagawa, 2004). A consistent observation was that there was a stronger relationship for objective measures of fitness or movement, compared to self-report physical activity, indicating that the many self-report studies may underestimate the association (LaMonte and Blair, 2006; Nocon et al., 2008). Although moderate activity does, by itself, confer much of the preventive benefit, adding some vigorous activity further increases the benefit (Gebel et al., 2015).

One additional concept is the notion of the burden of disease, or the attributable fraction for disease; in other words, what proportion of diseases could be prevented if the risk factor was reduced or eliminated. According to the World Health Organization (2009), 57% of cardiovascular deaths can be traced back to one of the lifestyle risk factors and conditions (physical inactivity, smoking, unhealthy diet, hazardous alcohol use, and the resultant hypertension, obesity, and hypercholesterolemia). Physical inactivity is responsible for 3–5 million preventable deaths per year, and is the fourth ranked of these risk factors, ranking ahead of overweight and obesity (especially for CVD risk, WHO, 2009). These physical inactivity-related deaths and cardiovascular events occur in high and middle income countries, and especially in middle income countries may lead to premature mortality of people of middle age, which has national economic and productivity implications in many countries.

Given the strong evidence base, many Government and non-Government organizations have developed and adopted physical activity guidelines for preventing heart disease. The recent guidelines by the American Heart Association point to the benefits of at least half an hour of moderate-vigorous intensity physical activity on most days of the week (Haskell et al., 2007); the World Health Organization has a similar recommendation, of 150–300 min MVPA per week. For most patients with heart disease, the same recommendation, 30–60 min of activity on most days of the week remains current. In addition, sedentary behavior guidelines, to reduce prolonged and uninterrupted sitting time, are being developed, although the exact cut-points and nature of sitting-related risk remains uncertain (Young et al., 2016).

The Role of Exercise as a Secondary Prevention in Managing CVD

Exercise and physical activity are evidence-based approaches to the management of CVD, and in particular to cardiac rehabilitation following a cardiac event (Anderson et al., 2016). The physiological mechanisms underlying the beneficial effects of exercise in the secondary prevention for patients with CHD are well established. Exercise remains an important component of recovery from cardiac events, because it protects against cardiac ischemia/reperfusion injury (Boden et al., 2013). A number of reviews showed that regular moderate or vigorous-intensity exercise benefits people who have CHD by: (i) preventing the coronary arteries from further narrowing (antiatherosclerotic), (ii) inhibiting blood clotting (antithrombotic), (iii) promoting perfusion (antiischaemic), (iv) maintaining a normal heart rhythm (antiarrhythmic), (v) positively impacting on established cardiovascular risk factors (antiatherogenic), (vi) lowering inflammatory markers (antiinflammatory), and (vii) improving autonomic and vascular endothelial function (Boden et al., 2013; Fletcher et al., 2013; Leon et al., 2005). To exemplify this, exercise evokes antithrombotic effects by increasing fibrinolysis and decreasing platelet adhesiveness, fibrinogen, and blood viscosity (Fletcher et al., 2013). Moreover, the antiarrhythmic effect of exercise is stimulated through reduced adrenergic activity and increasing both vagal tone and heart rate variability (Fletcher et al., 2013). These changes help to decrease the load on the heart at rest and during exercise, which diminishes some of the symptoms of CHD (Fletcher et al., 2013).

It is well documented that people diagnosed with CHD who engage in aerobic exercise generate cardiac and peripheral adaptations including improvements in peak oxygen uptake, cardiac output, myocardial oxygen demand, and autonomic function (Fletcher et al., 2013; Mezzani et al., 2013). Regular, long-term exercise generates a range of morphological and phenotypic alterations in the myocardium such as resting bradycardia, left ventricle hypertrophy, cellular growth/adaptations in cardiac myocytes, and modified coronary vascular function (Brown et al., 2003; Fletcher et al., 2013). Moreover, participants diagnosed with heart disease who engaged in aerobic exercise frequently demonstrate an increase in cardiorespiratory fitness (VO_2 max levels, Mezzani et al., 2013). In addition, exercise may increase cardiac output which leads to improved vasomotor function (e.g., coronary dilatation) resulting in improved myocardial oxygen delivery to the myocardium leading to increased stroke volume (Leon et al., 2005). People with CHD who exercise at high-intensity levels show improvements in their cardiac function such as increased ejection fraction, stroke volume, diastolic function, and wall motion parameters (Fletcher et al., 2013). Exercise also promotes lowered myocardial oxygen demand after training for participants with CHD, leading to lower heart rate, systolic blood pressure, and circulating catecholamines (Fletcher et al., 2013; Leon et al., 2005). In summary, a plethora of evidence confirms a dose-response relationship between exercise and cardiovascular-related physiology and risk (Varghese et al., 2016).

When CHD is established, exercise and physical activity play a beneficial and significant role in the disease management. This is primarily because increased physical activity levels and cardiorespiratory fitness appear to mitigate CHD progression (Boden et al., 2013; Leon et al., 2005). Exercise also favorably influences some CHD risk factors, including insulin resistance, glycaemic control, blood pressure, lipid profile, fibrinolysis, endothelial function, inflammatory defense systems, and hemostatic factors

(Fletcher et al., 2013; Varghese et al., 2016). Exercise improves cardiorespiratory fitness, muscle strength, body composition, and flexibility—which ultimately lead to enhanced functional capacity and improvements in the functionality of the cardiovascular and endocrine systems (Varghese et al., 2016). Recent meta-analyses reported a 26% reduction in the risk of cardiac mortality (Anderson et al., 2016), a 13% reduction in all-cause mortality (Heran et al., 2011), and an 18% reduction in hospital admissions (Anderson et al., 2016), at 12 months in participants who undertook exercise-based cardiac rehabilitation compared to those who did not undertake cardiac rehabilitation. The meta-analyses demonstrated improvements in CHD risk factors, a greater increase in functional capacity, and enhanced health-related quality of life (Anderson et al., 2016; Taylor et al., 2015). Structured exercise for 12 months in patients with stable angina may even produce better outcomes than the standard therapy of percutaneous coronary intervention as measured by greater event-free survival, reduced hospitalization, and repeated vascularization procedures (Hambrecht et al., 2004). These findings reinforce the importance of exercise and physical activity as a therapeutic approach in the management of people diagnosed with CHD.

Given the numerous benefits of exercise-based cardiac rehabilitation, it forms part of national health policy recommendations (Achttien et al., 2013; Anderson et al., 2016). Most programs include supervised exercise sessions with an exercise physiologist and education on physical activity recommendations (Achttien et al., 2013). Guidelines for cardiac rehabilitation recommend that during phase II of the program, exercise sessions should be offered twice weekly for a minimum of 6 weeks (Achttien et al., 2013). The guidelines suggest that cardiac rehabilitation should be individualized and include a review of progress at each contact, plus follow-up telephone contact to facilitate long-term exercise behavior change (Piepoli et al., 2016). Guidelines also recommend counseling interventions for patients that promote setting specific and short-term goals; offer feedback on progress; advocate behavioral self-monitoring; and use tailored and personalized interventions, built on readiness to change, motivational interviewing, and enhanced patient self-efficacy (Piepoli et al., 2016; Varghese et al., 2016). Physical activity should comprise at least 150 min of moderate-intensity per week (or for 30 min, 5 days/week, overall) or 75 min of vigorous-intensity per week or equivalent combinations of moderate and vigorous activity, along with 2–3 days per week of resistance and flexibility exercise (Piepoli et al., 2016).

Health care professionals are encouraged to evaluate patients' physical activity levels at every visit and prescribe exercise at suitable dosages along with the use of mobile wearable physical activity devices and use social media platforms to increase physical activity awareness and motivation, and to self-monitor exercise progress (Kaminsky et al., 2016). It is also important to note that when cardiac rehabilitation is completed, exercise should be promoted as a lifetime habit (Achttien et al., 2013).

Exercise and Heart Failure

Exercise reduces the risk of HF, but the type and intensity of exercise have different effects on risk, and the effects persist into later life. For example, walking pace and leisure activity reduce the risk of developing HF, but the intensity of exercise does not. Reduced risk for HF occurs when walking at a moderate pace, versus a slow pace (>3 vs. 2 miles/hour, hazard ratio (HR): 0.74, 0.63–0.86). Higher total leisure time physical activity also reduces the risk of developing HF in comparison to lower physical activity levels (>845 vs. <845 kcal/week, HR 0.78; 0.69–0.87) (Del Gobbo et al., 2015). The risk of HF is reduced by exercise through multiple pathways; both direct, in terms of improved cardiac endothelial cell function and nitric oxide availability for example, and indirect, through the prevention of hypertension, left ventricular hypertrophy, CHD, and type 2 diabetes, all major risk factors for HF (Schuler et al., 2013). Even among older people (>65 years) exercise can have benefits for reducing the risk of HF. The mechanisms for the benefit of exercise in primarily through decreasing arterial stiffening and left ventricular changes commonly associated with aging, although there are no effects on cardiac stiffening (Fujimoto et al., 2010).

When HF occurs, there are marked effects on exercise tolerance leading to reduced capacity to complete normal daily activities and reduced health-related quality of life, and in the longer-term HF results in admissions to hospital and mortality. Reduction in exercise capacity occurs regardless of whether ejection fraction is preserved or reduced. Exercise training improves exercise intolerance and is recommended globally for HF patients as an effective and safe component of HF management (McMurray et al., 2012; O'Gara et al., 2013; National Clinical Guideline, 2014). Exercise training is usually delivered as a component of comprehensive cardiac rehabilitation programs. While exercise-based cardiac rehabilitation does not reduce mortality in HF patients, there are significant benefits for patients in terms of risk of hospital admission for any cause (RR 0.75; 0.62–0.92) and HF-specific cause (RR 0.61, 0.46–0.80), as well as clinical improvements in HF-specific health-related quality of life (mean difference −5.8 points, −9.2 to −2.4) (Sagar et al., 2015). The specific quantum or type of exercise training required to achieve these effects was not identified, as the programs varied greatly. The effects noted were found to be independent of the type of exercise (aerobic or resistance) and the dose of exercise, as well as the length of follow-up. However, post hoc analyses of the HF-ACTION trial indicated that 3–7 metabolic-equivalent hours/week is needed to achieve a clinical benefit in HF (Keteyian et al., 2012).

The mechanism of the effects of exercise training in HF patients is through improved oxygen extraction by skeletal muscle rather than by improving cardiac compliance or ventriculo-arterial coupling, both hallmarks of HF (Haykowsky et al., 2012). Further, the evidence for the effects of exercise in HF patients applies best to chronic, stable HF patients as patients with comorbid conditions and/frailty are excluded from trials. Given the greater prevalence and severity of comorbid conditions, such as diabetes, musculoskeletal disorders and cerebrovascular disease, in HF, this is an important limitation (Fleg et al., 2015).

Exercise and Atrial Fibrillation

The relationship between physical activity and risk of incident AF is complex: it appears a U-shaped risk-curve exists in men, and a dose-response association among women (Mohanty et al., 2016). Sedentary lifestyles are associated with an increased risk of incident AF, odds ratio (OR) 2.47 (Mohanty et al., 2016). Moderate physical activity lowers AF risk for both women (OR 0.91) and men (OR 0.81) (Mohanty et al., 2016); with a graded reduction in cardiac arrhythmias of 4.8% for every metabolic equivalent (MET)h/day run or walked (Williams and Franklin, 2013). Physical activity is thought to protect against AF through lowering systemic inflammation, lowering resting heart rate, blood pressure, and improving lipoprotein profiles (Aizer et al., 2009).

Some associations are not necessarily protective. Previous meta-analyses associated high-intensity exercise with increased AF risk in endurance athletes (OR 5.29, Abdulla and Nielsen, 2009), but recent analyses suggest this increased risk is only for males (OR 3.30). Excess risk is highest in men aged 30–50 years performing >5 sessions/week, with risk reducing with increasing age (Aizer et al., 2009; Abdulla and Nielsen, 2009; Drca et al., 2014). Endurance exercise is thought to increase AF risk through left atrial enlargement, longer p-wave duration, increased vagal tone affecting the atrial refractory period, increased atrial ectopy, and chronic systemic inflammation (Wilhelm et al., 2011; Swanson, 2006).

Improving cardiorespiratory fitness is likely to reduce AF risk. For each additional 1 MET achieved on baseline-exercise testing, AF risk reduces by 7% (Qureshi et al., 2015); and each 1 MET increase is associated with a decreased risk of AF recurrence (HR 0.87) (Pathak et al., 2015a,b). Recent evidence suggests possible reversal of AF, and significant improvement in AF recurrence, symptom burden, and arrhythmia-free survival, through both weight reduction (Abed et al., 2013; Pathak et al., 2015a) and increasing cardiorespiratory fitness >2 METs from baseline (Pathak et al., 2015a,b), in patients with paroxysmal and persistent AF. Structured exercise, ranging from aerobic exercise to walking and yoga, are demonstrated to be safe for people with AF, and can improve exercise capacity, resting heart rate, quality of life (Lowres et al., 2012; Wahlstrom et al., 2017), management and treatment of AF, and may even reduce the incidence of AF (Giacomantonio et al., 2013).

Sedentary Behavior and CVD: Current Evidence Overview

Sedentary behavior SB is defined as a low-energy expenditure rate (<1.5 MET) in a sitting or reclining posture (SBRN, 2012). In general, research on the health risks of SB is mostly concerned with prolonged "sitting time". Despite the substantial research progress in the last decade, our understanding of the links between SB and cardiovascular health is not fully developed. Multiple methodological issues, such as poor measurement and considerable heterogeneity between studies hinder a confident translation of the evidence into quantitative sedentary behavior guidelines. For example, international multicountry studies using questionnaires have estimated a sitting median time of around 5 h in adults (Bauman et al., 2011; Bennie et al., 2013), which is a substantially less than that international estimates using objective assessment of sitting time, that reported medians of 8–9 h of sitting time per day (Matthews et al., 2008; Stamatakis et al., 2012; van der Berg et al., 2016; Clark et al., 2015).

In children and adolescents the overwhelming majority of the evidence examining the links between objectively assessed sedentary behavior and surrogate CVD markers is cross-sectional; the results of the few available prospective studies point toward weak or limited associations (Ekelund et al., 2012; Stamatakis et al., 2015). In adults, a meta-analysis of nine prospective cohort studies ($n = 720{,}425$) examining the dose-response association between self-reported sitting and CVD risk, and found a nonlinear association; only very high amounts of daily sitting in excess of 10 h/day were linked to increased risk (Pandey et al., 2016).

The supposition that SB is associated with adverse cardiovascular outcomes independently of physical activity has been widely proposed in recent years (Hamilton et al., 2008; Young et al., 2016). However, a substantial meta-analysis in a pooled sample with greater than 849,000 adults (Ekelund et al., 2016) found that among those who reported >35 MET-hours of physical activity per week (just over 1 h of moderate intensity physical activity per day) eliminated the association between sitting time and CVD mortality. Although these results are encouraging in highlighting that the risks of prolonged sitting can be offset by being more active, it is worth noting that this amount of physical activity is achieved only by a minority of adult populations in developed countries. Smaller previous prospective studies also found that average daily amounts of physical activity roughly equivalent to (Petersen et al., 2014) or greater than (Chomistek et al., 2013) meeting the public health recommendations attenuate the CVD risk associated with sitting.

The introduction of frequent and regular interruptions of continuous bouts of sitting (termed as "sedentary breaks") has been proposed as a promising intervention to counteract the cardiometabolic risks of prolonged sitting (Healy et al., 2008). Currently there are no prospective epidemiological studies examining the association between sedentary breaks and cardiovascular biomarkers (Chastin et al., 2015) and even cross-sectional studies showed inconsistent or no associations (Healy et al., 2011; van der Berg et al., 2016). Some laboratory trials in type 2 diabetic patients have showed beneficial effects of frequently interrupting sitting time with simple resistance activities (Dempsey et al., 2016) or light intensity walking on resting blood pressure and nonesterified fatty acids (Dempsey et al., 2016; Henson et al., 2015).

In summary, sedentary behavior is associated with CVD risk in adults who report relatively large amounts of sitting per day (>10 h/day) and do not report relatively high amounts of physical activity (approx. 1 h/day). To date, all prospective studies in this area estimated sitting times using questionnaires that appear to be subject to underreporting; it is therefore important that future studies using objective measures of sitting and physical activity revisit these thresholds and associations, as self-report sitting time

might underestimate the epidemiological risk. The concept of sedentary breaks for CVD risk reduction is promising but needs further evidence through large prospective studies with objective measures of sitting and physical activity.

Conclusion

Physical activity, and its more structured form, exercise, are useful in the treatment of people with heart disease, through cardiac rehabilitation programs. New forms of telehealth and remote contact cardiac rehabilitation are required, such that more people can access physical activity programs after cardiac events (Huang et al., 2015). The role of exercise in HF is also well established, with increasing interest in the role of activity in AF.

Other forms of CVD are outside the scope of this review, but may be strongly related to physical activity, such as the prevention of stroke (McDonnell et al., 2013; Diep et al., 2010), and the clinical treatment of stroke and maintenance of functional capacity (Billinger et al., 2014). There are a subset of cardiac diseases where excessive exercise may pose hazards, for example, extreme exercise in athletes (La Gerche, 2016; Merghani et al., 2016). Further, a small number of young people with inherited cardiac disease may be susceptible to sudden cardiac death, and need early identification and special provision made for participation in physical activity (Diller and Baumgartner, 2016; Hammond-Haley et al., 2016).

In summary, physical activity and exercise remain substantial risk factors for the development of heart disease, and substantial elements of managing many people with CVDs. In terms of primary prevention, maintaining lifelong regular and active lifestyles has consistent and strong associations with reduced CHD morbidity and mortality. It is still somewhat neglected in clinical practice, as many professionals focus on clinical prevention, biomarker-improving pharmacotherapy, and the main areas of advice are around tobacco cessation. Physical activity is as important in global health as either tobacco control or obesity control (Lee et al., 2012), especially in the prevention and management of CVD.

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Extracorporeal Membrane Oxygenation

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Introduction

Extracorporeal membrane oxygenation (ECMO) has its roots with the development and idea of supporting the cardiopulmonary system by way of an artificial oxygenator and ventilator as well as a perfusion circuit to provide circulatory support. This became a reality in the 1950s through the work of Gibbon and his heart–lung machine and the development of a bubble oxygenator following early experience with cross circulation by Lillehei (Gibbon, 1954; Lillehei, 1982). These were designed to provide cardiopulmonary support for the performance of cardiac surgery and not as a temporary support. The ability to use ECMO as a standalone tool to provide cardiopulmonary support for recovery was first successfully performed in 1972 for an adult suffering from respiratory failure (Lewandowski, 2000). In the 1970s, a government-funded randomized trial to evaluate ECMO efficacy in adults with respiratory failure versus traditional mechanical ventilatory support failed to show a benefit of ECMO (Zapol et al., 1979). However, there was evidence of success with ECMO in neonates with the first successful therapy being instituted in 1975 (Bartlett et al., 1976). Building upon Bartlett and his colleagues' early success, the Extracorporeal Life Support Organization (ELSO) was founded in 1989 as an international, multiinstitutional effort in pushing the field forward. More recently with improvements in technology, advancements in critical care medicine, and understanding of physiology combined with success with its use for the H1N1 epidemic in late 2000s, ECMO has been thrusted into the mainstream of therapeutic consideration for patients with cardiopulmonary collapse (Peek et al., 2009).

Indications

ECMO is the tool by which to provide extracorporeal life support (ECLS) in those patients suffering from cardiopulmonary collapse. The three main indications for ECLS are for patients suffering from cardiac failure, respiratory failure, and a combination needing extracorporeal cardiopulmonary resuscitation (ECPR).

Patients requiring ECMO for strictly respiratory failure by definition have an intact cardiovascular system and the mode for delivering ECMO is via veno-venous cannulation (VV-ECMO). This provides only the ability to oxygenate and ventilate a patient with hemodynamic support and circulation to the body remaining by way of the patients own cardiovascular system. The main indications for this therapy are worsening hypoxia and/or hypercarbia refractory to maximal medical and ventilator therapy secondary to a reversible etiology. The most common causes of this are patients with pneumonia, vasculitis, acute or chronic graft failure following lung transplantation, chemical pneuomonitis, pulmonary hemorrhage or contusion, pancreatitis, and massive pulmonary embolism. The advantage of VV-ECMO for these patients is the avoidance of instrumentation of a large cannula into a systemic artery, thereby preventing systemic thromboembolic phenomenon as well as ischemia to the peripheral arterial system where the cannula is placed. In this article, we will not discuss VV-ECMO further, but concentrate on the use of ECMO for patients with primary cardiogenic shock and/or failure requiring ECLS.

Venoarterial ECMO (VA-ECMO) is required for patients suffering from either cardiopulmonary failure or primarily cardiac failure. Patients with cardiopulmonary failure are those patients with witnessed in-hospital cardiac arrest whose indication for ECMO is for ECPR. Although not universal, the generalized indications for institution of VA-ECMO in these patients are for adults who are in cardiac or respiratory arrest following a witnessed, in-hospital arrest where effective, uninterrupted, and ongoing CPR without evidence of hypoxia and/or anoxic brain injury have occurred. Additionally, patients should be immediately evaluated for initiation of ECPR as best results are obtained in patients for whom VA-ECMO is instituted within 10–15 min of cardiopulmonary arrest.

Primary cardiovascular collapse can be an immediate phenomenon or may take several days to develop. Those patients with evidence of hypotension that is medically refractory and does not respond to inotropes, vasopressors, or mechanical circulatory support (intraaortic balloon pump, percutaneous ventricular offloading device, etc.) to maintain appropriate perfusion to end organs warrants screening for VA-ECMO. Decreased organ perfusion can be seen in patients with persisting lactic acidosis >3.2 mmol/L, decreasing urine output, cool and diaphoretic extremities, altered mental status, rise of creatinine of >1 mg/dL in 24 h, elevation in transaminases or development of pulmonary edema or hypoxia should raise concern, and profound cardiogenic

shock should be diagnosed in patients requiring high dose of one or more inotropes, persistent cardiac index <1.8 L/min/kg with concomitant hypotension despite fluid resuscitation. The etiology for cardiogenic shock may include acute on chronic decompensated heart failure, acute decompensated heart failure, acute coronary syndrome, peripartum cardiomyopathy, fulminant myocarditis, or cardiac allograft failure. Other less common indications include refractory ventricular tachycardia or ventricular fibrillation unresponsive to conventional therapy, hypothermia, acute anaphylaxis, pulmonary embolism, sepsis-related cardiac dysfunction, and drug overdose.

The modified vasoactive-inotropic score (VIS), a calculation utilizing dosage of vasopressors and inotropes calculated into a score, is an objective measure that can guide the need for ECMO (Gaies et al., 2014). As the indications for VA-ECMO are quite varied with multiple disciplines involved, there are no standardized, absolute contraindications to VA-ECLS. It is important though to consider the futility of therapy in those patients who have advanced age (>75 years), life expectancy from end-stage/active malignancy with less than 1 year, severe peripheral vascular disease, end-stage renal disease on dialysis, advanced liver disease, current intracranial hemorrhage or other contraindication to systemic anticoagulation, unwitnessed cardiopulmonary arrest with ongoing cardiopulmonary resuscitation, and witnessed cardiopulmonary arrest with prolonged cardiopulmonary resuscitation (>30 min) without return of spontaneous circulation.

Components of ECMO

The VA-ECMO circuit consists of a pump, a membrane oxygenator, a controller, cannulas for venous return from patient to circuit, and arterial outflow from pump to patient as well as tubing (**Fig. 1**) (Rodriguez-Cruz et al., n.d.). The pump is usually a centrifugal pump that can generate up to 4000 rotations per minute and flow up to 8 L per minute. The oxygenator functions not only to oxygenate blood but also to eliminate carbon dioxide. The controller for the oxygenator has the ability to alter the fraction of inspired oxygen into the system as well as the sweep in liters per minute for the purpose of carbon dioxide removal. The controller displays information to the clinician and allows for adjustments to pump speed in rotations per minute and ultimately flow through the entire system.

In determining the best method for cannulation in the initiation of VA-ECMO, it is imperative to consider the indications, patient baseline characteristics, and anatomic pitfalls. In the most simplistic terms, there is a drainage cannula that takes blood from the patient to the circuit and a return cannula that takes blood from the circuit back to the patient. Dual lumen, single cannulas can be employed for veno-venous support, but is beyond the scope of this article. The drainage cannula is inserted into the venous system of the patient and is typically a large diameter (21–25 French) and longer (up to 60 cm) than its arterial counterpart.

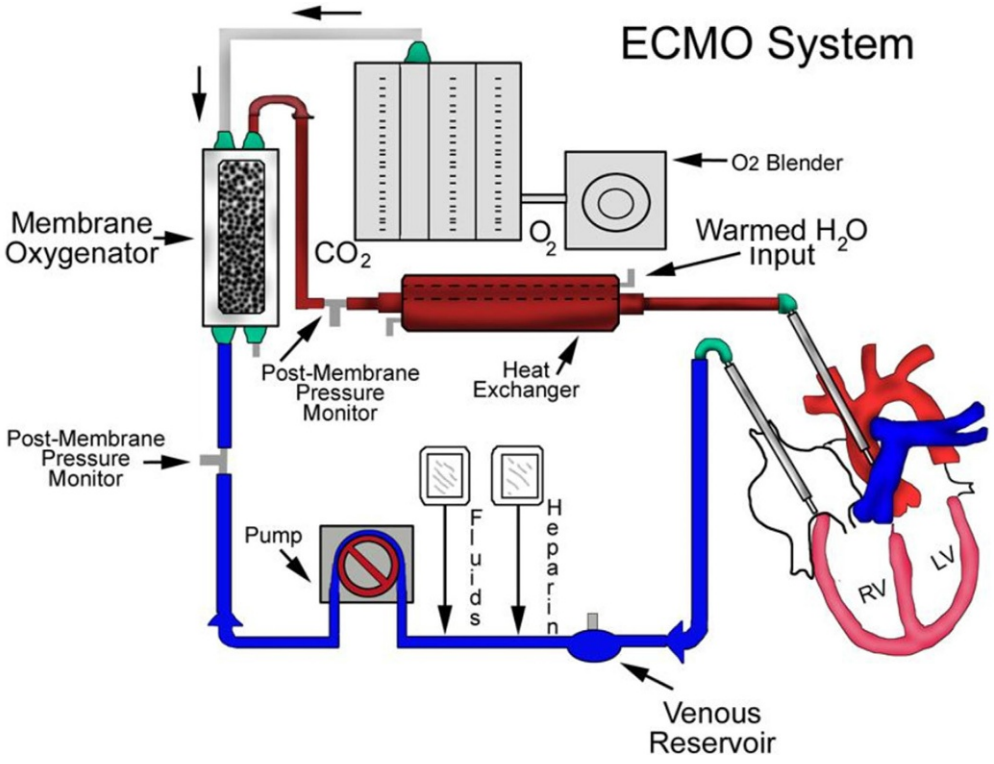

Fig. 1 The extracorporeal membrane oxygenation circuit. Courtesy of *EMedicine.com*.

The venous drainage cannula can be either single or multistage to facilitate drainage and hence inflow into the ECMO circuit. The return cannula is inserted into the arterial system, is typically 15–19 French in diameter, and is shorter with lengths of 20–25 cm when inserted peripherally.

The initiation of VA-ECMO in patients suffering from postcardiotomy shock, inability to wean from cardiopulmonary bypass, or in cases of emergent reentry sternotomy in a recent cardiac surgery patient represents arguably the most straightforward cannulation decision. Central cannulation with a drainage (venous) cannula inserted directly into the right atrium and a return (arterial) cannula directly into the ascending aorta are the most efficient and least problematic cannulation strategy. This is how cardiopulmonary bypass during routine cardiac surgery is performed and should be second nature to the cardiothoracic surgeon. For most patients, the chest is left open with packing material and cannulas tunneled away from the midline wound. In certain instances where aortic insufficiency may be present and/or left ventricular distension is seen on echocardiography, a left ventricular vent can be directly inserted into the left atrium and "y-ed" into the drainage cannula to facilitate left ventricular offloading and stagnation.

In the patient who is not status post recent cardiac surgery, peripheral VA-ECMO is the most common cannulation strategy. Peripheral cannulation requires vessels that can accommodate appropriate diameter cannulas for both drainage and perfusion. For these reasons and ease of access, the femoral artery and vein are the most frequently utilized vessels. Although there can be multiple configurations of VA-ECMO that can be employed, the most common indication and clinical scenario is one where emergent VA-ECMO is required during active cardiopulmonary resuscitation. As this is a hectic time and no adequate pulsatility and/or oxygenation exists, it is unreliable to use the usual measures to delineate venous versus arterial entry such as palpation of pulses, color of blood return, and presence of pulsatile flow. In this setting, the optimal configuration for cannulation is the ipsilateral femoral venous and femoral arterial access as the first cannula can act as the anatomic landmark for the second cannula. If an intraaortic balloon pump is already in place, one can utilize the ipsilateral femoral venous access based on relative anatomy and the contralateral side for femoral arterial access.

Other, less expeditious methods for return (arterial) cannulation include placement of a 8 mm graft (woven double velour vascular graft, polyester, or polytetrafluorethylene) sewn to the axillary artery or femoral artery. The advantage is to prevent distal limb ischemia and prevent high line pressures, and the disadvantage is the time necessary to dissect out the appropriate vessel and make a hemostatic anastomosis. Important considerations are to ensure systemic anticoagulation immediately once access is obtained and commencement of VA-ECMO.

This is often the time to pause and ensure appropriate circuit function. An activated clotting time (ACT) >220 s or an activated partial thromboplastin time (aPTT) >70 (depending on local laboratory normalization values) will ensure appropriate thinness of the blood for the circuit. The patient should be maintained on intravenous heparin infusion to prevent thrombus formation in the oxygenator and the need for an urgent circuit replacement.

Cannula position should be immediately evaluated using an array of diagnostics. Arterial line pressures >200 mm Hg can signal vascular injury, dissection, malpositioning, as well as an impending retroperitoneal hematoma. To further reduce the chance of vascular ischemia to the lower extremity of the femoral artery used, a 7 French armored (wire wound) catheter in the superficial femoral artery placed in an antegrade manner after the patient has been initiated on VA-ECMO support. Lack of venous return can signal hemorrhage, inadequate volume resuscitation, vasoplegia, or malpositioned venous cannula. In peripherally inserted cases, the tip of the venous cannula should be in the distal inferior vena cava by the cavoatrial junction. This position can be confirmed by echocardiography or chest X-ray postprocedure. Other methods of providing venous drainage access have been via internal jugular vein in cases of inferior vena cava thrombosis or inferior vena cava filters. It is imperative to ensure that cannulas are secure as to prevent migration and dislodgement. This should be done by a pursestring suture at the entrance site, multiple heavy sutures along the skin exit site, as well as course of cannulas to prevent any tension in addition to securing all tubing connection sites.

In some cases, where left ventricular distension is occurring either via intracardiac shunt, inappropriate venous drainage, or intracardiac shunt, adjunctive measures can be taken to decompress the left ventricle. These include left atrial or ventricular vents, intraaortic balloon pumps, and percutaneous ventricular offloading devices. The central idea is to allow reduction of myocardial wall stress and oxygen demand and ultimately improving the chance of ventricular recovery.

Outcomes

Along with the increased use of ECMO in the last decade, there has been an increase in the scrutiny of outcomes, definition of success or futility, and the cost to benefit ratio of this technology (Shah, 2016). Secondary to the inherit differences for the indication for ECMO initiation, center-specific guidelines on utilization of the technology, and definition of success, there are quite varied reports of outcomes following ECMO. Outcomes, as defined by ELSO, can be reported as survival to decannulation or survival to discharge. According to the ELSO database for those patients supported with ECMO in the registry between 1990 and 2015, 66% and 58% survived to decannulation and survival for respiratory indication for support ($n=9102$), 56% and 41% for patients supported for cardiac reason ($n=7850$), respectively, and 40% and 30% for patients with ECPR ($n=2374$), respectively. When compared with conventional CPR rates of 17% survival, these outcomes are encouraging as are the outcomes for respiratory and cardiac reasons for initiation (Extracorporeal Life Support Organization Registry Report, n.d.). Even in patients supported for cardiac reasons alone, there are variances in outcomes reported. In patients where there exists a reversible insult such as acute fulminant myocarditis, a multicenter analysis demonstrated cardiac recovery and weaning from VA-ECMO in up to 76% of cases with survival to hospital discharge of 72% (Lorusso et al., 2016). In cases of ECMO institution following the postcardiac surgery

phase, a single institution series of 233 patients demonstrated a much lower survival rate to hospital discharge of only 36% (Elsharkawy et al., 2010).

An equally important consideration in addition to survival to decannulation and/or discharge from the hospital is the absence of complications. In particular, ELSO definitions of quality measures include mechanical complications of ECMO (cannula, oxygenator clots), hemorrhagic complications, neurologic complications, renal failure, metabolic complications, infectious complications, and cardiopulmonary complications. Aside from nonrecovery of cardiac function and multiorgan failure, vascular complications are the most frequent cause of death with utilization of VA-ECMO. The rate of hemorrhagic complications can be as high as 40%, malperfusion with critical lib ischemia can occur in 15%–20% (10% fasciotomy requirement, 5% amputation rate), acute kidney injury in 55% (45% with need for renal replacement therapy), neurologic complications in 10%–15%, and infection in 30% of patients supported with ECMO (Cheng et al., 2014).

Discussion

In patients suffering from witnessed in-hospital cardiopulmonary arrest, respiratory failure refractory to mechanical ventilation with advanced maneuvers, and those in cardiogenic shock, ECMO can be a life-saving intervention. The moribund nature of these patients has to be remembered when trying to determine the success or futility of this therapy. In fact, ELSO recommendation for the consideration for ECMO is the presence of a 50% mortality with initiation of therapy when 80% mortality risk exists in appropriate patients. ECMO has become an increasingly prominent treatment modality in the armamentarium of clinicians who treat patients with acute, medically refractory circulatory failure. Although general guidelines and techniques exist for this therapy, there are no large randomized, controlled studies that can serve as unbiased studies in cardiogenic shock. Indeed, this form of therapy is a last resort in patients who are in desperate need of cardiopulmonary support.

It is crucial to develop a multidisciplinary institutional team in the management and selection of patients that will benefit from this therapy. It is the hope in all cases that there exists a reversible cause for patient deterioration, but a realistic time frame (i.e., 14 days) for support of these patients needs to be made in conjunction with patient and their families. Any steps to aid in recovery or escalate therapy such as suitability for decannulation, more permanent mechanical support, an ablation procedure, a revascularization procedure, or transplantation should be considered on an ongoing and daily basis. Undoubtedly, there will always exist a significant risk of mortality and morbidity in these patients. Clear goals of care with constant communication with patient and their family in conjunction with help from palliative care medicine can stress the importance of not just treating a pathology but the patient.

A healthy understanding of the indications and management will be essential in continuing to optimize survival while reducing the complication rates of this technology. Furthermore, suprainstitutional networks of early referral and transfer of patients to tertiary care centers for consideration for ECMO can improve outcomes in patients prior to the onset of multiorgan failure (Aubin et al., 2016). In cases of ECPR, the education of first line respondents such as emergency room, critical care, and interventional cardiology physicians are paramount to increase survival from patients in shock. Despite the ongoing challenges of ECMO, there is no doubt that it has already saved thousands of critically ill patients' lives and will continue to do so in the foreseeable future.

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F

Familial Hypercholesterolemia

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Introduction

Autosomal dominant hypercholesterolemia (ADH) is a genetic disorder affecting low-density lipoprotein cholesterol (LDL-C) metabolism, characterized by very high levels of circulating LDL-C and premature cardiovascular disease, myocardial infarction or death early in the life, especially in untreated subjects (Nordestgaard et al., 2013; Cuchel et al., 2014; Wiegman et al., 2015; Krogh et al., 2016). Despite the current availability of several cholesterol-lowering drugs, which appear to be very effective also in the treatment of genetically determined hypercholesterolemia, a main gap in the management of this disease to prevent its cardiovascular complications is the detection of familial hypercholesterolemia (FH) subjects. In fact, while the most severe forms are generally characterized by the presence of unequivocal physical features, the less severe forms may remain undetected until the first cardiovascular event occurs. Due to the high burden of cholesterol exposure from the birth, the early detection of such subjects is essential so that they can be treated from childhood to reduce their cardiovascular risk and gain decades of life free of cardiovascular events. In this chapter, we will describe the features of FH disease and present the results of clinical trials of currently available drugs approved for the treatment of FH patients.

Genetics of Familial Hypercholesterolemia

Several genetic defects cause ADH; FH (or ADH1) is the most common form of ADH and is due to mutations in the *LDLR* gene, encoding for the LDL receptor (LDLR), responsible for the binding and uptake of LDL particles in the liver (**Fig. 1**) (Sniderman et al., 2014). Mutations in the *APOB* gene, which block the association of the LDL particle to LDLR, are the cause of familial defective apolipoprotein B100 (FDB, or ADH2) (**Fig. 1**; Whitfield et al., 2004; Andersen et al., 2016); gain-of-function (GOF) mutations in *PCSK9* gene reduce LDLR recycling and accelerate its lysosomal degradation, causing a condition referred to as ADH3 (**Fig. 1**; Sniderman et al., 2014). Finally, a rare recessive form of hypercholesterolemia (autosomal recessive hypercholesterolemia, ARH), caused by the loss-of-function (LOF) mutations in the *LDLRAP1* gene (encoding for a protein promoting the hepatic internalization

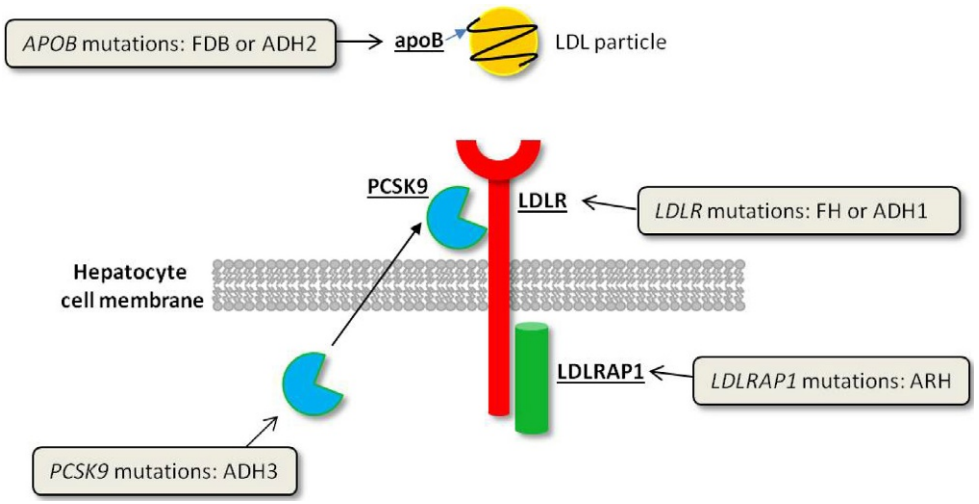

Fig. 1 Genetic causes of autosomal dominant hypercholesterolemia or autosomal recessive hypercholesterolemia.

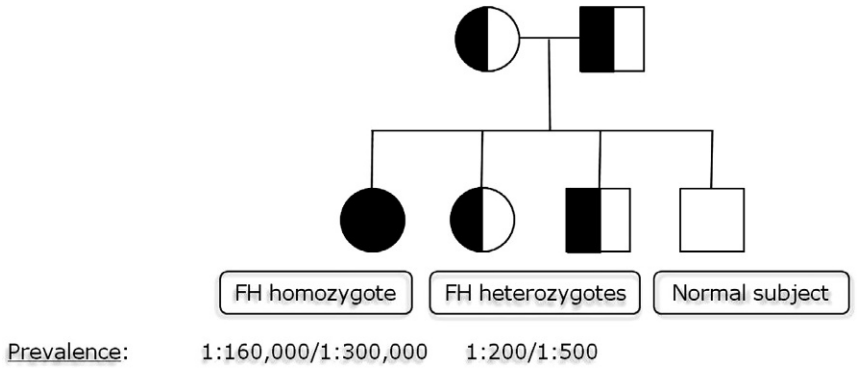

Fig. 2 Inheritance of homozygous or heterozygous familial hypercholesterolemia in a pedigree and prevalence in the general population.

of LDLR/LDL complex), has also been described (**Fig. 1**; Garcia et al., 2001; Fellin et al., 2015). All these conditions are commonly referred to as FH.

Being a genetic disorder, FH can present either in the less severe heterozygous form (HeFH) or in the rarer homozygous form (HoFH) (**Fig. 2**; Cuchel et al., 2014; Singh and Bittner, 2015). HeFH has a prevalence of ~1/500–1/200 in the general population and is characterized by a reduced LDLR activity (~50%) which translates into a two- to threefold increase of LDL-C levels and the occurrence of coronary heart disease (CHD) before the age of 55 (60 for women) (Nordestgaard et al., 2013). HoFH includes the true homozygotes, carrying the same mutation in both gene alleles, compound heterozygotes, carrying two distinct mutations in the two alleles of the same gene, and the rare form of double heterozygosity, due to the presence of mutations in two different genes (Nordestgaard et al., 2013; Cuchel et al., 2014). HoFH, with a prevalence of ~1/160,000–1/300,000, is characterized by a significantly reduced LDLR activity and includes either receptor-defective subjects (2%–30% residual LDLR activity) or receptor-negative subjects (<2% LDLR activity) having more severe cardiovascular conditions compared with subjects with receptor deficiency (Cuchel et al., 2014). Being exposed to very high levels of LDL-C (>500 mg/dL) from birth, receptor-negative HoFH may present clinical signs of heart disease very early and may experience myocardial infarction before the age of 20 if untreated (Cuchel et al., 2014; Sniderman et al., 2014). Subjects with FDB present a less severe hypercholesterolemia, lower occurrences of tendon xanthoma, and a lower incidence of coronary artery disease compared with FH (Whitfield et al., 2004). Heterozygous and homozygous FH determined by *PCSK9* mutations exhibit a milder phenotype compared with FH caused by *LDLR* mutations (Mabuchi et al., 2014).

Other Lipid Abnormalities in FH: Focus on Lipoprotein(a) Levels

Lipoprotein(a) (Lp(a)) is an LDL-like particle to which apolipoprotein(a) is linked to apoB via a disulfide bond; several epidemiological studies have suggested that elevated plasma levels of Lp(a) represent a cardiovascular risk factor independently of LDL-C levels (Nordestgaard et al., 2010). It has been shown that FH patients, particularly those with cardiovascular disease, exhibit

significantly higher plasma levels of Lp(a) compared with their nonaffected relatives (Alonso et al., 2014), although no difference was observed between FH patients carrying *PCSK9* GOF mutations and FH patients carrying *LDLR* mutations (Tada et al., 2016). Furthermore, Lp(a) represents an independent predictor of CVD in these subjects (Alonso et al., 2014). Lp(a) levels are higher in FH subjects with early CHD events than in FH subjects with late or no CHD (Nenseter et al., 2011). Finally, although statin therapy has significantly improved the prognosis of FH patients, a considerable degree of CAD in asymptomatic FH under long-term statin treatment has been observed, suggesting that additional risk factors independent of LDL-C levels may contribute to the residual CVD risk in treated FH patients, and Lp(a) may play a role. Experimental and clinical evidence indicate a desirable Lp(a) levels below 50 mg/dL (Nordestgaard et al., 2010).

An association between Lp(a) levels and c-IMT has been observed both in the general population and in hypercholesterolemic subjects (Baldassarre et al., 1996; Schreiner et al., 1993). Surprisingly, when analyzed in FH, patients with high or low Lp(a) levels (>30 mg/dL and ≤30 mg/dL, respectively) had the same plaque prevalence and similar c-IMT values (Bos et al., 2015). A possible explanation for this finding is that patients were relatively young (mean age 48 years) and treated with aggressive statin therapy. On the other hand, another study reported an association between Lp(a) plasma levels and aortic valve calcification (which is a significant risk factor for aortic valve stenosis and cardiovascular disease), but not with coronary artery calcification (Vongpromek et al., 2015). Although statin therapy attenuates the progression of CAC, as a result of LDL-C lowering, it does not appear sufficient to provide better clinical outcomes in patients with aortic valve calcification, possibly because it does not reduce Lp(a) levels (Vongpromek et al., 2015).

In fact, while it is possible that LDLR is involved in Lp(a) uptake and degradation, the observations that non-FH subjects have lower levels of Lp(a) compared with FH patients, that carriers of null LDLR mutations have higher Lp(a) levels compared with those with defective mutations (Alonso et al., 2014), and that HoFH have higher Lp(a) levels compared with HeFH suggest a gene-dosage effect (Kraft et al., 2000). However the effect of statins (which upregulate LDLR) on Lp(a) levels is inconsistent (Tziomalos et al., 2009). On the contrary, PCSK9 inhibitors, which act also on LDLR expression, seem to reduce significantly also Lp(a) levels.

Altogether these observations suggest that Lp(a) levels should be assessed in FH patients to identify those who could benefit from more aggressive lipid-lowering treatments.

Diagnosis of Familial Hypercholesterolemia

The diagnosis of FH can be based on either clinical criteria or genetic tests. The last provide a definitive diagnosis of FH, although some patients presenting with clinical characteristics of FH may have a negative genetic test, suggesting the possible involvement of unknown genes. Several criteria exist for the diagnosis of FH, including the Simon Broome register (Scientific Steering Committee on behalf of the Simon Broome Register Group, 1991), the WHO criteria (World Health Organization, 1999), and the Dutch Lipid Clinic Network (DLCN), the latter being the most commonly used. DLCN criteria establish a score that gives the probability that a subject has FH (**Table 1**). The score is calculated based on family history of premature CHD or hypercholesterolemia, on patient history of premature CHD or presence of high levels of LDL-C, on the presence of physical signs such as tendon xanthomas or corneal arcus (**Table 1**). HoFH can be diagnosed based on the clinical conditions of the patient, including very high levels of untreated LDL-C (>13 mmol/L or >500 mg/dL) or treated LDL-C ≥8 mmol/L or ≥300 mg/dL, the presence of cutaneous or

Table 1 Dutch Lipid Clinic Network (DLCN) criteria for the diagnosis of FH

Criteria	*Score*
Family history	
First degree relative with known premature (<55 years for men, <60 years for women) coronary heart disease	1
OR	
First degree relative with known LDL-C >95th percentile by age and gender for country	
First degree relative with tendon xanthomas and/or corneal arcus	2
OR	
Children <18 yeards with LDL-C >95th percentile by age and gender for country	
Clinical history	
Subject with premature (<55 years for men, <60 years for women) coronary heart disease	2
Subject with premature (<55 years for men, <60 years for women) cerebral or peripheral vascular disease	1
Physical examination	
Tendon xanthomas	6
Corneal arcus in a subject <45 years	4
Untreated LDL-C levels	
>8.5 mmol/L (>325 mg/dL)	8
6.5–8.4 mmol/L (251–325 mg/dL)	5
5.0–6.4 mmol/L (191–250 mg/dL)	3
4.0–4.9 mmol/L (155–190 mg/dL)	1
Genetic test	
Causative mutation in *LDLR*, *APOB*, or *PCSK9* genes	8

tendon xanthomas before the age of 10, or the presence of high levels of LDL-C in both parents, or the genetic confirmation of two mutants alleles in *LDLR*, *apoB*, *PCSK9*, or *LDLRAP1* genes (Cuchel et al., 2014). However, the discrimination between HeFH and HoFH may be not always simple, particularly if based only on LDL-C plasma levels, as a high phenotypic heterogeneity has been observed due to the high heterogeneity of underlying genetic defects.

Cardiovascular Risk and Complications in Patients With FH

Due to the exposure to high levels of circulating LDL-C from birth, patients with FH are at high risk for cardiovascular disease; HeFH have a 3.5–16-fold higher risk of CAD compared with non-FH subjects (Hovingh and Kastelein, 2016), and homozygous have a further increased risk (Cuchel et al., 2014). HoFH patients have an accelerated atherosclerosis that impairs not only coronary arteries and the aortic root, but also carotid, descending aorta, and peripheral arteries (Raal and Santos, 2012). Clinically diagnosed HoFH commonly present their first cardiovascular event, including angina pectoris and myocardial infarction, during adolescence, but may also occur in early childhood when the genetic defect leads to an LDLR-negative phenotype (Cuchel et al., 2014). If untreated, they usually die before the age of 20; LDLR-defective HoFH have a better prognosis, as they manifest their first cardiovascular event by the age of 30 (Cuchel et al., 2014). The underlying genetic defects determine the severity of the disease; thus, the phenotype of a severe HeFH condition may be similar to a HoFH phenotype (Snozek et al., 2009).

A major limitation in the management of FH patients is that, while HoFH often present with unequivocal physical symptoms, HeFH may be undetected until they present with their first coronary event. Furthermore, due to the overall "milder" phenotype compared with HoFH, they may present with LDL-C levels unnecessarily extremely high and thus may be confounded with "normal" hypercholesterolemic subjects. However, the presence of genetic defects per se increases the cardiovascular risk, as it implies a life-long exposure to elevated LDL-C levels; thus, FH subjects with LDL-C $\geq$190 mg/dL have a threefold increased risk compared with non-FH subjects with the same LDL-C plasma level (Khera et al., 2016). This reinforces the idea that the genetic testing may provide risk information beyond the LDL-C levels.

FH in Childhood

Due to the high risk of early cardiovascular events in FH subjects, the identification of children and adolescents with FH, followed by lifestyle changes and lipid-lowering therapy, represents an important step in the prevention of premature coronary complications. In fact, increased c-IMT can be observed as early as 7 years in FH children, as well as endothelial dysfunction and thickening of the arterial vessel wall (Wiegman et al., 2004a; de Jongh et al., 2002; Narverud et al., 2014); if untreated, they may experience their heart attack very early in the life (Kusters et al., 2014). As for adults, FH may be diagnosed either based on phenotypic criteria or by positive genetic testing; plasma Lp(a) levels may add prognostic value (Stock, 2015).

The management of young FH patients includes lifestyle changes and cholesterol-lowering therapy, that should be started at the lowest dose, with possible titration based on individual response, and a rigorous monitoring (Stock, 2015). Lifestyle changes include a low saturated fat diet devoid in trans-fat and high in fruits, vegetables, and fiber; the expected reduction in LDL-C levels is related to the baseline lifestyle behaviors and the adherence to the recommendations (de Ferranti, 2015). Lipid-lowering therapy has as first-line agent statins, but may also include bile acid binding resins or ezetimibe (de Ferranti, 2015); a major issue is the safety of these pharmacological therapies in children.

Children with FH are also characterized by a pro-inflammatory profile, with increased levels of TNFα and reduced levels of IL-10 (Narverud et al., 2011). It can be hypothesized that the early vascular lesions present in these subjects may contribute to a low-grade inflammation; this may further activate inflammatory pathways, leading to a persistent inflammation that contributes to accelerate the atherosclerotic process. In agreement with this observation, FH children with reduced HDL-C levels ($<$1.3 mmol/L, $<$50 mg/dL) exhibited monocytosis and a shift in the monocyte subpopulations, characterized by increased pro-inflammatory, nonclassical monocytes, and reduced levels of classical monocytes (Christensen et al., 2016; Tolani et al., 2013), suggesting a very early activation of monocytes.

A selective screening of children with family history of early atherosclerotic events and/or high LDL-C levels is recommended; a child of an FH subject has a 50% probability of inheriting FH and thus the cascade screening of families allows the early identification of these children. If the mutation in the family member has been identified, the molecular testing is the best method for the definite classification; in alternative, a phenotypic strategy should be used, including the measurement of LDL-C levels (Wiegman et al., 2015). An universal screening of children for hypercholesterolemia might be also proposed, thinking to perform it during routine health visits, so that the early identification and the optimal management may provide them decades of healthy life (Wiegman et al., 2015; Wald et al., 2016).

Guidelines for the Management of Familial Hypercholesterolemia

Clinical complications in FH subjects are related not only to the type and severity of the mutation causing the disease but also to the effectiveness of pharmacological treatments and the time of therapy initiation (Kolansky et al., 2008; Al-Shaikh et al., 2002). Based

Table 2 Recommended LDL-C targets in FH subjects

Category	*LDL-C levels*
Adults	<100 mg/dL (<2.5 mmol/L)
Adults with known CHD/diabetes	<70 mg/dL (<1.8 mmol/L)
Children	<135 mg/dL (<3.5 mmol/L)

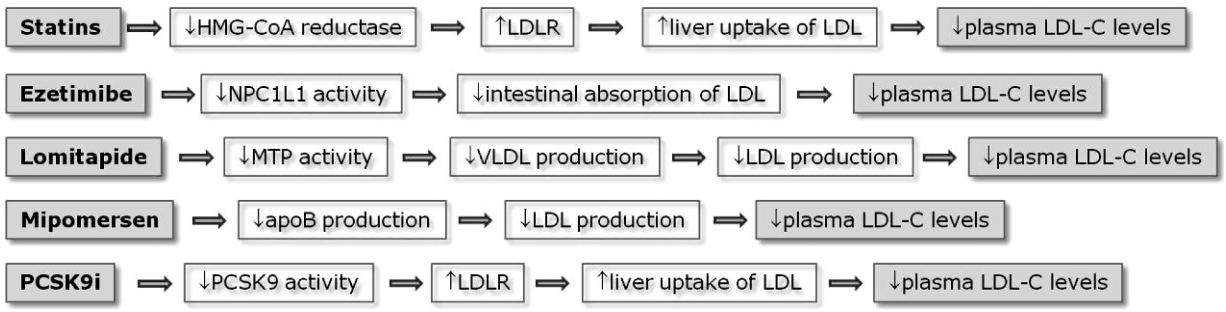

Fig. 3 Mechanisms of action of available drugs approved for the treatment of familial hypercholesterolemia.

on these observations, current guidelines suggest to lower LDL-C as early as possible, to reduce the cumulative burden of high levels of LDL-C, and to delay the onset of cardiovascular events (Cuchel et al., 2014; Kolansky et al., 2008; Raal et al., 2011). Recommended LDL-C goals for FH patients are <100 mg/dL (<2.5 mmol/L) for adults, or <70 mg/dL (<1.8 mmol/L) for adults with CHD or diabetes, and <135 mg/dL (<3.5 mmol/L) for children (**Table 2**; Nordestgaard et al., 2013).

Lifestyle interventions are recommended, but a strong reduction of LDL-C levels may be achieved only with pharmacological treatments associated with LDL apheresis, especially in HoFH or in nonresponders to therapy. Several cholesterol-lowering drugs are available; statins are the first choice not only in adults but also in children, in whom only those proven to be relatively safe should be used (Nordestgaard et al., 2013; McCrindle et al., 2003; Wiegman et al., 2004b). FH patients, in particular HoFH, usually cannot reach the recommended LDL-C level goals with statin monotherapy, thus requiring combination therapies in which statins are associated with ezetimibe or more recent cholesterol-lowering drugs such as mipomersen and lomitapide, or with anti-PCSK9 inhibitors. The combination of these drugs allows a greater LDL-C level reduction, taking advantage of distinct mechanisms of action (**Fig. 3**).

Current Treatments of Familial Hypercholesterolemia

Liver Transplantation

As 80% of total LDLR is localized in hepatic cells, liver transplantation represents the only definitive option for HoFH, resulting in the almost complete replacement of dysfunctional LDLR (Alim et al., 2016; Mansoorian et al., 2015). Liver transplantation determines an almost immediate massive improvement of lipid profile, with a significant decrease of LDL-C levels (Page et al., 2014); however, if this translates into a long-term cardiovascular benefit remains unclear, due to the low number of cases and short follow-up available. A 20-year follow-up of a case of combined heart and liver transplantation in a HoFH woman who presented with severe heart failure and advanced atherosclerotic disease showed that LDL-C levels were dramatically decreased 10 days posttransplantation (from 13 to 2.1 mmol/L) (Ibrahim et al., 2012); 20 years later LDL-C levels (with the support of lipid-lowering therapy) were only modestly abnormal (4.3 mmol/L), and tendon xanthomata were extensively reduced (Ibrahim et al., 2012). No signs of cardiovascular disease were observed (Ibrahim et al., 2012).

Due to the significant challenges regarding transplantation (limited availability of donors, high postoperative complication risk, and the need of life-long treatment with immunosuppressants), it remains an option only for patients with very severe phenotypes and refractory to existing cholesterol-lowering therapies.

LDL Apheresis

Lipoprotein apheresis is the physical removal of cholesterol-rich lipoproteins from plasma and represents an important tool for the treatment of FH, in particular for patients with HoFH or severe HeFH who do not respond to statins. A single procedure of apheresis (usually repeated every 2 weeks) significantly decreases LDL-C levels (50%–70%), which however return to normal within 14 days. Several reports have observed a reduced mortality among FH subjects treated with LDL apheresis compared with those untreated (Keller, 2009; Thompson et al., 1985; Mabuchi et al., 1998; Thompson, 2013), with data suggesting that the earlier apheresis is started, the better the prognosis. LDL apheresis is usually well tolerated, with side effects including hypotension, nausea, and

vomiting; the major limit of this procedure is the rebound of LDL-C levels after apheresis that requires continuous treatments to maintain low LDL-C levels, raising issues regarding the impact on the patient life quality and cost and accessibility of the treatment (Wang et al., 2016). The current availability of new cholesterol-lowering drugs may allow FH patients to reduce the frequency or even delay apheresis treatments.

Statins

Statin therapy represents the first step in the management of hypercholesterolemia also in FH patients. Due to the mechanism of action of statins, which inhibit the endogenous cholesterol synthesis thus resulting in increased expression of LDLR and reduced LDL-C levels, it is expected that statins would not be effective in homozygous FH patients with null mutation on LDLR gene (receptor-negative). Despite this, also these patients are responsive, although to a lesser extent (Feher et al., 1993; Raal et al., 1997), which suggests alternative mechanisms of action of statins, such as a reduction of VLDL production, the major source of circulating LDL. Thus, atorvastatin reduced LDL-C levels in either HoFH with residual LDLR activity or LDLR-negative HoFH (Raal et al., 2000). The LDL-C reductions achieved in FH patients with statin therapy, although not massive (~20%, compared with 40%–60% achieved in nongenetically determined hypercholesterolemia (Hovingh et al., 2013)), may have a clinical relevance, particularly when statin therapy is started early in the life, as it may translate into a delayed occurrence of cardiovascular events and prolonged survival (Raal et al., 2011).

An important observation is a plateau effect observed in FH patients, with no further LDL-C reduction by increasing atorvastatin dose, which suggests a limit to the ability of statins to reduce LDL-C levels in FH patients and the need of combination therapies including drugs with different mechanisms of action (Raal et al., 2000).

The need to start as early as possible with a pharmacological therapy in FH children, especially if homozygous, raised a question regarding the safety of statins in children. Thus, a meta-analysis of six studies with 798 children showed that statins significantly reduced LDL-C, total cholesterol, and apoB, with an incidence of adverse events comparable to that observed in children receiving placebo (Avis et al., 2007). More recently, two studies have demonstrated the efficacy and safety of rosuvastatin and pitavastatin, not included in the previous meta-analysis, in FH children, and the lack of adverse effects on growth and sexual maturation (Braamskamp et al., 2015a,b).

Despite all these considerations, a very small number of FH patients achieve the recommended LDL-C level target with statin monotherapy, suggesting the need of adding other drugs able to reduce LDL-C levels by a different mechanism of action (Nordestgaard et al., 2013; Hovingh et al., 2013).

Ezetimibe

Ezetimibe is an inhibitor of the intestinal uptake transporter Niemann-Pick C1 Like 1 and is a useful cholesterol-lowering drug often given in combination with a statin for the management of FH patients; this results in an additional reduction of LDL-C levels of about 10%–15% (Pisciotta et al., 2007; Gagne et al., 2002; Kastelein et al., 2008), which may translate in a further decrease of cardiovascular event incidence. Despite the addition of ezetimibe to statin therapy further reduces LDL-C levels, contrasting results have been reported on the effect on intima-media thickness progression (Kastelein et al., 2008; Kalogirou et al., 2010; Avellone et al., 2010).

The combination ezetimibe/simvastatin has been tested also in adolescents with HeFH, showing a greater reduction of LDL-C levels compared with simvastatin alone (−49.5% vs. −34.4%, $P<0.01$) (van der Graaf et al., 2008). The combination was well tolerated throughout 53 weeks (van der Graaf et al., 2008).

Lomitapide

The microsomal triglyceride transfer protein (MTP) is a lipid transfer protein involved in the assembly and secretion of apolipoprotein-B-containing lipoproteins (Hooper et al., 2015); it transfers neutral lipids (triglycerides and cholesteryl esters) from the endoplasmic reticulum membrane to the nascent apoB (Hooper et al., 2015). Subjects carrying mutations in the gene encoding for MTP (*MTTP*) exhibit inadequate formation of VLDL and chylomicrons and increased apoB degradation, resulting in a significant reduction of circulating VLDL and LDL (Goldberg, 2013); this suggested MTP as a possible pharmacological target to treat hypercholesterolemia.

Lomitapide is an MTP inhibitor that binds to this protein and inhibits the MTP-mediated synthesis of both hepatic VLDL and intestinal chylomicrons, resulting in significant reductions of LDL-C levels (**Fig. 3**). Lomitapide is effective in HoFH patients, even those LDLR-negative, and reduces LDL-C levels dose-dependently, with a good tolerance and gastrointestinal disorders (that can be prevented by a low-fat diet) as the most common side effects (Cuchel et al., 2007; Raper et al., 2015; Averna et al., 2016); hepatic enzymes as well as liver fat content were however increased following the treatment (Cuchel et al., 2007; Averna et al., 2016). To assess the relevance of the observed hepatic fat accumulation, lomitapide has been evaluated in HoFH in single-arm, open-label, phase 3 study (Cuchel et al., 2013; **Table 3**). This study, that included a 26-week efficacy study and a 52-week safety study, showed a significant LDL-C reduction (−50%) at week 26, with some patients being able to discontinue or delay LDL apheresis (Cuchel et al., 2013). At week 78, LDL-C levels, although significantly lower compared with baseline, were slightly higher compared with the values at the end of efficacy study, probably due to changes in the concomitant lipid-lowering therapies or to the reduction of

Table 3 Effects of lomitapide and mipomersen in FH

Clinical trial (ref.)	*Subjects*	*Duration*	*LDL-C (% change from baseline)*	*Lp(a) (% change from baseline)*
Lomitapide				
Cuchel et al. (2007)	HoFH	4 weeks for each dose	−24.7% (0.3 mg) −50.9% (1.0 mg)	Unchanged
Cuchel et al. (2013)	HoFH	26 + 52 weeks	−50% at week 26 −38% at week 78	−15% at week 26 −0.5% at week 78
Roeters van Lennep et al. (2015)	HoFH		−35% to −73%	
NCT02399852	HoFH	5 years	Ongoing	
Mipomersen				
Akdim et al. (2010b)	HeFH	13 weeks	−37% (300 mg)	−29% (300 mg)
Stein et al. (2012a)	HeFH with CAD on maximally tolerated statin, LDL-C ≥ 100 mg/dL	28 weeks	−28% (200 mg)	−21.1% (200 mg)
Raal et al. (2010)	HoFH	28 weeks	−24.7% (160 mg)	−31.1% (160 mg)
Santos et al. (2015)	FH or severe hypercholesterolemia	4.5 years	−28% at week 104	−14% at week 104
FOCUS FH (NCT01475825)	Severe HeFH	60 weeks	Completed	

lomitapide doses in patients reporting adverse effects (Cuchel et al., 2013). Other lipids were significantly decreased, including VLDL-C, non-HDL-C, and TG levels (Cuchel et al., 2013). Some patients experienced an increase of hepatic enzymes, but such changes were transient and did not associate with deterioration of liver function (Cuchel et al., 2013); mean hepatic fat increased during the efficacy phase (from 1.0% to 8.6% at week 26), but it remained stable throughout the study (8.3% at week 78) (Cuchel et al., 2013). The long-term clinical relevance of this increase of liver fat is still unclear, requiring further investigations to establish whether it can progress to hepatic fibrosis.

Overall, these studies support, in one hand, the importance of low-fat diet adherence to lessen the impact of gastrointestinal disorders, thus increasing the tolerability and success of lomitapide therapy, and the relevance of a close monitoring of the hepatic enzymes during the treatment to prevent the impairment of the hepatic function and discontinuation of the therapy (Roeters van Lennep et al., 2015). Longer follow-up with clinical outcomes is required to establish whether lomitapide therapy may translate into a reduced cardiovascular risk. In this context, LOWER (Lomitapide Observational Worldwide Evaluation Registry) is a noninterventional study aimed at evaluating the long-term safety and efficacy of lomitapide in clinical practice by following patients for at least 10 years (Blom et al., 2016). Furthermore, an ongoing study is evaluating the effect of lomitapide treatment on carotid and aortic atherosclerosis in HoFH patients (NCT02399852) and has as primary outcome the percent change from baseline in carotid vessel wall area at the 2-year evaluation and as secondary outcome the percent change from baseline in carotid aortic vessel wall area and thickness at 1- and 5-year intervals (Table 3).

Lomitapide can be given in combination with other drugs taking advantage of their different mechanisms of action to further decrease LDL-C levels, particularly in FH subjects. It is worth noting that lomitapide is a weak inhibitor of CYP3A4 and may thus increase the exposure to statins metabolized by this enzyme, including simvastatin and atorvastatin (Tuteja et al., 2014; Aegerion, n.d.); although rosuvastatin is mainly excreted unaltered and only a minor part (<10%) is metabolized through CYP2C9, a significant increase of rosuvastatin exposure (32%) has been reported (Tuteja et al., 2014). Furthermore, lomitapide is metabolized primarily via CYP3A4, thus if administered concomitantly with moderate or strong CYP3A4 inhibitors they may significantly increase lomitapide exposure, and a reduction of lomitapide dose may be required (Aegerion, n.d.).

Mipomersen

High plasma levels of apoB, the main apoprotein of atherogenic lipoproteins (LDL, VLDL, chylomicrons, and Lp(a)), represent an established risk factor in atherosclerosis (Sniderman et al., 2011). Thus, apoB inhibition may represent a valuable tool for the treatment of hypercholesterolemia.

Mipomersen is a second-generation antisense oligonucleotide targeting the coding region of human apoB mRNA; this results in the mRNA degradation and reduction of apoB production, with consequent reduction of LDL-C (**Fig. 3**) (and other apoB-containing lipoproteins) levels independent of LDLR function (Crooke and Geary, 2013). Mipomersen is not metabolized through the cytochrome system and it does not inhibit major CYP isoenzymes, resulting in a lack of relevant clinical interactions with statins or ezetimibe (Yu et al., 2009).

Several studies have shown the cholesterol-lowering efficacy of mipomersen both as monotherapy and in combination with other drugs (Akdim et al., 2010a; Akdim et al., 2010b; Kastelein et al., 2006; McGowan et al., 2012; Raal et al., 2010; Stein et al., 2012a; Akdim et al., 2011; Visser et al., 2012). In HeFH patients, mipomersen injection for up to 13 weeks resulted in a dose-dependent reduction of LDL-C (up to −37%, $P < 0.001$) and Lp(a) (up to −29%, $P < 0.05$) (Akdim et al., 2010b; **Table 3**); all the included patients were on conventional cholesterol-lowering therapies and the addition of mipomersen induced an further

reduction of LDL-C, due to the different mechanism of action. In phase 3 studies the efficacy of mipomersen was evaluated in either HoFH or HeFH patients. In HoFH on low-fat diet and maximal tolerated cholesterol-lowering therapy, mipomersen reduced LDL-C by 21.3% ($P=0.0003$) and Lp(a) by 23.2% ($P=0.0013$) compared with placebo (Raal et al., 2010; **Table 3**); concomitant significant reductions were observed also in other lipid parameters, including non-HDL-C and VLDL-C (Raal et al., 2010). The most common adverse events were injection-site reactions (Raal et al., 2010). HeFH patients with coronary artery disease and LDL-C $\geq$ 100 mg/dL despite aggressive cholesterol-lowering therapy were treated with mipomersen for 26 weeks (Stein et al., 2012a); LDL-C significantly decreased (−28% difference between mipomersen and placebo, $P<0.001$) and also Lp(a) was reduced (−21.1%, $P<0.001$) as well as other lipids, including VLDL-C (−13.8%, $P=0.023$), total cholesterol (−19.4%, $P<0.001$), and non-HDL-C (−25.0%, $P<0.001$) (Stein et al., 2012a; **Table 3**). Some patients experienced elevations in alanine aminotransferase, the clinical significance of which is still unclear (Stein et al., 2012a). This effect has been evidenced also by two meta-analyses which confirmed the beneficial effect of mipomersen on LDL-C levels and reported elevation of ALT (>3X ULN), hepatic steatosis and flu-like symptoms as major adverse events associated with mipomersen (besides the risk of injection-site reactions) (Li et al., 2014; Panta et al., 2015). Although short-term studies reported increased steatosis (whose clinical relevance was considered low) in mipomersen-treated subjects, long-term (up to 104 weeks) clinical trials have indicated a return to normal of hepatic TG levels (Sahebkar and Watts, 2013; Toth, 2013). Biopsies of liver from patients treated with mipomersen showed a histopathological feature of simple steatosis without signs of inflammation or fibrosis (Hashemi et al., 2014). An interim report of a long-term open-label extension trial with mipomersen on HeFH/HoFH patients who completed phase 3 studies has confirmed these results (Santos et al., 2015). In fact, the treatment for up to 2 years on top of maximally tolerated dose of lipid-lowering therapy resulted in sustained reduction of apoB (−31%), LDL-C (−28%), and Lp(a) (−14%) levels, being safety issues similar to those reported for 26-week treatment (Santos et al., 2015; **Table 3**). Data from the 4-year time point as well as results from the FOCUS FH study (NCT01475825) are awaited to substantiate these findings.

PCSK9 Inhibitors

Despite commonly used cholesterol-lowering drugs have significantly improved the management of FH patients, particularly when used in combination, a large proportion of FH patients still do not achieve the recommended LDL-C level target even with maximally tolerated doses of drugs. Thus, there is the need of additional drugs that can efficiently reduce LDL-C levels below those achievable with the common cholesterol-lowering drugs.

GOF mutations in the *PCSK9* gene have been identified as causes of FH (Abifadel et al., 2003) and increased cardiovascular risk (Davignon et al., 2010), while LOF mutations are associated with reduced LDL-C plasma levels and lower prevalence of coronary disease (Cohen et al., 2006; Benn et al., 2010). These observations have suggested PCSK9 as a possible pharmacological target, leading to the development of PCSK9 inhibitors in the attempt to increase residual LDLR activity. Such inhibitors are monoclonal antibodies which act by binding PCSK9, thus preventing its interaction with surface LDLR. Several clinical trials have evaluated the efficacy of these antibodies, named evolocumab and alirocumab, in special groups such as FH patients that should be the most sensitive for the treatment with these agents. The RUTHERFORD study evaluated the effect of evolocumab in HeFH patients who did not achieve the LDL-C target <100 mg/dL despite statin therapy with or without ezetimibe (Raal et al., 2012). Rapid and significant reductions of LDL-C were observed (−43.8% and −56.4% vs. placebo with two different doses of evolocumab) (Raal et al., 2012; **Table 4**); after 12-week treatment, a high percentage of patients (>70%) reached LDL-C levels <100 mg/dL (<2.6 mmol/L) and many of them reached LDL-C <70 mg/dL (<1.8 mmol/L) (Raal et al., 2012). Other lipid parameters were also significantly reduced, including TC, apoB, and Lp(a) (Raal et al., 2012). The phase 3 RUTHERFORD-2 trial showed similar reductions (Raal et al., 2015a; **Table 4**). After the genetic test, seven patients clinically defined as HeFH were reclassified as HoFH or compound heterozygous, but, unexpectedly, they responded to evolocumab similarly to HeFH, even when receptor-negative mutations were identified (Raal et al., 2015a). This finding suggests that LDLR-independent mechanisms might have a role in the regulation of LDL-C levels in these patients, as already suggested by the heterogeneous responses observed in patients carrying the same mutation. HoFH enrolled in the TESLA part A study had a mean reduction of LDL-C of 17%, but the two patients carrying LDLR-negative mutations did not respond to the treatment (Stein et al., 2013; **Table 4**). This finding was not confirmed in the TESLA part B (Raal et al., 2015b), in which HoFH patients not receiving apheresis were administered evolocumab for 12 weeks in addition to their current lipid-lowering therapy. LDL-C levels were overall reduced by 30.9%; the LDLR-negative patient and the patient with ARH did not respond to the treatment, and patients homozygous for the same LDLR mutation had a heterogeneous response to the treatment (−7.1% up to −56%) (Raal et al., 2015b). It appears that the response to evolocumab is determined by the genetic defects, ranging from 0% in LDLR negative patients up to 40.8% in more than half patients carrying one or two defective LDLR mutations. The ongoing open-label TAUSSIG study (NCT01624142) will evaluate the long-term (5 years) efficacy and safety of evolocumab in HoFH patients not controlled by current lipid-lowering therapy (statin with/without ezetimibe) and will be completed in March, 2020. The interim results show a significant reduction of LDL-C and Lp(a) and indicate a potential reduction of cardiovascular event incidence (Raal et al., 2016a; Stein et al., 2016).

The other monoclonal antibody under current clinical evaluation is alirocumab. The first phase 2 study showed a substantial decrease of LDL-C levels (−67.9%) in a group of HeFH patients having LDL-C baseline levels $\geq$2.6 mmol/L ($\geq$100 mg/dL) on stable diet and statin dose with or without ezetimibe (Stein et al., 2012b; **Table 4**). This translated into a very high percentage of patients reaching the prespecified goals of <2.6 mmol/L (<100 mg/dL) and 1.8 mmol/L (<70 mg/dL) (Stein et al., 2012b). A group of studies within the ODYSSEY program (FH I, FH II, and HIGH FH) have evaluated the effects of alirocumab

Table 4 Effects of PCSK9 inhibitors evolocumab and alirocumab in FH

Clinical trial (duration)	*Subjects*	*Background LLT*	*Intervention*	*LDL-C (% change from baseline)*
Evolocumab				
RUTHERFORD (Raal et al., 2012) 12 weeks	HeFH LDL-C $\geq$100 mg/dL	Statin $\pm$ ezetimibe	350 mg Q4W 420 mg Q4W	−43.8% −56.4%
RUTHERFORD-2 (Raal et al., 2015a) 12 weeks	HeFH LDL-C $\geq$100 mg/dL	Statin $\pm$ ezetimibe	140 mg Q2W 420 mg Q4W	−59.2% −61.3%
TESLA part A (Stein et al., 2013) 36 weeks	HoFH; LDL-C $\geq$130 mg/dL	Stable LLT for at least 4 weeks	420 mg Q4W for 24 weeks +420 mg Q2W for 12 weeks	Q4W: −16.5% Q2W: −13.9%
TESLA part B (Raal et al., 2015b) 12 weeks	HoFH; LDL-C $\geq$130 mg/dL	Stable LLT for at least 4 weeks	420 mg Q4W	−30.9%
TAUSSIG (Raal et al., 2016a) 5 years	Severe FH	Stable LLT	Evo Q2W or Q4W	Ongoing
Alirocumab				
ODYSSEY FH I and FH II (Kastelein et al., 2015) 78 weeks	HeFH, LDL-C $\geq$100 mg/dL (for primary prevention) or LDL-C $\geq$70 mg/dL (for secondary prevention)	Max tolerated statin $\pm$ other LLT	75 mg Q2W (increased at 150 mg Q2W if LDL-C $\geq$70 mg/dL at week 8)	FH I: −57.9% FH II: −51.4%
ODYSSEY HIGH FH (Ginsberg et al., 2016) 78 weeks	HeFH LDL-C $\geq$160	Stable LLT	150 mg Q2W	−39%
ODYSSEY LONG TERM (Robinson et al., 2015) 78 weeks	HeFH or established CHD or CHD equivalent LDL-C $\geq$70 mg/dL	Max tolerated statin $\pm$ other LLT	150 mg Q2w	−61.9%
ODYSSEY OLE 176 weeks	HeFH who have completed one of the four parent studies		Ongoing	

administration in HeFH patients (Kastelein et al., 2014). ODYSSEY FH I and FH II studies showed a great efficacy of alirocumab in patients who did not achieve their recommended LDL-C level target despite maximal tolerated dose of statin (Kastelein et al., 2015; **Table 4**). The ODYSSEY HIGH FH reported a reduction of about 50% in HeFH patients with LDL-C $\geq$ 4.1 mmol/L ($\geq$ 160 mg/dL) despite maximal tolerated lipid-lowering therapy, with 41% of patients reaching the prespecified LDL-C goals (Ginsberg et al., 2016; **Table 4**).

The ODYSSEY LONG TERM evaluated a 86-week treatment with alirocumab in HeFH patients not adequately controlled and reported a −63.2% reduction of LDL-C level (Robinson et al., 2015; **Table 4**), a reduction comparable to that observed in non-HeFH patients (Robinson et al., 2015). A post-hoc analysis observed a lower incidence of major cardiovascular events in patients treated with alirocumab than in placebo group (1.7% vs. 3.3%, HR 0.52, nominal $P = 0.02$) (Robinson et al., 2015). The ongoing ODYSSEY OLE (NCT01954394) is evaluating the long-term safety of alirocumab up to 176 weeks in HeFH patients.

PCSK9 Inhibition and Lp(a) Plasma Levels

In contrast to statins, PCSK9 inhibitors have been shown to reduce Lp(a) by as much as 30% (Raal et al., 2014; Gaudet et al., 2014), although the exact mechanism by which such reduction takes place is still unclear. Reductions of LDL-C and Lp(a) levels following administration of PCSK9 inhibitors occur concomitantly, and greater Lp(a) reductions have been observed in patients who achieved lower LDL-C levels (Raal et al., 2016b); furthermore, LDLR has been shown to mediate the effects of PCSK9 on Lp(a) internalization in vitro (Romagnuolo et al., 2015). The possibility that other receptors modulated by PCSK9 may be involved in Lp(a) uptake cannot be excluded, however, especially based on the observation that the effects of statins on Lp(a) levels are inconsistent, despite they increase LDLR expression.

Based on these observations, Lp(a) levels have been evaluated in FH patients treated with evolocumab or alirocumab. Both mAbs significantly reduced Lp(a) levels. The RUTHERFORD studies, in fact, reported reductions ranging from −23% up to −31.6% versus placebo (Raal et al., 2012; Raal et al., 2015a) and similar results were reported by the studies of the ODYSSEY program performed in FH subjects, with reductions ranging from −14.8% to −25.6% versus placebo (Kastelein et al., 2015; Ginsberg et al., 2016; Robinson et al., 2015). Significant reductions were observed also in HoFH, apparently independent of the genetic defect (defective LDLR: −20.0%; negative LDLR: −22.7%) (Stein et al., 2013; Raal et al., 2015b). The clinical relevance of Lp(a) reduction remains however to be addressed.

Conclusions

The diagnosis and therapy of FH has dramatically improved in the last few years; new diagnostic tools as well as number of therapeutic options are now available even for those patients with the most severe forms such as HoFH. In the field of lipidology FH patients have been the front runners in the study of innovative therapeutic approaches that are now being developed for other genetic and nongenetic conditions.

The scene is set now for clinicians to better identify and treat these patients.

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Frailty in Heart Failure Patients

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Introduction

Frailty is relatively common in the general elderly population, and even more so in patients with heart failure. Identifying frailty is an important aspect of care for the heart failure patients, as it may be a result of the heart failure itself, or comorbid to heart failure. As with the general population, frailty correlates with poorer outcomes in advanced heart failure management.

In this article we will review the concept of frailty, approaches to assessment, and potential management strategies for both general frailty and frailty in the setting heart failure.

Definition

Frailty is defined as a clinical syndrome of increased vulnerability in older adults due to dysregulation of energetics balance and multiple physiologic systems causing subclinical organ dysfunction (Goldwater and Pinney, 2015). Frail older adults are at increased risk of hospitalization, falls, disability, and mortality. This physiological dysregulation is chronic and progressive until it reaches a critical threshold. Since there is a strong relationship between frailty and negative outcomes, routinely screening for risk stratification and prognosis discussion should be implemented as clinical practice. The risk of mortality is over 6–12 months on patients who have advanced frailty.

The intrinsic aging process is associated with frailty, where sarcopenia, or loss of lean body mass and strength are key predictors of primary frailty. Nonetheless, similar vulnerability is associated with advanced stages of diseases where inflammation and wasting are present such as cancer, heart failure, COPD, and HIV/AIDS. This is known as secondary frailty (Fried et al., 2001).

There is a higher incidence of frailty and cardiovascular disease even though it is already particularly high in the elderly population (twofold to threefold higher). Likewise, the incidence of cardiovascular disease such as congestive heart failure (CHF) increases up to sevenfold in frail patients (Jha et al., 2015).

Pathobiology

CHF and frailty may share some pathophysiologic mechanisms. They are both systemic syndromes. There are several emerging biomarkers for frailty, which include interleukin-6 levels, tumor necrosis factor alpha, increased C-reactive protein sensitivity, and low vitamin D levels (Walston et al., 2002). Likewise, proinflammatory cytokines can contribute to the progression and side effects of CHF and in turn can be associated with skeletal muscle loss, cardiac cachexia, and declining physical function, which are all important markers of frailty (Anker and von Haehling, 2004). Hormonal changes seen in frailty that are relevant to CHF include dysregulation of the growth hormone/insulin-like growth factor I pathway, low DHEA-s, increased cortisol, low testosterone, increased insulin resistance with appearance of a catabolic state, muscle loss and, specifically for heart failure, decrease in cardiopulmonary exercise testing (Cicoira et al., 2003). Sarcopenia and cachexia are both paramount in the definition of frailty and very frequently present in CHF. Similarities between the two processes from the biological standpoint also explain why they may coexist frequently in the clinical setting.

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Frailty Phenotype and Assessment

There are no standardized measures of frailty in CHF patients. The most commonly used assessments are the FP (FP), developed and validated by Fried and colleagues in the cardiovascular health study (CHS). It identified five physical indicators: sarcopenia, slowed motor performance, poor nutritional status, decreased physical activity, and worsened exercise tolerance (Fried et al., 2001). When three or more attributes are present a patient is considered frail. However, patients could experience some of the indicators without fulfilling the full definition of frailty. The comprehensive geriatric assessment (CGA) has also been used to determine frailty in heart failure population. This evaluation includes several different additional measures that have a more holistic approach to the diagnosis of frailty as it includes assessment of autonomy, cognitive deterioration, and emotional well-being. One of the criticisms for CGA is the fact that it evaluates not only frailty but also disability which may be considered two distinct concepts that belong on a continuum. This is important as both instruments have been used to evaluate frailty in people with CHF but prevalence varies depending on the instrument used. In the previously mentioned CHS, where the FP was used, prevalence of 6.3% was reported (Walston et al., 2002). On the other hand, when using the CGA as a tool to screen for frailty, we see that prevalence has consistently been higher and raging between 40% and 42% (Altimir et al., 2005; Lupon et al., 2008). Independent of the tool used, there appears to be a strong relationship between age and frailty in patients with CHF. Likewise, female gender has also been identified as a variable associated with higher prevalence of frailty (Altimir et al., 2005; Lupon et al., 2008).

It is very important to identify frailty in the CHF population for several reasons. Frail patients with CHF have higher mortality (Cacciatore et al., 2005; Lupon et al., 2008; Pulignano et al., 2010) and a higher hospitalization rate (Pulignano et al., 2010). These observations are not necessarily different from the general elderly population making the use of a frailty index a more interesting measure in younger patients with heart failure. Another important aspect is the intricate relationship that frailty may have with prognosis and response to invasive measures aimed at treating CHF. Similar to the observation of higher morbidity and mortality in CHF, elderly patients who are frail also have worse outcomes after cardiac surgery (Lee et al., 2010). One especially interesting population is those patients considered for destination therapy left ventricular assist device treatment (DT LVAD). Most of them are elderly and have multiple comorbidities that make them poor candidates for heart transplantation. Measures to determine outcomes after DT LVAD remain limited (Cowger et al., 2013) but there is growing evidence that patients who are deemed to be frail at the time of LVAD implantation have increased risk of death as well as higher incidence of hospital readmission (Dunlay et al., 2014). One unique aspect about the relationship between frailty and cardiovascular diseases is the concept that it may be reversible with adequate therapy. Heart failure itself can result in a multitude of changes in the body, which in turn may increase frailty on individuals. Some studies looking at different frailty measures such as hand grip strength and sarcopenia have identified patients who may experience improvement of these measures that can also be associated with resolution of some metabolic abnormalities that are present in chronic heart failure patients (Chung et al., 2014; Khawaja et al., 2014).

Because of the close relationship between frailty and CHF, one attractive pathway is to intervene on modifiable risk factors contributing to frailty in order to achieve better outcomes in CHF. Even though this is a controversial concept (frailty reversal), a recent trial has shown that improvement of frailty parameters is possible on an early population with interventions directed to specific components of the frailty index.

Prevalence

Over the past 15 years, studies have found the prevalence of frailty to range from 4% to 16% in patients over 65 years of age (Fried et al., 2001; Woods et al., 2005; Bandeen-Roche et al., 2006; Cawthon et al., 2007; Kiely et al., 2009). These studies mostly utilized the FP criteria, though definitions of the five criteria varied somewhat from study to study. Frailty is even more common in patients with heart failure, with a prevalence ranging from 18% to 54% in the outpatient setting (Jha et al., 2015). In a separate set of studies focusing on advanced heart failure patients (generally classified as New York Heat Association III–IV and potentially undergoing interventional management), a fairly similar prevalence of 22%–34% was observed (Dunlay et al., 2014; Chung et al., 2014; Dominguez-Rodriguez et al., 2015; Jha et al., 2016). These populations were evaluated either with the FP or through measuring grip strength as a surrogate for the FP. Of note, a portion of the advanced heart failure studies included patients as young as 53 years old, whereas most prevalence studies in the general population have a cut off of 65 years or older.

Therapeutics

There are four proposed frailty interventions for the general population including exercise, nutritional support, vitamin D supplementation, and polypharmacy control. In cardiac patients, optimal control of cardiac cachexia has not been established but there are recommendations about nutritional interventions in frail patients (Turner et al., 2014). HF patient participation in cardiac rehabilitation reduced hospitalization and improved quality of life (Davies et al., 2010).

One of the primary therapies for frailty is exercise and caloric and protein supplementation. There is little available evidence for effective therapies for cardiac cachexia, but there is some support for nutritional supplementation (Uchmanowicz et al., 2014).

In addition to nutritional supplementation, exercise is a principal therapy for heart failure-related frailty. In a meta-analysis, cardiac rehabilitation has been shown to effectively reduce hospitalizations and improve quality of life in heart failure patients (Davies et al., 2010). Rehabilitation may increase mobility and help reduce caregiver burden. Both short- and long-term cardiac rehab programs have been found to be effective.

Evidence shows that 25-OH vitamin D levels and hsCRP levels are correlated with lower exercise capacity in frail patients with heart failure. Biophysical markers associated with frailty were correlated with 6 min walk, and 25OHD and hsCRP correlated with a lower 6 min walk distance and predicted frailty status as measured by FP (Boxer et al., 2008). Although there is no data specific to frailty, vitamin D supplementation has been shown to reduce falls, hip fractures, and mortality in elderly patients (Morley et al., 2013).

Polypharmacy is a growing concern for patients with heart failure. Frequent hospitalizations lead to frequent changes in medical plans and patients are at a high risk for polypharmacy. 90% of patients admitted to a hospital for heart failure are taking at least four medications. It is critical that clinicians review medication lists frequently and eliminate duplicate medications or medications that carry a negative side effect profile in the frail population (Uchmanowicz et al., 2014). Guidelines such as the Beers Criteria can be helpful in identifying potentially problematic medications (Panel, 2015).

Utilization of a multidisciplinary team is essential in providing these interventions as well as minimizing potential frailty related incidents by identifying environmental risks and level of social support. In addition to specific frailty interventions, potentially reversible or manageable conditions such as depression, visual or auditory impairment, neurologic disease, and endocrine disorders should be investigated and optimized as part of the overall management of frailty.

Reversibility in Heart Failure

Technology is changing rapidly in the field of heart failure. Many patients are now eligible for advance cardiac therapies which include ambulatory inotropic support, DT LVADs, and heart transplant. Frailty related to heart failure is unique from frailty in the elderly because it is due to single organ failure. Therefore, with adequate treatment of the underlying condition, there is the possibility that intervention with advance cardiac therapies may reverse the underlying frailty. The key to success is determining if the source of the underlying frailty is multifactorial or related to single organ failure and ultimately reversible.

Due to the overlap of heart failure-related frailty and general frailty, it is critical to determine if a patient has "LVAD Responsive" heart failure during the selection process. LVAD responsive frailty is associated with systolic and diastolic dysfunction, high pulmonary capillary wedge pressure, and low cardiac output leading to the cascade of proinflammatory cytokines and the FP. LVAD-independent frailty will not change with implantation of an LVAD and is usually related to aging, comorbid conditions, and multiorgan dysfunction. Clinicians should critically assess patients prior to LVAD implementation to determine if they have LVAD responsive frailty. These patients have low risk for complications and they can expect and improve in functional status. Patients with LVAD unresponsive frailty are at high risk for premature death and complications with low ability to rehab and regain a high functional status (Flint et al., 2012).

A study by Dunlay et al. assessed frailty with the Deficit Index in 99 patients receiving LVAD implementation. After a 1.9-year follow-up, 79% of patients were hospitalized and 45% had died. They grouped patients into subcategories of frail, intermediate frailty, and not frail. There was a statistically significant correlation with frailty and survival after LVAD implementation. Mean days of lives were 250, 266, and 293 in the respective categories (Dunlay et al., 2014).

Another group looked at the biophysical markers of frailty prior to LVAD implantation. They assessed psoas muscle area of 100 patients via CT scan. Patients with lowest tertile area by gender were labeled as sarcopenic, a primary phenotypic feature of frailty. Sarcopenia was present in 32% of the heart failure population and was associated with prolonged hospitalization and higher risk of post LVAD mortality (Heberton et al., 2016).

In summary, clinicians should consider frailty as a key predictor of hospitalizations and increased risk of mortality. As patients are considered for advanced cardiac therapies the underlying cause of the frailty should be evaluated. Patients who have frailty associated with heart failure should be optimized with exercise, nutrition, vitamin D supplementation, and reducing polypharmacy. For those who have single organ failure or heart failure with sources of frailty that are thought to be reversible are good candidates for advanced cardiac therapies. Patients with sarcopenia and high frailty scores will be at high risk for prolonged hospitalizations and higher risk of mortality.

Conclusion

Frailty is a common syndrome found in heart failure patients. Though standardization of the assessment of frailty in heart failure is currently lacking, it is already clear recognition and management of frailty in patients with heart failure will help and may be a useful marker in the future when assessing risk in patients considering advanced therapies.

Example Cases

Case A

John Smith is a 56-year-old male with a history of acute chronic systolic heart failure secondary to nonischemic cardiomyopathy. He was recently admitted to the cardiac service for heart failure exacerbation. His heart failure was first diagnosed 7 years ago and a

recent echo revealed a left ejection fraction of <20%. He reports weight loss of 12 lbs over the past 8 months and minimal physical activity in the last month due to frequent exhaustion. His BMI is now 24. Mr. Smith underwent right heart catheterization and was found to have a low cardiac index.

Mr. Smith was evaluated by a multidisciplinary team including a cardiologist, a cardiothoracic surgeon, a social worker, and the palliative care team. He was offered left ventricular assist device implantation and underwent a successful surgery. Post op the patient improved significantly, with improved appetite, weight gain, and ability to tolerate physical activity. He was fortunate and did not experience significant complications, allowing him four relatively symptom-free years prior to onset of right heart failure.

Case B

Mary Barton is a 73-year-old female with a history of heart failure with reduced ejection fraction, diabetes, chronic kidney disease, and cognitive decline who is followed for heart failure management. Her medications include Lisinopril, metoprolol, furosemide, pravastatin, and zolpidem. She lives alone and a neighbor assists her in getting to appointments. During a recent office visit, Ms. Barton's neighbor expressed concerns about Ms. Barton falling more frequently. She had also lost 13 lbs over the last year and states that she feels exhausted all the time.

The patient and neighbor report good compliance with mediations and she feels that her intermittent shortness of breath on exertion is stable. Noting that her frailty posed a large risk to the patient, her care team began to focus on interventions to reduce her risk of adverse events. Her 25-OH vitamin D level was found to be 10 ng/mL and she was started on vitamin D supplementation. Her zolpidem was also discontinued and sleep hygiene education was provided. She was also provided with a supplemental nutrition drink and referred to a dietician. Ms. Barton returned for a follow-up visit feeling improved, with the neighbor reporting no falls and weight gain of 2 lbs.

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G

Genetic Atrial Fibrillation

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Introduction

As a bearer of significant morbidity and mortality, atrial fibrillation (AF) presents one of the major therapeutic dilemmas in modern clinical practice. Despite significant advances in catheter-based and surgical therapies for AF, failure to maintain sinus rhythm remains high at ~25% with significant divergence in response to therapy. Possible explanations for this include the heterogeneous nature of AF, limited understanding of the molecular mechanisms of the arrhythmia, and inadequacy of current available therapies. Despite these imperfections, advancements over the last decade have brought into light the genetic architecture of AF. Linkage analyses, candidate gene approaches, and next generation sequencing (NGS) have identified mutations encoding cardiac ion channels including transcription factors and signaling proteins linked to early-onset familial AF. Simultaneously, large population-based studies have exposed common single nucleotide polymorphisms (SNPs) that modulate AF risk with the strongest association at the chromosome (chr) 4q25 locus (Darbar and Roden, 2013).

Genetic approaches to AF have not only provided important insights into the underlying mechanisms of AF but also defined responses to therapy. Nonetheless, bedside application of these insights has so far not been fully realized. Promising data from proof-of-concept studies support the hypothesis that genotype-directed therapies for AF may target therapy to those most likely to benefit. In addition, continued advancement of more mechanism-based therapies for AF is a path to enhanced therapeutic effectiveness and reduction in adverse side effects.

Mechanisms of AF

In recent times, there has been a tremendous progress in understanding the underlying mechanisms of AF. However, attaining a unifying underlying mechanism for AF i.e. Moe's multiple wavelet hypothesis (Moe and Abildskov, 1959) has been challenging. This is in part related to the heterogeneity of the processes propagating and maintaining AF, often times occurring concomitantly in order to manifest the arrhythm. This is evidenced by the tendency of AF to occur as age and risk factors accrue, arguing that numerous independent processes must occur concurrently to trigger the arrhythmia- genetic and acquired.

Preceding AF initiation, tissue level changes are vital to maintain reentry or rapid focal ectopic firing. Abnormal impulse formation is driven by focal ectopic firing resulting from increased automaticity. This is likely secondary to decreased cardiac inward rectifier potassium current (I_{K1}), heightened activation of the cardiac pacemaker "funny" current (I_f), or triggered activity (Stillitano et al., 2008). Early after depolarizations may be the driving force behind this as they increase L-type Ca^{2+} current (I_{CaL}). Alternatively, the process could be set in motion by delayed after depolarizations (DADs) instigated by functionally defective ryanodine receptors (RyR) or Ca^{2+} excess in the sarcoplasmic reticulum (Yeh et al., 2008; Voigt et al., 2012).

Concerning catheter ablation for AF, inadequacies in the therapy have been created due to the unintentional formation of gaps in ablation lines, causing anatomical barriers that develop reentry circuits. This, in turn, leads to persistent macroreentrant atrial tachycardias and ultimately, AF. Scenarios in which action potential duration (APD) and atrial effective refractory period (ERP) are shortened, or conduction velocity (CV) is slowed, can lead to the initiation and maintenance of AF (Nattel, 2002). Destructive structural remodeling, which leads to the development of fibrosis, activation of inflammatory pathway, neural restructuring, and electrical modifications, have also been reported to promote further AF by means of a reentrant substrate which is needed to sustain the rhythm (Nattel et al., 2008; Shiroshita-Takeshita et al., 2009). The evolution of the atrial remodeling varies in its time course from patient to patient, and the goal of modern therapies is largely to dampen the progression of the various pathogenic processes that proliferate the disease.

Genetic Basis of AF

In AF, Mendelian inheritance patterns have been recognized since the 1940's (Wolff, 1943a,b) Brugada et al. reported three families with autosomal dominant AF in 1987, and subsequently identified a genetic locus (10q22-24) linked with AF in these kindreds. (Brugada et al., 1997) Although the gene responsible for AF has not been identified (Darbar et al., 2003a; Shah et al., 2002), the report had initially suggested that AF could be a heritable and a monogenic disorder. However, further observation of incomplete penetrance of the phenotype within family members would ultimately suggest AF to be rather a multigenic and genetically heterogeneous disorder.

Most cases of AF in the general population are sporadic, and the likelihood of developing AF increases with age, as well as the presence of other risk factors such as hypertension, diabetes, obesity, sleep apnea, heart failure, and metabolic syndrome (Darbar and Roden, 2013). Nevertheless, at least 15% of patients develop sporadic AF at a relatively early age without such risk factors, a condition previously referred to as 'lone' but now better defined as early-onset AF (Ellinor et al., 2005). Although analysis of rare disease-causing variants with high penetrance can be insightful, early-onset familial AF accounts for only a minority of the overall prevalence of the arrhythmia.

Family history of AF itself has been shown to be an independent risk factor for developing AF in the general population. As seen in the Framingham study, offspring of parents with AF had double the 4-year risk of developing de novo AF even after accounting for established AF risk factors.(Fox et al., 2004) Similarly, in Icelandic population studies, relatives of an affected individual were at increased risk of developing AF, but this risk diminished as the degree of relation increased (Arnar et al., 2006). In the Danish Twin Study, the risk of developing AF was nearly twice as high for monozygotic twins compared to dizygotic twins (Christophersen et al., 2009). Taken altogether, it is quite likely that an individual's genetic predisposition also plays a role in polygenic AF. This observation has driven research into understanding the link between genetics and AF.

Insights Into Genetics of AF

Given the clinical and genetic heterogeneity of AF, the arrhythmia should best be viewed as the common final phenotype of multiple diverse pathways (Darbar et al., 2003b). The pathophysiology of AF is highly complex and even in the absence of identifiable risk factors, multiple structural or ionic mechanisms may be operant in a single patient with AF. Organized propagation of electrical conduction across myocardial cells is dependent upon a balanced interplay between structural features and ionic components of the atrium, which are potentially determined by a combination of genetic, environmental, and acquired factors. Currently, most identified mutations appear to affect pathways involving cardiac development, generation and propagation of electrical impulses, inflammation signaling pathways, atrial remodeling, and development of fibrosis. Ultimately, conditions that slow CV, and shorten APD or atrial ERP favor development of AF (Schotten et al., 2011).

Modulation of APD

Data gathered from candidate genes as well as in vitro functional studies have strongly suggested that mutations altering APD in cardiac ion channel can increase susceptibility to AF. For instance, in persistent and permanent AF, cellular and electrophysiological studies have demonstrated marked reductions in the density of several channels, including ultra-rapid delayed rectifier potassium current (I_{Kur}), L-type voltage-gated ($I_{Ca,L}$), and muscarinic potassium current (I_{KACh}).(Van Wagoner, 2003).

In familial AF, the first mutation identified was a S140G mutation in *KCNQ1*, which encodes the pore forming α-subunit of the delayed rectifier potassium current (I_{Ks}). This gain-of-function mutation ultimately shortens APDs in the atrium (Darbar and Roden, 2013). The pathogenic role of APD shortening by mutations in I_{Ks} has been reinforced by the identification of additional

mutations, such as *KCNQ1* (Das et al., 2009) and R27C mutations of the *KCNE2* gene. These mutations encode the shared β-subunits of I_{Ks} and I_{Kr} (Yang et al., 2004).

In patients with nonfamilial AF, a missense L65F mutation in *KCNE5* has been identified. *KCNE5* is believed to downregulate I_{ks} activity by competing with the *KCNE1* β-subunit for the *KCNQ1* α-subunit. In vitro transfection of KCNE5-L65F into Chinese hamster ovary cells failed to suppress I_{ks} activity, suggesting shortening of APD is the underlying mechanism by which this mutation causes AF (Ravn et al., 2008).

The first nonion channel gene linked with familial AF was identified in 2008. It involved a frameshift mutation in the *NPPA* gene that resulted in encoding of a mutant atrial natriuretic peptide (ANP) that, when infused in a rat whole-heart Langendorff's preparation, shortened the monophasic APD and ERP (Hodgson-Zingman et al., 2008). *NPPA* may also be involved in inflammatory pathways and regulation of electrical and structural remodeling (Darbar and Roden, 2013).

Finally, mutations in sodium channel genes may also play an important role in the pathogenesis of AF. Screening for *SCN5A* variants in a large AF cohort found mutations in 5.9% of those with AF (Darbar et al., 2008). A recent metaanalysis of over 7000 African American patients across five cohorts found that the common *SCN5A* variant rs7629265 was associated with increased AF risk and shorter PR interval. Other variants, such as rs6795970 for *SCN10A*, which encodes the Nav1.8 channel, have also been found to be associated with AF. In particular the A1073 genetic variant, which confers gain-of-function to Nav1.8, prolongs APD, and thereby increases susceptibility to AF (Ilkhanoff et al., 2014).

Modulation of Gap Junction Expression

Aging, structural remodeling, and development of fibrosis are all known to increase the likelihood of developing AF as the disturbance of uniform impulse propagation from cell to cell and the variability in regional cardiac velocity creates a substrate for reentry (Jennings, 2013). Both germ-line and somatic mutations in the *GJA5* gene, which encodes connexin-40, and a common variant in the promotor region of this gene have been shown to be associated with early-onset lone AF (Gollob et al., 2006; Wirka et al., 2011). Further, mutations in *GJA5* have also been identified in patients with familial AF (Yang et al., 2010), as knock-out mouse models appear to exhibit increased vulnerability to atrial arrhythmias. Similar to acquired cases of AF, genetic variants for genes encoding gap junction proteins likely mediate increased risk for AF by reduced expression of gap junctions, leading to slower and less uniform atrial conduction.

Role in Atrial Fibrosis and Inflammatory Pathways

Histologic evidence linking myocarditis to early-onset AF and the high incidence of AF following cardiac surgery suggests that pathways involved with inflammation and fibrosis play a role in the pathogenesis of AF (Gallagher et al., 1997; Patti et al., 2006). In animal studies, induction of atrial fibrosis has been associated with increased risk for atrial arrhythmias, and in humans, the extent of atrial fibrosis has correlated with success of ablation therapy (Akoum et al., 2011). Although the role of many common AF risk alleles has not been fully elucidated, there is now increasing evidence that atrial fibrosis may be a key mediator for increased risk of developing AF.

Besides its role in the development of the pulmonary vein myocardium, evidence suggests PITX2 may play a role in structural and electrical remodeling of the atria. PITX2 encodes for the homeobox transcription factor PITX2c, which is expressed 100-fold higher in the left than the right atrium (Kirchhof et al., 2011). *Pitx2c*$^{-/-}$ knock-out mice develop four-chamber cardiac enlargement, histological evidence of atrial fibrosis, and upregulation of collagen precursor genes. In contrast, *Pitx2c*$^{+/-}$ heterozygous mice have no evidence of cardiac enlargement but have increased expression of genes regulating Wnt signaling, a key fibrosis pathway (Kirchhof et al., 2011). Common SNPs at the chr7q31 AF locus encode caveolin-1, which internalizes transforming growth factor (TGF)-β receptors leading to suppression of this signaling pathway and prevention of atrial fibrosis. Variants in *CAV1* have not only been associated with myocardial fibrosis but also pulmonary hypertension.

The zinc finger homeobox 3 transcription factor encoded by ZFHX3, also plays an important role when responding to TGF-β signaling (Berry et al., 2001). SYNP02L and SYNE2 encode cytoskeletal and nucleoproteins that link the nucleoskeleton to the nuclear membrane structures and may play an important role in cardiac development (Beqqali et al., 2010). SYNE2 and emerin interact with a-catenin regulating the Wnt signaling pathway. PRRX1 is a transcription factor that is thought to be important for normal lung development and has been associated with pulmonary fibrosis. Scleroderma fibroblasts stimulated with TGF-β show increased expression of C90RF3. Another possible mechanism by which *NPPA* variants may mediate increased risk for AF is by causing both electrical and structural remodeling of the atria with the development of atrial fibrosis. Although overexpression of the humanized *NPPA* variant in mice does not seem to cause atrial enlargement, a different *NPPA* mutation identified in four Italian families was associated with an atrial myopathy, massive biatrial enlargement, and atrial standstill (Disertori et al., 2013).

Genetic Approaches to AF

Linkage Analysis and Candidate Gene Studies

Positional cloning and linkage analysis have proven to be powerful tools for detecting the chromosomal location of disease-associated genes and is based on the assumption that nearby genes on a chromosome tend to remain linked during meiosis (**Fig. 1**).

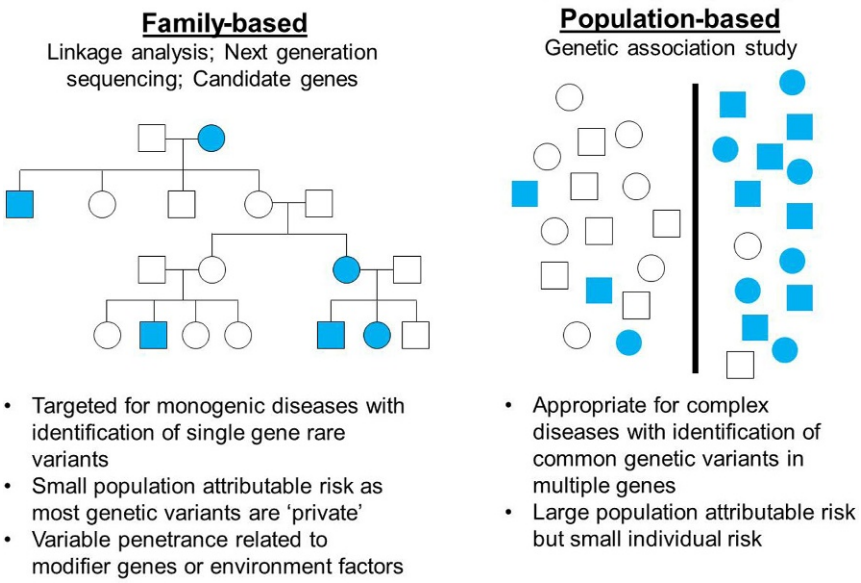

Fig. 1 Genetic approaches to defining the genetic architecture of atrial fibrillation (AF). Linkage analysis, next generation sequencing and candidate gene approaches have identified rare genetic variants in single genes in large familial kindreds with early-onset AF. Conversely, genetic association studies, that is, genome-wide association studies have uncovered common genetic variants associated with AF in many genes. (Filled in symbols indicate individuals with AF and unfilled symbols represent controls.)

While these disease-associated loci are expected to have stronger effect size than common genetic variants, a major limitation of this approach is the limited availability of large AF kindreds with multiple affected individuals. Regardless, positional cloning and linkage analysis have helped identify AF loci and genes with large effect size, including 11p15.5, 21q22, 17q, 7q35-36, 5p13, 6q14-16, and 10q22-24, although the responsible genes have not been identified.

The first gene to be linked to familial AF and to be validated in functional studies was *KCNQ1*, which encodes the -subunit of the delayed rectifier potassium channel (*KCNQ1*). Using a candidate gene approach, investigators have screened for other cardiac ion channel genes hypothesized to be mechanistically linked to AF including *KCNE2*, *KCNJ2*, *KCNE5*, *SCN5A*, and *SCN10A*. Otway et al.(Otway et al., 2007) examined 50 families with a history of AF and identified a single mutation (R14C) in *KCNQ1* in one family which did not have any direct effect on KCNQ1 and KCNE1 channels and corresponding current amplitudes in cultured cells. However, exposure to hypotonic solution led to swelling of atrial cells and mutant channels then exhibited a marked increase in current amplitude compared with wild-type channels. Further, only patients with dilated LA chambers carry the mutation developed AF. The concept that certain acquired or environmental conditions must be met before a genetic trait manifests itself, could perhaps explain why variable penetrance is observed in AF families carrying rare variants with large effect size (Darbar and Roden, 2013).

A significant limitation of the candidate gene approach is the low pretest probability due to the overall frequency of common polymorphisms within the genome and single variant associations are often difficult to replicate.

Genome-Wide Association Studies (GWAS)

Although many patients with non-familial AF have known risk modifiers (e.g., hypertension, diabetes, metabolic syndrome), most people in general population do not go on to develop AF despite carrying the same risk factors. A concept, known as the "two-hit hypothesis," has been proposed whereby common genetic variants are thought to increase susceptibility to AF in patients harboring other identifiable risk factors (Darbar and Roden, 2013). NGS has made it possible to sequence hundreds of thousands of SNPs across the human genome in a feasible manner to test for association between affected and unaffected subjects. Given that such a large number of genes are tested without a priori assumption regarding their potential disease association, the effect size of many disease-associated SNPs tend to be smaller and their association may be difficult to replicate, especially across different ethnic populations.

In the first GWAS performed in 2007, two noncoding SNPS on the chr4q25 locus were found to be independently associated with AF in an Icelandic population. This association was then replicated in Asian (Shi et al., 2009) and European populations (Gudbjartsson et al., 2007) and in patients after coronary artery bypass graft (CABG) surgery (Body et al., 2009). The nearest gene to this locus is the paired-like homeodomain transcription factor 2 (*PITX2*) gene, which encodes a transcription factor important for PV development (Mommersteeg et al., 2007a), determination of left and right atrial asymmetry during embryogenesis (Logan et al., 1998), and suppression of formation of the LA sinus node (Mommersteeg et al., 2007b). Nine additional AF loci and five novel AF genes were identified when a metaanalysis of AF GWAS was recently conducted (**Table 1**) (Ellinor et al., 2012; Sinner et al., 2014). Interestingly, the severity of cardiac developmental malformations appears correlated to the degree of PITX2 expression (Gage et al.,

Table 1 Common atrial fibrillation (AF) susceptibility loci identified by genome-wide association studies

SNP	Locus	Closest gene	Functional effect	Minor/ major allele	MAF (%)	RR (95% CI)	P value
rs2200733	4q25	*PITX2*	Development of pulmonary vein myocardial	T/C	25.8	1.71 (1.54–2.21)	6.1×10^{-41}
rs10033464	4q25	*PITX2*	sleeve	G/C	13.1	1.42 (1.13–1.77)	3.1×10^{-11}
rs7193343	16q22	*ZFHX3*	Unknown	T/C	17.6	1.25 (1.17–1.3)	1.8×10^{-15}
rs3903239	1q24	*PRRX1*	Development of great vessels	G/A	44.7	1.14 (1.10–1.18)	9.1×10^{-11}
rs3807989	7q31	*CAV1*	Atrial signal transduction protein	A/G	40.4	0.88 (0.84–0.91)	9.6×10^{-11}
rs10821415	9q22	*C9orf3*	Unknown	A/C	42.4	1.13 (1.08–1.18)	7.9×10^{-9}
rs10824026	10q22	*SYNPO2L*	Cardiac protein that localizes to Z-disc	G/A	15.8	0.85 (0.81–0.9)	1.7×10^{-8}
rs1152591	14q23	*SYNE2*	Structural protein of cardiac sarcomere	A/G	47.6	1.13 (1.09–1.18)	6.2×10^{-10}
rs7164883	15q24	*HCN4*	Ion channel (I_f) involved in cardiac pacemaking	G/A	16.0	1.16 (1.10–1.22)	1.3×10^{-8}

AF, Atrial fibrillation; *CI*, Confidence interval; *MAF*, Minor allele frequency; *RR*, Relative risk; *SNP*, Single nucleotide polymorphism;

1999) as complete absence of Pitx2 function in mice leads to embryonic lethality while homozygous null embryos exhibit severe cardiac defects (Kitamura et al., 1999). Heterozygous $Pitx2^{+/-}$ mice, which are fully viable, have higher rates of atrial arrhythmias with programmed stimulation. The role of *PITX2* in the development of the left atrial and pulmonary veins fits well with our understanding that in many cases of paroxysmal AF the arrhythmia is triggered by ectopic foci originating from pulmonary vein myocardial sleeves.

Clinical Applications of Genetic Discoveries

Current treatment paradigms for AF remain largely empiric given the lack of evidence-based data to support mechanism-based treatment approaches. The identification of rare and common susceptible variants through genetic approaches has unlocked new areas of investigation that involve specific biological pathways. These pathways are responsible for the pathophysiology of AF, including dysfunctional cardiac ion channels, signaling pathways, and gap junction proteins. Although in vivo and in vitro models offer opportunity for functional validation and potential pharmacological development, transition from the bench to meaningful outcomes in clinical practice has been limited.

GWAS studies have identified numerous common genetic variants associated with AF, however the effect size of many of these SNPs is small and the contribution to the pathophysiology of AF remains unclear for many of them. Given the overall breadth of the human genome, one difficulty has been distinguishing benign SNPs from pathogenic mutations. Numerous approaches have been proposed including examining the variant type and location, segregation of the variant with phenotype in affected family members, and analyzing the degree of conservation of encoded amino acids across species. Multiple "heuristic" prediction algorithms have also been developed to determine the likelihood of a rare variant being pathogenic.

While it has become clear that an individual's genetic background can influence the risk of developing AF (Everett et al., 2013; Tada et al., 2014) incorporation of genetic analysis into treatment strategies still remains limited. Our inability to readily identify specific mechanisms responsible for AF in an individual, difficulties determining response to therapies, and lack of genotype-directed prospective clinical trials, are all factors that prevent the integration of these genetic discoveries into our clinical practice.

Identification of Genetic Subtypes and Response to Therapy

Genetic studies have not only uncovered common and rare genetic variants associated with AF but have also identified AF subtypes with differential responses to therapy. One of the first studies assessing genotype influence on response to therapy involved the use of antiarrhythmic drugs (AADs). In a study published in 2012, Parvez et al. (Parvez et al., 2013) assessed whether the common AF risk loci on chr4q25 (near *PITX2*), 16q22 (in *ZFHX3*), and 1q21 (in *KCNN3*) modulated response to AADs in patients with symptomatic AF. The study determined that carriers of the chr4q25 SNP (rs10033464) were significantly associated with successful symptom control (odds ratio [OR], 2.97; 95% confidence interval, 1.42–6.21; $P = 0.003$). Further, individuals with the chr4q25 SNP responded better to class I versus class III antiarrhythmic drugs in both the discovery and validation cohorts.

Interestingly, it has been shown in multiple studies that carrying a chr4q25 SNP predicts poorer response to AF catheter ablation compared to the presence of the wild-type SNP (Benjamin Shoemaker et al., 2013; Husser et al., 2010). The chr4q25 SNPs have also been seen to be independent predictors of AF recurrence following restoration of sinus rhythm after electrical cardioversion (Parvez et al., 2013). Collectively, these data support the concept of selecting AF therapies based on a patient's genotype and predicted response. Also important is that although the effect size of common genetic susceptibility markers for AF have not been large (OR 1.1–1.7), in studies evaluating whether AF risk SNPs modulate response to therapy, the ORs were markedly higher than those used to determine predilection for AF itself.

While the possibility of tailoring AF therapy based on genotype rather than continuing current empiric approaches is exciting, prospective clinical trials are needed to validate the role of subtyping of AF by chr4q25 genotyping. Thus far, only a few centers have conducted such studies and replication in different ethnicities is essential as outcomes may be different among populations. In 2009, enrollment started for the CABANA Trial, which is the largest prospective international study yet to date comparing catheter ablation to pharmacologic rate/rhythm control. Along with the main trial, a substudy called CABANAgene will be performed concurrently and will provide a large multinational cohort in which replication studies can be performed.

Novel Approaches to Therapy

Although AF is a complex and multigenic disorder, insight into the genetic mechanisms of AF have resulted in proof-of-concept studies revealing novel pathways that may provide suitable targets for pharmacologic therapies. However, many promising new therapies have failed to show efficacy in prospective clinical studies (Podd et al., 2015), which can be discouraging considering the time and resources invested into the development of new drugs. Knowledge of a patient's genotype may potentially enhance selection of patients most likely to benefit from new mechanism-targeting therapies and aid in the execution of future clinical studies.

Pharmacologic agents targeting cardiac ion channels

Potassium channel antagonist

Research on familial AF-related mutations, especially WT-*KCNQ1* and *KCNQ1-S140G*, has reported that these two mutations possess distinct pharmacological properties that may enable inhibition of the I_{Ks}-selective blocker HMR-1556 (Campbell et al., 2013). Although a low concentration of the blocker had little effect on WT-I_{Ks}, it was capable of inhibiting the mutant channel. In cultured left atrial myocytes of adult rabbits, expression of S140G-I_{Ks} shortened APD; however, cells expressing WT-I_{Ks} did not. The familial *KCNQ1-S140G* gain-of-function mutation demonstrated increased sensitivity for the I_{Ks} blocker, suggesting the possibility of selective therapeutic targeting.

On the chr1q21 locus, AF risk SNPs have been identified in *KCNN3*, which encodes the SK channel, a member of the Ca^{2+}-activated potassium channel family. These channels are believed to contribute to the shortening of atrial APD. In large animal models, administration of the selective SK inhibitor, NS8593, led to termination of pacing-induced AF (>15 min of continuous AF), and decreased AF duration and vulnerability without affecting ventricular conduction and repolarization (Haugaard et al., 2015).

Ryanodine receptor antagonist

Mutations in the ryanodine receptor (*RyR2*) and sarcoplasmic reticulum Ca-binding protein, calsequestrin (*Casq2*), have been found to increase the risk for AF. Investigators at Vanderbilt examined the underlying mechanisms of AF associated with loss of Casq2, and tested a mechanism-based drug therapy for AF (Faggioni et al., 2014). Atrial burst pacing was used to quantify AF susceptibility in isolated hearts from *Casq2*$^{-/-}$ and *Casq2*$^{+/+}$ mice. AF was induced in 11/12 *Casq2*$^{-/-}$ mice hearts but not in *Casq2*$^{+/+}$ hearts.

The dual RyR2 and sodium channel blocker, R-propafenone, significantly reduced the frequency and amplitude of subthreshold diastolic calcium elevations, and DADs in atrial myocytes, and ultimately prevented induction of AF. In contrast, S-propafenone, an equipotent sodium channel blocker but much weaker RyR2 inhibitor, only partially reduced subthreshold diastolic calcium elevations and DADs, hence, failing to prevent AF.

These findings strongly suggest that targeting AF with R-propafenone may be a more mechanism-based approach to treating AF. A multisite, prospective human study using such a mechanism based approach for the treatment of paroxysmal AF is planned for the near future. Ranolazine, a late sodium channel blocker that blunts diastolic calcium accumulation in the cytosol, has been shown to have efficacy for prevention of postoperative AF, treatment of paroxysmal AF, and facilitation of electrical CV, both alone and in combination with amiodarone and dronaderone (Tsu and Lee, 2014; Murdock et al., 2012; Reiffel et al., 2015).

Gap junction modulators

Mutations in gene expressing connexin-40 and connexin-43 in familial and nonfamilial AF, as well as mice knock-out of GJA5 expression models, have been reported to increase vulnerability for atrial arrhythmias. Similarly, in canine models, extensive left and right atrial ablation is associated with lower acute recurrence of AF, but it is also associated with decreased connexin-43 near the ablation site. This suggests that worsened or irregular dispersion of CV may be proarrhythmic (Elvan et al., 1997).

Rotigaptide is a peptide that is believed to modulate gap-junction function by increasing PKC-dependent connexin-43 phosphorylation, which has been shown to reduce AF in chronic mitral regurgitation and acute ischemia models (Guerra et al., 2006; Shiroshita-Takeshita et al., 2007). Further, danegaptide, a dipeptide which behaves like rotigaptide, has also been shown to reduce AF inducibility in canine models (Dobrev et al., 2012).

Adenovirus-mediated gene transfer of connexin-40 and -43 was shown to reduce AF occurrence through normalization of connexin expression and CV pattern in swine models (Igarashi et al., 2012). While the clinical applicability of these approaches for AF in humans is still to be determined, genotype-based selection of patients with known mutations in connexin 40 and 43 genes may improve outcomes with these therapies compared to the general population.

Attenuation of the autonomic nervous system response

It is well known that autonomic nervous system is an important modulator of AF susceptibility. One clinical subtype, known as vagally-mediated AF, is thought to be caused by release of acetylcholine (Ach) from the vagus nerve and cardiac GP. This leads to enhanced I_{KACh} and heterogenous dispersion of APD shortening, potentially creating the electrical substrate for stabilizing reentrant rotors. I_{KACh} is composed of two homologous GIRK channel subunits: GIRK1 and GIRK4. In an animal model simulating sick sinus syndrome in $Ca_V1.3$ mice, inactivation of GIRK4 led to decreased inducibility of AF, suggesting that pharmacologic attenuation of I_{KACh} may be useful in vagally-mediated AF. Unfortunately, no human genetic variants associated with AF that affect I_{KACh} expression have been identified thus far.

Genetic variants in SCN10A encoding Nav1.8, which is expressed in peripheral nerve tissue, have been associated with early onset AF. In an animal study, administration of a Nav1.8 blocker resulted in blunting the effect of vagal nerve stimulation on CV and AF inducibility. This is presumably due to inhibition of neural activity of the cardiac GP. Further, in a small double-blinded clinical trial of 20 patients with paroxysmal AF and pacemakers, administration of BMS 914392 (a selective oral inhibitor of I_{KACh} current) did not reduce overall AF burden compared to controls (Podd et al., 2015). Identification of a pathologic genetic variant for I_{KACh} would potentially also allow tailoring of current AADs choices, such as disopyramide and dronaderone, which are potent inhibitors of I_{KACh}. Ultimately, suppression of autonomic signaling with pharmacologic therapy may also contribute to the efficacy of PV-directed ablation procedures for AF as the effect of GP-targeting ablation strategies depend on successful modification of autonomic innervation patterns.

Utilizing Genetics to Improve Catheter Ablation Outcomes

Outcomes following AF ablation remain modest despite advances in catheter technology and use of new mechanism-based approaches, with freedom from AF being only 20%–50% at 1 year. Investigators have sought to assess whether using common AF-susceptibility alleles can help identify genetic subtypes of AF with differential responses to ablation therapy. In 2010, Husser et al. evaluated whether the common AF-susceptibility SNPs on the chr4q25 locus modulated response to catheter ablation (Husser et al., 2010). While none of the clinical or echocardiographic parameters predicted the response to ablation, the presence of one of the 4q25 variant SNPs did positively associate with AF recurrence after catheter ablation (OR 4.1). This finding was replicated in a large cohort of patients with 'typical' AF (associated with known risk factors), where the overall recurrence rate was 53% over an 18-month period (Benjamin Shoemaker et al., 2013). Further, the presence of the 4q25 SNP risk allele predicted a 24% shorter recurrence-free time (survival time ratio 0.76) than did the presence of the WT SNP (Benjamin Shoemaker et al., 2013). For this, carrying one or two copies of the chr4q25 AF-risk alleles is one of the strongest predictors of 1-year recurrence of AF after catheter ablation (Fig. 2). It should be noted however, that a recently published study of patients of solely Asian descent did not demonstrate a significant correlation between AF-associated SNPs and AF recurrence following catheter ablation seen in other reports (Choi et al., 2015).

In a recent report, Mohanty et al. sought to examine the association between common AF risk SNPs, non-PV triggers, and prevalence of LA scar in 371 patients who underwent catheter ablation for AF (Mohanty et al., 2015). Of the common variants examined, only the chr16q22 (near *ZFHX3*) SNP rs7193343 was associated with a high risk for non-PV triggers and atrial scar formation. While the transcription factor zinc finger homeobox 3 (encoded by *ZFHX3*) is a tumor suppressor-suppressor gene, it associates with another transcription factor (runt-related transcription factor 3 [RUNX3]), and both translocate to the nucleus in response to transforming growth factor (TGF)–β signaling, an important mediator of fibrosis (Verheule et al., 2004; Mabuchi et al., 2010). Seven of the 16 SNPs examined in the study were associated with atrial fibrosis during endocardial scar mapping. It is increasingly appreciated that atrial fibrosis not only plays a prominent role in the pathogenesis of AF but may also be a plausible link between genetic variants and development of AF.

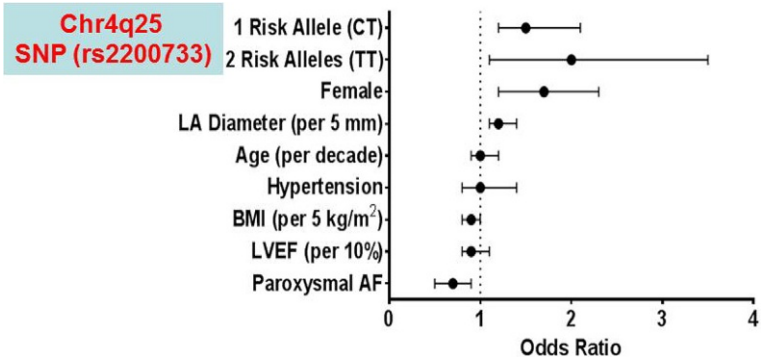

Fig. 2 Modulation of response to catheter ablation of AF by common genetic variants. Predictors of 1-year recurrence of AF after catheter ablation. *BMI*, Body mass index; *Chr*, Chromosome; *LA*, Left atrial; *LVEF*, Left ventricular ejection fraction; *SNP*, Single nucleotide polymorphism.

Risk Model Score

Ethnicity and AF

Although the risk of AF across different races has been addressed in a few studies, the focus is black versus white populations. However, there is paucity of data related to other races such as Asians, or Hispanic/Latinos. Reports suggest that black individuals have a lower prevalence and incidence of AF compared with white individuals. As seen in the Cardiovascular Health Study (CHS), the incidence is slightly lower in black participants than in white participants (12.0 vs 19.5 per 1000 person-years, respectively; $P>0.10$) (Psaty et al., 1997). Further, in a multivariate Cox proportional hazards model adjusted for age and traditional cardiovascular risk factors, black race/ethnicity was associated with lower risk of incident AF (HR: 0.47; 95% CI: 0.22–1.01) compared with white race/ethnicity.

Data regarding AF incidence in Hispanic/Latino and Asian populations is much more limited. A metaanalysis of data from seven randomized clinical trials reported that Asian race/ethnicity was associated with significantly lower rates of AF (OR: 0.65; 95% CI: 0.50–0.84; $P=0.001$) compared with white race/ethnicity. These findings are consistent with a previous report which demonstrated that AF is less common in patients of South Asian origin who were newly hospitalized for heart failure compared to white patients (15 vs. 31%; $P=0.0002$). In the Northern Manhattan Stroke Study, AF was more common in white patients (29%) than in either black (11%) or Hispanic (11%) patients. Similarly, in a large study of US Veterans, the prevalence of AF was 5.7% in White, 3.4% in Black, 3.0% in Hispanic, 5.4% in Native American/Alaskan, 3.6% in Asian, and 5.2% in Pacific Islander participants ($P<0.001$).

Conclusions

Advances in Next Generation Sequencing (NGS) have uncovered the genetic architecture of AF, provided important insights into the underlying mechanisms, defined response to pharmacologic and ablation therapy, and identified novel therapeutic targets. While the impact of these discoveries thus far has been limited, we envision a scenario in the near future where an individualized approach to the management of AF is a reality. Such an approach will not only improve efficacy but also minimize adverse events.

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Genetic Disorders Involving Valve Function

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Introduction

Cardiovascular disease is the leading global cause of death and is responsible for one in three deaths in the United States (Benjamin et al., 2017). Valvular heart disease is one of the most common forms of cardiovascular disease and its prevalence increases with age. Using hospital admission and discharge data for 2010, valvular heart disease was identified as the cause of admission in 85,000 cases, and was associated with over 25,000 deaths in the United States. Based on prospective echocardiographic data from almost 12,000 participants, the prevalence of valvular disease has been suggested to be over 4.4% in individuals over the age of 65, and over 11% in those above the age of 75 (Benjamin et al., 2017). After adjustments for age and sex, this corresponds to a prevalence of 2.5% in the US population for any valve disease with no significant difference between males and females. To date, no effective medical therapies have been discovered to prevent or slow down the progression of valve deterioration. As a result, the only treatment for any type of valve disease is valve repair or replacement, a costly procedure that is associated with considerable morbidity and mortality. Despite clear evidence for the high burden of valvular disease, the pathophysiology and most importantly the genetic basis of many forms of valvular abnormalities remain poorly understood. In this article, we discuss the recent evidence surrounding valvular disease focusing on its epidemiology and genetic basis.

Aortic Valve Disease

The relationship between aortic valve structure and blood flow was first described in the 15th century Italy, by Leonardo Da Vinci. It has been reported that Da Vinci (1452–1519) used the motion of waterborne grass seeds flowing through glass models of aortas to describe this relationship (Dorn, 2013; Boon, 2009). Aortic stenosis (AS) has since been identified as the most common form of valvular heart disease in high-income countries of the Western world (Benjamin et al., 2017). Worldwide, rheumatic valve disease continues to be the most common cause of AS (Magnati et al., 2010). The prevalence of AS of any severity in the general US population is about 0.4%, corresponding to over 1.2 million Americans based on the 2016 US population data (Benjamin et al., 2017; Nkomo et al., 2006). The prevalence of AS rises from 1.3%–1.4% in those above the age of 65 to 2.6%–4.6% in individuals above the age of 75 (Nkomo et al., 2006; Lindroos et al., 1993; Stewart et al., 1997), and its associations with cardiovascular morbidity and death are well supported (Otto et al., 1999). In addition to age, male sex has also been reported as a risk factor for AS, but this has not been consistently noted (Stewart et al., 1997). Moreover, evidence suggests the presence of higher prevalence of

coronary artery disease (CAD), renal insufficiency, hypertension, smoking, and dyslipidemia among patients with AS (Nkomo et al., 2006; Stewart et al., 1997; Otto et al., 1999; Thanassoulis et al., 2010). However, it is unclear if the presence of these comorbidities is the result of a common pathophysiological process or not.

The causes of valvular AS include bicuspid valve (BAV), calcification, and rheumatic disease (which is often concomitantly associated with mitral valve disease), and less common processes such as alkaptonuria (Phornphutkul et al., 2002) or familial hypercholesterolemia (Rajamannan et al., 2003). AS requiring intervention is rare in those under the age of 70. In one study, more than two thirds of patients who underwent aortic valve replacement before the age of 70 were noted to have BAV (Roberts and Ko, 2005), suggesting that AS is fairly uncommon in the younger population in the absence of a congenital valve abnormality. Aortic sclerosis defined as aortic valve leaflet thickening, and aortic valve calcification, are the two processes that pave the way for AS and have a high prevalence among the elderly (Otto et al., 1999). These two clinically silent processes were previously thought to be benign, but new evidence suggests that they also increase the risk of cardiovascular death in patients (Otto et al., 1999).

Calcific Aortic Valve Disease

Clinical significance

Calcific aortic valve disease (CAVD), which includes aortic sclerosis and calcifications as well as clinical AS, is thought to be the most common cause of valve stenosis in the Western world (Benjamin et al., 2017). It is estimated to affect over five million individuals in North America (Bach et al., 2007), and has a huge burden on health care with direct health care costs exceeding $1 billion/year (Benjamin et al., 2017). Currently, it serves as the third most common cardiovascular disease after ischemic heart disease and hypertension (Thanassoulis, 2016). Data suggest that age serves as an important risk factor for aortic valve sclerosis/calcification with over 50% of patients above the age of 75 suffering from at least mild aortic valve sclerosis/calcification (Lindroos et al., 1993). Aortic sclerosis is currently a progressive and irreversible process that leads to AS (Owens et al., 2010).

Pathogenesis and current evidence

CAVD initially has a silent clinical phase that involves hardening and calcification of the aortic valve leaflets, known as sclerosis, with no hemodynamic compromise. This stage of the disease has a prevalence of 26% in those older than 65 years of age, and it increases to about 40% after the age of 75 (Lindroos et al., 1993). Previously, aortic valve sclerosis was thought to be benign. However, recent evidence suggests that patients with sclerosis have a 50% increased risk of cardiovascular death and myocardial infarction at five years (Otto et al., 1999; Taylor et al., 2005; Carabello, 1999). Sclerosis shares many of the risk factors with vascular atherosclerosis, such as elevated low-density lipoprotein cholesterol (LDL-C), lipoprotein(a) (Lp[a]), diabetes, smoking, and hypertension (Nkomo et al., 2006; Stewart et al., 1997; Otto et al., 1999; Thanassoulis et al., 2010). Despite similar risk factors, data suggests that aortic valve sclerosis and vascular atherosclerosis are two different disease states (Otto et al., 1994; Demer and Tintut, 2011; Kvidal et al., 2000; Chan et al., 2010; Cowell et al., 2005; Rossebo et al., 2008). A study by Otto and colleagues provided histologic and immunochemical evidence of the similarities and differences between aortic sclerosis and atherosclerosis, highlighting the presence of early mineralization phase and less smooth muscle cells in valve sclerosis compared to atherosclerosis (Otto et al., 1994). In valve sclerosis, calcification develops early and is thought to be independent of atherosclerosis (Demer and Tintut, 2011). In fact, of patients undergoing aortic valve replacement for noncongenital stenotic valve, only about 40% of the subjects required concomitant coronary bypass surgery again highlighting that although valve stenosis and vascular atherosclerosis share several risk factors, unique differences in pathophysiology must be at play (Kvidal et al., 2000). It is also important to note that trials using lipid-lowering agents (i.e., statins) have not shown any benefit in preventing aortic valve disease despite their established role in preventing the progression of atherosclerosis (Chan et al., 2010; Cowell et al., 2005; Rossebo et al., 2008).

The exact mechanism responsible for valvular calcification is not fully understood. Some suggest that cholesterol crystals, elastin fibers, and collagen may serve as locations that allow for mineralization (Towler, 2013; Laird et al., 2006; Fartasch et al., 1990). The process of valve calcification is thought to be similar to bone formation. Noncollagenous matrix proteins involved in bone formation, namely osteopontin, osteocalcin, and osteonectin, have been suggested to be expressed in cells that are in close proximity to valvular zones of calcification undergoing mineralization and could potentially serve as regulators of valvular calcification (Srivatsa et al., 1997). Moreover, early evidence pointed to coexpression of collagen and alkaline phosphatase (ALP) to be sufficient enough for promotion of mineralization (Murshed et al., 2005). Matrix vesicles that are involved in organization of mineral deposition associated with elastin and collagen shed ALP that can then inactivate osteopontin, and inorganic phosphates, which are putative natural inhibitors of calcification (Towler, 2013). Another matrix vesicle enzyme called Phospho1, has also been thought to be involved in biomineralization (Kiffer-Moreira et al., 2013). In addition to ALP and Phospho1, matrix vesicles also contain molecules that are able to bind calcium and promote mineralization even without ALP being active. These molecules are called annexins A5, annexins A6, and phosphatidyl serine (Kapustin et al., 2011). In addition, cells from the myeloid lineage and especially those expressing CD45 and type I collagen have also been suggested to be involved in calcification based on histopathologic studies that have noted their presence especially in the late stages of CAVD (Egan et al., 2011; Fadini et al., 2011). Despite extensive research, our understanding of the pathobiology of AS and CAVD remains incomplete. Ongoing research is currently looking into identifying novel genes that could shed light into molecular pathways that may be involved in this process, which could serve as potential therapeutic targets to prevent the progression of valvular calcification.

Genetics of calcific aortic valve disease

Heritability

With regard to the genetics of CAVD, evidence has highlighted familial clustering of this disease (Horne et al., 2004; Bella et al., 2007; Probst et al., 2006). Data from the Utah Population Database for cases of death attributable to nonrheumatic aortic and mitral valve disease initially suggested high degree of relatedness that extended beyond second-degree relatives among individuals with nonrheumatic valvular mortality (Horne et al., 2004). Researchers from the Hypertension Genetic Epidemiology Network (HyperGEN) Study, evaluated genotype and echocardiographic data from 1871 siblings and identified 142 affected siblings of which 78 sibships from 68 families suffered from at least two affected siblings with one or more types of valve calcification (Bella et al., 2007). The data from the HyperGEN study showed a modest degree of familial aggregation with sibling recurrence risk and sibling recurrence risk ratio for aortic valve sclerosis being 0.25 ($P=0.035$) and 2.31 (95% CI 1.72–3.11). Moreover, a study from France by Probst and colleagues detected specific regions in France with high rates of AS supporting the notion that CAVD may be heritable (Probst et al., 2006). The investigators identified 48 cases of severe AS, 13 of which were within the same family, and traced them all back to a single common ancestor born in 1650, 13 generations prior (Probst et al., 2006). None of the families analyzed had evidence of renal failure or familial hypercholesterolemia, which are considered confounding disorders for aortic valve disease. Moreover, there was no association between AS cases and carriage of Vitamin D receptor (VDR) or Apolipoprotein E variants. Based on their analysis, there was 33% first-degree recurrence rate, providing the most definitive evidence for heritability of CAVD.

Candidate gene studies

One of the main hypotheses surrounding the pathogenesis of aortic valve disease has been regarding the role of atherosclerosis and lipids in valve disease. Hence, there have been many genetic studies that have focused on the association between lipid genes and AS with conflicting results. Candidate gene studies for CAVD have been summarized in **Table 1**. Work by Avakian et al. using a case–control study design of apolipoprotein A1, B, and E polymorphisms in 122 subjects (62 controls and 62 cases with severe AS),

Table 1 Summary of candidate gene association studies for calcific aortic valve disease

Gene	*Polymorphism*	*Risk variant*	*Ethnicity*	P-*value*
Apolipoprotein B (*APOB*) (Avakian et al., 2001)	2p24.1 *Xbal* restriction enzyme	Xbal X+/X +	Brazilian	0.007
Apolipoprotein B (*APOB*) (Gaudreault et al., 2011)	2p24.1 E4181K, 3′ untranslated region	–	French Canadian	1.03×10^{-5}; 1.32×10^{-5}
Apolipoprotein E (*APOE*) (Avakian et al., 2001)	19q13.2 *HHa1* restriction enzyme	E2 allele	Brazilian	0.034
Apolipoprotein E (*APOE*) (Novaro et al., 2003)	19q13.2 *HHa1* restriction enzyme	E4 allele	Predominantly White European	0.046
Estrogen Receptor α (*ESR1*) (Nordstrom et al., 2003)	6q25.1 *Pvull* and *Xbal* restriction enzymes	p allele	Postmenopausal White European Women	0.03
VDR (Ortlepp et al., 2001)	12q13.11 *Bsml* restriction enzyme	B allele	–	0.001
Interleukin-10 (*IL10*) (Ortlepp et al., 2004)	1q31-32 G1082A, C819T, and C592A	A, T, and A alleles	–	0.026; 0.027; and 0.028
Interleukin-10 (*IL10*) (Gaudreault et al., 2011)	1q31-32 6 polymorphisms in 5′ region, introns, and 3′ region	–	French Canadian	$0.016–6.21\times10^{-11}$
Connective Tissue Growth Factor (*CTGF*) (Ortlepp et al., 2004)	6q23.1 G447C	GC	–	0.099 (0.037 in combination with CCR5 polymorphism)
Chemokine (C–C motif) Receptor 5 (*CCR5*) (Ortlepp et al., 2004)	3p21.31 Δ32 deletion	Deletion	–	0.213 (0.037 in combination with CTGC polymorphism)
Parathyroid Hormone (*PTH*) (Schmitz et al., 2009)	11p15.3-p15.1 rs6254	AA	–	0.007
Myosin VIIA (*MYO7A*) (Ellis et al., 2012)	11q13.5 rs2276288	AA	–	0.001
Angiotensin II Receptor, Type 1 (*AGTR1*) (Ellis et al., 2012)	3q24 rs5194	AA	–	0.004
Elastin (*ELN*) (Ellis et al., 2012)	7q11.23 rs2071307	AA	–	0.005
Lipoprotein(a) (*LPA*) (Thanassoulis et al., 2013)	6q26-27 rs10455872	G allele	White European	9.0×10^{-10}

Adapted from Mauer, A. C., Owens, D., O'Donnell, C. J., Post, W. S. and Thanassoulis, G. (2015). Genetics of valvular heart disease. In: Shah, S. J. and Arnett, D. K. (eds.) *Cardiovascular genetics and genomics in clinical practice*. New York: Demos Medical.

identified the apolipoprotein B XbaI restriction site polymorphism to be in excess among their AS cases along with the apolipoprotein E2 allele (Avakian et al., 2001). Novaro and colleagues evaluated the role of apolipoprotein E (*APOE*) gene in 802 individuals with 43 cases (Novaro et al., 2003). The investigators found that age and the APOE4 allele were independent predictors of AS, but not the E2 allele previously identified by Avakian and colleagues.

Given the previously demonstrated association of AS with age and atherosclerosis (Lindroos et al., 1993; Thanassoulis et al., 2010), Nordstrom and colleagues evaluated the role of postmenopausal hormone variations, such as polymorphisms in the estrogen receptor alpha (*ESR1*), and suggested an association with AS (Nordstrom et al., 2003). In addition, based on the suggested involvement of transforming growth factor beta1 (*TGFB1*) in fibrosis and atherosclerosis, Nordstrom et al. studied its effect in AS (Nordstrom et al., 2003). A mutation in the intron region of the estrogen receptor gene was identified in 41 women and was associated with increased risk of AS in their population. While a genotype resulting from the combination of this polymorphism and two additional *TGFB1* restriction sites was categorized as high-risk, no high-risk *TGFB1* polymorphisms were identified, ruling out a potential association between *TGFB1* and AS.

Based on recent evidence supporting the involvement of inflammatory pathways in the pathogenesis of CAD, Ortlepp and colleagues studied the effect of inflammation on AS (Ortlepp et al., 2004). The authors evaluated 187 patients undergoing surgical aortic valve replacement and surprisingly identified a haplotype made up of IL-10 gene polymorphisms that is associated with elevated IL-10 levels, an antiinflammatory cytokine, to be associated with AS (Ortlepp et al., 2004). This was however not replicated by a subsequent study that identified the alternate proinflammatory haplotype to be associated with AS (Gaudreault et al., 2011).

Given the suggested similarities between bone mineralization and valvular calcification, Ortlepp et al. studied a VDR gene polymorphism (BsmI polymorphism), that has been associated with bone mineral mass (Ortlepp et al., 2001). In their study, they reported that the BsmI B variant was present with a much higher frequency among the 100 patients with AS compared to controls. To investigate a similar hypothesis, Schmitz and colleagues studied *VDR* and the parathyroid hormone gene (*PTR*), however the *VDR* variant was only marginally significant, but an intronic *PTR* mutation was associated with aortic valve stenosis (Schmitz et al., 2009).

It is important to highlight that most of these candidate–gene associations have not been subsequently replicated, making the involvement of these genes in CAVD debatable. Gaudreault and colleagues tried to replicate the associations of several of these candidate genes with AS in 457 patients undergoing aortic valve replacement using more modern approaches and were only able to reproduce the association with two polymorphisms in the *APOB* gene (Gaudreault et al., 2011), which supports the LDL-C hypothesis in CAVD.

Ellis and colleagues were able to identify novel association between AS and *MYO7A* (a myosin variant), *ELN* (elastin), and interestingly *AGTR1* (angiotensin receptor II, type 1) genes by studying 660 single nucleotide polymorphisms (SNPs) in 265 cases of AS (Ellis et al., 2012). Of the genes identified in this study, the association with *AGTR1* is of most interest given the angiotensin may play a role in AS (Helske et al., 2004), as well as the early clinical evidence that has suggested improved survival in patients with AS who receive angiotensin-converting enzyme (ACE) inhibitors (Nadir et al., 2011).

Mutations in the *NOTCH1* gene at chromosome 9q34.3 that acts as a transcriptional regular and have been associated with familial forms of AS (including BAV) have also been linked to the development and progression of CAVD (Garg et al., 2005). As a result, Ducharme and colleagues genotyped *NOTCH1* in 457 patients with severe tricuspid aortic valve stenosis (Ducharme et al., 2013) and were able to identify one of the previously reported *NOTCH1* mutations. In addition, the authors were able to identify 32 other individuals who were heterozygous for two other polymorphisms. An association was initially found between AS and one polymorphism in intron 2, however this was not significant after correction for population stratification. *NOTCH1* represses the activation of *Runx2*, a transcription factor regulating the expression of osteoblast-specific genes that is thought to be upregulated in valvular calcification of animal models (Garg et al., 2005). In addition, *NOTCH1* was found to regulate the osteogenic pathways in murine aortic valves via bone morphogenic protein 2 (*BMP2*) (Nigam and Srivastava, 2009). Thus, there remains significant interest surrounding the potential involvement of the *NOTCH1* gene in CAVD.

Genome-wide association studies and Mendelian randomization

To date, the best evidence surrounding the genetics of aortic valve disease comes from only one genome-wide association study (GWAS) by Thanassoulis and colleagues (Thanassoulis et al., 2013). GWAS is a new technique in use since the early 2000s that was made possible with complete characterization of the human genome sequence. In their study, Thanassoulis et al. used genome-wide genotyping in almost 7000 individuals in three cohorts with computed tomographic (CT) evaluation of aortic valve calcium to identify variants associated with CAVD and subsequently replicated their results in several additional cohorts (Thanassoulis et al., 2013). Only one SNP that is located within intron 25 of the apolipoprotein(a) gene (*LPA*) reached genome-wide significance and was noted to double the odds of CAVD. This SNP was also validated prospectively in additional cohorts. This SNP is thought to lead to higher levels of Lp(a) because of its linkage disequilibrium with fewer Kringle IV- type 2 (KIV-2) repeats, which is an important regulator of *LPA* expression. Using Mendelian randomization, Thanassoulis et al. demonstrated that Lp(a) levels, which are genetically determined by the *LPA* genotype, are strongly associated with AS and increased risk by almost 70% per risk allele; providing robust evidence for a causal relationship between variation at the *LPA* gene and development of AS (Thanassoulis et al., 2013). These results have been replicated by several independent groups (Arsenault et al., 2014; Kamstrup et al., 2014; Cairns et al., 2017) demonstrating that the association between *LPA* variants, circulating Lp(a), and CAVD is robust and pointing to a novel therapeutic target for AS.

Subsequent work by Smith and the extracoronary calcium working group of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium, used Mendelian randomization to investigate the effect of genetic elevations in

plasma lipids on aortic valve calcium and AS (Smith et al., 2014). The authors were able to demonstrate that genetic elevations in plasma LDL-C levels, but not high-density lipoprotein cholesterol (HDL-C) or triglycerides, was significantly associated with the presence of aortic valve calcium and incident AS, confirming prior evidence from observational studies that had suggested a causal association between LDL-C and AS (Smith et al., 2014).

Bicuspid Aortic Valve Disease

Clinical significance

Although Harvey was the first to describe blood circulation in the 16th century, Leonardo Da Vinci is credited as the first person to describe the mechanics of aortic valve and the effect of having a bicuspid versus a tricuspid valve on flow in the 15th century (Dorn, 2013). Later in the 1800s, Sir William Osler reported 18 cases of BAV in more than 800 autopsies (Osler, 1886). Since then, BAV has been identified as the most common congenital cardiac abnormality in the world, with the largest health burden of all congenital heart defects combined (Siu and Silversides, 2010). It affects about 1%–2% of the population, with a higher predominance of 3:1 in males versus females (Tutar et al., 2005; Movahed et al., 2006; Basso et al., 2004). It is the cause of AS in up to 85% of cases in the pediatric population and in about 50% of AS cases in adults (Schoen, 2008; Cripe et al., 2004). BAV is the result of the aortic valve developing with two leaflets rather than its normal trileaflet configuration. In over 60% of the cases, it is the result of congenital fusion of the right and left coronary cusps, identified as type A, with most of the remaining cases being the result of the fusion of the right coronary cusp with the noncoronary cusp, known as type B. The fusion of the left coronary cusp and noncoronary cusp, or type C BAV, is rarely seen (Fernandes et al., 2004; Sabet et al., 1999a; Russo et al., 2008; Schaefer et al., 2007, 2008; Ciotti et al., 2006).

Studies have suggested that patients with a BAV have the same life expectancy as those with a normal trileaflet aortic valve (Tzemos et al., 2008; Michelena et al., 2011). Despite this, more than 35% of those with bicuspid aortic valve develop severe complications that include aortic valve regurgitation, aortic valve stenosis, infective endocarditis, aortic coarctation, ascending aortic aneurysms, and aortic dissection (Michelena et al., 2011; Larson and Edwards, 1984; Roberts et al., 2012; Sabet et al., 1999b; LaHaye et al., 2014; Padang et al., 2012a). Moreover, having BAV increases the risk of premature valvular calcification. BAV has been shown to significantly increase the risk of aortic dissection by as much as eightfold, along with 26% increased risk of aortic aneurysm formation, and 53% risk of valve replacement over 25 years (Michelena et al., 2011). Although BAV can be present as an isolated abnormality, it has also been associated with a number of other diseases including coarctation of the aorta, ventricular septal defect (Duran et al., 1995), Turner syndrome (Hou et al., 1993; Sybert, 1998), Williams–Beuren syndrome (Hallidie-Smith and Karas, 1988; Del Pasqua et al., 2009; De Reubens et al., 2008), Rubinstein–Taybi syndrome (Stevens and Bhakta, 1995), Andersen syndrome (Andelfinger et al., 2002), Williams syndrome (Lopez-Rangel et al., 1992), and others.

Pathogenesis and current evidence

Studies since the 1970s have highlighted the familial clustering of BAV and point to the high likelihood of a genetic basis. In the first family study of BAV published in 1978, Emanuel and colleagues studied the families of 41 patients with surgically proven isolated BAV (Emanuel et al., 1978). They estimated a minimal familial incidence of 14%–32% in first-degree relatives of their patients and provided systemic evidence that BAV is a heritable disorder. Further evidence to support this theory was later obtained by reports of BAV in pairs of identical twins, and also families with multiple members in different generations affected (Godden et al., 1987; Brown et al., 2003; Clementi et al., 1996; Glick and Roberts, 1994). Current evidence suggests that the prevalence of BAV among first-degree relatives of patients with known disease is about 9%, and the prevalence increases to as much as 24% in families with more than one individual affected, and over 35% in families with at least two affected members (Padang et al., 2012a; Huntington et al., 1997). This is based on work by Huntington and colleagues who identified 30 patients with documented BAV on echocardiography and evaluated their relatives using echocardiography (Huntington et al., 1997). Of the 186 first-degree relatives that they evaluated, 17 had BAV. They also identified 11 families out of the initial 30 who had at least one additional member with BAV. In a more recent study of 100 families in a Mediterranean population, the prevalence of BAV in first-degree relatives was noted to be much lower at 4.6%, highlighting that the prevalence of BAV likely differs in different populations or geographic areas (Robledo-Carmona et al., 2013). Lastly, to estimate the heritability of BAV, Cripe et al. evaluated over 300 individuals from families of 50 patients with documented BAV using echocardiography (Cripe et al., 2004). They estimated the heritability of BAV at 89% with a prevalence of 24%. Furthermore, when BAV was present with another cardiac abnormality, they reported the combined prevalence of 31% with heritability of BAV with another malformation at 75%.

Despite knowledge of its existence since the 15th century, its high prevalence, heritability, and the large burden it imposes on healthcare systems around the world, little is known about the pathogenesis of BAV. Familial studies that highlighted the clustering of BAV in families suggest a pattern of autosomal dominant inheritance, but with reduced penetrance, an observation that was confirmed in linkage and candidate gene studies (Garg et al., 2005; Godden et al., 1987; Brown et al., 2003). Multiple genetic mutations including *GATA5* missense mutations in nonsyndromic BAV patients (Foffa et al., 2013; Padang et al., 2012b), *KCNJ2* mutation in those with Anderson syndrome (Andelfinger et al., 2002), *ACTA2* mutation in patients with ascending aortic aneurysm and dissection syndrome (Guo et al., 2007, 2009), fibrillin 1 mutation in Marfan patients (Faivre et al., 2007; Lemaire et al., 2011), elastin mutation in William–Beuren syndrome (Hallidie-Smith and Karas, 1988; Del Pasqua et al., 2009; De Reubens et al., 2008; Pober et al., 2008), and 45XO karyotype in Turner syndrome (Hou et al., 1993; Sybert, 1998; Bondy et al., 2013) are some of the genetic abnormalities that have been associated with BAV (Padang et al., 2012a).

However to date, the strongest genetic evidence comes from a study by Garg and colleagues in which they studied a family of European-American descent spanning five generations with six cases of isolated BAV out of nine cases of congenital aortic valve disease with an autosomal dominant pattern of inheritance (Garg et al., 2005). They used genome-wide linkage analysis and were able to identify a locus on chromosome 9q34-35, a region that includes the *NOTCH1* gene, to be associated with bicuspid aortic valve disease (Garg et al., 2005). Sequencing of the region revealed a mutation that resulted in a premature stop codon in the *NOTCH1* gene of affected family members. In the same study, the authors performed direct sequencing of *NOTCH1* in an unrelated Hispanic family with aortic valve disease and identified a second mutation that segregated with family members suffering from BAV. The authors then used murine models to demonstrate a role for *NOTCH1* gene in embryonic valve development (Garg et al., 2005). *NOTCH1* functions as a transcriptional regulator and is involved in cell differentiation during organogenesis including cardiac development (Artavanis-Tsakonas et al., 1999; De La Pompa and Epstein, 2012; High and Epstein, 2008). The NOTCH family of proteins are transmembrane proteins that once bound to their ligand proteins, become activated to modify gene expression (Artavanis-Tsakonas et al., 1999; De La Pompa and Epstein, 2012; High and Epstein, 2008). The transcripts of the *NOTCH1* gene have been found in the mesenchyme of the outflow tract and the developing aortic valve leaflets, highlighting its importance for aortic valve development (Garg et al., 2005; Krebs et al., 2000; Timmerman et al., 2004). Mutations in the *NOTCH1* gene cause dysregulation in this pathway and have been found in affected family members of those with BAV. Moreover, evidence suggests that *NOTCH1* mutations are not only involved in defective development of the aortic valve, but also can lead to rapid progression of aortic valve calcification discussed in the previous section of this article.

In contrast to CAVD, currently there are no GWAS studies reported for BAV. This is likely due to the low prevalence of BAV in the general population and the difficulty in clearly identifying the presence of a BAV when the valve is calcified and/or thickened, and the diversity of BAV phenotypes observed. Given these difficulties, Wooten and colleagues used a used gene network-based analysis techniques in 68 patients with BAV and identified a concentration of SNPs associated with BAV located at 16p13.3, a region encompassing two genes called *AXIN1* and *PDIA2* (Wooten et al., 2010). *AXIN1* (Axis Inhibitor 1) is a crucial member of signaling pathways involved in regulation of cardiac neural crest development (Niessen and Karsan, 2008) and heart valve formation (Armstrong and Bischoff, 2004). *PDIA2* (protein disulfide isomerase family A member 2), on the other hand, has no known involvement in cardiac valve formation. In the same study, they also identified a second gene, *ENG* (Endoglin), which is involved in neural crest differentiation of cells that inhabit the aorta (Mancini et al., 2007), and more importantly, defects in the *NOTCH* signaling pathway result in downregulation of this gene (Benedito et al., 2008). A summary of candidate gene studies for BAV can be found in **Table 2**.

Rare Forms of Aortic Valve Disease

Although some cases of aortic valve disease follow an autosomal dominant/reduced penetrance mode of inheritance, most cases do not follow a clear pattern. However, there are certain rare Mendelian syndromes that are an exception to this rule. These cases are extremely rare and have very little in common with the cases of AS that are encountered clinically.

Familial hypercholesterolemia

The association of familial hypercholesterolemia (FH) and AS has long been known, but the exact mechanisms are not well defined (Rajamannan et al., 2003; Barr et al., 1954). Phenotypes in FH differ from the typical presentation of AS. For example, FH patients often develop supravalvular, rather than valvular, aortic calcification (Brook et al., 1989).

Aortic insufficiency

Similar to AS, aortic insufficiency (AI) is also a characteristic of many connective tissue diseases including Marfans (Buntinx et al., 1991; Disabella et al., 2006), Loeys-Dietz (Attias et al., 2009), Ehlers-Danlos (Schwarze et al., 2004), and osteogenesis imperfecta (Hortop et al., 1986). Development of AI in these conditions appears to be a secondary consequence of primary aortic pathology.

Alkaptonuria

Alkaptonuria is a rare autosomal recessive disorder that is due to a mutation in the homogentisate 1,2 dioxygenase (*HGD*) gene, resulting in abnormalities of tyrosine catabolism and tissue deposition of homogentisic acid. The tissue deposition of this compound causes black pigmentation of collagenous tissues, in addition to arthritis, urolithiasis, vascular, and aortic valve mineralization that lead to premature AS (Phornphutkul et al., 2002; Hannoush et al., 2012).

Mitral Valve Disease

Mitral Valve Prolapse

Clinical significance

Mitral valve prolapse (MVP), also known as Barlow's disease, is a common valvular abnormality affecting approximately 2.4% of the population based on the most widely accepted definition of this disorder (Freed et al., 1999). There is some evidence for a higher prevalence in women compared to men (Freed et al., 1999). The mitral valve is composed of an anterior and a posterior leaflet. These leaflets are attached to the oval-shaped mitral annulus via their bases with their tips attached to the chordae that join the

Table 2 Summary of candidate gene association studies for bicuspid aortic valve

Mode of Inheritance	*Gene*	*Polymorphism*	*Population*
Familial	Notch1 (*NOTCH1*)	9q34.3 (Garg et al., 2005) R1108X	European-American
		9q34.3 (Garg et al., 2005) H1505del	Hispanic
		9q34.3 P284L (Foffa et al., 2013)	Italian
		9q34.3 (Foffa et al., 2013) Y1619X	Italian
	GATA-binding protein 5 (*GATA5*) (Padang et al., 2012b)	20q13.33 G166S	Predominantly Caucasian
	Transforming growth factor, β receptor II (*TGFBR2*) (Girdauskas et al., 2011)	3p22 V387M	–
Sporadic	Notch1 (*NOTCH1*)	9q34.3 (McBride et al., 2008) G661X	European-American
		9q34.3 (McBride et al., 2008) V2536I	European-American
		9q34.3 (McKellar et al., 2007) A1343V	–
		9q34.3(McKellar et al., 2007) P1390T	–
		9q34.3 (McKellar et al., 2007) R1350L	–
		9q34.3 (McKellar et al., 2007) P1337S	–
		9q34.3(Mohamed et al., 2006) T596M	German
		9q34.3 (Mohamed et al., 2006) P1797H	German
	GATA-binding protein 5 (*GATA5*)	20q13.33 (Padang et al., 2012b) Q3R	Predominantly Caucasian
		20q13.33 (Padang et al., 2012b) S19W	Predominantly Caucasian
		20q13.33 (Padang et al., 2012b) Y142H	Predominantly Caucasian
	SMAD family member 6 (*SMAD6*) (Tan et al. 2012)	15q21-q22 C484F	Caucasian/British
		15q21-q22 P415L	Caucasian/British

Adapted from Mauer, A. C., Owens, D., O'Donnell, C. J., Post, W. S. and Thanassoulis, G. (2015). Genetics of valvular heart disease. In: Shah, S. J. and Arnett, D. K. (eds.) *Cardiovascular genetics and genomics in clinical practice*. New York: Demos Medical.

papillary muscles. The anterior leaflet is described as long and narrow, while the posterior leaflet is short and wide. The posterior leaflet occupies a larger portion of the valvular area and based on Carpentier's description, it is divided into the anterolateral (P1), middle (P2), and postero-medial (P3) scallops. The anterior leaflet is divided into three segments (A1–A3) based on their corresponding posterior leaflet scallop (McCarthy et al., 2010).

The basic definition of MVP is the extension of its leaflet(s) beyond the mitral valve annulus and into the left atrium during ventricular systole when intracardiac pressures are elevated (Guy and Hill, 2012; Hayek et al., 2005; Delling and Vasan, 2014). This could then lead to disruption of the leaflet coaptation and result in mitral regurgitation, often starting in mid-to-late systole. Hence, MVP has been identified as the most common cause of mitral valve regurgitation requiring surgical repair. This valvular abnormality has also been shown to predispose patients to infective endocarditis, congestive heart failure, arrhythmia, and even sudden cardiac death (Freed et al., 1999; Guy and Hill, 2012; Hayek et al., 2005; Delling and Vasan, 2014). When isolated and in the absence of an identifiable connective tissue disease, MVP is classified as primary, which can be sporadic or familial. Secondary MVP is often associated with an identifiable connective tissue disease such Marfan, Ehlers–Danlos, osteogenesis imperfecta, pseudoxanthoma elasticum, Loeys–Dietz, or Williams syndromes (Hayek et al., 2005).

The exact echocardiographic diagnostic criteria for MVP have become more uniform in recent years based on results published from the Framingham Heart Study (Freed et al., 1999). Currently, MVP is diagnosed by abnormal displacement of one or more portions of the mitral valve leaflets ≥ 2 mm above the annular plane into the left atrium during systole, also called billowing, and best identified in a long axis view. This can occur with or without thickening of the leaflets, which is identified as having a thickness

of $\geq$5 mm or not. MVP with leaflet thickness is called classic MVP, where as a valve without thickened leaflets is identified as nonclassic MVP. Moreover, when MVP is present with extracardiac manifestations such as pectus excavatum (Bon Tempo et al., 1975), it is classified as syndromic. In this article, we will focus on familial and sporadic forms of MVP in nonsyndromic individuals. The prevalence of nonsyndromic MVP has been estimated as 2.4% in the general population (Freed et al., 1999).

Pathogenesis and current evidence

Histologically, distinct layers identified as atrialis, spongiosa, fibrosa, and ventricularis form normal mitral valve leaflets. These layers are comprised of fibroblasts, connective tissue fibers, and collagen. The atrialis and ventricularis layers are each covered by a layer of endothelial cells overlying them that is continuous with the luminal surface of the atrium and the ventricle. The diseased valve has been shown to have excessive proteoglycan deposition and expansion of the spongiosa layer (McCarthy et al., 2010). This along with other changes such as overexpression of proteolytic enzymes and diminished collagen fibers, results in pathologic remodeling of the valvular leaflet (Padang et al., 2012a).

The majority of our knowledge regarding the genetics of MVP comes from initial investigations into the various connective tissue disorders associated with MVP (Hayek et al., 2005). One example of such disorders is Marfan syndrome, which is secondary to a mutation in the Fibrillin 1 (*FBN1*) gene, a major component of the extracellular matrix microfibrils and a regulator of transforming growth factor-beta (TGF-β) (Ng et al., 2004). TGF-β has been suggested as a key player in valvular development during the embryonic period and remodeling in adult life seen in MVP (Coombs and Yutzey, 2009). Such mutations, however, have not been identified in isolated nonsyndromic MVP.

The identification of genetic factors in nonsyndromic MVP is the result of findings from familial studies. Evidence suggests that the most common method of transmission in familial primary MVP is autosomal dominant, but X-linked patterns of inheritance have also been described (Levine and Slaugenhaupt, 2007; Grau et al., 2007; Cooper and Abinader, 1981; Weiss et al., 1975; Shappell et al., 1973), with variable age and sex-dependent penetrance (Weiss et al., 1975; Devereux et al., 1982). Prior work estimated the prevalence of MVP in first-degree relatives to as much as 50% (Weiss et al., 1975; Devereux et al., 1982; Strahan et al., 1983). However, most of this work was done prior the adoption of clear and uniform diagnostic criteria and phenotyping. Further evidence supporting a genetic pattern of inheritance comes from twin studies, which suggested that the prevalence of MVP was higher among twins compared to controls (Rizzon et al., 1973; Girard and Girard, 1977). More recently, Delling and colleagues used a community-based sample in Framingham and identified parental MVP to be significantly associated with higher prevalence of MVP in offsprings. In addition, the authors highlighted the importance of nondiagnostic MVP morphologies by suggesting their association with increased prevalence of MVP in the offspring (Delling et al., 2015).

MVP with X-linked inheritance is a rare form of the disease that was first reported in 1960s by Monteleone and colleagues after they identified a family in which only male members in three generations had evidence of MVP (Monteleone and Fagan, 1969). More recent work by Kyndt et al. in a family with cosegregation of mild hemophilia A and MVP phenotype, allowed for mapping to the Xq28 locus (Kyndt et al., 1998). Later work identified a mutation in the filamin A (*FLNA*) gene at this locus to be responsible for this X-linked myxomatous valvular dystrophy (*XMVD*) (Kyndt et al., 1998; Lardeux et al., 2011). The Filamin A protein is involved in localization and activation of Smad proteins, including Smad2, and regulation of TGF-beta (Kyndt et al., 2007).

Family studies have been useful in identifying genes that are involved in familial forms of MVP. However, as stated previously, MVP can also occur sporadically. In nonsyndromic, sporadic MVP, association of disease with SNPs has been evaluated. Given that MVP is often present with connective tissue disease, particular attention has been given to genes involved in extracellular matrix remodeling, collagen metabolism, and the renin–angiotensin–aldosterone system (RAAS). Genome-wide linkage analysis in large families and recent diagnostic criteria for MVP have shed light on 3 potential loci called myxomatous mitral valve prolpase (*MMVP*) loci on chromosomes 16p11.2-p12.1 (*MMVP-1*), 11p15.4 (*MMVP-2*), and 13q31.3-32.1 (*MMVP-3*) (Disse et al., 1999; Freed et al., 2003; Nesta et al., 2005). Disse and colleagues identified the first of these loci in 1999 (Disse et al., 1999). They used four pedigrees of autosomal dominant, heritable MVP, of which, one pedigree involved 24 subjects over three different generations, with linkage to the region on chromosome 16, later called *MMVP-1* on locus 16p11.2-12.1. There were able to replicate this finding in a second pedigree, but not in the other two pedigrees likely due to genetic heterogeneity of MVP. Shortly after, Freed and colleagues identified the second locus (*MMVP-2*) located on chromosome 11p15.4, using a single large pedigree (Freed et al., 2003). In 2005, Nesta et al. used a pedigree of 46 subjects with living members in three generations and identified *MMVP-3* on the long arm of chromosome 13 (Nesta et al., 2005). It is worth highlighting that all three of these studies had a limited ethnic diversity and very small number of pedigrees. In addition, the causative genes on these loci have yet to be identified.

Based on evidence that *MMVP2*, a locus associated with nonsyndrome MVP was located on chromosome 11, Durst and colleagues identified four individuals from a large multigenerational family segregating a nonsyndromic MVP and performed capture sequencing of the linked interval on chromosome 11 (Durst et al., 2015). Their work identified a missense mutation in the *DCHS1* gene that segregated with MVP. Knockdown zebrafish models of this gene developed a cardiac atrioventricular canal defect that was only rescued with the wild-type human *DCHS1*, but not with the familial mutant form of the gene. Durst et al. were also able to identify a second mutation in *DCHS1* that segregates with MVP based on genetic data in two additional families. Both of the identified loss-of-function mutations in *DCHS1* are thought to reduce protein stability during valve morphogenesis (Durst et al., 2015). This work highlighted the potential involvement of *DCHS1* in MVP pathogenesis.

A recent meta-analysis of two independent French genome-wide association studies by Dina and colleagues with 1412 MVP cases and 2439 controls led to identification of few loci, which after replication in 1422 cases and 6779 controls provided genetic and functional evidence for two candidate genes called LIM and cysteine-rich domains 1 (*LMCD1*) on chromosome 3 and tensin 1

Table 3 Summary of candidate gene association studies for mitral valve prolapse (MVP)

Gene	*Polymorphism*	*Population*	*Phenotype*	*Risk variant*	*Odds ratio*	P-*value*
Fibrillin-1 (*FBN1*) (Chou et al., 2003a; Ozdemir et al., 2012)	15q21.1 Exon 15	Taiwan Chinese Han	MVP	TT	2.40 (1.32, 4.39)	0.01
	15q21.1 Exon 27	Taiwan Chinese Han		GG	3.61 (1.01, 12.89)	0.04
	15q21.1 Intron 56	Turkish children		GC	5.04 (2.60, 9.76)	0.0001
Collagen IIIα1 (*COL3A1*) (Chou et al., 2004a)	2q31 Exon 31	Taiwan Chinese Han		GG	7.42 (4.40, 12.52)	0.0001
Transforming Growth Factor-β1-β1 (*TGFB1*) (Chou et al., 2002a)	19q13.1 C509T, T869C	Taiwan Chinese Han		–	–	NS
Urokinase Plasminogen Activator (*PLAU*) (Chou et al., 2004b)	10q22.2 T4065C	Taiwan Chinese Han		TC	6.03 (2.11, 14.83)	0.0001
Dachsous cadherin-related 1 (*DCHS1*) (Durst et al., 2015)	11p15.4 Insertion/deletion	European		–		
LIM and cysteine-rich domains 1 (*LMCD1*) (Dina et al., 2015)	3p13 rs171408	European		G	1.32 (1.22–1.43)	1.3×10^{-11}
Tensin 1 (*TNS1*) (Dina et al., 2015)	2q35 rs12465515	European		–	1.25	3.11×10^{-11}
Matrix Metallo-proteinase (*MMP1*) promoter (Lin et al., 2013)	11q22.3 1607 1G/2G	Taiwanese	MCTR + MVR	1G/1G	3.22	0.009
Angiotensin II receptor, type 1 (*AGTR1*) (Szombathy et al., 2000; Chou et al., 2002b)	3q24 A1166C	White European	MVP syndrome	C allele	4.0 (1.4, 12.1)	–
	3q24 A1166C	Taiwan Chinese Han		CC	–	0.89
Angiotensinogen (*AGT*) (Chou et al., 2002c)	1q42.2 M235T	Taiwan Chinese Han		TT	8.55 (4.51, 16.2)	<0.001
Angiotensin-I converting enzyme (*ACE*) (Chou et al., 2003b)	17q23.3 Insertion/deletion	Taiwan Chinese Han		I/I	2.14 (1.20, 3.80)	–

MCTR, Mitral chordae tendinae rupture; *MVP*, mitral valve prolapse; *MVR*, mitral valve replacement

Mauer, A. C., Owens, D., O'Donnell, C. J., Post, W. S. and Thanassoulis, G. (2015). Genetics of valvular heart disease. In: Shah, S. J. and Arnett, D. K. (eds.) *Cardiovascular genetics and genomics in clinical practice*. New York, NY: Demos Medical.

(*TNS1*) on chromosome 2, both of which are involved in cell proliferation and migration possibly during valve development (Dina et al., 2015). The strongest overall association was observed at 3p13 for rs171408 that maps *LMCD1* (OR 1.32, $P = 1.29 \times 10e{-}11$). *LMCD1*, also known as dyxin, encodes a protein that belongs to the LIM-domain family of zinc-finger proteins and is involved in coregulation of transcription. This gene is highly expressed in mouse cardiac tissue and is a direct repressor of *GATA6*, an important regulator of cardiac development. In knockdown models of *LMCD1* in zebrafish, a significant increase in the incidence of atrioventricular valve regurgitation was noted (Rath et al., 2005). Another strong association was noted to be at 2q35 for rs12465515 that lies within the region for *TNS1* (OR 1.25, $P = 3.1 \times 10e{-}11$), a second candidate gene encoding a focal adhesion protein called tensin 1 involved in cytoskeleton organization. Similar to filamin A, tensin 1 interacts with actin and its variants can cause a rare X-linked form of MVP (Kyndt et al., 2007). The important work by Dina et al. also highlighted few other susceptibility loci including *IGFBP5*, *SMG6*, *PITPNB*, *PCNX*, *SIPA1L1*, *SETD4*, *CBR1*, and *MN1* in addition to the already discussed *LMCD1* and *TNS1* (Dina et al., 2015). A summary of these studies and other candidate gene association involving MVP can be found in **Table 3**.

Mitral Annular Calcification

Clinical significance

Mitral annular calcification (MAC) is defined by calcium deposition along the mitral annulus, valvular, and/or subvalvular structures. It is routinely identified by cardiac imaging among the elderly and is suggested to be independently associated with cardiovascular events (Fox et al., 2003, 2004; Barasch et al., 2006a). It has also been suggested to precede the development of clinically significant mitral valve regurgitation and stenosis (Labovitz et al., 1985; Movahed et al., 2007; Korn et al., 1962). Prevalence of MAC varies based on the modality used and ranges between 3% and 42%, and increases significantly with age (Fox et al., 2004; Savage et al., 1983; Barasch et al., 2006b). There are a number of clinical factors that have been associated with the presence and severity of MAC, including traditional cardiovascular risk factors (Thanassoulis et al., 2010; Abramowitz et al., 2015; Kanjanauthai et al., 2010; Elmariah et al., 2013), end-stage renal disease (Maher et al., 1987; Asselbergs et al., 2009), and chronic inflammatory conditions (Molad et al., 2006; Sherif, 2009).

Pathogenesis and current evidence

The development and progression of MAC is now recognized as a tightly regulated process involving complex biological pathways that cause progressive calcification. There is a shortage of studies investigating the heritability of MAC given that it has been presumed to be an age-related, degenerative disease. One of the best evidence for its heritability comes from the HyperGEN study, involving a total of 78 sibships in 68 families with at least 2 affected siblings, which suggested that despite the contribution of environmental factors to MAC, there likely is a significant genetic influence involved in the process (Bella et al., 2007). In another study, Novaro and colleagues evaluated the association between *APOE* alleles and MAC in over 800 participants, but were unable to identify any significant difference in genotype distributions between the cases and controls (Novaro et al., 2003).

Thanassoulis et al. used data from the CHARGE Consortium collaboration to better evaluate the associations of MAC (Thanassoulis et al., 2013). The authors identified MAC using CT scans among subjects of White European descent within the Multi-Ethnic Study of Atherosclerosis ($n = 2527$, 12%) and the Framingham Heart Study ($n = 1298$, 20%), and suggested an association between MAC and genetic variants near the *IL1F9* gene, which was also replicated among Hispanic Americans (Thanassoulis et al., 2013). Recently, the same group used Mendelian randomization to demonstrate that a genetically elevated triglyceride plasma level is strongly associated with the presence of MAC (Afshar et al., 2017). MAC was significantly associated with the triglyceride genetic risk score (GRS), a measure of the genetic predisposition to elevations in plasma triglyceride levels. Moreover, they did not find such an association between MAC and HDL-C or LDL-C. This work highlights the importance of genetic predisposition to elevated triglyceride level, and its association with the presence of MAC, suggesting a causal association between triglycerides and mitral valve disease (Afshar et al., 2017).

Conclusions and Future Directions

Valvular heart disease is a common cardiovascular condition and its prevalence appears to increase with age. Until recently, the genetic etiology of these disorders was poorly understood. However, recent advances have pointed to a variety of altered molecular and genetic mutations as a common pathway for familial and sporadic forms of valvular heart disease. The use of cardiac CT which provides excellent resolution of valve calcium and likely represents a "deep-phenotype", has been particularly useful in identifying new genomic insights for aortic valve and MAC. Using new imaging modalities, beyond echocardiography and CT, such as positron emission tomography (PET) may provide an opportunity to better evaluate valvular inflammation and osteoblast activity (Marincheva-Savecheva et al., 2011; Dweck et al., 2012), relevant biologic phenotypes that may be more amenable to genetic dissection. In addition, due to the relative rarity of valve disease in the community, the increasing availability of large cohorts derived from hospital-based electronic health records (where valve disease is enriched) as well as very large population cohorts with linkage to administrative data will provide the requisite sample size to identify new variants for valvular heart disease.

Finally, the discovery of *LPA* variants in AS has provided a highly robust pharmacological target to prevent disease progression and randomized trials for Lp(a) lowering are highly anticipated. The availability of larger sample sizes and new technologies for genetic analysis (including whole genome sequencing) as well as emerging integrative –omics approaches, will provide a deeper understanding of the molecular pathways involved in the genesis of valvular heart disease and will create unique opportunities for the development of novel pharmacotherapies to reduce the burden of valvular heart disease.

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Genetic Disorders of the Vasculature

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Introduction

The vascular system, which develops from a complex process of vasculogenesis and angiogenesis (Arboleda-Velasquez and D'Amore, 2014), represents an intricate network that serves a crucial role in the maintenance of life: the delivery of nutrients to

and the removal of waste products from our tissues (Sumpio and Chin, 2014). However, it has become abundantly clear that the vasculature is not merely a static conduit for blood flow but a dynamic system that responds to both physiologic and pathologic phenomena (Sumpio and Chin, 2014).

This dynamic system can be disrupted by genetic disorders that alter the different components of the vessel wall. While the relative size and the composition of the different layers of blood vessels may vary between arteries and veins, and among the different types of arteries (elastic and muscular), the basic organizational framework remains the same. This includes the tunica intima (composed of endothelial cells supported by an internal elastic lamellae), the tunica media (composed of smooth muscle cells (SMCs) and connective tissue), and the tunica adventitia (composed of connective tissue, vasa vasorum that supply nutrients to the media, and nerves) (Sumpio and Chin, 2014).

By altering endothelial cells, SMCs, and/or the extracellular matrix (ECM) of the vessel wall, the primary manifestations that result from underlying genetic disorders include arterial aneurysms and dissections, vascular malformations, and other vasculopathies that mainly lead to vessel rupture and/or downstream tissue ischemia. This article will focus on our current understanding of the genetics of arterial aneurysms and dissections, with a particular focus on thoracic aortic aneurysm and dissection (TAAD), abdominal aortic aneurysms (AAAs), and intracranial aneurysms (IAs), as well as the genetics of inherited vascular malformations and other inherited vasculopathies.

Genetics of Aneurysmal Disease

An aneurysm is defined as a focal pathologic dilatation of an artery to greater than 1.5 times its normal diameter (Norman and Powell, 2010). While TAAD, AAAs, and IAs may have differing underlying pathophysiology and risk factors (**Table 1**), the overall pathologic process that occurs with degenerative aneurysms, namely increased proteolysis, inflammation, and loss of arterial wall

Table 1 Epidemiologic characteristics and pathophysiology of TAAs, AAAs, and IAs

Characteristic	*TAAs*	*AAAs*	*IAs*
Mode of inheritance	20% of cases exhibit Mendelian inheritance (primarily autosomal dominant) (Biddinger et al., 1997; Coady et al., 1999)	Genetic heterogeneity (Kuivaniemi et al., 2015a; Ruigrok et al., 2008)	Genetic heterogeneity (Ruigrok et al., 2008)
Incidence/ prevalence	Incidence: 10.4/100,000 person-years (Ramanath et al., 2009)	Prevalence: 1.3%–8.9% in men, 1.0%–2.2% in women (Sakalihasan et al., 2005)	Prevalence: 2%–5% (Nasr and Brown, 2016; Etminan and Rinkel, 2017; Vlak et al., 2011)
% of individuals with a positive family history	20% (Biddinger et al., 1997; Coady et al., 1999)	Average = 12% (higher among brothers) (Kuivaniemi et al., 2015a)	9% (Ronkainen et al., 1997, 1998) (range from 7% to –20% (Thompson et al., 2015))
Major risk factors	HTN, BAV, FH/inherited mutation in TAAD-causing gene (Milewicz et al., 2016)	Smoking, FH/certain hereditary diseases, older age, male sex (Kuivaniemi et al., 2015a)	Smoking, HTN, female sex, FH/certain hereditary diseases, older age, multiple aneurysms (Etminan and Rinkel, 2017)
Male/female ratio	1.6:1 (Balistreri, 2015)	4–6:1 (Kent, 2014; Saratzis and Bown, 2014)	1:3 (Nasr and Brown, 2016)
Typical age of onset	>60 years (average = 69 years (Ramanath et al., 2009))	>50 for men, >60–70 for women (Kent, 2014) (average = 75 years (Balistreri, 2015)	40–60 years (Etminan and Rinkel, 2017)
Complication rates	Annual risk for rupture/dissection/death = 4.7%, 7.3%, and 12.1% for ascending aortas of 5, 6, and 7 cm, respectively (Elefteriades et al., 2015)	Annual rupture risk ≤1% for AAAs <5.5 cm and 32.5% for AAAs > 7.0 cm (Kent, 2014)	Overall rupture risk = 0.5–1.4%/year (Nasr and Brown, 2016)
Size for intervention	Standard: 5.0–5.5 cm ascending aorta (Hiratzka et al., 2010)	5.5 cm infrarenal aorta (Ahmed et al., 2016)	No definitive size; PHASES score estimates 5-year rupture risk (increased risk >0.7 cm (Nasr and Brown, 2016; Greving et al., 2014))
Typical shape	Fusiform (van 't Hof et al., 2016)	Fusiform (van 't Hof et al., 2016)	Saccular (van 't Hof et al., 2016)
Location	60% root/ascending, 10% arch, 40% descending, 10% thoracoabdominal (some involve >1 segment) (Isselbacher, 2005)	Primarily infrarenal (Kuivaniemi et al., 2015b)	90% anterior circulation (30%–35% ACA, 30% ICA + branches, 20% MCA), 10% posterior circulation (Fennell et al., 2016)
Pathophysiology/ histology	Cystic medial degeneration due to dysregulated TGF-β signaling and disruption of elastin-contractile unit, inflammation, cytokine activity (Karimi and Milewicz, 2016; Andelfinger et al., 2016; Elefteriades et al., 2011)	Inflammation, autoimmunity, ECM degradation, oxidative stress, VSMC apoptosis (Kuivaniemi et al., 2015b; Kent, 2014)	Degeneration of internal elastic lamina, disorganization of muscular media, intimal hyperplasia, irregularity of luminal surface, inflammation, hemodynamic stress (Etminan and Rinkel, 2017; Fennell et al., 2016)

AAA indicates abdominal aortic aneurysm; ACA, anterior communicating artery; BAV, bicuspid aortic valve; ECM, extracellular matrix; FH, family history; HTN, hypertension; IA, intracranial aneurysm; ICA, internal carotid artery; MCA, middle cerebral artery; TAA, thoracic aortic aneurysm; TAAD, thoracic aortic aneurysm and dissection.

matrix, has been observed with all three types of aneurysms (Norman and Powell, 2010). Whereas the most devastating complication associated with AAAs and IAs is rupture, an equally important complication with thoracic aortic aneurysms (TAAs) is aortic dissection, which primarily determines life expectancy in TAA-affected individuals (Gillis et al., 2013).

Since the original description of arterial aneurysms in the writings of ancient Egyptians in 2000 BC (Karimi and Milewicz, 2016), the familial nature of TAAD (Biddinger et al., 1997; Coady et al., 1999; Albornoz et al., 2006), AAAs (Clifton, 1977; Kuivaniemi et al., 2015a), and IAs (Ullrich, 1960; Nahed et al., 2007; Tromp et al., 2014) has now become well established. However, the familial clustering of aneurysms does not necessarily indicate that the observed phenotype is due to an underlying genetic etiology. For instance, Korja and Kaprio (Korja and Kaprio, 2016) argue that the observed clustering of IAs and subarachnoid hemorrhage (SAH) in families may be related to the familial aggregation of risk factors, particularly smoking and hypertension.

Nevertheless, research over the past two decades has led to an explosion in our understanding of the genetics of aneurysms. In particular, it has become clear that syndromic and familial TAAD have a strong monogenic component (Luyckx and Loeys, 2015; Milewicz et al., 2016), while AAAs and IAs are considered complex diseases with a genetic contribution (Kuivaniemi et al., 2015a; Nahed et al., 2007). Thus, while familial TAAD is primarily inherited via an autosomal dominant mechanism with reduced penetrance and variable expressivity (Albornoz et al., 2006; Milewicz et al., 2014), the inheritance pattern of AAAs and IAs is unclear, aside from the syndromic conditions that can manifest with AAAs or IAs or the familial cases of co-occurring aortic aneurysms and IAs. In fact, studies have described the phenotypes of individuals with likely autosomal dominant inherited TAAs with saccular IAs (Regalado et al., 2011a), as well as AAAs with saccular IAs (Kim et al., 2005). Furthermore, other studies have demonstrated the co-occurrence of TAAs and AAAs (Larsson et al., 2011; Svensjo et al., 1996), as well as AAAs with IAs (Miyazawa et al., 2007; Norrgard et al., 1987). A recent study of 1081 individuals with aortic aneurysms found that 11.8% had an associated IA with no significant difference in the rates of IAs observed between those with AAAs and TAAs (Rouchaud et al., 2016). These results provide evidence for a possible genetic overlap between these aneurysm conditions, which is further supported by a genome-wide association study (GWAS) mega-analysis that identified 4 chromosomal loci (9p21, 18q11, 15q21, and 2q33) significantly associated with all three aneurysm types (van 't Hof et al., 2016). Additionally, one genome-wide linkage analysis of 26 IA and TAA or AAA families found linkage peaks at chromosomal loci 6p23 and 11q24 (Worrall et al., 2009). A review of all whole genome linkage studies identified five chromosomal regions that are associated with at least two of the types of aneurysms, with the 11q24 region being associated with all three (Ruigrok et al., 2008). The differences in identified chromosomal loci between GWAS and DNA linkage studies can possibly be explained by the fact that there may only be a genetic overlap between all three aneurysm types in familial as opposed to sporadic cases (van 't Hof et al., 2016).

Furthermore, since the ascending aorta and intracranial arteries are both derived from neural crest cells (Weston and Thiery, 2015), several studies have evaluated aortic root diameters in individuals with IAs. Shin et al. found that aortic root dimensions were larger among individuals younger than 55 with at least one ruptured IA, an IA $\geq$7 mm in size, or multiple IAs than among those not meeting these criteria (Shin et al., 2014). Additionally, Can et al. found that individuals with ruptured fusiform IAs had larger aortic root diameters than those with ruptured saccular IAs (Can et al., 2015). Another study discovered that anterior circulation IAs were more common among patients with ascending aortic aneurysms, whereas internal carotid IAs were more common among patients with infrarenal AAAs (Shin et al., 2015). Perhaps ascending aortic aneurysms and anterior circulation IAs are linked genetically, which may partially explain the increased role that genetic risk factors play in the development of middle cerebral artery aneurysms (an anterior circulation IA) as compared to IAs at other sites (van 't Hof et al., 2014). While these results are intriguing, as Sattur et al. argue, it is premature to state that the observed link between IAs and aortic root diameters reflects an underlying genetic or developmental disease with diffuse vascular effects, as risk factors, such as hypertension, may confound the association (Sattur et al., 2016).

Genetics of TAAD

TAAs are typically asymptomatic yet potentially fatal, as their natural history is to progressively expand until rupture or dissection occurs. It is estimated that 10.4 per 100,000 people develop TAAs each year (Ramanath et al., 2009) and that the annual incidence of aortic dissection and rupture are each 3.5 per 100,000 patients (Clouse et al., 2004). However, this may underestimate the true burden of aortic disease, as patients with aortic dissection may be misdiagnosed up to 30% of the time (Ramanath et al., 2009). Treatment is targeted at preventing the complications of dissection or rupture via prophylactic surgery (Chau and Elefteriades, 2013). However, almost 60% of patients with type A dissections have aortic diameters $<$5.5 cm, the commonly used cutoff for surgical intervention, and 40% of patients have diameters $<$5 cm (Pape et al., 2007). Although size is a strong predictor of the complications of TAAs (Elefteriades et al., 2015), it is imperative that clinicians possess a better means of risk stratifying affected individuals to hopefully prevent these devastating complications. As this section of the article demonstrates, genetics plays a critical role in determining who is at risk of aortic dissection at aortic diameters $<$5.0 cm (Milewicz and Regalado, 2015).

Since the discovery of Marfan syndrome (MFS) in 1896 and the recognition that aortic disease is a chief characteristic of the disease in 1912 (Andelfinger et al., 2016), more than 30 heritable conditions have been described that can manifest with aortic or arterial aneurysms (Bradley et al., 2016a). However, the familial nature of non-syndromic TAAD was not elucidated until the late 1990s, when studies demonstrated that 21% of TAAD patients had an affected family member (Biddinger et al., 1997; Coady et al., 1999). This percentage most likely represents a marked underestimate though, since routine imaging of family members of affected patients would probably yield a higher percentage (Elefteriades and Farkas, 2010). Further studies determined that the primary

mode of inheritance of familial TAAD is autosomal dominant with reduced penetrance and variable expressivity (Albornoz et al., 2006; Milewicz et al., 1998), indicating that a single gene is most likely responsible for the disease (Milewicz et al., 2014).

TAAD can currently be classified into two categories: syndromic (associated with abnormalities of other organ systems) and non-syndromic (manifestations restricted to the aorta) (Pomianowski and Elefteriades, 2013; El-Hamamsy and Yacoub, 2009). Individuals with syndromic TAAD suffer from MFS, Ehlers–Danlos syndrome (EDS), Loeys–Dietz syndrome (LDS), arterial tortuosity syndrome (ATS), and Cutis laxa, among others (Bradley et al., 2016a; Pomianowski and Elefteriades, 2013; El-Hamamsy and Yacoub, 2009). These individuals tend to have abnormalities of the integumental, musculoskeletal, ocular, and/or craniofacial systems in addition to aortic and other cardiovascular disease (Isselbacher et al., 2016a). On the contrary, non-syndromic TAAD encompasses those with familial TAAD (more than one family member affected) and sporadic TAAD (no affected family members) (Pomianowski and Elefteriades, 2013). Albornoz et al. demonstrated that familial TAAD appears to be a more virulent disease than sporadic TAAD, as familial TAAD patients present at an earlier age (58.2 vs. 65.7) and have aneurysms that grow at faster rates (0.21 cm/year vs. 0.16 cm/year) (Albornoz et al., 2006). However, sporadic TAAD is estimated to account for 80% of all TAAD cases (Luyckx and Loeys, 2015). In a study of 760 individuals with either MFS, non-syndromic TAA (age <60 with cystic medial degeneration demonstrated on pathology or a positive family history), or bicuspid aortic valve (BAV), Sherrah et al. found that the calculated 10-year mortality rates for MFS (8.7%) and non-syndromic TAA (7.8%) were similar but significantly worse than for BAV patients (3.5%) (Sherrah et al., 2016). Sherrah et al. also demonstrated that aortic dissection is often the initial presentation for non-syndromic TAA and that the type of aortopathy, a family history of dissection, and age at presentation can be used to risk stratify patients (Sherrah et al., 2016).

Despite the categories presented above, there is significant overlap between syndromic and nonsyndromic familial TAAD, since the same gene can cause both forms of the disease (Bradley et al., 2016a). Furthermore, there is substantial variability in many of the syndromic causes of TAAD, which can lead to systemic features in certain family members that are absent in others (Bradley et al., 2016a). It is possible that unidentified genetic modifiers contribute to the wide variability in clinical presentation among individuals with the same mutation (Luyckx and Loeys, 2015).

There are at least 29 genes that have been associated with TAAD (**Table 2**), with the majority of them encoding proteins involved in the ECM, SMC contraction or metabolism, or the TGF-β signaling pathways. These identified genes will be presented according to this categorization, along with clinical implications specific for each gene (**Table 3** and **Fig. 1**).

ECM Genes

The hallmark pathologic change in the aortas of TAAD patients is medial degeneration, otherwise known as cystic medial necrosis, which is characterized by SMC loss, fragmentation and depletion of elastic fibers, and accumulation of proteoglycans and glycosaminoglycans within cell depleted areas of the aortic media (Wu et al., 2013). However, medial degeneration is not the root cause of an aortic dissection but is rather a pathologic consequence of a primary process (Gillis et al., 2013; Yuan and Jing, 2011).

Since the ECM of the aortic wall is a dynamic environment, mutations that alter its structure are predicted to disrupt the molecular pathways that govern aortic wall homeostasis (Wu et al., 2013).

The aortic media is composed of greater than 50 alternating layers of elastic lamellae and SMCs (Karimi and Milewicz, 2016). Elastin fibers are composed of elastin surrounded by microfibrils and associated proteins. These microfibrils attach to the surface of SMCs via focal adhesions, the same sites where components of the contractile unit within the SMC attach (Karimi and Milewicz, 2016). Disruption of this elastin-contractile unit, which is the structural and functional unit of the aortic media, is believed to underlie TAAD (Karimi and Milewicz, 2016).

FBN1

FBN1, the causative gene of MFS (OMIM 154700), encodes fibrillin-1, an important component of microfibrils (Karimi and Milewicz, 2016). MFS, which has an incidence of 2–3 cases per 10,000 individuals (Judge and Dietz, 2005), is a multisystem disease characterized by changes to the skeletal system (disproportionate overgrowth, joint laxity, vertebral column deformity), the ocular system (ectopia lentis), and the cardiovascular system (TAAD, mitral/aortic valve disease) (Gillis et al., 2013). The diagnosis of MFS is based on the revised Ghent nosology, which utilizes clinical criteria, family history, and genetic testing of the *FBN1* gene (Loeys et al., 2010) (**Table 4**). The primary cardiovascular manifestation of MFS is aneurysm of the aortic root (annuloaortic ectasia), which can extend to the ascending aorta (Isselbacher et al., 2016a). This places patients at increased risk for type A aortic dissection, as the two most important risk factors for dissection are aortic size and a family history of dissection (Judge and Dietz, 2005). Furthermore, MFS patients are also at increased risk for type B dissections even among those with prophylactic ascending aortic surgery and a normal or minimally dilated descending aorta ($\geq$2.7 cm) (den Hartog et al., 2015; Weinsaft et al., 2016) and for recurrent aortic dissections, which are more commonly type B, especially in MFS patients (Isselbacher et al., 2016b). Generally, aortic dissection or the requirement for prophylactic aortic root surgery does not occur until the third or fourth decade of life, although they can occur earlier (Bradley et al., 2016a).

MFS is primarily caused by heterozygous mutations in the *FBN1* gene, and to this date, approximately 1850 mutations have been identified within this gene (Collod-Beroud et al., 2003). While the majority of *FBN1* mutations are associated with MFS, Regalado et al. found that 3% of familial TAAD patients manifest mutations in the *FBN1* gene (Regalado et al., 2016). It is possible that wild

Table 2 Genes associated with syndromic and non-syndromic thoracic aortic aneurysm and dissection

Gene	*Location*	*Protein*	*Syndromic TAAD*	*Non-syndromic FTAAD*	*Associated disease/syndrome*	*Inheritance*	*OMIM No. (Phenotype)*
ACTA2	10q23.31	Smooth muscle α-actin	+	+	AAT6, mulstisystemic smooth muscle dysfunction, MYMY5	AD	611788 613834 614042
BGN	Xq28	Biglycan	+	−	Meester-Loeys syndrome	X-linked	300989
COL1A2	7q21.3	Collagen 1 α2 chain	+	−	Ehlers–Danlos syndrome, arthrochalasia type (VIIb) and cardiac valvular type	AD, AR	130060 225320
COL3A1	2q23.2	Collagen 3 α1 chain	+	−	Ehlers–Danlos syndrome, vascular type (IV)	AD	130050
COL5A1	9q34.3	Collagen 5 α1 chain	+	−	Ehlers–Danlos syndrome, classic type (I)	AD	130000
COL5A2	2q23.2	Collagen 5 α2 chain	+	−	Ehlers–Danlos syndrome, classic type (II)	AD	130000
EFEMP2	11q13.1	Fibulin-4	+	−	Cutis laxa, AR type Ib	AR	614437
ELN	7q11.23	Elastin	+	−	Cutis laxa, AD	AD	123700
EMILIN1	2p23.3	Elastin microfibril interfacer 1	+	−	Unidentified CTD	AD	Unassigned
FBN1	15q21.1	Fibrillin-1	+	+	Marfan syndrome	AD	154700
FBN2	5q23.3	Fibrillin-2	+	−	Contractural arachnodactyly	AD	121050
FLNA	Xq28	Filamin A	+	−	Periventricular Heterotopia	XLD	300049
FOXE3	1p33	Forkhead box 3	−	+	AAT11	AD	617349
LOX	5q23.1	Lysyl oxidase	−	+	AAT10	AD	617168
MAT2A	2p11.2	Methionine adenosyltransferase II alpha	−	+	FTAA	AD	Unassigned
MFAP5	12p13.31	Microfibril-associated glycoprotein 2	−	+	AAT9	AD	616166
MYH11	16p13.11	Smooth muscle myosin heavy chain	−	+	AAT4	AD	132900
MYLK	3q21.1	Myosin light chain kinase	−	+	AAT7	AD	613780
NOTCH1	9q34.3	NOTCH1	−	+	AOVD1	AD	109730
PRKG1	10q11.2-q21.1	Type 1 cGMP-dependent protein kinase	−	−	AAT8	AD	615436
SKI	1p36.33-p36.32	Sloan Kettering proto-oncoprotein	+	−	Shprintzen-Goldberg syndrome	AD	182212
SLC2A10	20q13.12	Glucose transporter 10	+	−	Arterial tortuosity syndrome	AR	208050
SMAD2	18q21.1	SMAD2	+	−	Unidentified CTD with arterial aneurysms/dissections	AD	Unassigned
SMAD3	15q22.33	SMAD3	+	+	Loeys–Dietz syndrome type 3	AD	613795
SMAD4	18q21.2	SMAD4	+	−	JP/HHT syndrome	AD	175050
TGFB2	1q41	TGF-β2	+	+	Loeys–Dietz syndrome type 4	AD	614816
TGFB3	14q24.3	TGF-β3	+	−	Loeys–Dietz syndrome type 5	AD	615582
TGFBR1	9q22.33	TGF-β receptor type 1	+	+	Loeys–Dietz syndrome type 1, AAT5	AD	609192
TGFBR2	3p24.1	TGF-β receptor type 2	+	+	Loeys–Dietz syndrome type 2, AAT3	AD	610168

A "+" symbol in the syndromic TAAD column indicates that the gene is causative of syndromic TAAD (same for the non-syndromic TAAD column). A "−" symbol in the syndromic TAAD column indicates that the gene is not known to cause syndromic TAAD (same for the non-syndromic TAAD column).

AAT indicates aortic aneurysm, familial thoracic; AD, autosomal dominant; AOVD, aortic valve disease; AR, autosomal recessive; BAV, bicuspid aortic valve; CTD, connective tissue disease; FTAA, familial thoracic aortic aneurysm; FTAAD, familial thoracic aortic aneurysm and dissection; HHT, hereditary hemorrhagic telangiectasia; JP, juvenile polyposis; MYMY, moyamoya disease; OMIM, Online Mendelian Inheritance in Man; TGF, transforming growth factor; TAAD, thoracic aortic aneurysm and dissection; TGFBR, TGF-β receptor; XLD, X-linked dominant.

type *FBN1* expression levels contribute to the variability in intrafamilial clinical presentation (Aubart et al., 2015; Hutchinson et al., 2003; Verstraeten et al., 2016). The majority of mutations causing MFS are missense, though frameshift mutations, nonsense mutations, splicing errors, and complete deletion of the FBN1 gene have also been shown to lead to the disease (Karimi and Milewicz, 2016). Whereas MFS typically follows an autosomal dominant pattern of inheritance with high penetrance and variable inter/intrafamilial expressivity (Pomianowski and Elefteriades, 2013), 25% of cases arise de novo and rare cases of homozygous recessive or compound heterozygous mutations have been reported (Verstraeten et al., 2016; Hogue et al., 2013; Hilhorst-Hofstee et al., 2010; Van Dijk et al., 2009; de Vries et al., 2007; Karttunen et al., 1994). While initial reports indicated that homozygous and compound heterozygous individuals exhibited more severe phenotypes than their heterozygous relatives (Verstraeten et al., 2016), a recent study of four homozygotes and five compound heterozygotes found that their phenotypes were not more severe than those with only one mutated allele (Arnaud et al., 2017). It is thought that mutations in *FBN1* act via haploinsufficiency (by reducing the

Table 3 Associated vascular characteristics and size criteria for elective surgical intervention for each gene associated with thoracic aortic aneurysm and dissection

Gene	*Associated disease/syndrome*	*Associated clinical characteristics of the vasculature*	*Ascending aorta size (cm) for surgical intervention*
ACTA2	AAT6, mulstisystemic smooth muscle dysfunction, MYMY5	TAAD, early aortic dissection*, CAD, stroke (Moyamoya disease), PDA, pulmonary artery dilation, BAV (Milewicz et al., 2016; Bradley et al., 2016a)	4.5–5.0[a] (Andelfinger et al., 2016; Guo et al., 2007; Disabella et al., 2011)
BGN	Meester-Loeys syndrome	ARD, TAAD, pulmonary artery aneurysm, IA, arterial tortuosity (Meester et al., 2017)	Standard
COL1A2	Ehlers–Danlos syndrome, arthrochalasia type (VIIb) and cardiac valvular type	Borderline aortic root enlargement (Bradley et al., 2016a; Schwarze et al., 2004)	Standard
COL3A1	Ehlers–Danlos syndrome, vascular type (IV)	TAAD, early aortic dissection*, visceral arterial dissection, vessel fragility, IA (Germain, 2007; De Paepe and Malfait, 2012; Erbel et al., 2014)	5.0[b] (Erbel et al., 2014)
COL5A1	Ehlers–Danlos syndrome, classic type (I)	ARD, rupture/dissection of medium-sized arteries (Wenstrup et al., 2002; Mehta et al., 2012; Monroe et al., 2015)	Standard
COL5A2	Ehlers–Danlos syndrome, classic type (II)	ARD	Standard
EFEMP2	Cutis laxa, AR type Ib	Ascending aortic aneurysms, other arterial aneurysms, arterial tortuosity and stenosis	Standard
ELN	Cutis laxa, AD	ARD, ascending aortic aneurysm and dissection, BAV, IA possibly associated with SVAS (Szabo et al., 2006; Callewaert et al., 2011; Jelsig et al., 2017)	Standard
EMILIN1	Unidentified CTD	Ascending and descending aortic aneurysms (Capuano et al., 2016)	Standard
FBN1	Marfan syndrome	ARD, TAAD, AAA, other arterial aneurysms, pulmonary artery dilatation, arterial tortuosity (Morris et al., 2011)	5.0 (Andelfinger et al., 2016; Hiratzka et al., 2010)
FBN2	Contractural arachnodactyly	Rare ARD and aortic dissection (Takeda et al., 2015), BAV, PDA	Standard
FLNA	Pervientricular heterotopia	Aortic dilatation/aneurysms, peripheral arterial dilatation (Reinstein et al., 2013), PDA, IA (Lange et al., 2015), BAV	Standard
FOXE3	AAT11	TAAD (primarily type A dissection) (Kuang et al., 2016)	Standard
LOX	AAT10	TAAD, AAA, hepatic artery aneurysm, BAV, CAD	Standard
MAT2A	FTAA	Thoracic aortic aneurysms (Guo et al., 2015)	Standard
MFAP5	AAT9	ARD, TAAD	Standard
MYH11	AAT4	TAAD, early aortic dissection*, PDA, CAD, peripheral vascular occlusive disease, carotid IA	4.5–5.0 (Andelfinger et al., 2016; Pannu et al., 2007)
MYLK	AAT7	TAAD, early aortic dissection*	4.5–5.0[a] (Andelfinger et al., 2016; Wang et al., 2010)
NOTCH1	AOVD1	BAV/TAAD (McKellar et al., 2007; Proost et al., 2015)	Standard
PRKG1	AAT8	TAAD, early aortic dissection*, AAA, coronary artery aneurysm/dissection, aortic tortuosity, small vessel CVD	4.5–5.0 (Guo et al., 2013)
SKI	Shprintzen-Goldberg syndrome	ARD, arterial tortuosity, pulmonary artery dilation, other (splenic) arterial aneurysms (Doyle et al., 2012)	Standard
SLC2A10	Arterial tortuosity syndrome	ARD (Callewaert et al., 2008), ascending aortic aneurysms (Callewaert et al., 2008), other arterial aneurysms, arterial tortuosity, elongated arteries aortic/pulmonary artery stenosis	Standard
SMAD2	Unidentified CTD with arterial aneurysms/dissections	ARD, ascending aortic aneurysms, vertebral/carotid aneurysms and dissections (Micha et al., 2015)	Standard
SMAD3	Loeys–Dietz syndrome type 3	ARD, TAAD, early aortic dissection*, AAA, arterial tortuosity, other arterial aneurysms/dissections, IA, BAV (van de Laar et al., 2012; van der Linde et al., 2012)	4.0–4.2 (Andelfinger et al., 2016; Hiratzka et al., 2010)
SMAD4	JP/HHT syndrome	ARD, TAAD, AVMs, IA (Wain et al., 2014; Heald et al., 2015)	Standard
TGFB2	Loeys–Dietz syndrome type 4	ARD, TAAD, arterial tortuosity, other arterial aneurysms, BAV (Lindsay et al., 2012; Boileau et al., 2012)	4.5–5.0[c] (Renard et al., 2013b)
TGFB3	Loeys–Dietz syndrome type 5	ARD, TAAD, AAA/dissection, other arterial aneurysms, IA/dissection (Bertoli-Avella et al., 2015)	Standard

Table 3 (Continued)

Gene	*Associated disease/syndrome*	*Associated clinical characteristics of the vasculature*	*Ascending aorta size (cm) for surgical intervention*
TGFBR1	Loeys–Dietz syndrome type 1, AAT5	TAAD, early aortic dissection*, AAA, arterial tortuosity, other arterial aneurysms/dissection, IA, PDA, BAV (MacCarrick et al., 2014)	4.0–4.5[d] (Andelfinger et al., 2016; Hiratzka et al., 2010; Jondeau et al., 2016)
TGFBR2	Loeys–Dietz syndrome type 2, AAT3	TAAD, early aortic dissection*, AAA, arterial tortuosity, other arterial aneurysms/dissection, IA, PDA, BAV (MacCarrick et al., 2014)	4.0–4.5[d] (Andelfinger et al., 2016; Hiratzka et al., 2010; Jondeau et al., 2016)

It is important to note that since mutations in many of these genes are rare and have only recently been implicated in TAAD, there is a lack of adequate prospective clinical studies of individuals affected by mutations in the majority of these genes. Therefore, it is difficult to establish threshold diameters for intervention for TAAs. One must consider each individual on a case by case basis, taking into account the rate of change in aneurysm size (>0.5 cm per year is considered rapid) and any family history of aortic dissection.

A reference is provided for each of the associated vascular characteristics not reported in the OMIM entry for that gene.

AAA indicates abdominal aortic aneurysm; AAT, aortic aneurysm, familial thoracic; AD, autosomal dominant; AOVD, aortic valve disease; AR, autosomal recessive; ARD, aortic root dilatation; AVM, arteriovenous malformation; BAV, bicuspid aortic valve; CAD, coronary artery disease; CTD, connective tissue disease; FTAA, familial thoracic aortic aneurysm; HHT, hereditary hemorrhagic telangiectasia; IA, intracranial aneurysm; JP, juvenile polyposis; MYMY, moyamoya disease; PDA, patent ductus arteriosus; SVAS, supravalvular aortic stenosis; TAAD, thoracic aortic aneurysm and dissection; XLAS, X-linked Alport Syndrome.

Standard = surgical intervention at 5.0–5.5 cm.

Early aortic dissection* = dissection at aortic diameters < 5.0 cm.

[a]Individuals with MYLK and ACTA2 mutations have been shown to have aortic dissections at a diameter of 4.0 cm (Disabella et al., 2011; Wang et al., 2010).

[b]There *are* no data to set threshold diameters for surgical intervention for Ehlers–Danlos Syndrome type IV (Erbel et al., 2014). The Canadian guidelines recommend surgery for aortic root sizes of 4.0–5.0 cm and ascending aorta sizes of 4.2–5.0 cm for EDS type IV, though these patients are at high risk of surgical complications due to poor-quality vascular tissue (Boodhwani et al., 2014).

[c]There *are* limited data concerning the timing of surgical intervention for Loeys–Dietz Syndrome type 4. However, there has been a case of type A aortic dissection at an aortic diameter < 5.0 cm (Renard et al., 2013b), hence the recommended threshold range of 4.5–5.0 cm.

[d]According to the European guidelines, more clinical data *are* required to determine the appropriate threshold for surgical intervention for Loeys–Dietz syndrome (LDS) types 1 and 2 (Erbel et al., 2014). TGFBR2 mutation carriers have been shown to have similar outcomes to FBN1 mutation carriers once their disease is diagnosed (Attias et al., 2009), and the clinical course of LDS 1 and 2 does not appear to be as severe as originally reported (Tran-Fadulu et al., 2009; Jondeau et al., 2016; Teixido-Tura et al., 2016). Therefore, as Milewicz et al. assert, medically managed adult patients with LDS 1 or 2 may not require prophylactic surgery at ascending aortic diameters of 4.0–4.2 cm (Milewicz et al., 2016). Studies have demonstrated that individuals with TGFBR2 mutations are more likely to have aortic dissections at diameters < 5.0 cm than those with TGFBR1 mutations (Tran-Fadulu et al., 2009; Jondeau et al., 2016). Therefore, Jondeau et al. propose that utilizing the presence of TGFBR2 mutations (vs. TGFBR1 mutations), the co-occurrence of severe systemic features (arterial tortuosity, hypertelorism, wide scarring), female gender, low body surface area, and family history of dissection or rapid aortic root enlargement, which are all associated with an increased risk for aortic dissection, to determine the appropriate time for surgical intervention may be beneficial for LDS 1 and 2 patients to avoid unnecessary surgery at small aortic diameters (Jondeau et al., 2016). Therefore, in LDS 1 or 2 individuals without the above features, Jondeau et al. contend that 4.5 cm may be an appropriate threshold, but females with TGFBR2 mutations and severe systemic features may benefit from surgery at 4.0 cm (Jondeau et al., 2016).

amount of fibrillin-1) and/or a dominant negative mechanism (by altering the deposition of fibrillin-1 within microfibrils) (Verstraeten et al., 2016) and thereby disrupt the elastin-contractile unit (Karimi and Milewicz, 2016). Furthermore, fibrillin-1interacts with the latent TGF-β binding protein, which is a component of the large latent complex (LLC) along with the TGF-β ligand and the TGF-β propeptide (Verstraeten et al., 2016). By causing fibrillin-1 deficiency, mutations in *FBN1* alter ECM targeting of the LLC, which leads to an increase in the release of TGF-β (Verstraeten et al., 2016). It is now well established that upregulated TGF-β signaling is the inciting event for TAA formation in MFS and is a principal characteristic of syndromic and nonsyndromic TAAD (Andelfinger et al., 2016).

Studies of MFS patients have demonstrated that there are genotype–phenotype correlations that may have important clinical ramifications. Mutations in exons 24–32 have been linked to either neonatal MFS (Tiecke et al., 2001) or severe phenotypes, including increased probability of ascending aortic dilatation (Faivre et al., 2007). Additionally, truncating and splicing mutations in *FBN1* have been shown to be associated with higher rates of aortic events (dissection/surgery) that occur at younger ages (29 years vs. 51 years for missense mutations) among patients who satisfy the revised Ghent criteria (Baudhuin et al., 2015a). Although dominant negative mutations are generally more likely to result in severe pediatric presentations than haploinsufficient mutations (Dietz, 2015), a recent prospective study of 570 MFS patients found that individuals with haploinsufficient mutations had a 2.5-fold increased risk of cardiovascular death and a 2.4-fold increased risk of the combined endpoint of death or dissection than patients with dominant-negative mutations (Franken et al., 2016). Furthermore, Franken et al. report that over a period of 3 years, daily losartan significantly reduced the aortic dilatation rate only among MFS patients with haploinsufficient mutations but not among those with dominant negative mutations (Franken et al., 2015). However, as Dietz maintains, an increased baseline severity of aortic disease in the haploinsufficiency group in this study may have contributed to the observed differences in response to losartan therapy (Dietz, 2015).

Despite the 2010 revised Ghent nosology, Baudhuin et al. demonstrated that 27% of patients with a pathogenic or likely pathogenic variants did not fulfill the criteria (Baudhuin et al., 2015b). Given that these criteria are not completely sensitive or specific, the authors therefore suggest that utilizing genetic testing as opposed to purely clinical features for diagnosing MFS is important (Baudhuin et al., 2015b). Baudhuin et al. also observed a lower rate of missense mutations (41%) in those meeting the revised Ghent criteria as compared to prior studies using the old Ghent criteria, which conflicts with the belief that MFS patients with

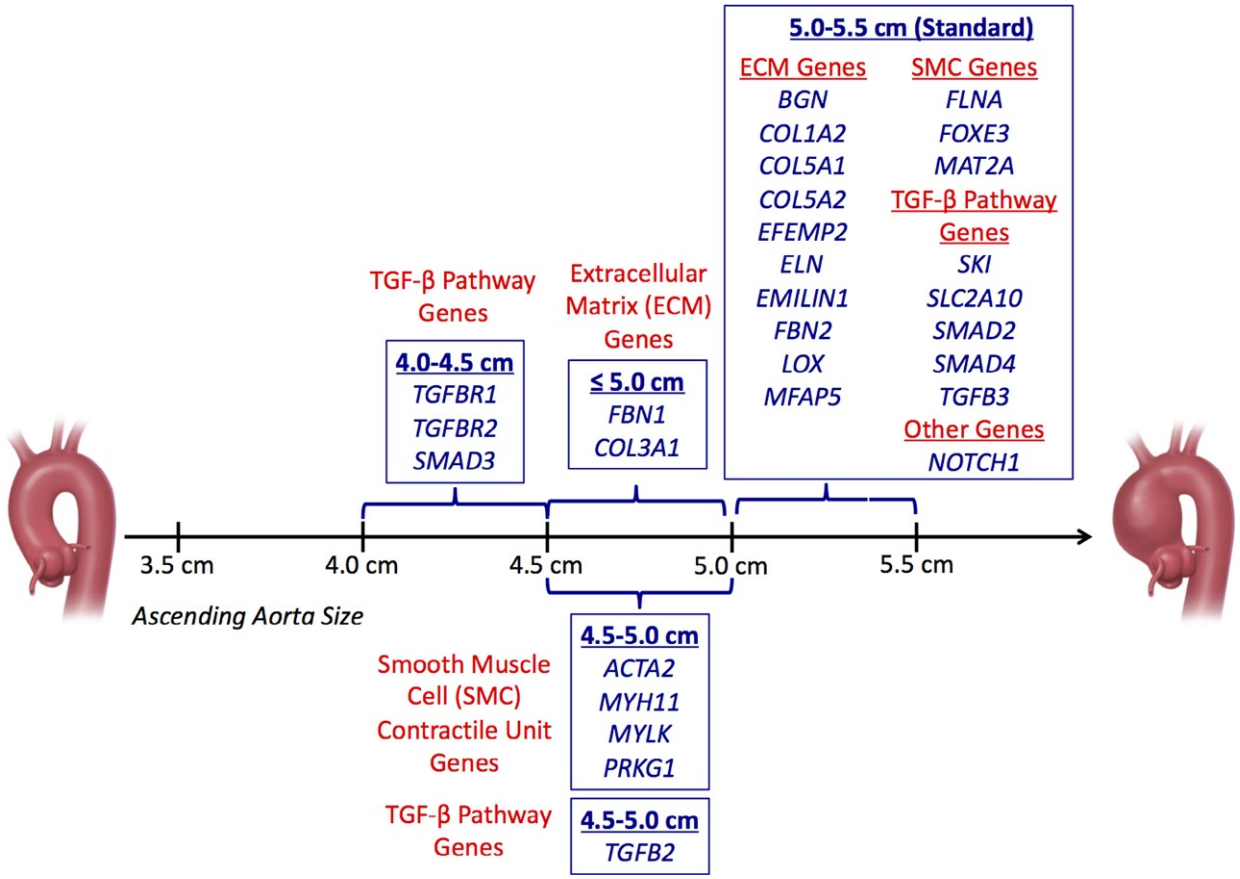

Fig. 1 Ascending aorta dimensions for prophylactic surgical intervention (Data derived from **Table 3**).

Table 4 2010 revised Ghent nosology for Marfan syndrome

In the absence of FHx:
- Ao ($Z \geq 2$) and EL = MFS
- Ao ($Z \geq 2$) and FBN1 mutation = MFS
- Ao ($Z \geq 2$) and Syst (≥ 7 pts) = MFS
- EL and FBN1 mutation with known Ao enlargement = MFS

In the presence of FHx
- EL and FH of MFS = MFS
- Syst (≥ 7 pts) and FHx of MFS = MFS
- Ao ($Z \geq 2$ above 20, ≥ 3 below 20 years) + FHx of MFS = MFS

Systemic score: 7 or higher is considered positive
- Wrist and thumb sign: 3 points
- Wrist or thumb sign: 1
- Pectus carinatum: 2
- Pectus excavatum: 1
- Hindfoot deformity: 2 (med rotation of med malleolus)
- Pes planus: 1
- Pneumothorax: 2
- Dural ectasia: 2
- Reduced upper/lower segment and increased armspan/height and no severe scolosis: 1
- Scoliosis or kyphosis: 1
- Reduced elbow extension: 1
- 3 of 5 facial features[a]: 1
- Skin striae: 1
- Myopia: 1
- Mitral valve prolapse: 1

Ao, aortic diameter at sinus of Valsalva; EL, ectopia lentis; FHx, family history; med, medial; MFS, Marfan syndrome; syst, systemic score; Z, *z*-score.

[a]Facial features: dolichocephaly, downward slanting palpebral fissures, enophthalmos, retrognathia, and malar hypoplasia.

Data derived from Loeys, B.L., Dietz, H.C., Braverman, A.C. et al. (2010). The revised Ghent nosology for the Marfan syndrome. *Journal of Medical Genetics* **47**, 476–485.

non-missense variants have a milder disease course (Baudhuin et al., 2015b). Once the diagnosis is established, an ascending aortic diameter threshold of 5.0 cm for prophylactic aortic surgery is adequate given the low rate of complications at smaller diameters (Andelfinger et al., 2016) unless the aorta dilates rapidly (>0.5 cm/year) (Hiratzka et al., 2010). However, since some MFS patients have been observed to dissect their aortas at diameters <5.0 cm (Gott et al., 1999), a family history of aortic dissection may justify prophylactic surgery at smaller aortic sizes (Andelfinger et al., 2016).

FBN2

FBN2, the causative gene in the autosomal dominant connective tissue syndrome congenital contractural arachnodactyly (OMIM 121050), otherwise known as Beals-Hecht syndrome (Putnam et al., 1995) encodes fibrillin-2, another component of microbils. The principal characteristics of this disease are external ear abnormalities, a marfanoid habitus with arachnodactyly, contractures, and progressive scoliosis (Callewaert et al., 2009), with aortic root dilatation occurring occasionally (Callewaert et al., 2009; Gupta et al., 2002). The majority of mutations in affected individuals occur between exons 24 and 35 of the gene (Frederic et al., 2009). A severe form has been described in a patient with a splicing mutation of exon 34, resulting in death during infancy from cardiovascular and gastrointestinal anomalies (Wang et al., 1996). While type A aortic dissection has been reported in a patient with a variant that led to deletion of exon 32 of the *FBN2* gene (Takeda et al., 2015), prospective studies are needed to assess the risk of aortic dissection among affected patients (Callewaert et al., 2009). It is recommended that patients with *FBN2* mutations be regularly screened for aortic dilatation via cardiac imaging (Bradley et al., 2016a).

COL3A1

COL3A1 is the causative gene of EDS type IV (EDS 4) (OMIM 130050), otherwise known as the vascular subtype of EDS. It is inherited in an autosomal dominant manner with penetrance close to 100% for missense and exon-skipping mutations (Pepin et al., 1993), though rare cases of biallelic disease causing variants leading to a more severe phenotype have been observed (Jorgensen et al., 2015). Taken together, all EDSs have an estimated prevalence of 1:10,000–1:25,000, with EDS 4 accounting for 5%–10% of all cases (Germain, 2007). The four major clinical diagnostic criteria are (1) thin, translucent skin, (2) extensive bruising, (3) characteristic facial appearance, (4) and arterial, digestive, or uterine fragility or rupture (Germain, 2007). The majority of initial clinical presentations are attributable to arterial or gastrointestinal complications, with approximately 80% of affected individuals having a complication by age 40 (Pepin et al., 2000). Arterial ruptures or dissections account for the majority of deaths (Germain, 2007), with the mean age of death being 48 years (Pepin et al., 2000). In a natural history study of over 1200 individuals (630 index cases and 601 relatives) with EDS 4, Pepin et al. found that disease of the aorta (aneurysm, dissection, and rupture), which occurred at a mean age of 35.7 years and was the leading vascular complication (22%), led to death in 68% of cases (Pepin et al., 2014).

COL3A1 encodes the pro-α1 chain of type III collagen (Smith et al., 2011), which is an essential component, along with type I collagen, of the aortic media (Karimi and Milewicz, 2016). It is thought that mutations in *COL3A1* alter the triple helix structure of type III collagen, thereby predisposing patients to arterial tears or dissections (Germain, 2007). The triple helix amino acid structure consists of repeated glycine-X-Y sequences (Germain, 2007), with the majority of mutations being missense mutations that lead to glycine substitutions (Shalhub et al., 2014). In fact, Pepin et al. found that over 90% of identified mutations in the *COL3A1* gene were missense or splice site variants (Pepin et al., 2014). More than 600 *COL3A1* mutations have been identified to cause EDS type IV, with 50% being de novo and 50% being inherited (Shalhub et al., 2014).

Just as with MFS, genotype–phenotype correlations have been described. Specifically, Pepin et al. demonstrated that the median survival rates for splice site acceptor, splice site donor, and glycine substitution mutations were 52, 37, and 51 years, respectively (Pepin et al., 2014). All three mutations types had a significantly higher hazard ratio compared to those with null mutations (patients with heterozygous mutations that caused loss of the stable mRNA transcript from one allele), with donor site mutations having the highest hazard ratio. Furthermore, among those with glycine substitution mutations, the specific amino that replaced glycine was important for survival, with valine substitutions having the highest hazard ratio. Null mutations have also been shown to be inherited with reduced penetrance compared to missense or exon-skipping mutations, and individuals with null mutations have a delayed onset of the first complication by almost 15 years (Leistritz et al., 2011). In another study, Shalhub et al. categorized patients into two groups based on mutations that led to the production of either 10%–15% (MIN), which included those with glycine substitution mutations, or 50% (haploinsufficiency) normal collagen (Shalhub et al., 2014). They found that patients in the haploinsufficiency group had significantly increased rates of aortic pathology, including dissection, though they presented at an older age than those in the MIN group (Shalhub et al., 2014). Similarly, Frank et al. demonstrated that patients with haploinsufficiency had higher rates of aortic complications compared to those with glycine substitutions and splice site mutations (Frank et al., 2015). Despite the higher rates of aortic complications in those with haploinsufficiency, these patients, along with those with variants in the N- or C-terminal region of the protein, were shown to have milder disease overall than those with glycine substitutions and splice site mutations (Frank et al., 2015).

Even with these observed genotype–phenotype correlations, it is challenging to counsel patients given the significant intra- and interfamilial variability in survival for the same disease causing variant (Pepin et al., 1993). Additionally, since aortic dissections have been shown to occur even without significant development of an aortic aneurysm in EDS type IV patients (Gillis et al., 2013), it is difficult to determine the appropriate time for prophylactic aortic surgery. This is further complicated by the fact that surgery can be quite dangerous in these patients as they have excess vessel fragility (Pomianowski and Elefteriades, 2013).

Other Collagen Genes

COL1A1 and *COL1A2*, the causative genes in EDS arthrochalasis type (OMIM 130060) and osteogenesis imperfecta, respectively, encode pro-α1 and 2 chains of type I collagen, the most abundant ECM protein in humans (Malfait et al., 2007, 2013). While the majority of mutations in *COL1A1* and *COL1A2* cause osteogenesis imperfecta (Malfait et al., 2007), a specific type of mutation that leads to partial or complete skipping of exon 6 is the cause of the EDS arthrochalasis type, leading to incomplete processing of the procollagen chains (Malfait et al., 2013). The syndrome is characterized by severe joint hypermobility with joint dislocations, muscular hypotonia, skin hyperextensibility, and mild dysmorphic features (Malfait et al., 2013; Klaassens et al., 2012). EDS arthrochalasis type can be inherited via an autosomal dominant or an autosomal recessive mechanism (Klaassens et al., 2012). Mutations in *COL1A1* have been associated with dissection of the infrarenal aorta as well as the iliac, femoral, and cervical arteries (Malfait et al., 2007; Mayer et al., 1996). Mutations in *COL1A2* are involved in the development of cardiac valvular disease and borderline aortic root enlargement (Bradley et al., 2016a; Schwarze et al., 2004).

Mutations in *COL5A1* and *COL5A2*, which encode components of type V collagen, cause the classical type of EDS (OMIM 130000) (De Paepe and Malfait, 2012). The major diagnostic criteria for the classical type of EDS are skin hyperextensibility, widened atrophic scars, joint hypermobility, and a positive family history (Malfait et al., 1993). Approximately 50% of individuals with the classical type of EDS have pathogenic variants within *COL5A1* and *COL5A2* (Malfait et al., 1993), with the majority being null mutations in *COL5A1* (Malfait et al., 2005). Aortic root dilation was diagnosed in classical EDS in 33% of patients at a mean age of 23.4 in one study (Wenstrup et al., 2002), but progression to aortic dissection is rare (Hiratzka et al., 2010). While mutations in *COL5A1* have been shown to cause similar clinical presentations to the vascular type of EDS caused by *COL3A1* mutations, including arterial rupture and dissection of medium-sized arteries (Mehta et al., 2012; Monroe et al., 2015), variants in *COL5A2* have not been associated with aortic dissection (Meienberg et al., 2010). However, one mutation that resulted in deletion of both the *COL3A1* and *COL5A2* genes causing haploinsufficiency of both genes was associated with aortic dissection (Meienberg et al., 2010). The authors attributed this clinical presentation primarily to deletion of the *COL3A1* gene. Despite the rarity of aortic and other arterial dissection/rupture in patients with mutations in collagen genes other than *COL3A1*, it may be prudent to consider screening these patients via baseline echocardiogram to assess for aortic dilatation.

EFEMP2

EFEMP2 (*FBLN4*), the causative gene of autosomal recessive cutis laxa type IB (ARCL1B) (OMIM 614437), encodes fibulin-4, an ECM protein that plays an important role in the assembly and function of elastic fibers (Renard et al., 2010). Homozygous or compound heterozygous mutations in *EFEMP2* lead to ascending aortic aneurysms, arterial tortuosity and stenosis, and cutis laxa (loose, redundant, and inelastic skin) (Renard et al., 2010; Hucthagowder et al., 2006; Hebson et al., 2014), and thus there is significant overlap with LDS and ATS (Renard et al., 2010). However, ARCL1B is highly variable in its clinical presentation, with some affected patients exhibiting perinatal cardiopulmonary failure while others have manifestations restricted to the vascular and craniofacial system and have minimal skin involvement (Loeys et al., 1993). Renard et al. reported for the first time survival beyond early childhood to ages 7 and 20 for two affected patients (Renard et al., 2010). Not only is fibulin-4 critical for elastogenesis but mouse studies have shown that it plays an important role in collagen maturation (Papke et al., 2015; Igoucheva et al., 2015). Additionally, mutations in *EFEMP2* have been associated with increased TGF-β signaling in aortic tissue of affected patients (Renard et al., 2010). There have been no reported cases of aortic dissection, though most patients present in infancy or at very young ages.

ELN

Heterozygous mutations in *ELN*, which encodes the protein tropoelastin, are the cause of autosomal dominant cutis laxa-1 (ADCL-1) (OMIM 123700) (Szabo et al., 2006), which is characterized by generalized lax skin folds (Callewaert et al., 2011). Although it was originally thought that affected patients did not exhibit systemic features (Loeys et al., 1993), several studies have shown that mutations within *ELN* are associated with dilatation of the aortic root and aneurysm formation, as well as pulmonary features, including emphysema (Szabo et al., 2006; Callewaert et al., 2011). In one of these studies, one of the probands' uncles suffered from an aortic dissection at age 26, though he did not exhibit skin laxity while alive (Szabo et al., 2006). Culturing of patients' fibroblasts by Callewaert et al. demonstrated increased TGF-β signaling, which the authors speculated is due to the inability of disorganized elastic fibers to sequester TGF-β (Callewaert et al., 2011). Interestingly, Callewaert et al. also observed a significant association of ADCL-1 with BAV (Callewaert et al., 2011). Additionally, one study involving patients with a 7q11.23 microduplication and another involving patients with a 7q11.23 triplication, a region of the chromosome that encompasses the *ELN* gene, found that almost all patients had aortic dilation or an ascending aortic aneurysm (Parrott et al., 2015; Guemann et al., 2015). Therefore, though longitudinal studies are lacking, regular aortic imaging in these patients is required to monitor for any development of aortic dilatation or aneurysm formation (Szabo et al., 2006).

Mutations in *ELN* can also lead to supravalvular aortic stenosis (SVAS) (OMIM 185500), an autosomal dominant systemic arteriopathy that leads to narrowing of the ascending aorta as well as other arteries (most commonly branches of the pulmonary and coronary arteries) due to intimal or medial thickening (Merla et al., 2012). While ADCL-1 is most commonly due to mutations at the 3′ end of the *ELN* gene, which are predicted to disrupt the C-terminal region of tropoelastin (a critical region for binding microfibril-associated glycoproteins and glycosaminoglycans), haploinsufficiency of the *ELN* gene due to premature stop codons is believed to lead to SVAS (Merla et al., 2012). A novel frameshift mutation leading to a stop codon was just recently described in a three-generation family with SVAS, in which two family members suffered from sudden death of unknown etiology and one

confirmed mutation carrier suffered from a ruptured IA (Jelsig et al., 2017). SVAS has also been described in an infant with autosomal recessive cutis laxa type IA (OMIM 219100), which is due to mutations in *FBLN5* (fibulin-5) (Loeys et al., 2002).

EMILIN1

The elastin microfibril interface 1 (*EMILIN1*) gene encodes an ECM protein that associates with microfibrils and elastic fibers and is involved in elastogenesis, vessel assembly, and regulation of TGF-β1 (Capuano et al., 2016). Capuano et al. identified a proband with one ascending and one descending aortic aneurysm, as well as bilateral lower leg and foot sensorimotor neuropathy, arthropathy, and increased skin elasticity (Capuano et al., 2016). He was found to have a variant in the signal peptide cleavage site of *EMILIN1*, which led to the abnormal accumulation of the protein in the endoplasmic reticulum and subsequent cellular apoptosis and altered ECM integrity (Capuano et al., 2016). The variant was found to segregate with disease in the family in an autosomal dominant manner, although there was marked intrafamilial variability since the patient's mother and son exhibited mild impairment with no history of aortic aneurysms (Capuano et al., 2016). Further studies are needed to confirm the role of *EMILIN1* in the formation of TAAs, as this is the first reported case involving this gene (Karimi and Milewicz, 2016).

MFAP5

MFAP5 (OMIM 616166) encodes microfibril-associated glycoprotein 2 (MAGP-2), a structural constituent of the ECM that has been shown to interact with other components of the ECM, including fibrillin-1 and tropoelastin, as well as to bind to both TGF-β1 and β2 (Barbier et al., 2014). Utilizing whole-exome sequencing, Barbier et al. identified two distinct mutations (one nonsense and one missense) within *MFAP5* that segregated with disease in two separate families, with one of the probands presenting with a type A aortic dissection at 58 years of age and other family members exhibiting aortic root dilatation (Barbier et al., 2014). Given that affected individuals only occasionally displayed mild skeletal features of MFS and LDS, mutations in *MFAP5* are believed to result in non-syndromic familial TAAD (Barbier et al., 2014). Consistent with what is seen with other TAAD causative genes, aortic tissue collected from the proband with aortic dissection showed enhanced TGF-β signaling (Barbier et al., 2014). Additionally, lone paroxysmal atrial fibrillation was present in multiple affected members of one of the two families studied. Given the clear possibility for aortic dissection in affected patients, regular cardiac imaging to monitor any aneurysmal growth would be prudent.

LOX

LOX (OMIM 617168) encodes a member of the lysyl oxidase protein family, a group of extracellular copper enzymes that cross-link collagens and elastin (Guo et al., 2016a). Two recent reports show that mutations in *LOX* resulted in familial TAAD, with one patient presenting at age 11 with an ascending aortic aneurysm and several patients presenting with aortic dissections in their 50s and 60s (Guo et al., 2016a; Lee et al., 2016). The identified mutations resulted in either haploinsufficiency or alteration of a highly conserved catalytic domain (Guo et al., 2016a). Since *LOX* encodes the enzyme primarily responsible for the cross-linking of collagen and elastin in the aorta, diminished enzymatic activity is thought to result in abnormal aortic development with decreased and fragmented elastic fibers (Guo et al., 2016a). Furthermore, *LOX* has been shown to bind to and inactivate free TGF-β via its amine oxidase activity (Atsawasuwan et al., 2008; Lindsay and Dietz, 2014). Since fibulin-4 is crucial for recruiting LOX to fibrillin microfibrils, decreased enzymatic inactivation of TGF-β by LOX as a result of fibulin-4 deficiency may also contribute to the predisposition toward aortic aneurysms in individuals with *FBLN4* (*EFEMP2*) mutations (Lindsay and Dietz, 2014). Interestingly, 3 of 20 *LOX* mutation carriers in one of the studies had BAVs (Guo et al., 2016a). More clinical data are needed to determine the risk of aortic dissection in *LOX*-mediated TAAD.

BGN

BGN, an X-linked gene, encodes the biglycan protein, which is a small leucine-rich proteoglycan involved in the maintenance and assembly of the ECM (Meester et al., 2017). Biglycan has been shown to interact with components of the ECM, including collagens and elastin, and with growth factors and cytokines, such as TGF-β (Meester et al., 2017). Meester et al. recently identified loss-of-function mutations in *BGN* in individuals with syndromic TAAD from five separate families (Meester et al., 2017). These individuals presented with TAAD at young ages (earliest cases of aortic aneurysm and aortic dissection were at age 1 and 15, respectively), along with several MFS and LDS-like extracardiovascular characteristics, including hypertelorism, pectus deformity, joint hypermobility, contractures, and mild skeletal dysplasia, among others (Meester et al., 2017). Furthermore, the aortic walls of affected individuals demonstrated increased TGF-β signaling, especially within the adventitia, which is the aortic wall layer where biglycan is mostly deposited (Meester et al., 2017; Heegaard et al., 2007). *BGN* and *FLNA* are the only genes shown thus far to lead to syndromic X-linked TAAD.

SMC Contractile Unit and Metabolism Genes

The SMC plays a critical role in the function and maintenance of the aortic media. The contractile unit of the SMC comprises actin thin filaments and myosin thick filaments, which interact to generate the force of contraction, as well as regulatory proteins including tropomyosin (Karimi and Milewicz, 2016). The components of the SMC contractile unit interact with the cytoskeleton

and the nuclear membrane, thereby allowing SMCs to convert mechanical stimuli into resultant biochemical activity (Karimi and Milewicz, 2016). It is thought that mutations in genes that disrupt these linkages lead to impaired sensation of mechanical stress in the aortic wall by SMCs, thereby diminishing their ability to adequately regulate and remodel the ECM (Karimi and Milewicz, 2016).

MYH11

MYH11 (OMIM 132900) encodes the smooth muscle myosin heavy chain, an essential contractile protein within SMCs (Zhu et al., 2006). In 2006, Zhu et al. found that two families with familial TAAD and patent ductus arteriosus (PDA) had mutations in *MYH11*, with one being a substitution at the splice donor site of intron 32 and the other being a missense mutation in exon 37 leading to substitution of glutamine for arginine (Zhu et al., 2006). These mutations are believed to affect the C-terminal coiled-coil region of the SMC myosin heavy chain (Zhu et al., 2006). Zhu et al. further demonstrated that *MYH11* mutation carriers had increased aortic stiffness compared to noncarriers, as evidenced by their decreased aortic compliance and increased pulse wave velocities (Zhu et al., 2006). In 2007, Pannu et al. identified novel missense mutations in two families affected by TAAD and PDA, though there was variable expressivity of both TAAD and PDA, as well as decreased penetrance of the mutation (Pannu et al., 2007). Interestingly, pathology from two affected patients revealed SMC disarray and focal hyperplasia of SMCs with evidence of dysregulation of insulin-like growth factor 1 and angiotensin II, as opposed to TGF-β (Pannu et al., 2007). This pathology is distinct from typical TAAD pathology and may contribute to the increased rates of vascular occlusive disease in these patients (Pannu et al., 2007).

In two further studies (Harakalova et al., 2013; Imai et al., 2015), the same deletion variant in *MYH11* was identified in a Dutch family and two Japanese families, though this variant only segregated with disease in the Japanese families. Additionally, a heterozygous missense variant identified in a Dutch family also did not segregate with disease entirely (Harakalova et al., 2013). Harakalova et al. therefore postulate that unidentified genetic modifiers may be contributing to the clinical phenotype observed in these Dutch families, which may explain the phenotypic variability and reduced penetrance observed (Harakalova et al., 2013). In addition to causing familial TAAD, it is possible that *MYH11* may contribute to sporadic TAAD, since a chromosomal duplication of 16p13.1, which contains *MYH11*, is estimated to impart an eightfold higher risk for development of TAAD (Luyckx and Loeys, 2015; Kuang et al., 2011). However, it is not established whether MYH11 is the culprit gene in this chromosomal duplication (Luyckx and Loeys, 2015).

For patients with familial TAAD with *MYH11* mutations, prophylactic aortic surgery is warranted at ascending aortic diameters of 4.5–5.0 cm (Andelfinger et al., 2016). Yet, Harakalova et al. maintain that family members of affected patients who do not carry the *MYH11* mutations should still participate in regular cardiac imaging given that the mutations they identified did not segregate with disease (Harakalova et al., 2013).

ACTA2

ACTA2 (OMIM 611788) encodes the smooth muscle α-actin, which polymerizes to form thin filaments that are crucial for SMC contraction (Karimi and Milewicz, 2016). Several studies have now shown that *ACTA2* mutations account for 12%–21% of FTAAD (Guo et al., 2007; Morisaki et al., 2009; Disabella et al., 2011). The majority of identified mutations are missense and result in a dominant negative mechanism that decreases actin filament assembly (Guo et al., 2007), though nonsense mutations have been identified as well (Renard et al., 2013a). Consistent with what is observed in syndromic TAAD, Renard et al. demonstrated increased TGF-β signaling in patients with an *ACTA2* mutation as well as those with an *MYH11* mutation (Renard et al., 2013a). Interestingly, this is in contrast to a prior study that did not observe increased TGF-β signaling in the aortas of *MYH11* mutation carriers (Pannu et al., 2007). Renard et al. postulate that enhanced TGF-β signaling may be a more prominent feature of *MYH11*-mediated TAAD than *ACTA2*-mediated TAAD, as not all biopsies from *ACTA2* mutation carriers showed increased TGF-β signaling, which was the case for *MYH11* mutation carriers (Renard et al., 2013a). Specific mutations in *ACTA2* have also been linked to early-onset coronary artery disease, stroke, iris flocculi, and livido reticularis (Disabella et al., 2011; Guo et al., 2009). Furthermore, one heterozygous de novo missense mutation, R179H, has been associated with severe multisystemic smooth muscle dysfunction (OMIM 613834), with all five patients in the study presenting with ascending aortic aneurysms at ages ranging from 11 to 26 years (Milewicz et al., 2010).

The most common presentation among family members with *ACTA2* mutations is thoracic aortic dissection, which has a penetrance of 50% (Luyckx and Loeys, 2015). One study of 277 patients with various *ACTA2* mutations found a lifetime cumulative risk of aortic events (dissection or repair) of 76% by age 85 (Regalado et al., 2015). The majority of patients presented with thoracic aortic dissections (88%) at a median age of 35 years with more type A than type B dissections (54% vs. 21%), though patients with type B dissections presented at significantly younger ages (27 vs. 36 years). Interestingly, the p.R179 and p.R258 substitution mutations were associated with increased risk for aortic events and at younger ages (100% by age 25 and 90% by age 63, respectively) (Regalado et al., 2015). On the contrary, p.R185Q and p.R118Q mutations were associated with a significantly decreased risk for aortic events relative to other *ACTA2* mutations. In fact, p.R185Q mutation carriers had a cumulative risk of aortic events of only 57% by age 77.

The specific *ACTA2* mutation also determines the presence and severity of extra-aortic vascular disease (Milewicz et al., 2016). In prior studies, the p.R179 and p.R258 mutations have been associated with increased severity of vascular disease, including PDA, early onset stroke, and tortuosity of small arteries of the retina (Guo et al., 2009; Munot et al., 2012; Moller et al., 2012). These more severe mutations have also been associated with dilatation of the pulmonary arteries and first-order branches of the thoracic aorta

(Milewicz et al., 2016). The Arg179 (primarily Arg179His) and Arg258 mutations produce a distinctive cerebrovascular phenotype (OMIM 614042), including dilatation of the proximal internal carotid artery, occlusive disease of the terminal internal carotid artery, a straight course of intracranial arteries, and no basal collaterals (unlike what is observed with Moyamoya disease) (Munot et al., 2012). Interestingly, this transition from a dilated to stenotic internal carotid artery occurs at the cavernous portion of the internal carotid artery, which is where the external elastic lamina disappears from the arterial wall (Munot et al., 2012). The Arg179Cys mutation has been observed to recapitulate this neurovascular pattern and has also been implicated in anomalies of brain development (de Grazia et al., 2017; Meuwissen et al., 2013). Despite the observed cerebrovascular anomalies in these patients, there is a relatively low frequency of IAs (1.8%) in those with *ACTA2* mutations (Milewicz et al., 2016). Additionally, Arg149 and Arg118 mutations have been linked to early onset coronary artery disease (Milewicz et al., 2016).

Importantly, Regalado et al. demonstrated that aortic dissection occurred at aortic diameters <5.0 cm in 1/3 of patients with *ACTA2* mutations who suffered from type A dissections (Regalado et al., 2015). Moreover, in another study, for three out of eight women with *ACTA2* mutations who had peripartum aortic dissections, their aortic diameters at the time of dissection ranged from 3.8 to 4.7 cm (Regalado et al., 2014). Therefore, patients with *ACTA2* mutations warrant surgical intervention at aortic diameters between 4.5 and 5.0 cm (Andelfinger et al., 2016) to hopefully mitigate their increased risk for dissection.

MYLK

MYLK (OMIM 613780) encodes myosin light chain kinase, a ubiquitously expressed kinase that phosphorylates regulatory light chain (RLC), thereby initiating SMC contraction (Wang et al., 2010). Through a candidate gene approach in 2010, two variants in *MYLK*, p.R1480X and p.S1759P, were found to segregate with aortic dissection in two families (Wang et al., 2010). Wang et al. found that these variants resulted in decreased kinase activity and therefore decreased SMC contraction (Wang et al., 2010). Importantly, affected patients were found to have aortic dissections at aortic diameters that were minimally dilated (even at 4.0 cm) (Wang et al., 2010). A recent study by Hannuksela et al., which identified a novel 2-bp deletion variant leading to a premature stop codon, also found that affected individuals suffered aortic dissections across a wide range of ages 23-77 and at diameters <5.0 cm (Hannuksela et al., 2016). Interestingly, Hannuksela et al. showed that carriers of this novel deletion variant did not differ from noncarriers in aortic diameter or aortic stiffness (Hannuksela et al., 2016). Although we recommend that patients with MYLK mutations should undergo prophylactic surgery at ascending aortic diameters between 4.5 and 5.0 cm (Andelfinger et al., 2016), it is likely that this criterion will not protect all patients, as individuals with this mutation can dissect at even smaller diameters.

PRKG1

PRKG1 (OMIM 615436) encodes type I cGMP-dependent protein kinase (PKG-1), which is activated upon binding of cGMP and leads to SMC relaxation via phosphorylation and activation of the phosphatase that dephosphorylates and thereby inactivates myosin RLC (Karimi and Milewicz, 2016). Only one variant (c.530G>A), which was identified in four families, has been shown to cause FTAAD (Guo et al., 2013). By altering the cGMP binding site of PKG-1, this gain of function mutation leads to constitutive activation of the kinase, leading to decreased phosphorylation of the myosin RLC, promoting SMC relaxation (Guo et al., 2013). Therefore, this gain of function mutation in *PRKG1* and loss of function mutations in *MYLK* both decrease phosphorylation of the RLC, thereby decreasing SMC contraction (Karimi and Milewicz, 2016). Guo et al. found that 63% of patients with this *PRKG1* mutation presented with aortic dissections at an average of 31 years, with the youngest being 17 years old (Guo et al., 2013). For the two aortic measurements available at the time of dissection, one patient had an ascending aortic diameter of 4.3 cm and the other had an aortic root diameter of 5.7 cm. Affected family members also exhibited other vascular abnormalities, including enlargement of the descending thoracic aorta, abdominal aorta, and other arteries, including the coronaries (Guo et al., 2013). Although there are limited data on *PRKG1* mutations, affected patients probably would benefit from prophylactic surgeries at the same aortic diameter as patients affected by mutations in other SMC genes, namely 4.5–5.0 cm.

FLNA

FLNA encodes filamin A, an important anchoring protein that links actin filaments in SMCs to transmembrane integrin molecules in focal adhesions of the cell membrane and thereby playing a prominent role in maintaining cell shape and cell signaling (Karimi and Milewicz, 2016). *FLNA* mutations are the cause of X-linked dominant periventricular heterotopia (XL-PH) (OMIM 300049), a neuronal migration disorder that primarily affects females and can lead to seizures, aortic aneurysms, PDA, and EDS-like features, including hyperextensible joints and skin laxity (Sheen et al., 2005). In a cohort of 47 affected individuals, Lange et al. found that the vast majority had truncating mutations and only 1 out of 34 patients had aortic dilation (Lange et al., 2015). However, other cohorts have described several patients with aortic dilation/aneurysms, so the estimated incidence of aortic dilation in XL-PH is unknown (Sheen et al., 2005; Reinstein et al., 2013). As far as we are aware, no cases of aortic dissection have been reported to date. In addition to causing aortic manifestations, XL-PH can also lead to dilatation/aneurysm of subclavian and pulmonary arteries (Reinstein et al., 2013). Interestingly, arterial tortuosity, which is a marker of adverse cardiovascular outcomes in MFS and LDS (Morris et al., 2011), was not present within a family of patients with XL-PH that manifested with an aortic aneurysm in one member and a subclavian artery aneurysm in another (Reinstein et al., 2014). Given the variable expressivity of vascular features, cardiac imaging in these patients is warranted to screen for aortic and pulmonary dilatation (Reinstein et al., 2013).

MAT2A

MAT2A encodes methionine adenosyltransferase II alpha (MAT-IIα), which catalyzes the production of *S*-adenosylmethionine (SAM), a critical enzyme involved in cellular metabolism (Guo et al., 2015). Guo et al. recently implicated two mutations in *MAT2A*, c.10131A>C (p.Glu344Ala) and c.1067G>A (p.Arg356His), in familial TAAD (Guo et al., 2015). While the exact mechanism by which *MAT2A* mutations lead to TAAD is unclear, it is thought that these mutations disrupt the enzyme activity of MAT-IIα, thereby altering SMC metabolism, perhaps by increasing oxidative stress or decreasing methylation activity within SMCs (Guo et al., 2015). In this study, 8 out of 18 (44%) of mutation carriers had dilatation of the ascending aorta and/or aortic root at a median age of 50 years. There were no reported cases of aortic dissection, though one obligate carrier died at the age of 32 suddenly from unknown causes (Guo et al., 2015). While there are limited data on the clinical phenotypes of *MAT2A* mutation carriers, the data suggest that affected patients may have a more benign presentation than other familial TAAD patients (i.e., no episodes of aortic dissection and youngest presentation with TAA at age 37). Therefore, Guo et al. postulate that it is possible a second environmental or genetic factor may be required for patients with *MAT2A* mutations to develop TAAD (Guo et al., 2015).

FOXE3

FOXE3, which encodes a forkhead transcription factor, was recently implicated in familial TAAD in a large family with eight identified cases of acute aortic dissection (Kuang et al., 2016). Seven out of eight family members presented with a type A aortic dissection at an average age of 45.6 years, with one family member having prior normal aortic imaging. Interestingly, decreased penetrance was observed in women, since only men were affected. Through exome and Sanger sequencing of this family and 564 unrelated familial TAAD probands, four likely pathogenic *FOXE3* variants were identified, which were all missense mutations that changed amino acids at the C-terminal end of the DNA-binding domain (Kuang et al., 2016). Through knockout mouse studies, Kuang et al. demonstrated that *FOXE3* is crucial for populating the ascending aorta with differentiated SMCs during development and for SMC response to aortic wall stress (Kuang et al., 2016). Further clinical studies are needed to determine the risk of aortic dissection in these patients and the timing of prophylactic aortic surgery to prevent such complications.

TGF-β Pathway Genes

Studies have consistently shown that increased TGF-β signaling is a feature of both syndromic and nonsyndromic TAAD (Andelfinger et al., 2016; Renard et al., 2013a; Loeys et al., 2005, 2006; Gomez et al., 2009). TGF-β acts via the canonical and noncanonical pathways (**Fig. 2**). The canonical pathway involves activation of the TGF-β receptor, phosphorylation of regulatory SMADs (SMAD2 and 3), recruitment of SMAD4 to form a SMAD2/3–SMAD4 complex, importation into the nucleus, and activation of gene transcription (Andelfinger et al., 2016; Wu et al., 2013). Several explanations have been proposed for why there is paradoxical upregulation of TGF-β signaling in the aortas of patients with TAAD despite their loss-of-function mutations in TGF-β pathway genes (Verstraeten et al., 2016). First, there is normally an autoregulatory feedback loop on TGF-β signaling provided by the canonical pathway (Lindsay and Dietz, 2011). However, it is believed that mutations in canonical TGF-β pathway genes lead to a loss of this feedback inhibition, thereby causing an upregulation of TGF-β signaling especially via the noncanonical pathways (Andelfinger et al., 2016; Lindsay and Dietz, 2011; Bolar et al., 2012). Second, the differential expression of TGF-β cytokines may play a role in the pathogenesis of TAAD, as increased *TGFB1* expression has been observed in LDS and Shprintzen–Goldberg syndrome (SGS) patients (Verstraeten et al., 2016; Doyle et al., 2012; Lindsay et al., 2012; van de Laar et al., 2011). Lastly, the interaction between second heart field-derived SMCs and neural crest cell-derived SMCs of the ascending aorta may play an important role (Lindsay and Dietz, 2011). Namely, it is thought that second heart field-derived SMCs are more susceptible to underlying TAAD-causing mutations than neural crest-derived SMCs and may therefore secrete excess TGF-β to compensate, which may have a paracrine effect on the surrounding neural crest-derived SMCs, leading to enhanced TGF-β signaling within these cells (Verstraeten et al., 2016; Lindsay and Dietz, 2011; Gallo et al., 2014). Regardless of the mechanism, the resulting upregulation of TGF-β signaling is thought to be a crucial element in the formation of TAAD.

TGFBR1 and *TGFBR2*

TGFBR1 and *2*, the causative genes in LDS type 1 (OMIM 609192) and 2 (OMIM 610168), respectively, encode the two major human TGF-β receptor subunits (Isselbacher et al., 2016a). LDS, which was originally described in 2005 (Loeys et al., 2005, 2006), is an autosomal dominant disorder with 3 characteristic features: (1) arterial tortuosity/aortic aneurysms, (2) bifid/broad uvula or cleft palate, and (3) hypertelorism (Loeys et al., 2005). Patients often exhibit features that overlap with MFS, including cardiovascular, craniofacial, and skeletal abnormalities (Gillis et al., 2013). Originally, LDS was classified based on the severity of craniofacial (type 1—typical dysmorphic features of hypertelorism, craniosynostoses, cleft palate) or cutaneous features (type 2—mild dysmorphic features with velvety, translucent skin and easy bruising) (MacCarrick et al., 2014). However, regardless of the severity of systemic features, LDS patients are at significantly increased risk of aortic dissection and aneurysm rupture at relatively young ages and small aortic diameters (MacCarrick et al., 2014). Therefore, a revised nosology has been proposed based on the causative gene: type 1 (*TGFBR1* mutations); type 2 (*TGFBR2* mutations); type 3 (*SMAD3* mutations)—previously known as Aneurysm

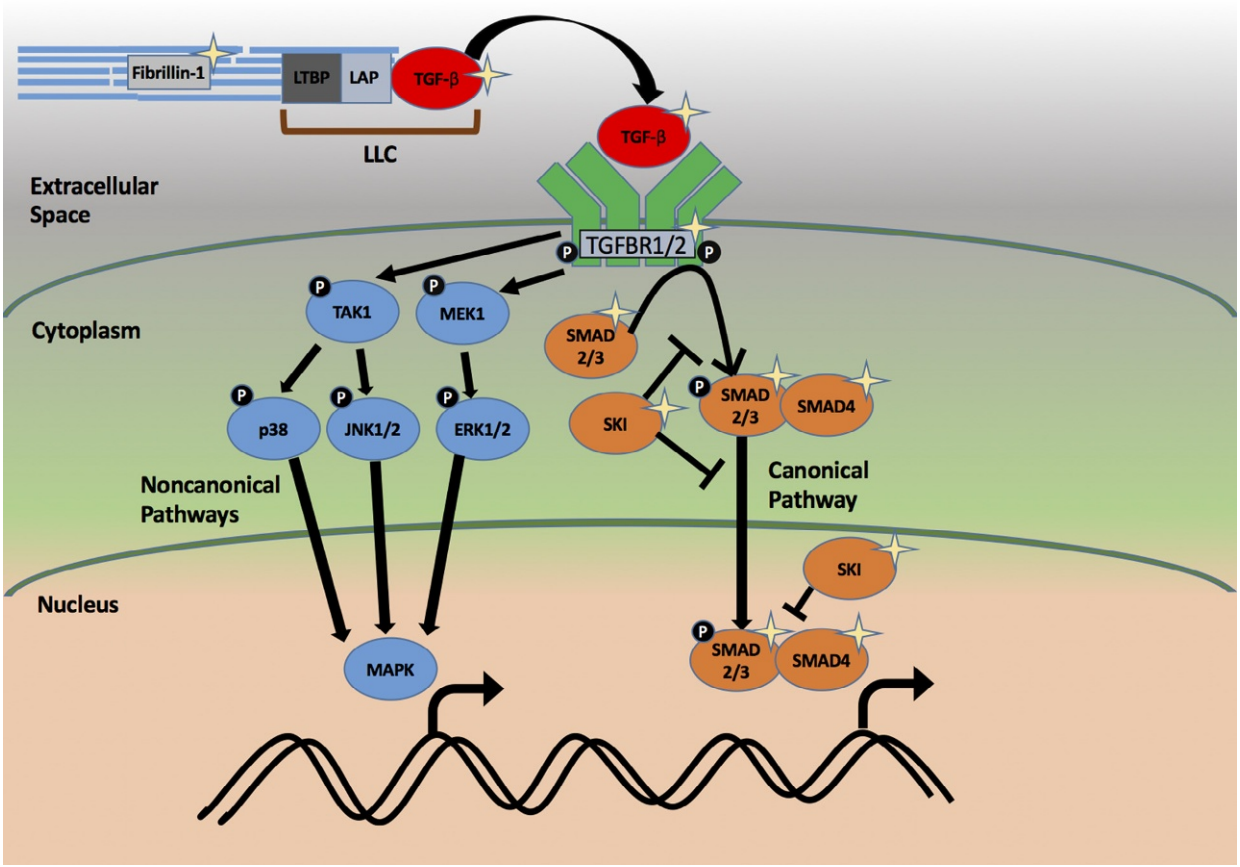

Fig. 2 Overview of canonical and noncanonical TGF-β signaling pathways. By interacting with the large latent complex (LLC) of TGF-β, comprised of the latent TGF-β binding protein (LTBP) and the latency-associated protein (LAP) (40), microfibrils composed of fibrillin-1 help sequester TGF-β, preventing it from acting upon cells. Once TGF-β is released, it can activate both the canonical (orange) and noncanonical (blue) pathways. The canonical pathway involves activation of the TGF-β receptor, phosphorylation of regulatory SMADs (SMAD2 and -3), recruitment of SMAD4 to form a SMAD2/3–SMAD4 complex, importation into the nucleus, and activation of gene transcription. SKI, a negative regulator of the canonical signaling pathway, acts by inhibiting many of these processes, including SMAD2/3 phosphorylation, importation into the nucleus, and activation of gene transcription (120). TGF-β receptors also activate noncanonical pathways, which involve multiple different SMAD-independent cascades (59). Upregulation of the noncanonical pathways is believed to play an important role in the development of aortic aneurysms (40). As depicted by the yellow stars, only mutations in the genes that encode components of the canonical signaling pathway have been shown to lead to thoracic aortic aneurysms and dissections.

Osteoarthritis Syndrome (AOS); type 4 (*TGFB2* mutations) (MacCarrick et al., 2014). This revised nosology indicates that a mutation in any of these genes plus presentation with an aortic aneurysm or dissection is sufficient for diagnosis regardless of the severity of systemic features (MacCarrick et al., 2014). Since this revised nosology was released, mutations in *TGFB3* are now considered to result in LDS type 5.

Mutations in *TGFBR1* and *TGFBR2* are almost exclusively missense and usually occur in the kinase domain of the receptors (Isselbacher et al., 2016a). Studies have demonstrated that mutations in both *TGFBR1* and *2* cause not only LDS but also nonsyndromic familial TAAD (Pannu et al., 2005; Tran-Fadulu et al., 2009), illustrating the wide range of clinical phenotypes seen with mutations in these genes (Luyckx and Loeys, 2015). In fact, identical *TGFBR2* mutations have been reported in familial TAAD patients and LDS patients displaying the typical characteristics of the syndrome (Luyckx and Loeys, 2015). LDS patients can also manifest with aneurysms of all portions of the aorta as well as different arterial branches of the aorta (MacCarrick et al., 2014). Additionally, type B dissections have been shown to occur at minimally or non-dilated aortic diameters of 3.7–4.2 cm, as has rapid expansion of aneurysms after a dissection in those with LDS 1–3 (MacCarrick et al., 2014).

Although initial reports did not show a difference in clinical presentation between LDS patients with *TGBR1* versus *TGFBR2* mutations (Loeys et al., 2006), one study of multigenerational TAAD families with *TGFBR1* ($n=30$ individuals) or *TGFBR2* ($n=77$ individuals) mutations did find differences in clinical presentations (Tran-Fadulu et al., 2009). In this study, the average age of presentation with vascular disease, including aortic aneurysms and dissections, was significantly younger in the *TGFBR1* cohort than in the *TGFBR2* cohort (31.4 vs. 45.6 years) (Tran-Fadulu et al., 2009). Interestingly, men with *TGFBR1* mutations presented primarily with ascending aortic disease, whereas women with *TGFBR1* mutations often presented with aneurysms and

dissections of other arteries. This led to lower rates of survival in men with *TGFBR1* mutations than women with *TGFBR1* mutations, which was not seen in the *TGFBR2* cohort. Finally, no individuals presented with aortic dissections at aortic diameters <5.0 cm with *TGFBR1* mutations, but three individuals did with *TGFBR2* mutations. Based on these results, Tran-Fadulu et al. suggest that individuals with *TGFBR1* mutations may not require prophylactic surgery for ascending aortic aneurysms at aortic diameters <5.0 cm due to their low risk of dissection at small diameters, though further data are required (Tran-Fadulu et al., 2009).

The recently published Montalcino Aortic Consortium (MAC) study of 441 *TGFBR1* or *2* mutation carriers further corroborated several of the findings by Tran-Fadulu et al., including no difference in overall survival between *TGFBR1* (median age of death 28.4) and *TGFBR2* (median age of death 34.8) mutation carriers but more aggressive disease in TGFBR2 mutation carriers (Jondeau et al., 2016). Among the seven patients who had aortic root diameters ≤4.5 cm within 1 year of dissection (only 22 patients had measurements available within 1 year or at time of dissection), all were women and six had *TGFBR2* mutations (average diameter 5.18 ± 1.34 cm for *TGFBR2* and 6.83 ± 2.30 cm for *TGFBR1*). Furthermore, as observed by Tran-Fadulu et al., the MAC study found that males with *TGFBR1* mutations had higher rates of type A dissection and aortic events than females with *TGFBR1* mutations, a differential which was not observed within the *TGFBR2* cohort. The MAC study also observed that type B dissections were a more frequent presenting feature of aortic disease in those with *TGFBR2* (6% of population) than in those with *TGFBR1* mutations (1.7% of population). Just as with MFS and vascular EDS, genotype–phenotype correlations were also observed in the MAC study, as the specific amino acid alteration in both *TGFBR1* and *TGFBR2* affected survival (Jondeau et al., 2016). In line with above studies demonstrating what appears to be a more severe disease phenotype in *TGFBR2* carriers, a recent cohort evaluating 33 genetically confirmed LDS adults over time found that those with *TGFBR2* mutations had a fivefold higher risk of requiring aortic surgery than those with *TGFBR1* mutations, with the surgeries tending to occur at younger ages (Teixido-Tura et al., 2016).

Initial reports indicated that the natural history of LDS involves early onset and rapid progression of TAAs with a mean age of death of 26.1 years, with aortic dissection being a major cause of death (Loeys et al., 2006; MacCarrick et al., 2014). However, other studies have challenged the concept that LDS patients always have a severe phenotype. In one study, Attias et al. found that when compared to patients with MFS, LDS two patients had similar rates of aortic dilatation (80%) and aortic dissection as well as similar rates of death once the disease had been diagnosed and medical care was provided (Attias et al., 2009). Furthermore, Texido-Tura et al. showed that the aortic dilation rate over a median period of 2 years among a cohort of 33 LDS adult patients was similar to previously calculated values for those with MFS (0.67 mm/year in LDS vs. 0.49 mm/year in MFS) (Teixido-Tura et al., 2016; Detaint et al., 2014). Texido-Tura et al. also observed a relatively milder aortic phenotype with a median age of first clinical presentation (surgery, death, dissection) of 50 as compared to the initial report describing a median age of 26 (Loeys et al., 2006). These differences may be explained by the inclusion of only adults in this recent study and the fact that the majority of patients had *TGFBR1* mutations (Teixido-Tura et al., 2016). The MAC study also confirms that survival is substantially better than originally thought, which may in part be explained by ascertainment bias (i.e., individuals with milder phenotypes were only reported after the initial description of patients with severe phenotypes and systemic features, which the MAC study demonstrates is associated with more severe aortic disease) and the implementation of better diagnostics and care (Jondeau et al., 2016). Nevertheless, these results indicate the remarkable heterogeneity in clinical severity among LDS patients, who exhibit considerable intra- and interfamilial variations (Teixido-Tura et al., 2016).

In sharp contrast to the increased risk of dissection in MFS patients at aortic diameters >5.0 cm, LDS 1–3 patients have been shown to dissect at aortic diameters of 3.9–4.0 cm (Loeys et al., 2006; MacCarrick et al., 2014). Therefore, current US guidelines indicate that those with LDS and mutations in *TGFBR1* or *TGFBR2* would benefit from prophylactic surgery at ascending aortic diameters between 4.0 and 4.2 cm (Andelfinger et al., 2016; Hiratzka et al., 2010; Boodhwani et al., 2014). However, as Milewicz et al. argue, these guidelines may be overly aggressive, particularly for medically treated adult patients (Milewicz et al., 2016). Jondeau et al. of the MAC study propose a more nuanced approach to prophylactic surgical repair for these patients based on gender, the mutated gene, family history of dissection, body surface area, and the presence of systemic features (Jondeau et al., 2016). In their analysis, Jondeau et al. found that systemic features, including arterial tortuosity, hypertelorism, and wide scars were significantly associated with a risk for aortic dissection after multivariate analysis and that females with *TGFBR2* mutations were at increased risk for aortic dissection at small aortic diameters. Therefore, they propose a threshold of 4.5 cm for LDS one and two patients, which can be lowered to 4.0 cm especially for women with *TGFBR2* mutations who have severe systemic features and a low body surface area (Jondeau et al., 2016). As the European guidelines state, more research is required to establish definitive criteria for surgical intervention (Erbel et al., 2014). Furthermore, Jondeau et al. recommend that patients who require prophylactic surgery should undergo complete ascending aorta replacement when possible, as 10% of individuals in the MAC study who underwent aortic root aneurysm surgery later presented with a type A dissection (Jondeau et al., 2016).

TGFB2 and *TGFB3*

TGFB2, the causative gene in LDS 4 (OMIM 614816), encodes the TGF-β2 ligand (MacCarrick et al., 2014). In 2012, Boileau et al. identified two mutations (frameshift mutation in exon 6 and nonsense mutation in exon 4) predicted to lead to haploinsufficiency in two large families with FTAAD, along with IAs and SAH (Boileau et al., 2012). They found that the youngest case of aortic dissection occurred at age 31 (mean of 35 years) and no dissections occurred at minimal levels of aortic enlargement (Boileau et al., 2012). Further heterozygous mutations or deletions were identified by Lindsay et al. in eight families with autosomal dominant TAAD and variable clinical expression (Lindsay et al., 2012). Boileau et al. and Lindsay et al. both demonstrated increased TGF-β signaling in the aortic walls of affected patients. In another cohort of 146 TAAD patients, four novel heterozygous loss-of-function

mutations in *TFGB2* were found among six patients, including two premature truncating mutations and a missense mutation (Renard et al., 2013b). There were two cases of type A aortic dissection, with one occurring at an aortic diameter <5.0 cm, and four cases of significant mitral valve disease (Renard et al., 2013b). Multiple other reports have found novel *TGFB2* mutations in patients affected by familial TAAD with variable expressivity of extra-cardiovascular manifestations, including skeletal and craniofacial abnormalities (Gago-Diaz et al., 2014; Leutermann et al., 2014; Ritelli et al., 2014a).

TFGB3, which encodes the TGF-β3 ligand, has recently been implicated in syndromic TAAD (known as LDS 5) (OMIM 615582) with associated craniofacial, skeletal, and cutaneous anomalies (Bertoli-Avella et al., 2015). Three truncating mutations and in-frame splice site mutations predicted to cause loss of function of *TGFB3* and *2* missense mutations in regions critical for TGF-β3 activation by integrins and TGF-β3 processing were identified (Bertoli-Avella et al., 2015). In this study of 43 affected individuals from 11 families, multiple mutation carriers had vascular abnormalities, including ascending aortic aneurysms and aneurysms of cerebral, iliac, and subclavian arteries. Four patients presented with type A and six patients presented with type B aortic dissections, occurring at median ages of 51 and 44.5, respectively, though no cases of aortic dissections at small diameters were found (Bertoli-Avella et al., 2015). Other cases of *TGFB3* mutations have been reported in pubertal age children presenting with characteristics of both MFS and LDS (Rienhoff et al., 2013; Kuechler et al., 2015), with one of the patients presenting with a mildly dilated aortic root (Matyas et al., 2014). Given the risk for aortic aneurysm and dissection, Bertoli-Avella et al. recommend that patients affected with *TGFB3* mutations undergo yearly echocardiograms to evaluate the aortic root as well the aorta and its side branches (Bertoli-Avella et al., 2015).

SMAD3 and other *SMAD* genes

SMAD3, the causative gene in LDS 3 (OMIM 613795), previously known as AOS, encodes a critical component of the canonical TGF-β signaling pathway (Regalado et al., 2011b). LDS 3 is characterized by arterial aneurysms, dissections, and tortuosity associated with mild craniofacial, skeletal, and cutaneous anomalies as well as early onset osteoarthritis and osteochondritis dissecans (van de Laar et al., 2011). The majority of the more than 20 pathogenic *SMAD3* mutations that have been identified (Zhang et al., 2015) involve the MH2 domain of the protein (Wischmeijer et al., 2013), a well-conserved region that is important for forming a complex with SMAD4 (van de Laar et al., 2011). This interaction is critical for translocation of the SMAD complex into the nucleus, where it can alter gene transcription (van de Laar et al., 2011). As these mutations cause a premature stop of translation through a frameshift or a substitution of highly conserved amino acids, it is believed this leads to a loss of function of *SMAD3* (Wischmeijer et al., 2013).

In the initial description of LDS 3, 60% of affected individuals had an aortic aneurysm or dissection, with some of the dissections occurring at minimally dilated ascending aortas (4.0–4.5 cm) (van de Laar et al., 2011). In another cohort of 45 patients described by van de Laar et al., cardiovascular abnormalities were found in 89% of affected individuals, including primarily TAAD as well as aneurysms of other arteries, left ventricular hypertrophy (in the absence of hypertension), atrial fibrillation, and congenital cardiac anomalies (van de Laar et al., 2012). Seventy-two percent of these patients had aortic aneurysms, mainly at the level of the sinuses of Valsalva, and 33% suffered from aortic dissections at aortic diameters ranging from 4.0 to 6.3 cm.

In a cohort of 44 patients with LDS 3 described by van der Linde et al., 71% of patients had an aortic root aneurysm and 15 deaths occurred at a mean age of 54 years (van der Linde et al., 2012), with aortic dissection accounting for 60% of deaths. Additionally, cerebrovascular abnormalities were diagnosed in 56% of patients and aneurysms of other arteries in the thorax and abdomen were diagnosed in 33% (van der Linde et al., 2012). Importantly, Wischmeijer et al. reported a patient with a *SMAD3* mutation who underwent prophylactic ascending aortic surgery for a progressively enlarging aorta at the age of only 12 months (Wischmeijer et al., 2013). Remarkably, the reported family exhibited a wide range of age of onset of TAAD (ranging from 1 to 54 years), variability which the authors attribute to unidentified genetic or environmental factors (Wischmeijer et al., 2013).

In addition to syndromic TAAD, Regalado et al. determined that *SMAD3* mutations account for 2% of familial TAAD (Regalado et al., 2011b). In this study of 42 affected individuals, 50% presented with TAAD, with several others presenting with IAs and AAAs. There is clearly a wide spectrum of clinical phenotypes, as some mutation carriers exhibited the characteristic extracardiovascular features of MFS and LDS (Regalado et al., 2011b). Individuals with *SMAD3* mutations warrant extensive cardiovascular imaging via echocardiogram plus imaging of the cerebral, abdominal, and thoracic vasculature, repeated regularly to monitor the growth of any identified aneurysms (van der Linde et al., 2012). Since cases of dissection have been reported at minimally enlarged aortic diameters, patients with *SMAD3* mutations would benefit from prophylactic surgery at diameters between 4.0 and 4.2 cm (Andelfinger et al., 2016).

In addition to *SMAD3*, *SMAD4*, which also encodes a key player in the canonical TGF-β signaling pathway, has been linked to TAAD (Teekakirikul et al., 2013). Two chart review studies of patients with both juvenile polyposis syndrome and hereditary hemorrhagic telangiectasia (JPS–HHT) (OMIM 175050), which are associated with heterozygous loss-of-function mutations in *SMAD4*, reported cases of aortic dissection in middle-aged individuals and several cases of aortic dilatation (Wain et al., 2014; Heald et al., 2015). Therefore, individuals with *SMAD4* mutations and JPS–HHT would benefit from screening for aortic aneurysms via echocardiography and computed tomography (Teekakirikul et al., 2013). On the other hand, gain-of-function mutations in *SMAD4* have not been associated with JPS–HHT but have been found to cause the rare autosomal dominant Myhre syndrome (OMIM 139210) (Lin et al., 2016). This syndrome has been shown to present with cardiovascular anomalies in 70% of patients, most commonly congenital heart disease, but also including juxtaductal aortic coarctation (15%), patent ductus arteriosus (20%), and diffuse descending aortic hypoplasia (6%) (Lin et al., 2016).

SMAD2, which encodes a SMAD protein that forms a complex with SMAD3 after its phosphorylation by the TGF-β receptor, has also been associated with TAAD (Micha et al., 2015). In this study, Micha et al. report four individuals from three families who were found to have *SMAD2* missense mutations located in the MH2 domain, the same evolutionarily conserved region where mutations are found to cause *SMAD3*-related TAAD (Micha et al., 2015). One of the probands, who developed a de novo *SMAD2* mutation, is a 17-year-old woman who was suspected of having MFS and presented with aortic root dilatation. Another affected individual presented with an ascending aortic aneurysm of 5.0 cm at age 46, while a third was found to have vertebral/carotid aneurysms and dissections; also, this patient's brother died from an abdominal aortic dissection at age 50 (Micha et al., 2015).

SKI

SKI is the causative gene in SGS (OMIM 182212), which is characterized by similar craniofacial, skin, skeletal, and cardiovascular features as MFS and LDS, along with intellectual disability and skeletal muscle hypotonia (Doyle et al., 2012; Shprintzen and Goldberg, 1982; Robinson et al., 2005). *SKI*, which encodes the Sloan Kettering proto-oncoprotein (SKI), serves as a negative regulator of the canonical TGF-β signaling pathway in several ways: by hindering SMAD2 and SMAD3 activation, preventing translocation of the activated SMAD2/3–SMAD4 complex into the nucleus, and by inhibiting transcription of TGF-β target genes (Doyle et al., 2012). To date, 17 different mutations in SGS patients have been identified, with all of them occurring in either the SMAD2/3 binding domain or the Dachshund-homology domain, which is crucial for binding to transcriptional corepressors (Schepers et al., 2015). Interestingly, all mutations occur in exon 1 and 73% of all affected patients have mutations affecting four adjacent SKI amino acid residues (Schepers et al., 2015). Though aneurysmal disease of the aortic root appears to be more benign (no cases of dissection) and less penetrant in SGS as compared to MFS and LDS, baseline echocardiography is recommended, with yearly screening in children followed by every 2–3 years in adult life (Schepers et al., 2015).

SLC2A10

SLC2A10, the causative gene in ATS (OMIM 208050), is a rare autosomal recessive disorder (approximately 100 affected individuals described to date) characterized by tortuosity of the aorta and medium-sized arteries with increased risk of aneurysm and dissection of the aorta and focal stenosis of the aorta and pulmonary artery (Bradley et al., 2016a; Coucke et al., 2006). ATS is also characterized by increased risk for ischemic vascular events, craniofacial abnormalities (high palate with dental crowding), musculoskeletal abnormalities (arachnodactyly, joint laxity, pectus excavatum, and hypotonia), hernias, and ocular involvement (Bradley et al., 2016a; Callewaert et al., 1993). Though early studies indicated severe disease with a mortality rate of 40% by 5 years of age (Wessels et al., 2004), more recent cohorts have demonstrated that patients live longer than previously thought (Callewaert et al., 2008; Ritelli et al., 2014b). One cohort of 3 families shows that 11 patients are alive with a mean age of 23 years (Ritelli et al., 2014b).

Mutations in *SLC2A10*, which encodes GLUT10, a facilitative glucose transporter, have been shown to lead to upregulation of TGF-β signaling (Coucke et al., 2006). Though the mechanism underlying this is not entirely understood, it is believed that loss-of-function mutations in *SLC2A10* lead to downregulation of decorin (its promoter contains a glucose response element), a natural inhibitor of TGF-β signaling, in VSMCs of ATS patients (Pomianowski and Elefteriades, 2013; Coucke et al., 2006). A total of 23 mutations have been identified to cause ATS, including 12 missense mutations, 5 nonsense mutations, 5 deletions leading to premature termination of translation, and 1 splice site mutation (Ritelli et al., 2014b). Ritelli at al. observed that 2 out of 12 patients had ascending/aortic root aneurysms (Ritelli et al., 2014b), while Callewaert et al. found that two adults (aged 23 and 35) out of 16 total patients had rapidly progressive aortic root dilation (Callewaert et al., 2008). However, given that the mean ages of these cohorts were 15 and 12.9 years, respectively, these patients may generally be too young to manifest with aortic root dilatation, which occurs more frequently in adulthood (Ritelli et al., 2014b). Affected patients require baseline full body vascular imaging with follow-up imaging depending on the results (and any family history of progressive aneurysmal disease); regular echocardiograms to determine aortic root diameters are also recommended (Callewaert et al., 2008; Castori et al., 2012).

Other Syndromes Associated With TAAD

In addition to the connective tissue disorders described above, several other syndromes have been associated with TAAD. First, Turner syndrome (TS), a sex aneuploidy syndrome due to loss of an X chromosome, can present with cardiovascular disease, including BAV, coarctation of the aorta, and TAAD (Halushka, 2014). One study found that 6.3% of TS patients had aortic dilatation or dissection (Lin et al., 1998), with many individuals presenting with TAAD at a young age (>50% of TS patients with aortic dissection were <30 years of age in another study (Carlson and Silberbach, 2007)). Second, Noonan syndrome (NS) (OMIM 163950), an autosomal dominant cardiofacial syndrome most commonly due to mutations in protein-tyrosine phosphatase, nonreceptor-type 11 (*PTPN11*), presents with cardiovascular involvement in 80%–90% of cases, most commonly pulmonary valve stenosis and hypertrophic cardiomyopathy (Halushka, 2014; Cornwall et al., 2014). In a cohort of 38 affected individuals, Cornwall et al. found that eight individuals (21.6%) had aortic root aneurysms, seven of which progressively dilated over an average follow-up interval of 6.8 years (Cornwall et al., 2014). Third, Alagille syndrome (OMIM 118450), an autosomal dominant disorder due to mutations in the NOTCH ligand jagged 1 (*JAG1*), can manifest with multiple cardiovascular abnormalities, including aortic

coarctation, pulmonary artery stenosis, large artery aneurysms, and moyamoya disease (Halushka, 2014). In a retrospective study of 268 affected patients, 16 had vascular abnormalities, including three with aortic aneurysms and several with IAs or intracranial artery anomalies (Kamath et al., 2004). Fourth, nonsense mutations in *COL4A5*, which encodes a component of type IV collagen and is the causative gene in X-linked Alport syndrome (XL-AS) (OMIM 301050), have been associated with aortic aneurysm and dissection (Kashtan et al., 2010; Earl et al., 2012). XL-AS primarily causes early-onset renal failure, sensorineural hearing loss, and ocular abnormalities (Halushka, 2014), although three of five patients in one study developed aneurysm and dissection of the thoracic aorta at ages ranging from 25 to 32 (Kashtan et al., 2010). Fifth, mutations in lysyl hydroxylase (*PLOD1*), the causative gene in EDS type VI (OMIM 225400), have been linked to aortic rupture and possibly aortic dissection, as well as rupture of other arteries (Wenstrup et al., 1989; Beighton, 1970). Last, autosomal dominant polycystic kidney disease (ADPKD), which is discussed more thoroughly in the IA section, has also been associated with TAAD.

BAV and TAAD

BAV, which affects 1%–2% of the general population, is the most common congenital heart defect (Luyckx and Loeys, 2015). It is estimated that BAV is associated with dilatation of the ascending aorta approximately 40%–50% of the time (Isselbacher et al., 2016a) and that 5% of individuals with a BAV will develop an aortic dissection (Elefteriades and Pomianowski, 2013). Therefore, BAV causes significantly more cases of aortic dissection than MFS (prevalence of 1/3–5000 people (Pyeritz, 2016)) despite the fact that there is a 40% risk of dissection in MFS patients (Elefteriades and Pomianowski, 2013). Aortic dissection associated with BAV typically occurs long before the aortic valve becomes significantly stenotic (Elefteriades and Pomianowski, 2013). The risk of aortic dissection in BAV patients is ninefold higher compared to patients with a normal tricuspid aortic valve, and 22% of aortic dissections in individuals younger than 40 are BAV related (Luyckx and Loeys, 2015).

Studies have clearly established the familial nature of BAV, which occurs in approximately 9% of first-degree relatives of affected patients (Huntington et al., 1997) and may have a heritability (an estimate of the genetic contribution to any phenotypic trait) as high as 89% (Andreassi and Della Corte, 2016; Cripe et al., 2004). It is believed that BAV is inherited via an autosomal dominant mechanism with decreased penetrance and variable expressivity, though the increased rates of BAV in individuals with TS provide evidence for a possible X-linked mechanism of inheritance as well (Andreassi and Della Corte, 2016). Unfortunately, however, the molecular genetics underlying BAV and associated TAAD remain unclear. While there are several monogenic causes of TAAD associated with BAV, including MFS (Nistri et al., 2012), LDS (Loeys et al., 2006; Girdauskas et al., 2011), *ELN*-related cutis laxa (Callewaert et al., 2011), *LOX*-related familial TAAD (Guo et al., 2016a), and *ACTA2*-related familial TAAD (Guo et al., 2007), these disorders collectively account for only a small percentage of all BAV cases (Isselbacher et al., 2016a). *NOTCH1* (Aortic valve disease 1 (AOVD1), OMIM 109730), which encodes a transmembrane receptor that functions as a transcriptional cofactor involved in cardiac embryogenesis and maintenance of the aorta, has been associated with BAV/TAAD (Garg et al., 2005; McKellar et al., 2007; Proost et al., 2015). Yet, mutations in *NOTCH1* are only estimated to contribute to 4% of BAV with or without TAAD (Andelfinger et al., 2016). Additionally, *NKX2.5* (Qu et al., 2014) and *GATA5* (Bonachea et al., 2014; Padang et al., 2012), which are both transcription factors critical for heart morphogenesis (Andreassi and Della Corte, 2016), have been implicated in BAV, though the same rare variants in *GATA5* have also been found in control patients (Foffa et al., 2013). *SMAD6* (AOVD2, OMIM 602931), which encodes an inhibitor of several important regulators of cardiogenesis that belong to the bone morphogenetic protein (BMP) family, has also been implicated in BAV (Tan et al., 2012). Several loci have been associated with BAV, including 18q, 5q, and 13q, though the specific genes within these chromosomal regions are yet to be identified (Andreassi and Della Corte, 2016; Martin et al., 2007) As Andelfinger et al. suggest, several factors may explain the difficulty with teasing out the genetic basis of BAV/TAAD, including its possible polygenic nature and its probable genetic heterogeneity, as well as the potential role for epigenetics and noncoding variants in contributing to the disease (Andelfinger et al., 2016).

Genomic Variation and TAAD

In addition to the established monogenic nature of TAAD, various single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) have been associated with an increased or decreased risk for TAAD. For instance, the rs2118181 SNP of the *FBN1* gene has been found to be highly associated with sporadic TAAD (LeMaire et al., 2011). Carriers of this SNP were shown to have a 76% increased risk of thoracic aortic dissection as compared to noncarriers (Iakoubova et al., 2014) (**Fig. 3**, panel A). Other SNPs of the *FBN1* gene have also been associated with increased risk for aortic dissection and increased risk for TAA formation (Lesauskaite et al., 2015). The Trp719Arg variant in the *KIF6* gene has also been linked to increased risk for aortic dissection. *KIF6* encodes a kinesin, which is a group of motor proteins that play a role in intracellular transport along microtubules (Iakoubova et al., 2008). This variant, which has also been found to be associated with myocardial infarctions and coronary artery disease, may alter the ability of KIF6 to transport its cargo by disrupting protein–protein interactions (Iakoubova et al., 2008). Carriers of this variant have a twofold higher risk of developing an aortic dissection than noncarriers (Iakoubova et al., 2016) (**Fig. 3**, panel B).

Moreover, Guo et al. discovered that the intronic SNP rs11172113 of the *LRP1* gene, which encodes low-density lipoprotein receptor-related protein 1, was associated with a decreased risk for sporadic thoracic aortic dissection (STAD) (Guo et al., 2016b). On the other hand, the same study found that the rs2272007 SNP of the *ULK4* gene, which encodes unc-51-like kinase 4, was

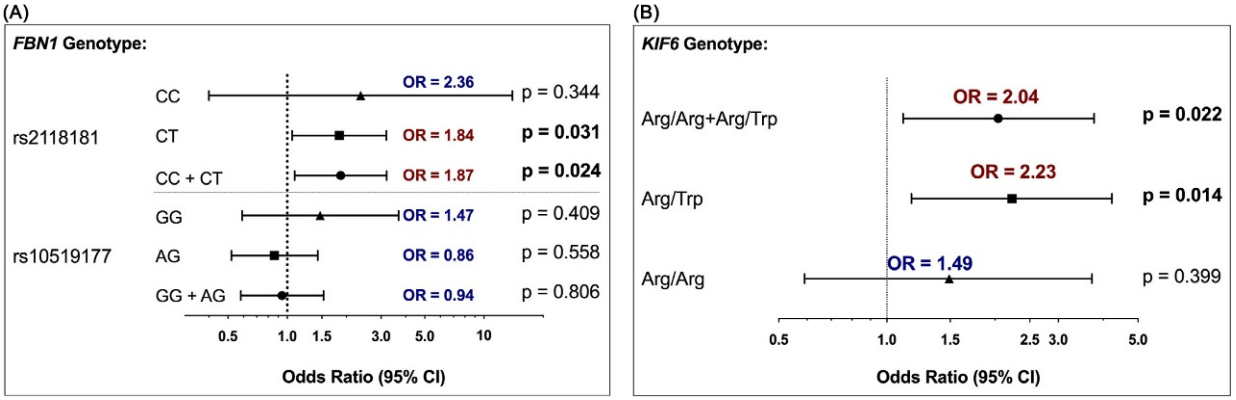

Fig. 3 Panel A—A substitution mutation at the rs2118181 locus on the *FBN1* gene predisposes twofold toward the development of aortic dissection; Panel B—The presence of the Arg/Trp and Arg/Arg + Arg/Trp alleles in the *KIF6* gene predispose toward the development of aortic dissection. (Panel A derived with permission from Iakoubova, O.A., Tong, C.H., Rowland, C.M., Luke, M.M., Garcia, V.E. and Catanese, J.J. et al. (2014). Genetic variants in FBN-1 and risk for thoracic aortic aneurysm and dissection. *PLoS One* **9**(*4*), e91437. Panel B derived with permission from Iakoubova, O.A., Tong, C.H., Catanese, J.J., Rowland, C.M., Luke, M.M. and Tranquilli, M. et al. (2016). KIF6 719Arg Genetic Variant and Risk for Thoracic Aortic Dissection. *Aorta (Stamford)* **4**(3), 83–90).

associated with an increased risk for STAD and that *ULK4* genomic deletions were associated with TAAD (Guo et al., 2016b). Other studies have also identified SNPs or haplotypes within matrix metalloproteinase genes that are associated with an increased risk for TAAD (Liu et al., 2016a; Wang et al., 2014; Kato et al., 2012; Chen et al., 2006). Additionally, TAAD has been associated with SNPs on 9p21 (near CDKN2B gene) and within the tumor necrosis factor-alpha gene (Du et al., 2016; Liu et al., 2010).

Furthermore, one study found that individuals with familial TAAD had a higher percentage of CNVs containing genes involved in SMC adhesion or contractility than those with sporadic TAAD (Prakash et al., 2010). In another study, Prakash et al. discovered 47 CNV regions in those with early onset TAAD (mean age 20, 55% with BAV), familial TAAD, and sporadic TAAD that were not present or were extremely rare in controls (Prakash et al., 2016). Patients with early onset TAAD were also more likely to have these rare CNVs than those with familial or sporadic TAAD. Therefore, Prakash et al. argue that, since the percentage of deleterious CNVs in cardiac and vascular developmental genes is increased among those with early onset TAAD, testing for the presence of these CNVs may help predict who is at increased risk for TAAD (Prakash et al., 2016).

Genetic Testing for TAAD

Though studies have clearly demonstrated that at least 20% of TAAD individuals have a positive family history, only one-quarter to one-third of familial TAAD can be explained by our current genetic understanding (Luyckx and Loeys, 2015). It is currently estimated that syndromic genes, including *FBN1* and LDS genes (*TGFBR1*, *TGFBR2*, *SMAD3*, *TGFB2*), account for 10% of familial non-syndromic TAAD, *ACTA2* accounts for 12%–21%, and all other known genes collectively account for 1%–2% (Milewicz et al., 2016). Therefore, there is undoubtedly a substantial degree of genetic heterogeneity contributing to TAAD. There are many more genes yet to be discovered, including those in different chromosomal loci that have previously been linked to TAAD without identification of a specific causal genes (Luyckx and Loeys, 2015), such as 5q13–14 (OMIM 607087) (Kakko et al., 2003; Guo et al., 2001), 11q23–24 (OMIM 607086) (Vaughan et al., 2001), 15q24–26 (Pannu et al., 2006), and 12q13–14 (Guo et al., 2011).

Given the clear genetic etiology of TAAD, next-generation sequencing, including whole exome sequencing (WES) (DNA sequencing the exons (coding regions) of all genes in the genome (Biesecker and Green, 2014)), is being utilized more frequently to identify causative mutations and genes (Milewicz et al., 2014; Proost et al., 2015; Ziganshin et al., 2015). WES of patients with TAAD has multiple advantages: (1) WES is likely to identify pathogenic mutations, since approximately 85% of causative mutations for Mendelian disease are found in coding regions or canonical splice sites (Milewicz et al., 2014), (2) WES is comprehensive, (3) data can be reanalyzed as new genes are discovered to cause TAAD, (4) WES is becoming more cost-effective than conducting single gene tests, and (5) WES allows for the identification of new potential causative genes and variants.

To date, the Aortic Institute at Yale-New Haven has completed WES for 211 patients with TAAD (mean age 56.5 ± 14.2 years (range 15–83)). 23 specific genes associated with TAAD were analyzed: *ACTA2*, *ADAMTS10*, *COL1A1*, *COL1A2*, *COL3A1*, *COL5A1*, *COL5A2*, *ELN*, *FBLN4*, *FBN1*, *FBN2*, *FLNA*, *MAT2A*, *MYH11*, *MYLK*, *NOTCH1*, *PRKG1*, *SKI*, *SLC2A10*, *SMAD3*, *TGFBR1*, *TGFBR2*, *TGFB2*. Of the 211 patients, 150 (71.1%) were found to carry no mutations in this panel of genes. Among the remaining 61 individuals, 68 variants were identified as potentially causative of TAAD with 65 of these variants occurring in the panel of tested genes. Three deleterious variants occurred in other genes, namely *TGFB3* ($n = 1$) and *MIB1* ($n = 2$). Among the 65 variants in TAAD-related genes, seven were found to be deleterious (*FBN1* ($n = 3$), *TGFBR2* ($n = 2$), *FLNA* ($n = 1$), *MYLK* ($n = 1$)), while 58 were classified as variants of unknown significance. Variants in *FBN1*, *NOTCH1*, and *MYH11* were the most commonly seen (17%, 15%, and 14%, respectively) (Fig. 4). These findings differ from prior reports that identified ACTA2 as the most commonly affected gene in FTAAD.

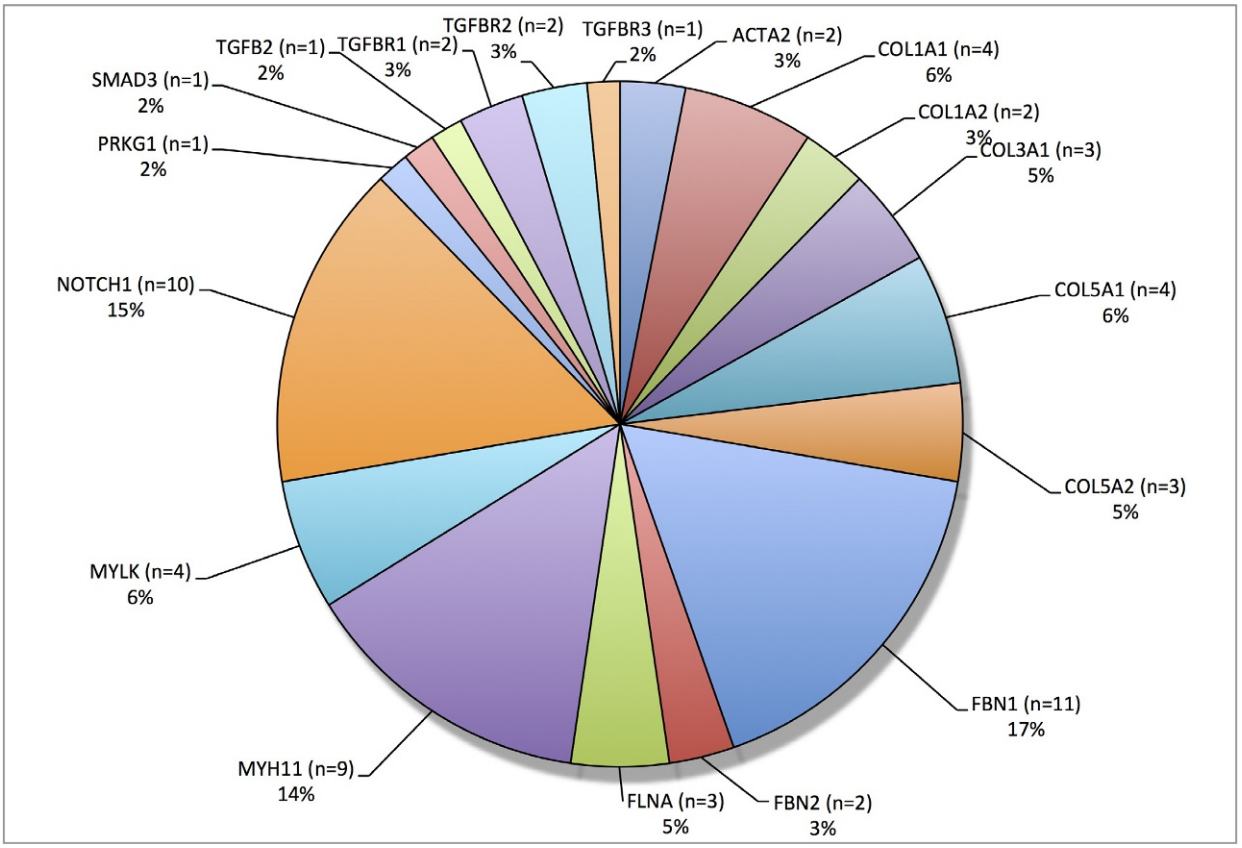

Fig. 4 Frequency distribution of genetic defects in TAAD-related genes based on the Yale Aortic Institute routine genetic testing program.

Genetics of TAAD: Conclusion

Since the identification of the *FBN1* gene as causative of MFS (Dietz et al., 1991), there has been a rapid advancement in our understanding of the genetics of TAAs and dissection. The familial nature of TAAD was a key turning point that led investigators to discover that multiple genes which alter the ECM, SMC contraction and metabolism, and TGF-β signaling pathways are involved in the pathogenesis of this disease. Studies have clearly demonstrated that the underlying causative gene in any given family can predict who is at risk for TAAD, how their aortic disease will manifest, and what is their risk for dissection at different aortic sizes (Milewicz et al., 2016). Therefore, we believe that personalized care based on the underlying mutation in any given individual is and will continue to be a critical component of effective and first-rate patient care. As genetic testing becomes more widely adopted, individuals at risk for TAAD will be identified earlier and thus provided with regular cardiovascular imaging, medical therapy, and timely prophylactic surgery to prevent the devastating complication of aortic dissection. Furthermore, WES will permit discovery of novel variants and genes, leading to construction of a comprehensive library of genetic variants linked to TAAD. Although, as surgeons, we emphasize the elegance and effectiveness of surgical therapies for TAAD, we recognize that the most important breakthrough in combating this fatal disease is currently, and will continue to be, on the genetic front.

Genetics of AAA

An AAA is defined as a permanent localized dilatation associated with an aortic diameter ≥ 3.0 cm (Kuivaniemi et al., 2015b). The natural history of AAAs is asymptomatic expansion until rupture occurs, which is the 16th leading cause of death in the United States (Go et al., 2014) and has an overall mortality rate of 85%–90% (Kent, 2014). The pathophysiology of AAAs involves inflammation, destruction of elastin and collagen in the media and adventitia, loss of SMCs, and neovascularization (Kent, 2014). It is also hypothesized that autoimmunity contributes to AAA formation and progression (Kuivaniemi et al., 2015b).

Studies have clearly demonstrated an overlap between AAAs and TAAs. While Larsson et al. found that 28% of individuals with AAAs had concomitant TAAs (Larsson et al., 2011), Chaer et al. observed that individuals with a AAA have a 10%–15% chance of

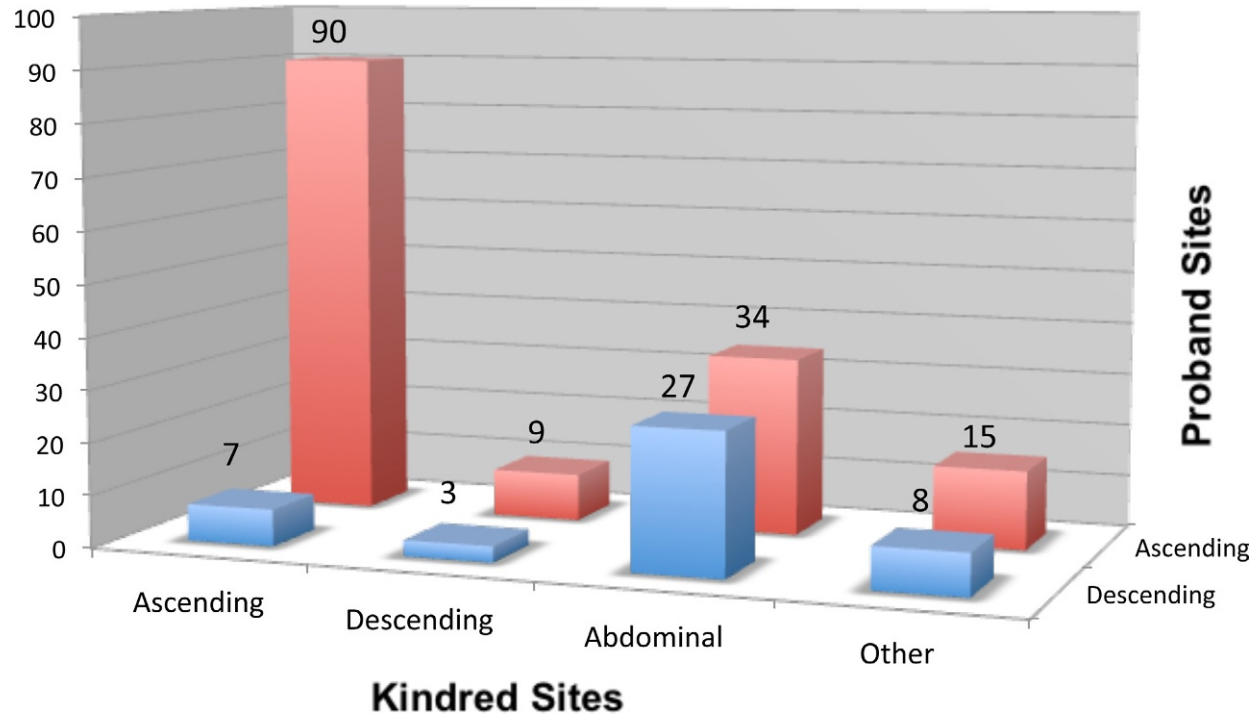

Fig. 5 Distribution of sites of arterial aneurysms and dissections in kindred of familial probands. (Modified with permission from Albornoz, G., Coady, M.A. and Roberts, M. et al. (2006) Familial thoracic aortic aneurysms and dissections—Incidence, modes of inheritance, and phenotypic patterns. *The Annals of Thoracic Surgery* **82**, 1400–1405).

having a synchronous (diagnosed within 2 years of AAA diagnosis) or metachronous (diagnosed >2 years after AAA diagnosis) TAA (Chaer et al., 2012). Despite these results, the vast majority of studies indicate that AAAs and TAAs independently segregate within families (Kuivaniemi et al., 2015b). In fact, Albornoz et al. demonstrated that individuals with ascending aortic disease were most likely to also have family members with ascending aortic disease, while individuals with descending aortic disease were most likely to have family members with AAAs (Albornoz et al., 2006) (**Fig. 5**). The fact that AAAs and TAAs appear to represent distinct disease processes may be related to the differing embryological origin of the ascending aorta (neural crest) versus the descending and abdominal aorta (mesoderm) (Liu et al., 2015; Cheung et al., 2012; Gittenberger-de Groot et al., 1999; Ruddy et al., 2013). While ascending aneurysms are highly familial, non-calcified, and non-atherosclerotic, descending and AAAs are highly associated with atherosclerosis and are often calcified and irregular with clot formation (Elefteriades and Pomianowski, 2013) (**Fig. 6**). Though there is significant overlap between the risk factors for AAA and for atherosclerosis (smoking, advanced age, male sex, and family history), there does not appear to be a strong common genetic background (Saratzis and Bown, 2014; Annambhotla et al., 2008).

Since the 1970s and 1980s, when the first studies of familial aggregation of AAAs were originally reported (Clifton, 1977; Tilson and Seashore, 1984a,b; Norrgard et al., 1984), it has become clear that family history, along with smoking, advanced age, and male sex, is a significant risk factor for AAA development (Kuivaniemi et al., 2015a). Though smoking is the most important risk factor for AAA development (Kuivaniemi et al., 2015b; Kent et al., 2010), having a family history increases an individual's risk for development of an AAA between 2- and 11-fold (Rolph et al., 2015). Two twin studies, one Swedish and one Danish, demonstrated higher concordance rates of AAAs between monozygotic twins than dizygotic twins (24% vs. 5% and 30% vs. 12%, respectively), with a calculated heritability of 70% and 77%, respectively (Wahlgren et al., 2010; Joergensen et al., 2016). While both interview studies and screening studies have observed a wide range estimating the familial nature of AAAs (average of 12%), Sakalihasan et al. found that approximately 10% of 618 AAA probands reported a positive family history in one study (Sakalihasan et al., 2014). In this analysis, ultrasound screening identified a prevalence of AAAs of 13% for all family members of AAA patients and a prevalence of 25% for brothers. Overall, the concordance rates between siblings varies between genders, as 20% of brothers and 5.8% of sisters of patients with AAA will develop one as well (Kuivaniemi et al., 2015a). Interestingly, in a cross-sectional analysis of a Danish population, Joergensen et al. found that first-degree relatives of a female patient with a AAA were greater than 2.5 times more likely to have a AAA than those with an affected male relative (OR = 2.65, 95% CI: 1.37–5.13) (Joergensen et al., 2014).

Furthermore, studies have demonstrated that familial AAAs may behave differently from sporadic AAAs. Individuals with familial AAAs are more likely to be female, present at a younger age, and manifest fewer cardiovascular risk factors and decreased carotid intima-media thickness (van de Luijtgaarden and Verhagen, 2015; van de Luijtgaarden et al., 2014a). Joergensen et al. demonstrated that individuals with familial AAAs have a significantly larger mean aortic diameter (20.5 mm) compared to those

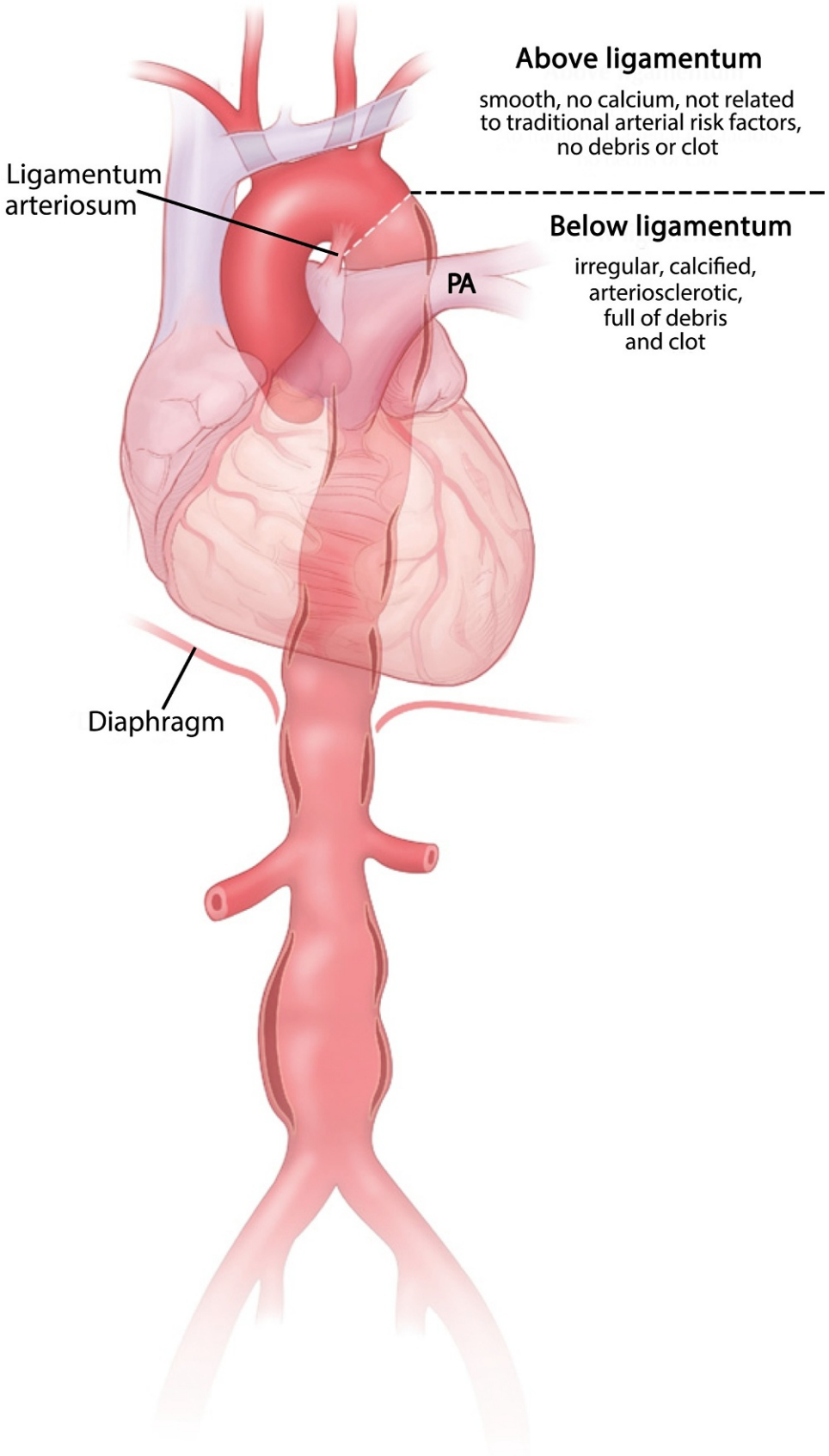

Fig. 6 Aortic aneurysm is really two diseases: ascending/arch disease differs markedly from descending/abdominal disease. (Reprinted with permission from Elefteriades, J.A. and Farkas, E.A. Thoracic aortic aneurysm clinically pertinent controversies and uncertainties. *Journal of the American College of Cardiology* **55**, 841–857).

with no family history (Joergensen et al., 2014), which, as van de Luijtgaarden et al. propose (van de Luijtgaarden and Verhagen, 2015), may explain the observed higher rate of AAA rupture in familial cases (Sakalihasan et al., 2014; Verloes et al., 1995). Moreover, Akai et al. found that the AAAs of those with a positive family history grew at more than two times the rate of those with a negative family history (4.2 mm/year vs. 2.0 mm/year, respectively; $P = .009$) (Akai et al., 2015). While Brewster et al. reported a trend toward increased aneurysm-related mortality after endovascular aneurysm repair (EVAR) in familial AAA patients (Brewster et al., 2006), other studies have identified increased rates of aneurysm-related complications, endoleaks, and secondary intervention following EVAR in familial AAA patients (van de Luijtgaarden et al., 2014b; Ryer et al., 2015). Similarly, Coscas et al. reported that having a family history of AAAs was significantly associated with an increased risk of late failure after open surgical aneurysm repair (Coscas et al., 2010). Therefore, as appears to be the case with TAAs, it is possible that familial AAAs represent a more virulent disease than sporadic AAAs.

Segregation analyses of affected families have demonstrated that AAAs appear to be genetically mediated and that familial AAAs exhibit either autosomal dominant or autosomal recessive modes of inheritance (Verloes et al., 1995; Majumder et al., 1991). In a study of 233 affected families, Kuivaniemi et al. demonstrated that 72% of families appeared to exhibit an autosomal recessive mode of inheritance, while 25% were consistent with an autosomal dominant mode of inheritance (Kuivaniemi et al., 2003). Despite these segregation studies, it has been challenging to identify causative genes of familial AAA given the late onset of this disease (van de Luijtgaarden and Verhagen, 2015). There are several monogenic connective tissue diseases, such as MFS, LDS, and EDS, that can be associated with AAA (Ahmed et al., 2016). Interestingly, a recent study investigating TAAD genes in familial and sporadic AAA patients found 47 rare heterozygous variants, including one pathogenic, segregating variant in *COL3A1* (p.Arg491X) in familial AAA, one likely pathogenic segregating variant in *MYH11* (p.Arg254Cys) in familial AAA, and one pathogenic variant in *TGFBR2* (c.1573delA) in sporadic AAA (van de Luijtgaarden et al., 2015). Therefore, while there may be monogenic causes of AAA, it is nevertheless considered a complex, multifactorial disease with a strong genetic component (Kuivaniemi et al., 2015a,b).

Over 100 genetic association studies have been conducted on AAAs using SNPs in biologically plausible genes, including genes involved in the ECM, cardiovascular system, immune system, and cellular signaling (Kuivaniemi et al., 2015b; Hinterseher et al., 2011). Genome-wide studies using family-based linkage analysis and GWAS have also been conducted to identify potential genes/loci involved in AAA pathogenesis (Kuivaniemi et al., 2015b). Linkage analysis has identified two chromosomal loci, 4q31 and 19q13 (Van Vlijmen-Van Keulen et al., 2005; Shibamura et al., 2004), demonstrating significant linkage, although the causative genes have not been identified (Kuivaniemi et al., 2015a). GWAS and candidate gene studies have identified a multitude of genes potentially linked to AAA, which are summarized in an extensive review by Hinterseher et al. (2011). However, a recent review by Bradley et al. illustrates that many of the observed associations of SNPs with AAAs are spurious due to biases, failed replication studies, or flawed methodology, including failure to correct for multiple testing, deviation from Hardy–Weinberg equilibrium, and flexible analysis (Bradley et al., 2016b). This article and others (Kuivaniemi et al., 2015a,b; Hinterseher et al., 2011) have extensively reviewed the most significant genetic associations for AAAs.

The identified genes with SNPs that are most significantly associated with AAAs encode either protein or RNA products involved in a wide variety of physiologic processes, such as cell growth, lipid metabolism, and inflammation (Kuivaniemi et al., 2015a,b). Two genes involved in cell growth have been significantly associated with AAAs. First, the rs10757278 SNP in *CDKN2BAS*, which encodes an antisense RNA that plays an important role in cell proliferation and apoptosis by regulating expression of cyclin-dependent kinase inhibitor 2A and 2B (Kuivaniemi et al., 2015a), was associated with both AAAs (OR = 1.31, $P = 1.2 \times 10^{-12}$) and IAs (OR = 1.29, $P = 2.5 \times 10^{-6}$) in a GWAS analysis (Helgadottir et al., 2008). Second, another GWAS identified a significant association of AAA with the rs7025486 SNP of *DAB2IP* (OR = 1.21, $P = 4.6 \times 10^{-10}$), which encodes DAB2-interacting protein, an inhibitor of cell proliferation and survival (Gretarsdottir et al., 2010). SNPs in five genes involved in lipid metabolism have also been significantly associated with AAAs, including *SORT1* (sortilin-1) (Jones et al., 2013), *LDLR* (low density lipoprotein receptor) (Bradley et al., 2013), *LRP1* (low density lipoprotein receptor-related protein 1) (Bown et al., 2011), *LPA* (apolipoprotein (a)) (Helgadottir et al., 2012), and *LRP5* (low density lipoprotein receptor-related protein 5) (Galora et al., 2013).Consistent with the role of inflammation in the pathogenesis of AAAs (Dale et al., 2015; Kuivaniemi et al., 2008), candidate gene studies have identified significant associations of a SNP in *IL6R* (Interleukin 6 receptor) (Harrison et al., 2013) and a SNP in *IL1RN* (Interleukin 1 receptor anatagonist) (Interleukin 1 Genetics C, 2015) with AAAs. Furthermore, a recent meta-analysis of 6 GWAS data sets verified the association of *SORT1*, *LDLR*, *IL6R*, *DAB2IP*, and *CDKN2BAS* with AAA and identified 4 novel loci significantly associated with AAA: 1q32.3 (*SMYD2*), 13q12.11 (*LINC00540*), 20q13.12 (near *PCIF1/MMP9/ZNF335*), and 21q22.2 (*ERG*) (Jones et al., 2017). Interestingly, these novel SNPs were not found to be associated with coronary artery disease, lipids, diabetes, or hypertension, indicating that while there is overlap between AAA and other cardiovascular diseases, as Jones et al. maintain, AAA nevertheless represents a distinct clinical phenotype (Jones et al., 2017). Though there have been multiple genes associated with AAAs, the causal relationship between many of these SNPs and AAA development needs to be investigated further (van de Luijtgaarden and Verhagen, 2015).

In a study of 3.1 million individuals, Kent et al. determined that more than 50% of persons predicted to have AAAs do not meet the established screening criteria set forth by the U.S. Preventative Services Task Force (men aged 65–75 ever smokers) (Kent et al., 2010). Given that more than 50% of AAA ruptures occur outside the target population stated above, scoring tools have been developed to detect large AAAs (>5.0 cm) in the general population (Greco et al., 2010). Currently, there are no risk scores or screening tests utilizing genetics that can be applied to clinical practice to help predict who will develop AAAs and which AAAs are more likely to rupture (Kuivaniemi et al., 2015a). Additionally, each allele in the genes described above may only increase an individual's risk of developing a AAA by 20% (van de Luijtgaarden and Verhagen, 2015). Since AAA is a disease with a complex

pathogenesis, however, the use of multiple polymorphisms in various genes may improve one's ability to risk stratify patients (Kuivaniemi et al., 2015b). For instance, Saracini et al. found that the presence of three or four polymorphisms in genes associated with AAA (*MMP2*, *MMP3*, *MMP13*, and *ELN*) was a strong and independent determinant of AAA disease (OR = 2.96, $P < .0001$) (Saracini et al., 2012). Furthermore, Ye et al. constructed a genetic risk score using SNPs in 4 genes significantly associated with AAA (*LRP1*, *DAB2IP*, *CDKN2A-2B*, and *SORT1*) and found that not only was the risk score associated with AAA independent of conventional risk factors and family history (OR = 1.06, $P < .001$) but also a higher risk score was associated with greater aneurysm growth rate (Ye et al., 2016).

Despite the well-established familial nature of AAAs (family history warrants early screening for men at age 55 and women at age 65 (Kuivaniemi et al., 2015a; Chaikof et al., 2009)), the complex nature of AAAs and the late onset of the disease have made it challenging to discover causative genes. More genetic studies are clearly required to improve our understanding of this potentially fatal disease, as the known genetic polymorphisms account for a small percentage of familial AAAs (Kuivaniemi et al., 2015a).

Genetics of IAs

Saccular IAs, which are estimated to affect 2%–5% of the adult population, are pathologic dilatations that commonly occur at proximal arterial bifurcations in the Circle of Willis (Nasr and Brown, 2016; Etminan and Rinkel, 2017; Vlak et al., 2011). IAs account for 80%–85% of non-traumatic SAH (Brown and Broderick, 2014; Kassell et al., 1990), which has a mortality rate of 42%–47% (Frosen, 2014; Karamanakos et al., 2012; Nieuwkamp et al., 2009). Though the pathogenesis of IAs is not entirely understood, it is thought that a combination of hemodynamic factors, including hemodynamic stress and vascular risk factors (hypertension, lipid accumulation, arteriosclerosis, and smoking), and genetic predisposition contribute to IA formation (Etminan and Rinkel, 2017). Therefore, IA is considered a complex disease with multiple modifiable and non-modifiable risk factors, including smoking (relative risk, 2.2–3.1), high blood pressure (relative risk, 2.5–2.6), heavy (>150 g/week) alcohol consumption (relative risk, 1.5–2.1), female gender (3:1 prevalence), older age (>50 years), ethnicity (increased risk among Finnish and Japanese descent), family history of IA or SAH, and the presence of certain hereditary diseases (Nasr and Brown, 2016; Bourcier et al., 2015).

Studies have clearly demonstrated that family history is an important risk factor for the development of IAs. In a study of 91 families with two or more affected family members without diagnosed heritable conditions, Ronkainen et al. found that the prevalence of IAs among first-degree relatives was 8.7% (Ronkainen et al., 1997). In a separate Finnish population study, the relative risk for unruptured IAs was 4.2 times higher in first-degree relatives of families with more than one IA case than in the general population (Ronkainen et al., 1998). In a cohort of patients in the familial intracranial aneurysm (FIA) study, among 303 individuals who were first-degree relatives of an individual with an IA, >30 years of age and had a history of smoking and/or hypertension, 19.1% were found to have an IA (Brown et al., 2008). Furthermore, in a long-term follow-up study of individuals with at least two first-degree relatives with SAH or unruptured IAs, family history was found to be a significant risk factor for IA at the first screening, when 11% were identified as having IAs (Bor et al., 2014). Although a personal history of IAs was the only risk factor for IA development at follow-up screening in this study, individuals with a positive family history are still at risk for development of IAs even after 10 years of follow-up (Bor et al., 2014). Consistent with these studies, the current guidelines recommend screening individuals with two or more first-degree relatives with SAH or unruptured IAs (Thompson et al., 2015).

Studies have also demonstrated that individuals with a family history of IAs are more likely to have multiple aneurysms and that the location of IAs are more likely to be shared among first-degree relatives (Mackey et al., 2012, 2013). Moreover, monozygotic twins from the FIA study exhibited higher rates of arterial location concordance than dizygotic twins, implying that genetics may influence IA location (Mackey et al., 2015). In addition to affecting the presence and possibly the location of IAs (familial IA occurs more commonly in the middle cerebral artery (Mackey et al., 2012)), family history of IAs is regarded as an important risk factor for IA rupture (Etminan and Rinkel, 2017). Broderick et al. demonstrated that individuals in the FIA study had IA rupture rates 17 times higher than comparative individuals with no family history of IAs (Broderick et al., 2009). Furthermore, the lifetime risk of SAH is increased 50-fold in those with ≥ 2 first-degree relatives with IAs compared to the general population (Etminan and Rinkel, 2017). Interestingly, geometric and morphologic characteristics associated with an increased risk for IA rupture (non-spherical shape, aspect ratio ≥ 1.6 (relation between length and neck of the IA), contact with or deformation by the perianeurysmal environment, and dominance of the posterior communicating artery in the case of IAs of that artery) cannot explain the increased rupture rates in familial IA patients (Mensing et al., 2016). Aneurysmal SAH patients with a positive family history have also been shown to present, at slightly younger ages (Bromberg et al., 1995a; Huttunen et al., 2010), to have poorer outcomes (Bromberg et al., 1995b) and to have larger aneurysms at the time of rupture (Ruigrok et al., 2004) than those with sporadic SAH. Despite these results, the largest twin study of SAH conducted to date estimated that the heritability of SAH is only 41%, implying that familial SAH is mostly attributable to environmental risk factors (Korja et al., 2010). Additionally, although Bor et al. found a strong relationship between the development of aneurysmal SAH and the presence of ≥ 2 affected relatives (odds ratio = 51 as compared to those with no affected relatives), only 0.2% of SAH patients had two or more affected relatives (Bor et al., 2008). Korja et al. therefore maintain that a positive family history only accounts for a small percentage of SAH and that the familial clustering of modifiable risk factors, including smoking and hypertension, may explain the increased rates of SAH seen in these individuals, as opposed to an underlying genetic etiology (Korja and Kaprio, 2016; Korja et al., 2010).

Nevertheless, it is currently thought that both genetic and environmental risk factors interact to contribute to the development of IAs (Etminan and Rinkel, 2017). Among affected individuals with a family history, autosomal dominant inheritance appears to be

the most common mode of transmission (Thompson et al., 2015), although one large study of 346 IA Finnish families found that autosomal recessive inheritance appeared to account for 57.2% of cases with the other cases being consistent with autosomal dominant inheritance (Wills et al., 2003). One study demonstrated that <10% of IA cases are associated with other conditions, such as BAV and aortic coarctation, or inherited syndromes, including ADPKD, EDS type 4, MFS, pseudoxanthoma elasticum (OMIM 264800), HHT, neurofibromatosis type 1 (OMIM 162200), alpha-1 antitrypsin deficiency (OMIM 613490), NS, alpha-glucosidase deficiency (OMIM 232300), and microcephalic osteodysplastic primordial dwarfism (OMIM 21072) (Thompson et al., 2015; Wiebers et al., 2003). ADPKD, EDS type 4, and microcephalic osteodysplastic primordial dwarfism are also associated with aneurysmal SAH (Thompson et al., 2015).

Autosomal dominant polycystic kidney disease (ADPKD), which is caused primarily by mutations in *PKD1* (OMIM 173900) or *PKD2* (OMIM 613095), is associated with aneurysms and dissections of almost every large artery, including the aorta and intracranial arteries (Perrone et al., 2015). IAs affect approximately 9%–12% of individuals with ADPKD (Perrone et al., 2015), and thus this disorder may increase the risk for IA formation 3- to 14-fold (Thompson et al., 2015). It appears that the risk of rupture of IAs in ADPKD patients is similar to that in the general population, although rupture tends to occur at younger ages (41 vs. 51 years, respectively) (Perrone et al., 2015). Interestingly, the same two base pair deletion variant in PKD1 was identified in two separate ADPKD + IA families (Rossetti et al., 2003; Watnick et al., 1999), suggesting that specific mutations may be associated with increased rates of IA formation among ADPKD patients (Perrone et al., 2015).

Among IA patients without an inherited syndrome, no genes have been identified yet that are strongly associated with IA development (Etminan and Rinkel, 2017). Multiple articles have reviewed the genes and chromosomal loci that have been associated IAs (Nahed et al., 2007; Tromp et al., 2014; Bourcier et al., 2015; Zhang and Claterbuck, 2008; Ruigrok and Rinkel, 2008; Caranci et al., 2013). Chromosomal loci identified via linkage studies, which have been replicated in different populations and are thus most strongly associated with IA formation (Bourcier et al., 2015), include 1p34.3–36.13 (Nahed et al., 2005), 7q11 (Onda et al., 2001), 19q13.13 (Mineharu et al., 2007), and Xp22 (Yamada et al., 2004). Though no specific genes at the 19q and Xp22 loci have been associated with IAs, a possible candidate gene at chromosomal locus 1p34.3–36.13 is *HSPG2* (Ruigrok and Rinkel, 2008). This gene encodes a heparin sulfate proteoglycan involved in arterial wall ECM maintenance, though only a moderate association was found (Bourcier et al., 2015; Ruigrok and Rinkel, 2008). Moderate associations were also identified at the chromosomal locus 7q11 for the *ELN* and *COL1A2* genes (Bourcier et al., 2015). A GWAS also identified the loci 12q22 and 20p12.1 as significantly associated with IAs (Yasuno et al., 2010).

In a meta-analysis of 61 GWAS and candidate gene association studies, Alg et al. identified 19 SNPs associated with IAs, with three chromosomal loci exhibiting the strongest associations: 9p21.3, 8q21.3, and 4q31.22 (Alg et al., 2013). The majority of genes implicated in this study are involved in vascular endothelial maintenance and ECM integrity (Alg et al., 2013). The chromosomal locus 9p21.3 contains the *CDKN2B/2A/2BAS* genes (Alg et al., 2013), which have also been associated with AAAs, TAAD, and coronary artery disease (Helgadottir et al., 2008). *CDKN2A* encodes a tumor suppressor protein that inhibits cyclin-dependent kinase 4, *CDKN2B* encodes a protein that prevents cell proliferation through induction by TGF-β, and *CDKN2BAS* is a noncoding RNA involved in transcriptional repression (Hussain et al., 2013). SNPs at the 9p21 locus have also been independently associated with IA rupture (Olsson et al., 2011). The 8q21.3 locus contains the *SOX17* gene, which plays a prominent role in the production and maintenance of vascular endothelial and hematopoietic stem cells (Alg et al., 2013). Through a meta-analysis, Foroud et al. further validated the association of the 9p21 and 8q21 chromosomal loci with IAs (Foroud et al., 2012). The 4q31 locus harbors *EDNRA*, which encodes a G-protein-coupled receptor for endothelins, which regulate vascular response after hemodynamic insult (Alg et al., 2013). Yasuno et al. also identified three chromosomal loci strongly associated with IA: 10q24.32, 13q13.1, and 18q11.2, which contain *CNNM2*, *STARD13*, and *RBBP8*, respectively (Yasuno et al., 2010). *CNNM2* encodes a protein involved in epithelial absorption of Mg^{2+} in the kidneys; *STARD13* is a tumor suppressor gene involved in endothelial cell migration and attachment; *RBBP8* encodes a protein that modulates cell cycle progression and DNA repair via interaction with retinoblastoma protein and P53 (Hussain et al., 2013).

Interestingly, one study of a genetic risk score calculated using 10 SNPs associated with IAs found that the risk score was only associated with the size of IAs as opposed to their presence in the general population (Peymani et al., 2015). The common variants in the above genes identified via GWAS only explain a fraction of the genetic risk for IAs (Farlow et al., 2015). In a recent WES study of 7 highly affected IA aneurysm families, 68 rare variants were identified in 68 genes segregating with disease, with only one gene being differentially expressed in aneurysmal tissue compared to control tissue: *TMEM132B* (Farlow et al., 2015). A heterozygous variant within this gene, which encodes a transmembrane protein of unknown function, was predicted to be pathogenic by altering a highly conserved amino acid (Farlow et al., 2015). Additionally, a recent study by Santiago-Sim et al. identified a nonsense mutation in Thrombospondin type 1 domain containing protein 1 (*THSD1*) that segregated with disease within a highly affected IA/SAH family as well as several missense *THSD1* mutations in 8 of 507 unrelated IA probands (Santiago-Sim et al., 2016). Functional analyses of *THSD1* demonstrated that disruption of the encoded protein leads to impaired endothelial cell adhesion to the basement membrane of cerebral arteries, which may explain the cerebral bleeding phenotype observed in the *Thsd1* loss-of-function animal models studied and possibly the development of IAs in affected patients (Santiago-Sim et al., 2016).

Further research is required to elucidate the complex genetics underlying IAs. Many of the SNPs associated with IAs occur in noncoding regions, indicating that studies are needed to address the regulatory function of noncoding SNPs and/or to identify the actual pathogenic variants within coding regions (Tromp et al., 2014). Further studies are required to replicate recent findings associating polymorphisms in multiple genes, including *RNF213* (Zhou et al., 2016), *ADAMTS* genes (Arning et al., 2016), *APOE* (Liu et al., 2016b), and *WWOX* (Fan et al., 2016) with IAs. According to Etminan et al., an unpublished systematic review by

Kleinloog et al. has identified 10 genetic factors associated with IA rupture (Etminan and Rinkel, 2017). Therefore, understanding the differential genetic risk between IA development and rupture through GWAS and next-generation sequencing approaches will hopefully permit the development of risk stratification tools based on underlying genetics. Furthermore, microarray expression data, which have strongly implicated immunologic genes in the pathogenesis of IA formation and rupture, can yield novel insights into the biology of IAs (Fennell et al., 2016).

Genetics of Vascular Anomalies

Vascular anomalies are localized defects in vascular development that are characterized by a focal increase in the number of vessels, which are abnormally tortuous and large (Boon et al., 2011). While the majority of vascular anomalies occur sporadically, there are a variety of inherited forms that most commonly exhibit autosomal dominant inheritance due to loss-of-function mutations (Nguyen et al., 2014). These disorders typically display incomplete penetrance and variable expressivity, which may be explained by Knudson's two-hit hypothesis: the combination of an inherited mutation and a post-zygotic somatic mutation leading to a localized loss of the gene is required for vascular anomaly development (Boon et al., 2011; Nguyen et al., 2014). This section will briefly describe the underlying genetics of inherited arteriovenous malformations (AVMs), capillary malformations (CMs), venous malformations (VMs), lymphatic malformations (LMs), and inherited complex syndromes manifesting with vascular anomalies.

Arteriovenous Malformations

AVMs are characterized by dilated vessels and arterialized veins due to a direct connection between arteries and veins that bypasses capillaries (Nguyen et al., 2014). Approximately 5% of AVMs occur as a result of inherited vascular disorders, primarily hereditary hemorrhagic telangiectasia (HHT) (Kim et al., 2016). HHT (incidence 1:5000–1:8000) is an autosomal dominant disorder that is diagnosed by satisfying three of the following four criteria: recurrent epistaxis, telangiectasias, visceral AVMs, and a positive family history (Shovlin et al., 2000). Six chromosomal loci have been linked to HHT, though only four genes, which all encode components of the TGF-β signaling pathway, have been identified as causative of the disease (Nguyen et al., 2014). Mutations in *ENG* (HHT1—OMIM 187300), which encodes the co-receptor endoglin, and *ACVRL1* (HHT2—OMIM 600376), which encodes the type I receptor activin kinase-like receptor-1 (ALK1), account for 80% of HHT cases (Nguyen et al., 2014; Shovlin, 2010). Interestingly, individuals with HHT1 suffer more commonly from cerebral and pulmonary AVMs, whereas HHT2 is more commonly associated with hepatic AVMs (Shovlin, 2010). As mentioned earlier, JPS–HHT is due to mutations in *SMAD4*, which accounts for 1%–2% of HHT cases (Shovlin, 2010). HHT5 (OMIM 615506) is caused by mutations in bone morphogenetic protein 9 (*BMP9*), which encodes a protein that exerts its effect on cells by binding to endoglin and ALK1 (Wooderchak-Donahue et al., 2013). No genes causative of HHT have been identified yet at chromosomal loci 5q31.3-q32 (HHT3—OMIM 601101) and 7p14 (HHT4—OMIM 610655) (Nguyen et al., 2014). Interestingly, in a recent WES study of one family with brain AVMs, Yilmaz et al. found a heterozygous mutation in *ACVRL1* in two siblings without any other HHT features, indicating the wide phenotypic variability with *ACVRL1* mutations (Yilmaz et al., 2017).

While approximately 10% of individuals with HHT have a brain AVM (Brinjikji et al., 2017), only 2% of brain AVMs are associated with HHT (Alvarez and Castillo, 2015). Given that there are reports of familial clustering of brain AVMs in those without HHT, there may be a genetic basis to the formation of brain AVMs outside of the HHT genes (van Beijnum et al., 2007, 2014). In a meta-analysis of six SNPs previously associated with sporadic brain AVMs (which constitute 95% of cases), only one SNP near the *ANGPTL4* gene was associated with AVM susceptibility (Kremer et al., 2016). *ANGPTL4* encodes the protein angiopoietin-like 4, which is thought to have both a proangiogenic and antiangiogenic effect via signaling independent of ACVRL1 and endoglin (Kremer et al., 2016). Additionally, the 9p21 locus, which has been linked with TAAs, AAAs, and IAs, has also been associated with sporadic brain AVMs (Sturiale et al., 2014). Currently, however, the role of genetic variants in sporadic brain AVMs is not well established (Yilmaz et al., 2017), and as Walcott et al. maintain, it is likely that underlying genetics, inflammation, and impaired blood–brain barrier integrity all contribute to brain AVM development (Walcott et al., 2016).

Capillary Malformations

CMs, which are commonly known as port-wine stains and are usually sporadic in occurrence, can be inherited via an autosomal dominant mechanism as part of the CM–AVM syndrome (Nguyen et al., 2014). CMs of CM–AVM are generally smaller than sporadic CMs, multifocal, and surrounded by a pale halo (Boon et al., 2011). These CMs, which can be associated with AVMs, AV fistulas, or Parkes Weber syndrome (OMIM 608355), are caused by mutations in *RASA1*, leading to loss of function of the encoded GTPase activating protein p120-RasGAP (Boon et al., 2011; Nguyen et al., 2014). Consequently, there is upregulation of the Ras/MAPK pathway, leading to abnormal cell growth, differentiation, and proliferation (Nguyen et al., 2014).

Cerebral cavernous malformations (CCMs), which are clusters of enlarged capillary caverns lined by a single layer of endothelial cells without normal intervening brain parenchyma (thus prone to rupture (346)), are inherited via an autosomal dominant mode in approximately 20% of cases (Nguyen et al., 2014). A double hit mechanism (inactivation of both alleles of a CCM gene) in one of

three discovered genes (*KRIT1*, *CCM2*, and *PDCD10*) linked to CCMs is required for their development (Nguyen et al., 2014; Pagenstecher et al., 2009; Akers et al., 2009). *KRIT1* (CCM1—OMIM 116860) encodes Krev Interaction Trapped-1, which plays a role in endothelial cell–cell junctions via the Delta-Notch pathway (Nguyen et al., 2014). *CCM2* (OMIM 60384) encodes the malcavernin protein, which serves as a scaffolding protein for mitogen-activated protein kinase kinase kinase and also interacts with KRIT1 (Nguyen et al., 2014; Kim et al., 2016). *PDCD10* (CCM3—OMIM 603285) encodes a protein involved in apoptosis that has been shown to interact with malcavernin (Kim et al., 2016). Though no gene has been discovered, a 4th CCM locus is possible at 3q26.3-q27.2 (Kim et al., 2016; Liquori et al., 2006). Interestingly, CCM1 patients may exhibit lower rates of hemorrhage than patients with CCM2 or 3 (Gault et al., 2006), while CCM3 patients may have a higher risk of cerebral hemorrhage (Denier et al., 2006).

Venous Malformations

VMs, which appear as dilated venous channels surrounded by thin, irregular layers of SMCs, are typically sporadic in nature but can be inherited in an autosomal dominant manner (Nguyen et al., 2014). The two forms that are inherited are cutaneomucosal venous malformations (VMCMs—OMIM 600195) and glomulovenous malformations (GVMs—OMIM 138000), which account for 1% and 5% of VMs, respectively (Nguyen et al., 2014). VMCMs are caused by mutations in *TIE2/TEK*, which is an extracellular receptor that binds angiopoietins and thus modulates angiogenesis and proliferation and maintains vessel wall stability (Nguyen et al., 2014). While 85% of mutations in sporadic VMs are attributable to the somatic L914F mutation in *TEK*, more than 50% of VMCMs are due to the R849W mutation (Nguyen et al., 2014). All mutations in *TEK* leading to VMCMs result in hyperphosphorylation of the receptor (Wouters et al., 2010), causing upregulation of PI3K/AKT signaling. This upregulation leads to decreased production of platelet-derived growth factor-beta, an important recruiter of SMCs (Nguyen et al., 2014).

GVMs, which are typically raised, nodular, multifocal, and hyperkeratotic, are histologically characterized by mural glomus cells in distended venous channels (Brouillard et al., 2013). GVMs are caused by loss-of-function mutations in glomulin (*GLMN*), which exhibit incomplete penetrance (90%) and variable expressivity, with no observed relationship between the specific mutation identified and the observed phenotype (Brouillard et al., 2013). It is thought that glomulin plays an important role in vascular SMC development (Nguyen et al., 2014). Brouillard et al. propose that the variable expressivity by family members with the same *GLMN* mutation may be related to the rate of somatic second-hit mutations that may vary among them (Brouillard et al., 2013).

Lymphatic Malformations

LMs, which consist of enlarged lymphatic vessels with fibrotic walls, only occur sporadically and are thus likely due to somatic mutations (Nguyen et al., 2014; Brouillard et al., 2014). However, lymphedema, which is characterized by the abnormal accumulation of interstitial fluid due to disruption of lymphatic vessels, can be primary (congenital) or secondary (acquired) (Nguyen et al., 2014). Primary lymphedema is most commonly sporadic, though 35% of cases are familial in nature and exhibit an autosomal dominant or recessive mode of inheritance (Nguyen et al., 2014; Brouillard et al., 2014). At least 21 genes have been linked to primary lymphedema, though only eight genes account for approximately 40% of familial lymphedema and 10% of sporadic lymphedema (Brouillard et al., 2014; Mendola et al., 2013). The other genes are known to cause rare syndromic diseases that manifest with lymphedema and thus only contribute to a small fraction of the cases of familial lymphedema (Brouillard et al., 2014). The majority of implicated genes encode proteins associated with the VEGFC/VEGFR-3 signaling pathway (Brouillard et al., 2014). Mutations can affect the function of this pathway at the level of the receptor/ligand (*VEGFR3*, OMIM 153100; *VEGFC*, OMIM 615907; *CCBE1*, OMIM 235510), modification of receptor activity (*PTPN14*, OMIM 613611), production of the receptor (*SOX18*, OMIM 607823 + 137940; *GATA2*, OMIM 614038), transcriptional regulation (*FOXC2*, OMIM 153400; *GATA2*), or a downstream target gene (*GJC2*, OMIM 613480; *VEGFR3*) (Mendola et al., 2013). *KIF11* (OMIM 152950), which encodes a protein involved in mitotic spindle assembly and has also been shown to be causative of primary lymphedema, has not been linked to the VEGFC/VEGFR-3 pathway, though inhibition of KIF11 activates the PI3K/AKT pathway (Brouillard et al., 2014; Mendola et al., 2013). Determining the underlying genetic etiology in familial primary lymphedema is crucial, as it helps clinicians predict the clinical prognosis of patients and their family members (Adamczyk et al., 2016).

Inherited Complex Syndromes

Vascular anomalies can also occur with several complex overgrowth syndromes, including Proteus syndrome (OMIM 176920), congenital lipomatous asymmetric overgrowth with vascular malformation, epidermal nevi, and skeletal anomalies (CLOVES—OMIM 612918), PTEN hamartoma tumor syndrome (PHTS), and Klippel–Trenaunay syndrome (KTS—OMIM 149000) (Nguyen et al., 2014). However, only PHTS, which is characterized by vascular anomalies, tissue overgrowth, gastrointestinal polyps, and macrocephaly, is an inherited condition (Nguyen et al., 2014). PHTS is caused by mutations in *PTEN*, a tumor suppressor gene that inhibits PI3K signaling (Nguyen et al., 2014). Rare chromosomal translocations have been associated with KTS, though this disorder, along with CLOVES, is most commonly due to somatic mutations in *PIK3CA*, whereas somatic mutations in *AKT1* are the cause of Proteus syndrome (Nguyen et al., 2014).

Other Vasculopathies

Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH), which is characterized by intimal thickening, SMC hypertrophy, adventitial fibrosis, and occlusion of the pulmonary vasculature (Han et al., 2013; Humbert et al., 2004), can be inherited in an autosomal dominant manner with reduced penetrance (estimated at 27%) (Best et al., 2014) in approximately 6% of cases (Ma and Chung, 2017). Several genes belonging to the TGF-β superfamily have been implicated in hereditary PAH (HPAH), including *BMPR2* (PPH1, OMIM 178600), *SMAD9* (PPH2, OMIM 615342), and the HHT 1 and 2 genes (*ENG* and *ACVRL1*, respectively) (Ma and Chung, 2017). Loss-of-function mutations in *BMPR2* are discovered in approximately 70% of hereditary PAH (HPAH) cases and in 10%–40% of idiopathic PAH (IPAH) cases (Ma and Chung, 2017). Interestingly, *BMPR2* mutation carriers appear to have more severe disease at diagnosis and are less likely to respond to acute vasodilator testing than individuals without *BMPR2* mutations (Rosenzweig et al., 2008). *CAV1* (PPH3, OMIM 615343), which encodes a membrane protein that plays a role in TGF-β, G-protein, and nitric oxide signaling, and *KCNK3* (PPH4, OMIM 615344), which encodes an outwardly rectifying K+ channel, are implicated in rare cases of HPAH (Ma and Chung, 2017). Clinical genetic testing for mutations in the above stated genes, along with several others associated with PAH (*SMAD4*, *KCNA5*, *BMP9*, and *NOTCH3*), is warranted for HPAH, IPAH, and pediatric PAH and is helpful for risk stratification for family members (Ma and Chung, 2017).

Metabolic Vasculopathy

Metabolic myopathies, which are disorders of skeletal muscle due to impaired carbohydrate, fatty acid, or energy metabolism, have been associated with dilatation or aneurysm of various arteries (Finsterer, 2012). In particular, Pompe's disease (glycogen storage disease II, OMIM 232300), an autosomal recessive disorder due to mutations in acid alpha-1,4-glucosidase, has been shown to manifest with dilative arteriopathy, especially of the basilar artery (Finsterer, 2012). The most common complications associated with vascular manifestations of Pompe's disease are bleeding or arterial wall dissection, leading to ischemic or hemorrhagic stroke, including subarachnoid bleeding (Finsterer, 2012). The pathophysiology of this disease involves excess storage of glycogen in vascular SMCs, which most commonly affects the cerebral vessels (Finsterer, 2012).

Mitochondrial disorders, which typically have multisystem manifestations due to mutations in mitochondrial DNA (maternally inherited) or mitochondria-related nuclear DNA, can also manifest with vasculopathy (Finsterer and Zarrouk-Mahjoub, 2016; El-Hattab et al., 2015). These disorders, which have the highest incidence among congenital metabolic disorders, are caused by mutations in more than 250 genes (Kohda et al., 2016). Primary mitochondrial vasculopathy is due to a direct effect of the underlying metabolic defect on the components of the arterial wall (endothelium, muscularis, adventitia, pericytes), while secondary mitochondrial vasculopathy is attributed to atherosclerotic risk factors that commonly affect individuals with mitochondrial disorders (Finsterer and Mahjoub, 2012). The vascular manifestations of mitochondrial disorders include microangiopathy (leukoencephalopathy, migraine-like headaches, stroke-like episodes, and peripheral retinopathy) and macroangiopathy (atherosclerosis, arterial ectasia, aneurysms, dissection, and spontaneous arterial rupture) (Finsterer and Zarrouk-Mahjoub, 2016). Mitochondrial disorders have been associated with aortic root ectasia (Brunetti-Pierri et al., 2011), carotid artery dissection (Sakharova et al., 2012), spontaneous rupture of the aorta (Tay et al., 2006), and AVMs (Fujitake et al., 2002). Finsterer et al. maintain that individuals with mitochondrial disorders should be systematically screened for mitochondrial vasculopathy in order to establish an early diagnosis and hopefully prevent any complications (Finsterer and Zarrouk-Mahjoub, 2016).

Inherited Neurovascular Disease

Neurovascular disease, which is typically multifactorial in nature, can also be caused by rare inherited disorders that can manifest with stroke as the principal characteristic or as part of a systemic disease (Tan and Markus, 2015). A number of monogenic causes of stroke have been described and reviewed extensively (Tan and Markus, 2015; Sam et al., 2015; Southerland et al., 2013). Many of these monogenic disorders affect either endothelial cells or SMCs (Sam et al., 2015) and cause one stroke subtype (ischemic vs. hemorrhagic and small vessel vs. large arterial disease), though several of the disorders can cause more than one subtype (Tan and Markus, 2015). Cerebral arteriopathy, autosomal dominant, with subcortical infarcts and leukoencephalopathy, type 1 (CADASIL1, OMIM 125310), which is the most common monogenic cause of cerebral small vessel disease (Tan and Markus, 2015), results from mutations in *NOTCH3* that lead to aggregation of NOTCH3 around vascular SMCs and consequently vascular SMC degeneration and cerebral arterial stenosis (Sam et al., 2015). Mutations in high temperature requirement serine peptidase A1 (*HTRA1*) have been shown to be causative of both CADASIL2 (OMIM 616779) and cerebral arteriopathy, autosomal recessive, with subcortical infarcts and leukoencephalopathy (CARASIL), which is characterized by a much earlier age of onset of stroke and cognitive decline (20–30 years) than the autosomal dominant forms (Verdura et al., 2015; Hara et al., 2009). *TREX1* and *FOXC1* mutations have also been linked to autosomal dominant syndromes (OMIM 192315 and 602482, respectively) causative of cerebral small vessel disease and subcortical infarcts (Tan and Markus, 2015).

Mutations in *COL4A1* have been shown to cause a spectrum of small vessel diseases that can manifest with ischemic or hemorrhagic strokes (HANAC, OMIM 611773; BSVD, OMIM 607595, Porencephaly 1, OMIM 175780), while mutations in *COL4A2* been linked to Porencephaly 2 (OMIM 614483) (Tan and Markus, 2015). Heterozygous mutations in *COL4A1* and *COL4A2* have also been associated with sporadic adult-onset hemorrhagic stroke (OMIM 614519) (Weng et al., 2012; Jeanne et al., 2012). Through a

cohort of 183 index patients primarily with porencephaly or infantile hemorrhage as well as a literature review, Meuwissen et al. determined that 50% of *COL4A1* and *COL4A2* mutations were inherited, while 25% were de novo and 25% were of unknown origin (due to lack of parental testing) (Meuwissen et al., 2015). Autosomal dominant inherited forms of cerebral amyloid angiopathy (OMIM 605714) can also cause hemorrhagic cerebral small vessel disease via deposition of amyloid fibrils in blood vessel walls (Tan and Markus, 2015). Fabry disease (OMIM 301500), pseudoxanthoma elasticum, neurofibromatosis 1, and homocystinuria (OMIM 236200) are all monogenic disorders causative of small and large ischemic arterial disease due to mutations in *GLA* (galactosidase alpha), *ABCC6* (multidrug resistance-associated protein 6), *NF1* (neurofibromin 1), and *CBS* (cystathionine beta synthase), respectively (Tan and Markus, 2015). Additionally, mutations in *CER1*, which encodes adenosine deaminase 2, were found to cause early-onset ischemic and hemorrhagic stroke, as part of the autosomal recessive disease known as childhood-onset polyarteritis nodosa (OMIM 615688) (Zhou et al., 2014).

Furthermore, there are several relatively common cerebrovascular arteriopathies, including moyamoya disease, cervical artery dissection, and fibromuscular dysplasia (FMD), that have a genetic basis as well (Southerland et al., 2013). Moyamoya disease, which is characterized by diffuse distal stenosis of intracranial carotid arteries due to intimal thickening leading to neovascularization (Fukushima et al., 2016), has been linked with six chromosomal loci (designated MYMY1–6). Moyamoya disease is associated with several genetic syndromes, including Down syndrome, Alagille syndrome, Neurofibromatosis 1, and sickle cell disease, though non-syndromic disease can also be inherited in families via an autosomal dominant mechanism with reduced penetrance (Wallace et al., 2016; Mineharu et al., 2006). A GWAS identified *RNF213* as a susceptibility gene for MYMY2 (OMIM 607151) (Kamada et al., 2011), and a recent study by Fukushima et al. found that the homozygous mutation p.Arg4810Lys in the *RNF213* gene resulted in pulmonary hypertension and Moyamoya disease in two patients (Fukushima et al., 2016). MYMY4 (OMIM 300845), which is an X-linked recessive syndrome characterized by Moyamoya disease, short stature, facial dysmorphism, and hypergonadotropic hypogonadism, is due to deletion of *BRCC3* and *MTCP1*/*MTCP1NB* on Xq28 (Miskinyte et al., 2011). MYMY5 (OMIM 614042), as previously mentioned, is due to heterozygous mutations in the *ACTA2* gene, though these patients do not exhibit the characteristic cerebrovascular features of typical Moyamoya disease, including collateral development (Guo et al., 2009). MYMY6, which is characterized by achalasia, hypertension, and Moyamoya disease, is caused by homozygous or compound heterozygous mutations in *GUCY1A3* that lead to disrupted nitric oxide signaling (Wallace et al., 2016; Herve et al., 2014). Additionally, Majewski osteodysplastic primordial dwarfism II (MOPD II) (OMIM 210720), an autosomal recessive disorder due to mutations in the pericentrin gene, can also manifest with cerebrovascular anomalies (19% of cases in one study), including Moyamoya disease and IAs (Hall et al., 2004).

Cervical artery dissection (CeAD), a major cause of ischemic stroke in young and middle-aged adults, can occur as a manifestation of several monogenic disorders, primarily vascular EDS, and possibly others, including different types of EDS, LDS, MFS, and osteogenesis imperfecta (Debette et al., 2014). Though some patients with CeAD present with other arterial anomalies and connective tissue aberrations, implying an underlying systemic vasculopathy (Debette and Leys, 2009), no consistent genetic association has been established to contribute to cervical artery dissection (Debette et al., 2014). However, via a GWAS of 1393 CeAD cases and 14,416 controls in the general population, Debette et al. demonstrated that the G allele of the rs9349379 SNP in the phosphatase and actin regulator 1 (*PHACTR1*) gene was associated with a significantly decreased risk for CeAD (OR = 0.75) (Debette et al., 2015). In a separate study, Debette et al. demonstrated that only 1% of 1934 patients with cervical artery dissection had a positive family history and that the inherited connective tissue syndromes listed above only accounted for a few cases (Debette et al., 2014). Therefore, cervical artery dissection appears to be a multifactorial disease (Debette et al., 2014). FMD, a non-atherosclerotic and noninflammatory vascular disease that can lead to stroke due to stenosis of extracranial carotid and vertebral arteries as well as arterial aneurysms and dissections, has also been associated with the rs9349379 SNP of the *PHACTR1* gene (OR = 1.39) (Kiando et al., 2016). Given that 7%–10% of FMD patients have a positive family history, current evidence indicates there is a genetic basis to FMD, which may be inherited via an autosomal dominant manner with incomplete penetrance (Olin et al., 2012, 2014; Pannier-Moreau et al., 1997; Guo et al., 2017). In a recent study, Guo et al. identified homozygous and compound heterozygous loss-of-function mutations in *YY1AP1* in several individuals with Grange syndrome (OMIM 602531), an autosomal recessive condition characterized by early-onset vascular disease similar to FMD (Guo et al., 2017). Interestingly, mutations in *YY1AP1* and *INO80D*, which both encode components of the INO80 chromatin remodeling complex, have been shown to lead to vascular disease (Guo et al., 2017; Shameer et al., 2014). In a WES study, Shameer et al. identified a homozygous missense mutation in *INO80D* in two siblings with aortic hypoplasia, early-onset atherosclerosis, and arterial stiffness (Shameer et al., 2014).

Conclusion

The vasculature represents a complex, interconnected fluid transport network which plays a dynamic and vital role in our maintenance and survival. Both rare monogenic and common polygenic disorders that disrupt the structure and thus function of the vasculature at the level of the endothelial cell, SMC, or connective tissue of the vessel wall can lead to aneurysm formation, arterial dissection, vascular malformations, and other vasculopathies, which can lead to rupture and/or downstream tissue ischemia. Unraveling the genetics of aneurysms, vascular malformations, and other vasculopathies via GWAS, genome-wide linkage studies, and next-generation sequencing has revolutionized our understanding of the biology of these diseases. We are already witnessing the impact of these discoveries in improving patient care and enabling personalized medicine based on underlying

clinical and molecular genetics. Through more widespread clinical genetic testing and continued genetic research, we will be able to offer prophylactic care at an earlier age to affected individuals and to design novel therapies for these potentially devastating diseases.

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Genetics of Heart Failure

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Introduction

Aging of the population and prolonged lifespan of patients with cardiovascular diseases due to contemporary therapeutic interventions has led to an increasing prevalence of heart failure (HF). Data from NHANES 2009 to 2012 estimates HF to affect more than 5.7 million American adults. Projections also show that the prevalence of HF will increase approximately by 50% from 2012 to 2030, resulting in greater than 8 million adults with HF (Writing Group Members et al., 2016). Despite recent advances in the therapeutic armamentarium, the mortality rate in patients with HF remains high (Heidenreich et al., 2013; Gerber et al., 2015).

HF with reduced ejection fraction (HFrEF) is categorized into ischemic and nonischemic causes, with the term dilated cardiomyopathy (DCM) often used interchangeably with nonischemic cardiomyopathy. Approximately 30%–40% of patients enrolled in registries have DCM and approximately 70% of these are classified as idiopathic (Writing Group Members et al., 2016; Heidenreich et al., 2013). Historically, a genetic origin of DCM had been suspected based on inheritance patterns observed in families with several members affected by the disease. Initial studies suggested an estimate familial involvement in 1%–2% of DCM (Codd et al., 1989). However, it has been recently recognized that about 20%–50% of patients with DCM have familial or a genetic cause of cardiomyopathy (Hershberger and Siegfried, 2011). The precise magnitude of the role of genetic predisposition, however, varies by disease type, race, and age of disease onset. As demonstrated by an analysis of the Framingham cohort (Ackerman et al., 2011), the relative risk of developing HF is 1.69 if one parent is affected and 1.92 if both parents have HF. Genetic predisposition for development of HF includes not only a Mendelian risk of developing a cardiomyopathy but also genetic variation that could lead to a maladaptive response to common risk factors such as hypertension or diabetes mellitus and potentially cardiotoxic chemotherapy drugs (Pereira and Weinshilboum, 2009). Genetics may also play a role in the variable clinical response to HF therapy (Vosberg, 1994). Moreover, in a subset of patients with HF, allele heterogeneity with variable penetrance leads to various phenotypes of inherited cardiomyopathies (Pereira and Weinshilboum, 2009).

The purpose of this review is to examine the role of genetics in the development and treatment of DCM, the use of genomic tools in identifying a cause of HF and variation in HF drug response, and discuss its implications for clinical practice.

Genetic Studies in HF: A Methodological Approach

Genetic traits associated with HF can be monogenic or complex. Monogenic traits demonstrate mutations, deletions, or insertions in the coding DNA of a single gene or mutations in noncoding regulatory regions in the 5′ or 3′ or intronic regions of the gene, or variants leading to alteration in gene splicing. Nevertheless, these disorders may not always be monogenic, and the clinical phenotype could also be influenced by mutations in additional genes.

Linkage Analysis

For most of these monogenic traits, direct DNA sequencing and/or linkage analysis has successfully yielded the causal genetic mutation. Linkage analysis was used to localize the chromosomal position of a causal gene, MYH7 or cardiac myosin heavy chain 7, for hypertrophic cardiomyopathy (HCM) (Richards et al., 2015). The key concept behind linkage analysis is that genetic loci are usually inherited together due to their location near one and other on the chromosome and if they are transmitted together from parent to offspring more often than expected under independent inheritance. Once markers across the genome of family members

Table 1 Genes associated with dilated cardiomyopathy

Gene	*Function*	*Approximate percentage of dilated cardiomyopathy cases*
LMNA-Lamin A/C	Nuclear membrane stability	6%
MYH6-Myosin 6	Muscle contraction	4.4%
MYH7-Myosin-7	Muscle contraction	4.3%
MYPN-Myopalladin	Muscle contraction	3.6%
TNNT2-Troponin T	Muscle contraction	3%
SCN5A-Sodium channel protein type 5-subunit A	Sodium influx	2.6%
MYBPC3-Myosin-binding protein C	Muscle contraction	2%
RBM20-RNA-binding protein 20	Spliceosome protein	2%
TMPO-Thymopoietin	Nuclear membrane stability-Lamin associated protein	1%
LAMA4-Laminin alpha 4	Transduction of contractile forces	1%
TCAP-Telethonin	Muscle contraction	1%
VCL-Metavinculin	Transduction of contractile forces	1%
LDB3-LIM domain-binding protein 3	Transduction of contractile forces	1%
DES-Desmin	Transduction of contractile forces	<1%
ACTC1-Actin	Muscle contraction	<1%
TPM1-Tropomyosin alpha-1 chain	Muscle contraction	<1%
PLN-Cardiac phospholamban	Sarcoplasmic reticulum Ca^{2+} regulator; inhibits SERCA2a pump	<1%
TNN3-Troponin I	Muscle contraction	<1%
ACTN2-Alpha actinin-2	Anchor for actin	<1%
TNNC1-Troponin C	Muscle contraction	<1%
CSRP3-Cysteine and glycine rich protein 3	Cytoskeletal	<1%
PSEN1/2-Presenilin 1/2	Transmembrane protein, γ-secretase activity	<1%
DMD-Dystrophin	Transduction of contractile forces	<1%
TAZ-Tafazzin	Mitochondrial	<1%
ILK-Integrin-linked kinase	Transduction of contractile forces	<1%
TTN-Titin	Muscle contraction	<1%
CRYAB-Alpha B crystaline	Cytoskeletal protein	<1%

have been identified, the estimated logarithm of the odds score represents the probability that a genetic marker and possible causal locus are linked in off springs as opposed to the null hypothesis of the absence of linkage in the pedigrees (Lohmueller et al., 2003). The initial linkage is validated by fine mapping linkage analyses (addition of markers to form a dense genotype set in the region of linkage). Although linkage analysis has been extensively used for identification of genes causally associated with monogenic diseases, its use for identification of susceptible loci responsible for complex traits in the absence of a familial transmission is limited. To perform linkage analysis it is essential to have at least a complete trio, an affected patient or proband, and his or her parents.

In the setting of DCM, mutations in the nuclear-envelope scaffolding protein lamin have been identified in 6% of inherited cases. The role of *LMNA* gene mutations in DCM was demonstrated by Fatkin and colleagues (Fatkin et al., 1999) who evaluated 11 families with autosomal dominant inheritance DCM and located a genetic variant on chromosome 1p1-q21, where nuclear-envelope proteins lamin A and lamin C are encoded by the *LMNA* gene. Since then several other gene mutations associated with DCM have been identified by linkage analysis of families with inherited cardiomyopathies (**Table 1**).

GWAS

A wide spectrum of common genetic variants both within the noncoding and coding regions of multiple genes and interaction with traditional risk factors or the environment could lead to modifications in gene expression, translation, and posttranslational products that in turn could contribute to the pathogenesis of complex traits (Ackerman et al., 2011; Pereira and Weinshilboum, 2009). Therefore, comprehensive analysis of susceptibility genetic loci for complex traits is more complicated. In contrast with monogenic traits, the correlation between genotype and phenotype is modest resulting in relative risks of up to 1.5 as demonstrated by genome-wide association studies (GWAS). This pattern of relatively weak genotype–phenotype correlation or "missing heritability" can perhaps be explained by epigenetic mechanisms such as DNA methylation and histone modification or by the presence of rare variants that are typically not assayed by GWAS platforms. Moreover, to identify susceptibility loci involved in complex traits by GWAS, large populations are required to achieve adequate statistical power. The importance of allele frequency (common versus rare) depends on the effect measure. As opposed to common variants (minor allele frequency (MAF) >5%), rare variants (MAF < 1%) may be associated with higher individual risk for a condition but the population attributable risk is low. Based on the common disease-common variant hypothesis complex traits result from interactions of multiple common genetic variants, most of which are shared by unrelated affected individuals and usually have lacked obvious functional association with the disease

and could be in linkage disequilibrium with functional variants (Bodmer and Bonilla, 2008). Conversely, the common disease-rare variant hypothesis (Pritchard, 2001) suggests that common disease susceptibility could be due to genetic variants with low population frequencies (MAF < 1%) usually with large effect sizes. Therefore unlike common variants, rare variants could be associated with greater risk for a disease condition, more likely to have relevance to gene function and the disease process (Bodmer and Bonilla, 2008).

In the past, the main approaches to identify susceptibility loci of complex traits were linkage analysis with fine mapping or candidate gene association studies. Candidate genes for association studies are usually selected on the basis of prior linkage study findings and/or prior biological knowledge of gene function as is relevant to disease pathogenesis. Subsequently, allele or haplotype (combination of polymorphic alleles that are located at closely linked loci and tend to be inherited together) frequencies between case and control groups are compared, and a statistical difference in frequencies between cases and controls provides evidence that the allele or haplotype is associated with the disease trait. The main limitation of this approach has been the lack or reproducibility and the inability to discover new disease-associated genes. The lack of reproducibility could be attributed to false positive tests, genetic and phenotypic heterogeneity (Goring et al., 2001; Hattersley and McCarthy, 2005).

GWAS test multiple genetic variants spread across the human genome for association with a specific trait and do not require prior knowledge of the biologic relevance of the tested genes. GWAS entail hybridization of DNA from a patient against an array of short oligonucleotide probes which are immobilized on the array. If any of the tested DNA sequences are complementary to probes on the array, they bind and become detected. The development of high-throughput genotyping technology and the analysis of high-resolution linkage disequilibrium maps of common genetic variants using data from the International HapMap Project (International HapMap Consortium, 2005) have facilitated the design and performance of case-control GWAS with large sample sizes and statistical power leading to identification of loci with known or unknown biologic function. Despite the increasing number of GWAS in the literature, a large number of susceptibility loci identified by the studies explain a small percentage of variability of complex traits, a phenomenon called "missing hereditability" (Maher, 2008). The residual variability can be explained by gene–gene interactions, epigenetic modifications, and the presence of rare alleles the detection of which require next-generation sequencing (NGS). Given the increasing number of GWAS in the literature, clinicians should be familiar with basic measures of validity of GWAS. Most importantly, the phenotype should be explicitly described, the genotype frequencies should follow the Hardy–Weinberg equilibrium, the level of significance should be adjusted for multiple comparisons (5×10^{-8} is usually considered a GWAS significant threshold), and finally the results should be replicated in other cohorts and a metaanalysis with these from other studies should confirm these findings.

Several studies have sought to determine the association of common susceptibility variants with the incidence and severity of HF. The first large-scale GWAS to investigate genetic factors that are associated with the onset of HF was the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) consortium. This metaanalysis included data from four community-based prospective cohorts of a total of 25,000 individuals followed up for about 11.5 years for the development of new-onset HF. After screening for approximately 2.5 million single-nucleotide polymorphisms (SNPs), two genetic loci were identified to be independently associated with incident HF. The first genome-wide significant locus was 58.8 kb from the gene *USP3* (encoding ubiquitin-specific peptidase 3) in individuals of European ancestry, and the second locus was 6.3 kb from the gene *LRIG3* (encoding leucine-rich repeats and immunoglobulin-like domains 3) in individuals of African ancestry (Smith et al., 2010). Although, GWAS have identified susceptibility loci for major risk factors of HF such as hypertension and coronary artery disease (Weintraub et al., 2017; Towbin et al., 2015), they have not been very successful in identification of loci directly related to HF, suggesting a complex interplay between traditional risk factors and genetic background.

The prospective metaanalysis from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium was the first large-scale GWAS to attempt to discover genetic risk variants for HF. The CHARGE study included about 24,000 individuals (21,000 subjects of European ancestry and almost 3,000 subjects of African ancestry) and studied 2.5 million genetic markers spread throughout the genome. Data from 4 large, population-based cohort studies that included cardiovascular outcomes as primary study endpoints were analyzed (Liggett et al., 2006; Cresci et al., 2009). The threshold for a genetic locus to be significantly associated with HF risk in each ethnic cohort was set at a significance level of 5×10^{-7}. In the European subset, the locus associated with HF risk was at chromosome15q22 near *USP3* (ubiquitin-specific peptidase 3 gene) with a hazard ratio that was determined to be 1.53 (p value: 1.4×10^{-8}) for the minor allele. In the African ancestry group, the locus associated with HF risk was found at chromosome 12q14 with a similar hazard ratio (p value: 6.7×10^{-8}). In both ethnic subsets several "high signal" SNPs were identified that did not reach the prespecified significance level. These results suggest that HF not unlike other complex diseases may be a polygenic disease process. However, to exclude false positive associations, these results will have to be replicated in other HF cohorts. Also, the identification of loci that are in linkage dysequilibrium with the identified SNPs and may be causally related to HF may require in depth resequencing.

In a large-scale candidate gene, case-control study of 20,926 European-ancestry participants with 2526 incident HF events, common SNPs in ≈2000 genes from the ITMAT/Broad CARe (IBC) cardiovascular SNP array, identified a genome-wide significant locus ($p = 1.4 \times 10^{-8}$) rs1739843, located in an intronic region of the *HSPB7* gene at 1p36, that encodes a heat shock protein, and was associated with incident HF (Fatkin et al., 1999). In this study SNPs in genes that were already known to have biological associations with various disease processes were evaluated. These results were replicated in another multicenter European study (Stark et al., 2010). The significant SNP in *HSPB7* gene was in linkage disequilibrium with a SNP (rs10927887, encoding Arg83Gly) in the *CLCNKA* gene (gene encoding a chloride ion channel), sequencing of the latter suggested significant association of a loss-of-function variant with HF (White et al., 2003) with an odds ratio of 1.27 per allele copy. These findings identify an independent

genetic risk factor for HF. The variant CLCNKA risk allele associated with linked variants in the adjacent HSPB7 gene uncovers a genetic mechanism affecting the cardiorenal axis and provides genetic evidence supporting clinical observations that individual differences in salt-sensitivity can predispose to cardiovascular complications.

These genotype–phenotype association studies described above include patients with HF of diverse etiology (ischemic and nonischemic) and type (systolic and diastolic). Recent studies have focused specifically on identification of susceptibility loci for DCM. DCM accounts for 30%–40% of HF cases in registries. A GWA study of 1179 patients with DCM and LVEF<45% without coronary artery disease and 1108 controls used a DNA-pooling approach and identified 2 SNPs at different loci showing some evidence of association with DCM (Palmer et al., 2003). A region on chromosome 1p.36.13 which included the gene *HSPB7*, previously associated with incident HF (Mestroni et al., 1999), and a nonsynonymous SNP (rs2234962) located within the coding sequence of *BAG3* (BCL2-associated athanogene 3) region on chromosome 10q26 were replicated in an independent cohort of 1165 DCM patients and 1302 controls ($p < 5.0 \times 10^{-7}$). In humans, variants in *BAG3* are associated with variable phenotypes including muscular dystrophy and cardiomyopathy (Huang et al., 2004).

A more recent large-scale case-control GWA that employed a three-staged design to analyze 4100 DCM cases and 7600 controls identified and replicated multiple SNPs on chromosome 6p21 (McNamara et al., 2001). The noncoding SNP located in *HCG22* was significantly associated with idiopathic DCM ($p = 4.9 \times 10^{-9}$). With the use of RNA transcriptome arrays the authors demonstrated that the SNP was an expression quantitative trait locus for class I and class II major histocompatibility complex heavy myosin proteins.

The largest GWAS of cardiac structure and function was performed among 6765 African Americans and identified four genetic loci related to left ventricular mass, interventricular septal wall thickness, left ventricular internal diastolic diameter, and ejection fraction, which reached genome-wide significance (Fox et al., 2013). However, none of these variants were replicated in the consortium of European ancestry (EchoGEN). Although these genetic variants have been associated with cardiac structure and function, they have not been linked to the development of HF.

Using a candidate gene approach in one large genotype–phenotype association study, 50,000 SNPs in approximately 2000 genes linked to cardiovascular disorders were assayed using the CardioMetabo Chip. In this study, two SNPs were identified to have significant association with advanced HF (Cappola et al., 2010). One of these two variants was in an intronic region of the *HSPB7* gene which encodes one of the small heat-shock proteins, expressed in both cardiac and skeletal muscle cells and plays a role in stabilizing sarcomeric proteins. GWA studies have shown the same locus to be associated with both DCM and HF. (Stark et al., 2010; Villard et al., 2011; Garnier et al., 2015). These studies may add further mechanistic explanations of the association of this genetic variant with both ischemic and nonischemic DCM.

Next-Generation Sequencing

Advances in DNA sequencing have allowed high-throughput in-depth sequencing of an individual's entire genome. Specifically, NGS uses parallel sequencing of multiple small fragments of DNA to analyze the entire DNA sequence or DNA sequence of interest. The first-generation sequencing methods (e.g., Sanger, Maxam, Gilbert) analyze the sequence of large DNA fragments by aligning a series of variable length products polymerized along the DNA template (Biesecker and Green, 2014). First-generation sequencing is used for sequence analysis of a specific gene or genes but is expensive and turnaround times for results are slow. In NGS multiple DNA fragments are sequenced in parallel and thus allow rapid DNA sequencing at a lower cost. The first step in preparation of NGS platforms is amplification and fragmentation of a patient's DNA, followed by isolation of the fragments by attachment to small beads. Sequencing is performed based on these fragments, and the results are computationally aligned against a "reference" genome, released by the National Center for Biotechnology Innovation (NCBI). The utility of NGS is the ability to perform multiple gene sequence analysis including the whole exome (WES) or genome (WGS). Third-generation sequencing uses parallel sequencing and single-DNA molecules rather than amplified DNA fragments as templates. The advantage over NGS is the elimination of potential errors in DNA sequencing that can occur during the DNA amplification process. In cases of Mendelian disorders with large number of candidate genes such as DCM and HCM, targeted gene panels are used that provide sequence data for a limited subset of candidate genes that have been previously demonstrated to be associated with the disease or mutations in which were considered pathogenic. These panels may be preferable to exome sequencing due to higher depth of coverage, lower cost, and lower likelihood of identification of variants with unknown significance. A number of validated gene sequencing panels with approximately 120 genes (**Table 1**) are used in the diagnosis of hereditary cardiomyopathies, and these continue to expand with inclusion of newly discovered causative genes. NGS with gene panels may provide false negative results in affected individuals who may have pathogenic variants in genes that are yet to be identified as causative in DCM. Performing WES in a trio, that is, the proband and both biological parents may have a higher diagnostic yield in identifying a previously unidentified pathogenic variant (Li et al., 2017).

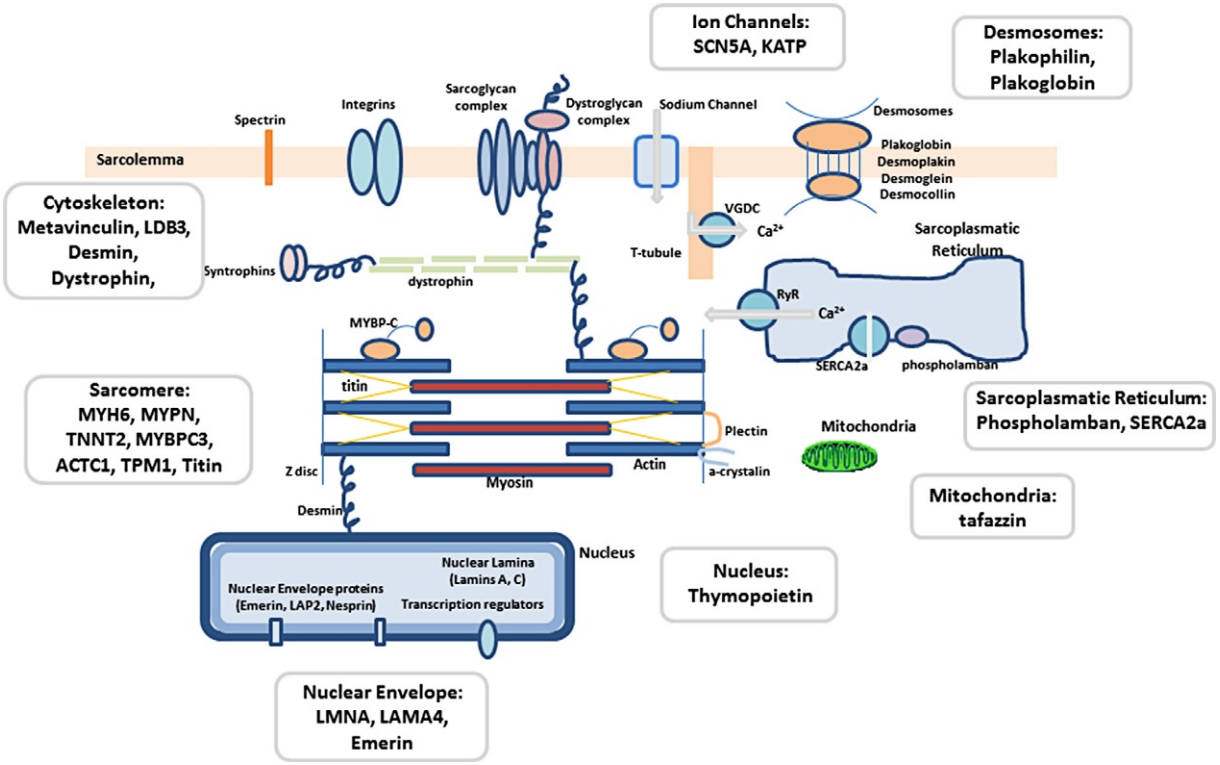
Genetics Causes of DCM

Familial DCM often displays an age-dependent penetrance, with patients typically manifesting disease in their fourth to sixth decades (Codd et al., 1989). The diagnosis is usually made when one or more first degree relatives are diagnosed with idiopathic DCM or if a first-degree relative had an unexplained sudden death under the age of 35 years. (Mestroni et al., 1999). The

prevalence of DCM has been estimated at approximately 1 in 2700 based on echocardiographic data from Olmsted County (Codd et al., 1989), but this is likely underestimated. Based on the same data the ratio of DCM to HCM prevalence was approximately 2 to 1.

Idiopathic DCM may be familial in approximately 1 in 5 patients with DCM when evaluated by obtaining a family history and by performing echocardiography. Obtaining a family history alone is insufficient to determine the familial nature of DCM as up to 40% of patients who have a familial pattern of transmission by echocardiography may be determined to not have a familial DCM. When patients with DCM are evaluated by genetic testing, pathogenic variants are found in up to 35% of cases indicating a high prevalence of familial DCM (Grunig et al., 1998).

Familial DCM is overall a very heterogeneous genetic disease, characterized by variable clinical presentation and age of onset, variable penetrance, and different modes of inheritance, depending on the gene/mutation involved. The most common pattern of inheritance observed is autosomal dominant inheritance. However, X-linked and mitochondrial inheritance also has been observed (Kamdar and Garry, 2016; Zaragoza et al., 2011). Mutations in greater than 40 genes have been implicated in familial DCM (**Table 1**, Figure). In total, these genes that are sequenced typically on standard cardiomyopathy gene panels account for 20%–48% of the molecular causes of the DCM (Grunig et al., 1998). It is important to note that the underlying genetic mutation should not override clinical diagnosis, since mutations in different genes can cause other cardiomyopathies such as HCM and left ventricular noncompaction as there appears to be an overlap in genetic causes of the various types of cardiomyopathy.



This concept can be exemplified by the phenotypic heterogeneity seen in the majority of HCM-susceptibility Z-disc genes that are also implicated in the pathogenesis of DCM (Bos and Ackerman, 2010). Similarly, the clinical presentations of both DCM and arrhythmogenic right ventricular cardiomyopathy (ARVC) can be caused by mutations in desmosomal protein genes (Elliott et al., 2010). Not only do mutations in the same gene yield distinct phenotypes, but sometimes a single mutation can manifest as HCM in one individual and DCM in another. One example of this phenotypic heterogeneity is the allelic heterogeneity seen in *TNNT2* gene, which encodes cardiac troponin T in DCM and HCM (Hershberger et al., 2013).

Mutations in the nuclear-envelope intermediate filament protein, LMNA, typically cause both DCM and progressive conduction disease, thus propagating the development of lethal arrhythmias and sudden cardiac death. Pathogenic variants in LMNA gene account for up to 5% of all cases of autosomal dominant DCM (van Tintelen et al., 2007). TTN, the gene encoding the sarcomere protein titin that represents the largest known protein in humans, genetic variation of which was associated with 25% of autosomal dominant cases of DCM (Schreiber and Kennedy, 2013). Most of the pathogenic variants found in the TTN gene were attributed mainly to truncation mutations, and the role of missense mutations in TTN is still unclear (Dahl et al., 2008). Sarcomere gene mutations account for up to 5% of all cases of DCM. These genes encode thick and thin filament sarcomere proteins and variants in which are also involved in the development of HCM and mainly include *MYH7*, *MYBPC3*, *ACTC1*, and *TNNT2* genes (Bonne et al., 1999). Pathogenic variances in mitochondrial DNA or in nuclear genes that code for mitochondrial components have also been

implicated in early-onset DCM. Patients with mitochondrial gene mutations often present in early childhood with DCM in conjunction with additional pathologies, including skeletal myopathies, neurological, or developmental diseases (Weintraub et al., 2017).

Patients with DCM as part of a neuromuscular syndrome classically have X-linked or autosomal recessive inheritance. Duchenne muscular dystrophy, Becker muscular dystrophy, and Emery–Dreifuss muscular dystrophy commonly present in childhood, with a predominant musculoskeletal phenotype with coexistent DCM. These dystrophies are caused by mutations in the DMD gene that encodes the protein dystrophin (Chen et al., 2013).

Genetic Testing in DCM: Recommendations and Implications

The role of genetic testing in DCM is a Class IIa recommendation (i.e., should be considered) (Hershberger and Siegfried, 2011) according to a HRS/EHRA expert consensus statement, since genetic testing can help confirm a molecular diagnosis of HF and facilitate screening of first-degree relatives (cascade screening). Cascade (also called stepwise) screening is a mechanism for identifying people at risk for a genetic disease by a process of systematic family evaluation. If a family member has abnormal cardiac screening, evaluation is then recommended in all first-degree relatives of this newly diagnosed patient. This stepwise screening should be continued until all consenting first-degree relatives of clinically affected have been evaluated.

The HF Society of America guidelines for evaluation of cardiomyopathies (Hershberger et al., 2009) recommend taking a comprehensive family history for three or more generations for all patients with cardiomyopathies (ARVC, DCM, HCM, LVNC, and restrictive), clinical screening for inherited cardiomyopathy in asymptomatic first-degree relatives, genetic and family counseling, consideration of genetic testing, and seeking further consultation with professionals who are expert in cardiovascular genetics. For DCM, clinical screening should consist of a history and examination, an electrocardiogram, an echocardiogram, and measurement of the creatine kinase level. Repeat clinical screening at regular intervals is also recommended and is outlined below. Genetic testing using a broad cardiomyopathy panel is typically offered to the most affected individual in a family or the proband to facilitate family screening. Genetic testing results may also affect clinical management of patients, for example, in patients with LMNA-associated cardiomyopathy who are susceptible to developing life-threatening arrhythmia regardless of left ventricular ejection fraction (LVEF), an implantable cardioverter–defibrillator (ICD) can be considered before the LVEF falls to below 35%.

Patients with DCM who have significant conduction tissue disease and/or a family history of premature sudden death, familial DCM due to mutations in the *LMNA* or *SCN5A* genes should be considered, and genetic testing in that situation is considered a Class I recommendation (Meune et al., 2006; Priori et al., 2015). In the presence of lamin A/C mutation the development of AV block is associated with poor outcomes and pacing therapy is insufficient to prevent SCD, thus supporting the use of prophylactic ICDs rather than pacemakers when cardiac involvement is present. Risk factors for sudden death and appropriate ICD therapy include nonsustained ventricular tachycardia, left ventricular ejection fraction <45%, male sex, and lamin A or C nonmissense mutations. Patients with laminopathies but with EF >30%–35% and indications for pacemaker or the aforementioned risk factors may be considered for an ICD (class IIa, Level of Evidence B) (Meune et al., 2006; Priori et al., 2015).

Identifying a pathogenic variant could help with counseling at-risk family members and determine the need and frequency of clinical evaluations. Due to the variable age of onset, a normal ECG and echocardiogram does not rule out the possibility of developing DCM in family members who are screened, and longitudinal follow-up of first-degree relatives should be performed (**Table 2**). Given the variable age of onset, clinical screening repeated at intervals is recommended, even if clinical genetic testing has not identified a disease-causing mutation in the family. The frequency of repeated screening is dependent on the results of genetic testing as well as on the type of cardiomyopathy (**Table 2**). If a disease-causing mutation is identified in the proband first degree relatives could be offered genotyping for the specific pathogenic mutation which would be far more cost-effective than performing an entire cardiomyopathy gene panel. The frequency of clinical screening in relatives known to be mutation positive should be performed at more frequent intervals due to an increased probability of those individuals developing cardiomyopathy (Hershberger et al., 2009). As a general rule, clinical screening of family relatives is recommended every 1–2 years up to age 20 years, every 2–3 years from 20 years to 40 years, and then every 3–5 years thereafter (Weintraub et al., 2017).

Table 2 Screening recommendations for inherited cardiomyopathies

Cardiomyopathy phenotype	*Screening interval (genetic testing and/or clinical family screening is negative)*	*Screening interval (mutation carrier)*	*Level of evidence*
Hypertrophic	Every 3 years until age of 30 (yearly during puberty); only if symptoms develop thereafter	Every 3 years until age of 30 (yearly during puberty); every 5 years thereafter	B
Dilated	Every 3–5 years beginning in childhood	Yearly in childhood; every 1–3 years in adults	B
ARVC	Every 3–5 years after age 10	Yearly after age 10 to 50 years of age	C
LVNC	Every 3 years beginning in childhood	Yearly in childhood; every 1–3 years in adults	C
Restrictive	Every 3–5 years beginning in adulthood	Yearly in childhood; every 1–3 years in adults	C

LVNC = left ventricular noncompaction.
ARVC = arrhythmogenic right ventricular cardiomyopathy.

Functional Significance of Genetic Variant Associated With DCM

The genes that have been implicated in DCM are diverse and encode the sarcomere, cytoskeleton, nuclear envelope, transcriptional pathways, and mitochondrial proteins. The mechanistic explanations of the development of DCM as a result of these mutations are complex. This can include alterations in force generation and transmission, altered metabolic profiles, abnormal nuclear integrity, altered protein degradation, and ion channel abnormalities which can finally lead to a final common pathway, resulting in LV dysfunction and HF (McNally et al., 2013).

Mutations in the nuclear-envelope intermediate filament protein, *LMNA*, typically cause DCM with progressive conduction disease, thus propagating the development of lethal arrhythmias and sudden cardiac death. Pathogenic variants in LMNA gene account for up to 5% of all cases of autosomal dominant DCM (van Tintelen et al., 2007). The lamin A/C gene (LMNA) encodes the A-type lamins A and C, which constitute the inner nuclear membrane. Several studies have shown that besides providing structural integrity to the cell nuclear envelope, particularly in heart and skeletal muscle, A-type lamins are involved in a number of other functions including mechanosensing, chromatin organization, DNA replication, transcription, and repair (Schreiber and Kennedy, 2013; Dahl et al., 2008). The variety of tissues involved, and variable functions of A-type lamins, may explain the increasing number of diseases associated with LMNA mutations, starting from the first case described in Emery–Dreifuss muscular dystrophy (EDMD) (Bonne et al., 1999) and then followed by more than 15 different laminopathy disorders described in man (Schreiber and Kennedy, 2013). The full range of functions of lamins and the mechanisms underlying these different phenotypes are complex and still incompletely understood. Genetic mutations of Lamin A gene causing cardiomyopathy are believed to cause loss-of-function and, either by haploinsufficiency or by dominant negative effect, lead to altered structural organization and/or altered protein expression and transcription factors critical for normal cardiomyocyte function (Schreiber and Kennedy, 2013; Dahl et al., 2008; Chen et al., 2013). A-type lamins form a molecular scaffold under the inner nuclear membrane and connect structurally with the cytoplasm by binding integral proteins of the nuclear envelope (Chen et al., 2013; Davidson and Lammerding, 2014). This network connects the nucleus to the extracellular matrix and provides nuclear anchorage, mechanical stress sensing, and resistance to pathologic deformation of the cell. Previous studies have shown that cells-expressing LMNA mutations exhibit disorganization of the proteinaceous meshwork, instability, and structural damage of the nuclear envelope. For example, the LMNA D192G mutation, which causes a severe form of DCM, is characterized by dramatic ultrastructural changes and disruption of the cardiomyocyte nucleus, as observed in heart tissue from patients and in cellular models (Sylvius et al., 2005). Further studies have suggested that cardiomyopathy mutations in LMNA lead to increased nuclear fragility resulting in cell death and progressive failure in tissues that are exposed to repetitive mechanical stress, such as seen in normal heart function (Davidson and Lammerding, 2014). Additional mechanisms associating LMNA mutations with DCM are through repetitive disruptions of the nuclear envelope, leading to impaired protein distribution into cell compartments, translocations of large amount of proteins to the cytoplasm that trigger cell apoptosis as well as impairment in gene expression derived from epigenetic changes in the chromatin that leads to disruption of intracellular signaling pathways important for cardiomyocyte function and survival (Tesson et al., 2014).

TTN, the gene encoding the sarcomere protein titin that represents the largest known protein in humans, was recently shown to explain over a 25% of all autosomal dominant cases of DCM (Gerull et al., 2002). Most of the pathogenic variants found in the TTN gene were attributed mainly to truncation mutations (Herman et al., 2012). Two main titin molecules exist in the sarcomere, and they are necessary for sarcomere assembly through there attachment at the Z-line and M-line (Knöll et al., 2002). Mutations that substantially disrupt the structure of full-length titin might cause DCM by means of several mechanisms. RNA- and protein-surveillance pathways most likely degrade some truncated titin peptides (Gramlich et al., 2009). Decreased titin levels could limit sarcomere formation and might cause cardiac dysfunction and remodeling. Truncated titin proteins in subjects with DCM would most likely be anchored at the Z-line but would not include the M-band residues that anchor titin to the middle of the sarcomere through myomesin, encode a kinase domain, and interact with many proteins. Moreover, the M-band portion of titin is implicated in sensing and modulating sarcomeric force. Therefore, loss of these interactions could lead to DCM through the disturbance of the normal regulation of sarcomeric force (Lange et al., 2005; Gräter et al., 2005). Further study of the functional consequences of TTN truncating mutations on myocardial physiological features and myocyte signaling is warranted. Elucidating the mechanisms underlying the functional effects of TTN truncating mutations and analysis of other classes of TTN genetic variations (e.g., missense variants) should improve our understanding of the importance of TTN in the pathogenesis of DCM.

Patients with DCM as part of a neuromuscular syndrome classically have X-linked or autosomal recessive inheritance. Duchenne muscular dystrophy, Becker muscular dystrophy, and Emery–Dreifuss muscular dystrophy commonly present in childhood, with a predominant musculoskeletal phenotype with coexistent DCM. These dystrophies are caused by mutations in the DMD gene that encodes the protein dystrophin (Towbin et al., 1993). Pathogenic variances in mitochondrial DNA or in nuclear genes that code for mitochondrial components have also been implicated in early-onset DCM. Patients with mitochondrial gene mutations often present in early childhood with DCM in conjunction with additional pathologies, including skeletal myopathies, neurological, or developmental diseases (Weintraub et al., 2017). Multiple genes were associated with the development of LVNC, predominantly inherited in an X-linked pattern. However, other patterns were described including mitochondrial inheritance. Gene mutations commonly involved in LVNC include the TAZ/G4.5 gene resulting in Barth syndrome, sarcomere-encoding gene mutations, most commonly in MYH7 and MYBPC3, as well as mutations in the SCN5A and DMD genes (Towbin et al., 2015).

Genetics to Assess Treatment Response in HF and Heart Transplantation

Standard medical therapy has dramatically improved survival in patients with HF. However patients often respond in a variable manner to medical therapy. Genetic variation may play an important role in this variable response to medications used to treat HF. Susceptibility loci within genes encoding proteins involved in neurohormonal pathways have been implicated in variable drug response. Although GWAS have identified drug–gene interactions in other cardiovascular conditions (Bruck et al., 2005), no such allelic variants have been associated with response to HF therapies.

Candidate gene analysis has identified the Arg389Gly variant in the beta-1 adrenergic receptor to play an important role in the variable response observed with bucindolol therapy.

The Arg389 variant is associated with greater agonist-promoted G protein coupling, resulting in a more active form of the beta-1 adrenergic receptor (Terra et al., 2005). Arg389 homozygotes exhibit enhanced chronotropy, contractility, and effective sympathetic blockade by bisoprolol (Terra et al., 2005). The gain-of-function Arg389 β1-adrenergic receptor polymorphism was associated with higher LVEF and peak oxygen consumption, better response to beta-blockade and overall improved survival with bucindolol therapy (Liggett et al., 2006; Cresci et al., 2009). However, these findings were not replicated in a substudy of the MERIT-HF trial (White et al., 2003). Polymorphisms Ser49Gly in the beta-1 adrenergic receptor gene and the Glu27Gln variant in the beta-2 adrenergic receptor gene have also been reported to modulate beta-blocker response in HF patients as homozygotes exhibit greater improvement in echocardiographic parameters with beta-blockers compared with wild-type subjects (Metra et al., 2010). Patients with HF who are homozygous for the Gln27 variant have a diminished response to carvedilol (Nelveg-Kristensen et al., 2015). However, subsequent larger studies have not confirmed these associations (White et al., 2003). Due to a lack of replication of findings of these candidate-gene and drug-response associations, genotyping to guide administration and dosing of beta-blockers in HF has not found clinical applicability.

Allelic variations of the genes encoding proteins of the renin–angiotensin–aldosterone axis have also been studied. One of the most commonly studied polymorphism is the insertion/deletion variant of the angiotensin-converting enzyme gene (*ACE I/D*). The deletion of a 250-bp DNA fragment was detected within the intron 16 of the angiotensin I-converting enzyme (*ACE*) gene that is associated with higher ACE activity, progression of left ventricular remodeling, and HF. Patients with this intronic deletion most benefit from administration of high doses of ACE inhibitors, suggesting that determination of ACE genotype may help to target therapy for patients with HF (Palmer et al., 2003; Huang et al., 2004; McNamara et al., 2001; Andersson et al., 1999; McNamara et al., 2004).

Prior studies in hypertensive patients demonstrated that DD homozygotes had increased plasma aldosterone levels compared with heterozygotes, despite long-term treatment with ACE inhibitors (McNamara et al., 2001). In the Genetic Risk Assessment of Cardiac Events Study of 479 patients with HF, the presence of the D variant was associated with lower transplant-free survival at 1 year, mainly in patients on low-dose ACE inhibitors or not treated with beta-blockers (McNamara et al., 2001; McNamara et al., 2004). These results were not replicated in a recent Danish cohort of 534 HF patients treated with ACE inhibitors which suggested no association between pharmacogenetic scores, including variants in genes encoding ACE, angiotensin II receptor type I, and bradykinin receptor, with mortality (Díaz-Molina et al., 2012).

As opposed to the SNPs in various genes encoding proteins of the neurohormonal axis, polymorphisms in genes encoding the hepatic P450 cytochrome enzymes CYP3A5 and CYP3A4 are important determinants of metabolism of tacrolimus which is the cornerstone of immunosuppressant therapy following heart transplant. A SNP in the third intron of *CYP3A5* (rs776746, 6986 G>A) affects the premessenger mRNA splicing, resulting in lack of the active protein. Individuals homozygous for this allele (*CYP3A5*3*) demonstrate reduced tacrolimus clearance and require lower medication doses to achieve the target plasma concentrations compared with carriers of the wild-type alleles (Elens et al., 2011). An additional SNP, the *CYP3A4*22* (rs35599367, intron 6 C>T) which results in reduced hepatic *CYP3A4* mRNA expression and enzyme activity, has been associated with decreased tacrolimus clearance and lower tacrolimus dose requirements (Deininger et al., 2016; Birdwell et al., 2015). Although the *CYP3A5* SNPs do not seem to affect prognosis after heart transplantation, they are increasingly being tested in heart transplant centers as they guide initial tacrolimus dosing requirements. The Clinical Pharmacogenetics Implementation Consortium [CPIC] dosing guideline for tacrolimus recommends increasing the starting dose by 1.5–2 times the recommended starting dose in patients who are CYP3A5 intermediate or extensive metabolizers, though total starting dose should not exceed 0.3 mg/kg/day (Parsa et al., 2011).

Genetics and HF Prognosis

Several SNPs have been demonstrated to be involved in HF phenotype and severity of the disease. A three-stage genome-wide, case-control, and case-only association study was performed, and 21 SNPs associated with cardiac hypertrophy were identified (Pugh et al., 2014). The control population consisted of 851 Amish individuals as part of the Heredity and Phenotype Intervention Heart (HAPI Heart) Study. The HF cohort consisted of unrelated prospectively enrolled patients from the University of Cincinnati College of Medicine ($n=1302$) and the University of Maryland School of Medicine ($n=308$), with left ventricular ejection fraction (LVEF) <35%.

One of the SNPs associated with hypertrophy (rs2207418, $p=8\times10^{-8}$) was associated with reduced ejection fraction (HFrEF) (RR 1.85, $p=0.0019$), and among HF patients, rs2207418 was associated with increased mortality (HR 1.51, $p=0.0004$). Among

HF patients in the European cohort of the CHARGE consortium population described above, analysis suggested a locus on chromosome 3p22 in the intron of a CKLF-like MARVEL transmembrane domain containing 7 (*CMTM7*), a chemokine-like factor gene to be associated with higher mortality, with an odds ratio about 1.5 ($p = 0.001$). No loci reached statistical significance for association with prognosis in HF patients of African ancestry (Morrison et al., 2010). An expansion of the CHARGE consortium analysis with data and follow-up from 2828 new HF cases from the Atherosclerosis Risk in Communities (ARIC) Study, the Cardiovascular Health Study (CHS), the Framingham Heart Study (FHS), the Health, Aging and Body Composition (Health ABC) Study, and the Rotterdam Study (RS) identified a SNP (rs9885413) on chromosome 5q22 associated with 36% increased risk of death ($p = 2.7 \times 10^{-9}$) but not with incidence of the disease in patients with HF. The SNP was associated with expression of *TSLP*, a gene encoding the cytokine TSLP that promotes Th2 immune response and is involved in angiogenesis and fibrosis (Smith et al., 2016).

Additional studies are needed to validate these finding and to investigate the use of this SNP in predicting severe HF phenotypes that may require more aggressive therapy.

Clinical Implications and Future Perspectives

Although the contribution of genetic variation to pathogenesis, progression, and outcomes of patients with HF is being increasingly accepted, the currently available data are limited by the heterogeneity in the phenotype of HF and the lack of reproducibility of the generally modest effect size (odds ratio ≤ 1.5) associated with susceptibility variants. One approach to overcome this limitation would be NGS (whole exome or whole-genome sequencing) to identify regions associated with HF risk; however this approach given the extent and complexity of data is fraught with statistical and bioinformatic interpretation complexity. Functional genomic studies to identify the role of noncoding or regulatory regions or loci in linkage disequilibrium to demonstrate the significance of genetic variants not only on gene but also on cardiac function are necessary. Applying genotyping and sequencing strategies to clinical practice is also challenging due to physician and patient perceptions, demonstration of incremental value of such information over existing clinical tests, insurance coverage, and stringent criteria applied to adopt such strategies into clinical practice guidelines.

In the case of inherited cardiomyopathies, when performing genetic testing, the standard approach is usually to perform a full screen of all known genetic variants in the index case (proband), and then perform focused genetic testing, if found to be pathogenic or likely pathogenic, in at-risk family members. The development of multigene panels and addition of new genes associated with cardiomyopathy enables a more accurate diagnostic yield. In one study, increasing the DCM gene panel from 5 to 46 genes increased the clinical sensitivity of the test from 10% to 37%, but at a cost of significant increase in variants of uncertain significance (VUS), with a parallel increase in inconclusive cases from 4.6% to 51% (Pugh et al., 2014).

However, whole-genome and -exome sequencing the cost of which is decreasing, information from which can be incorporated in the electronic medical record and the potential availability of this information as new pathogenic variants are discovered especially if standard cardiomyopathy gene panels are negative pose as attractive alternative testing strategies. Currently, clinical whole-exome sequencing is reserved for patients, and their parents (a trio) with undiagnosed syndromes or in families in whom targeted gene panels do not identify an etiologic genetic variant.

Irrespective of sequencing strategies, the importance of genetic counseling for all patients and first-degree relatives of patients with familial DCM, including discussion of risks, benefits, and options for such genetic testing should be emphasized, and if such expertise does not exist, referral of such patients to centers experienced in comprehensive genetic evaluation should be made.

Although several genomic studies as described above have identified multiple novel genes and loci implicated in HF and DCM, no novel drug targets have emerged from these studies. Gene editing is an emerging technique that can be applied to target pathogenic genes associated with DCM and may be useful especially in curing monogenic diseases. For example, Long et al have used gene editing to restore dystrophin expression in an animal model with muscular dystrophy with promising results (Long et al., 2016). The challenges lie in interpreting the massive amount of data generated from large genetic studies and in understanding which of these and how significant genetic variants directly lead to cardiomyopathy and HF especially if these are located in intergenic or intronic areas. However, these studies may shed light on to mechanistic pathways that may help in understanding the biology and pathogenesis of HF.

Conclusions

The role of rare genetic variation in the development, progression, and prognosis of cardiomyopathies is well established. However we currently lack evidence demonstrating the role of common genetic variation in complex forms of HF due in part to phenotypic heterogeneity and the need for additional studies of gene–gene, and gene–environmental interactions that may also influence susceptibility to develop HF. The decreased cost and increased availability of NGS, improved phenotype characterization, and incorporation of multiple "omics" platforms may allow the promise of an individualized approach to HF patients that would perhaps eventually improve outcomes in this complex and morbid disease process.

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Heart Development

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Formation of the Embryonic Cardiac Crescent and Heart Tube

In its earliest stages, the embryo exists as a bilayer disc, with two single cell thickness sheets of cells (epithelia) suspended between two fluid filled cavities, the yolk sac and the amniotic cavity (**Fig. 1A**). The ventral epithelium (facing the yolk sac) is the hypoblast, which will eventually be relegated to extraembryonic structures. The dorsal epithelium (facing the amniotic cavity) is the embryonic ectoderm, which will form the embryo. This single layer will transform into three embryonic germ layers: the ectoderm (nervous system and skin), mesoderm (heart, skeleton, muscle, and connective tissue), and endoderm (gut). Embryonic mesoderm and endoderm differentiate from the primitive ectoderm by a process known as gastrulation. Gastrulation can be observed in the embryo as a groove forming in the epiblast, starting at the tail end (caudal) and gradually extending toward the head (cranially) to an endpoint known as the prechordal plate (**Fig. 1B**). This groove is known as the primitive streak, and its leading edge is the primitive node. What forms this streak is cells detaching from the epiblast and invading the space between the epiblast and hypoblast. As this occurs, these cells transform into a new cell type (mesoderm) and are no longer part of an epithelium, but are migratory and dynamic cells. This is an example of a developmental process known as epithelial to mesenchymal transition (EMT). On the molecular level, these cells respond to EMT inducing signaling molecules including TGF-β and Notch (Mercado-Pimentel and Runyan, 2007; Luna-Zurita et al., 2010), losing cell polarity and downregulating cell–cell adhesion proteins such as E-cadherin to allow migration (Thiery and Sleeman, 2006). The heart forms from these migrating cells as a crescent-shaped structure cranial to the prechordal plate and extending along both sides. The cells making up this cardiac crescent are termed the lateral plate mesoderm

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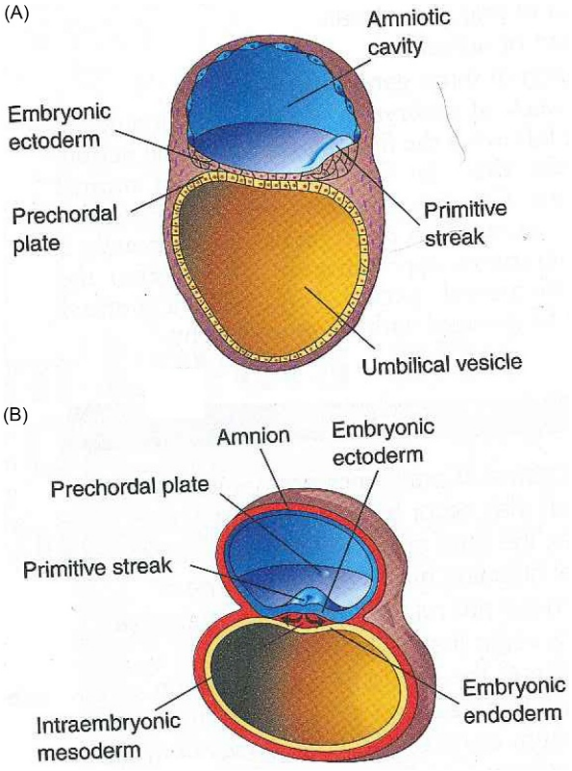

Fig. 1 The embryonic bilayer disc, and initiation of gastrulation; day 15–16 postconception. (A) Sagital section (cranial to the *left* of image, caudal to the *right*) through the embryo showing the embryo (ectodermal layer only) suspended between the amniotic cavity and the yolk sac. (B) Transverse section as the primitive streak begins at the caudal end of the embryo and extends cranially toward the prechordal plate. Cells delaminate from the ectoderm at the primitive streak and form the other two germ layers—mesoderm and endoderm- during the process of gastrulation. From Moore and Persaud, *The Developing Human*, *8th edition* Saunders Elsevier; 2008, p. 56.

(**Fig. 2A** and **B**). Fate mapping studies suggest that many cell fate decisions are set during gastrulation or even before (Fishman and Chien, 1997; Garcia-Martinez and Schoenwolf, 1993; Xin et al., 2013). For example, the apex of the cardiac crescent is formed from cells that have migrated through the primitive streak closest to the primitive node. These cells will contribute to the most anterior portion of the heart, connecting to the outflow tracts and the secondary heart field (see section "Formation of the Linear Heart Tube and Cardiac Looping"; **Fig. 2**; compare B and C). The cells on either side of this region have migrated through the midportion of the streak and will form the primitive ventricle (eventual left ventricle). Finally, the most lateral and caudal cells are those that have migrated through the most posterior part of the streak and will become the inflow and atrium. The exact details of when and how this occurs are still under debate and refinement as better techniques become available (Abu-Issa and Kirby, 2007; Cui et al., 2009; Ehrman and Yutzey, 1999). Fate-mapping studies using live-cell tracking and time-lapse imaging demonstrate that these cells move as a cohort (tissue motion) rather than individually migrating. In this way, the cells maintain their relative positions within a tissue and eventually within the organ (Cui et al., 2009). Although it is not yet apparent in the symmetric embryo at this early stage, the basic left–right asymmetry of the embryo is set during gastrulation. This occurs via concentration gradients of the factors sonic hedgehog (shh) and fibroblast growth factor 8 (fgf-8) created by ciliary motion at the primitive node. This in turn causes a cascade of downstream genes to be activated, including nodal, lefty, and pitx-2 (Capdevila et al., 2000; Rodriguez-Esteban et al., 2001; Tsukui et al., 1999) (**Fig. 2D**).

Formation of the Linear Heart Tube and Cardiac Looping

The cardiac crescent is a symmetric structure, and it will next fuse in the midline into an initially symmetric appearing heart tube as shown in **Fig. 2C, E** and **F**. This primitive heart tube is two cell layers thick. There is an inner endocardium and an outer myocardium. The endocardium will eventually form the inner lining of the heart and the valves, in processes that we will discuss in later sections. The myocardium is cardiac muscle, and will become thicker and organize into the contractile myocardium of the atria and ventricles. Fusion of the primitive heart tube occurs as the ectoderm dorsal to the crescent folds to create the neural canal, and the endoderm ventral to the crescent folds to create the foregut (**Fig. 2E**). As the foregut forms, it moves dorsally, pushing the cardiac crescent ventrally and medially, such that the two arms of the crescent fuse into a tube sitting directly in front of the foregut

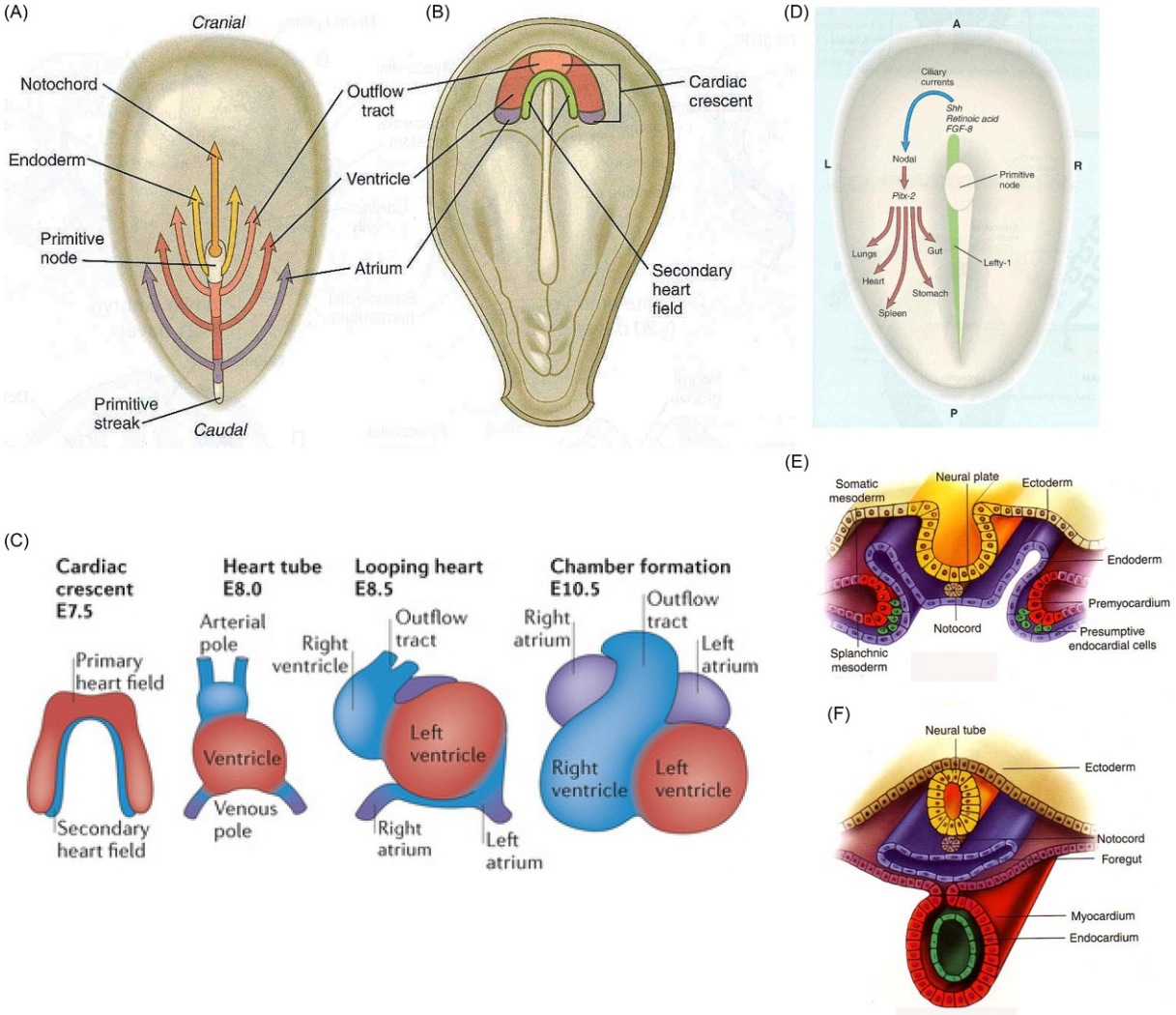

Fig. 2 Cell fate decisions in the heart are set at gastrulation, as is *left–right* asymmetry of the entire embryo. (A) Position of cells migrating through the primitive steak determines their eventual position in the developing heart. (B) Formation of the cardiac crescent, the first cardiac structure in the embryo, with primary and secondary heart fields. (C) Preservation of cell position during fusion of the limbs of the cardiac crescent into the primitive heart tube and after cardiac looping. See contributions from primary and secondary heart fields. (D) Genes regulating *left–right* asymmetry during gastrulation, with ciliary currents creating a gradient of Nodal expression to the *left* side of the embryo, initiating a cascade of other signaling molecules as shown. (E) and (F) Formation of the three germ layers and fusion of the primitive heart tube. Cardiomyocytes originate from mesodermal precursors (*red* and *green*). The heart tube fuses as it is displaced ventrally and to the midline by the folding gut tube (*blue*). (A, B) From Carlson, B. M. (2014). *Human embryology and developmental biology* (5th edn.), p. 105. Philadelphia: Elsevier Saunders. (C) From: Xin, M et al. (2013). Mending broken hearts: Cardiac development as a basis for adult heart regeneration and repair. *Nature Reviews Molecular Cell Biology* **14** (8), 529–541. (D) From: Carlson, B. M. (2014). *Human embryology and developmental biology* (5th edn.), p. 87. Philadelphia: Elsevier Saunders. (E) and (F) From: Mikawa, T. (1999). Cardiac lineages. In Harvey and Rosenthal (eds.). *Heart development*, p. 20. Academic Press.

(Fig. 2F). As fusion of the heart fields occurs, the heart begins to beat. Blood has not yet been formed by the embryo, but fluid is pumped through the heart as it begins to beat. The posterior/inferior portion of the tube retains two limbs, the sinus venosus or inlet portion of the tube (Fig. 2C), which draws the fluid into the heart tube. The fluid then passes through the atrial and ventricular segments and is pumped out through the anterior/superior outflow tract, which connects to aortic sac and paired dorsal aortae. In a human fetus, this happens at approximately 22 days gestation, but in a mouse embryonic on day 8.

Within four days of heart tube formation, the heart tube undergoes a dramatic change in a shape known as cardiac looping. Here, the heart tube loses its symmetry, and forms an "S" shape, with distinct left and right sides (Steding, 2009) (Fig. 3A–E). Much is known about the molecular signaling and gene expression patterns involved in looping. Several cardiac transcription factors have been shown to be important to looping, including Nkx2-5, MEF2, and Hand2 (Yamagishi et al., 2001; Karamboulas et al., 2006; Jamali et al., 2001a,b; Christoffels et al., 2006a; Hiroi et al., 2001; Cui et al., 2009; Srivastava et al., 1997; Han et al., 2006; Han and

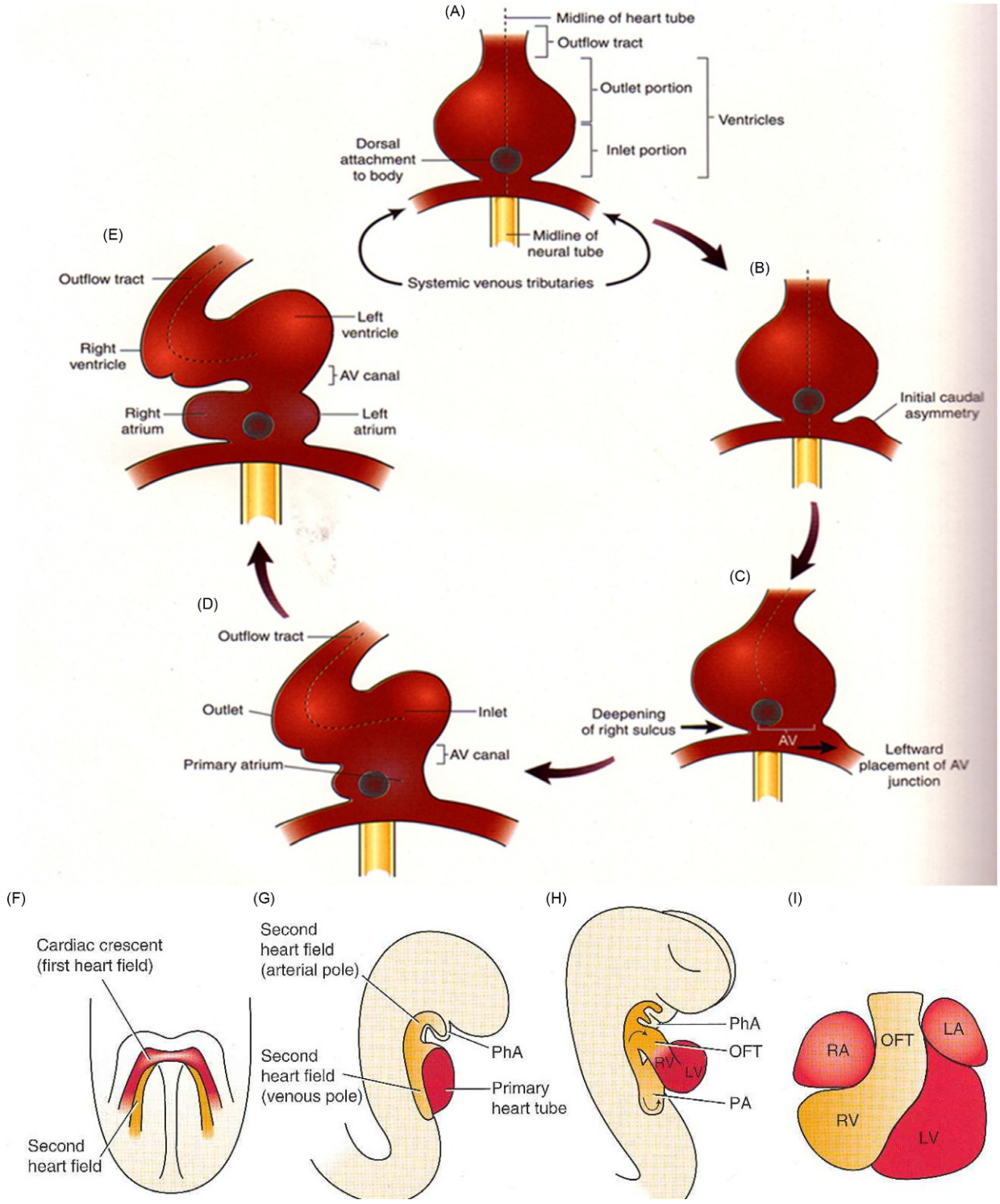

Fig. 3 Normal cardiac looping (D-looping) with contributions from the secondary heart field. (A–E) The morphologic changes of the heart as it progresses from the linear heart tube (A) to the looped heart, with inlet segment to the *left* and elongated outflow segments to the *right* (B–E). (F–I) The second heart field (*yellow*) forms medial to the cardiac crescent (F) These cells migrate into the looping heart, primarily in the outflow region, contributing to the outflow tracts and *right* ventricle as shown, primary heart field derivatives in *red* and secondary in *yellow*. (A–E) From: Brown, N. and Anderson, R. (1999). Symmetry and laterality in the human heart: Developmental implications. In Harvey and Rosenthal (eds.) *Heart development*, p. 458. Academic Press. (F–I) From Schoenwolf et al. (2015). *Larsen's human embryology* (5th edn.), p. 275. Philadelphia: Elsevier Churchill Livingstone.

Olson, 2005; McFadden et al., 2005; Tsukui et al., 1999). Disruption of any of these factors in mice by gene knockout causes embryonic lethality, due to abnormal looping. Cardiac looping also involves differential growth, with higher proliferation of myocytes along the outer curvature (outflow tract and ventricular segments) than the inner curvature (inflow tracts and atrial segment; **Fig. 3E**) (Moorman and Christoffels, 2003). Furthermore, there is a migration of cells from a specialized pool of cardiac precursors anterior and medial to the original heart field known as the secondary heart field, as shown in **Fig. 3F**; also see **Fig. 2C** (Kioussi et al., 2002). These cells populate the outflow tracts and part of the ventricular segment, specifically the eventual right ventricle (**Fig. 3G–I**). (Kelly and Buckingham, 2002; Mjaatvedt et al., 2001; Waldo et al., 2001; Christoffels et al., 2006b). Discovery of the secondary heart field was primarily using cell lineage tracing studies. In chick hearts marked just after fusion of the heart tube, a population of unmarked cells interposed themselves between marked cells of the distal and middle heart tube one day later (Mjaatvedt et al., 2001). Studies in mouse compared to chick suggest that the anterior heart field in mouse forms most of the right ventricle (Kelly and Buckingham, 2002; Zaffran et al., 2004). What differences are there in humans remain unknown. Both differential growth and addition of secondary heart field cells serve to elongate the outflow tract and enlarge the ventricles relative to the atria (Moorman and Christoffels, 2003). In addition, there are mechanical forces involving the cytoskeleton, including nonmuscle actin bundles (Itasaki et al., 1991) and myosin (Linask and Vanauker, 2007), the motor protein dynein (Basu and Brueckner, 2008; Brueckner, 2001; Supp et al., 1997), and microtubules (Icardo and Ojeda, 1984).

Normal looping occurs with the ventricular segment and outflow tract out-pouching anteriorly and to the right (D-looping), and the sinus venosus and atria shifting posteriorly and to the left. Importantly, the sinus venosus also moves cranially such that it lines up with the outflow tracts along the horizontal body axis. This repositioning is critical for proper atrioventricular and ventriculoarterial connections.

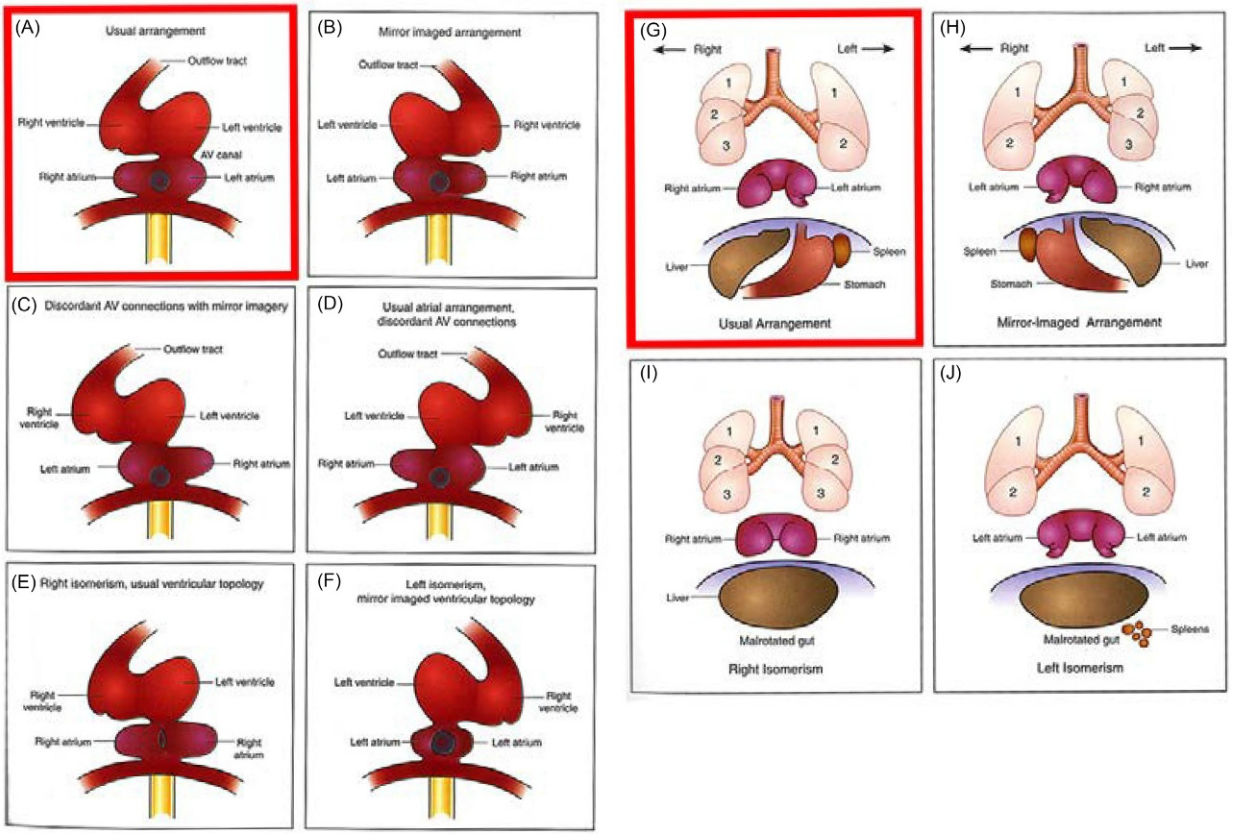

Fig. 4 Diagram of normal cardiac D-looping with normal AV connections (A). Also shown are several common abnormal looping patterns, including mirror image L-looping with normal AV connections (B), discordant AV connections with atrial situs inversus (C), and with normal atrial situs (D). Both C and D are usually associated with discordant ventriculoarterial connections, creating congenitally corrected transposition. Below are *right* and *left* atrial isomerism, or bilateral *right* sidedness (E) and bilateral *left* sidedness (F), generally found along with anomalies of the systemic and pulmonary veins, of cardiac septation and of the inflow and outflow tracts. *Right* and *left* isomerization syndromes also go along with abnormal sidedness on other asymmetric organs of the chest and abdomen, or heterotaxy. Shown in G is normal visceral organ arrangement. H shows mirror image, or situs inversus; I *right* isomerism and J *left* isomerism. Adapted from: Brown, N. and Anderson, R. (1999). Symmetry and laterality in the human heart: Developmental implications. In Harvey and Rosenthal (eds.) *Heart development*, p. 449 and 459. Academic Press.

Heart Defects Arising from Abnormalities of Looping

Sometimes, looping can occur to the left, with the venous pole and atria moving rightward and the ventricles and arterial pole moving leftward. This is referred to as L-Looping (**Fig. 4B**, D and F). The ventricles switch positions when L looping occurs: the right ventricle is on the left and left ventricle on the right. This is often associated with L malposition of the great arteries, in an interesting anomaly known as congenitally corrected transposition of the great arteries (CCTGA). Here the right atrium is connected to the left ventricle, which is then connected to the pulmonary artery (PA). The left atrium is connected to the right ventricle, which is connected to the aorta. Thus, the atrioventricular and ventriculoarterial connections are both incorrect, but the circulation of blood is actually correct—the two abnormal connections correct the circulation. CCTGA can be an isolated anomaly, or can occur with other congenital heart defects such as abnormal septation or abnormal valve development. Also in the category of abnormal laterality are global embryonic defects known collectively as heterotaxy (**Fig. 4H–J**). In heterotaxy, left–right assignments along the body axis are individually randomized. Thus, the heart can end up on the right side of the body instead of the left, sometimes with a right sided stomach and liver. In addition, it is possible to have an abnormal laterality within the heart, with the left and right atria switching positions (atrial situs inversus) or being bilaterally symmetric with two left atria, or two right atria. When laterality and/or looping are abnormal, each segment is independent of the last, leading to a complex set of possible anomalies.

Ventricular Inlet Septation: Endocardial Cushions

After cardiac looping has occurred, the orientation of the heart tube has changed, but the blood flow through it remains essentially the same: blood flows into the heart from the sinus venosus, through the atrial segment into the ventricle, then out the conotruncus to the aorta. Inflow and atrial segments are leftward, and after D looping, the ventricle and outflow segments are rightward. During the next phase of heart development, the atrioventricular (inlet) and ventriculoarterial (outlet) structures will realign and septate, forming four chambers, a right and left atrium and a right and left ventricle. All four chambers undergo extensive remodeling, with muscular septa dividing right and left atria and ventricles, and one-way valves between atrium and ventricle and between ventricles and outflow tracts. The four cardiac valves serve to ensure unidirectional flow through the heart as it pumps, opening and closing sequentially with the cardiac cycle. In human embryos, this occurs during week 7 of gestation (Dhanantwari et al., 2009; Steding, 2009).

The cardiac valves are formed primarily from a tissue known as the endocardial cushions. This is a second example of EMT in the developing heart. Within the primitive heart tube, even before looping, there are faint constrictions between the atrial and ventricular segments (AV groove) and in the forming conotruncus between the ventricular segment and the outflow tract. The endocardial cushions initially appear as swellings in the AV groove and conotruncus, caused by cells from the inner lining of the heart (endocardium) undergoing EMT and migrating into the extracellular matrix between the endocardium and the myocardium (**Fig. 5A, C** and D) (Lin et al., 2012). Signaling involving the TGF-β family of genes, BMP2, Notch1, and TBX20 among others, are important to this process. This signaling is back and forth between the myocardium and endocardium to initiate and maintain this process (Brown et al., 1996, 1999; Jiao et al., 2006; Combs and Yutzey, 2009).

In both the AV canal and in the conotruncus, four distinct cushions form, named by their anatomic locations. We will start with septation of the aterioventricular canal (**Fig. 5B**). In the AV canal, the cushions are superior, inferior, left, and right. The AV canal septates as the superior and inferior cushions grow toward one another and fuse in the midline. The AV valves themselves form from remnants of the four cushions, which thin out into acelluar fibroelastic structures called leaflets: a leftward mitral valve with two leaflets, and a rightward tricuspid valve with three. The developing tricuspid valve is shown in **Fig. 5D** and **E**. The valve delaminates from the ventricular wall as it forms, with the leaflets attached to a fibrous annulus or ring. The leaflets open on hingepoints at the annulus, allowing blood to flow freely from atrium to ventricle (ventricular filling phase, or diastole). They are supported by muscular (chordea tendinae) attached to the ventricular muscle, which ensures that they close tightly in the midline as the ventricle contracts and empties (ventricular contraction, or systole), to prevent backflow of blood. The septation of the inflow tracts, along with the formation of the atrial and ventricular septa (with contributions from endocardial cushion tissue, see below), forms the crux of the heart. This is the three-dimensional juncture between the four chambers: left (mitral), right (tricuspid), atrium, and ventricle.

Heart Defects Arising from Abnormalities of Inlet Septation and Endocardial Cushions

Abnormalities in the endocardial cushions are an important subtype of congenital heart lesions, known as endocardial cushion defects. Failure of complete fusion between the superior and inferior cushions in the midline results in a cleft within the anterior leaflet of the mitral valve. This is a milder form of an endocardial cushion defect, often associated with a primum atrial septal defect 888 and known as a partial atrioventricular canal. A more profound failure of proper AV cushion expansion and fusion results in a complete atrioventricular canal. Here the crux of the heart is unformed, as in **Fig. 5C**. There remains a common orifice overlying both the ventricles, with defects in the adjacent atrial and ventricular septa. The common atrioventricular valve has leaflet structure similar to the unseptated AV canal, **Fig. 5B**, with four or sometimes five separate leaflets.

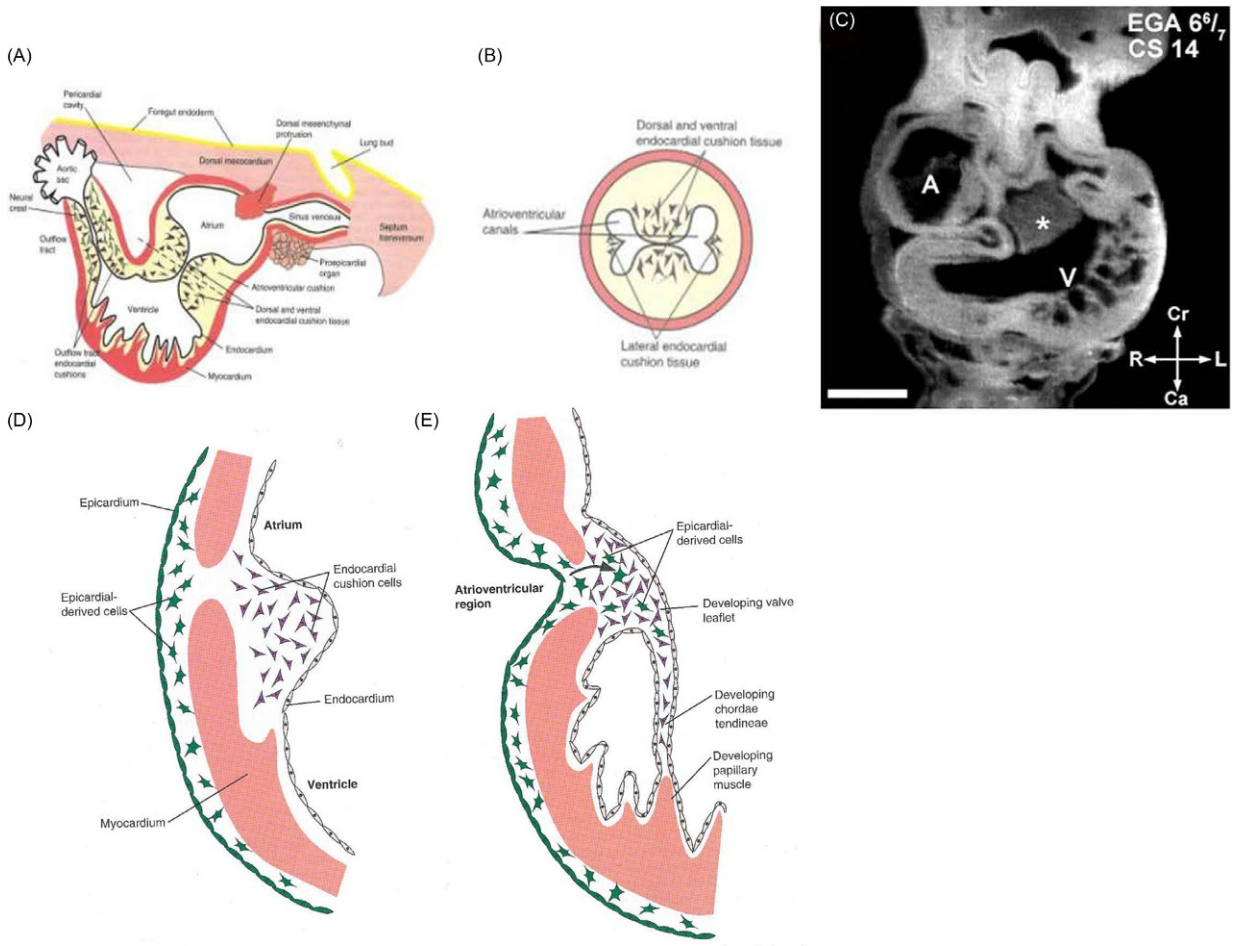

Fig. 5 Development of the endocardial cushions and atrioventricular valves: (A) Schematic diagram of cushion formation in the inflow and outflow tracts by EMT in the looped heart tube. Note continuity between the AV and conotruncal cushions along the inner curvature. Also shown are the neighboring tissues and cell populations contributing to the developing heart at this stage. (B) Schematic of the septating AV canal, leaflets named by position as shown. (D) and (E) are schematics of the subsequent delamination and formation of support structures of the AV valves (tricuspid valve is shown). (C) is an EFIC (episcopic fluorescence image capture) image of a sectioned paraffin-embedded human embryos at 6 6/7 weeks. Seen are atria (A), ventricles (V), and AV cushions (*). Scale bar = 0.515 mm. (A, B, D, E) From Schoenwolf et al. (2015). *Larsen's human embryology* (5th edn.), p. 284, 291. Philadelphia: Elsevier Churchill Livingstone. (C) From: Dhanantwari, P., Lee, E., Krishnan, A., et al. (2009). Human cardiac development in the first trimester: A high-resolution magnetic resonance imaging and episcopic fluorescence image capture atlas. *Circulation* **120** (4), 347.

Ventricular Outflow Tract Septation: Endocardial Cushions and Neural Crest

Septation of the outflow tract involves complex interactions between several resident and migrating cell types in a tightly regulated spatial and temporal manner. This includes the primary and secondary heart fields as already described, EMT of endocardium to form the conotruncal endocardial cushions, and addition of yet another population of external migrating cells, the neural crest. These populations are shown in **Fig. 6A**. Neural crest (shown in blue in **Fig. 6A**; secondary heart field in red) are ectodermal cells which originate from neural tissue in the anterior rhombencephalon and migrate through the pharyngeal region and into the aortic arches (Creazzo et al., 1998; Epstein et al., 2000; Li et al., 2003; Waldo and Kirby, 1993; Waldo et al., 1996). These neural crest cells are also important in the development of the nearby parathyroid, thyroid, and thymus glands. In addition, they innervate the heart and form much of the smooth muscle of the proximal aorta (Creazzo et al., 1998). In the conotruncus, recent data suggest a role for neural crest cells contributing to the development and patterning of the pulmonary and aortic valves as they begin to form (Phillips et al., 2013). In addition, neural crest cells surround the epithelia of the arch arteries and extend into the truncus and proximal conus, contributing cells to the truncal cushions (Neeb et al., 2013). The truncal cushions form the aorticopulmonary septum separating the two great arteries, the PA carrying blood to the lungs and the aorta carrying blood to the body (**Fig. 6D** and **E**). This occurs in a spiral, completing an almost 180 degree rotation (Carlson, 2004; Ya et al., 1998) (**Fig. 6C–H**). With this rotation, the PA is positioned directly anterior to the aorta at the level of the newly formed pulmonary and aortic valves, (**Fig. 6F** and **G**, level 3). Further along the outflow tract, the PA and aorta are more left-right to one another, as the spiral extends (**Fig. 6F** and **G**, level 2).

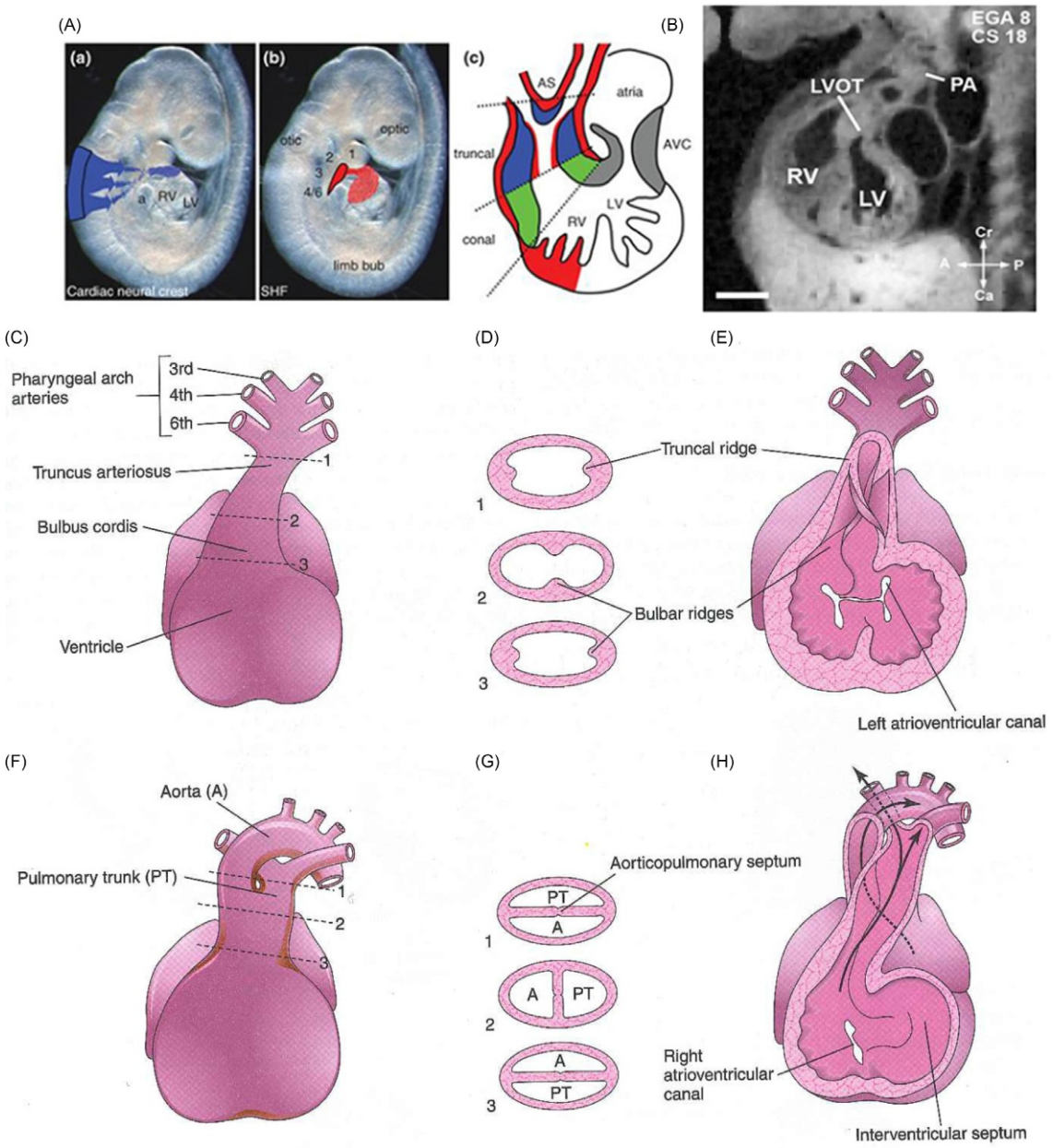

Fig. 6 Septation of the outflow tracts. (A) Contribution of neural crest (*blue*, A) and secondary heart field (*red*, B) migrating into developing outflow tracts in the mouse embryo. The schematic (C) shows the extent of these tissues, as well as the contribution from conal endocardial cushions (*green*). A, atria; RV, LV, *right* and *left* ventricles; 1–6 are numbered aortic arches; AS, aortic sac; AVC, atrioventricular canal. (B) EFIC image of an embryo at 8 weeks showing LV and developing LV outflow tract (LVOT) and the anterior RV and a portion of the PA. Scale bar = 1.35 mm. (C–H) Schematic of heart starting at 5 weeks gestation, with outflow tract beginning as a common truncus connected to the bulbus cordus and common ventricle. As the conotruncal cushions form, the aorticopulmonary septum forms in a spiral fashion as shown. *Dotted lines* in C, F represent levels of sections shown in D, G. (A) From: Neeb, Z., et al. (2013). Cardiac outflow tract anomalies. *WIREs Developmental Biology* **2**, 499–530. (B) From: Dhanantwari, P., Lee, E., Krishnan, A., et al. (2009). Human cardiac development in the first trimester: A high-resolution magnetic resonance imaging and episcopic fluorescence image capture atlas. *Circulation* **120** (4), 347. (C–H) From Moore and Persaud (2008). *The developing human* (8th edn.), p. 307. Saunders Elsevier.

As the aorta arches to the left, the proximal transverse arch passes anterior to the PA, completing a 180-degree turn of the spiral (Fig. 6F and G, level 1). This rotation not only involves the truncal cushions but also involves the rotation of the myocardial tube (Bajolle et al., 2006; Lomonico et al., 1986). With this rotation, the conus of the primitive heart tube is normally retained only by the right ventricle. The left ventricle connects directly to the aorta, as the aortic portion of the truncus shifts leftward. Continuity between the superior atrioventricular and left ventral conal cushions results in continuity between the mitral and aortic valves (see Fig. 5A, and schematic in Fig. 6A).

Heart Defects Arising from Abnormalities of the Ventricular Outflow Tracts

The outflow tracts of the heart are arguably the most complex area of the heart genetically and morphologically, and most prone to developmental defects.

If the 180-degree twist of the great artery septation does not occur or is incomplete, the result is transposition of the great arteries. This is defined by the aortic valve being anterior to the pulmonary valve. The left–right orientation of the valves is most commonly preserved (aortic rightward), although can vary within a 90-degree range, from directly anterior-posterior to directly side by side. L-transposition, aortic leftward, is most often associated with L-Looped ventricles or heterotaxy syndromes, and is rare in isolated transposition. TGF-beta signaling, important in left–right patterning and extracellular matrix remodeling and many other processes in normal development, appears to play an important role in this process both in mouse models and in human disease (Oh and Li, 1997; Rankin et al., 2000; Goldmuntz et al., 2002; Karkera et al., 2007).

Defects in expression of secondary heart field genes can lead to right ventricular hypoplasia and outflow tract abnormalities, including Tetralogy of Fallot and double outlet right ventricle (Ward et al., 2005; Zeisberg et al., 2005). These clinical entities are described in detail elsewhere in this text. Embryologically, malalignment of the conal and truncal cushions results in incomplete closure of the ventricular septum with malalignment of the overlying outflow tracts. In Tetralogy of Fallot, this results in an enlarged aorta overriding the ventricular septum, and an underdeveloped PA. If the malalignment results in both great arteries positioned over the right ventricle, this is known as double outlet right ventricle (van den Hoff et al., 1999; Waller et al., 2000). Clinically, a DORV includes a ventricular septal defect and presence of a conus segment of both ventricles (bilateral conus). As described above, the normal conus is a muscular "neck" within the right ventricle between the tricuspid and pulmonary valves that is not normally present in the left ventricle.

Defects in neural crest migration and/or survival also cause OFT heart defects. The importance of neural crest in outflow tract development has been well studied in animal models using neural crest ablation. Primarily among these are truncus arteriosus and interrupted aortic arch (Waldo et al., 1996; Waldo et al., 2005; Creazzo et al., 1998; Waldo and Kirby, 1993; Nishibatake et al., 1987; Kirby and Waldo, 1995). Again the clinical features of these defects are described elsewhere. Embryologically, truncus arteriosus is lack of separation of the aorta and PA, such that a common trunk persists connected to both ventricles, with a VSD allowing egress from both ventricles. The branch pulmonary arteries grow in from the lungs and connect directly to the common trunk. In interrupted aortic arch, the great arteries septate but malalignment of the conal septum results in hypoplasia of the left-sided outflow and malformation of the aortic arch. Neural crest defects also can also cause Tetralogy of Fallot, double-outlet right ventricle, and VSD as described above.

The genetics of DiGeorge syndrome and TBX1 mutation illustrates the complexity of conotruncal defects. DiGeorge syndrome has clinical features of a neural crest defect, with aortic arch abnormalities, parathyroid and thymic abnormalities (Liao et al., 2004). In human genetic studies, DiGeorge syndrome has been linked to a deletion of up to three megabases in chromosome 22 (22q11) based on kindred studies in families with the syndrome (Beaujard et al., 2009; Epstein, 2001; Merscher et al., 2001; Portnoi, 2009). The defect was recapitulated experimentally in mice, with identification of TBX1 as the specific responsible gene in these mice (Jerome and Papaioannou, 2001; Lindsay et al., 2001; Merscher et al., 2001). TBX1 is a transcription factor in a family known to be important in embryonic patterning; other than some differences in severity, suggesting a role for modifying genes, all of the features of human DiGeorge were seen in the mouse model (Jerome and Papaioannou, 2001). Yet isolated TBX1 mutations appear to be very rare as causes of clinical DiGeorge syndrome in humans (Beaujard et al., 2009; Portnoi, 2009; Rauch et al., 2010); most patients having a larger megabase deletion. Furthermore, TBX1 is not expressed by neural crest cells, but rather in the secondary heart field (Vitelli et al., 2002; Liao et al., 2008). Interestingly, overexpression of Tbx1 in mouse models are also associated with conotruncal defects, suggesting that there are critical levels of Tbx1 necessary for proper outflow tract development (Liao et al., 2004, 2008).

Development of the Ventricles and Ventricular Septum

The majority of the ventricular septum is muscular, and made up of a protrusion of ventricular myocardium that starts at the apex of the primitive ventricle and extends into the ventricular cavity (**Fig. 7A**, asterisk). As the septum extends upward, the separation becomes nearly complete (**Fig. 7B**, arrow). Remaining portions of the septum are closed by endocardial cushion tissue. First, the AV endocardial cushions form the posterior septum adjacent to the AV valves. Second, the conal cushions form the conal septum below the great arteries. An important feature of this process is that these regions of septum contain muscle as well as cushion tissue, and this muscle has its origin in the secondary heart field (Neeb et al., 2013).

Heart Defects Resulting from Incomplete Ventricular Septation

VSDs are among the most common heart defects seen clinically, and can be in isolation or in combination with more complex defects (Soto et al., 1980). The ventricular septum arises from several sources as already discussed. VSDs can be found as tissue defects or holes in any of these components: muscular, inlet (from deficiency of AV cushions), outlet (from deficiency of conotruncal cushions), or membranous, which is the portion of the septum at the juncture of the three components (Soto et al., 1980). The membranous portion of the septum is nonmuscular and made up of fibrous tissue, and is the most common of VSDs.

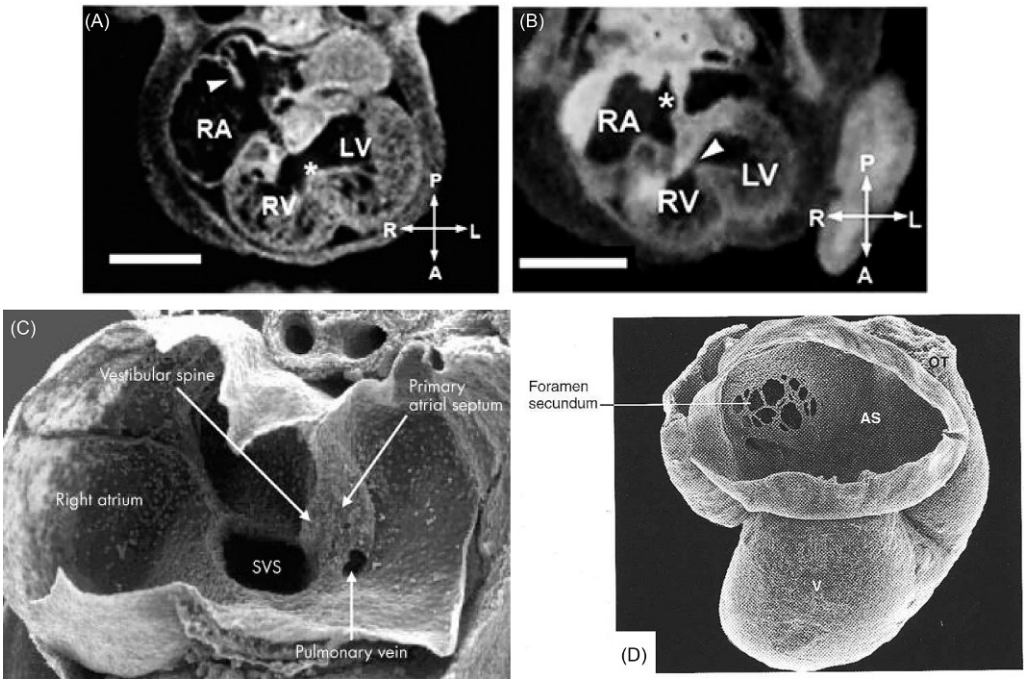

Fig. 7 Atrial and ventricular septation. (A) Human fetal MRI images in transverse plane, fetus at 7 5/7 weeks gestation. Shown are the four chambers of the heart (RA right atrium; RV right ventricle; LV left ventricle). Orientation as shown by the *arrows*: P (posterior), A (anterior), R (right), and L (left). The atrial septum primum is marked by the *arrowhead*, with the foramen primum below allowing shunting of blood from RA to LA. Between the ventricles, the muscular intraventricular septum forms but is not yet complete (*). Scale bar is 1.25 mm. (B) Fetus at 8 weeks gestation. Here the atrial septum primum (*) is fused with the endocardial cushions inferiorly; not seen is the foramen ovale that allows continues *right* to *left* atrial shunting in the fetus. The arrowhead shows a small residual ventricular septal defect in the *inlet* (posterior) septum, not yet closed by endocardial cushion tissue. Scale bar is 1.5 mm. (C, D) Further details of atrial septation. In C, note the close proximity of the systemic venous sinus (SVS) and pulmonary vein, with the primary atrial septum in between. Behind is the vestibular spine, or dorsal mesenchymal protrusion that extends the septum primum. (D) The foramen secundum, seen as perforations in the superior portion of the septum that appear as the septum primum extends toward the AV canal. (A, B) Adapted from: Dhanantwari, P., Lee, E., Krishnan, A., et al. (2009). Human cardiac development in the first trimester: A high-resolution magnetic resonance imaging and episcopic fluorescence image capture atlas. *Circulation* **120** (4), 345–346. (C) From: Anderson, R. H. et al. (2003). Development of the heart (2) septation of the atriums and ventricles. *Heart* **89** (8), 949–958. (D) From: Schoenwolf, et al. (2015). *Larsen's human embryology* (5th edn.), p. 287. Philadelphia: Elsevier Churchill Livingstone.

Development of the Atria and Atrial Septum

The original atrial segment in the heart tube is replaced during remodeling of the atria and is left as remnants on each side, termed the right and left atrial appendages. Most of the remodeled right atrium comes from incorporation of the right horn of the sinus venosus, as the systemic veins incorporate into their final drainage patterns to the heart. The same is true for the pulmonary veins connecting to the left atrium and incorporating into the back wall (see next two sections). The atria are separated by a muscular wall, the atrial septum. Like the ventricular septum, the atrial septum begins as a ridge of muscular tissue, posterior and midline, which expands to divide the right side of the atrium containing the orifice of the sinus venous from the left side of the atrium containing the orifice of the common pulmonary vein (**Fig. 7C**). This structure is known as the septum primum, and it grows toward the fusing AV cushions (Wessels et al., 2000; Anderson et al., 2003). The leading edge of the primum septum is a mesenchymal tissue (mesenchymal cap) with origin from endocardial EMT, similar to the endocardial cushions. Another mesenchymal ingrowth, termed the dorsal mesenchymal protrusion, adds tissue to the extending atrial septum from the sinus venosus region. This mesenchyme has its origin in the secondary heart field (Briggs et al., 2016; Burns et al., 2016; Snarr et al., 2007). This is a thicker tissue than the rest of the atrial septum and provides a buttressing effect inferiorly. Superiorly, small perforations appear, enlarge, and coalesce within the primum septum such that it never forms a complete barrier to flow between the atria; this is termed the foramen secundum, (**Fig. 7D**). A second structure of atrial septal tissue next begins to form, the septum secundum (**Fig. 7C**, green) forms a crescent on the right atrial side of the septum primum, extending alongside the septum primum (Anderson et al., 2003). This creates a flap, allowing shunting of blood away from the lungs in the fetus. The central portion of the crescent remains incomplete, forming the fossa ovalis.

Heart Defects Resulting from Incomplete Atrial Septation

Since an atrial communication is normal in fetal circulation, all newborn infants retain a small atrial shunt, or patent foramen ovale (PFO). As long as this is a trivial communication, it is considered a normal variant to retain a PFO even into adulthood. On the other

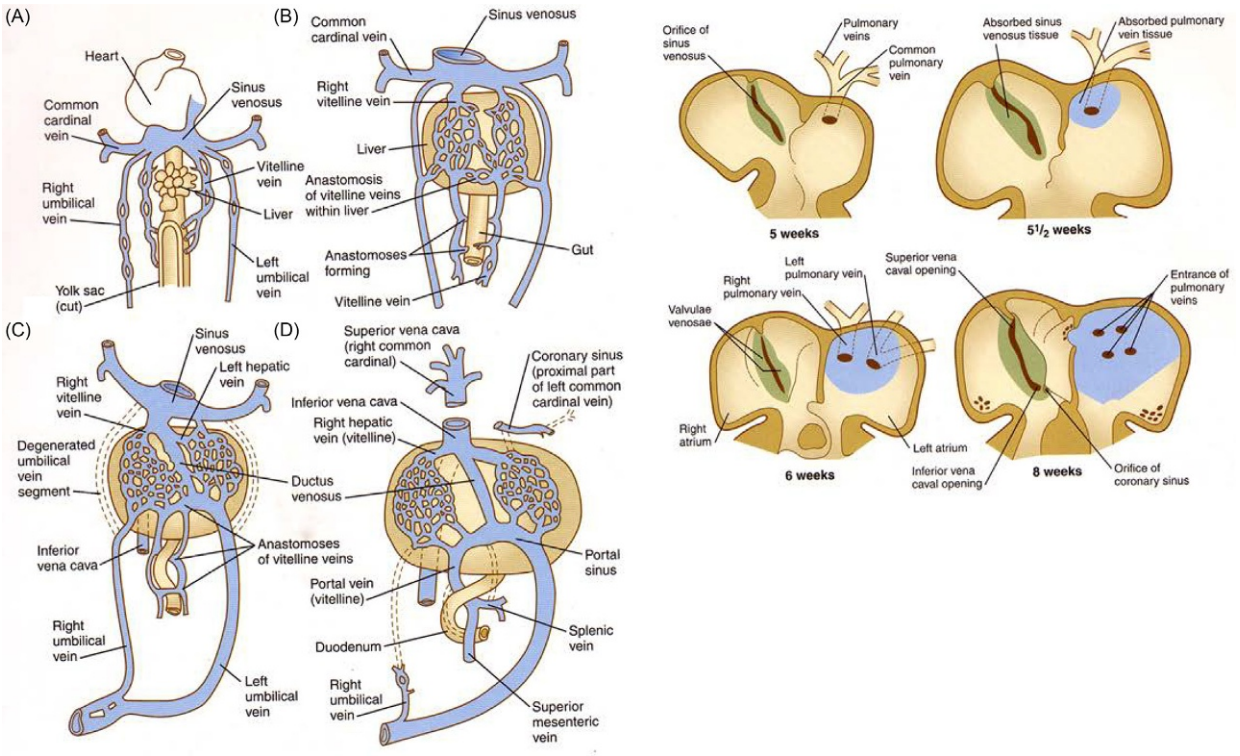

Fig. 8 Stages of development of the systemic veins (*left*) and pulmonary veins (*right*). From: Carlson, B. M. (2014). *Human embryology and developmental biology* (5th edn.), pp. 423–424. Philadelphia: Elsevier Saunders.

hand, a true deficiency in atrial septal tissue in the region of the foramen ovale constitutes a secundum ASD. Another type of ASD results from failure of the cushions to form the inferior-most part of the atrial septum results. This is termed a primum atrial septal defect, and is considered an endocardial cushion defect in the spectrum of the AV canal defects. Such defects do not close spontaneously, and may be associated with other endocardial cushion defects, most commonly an incompletely fused, or cleft mitral valve. Finally, there is a small contribution to the atrial septum near the junctions of the superior and inferior vena cava (IVC) (see below). Defects in this process result in a sinus venosus ASD, and there are superior or inferior types. Such defects again result from deficiency of atrial septal tissue, and do not close spontaneously.

Systemic Vein Development

The sinus venosus of the primitive heart tube is a symmetric structure, and in mammals receives three sets of paired (left and right) systemic veins: from the yolk sac (vitelline), placenta (umbilical), and embryo proper (cardinal) (Fig. 8A). The yolk sac is a structure that communicates with the primitive gut via the vitelline duct. The duct normally regresses completely, although the portion within the liver contributes to the hepatic and portal veins, including a small segment to the IVC (see Fig. 9A and below). The umbilical veins return from the placenta carrying oxygenated blood. These are the first to develop, by three weeks gestation. As the liver develops, the umbilical veins develop connections within the liver and the connection to the sinus venosus involutes. The cardinal veins carry the venous returning from the embryo to the heart. On each side, anterior and posterior cardinal veins join to form the common cardinal vein; the anterior carrying the venous returning from the upper body, and the posterior from the lower body. The right anterior and right common cardinal vein will form the superior vena cava in the mature circulation. The left common cardinal vein and left segment of the sinus venosus form the coronary sinus, which receives venous return from the coronary system. These structures are pulled rightward as the IVC forms, such that the opening (os) of the coronary sinus ends up in the right atrium once atrial septation is complete. The IVC is more complex, and made up of five segments, coming from urogenital, mesenteric and hepatic venous channels fusing together to the right of the spine as their leftward paired counterparts involute.

Heart Defects Resulting from Abnormal Systemic Vein Development

As numerous vessels go into making the IVC, interruption of the IVC can sometimes occur at various segments. This is most often in the setting of heterotaxy, but can occur in isolation. When the IVC is interrupted, a connection between the lower and upper will

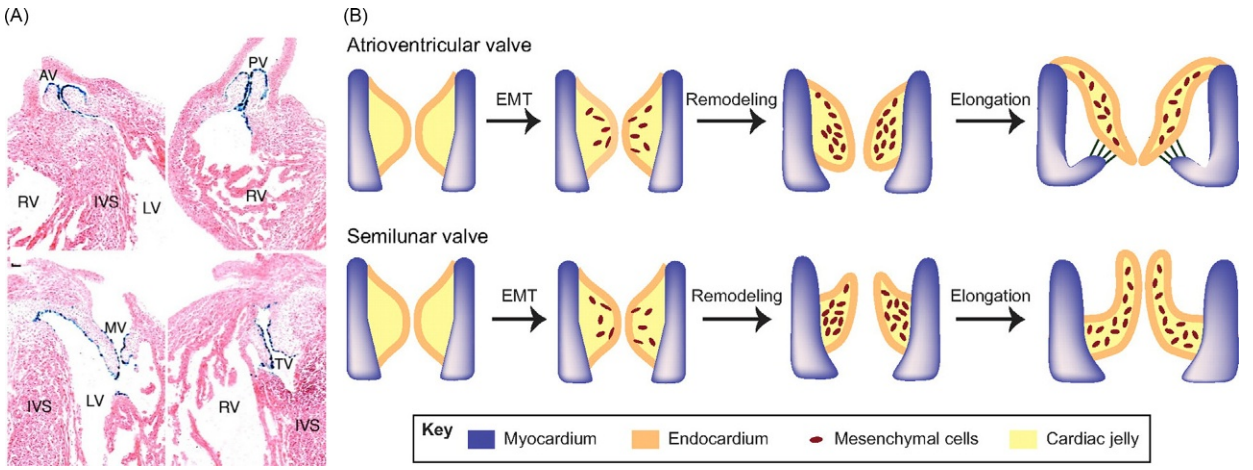

Fig. 9 Valve maturation. (A) Nfatc1 expression in the developing valves, as they begin to elongate. Note expression in the endocardial surface of the valves; mesenchymal cushion tissue in between. (B) Schematic of semilunar valve remodeling showing EMT, remodeling, and elongation of the valves. (A) From: Wu, et al. (2011). Nfatc1 coordinates valve endocardial cell lineage development required for heart valve formation. *Circular Research* **109** (2), 183–192. (B) From Lin, et al. (2012). Partitioning the heart: Mechanisms of cardiac septation and valve development. *Development* **139** (18), 3277–3299.

persist and enlarge, bringing the IVC blood up to the SVC. Sometimes both anterior cardinal veins persist, creating bilateral SVCs. A persistent left SVC most often drains into the coronary sinus (see above) but can drain directly to the roof of the left atrium as well. Most systemic venous anomalies in isolation are not a serious problem for the circulation, as all of the venous return ends up in the right atrium, and do not require surgical correction.

Pulmonary Vein Development

The pulmonary veins grow into the heart from the developing lung vasculature. A common pulmonary vein coming from both lungs fuses into the back of the left atrium (Wessels et al., 2000). As the atrium expands, the common pulmonary vein becomes further incorporated into the back wall, such that first two, then four of its branches eventually enter independently into the atrium, two from the left and two from the right lung (**Fig. 8B**).

Heart Defects Resulting from Abnormal Pulmonary Vein Development

As the common pulmonary vein approaches the back of the left atrium, there are nearly several structures that it can accidentally encounter and incorporate into. These include the coronary sinus, right atrium, and remnants of the cardinal system that normally regress. If this happens, these vessels will enlarge and persist, and the pulmonary vein will drain directly, or indirectly, to the right atrium. This is called total anomalous pulmonary venous return, and is life-threatening, generally requiring corrective surgery in the newborn. Partial anomalous pulmonary venous return is also possible, usually occurring in conjunction with an atrial septal defect (see other sections of this text for clinical details).

Cardiac Valve Maturation

Cardiac valve development begins with the endocardial cushions during week 7 of gestation, but maturation of the atrioventricular (mitral and tricuspid) and semilunar valves (aortic and pulmonic) takes several weeks for completion (Dhanantwari et al., 2009; Steding, 2009) (Combs and Yutzey, 2009; Hinton et al., 2006; Lincoln et al., 2004; Kanani et al., 2005; de Lange et al., 2004). Genes important in the maturation process include Wnt/β catenin, Notch 1, and Nfatc1. **Fig. 9A** shows Nfatc1 expression in the endocardial surface of all four of the developing valves. Targeted gene disruption studies show that Nfatc1 regulates EMT and proliferation of mesenchymal cells within the maturing valves (Wu et al., 2011). As the endocardial cushions enlarge, they protrude into the lumen of the heart. Here, subject to constant flow of blood, the protrusions condense and elongate (Lin et al., 2012). Endocardial cells overlying the cushions proliferate slowly, and there is programmed cell death (apoptosis) of mesenchymal cells underneath (**Fig. 9B**). The valves undergo extensive remodeling of the extracellular matrix; in the case of the semilunar valves, this results in a fibrous layer (primarily collagen) on the arterial side, a spogiosa layer (glycosaminoglycans) in the middle, and an elastic layer (elastin) on the ventricular side (**Fig. 9**) (Combs and Yutzey, 2009; Shelton and Yutzey, 2007; Hinton et al., 2006). In the case of the semilunar valves, this remodeling process of the endocardial cushions continues through the last trimester of pregnancy and into the neonatal period (Aikawa et al., 2006; Hinton et al., 2006).

Heart Defects Resulting from Abnormal Valve Development

Defects in valve development can be concomitant with defects in inflow or outflow tract development, or can occur later during valve maturations. Examples of valve defects that are a part of a broader developmental abnormality include the common atrioventricular valve in endocardial cushion defects, or the small pulmonary valve in Tetralogy of Fallot resulting from abnormal division of the outflow tract and hypoplasia of the rightward portion. Abnormal valves can also be found without other cardiac anomalies, with normal outflow tract sizes and position. These involve abnormal valve maturation resulting in abnormally partitioned valves, thickened valve leaflets, and/or incomplete leaflet delamination from the ventricular surface. A bicuspid aortic valve is a common example in which the aortic valve has two leaflets instead of three, which can result if either narrowing of the valve opening (stenosis) or incomplete closure with backflow of blood (insufficiency). Ebstein's anomaly of the tricuspid valve is an example in which the septal leaflet of the tricuspid valve incompletely delaminates, resulting in displacement of the valve into the body of the right ventricle. These clinical entities are covered elsewhere in this text. Genetic defects linked to abnormal valve development include the PTPN11 gene which Noonan's syndrome, characterized by a dysplastic and stenotic pulmonary valve (Tartaglia et al., 2001). The PTPN11 encodes for SHP-2, a signaling molecule important to growth factors, cytokines, and hormones (Tartaglia et al., 2001). This has been linked to a role in mediating growth (Chen et al., 2000), and interestingly appears to be a gain-of function mutation (Tartaglia et al., 2002). Mutations in the Notch1 receptor and its signaling pathway have been associated with the formation of aortic valves and with multiple stages of cardiac development (Garg et al., 2005; High and Epstein, 2008).

Aortic Arch Development

The primitive heart tube connects to the aortic sac, the precursor to the ascending aorta, and begins pumping to the developing systemic circulation of the embryo as soon as it is formed. The aortic sac connects to paired dorsal aortae posteriorly via an aortic root, which gives rise to a series of arches, the branchial arches (**Fig. 10A**). It is along these arches that the neural crest migrates into the conotruncus and support development of the arch arteries. Again these are paired structures, and there are a total of six pairs, although not all are patent at the same time. Arches 1–4 carry the blood flow in the 4-week embryo. Arches 1 and 2 mostly regress completely, although parts contribute to some of the arteries of the face. Arch 3 does not persist as an arch, but parts of it contribute to the formation of the carotid system. The left limb of arch 4 remains in continuity with the aortic root. Together they make up the aortic arch and its first branch, the brachiocephalic (innominate) artery. The right limb of arch 4 becomes part of the right subclavian artery, which retains its proximal connection with the brachiocephalic artery (Waldo et al., 1996). This arch remodeling occurred by 7 weeks of gestation. Arch 5 is small and not fully developed. Arch 6 forms with the developing PA vasculature, and makes up the proximal left and right pulmonary arteries and the ductus arteriosus (Waldo and Kirby, 1993). The ductal arch persists throughout fetal life as another means of right to left shunt, allowing blood from the right ventricle to bypass the pulmonary circulation and cross over into the descending aorta.

Heart Defects Resulting from Abnormal Aortic Arch Development

In some cases, either independently or as a part of another developmental cardiac defect, the right limb of arch 4 remains patent and the left forms the left subclavian artery — this forms a right aortic arch with mirror-image branching. Anomalies are seen with regression in any segment of the arch resulting in left or rightward arches with aberrant position of the subclavian arteries. No regression of an arch segment results in a double aortic arch, which can also be seen. Narrowing of the aortic arch, again either in isolation or in combination with other defects is known as coarctation of the aorta. Clinical features of coarctation are described elsewhere in this text.

Coronary Artery Development

The coronary arteries arise from the aorta just above the aortic valve, and have the important purpose of supplying blood to the heart muscle itself. The coronary arteries form first within the myocardium, then connect to the aorta via a coronary stem that forms within the aorta from peritruncal capillaries (Tian et al., 2013a, 2015; Liu et al., 2015; Mikawa and Fischman, 1992; Mikawa and Gourdie, 1996) (**Fig. 11A**). The coronary vessels form from several sources, as shown in **Fig. 11C–E**. Early in development, the proepicardial organ (PEO) appears as bilateral clusters of cells near the septum transversum, where the liver bud approaches the heart (**Fig. 11B** and **C**) (Mikawa and Gourdie, 1996; Reese et al., 1999, 2002). This is in close proximity to the sinus venosus, and there appears to be some movement of cells from one population to the other (Tian et al., 2015). The PEO is a transient structure that spreads out over the surface of the heart, forming an outer layer called the epicardium. Some of the epicardial cells delaminate and migrate into the myocardium, in another example of EMT in heart development. Cells from epicardial EMT contribute to coronary vessels as both endothelium (lining of blood vessels) and arterial wall smooth muscle (Mikawa and Gourdie, 1996; Reese et al., 2002). Recent studies have used detailed transgenic lineage tracing methods in mouse to show there are also important contributions to the coronary endothelium from the endocardium, also via EMT (Wu et al., 2012), and from the sinus venosus (Red-Horse et al., 2010; Tian et al., 2013b, 2014). To add to the complexity of cell lineages, this process appears to occur differently

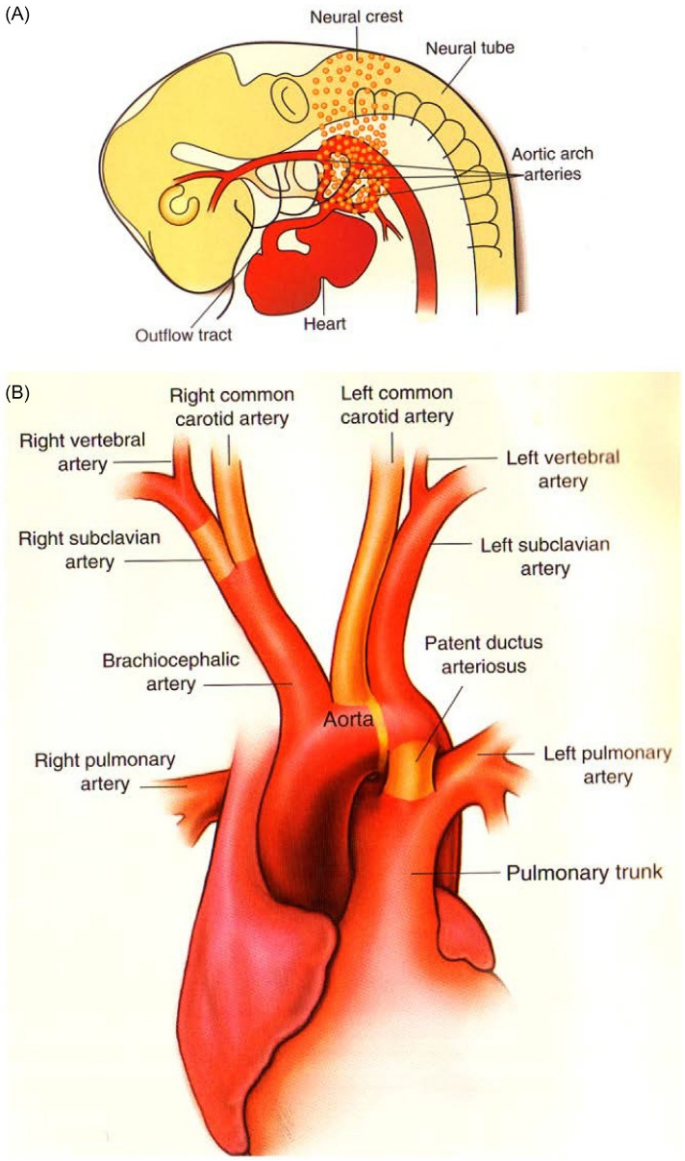

Fig. 10 Arch development. (A) Migration pattern of neural crest cells into pharyngeal arches 3, 4, and 6. (B) Normal aortic arch at term. Most of the pharyngeal arch segments have regressed. The third arch persists in part as the *left* and *right* common carotid arteries. The *left* fourth arch persists as the true aortic arch, while a portion of the *right* fourth arch makes up the proximal *right* subclavian artery. The sixth arch persists as the ductus arteriosus (ductal arch). From: Kirby, M. L. (1999). Contribution of neural crest to heart and vessel morphology. In Harvey and Rosenthal (eds). *Heart development*, pp.180–181. Academic Press.

in different segments of the ventricular myocardium (Tian et al., 2015; Wu et al., 2012). Regardless of the origin, the coronary vessels form, grow, and fuse with one another to form a working vasculature. Signaling from neural crest, epicardial-derived cells, and recently discovered intraaortic cardiomyocytes all appear to play an important guiding role (Arima et al., 2012; Eralp et al., 2005; Chen et al., 2014).

Heart Defects Resulting from Abnormal Coronary Development

Occasionally, a coronary artery (usually the left) can join into the PA instead of the aorta. This does not damage the fetal myocardium given the low oxygen state of the fetus, with little difference in oxygen tension between the aorta and PA bloodflow. After birth, however, ischemia sets in rapidly, causing myocardial ischemia and infarction in infancy (Huddleston et al., 2001). Other less dramatic examples are coronary arteries arising from the opposite sinus of the aortic root (left coronary from the right side) or from a single origin. This results in an abnormal course of the coronary artery over the heart, and may or may not be a clinical concern (Gersony, 2007).

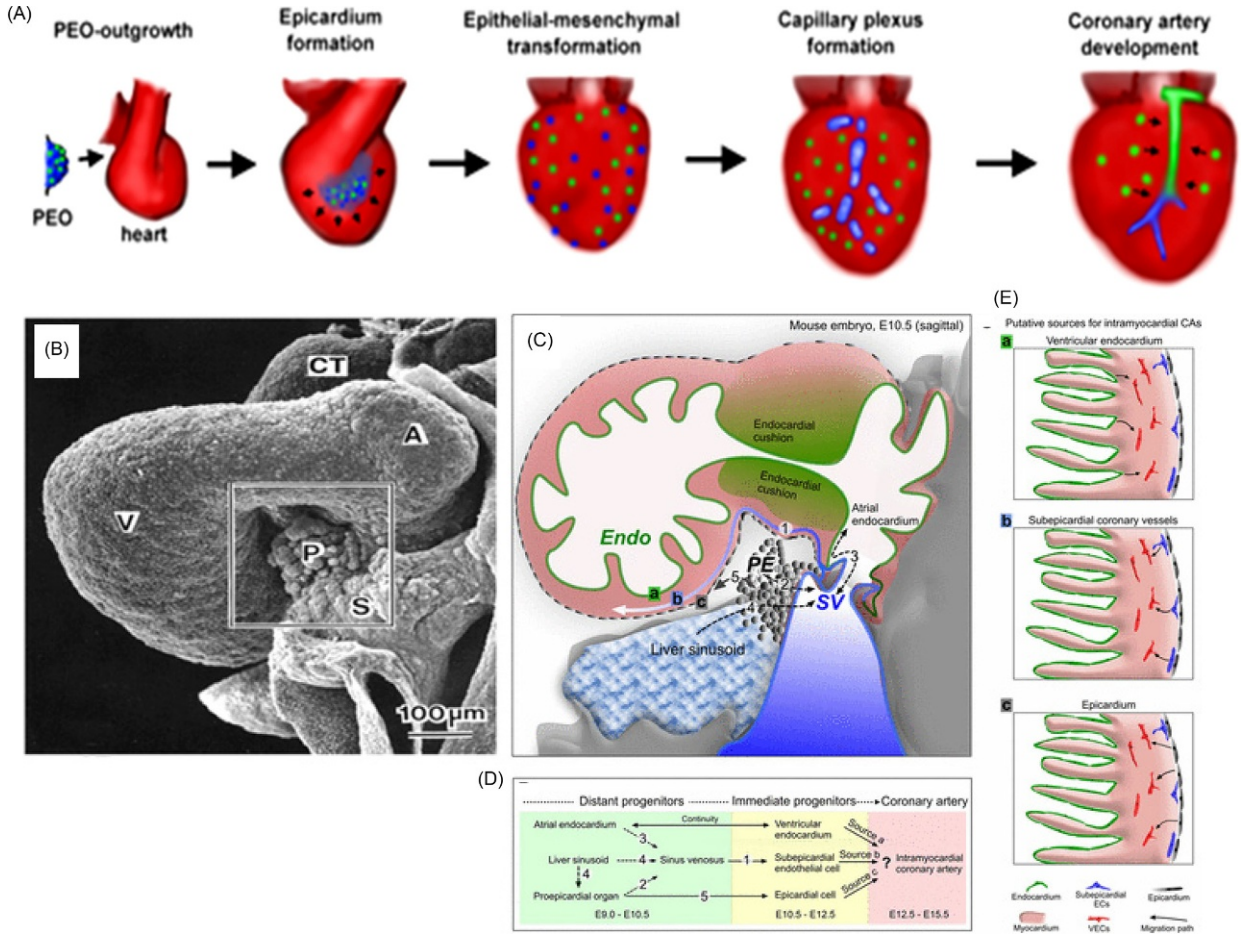

Fig. 11 Coronary origins. (A) Schematic of the basic steps of coronary artery differentiation, starting with the PEO and formation of the epicardium, with EMT of the coronary endothelium and formation of the coronary vasculature. (B) Scanning electron micrograph showing the developing PEO (P), Sinus venosus (S), atrium (A) ventricle (V), and conotruncus (CT). (C) Schematic of mouse embryo at the same stage (E10.5) showing the various origins of the coronary plexus, including endocardium (endo, *green*), PEO (PE, *gray*), and sinus venosus (SV, *blue*). (D) Differentiation path of the various cell types with timing in the mouse embryo; numbers correspond to migration paths shown in (A). (E) *Arrows* show migration paths of each cell type into the myocardium (*pink*), using the same color coding as above; epicardium arises from the PE (*black*) and subendocardial ECs are subendocardial endothelial cells arising from the sinus venosus (*blue*). In *red* are the vascular endothelial cells (VECs) that form the coronary vessels. (A) From Reese, et al. (2002). Development of the coronary vessel system. *Circular Research* **91** (9), 761–768. (B) From; Hiruma, T. and Hirakow, R. (1989). Epicardial formation in embryonic chick heart: Computer-aided reconstruction, scanning, and transmission electron microscopic studies. *American Journal of Anatomy* **184**, 132. (E) From: Tian, X., Pu, W. T. and Zhou, B. (2015). Cellular origin and developmental program of coronary angiogenesis. *Circulation Research* **116**, 517.

Conduction System Development

Cardiomyocytes constantly depolarize and repolarize in an automatic fashion, and this is the mechanism for their contractility. The conduction system of the heart includes special myocytes that conduct action potentials rapidly allowing for coordination of myocyte contraction. This includes the sinoatrial node, or intrinsic pacemaker of the heart, the atrioventricular node, a region of slowed conduction between the atria and ventricles, and the His bundles, which transmit the action potential to the ventricular myocardium. Lineage tracing studies show conclusively that the cells of the conduction system differentiate in situ from myocytes (Waldo et al., 1994; Hyer et al., 1999; Cheng et al., 1999; Gourdie et al., 1998; Moorman et al., 1997). The conduction system tends to develop alongside coronary vessels, and the developing coronary vasculature appears to be a source of signaling for this transdifferentiation (Hyer et al., 1999). Conduction system myocytes express different markers, junctions, and have different action potential profiles from myocytes that allow for automaticity (Alyonycheva et al., 1997; Hoogaars et al., 2007; Moorman et al., 1997; Wessels et al., 1992; Takebayashi-Suzuki et al., 2001). Recently, lineage tracing studies in early chick embryos showed that the SA node arises from a population of cardiac mesoderm outside of the primary and secondary heart fields that coalesce in the heart near the right sinus horn (Bressan et al., 2013). Activation mapping of embryonic chick heart conduction shows that the primitive SA node begins to function soon after cardiac looping (the site of earliest activation prior to this actually being in the left sinus horn)

(Bressan et al., 2013). Once active, the SA node maintains the fastest depolarization and to 'set the pace' for the heart rate. This starts at about 1 month gestation in humans (Phoon, 2001).

Heart Defects Resulting from Conduction System Development

Conduction system disease can result from many defects at the cellular level, including abnormal action potential channels which are beyond the scope of this article. Relevant to our discussion of cardiac development are atrioventricular accessory connections. These are bridges of muscle between the atrium and ventricle that should regress with atrioventricular valve development and creation of fibrous discontinuity at the mitral and tricuspid annulus. Persistent accessory connections can allow for reentrant tachyarrhythmias, in which the electrical impulse transmitted to the ventricles are able to circle back up into the atrium setting up a loop of rapid conduction. Sometimes, these accessory connections are large enough to be detected on an electrocardiogram, and this is the hallmark of an entity known as Wolff–Parkinson–White syndrome. These connections most often are found in structurally normal hearts, but can accompany Ebstein's anomaly. Heterotaxy is another setting in which the conduction system may be significantly abnormal, with randomization of left and right signals; the sinus and AV nodes are often malpositioned. For further discussion of arrhythmias, please see this section elsewhere in this text.

Conclusion

We have reviewed heart development, with attention to the morphology, cell biology, genetics, and physiology of the heart as it forms and remodels. The field of developmental biology of the heart continues to advance rapidly on all of these fronts.

Creative, integrative approaches continue to be developed and applied to the study of heart development. Animal models continue to be refined, including tissue specific gene expression and knockout studies, to create complex models of human disease at the cell and whole organ level. Advanced imaging, including techniques that allow earlier and better visualization of the developing human heart, are highly useful tools. Cardiac MRI can visualize a human fetus as early as week 6 gestation (Dhanantwari et al., 2009), and cardiac ultrasound by 10–11 weeks. The common goals to be applied to human disease are early recognition of congenital heart malformations, prevention where feasible, and state of the art intervention to allow an abnormal heart to function as normally as possible. In the future, gene therapy, cardiac stem cell grafting, and in vitro tissue engineering will likely be added to the therapeutic potentials for patients with congenital heart malformations.

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Heart Failure in African Americans

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Introduction

Heart failure is one of the leading causes of comorbidity and death among adults in the United States, with an estimated prevalence of 5.7 million people affected (Mozaffarian et al., 2015). African Americans have the highest incidence and prevalence of heart failure compared with other races (Husaini et al., 2011). Understanding what is currently known about the causes, prevention, and treatments of heart failure in this population is imperative to improving outcomes.

Epidemiology

The prevalence of heart failure among African Americans living in the United States has been estimated at 3.6%–5.9%, compared with 2.4% for Caucasians (Heidenreich et al., 2013; Gupta et al., 2013). Incidence rates vary among studies, with one study finding a rate of 4.6 per 1000 person years (2.4 for Caucasians) (Bahrami et al., 2008). However, data from the Atherosclerosis Risk in Communities (ARIC) study showed an even higher incidence rate of 9.1 for African American men (Caucasian men = 6) and 8.1 for African American women (Caucasian women = 3.4) (Loehr et al., 2008).

The majority of African Americans with heart failure have heart failure with preserved ejection fraction (HFpEF), with the ARIC study finding that 73% of African Americans had HFpEF and that HFpEF was more common in women (Gupta et al., 2013). They are less likely to have an ischemic etiology (Husaini et al., 2011). African American women are also at higher risk for peripartum cardiomyopathy (Kao et al., 2013).

In addition to being at higher risk for developing heart failure, African Americans as a group have more rapid and severe progression. The average age at the time of diagnosis and first hospitalization is younger (Jolly et al., 2010; Kheirbek et al., 2016), rates of hospitalization are higher, and cardiac event-free survival is low compared with Caucasians (Kheirbek et al., 2016; Wu et al., 2016; Wierenga et al., 2016; Husaini et al., 2016). Although overall mortality is higher, mortality rates after a hospitalization for heart failure have been found to be paradoxically lower or similar to Caucasians in some studies, which could suggest differences in disease severity at the time of hospitalization, in etiology, or in comorbidities affecting long-term survival (Loehr et al., 2008; Gordon et al., 2010).

The increased prevalence of heart failure, increased mortality, and increased hospitalization in African Americans are likely multifactorial. Possible explanations include a higher prevalence of hypertension, diabetes, and obesity, as well as poorer control of risk factors such as hypertension and DMII (Zhao et al., 2014; Elperin et al., 2014; Eaton et al., 2016). Chronic kidney disease and anemia may also be risk factors (Bansal et al., 2016; Wang et al., 2016; Astor et al., 2004). The prevalence of hypertension within the ARIC cohort was 59% among African Americans without heart failure and 85% in those with HFpEF (Gupta et al., 2013). Obesity is also a significant comorbid condition occurring in 45% of African Americans without HF in the ARIC cohort and 71% of those with HFpEF. A retrospective analysis of data from the Jackson Heart Failure study found that obesity and morbid obesity were also common among African Americans, and both were associated with a higher risk of heart failure and heart failure hospitalizations (Krishnamoorthy et al., 2016). Similarly, a prospective study using data from the Women's Health Initiative found that African American women had higher baseline rates of hypertension, obesity, diabetes mellitus, and MI (Eaton et al., 2016). Risk factors for both HFpEF and HFrEF included coronary disease, diabetes, hypertension, and smoking; HFpEF was also associated with obesity, especially among African American women (Eaton et al., 2016).

A pooled analysis of four cohort studies, including the ARIC cohort, found that African Americans have a higher prevalence of hypertension, obesity, and DM compared with Caucasians (Ahmad et al., 2016). By age 45, only 36.1% of African Americans have zero of these risk factors, compared with 55% of Caucasians; thus, by age 45, the majority of African Americans have at least one heart failure

risk factor. African Americans with none of these risk factors have an increased heart failure-free survival time of 12.9 years compared with those with all three risk factors at age 45, suggesting that prevention of these risk factors may be key (Ahmad et al., 2016).

Poorer control of risk factors such as hypertension or obesity may also contribute to increased mortality and hospitalization. Of note, a significant proportion of deaths, especially for those with HFpEF, may be due to noncardiovascular causes, which may contribute to the higher mortality rate in African Americans with heart failure as they often have more comorbidities as well (Gupta et al., 2013). This may help to explain the paradox of lower or similar risk of death post hospitalization for heart failure but higher long-term mortality.

Disparities in cardiovascular care likely exist, although quality initiatives may mitigate some of these disparities. African American patients treated at hospitals participating in a registry-based performance improvement program for heart failure (OPTIMIZE-HF) received largely similar provisions of quality measures compared with other races; they were as likely or more likely to receive evidence-based medications, although fewer received complete discharge instructions or smoking cessation counseling compared with other races (Yancy et al., 2008). African Americans treated at these hospitals had lower inpatient mortality after adjustment for risk factors but similar 60- to 90-day mortality (Yancy et al., 2008).

Socioeconomic status also impacts the risk of developing heart failure and morbidity and mortality after diagnosis. African Americans are four times more likely to live in the lowest socioeconomic neighborhoods, and this is associated with higher rates of diabetes and hypertension (Puckrein et al., 2015). African Americans with heart failure who have a lower financial status have a 4–6 times increased risk of cardiac events than higher income Caucasians (Wu et al., 2016). Self-care may affect outcomes among patients with heart failure, and African Americans report fewer self-care activities (Wu et al., 2016) and have lower heart failure knowledge on average (Dickson et al., 2015). African Americans with both HFpEF and HFrEF have been found to have lower rates of medication adherence (Dickson et al., 2015; Zhang and Baik, 2014). Improving home care through caregiver coaching programs may be one effective strategy to reduce hospitalizations (Piamjariyakul et al., 2015).

Genetics has been postulated to play a role, although specific genetic polymorphisms are largely uncharacterized. The presence of genetic markers linked to African ancestry has been found to be associated with worse parameters of diastolic function in patients with HFrEF, although the causal mechanism or possible genetic polymorphisms have not been identified (Bernardez-Pereira et al., 2016). One known variant is a mutation in the transthyretin gene that is present in 3%–4% of African Americans and is associated with late onset amyloid cardiomyopathy and an increased risk of heart failure after age 65 (Buxbaum et al., 2010). However, this is likely a small contributor as the overall prevalence of overt cardiac amyloid is low even in carriers (Quarta et al., 2015). African Americans are more likely to possess a polymorphism in the guanine nucleotide-binding protein β-3 subunit (GNB3 TT) that has been associated with worse left ventricular ejection fraction (LVEF) 6 and 12 months after diagnosis with peripartum cardiomyopathy (Sheppard et al., 2016).

Heart Failure With Reduced Ejection Fraction: Medication Guidelines

The current American College of Cardiology/American Heart Association (ACC/AHA) recommendations for African Americans with HFrEF are summarized in Table 1. For all patients with stage C HFrEF, the American College of Cardiology and the American Heart Association recommend treatment with an ACE inhibitor (ACEi), angiotensin receptor blocker (ARB), or ARNI in addition to a beta-blocker and an aldosterone antagonist in selected patients (Yancy et al., 2013, 2016). An ARNI is recommended over an ACEi or ARB if tolerated in appropriate patients (Yancy et al., 2016). Ivabradine may also be used to reduce heart failure hospitalization for patients with HFrEF NYHA class II–III and LVEF $\leq$35%, who despite receiving a beta-blocker at maximally tolerated doses have a heart rate above 70 bpm and who are also receiving other guideline-directed therapy for heart failure (Yancy et al., 2016). In addition, combined isosorbide dinitrate and hydralazine (I/H) are recommended for African American patients with symptomatic HFrEF currently receiving optimal medical therapy (Yancy et al., 2013).

HFrEF: Renin–Angiotensin System Blockade in African Americans

ACEi or ARB use (for patients intolerant to ACEi) is likely equally effective in reducing mortality and preventing rehospitalizations for African Americans compared with Caucasians. A retrospective study of patients with HFrEF including 619 African Americans found similar protective effects for death or heart failure rehospitalization among African Americans and Caucasians (El-Refai et al., 2015). In addition, similar benefit has been observed for adding an ACEi or ARB after an acute MI with LV systolic dysfunction in African Americans compared with Caucasians (Prisant et al., 2008). However, an earlier cohort study looking at data from the studies of left ventricular dysfunction prevention and treatment trials found a decrease in heart failure hospitalizations for Caucasians, but not for African Americans and no change in mortality for either group, so some data are mixed (Exner et al., 2001). One important point for clinicians to be aware of is that African Americans do have a higher incidence of angioedema with ACEi and ARNI, although this is still a rare side effect (Bahrami et al., 2008). Data on how often eligible African Americans are prescribed an ACEi/ARB/ARNI are limited. Although one small retrospective study did find that they were less likely to be prescribed an ACEi (Dickson et al., 2015), African Americans discharged from hospitals participating in a performance improvement program for heart failure were more likely to be prescribed an ACEi compared with other races. This suggests quality improvement measures may help ensure African Americans and other patients are receiving appropriate evidence-based therapies (Yancy et al., 2008).

Table 1 ACC/AHA/HFSA recommendations for stage C patients with heart failure with reduced ejection fraction (Yancy et al., 2013)

Medication class/ medications	*Target population*	*COR*[a]	*LOE*[b]	*Effect/additional information*
RAAS blockade	All patients with chronic HFrEF	I		
ACE inhibitor (ACEi)	Use if ARNI not tolerated	I	A	Reduce morbidity and mortality in RCTs
Angiotensin II receptor blocker (ARB)	Use if ACEi or ARNI not tolerated	I	A	Reduce morbidity and mortality in RCTs
Angiotensin II receptor blocker neprilysin inhibitor (ARNI)	Use instead of/substitute for ACEi or ARB if tolerated	I	B-R	Reduced composite end point of CV death or HF hospitalization by 20% in RCT compared with enalapril
Beta-blockers (BB)	All stable patients with stage C HFrEF	I	A	Reduce mortality; use bisoprolol, carvedilol, or metoprolol succinate ER
Aldosterone receptor antagonists	(1) NYHA class II–IV and LVEF $\leq$35%, (2) after acute MI with LVEF $\leq$40% and symptoms of HF or DM	I	(1) A (2) B	Reduce morbidity and mortality; avoid use if eGFR $\leq$30 or potassium $\geq$5
Hydralazine/ isosorbide dinitrate	(1) African Americans with NYHA class III–IV HFrEF on GDMT[c], (2) patients with HFrEF intolerant of ACEi/ARB/ARNI	(1) I (2)IIa	(1) A (2) B	Further reduced mortality and heart failure hospitalizations in African Americans already on ACEi/ARB/ARNI, BB, and, if indicated, aldosterone antagonist
Ivabradine	NYHA class II–III stable chronic HFrEF, receiving GDMT including BB, in sinus rhythm with resting HR $\geq$ 70 bpm	IIa	B-R	One RCT showed reduction in hospitalization for heart failure, presumed mechanism slowing of heart rate, important to titrate BB up to target dose prior to consideration
Diuretics	Patients with HFrEF and fluid retention	I	C	Improve symptoms and exercise tolerance, unknown mortality effects
Digoxin	Consider in persistently symptomatic patients with HFrEF on GDMT	IIa	B-R	No mortality benefit; placebo-controlled trials showed reduction in symptoms, improved QOL and exercise tolerance and may modestly reduce hospitalizations. Low doses just as beneficial for HF; risk of toxicity including cardiac arrhythmia

[a]COR, class (strength) of recommendation—class I, (strong) benefit ⊠ risk; class IIa, (moderate) benefit ≫ risk; class IIb, (weak) benefit ≥ risk; class III: no benefit, (moderate) benefit = risk; class III: harm, (strong) risk > benefit (Yancy et al., 2016).

[b]LOE, level (quality) of evidence: level A, high-quality evidence from multiple RCTs, meta-analyses of high-quality RCTs, or one of more RCTs corroborated by high-quality registry studies; level B-R (randomized), moderate-quality evidence from one or more RCTs or meta-analyses of moderate-quality RCTs; level B-NR (nonrandomized), moderate-quality evidence from one or more well-designed, well-executed nonrandomized, observational, or registry studies or meta-analyses of such studies; level C-LD (limited data), randomized or nonrandomized observational studies with limitations of design or execution, meta-analyses of such studies, or physiological or mechanistic studies in human subjects; level C-EO (expert opinion), consensus of expert opinion based on clinical experience (Yancy et al., 2016).

[c]GDMT, guideline-directed medical therapy that is optimal medical therapy as recommended by ACCF/AHA guidelines (Yancy et al., 2013).

The data for the efficacy of aldosterone antagonists are not as clear. An analysis of data from the RALES study found no reduction in a combined end point of death or hospitalization in the African American subgroup prescribed spironolactone and also found a lower incidence of hyperkalemia, suggesting spironolactone may be less efficacious in African Americans (Vardeny et al., 2013). However, a retrospective analysis of data from the African American Heart Failure Trial (A-HeFT) found decreased mortality with the use of spironolactone and a trend toward increased event-free survival and time to first heart failure hospitalization but only for African Americans already taking hydralazine–isosorbide dinitrate (Ghali et al., 2008). The addition of aldosterone antagonists remain, however, part of standard guideline-directed therapy in symptomatic patients with heart failure.

HFrEF: Beta-Blockers in African Americans

Studies support a benefit for beta-blocker use in African Americans with HFrEF, although it is possible the magnitude of the protective effect is not as large as for Caucasians. A study including 523 African American patients using data from the Carvedilol Heart Failure Registry found similar reductions in hospitalizations and improvement in symptoms for African Americans and Caucasians taking carvedilol (Abraham et al., 2007). A second retrospective study also found a lower risk of death or rehospitalization for African Americans with HFrEF prescribed beta-blockers but with a smaller protective effect compared with Caucasians (Lanfear et al., 2012). Another small retrospective study found an improvement in LVEF, left ventricular end-diastolic dimensions (LVEDD), and mitral regurgitation after 1 year of beta-blocker treatment in African Americans and Caucasians, although African Americans were slightly less likely to have improvement in LVEF and LVEDD compared with Caucasians (Kelesidis et al., 2013).

Differential responses to beta-blockade have been observed in the general population as well, and there are known genetic polymorphisms in the beta-adrenergic receptors and in G-protein receptor kinase 5 (GPRK5), which regulate beta-adrenergic signaling. One study found similar beta-blocker effects on survival when African American and Caucasian patients were matched for two polymorphisms, one in GPRK5 and the other in one of the beta-blocker receptors (ADRB1), suggesting genetic polymorphisms in the beta-blocker signaling cascade may be responsible for the observed differences in the magnitudes of the effect (Cresci et al., 2009).

HFrEF: Hydralazine and Nitrates

The A-HeFT trial evaluated adding a fixed-dose combination of I/H to standard therapy in African American patients with NYHA class III or IV HFrEF. The trial was terminated early due to a 40% reduction in mortality in the treatment group, as well as significant improvement in the primary end point, a combined end point of weighted values for death, first hospitalization for heart failure, and change in quality of life (Taylor et al., 2004). The trial was designed after the Vasodilator Heart Failure Trial II (V-HeFT II) found that enalapril was more effective than I/H for patients with HFrEF, except in African Americans, where the two were comparable.

The approval of I/H as the first race-based drug was controversial. The A-HeFT trial design was criticized, since by only including African Americans it failed to show that the addition of I/H to standard therapy was superior in African Americans compared with other races or that it was ineffective in other races. The use of self-identified race was also controversial, as race is a social construct and those who identify as African American may not all share genetic differences that could explain a disproportionate effect. Others, however, saw it as a breakthrough for a group with a high incidence of and mortality from heart failure who are often underrepresented in major trials (Maglo et al., 2014).

Several different mechanisms for a possible differential response to I/H in African Americans have been proposed. African Americans with one genotype for the guanine nucleotide-binding protein beta-3 subunit, which plays a role in alpha-2 adrenergic signaling (GNB3 TT), had lower rates of death and hospital stay for heart failure and improved quality of life when treated with I/H compared with those who had other alleles (McNamara et al., 2014). The same allele, which is more common in African Americans, has also been associated with worse outcomes after a diagnosis of peripartum cardiomyopathy (Sheppard et al., 2016), suggesting increased alpha adrenergic tone may be both a risk factor for development/progression of heart failure and one of the treatment targets for I/H. A role for hydralazine enhancing nitric oxide's effects on vasodilation and inhibition of platelet aggregation has also been proposed (Chirkov et al., 2010), although this is debated. It has been suggested that African Americans might have differences in nitric oxide bioavailability that could account for I/H's observed benefits (McNamara et al., 2009).

More studies are needed to further assess the utilization of I/H in practice. Some studies have found that only 4.5%–22% of eligible African Americans were discharged home after a hospitalization for HFrEF with a prescription for I/H; however, further study is needed to assess whether this is true across other cohorts and to determine which factors might affect prescribing decisions at discharge (including whether patients are already prescribed other optimal medical therapy) (Yancy et al., 2008; Golwala et al., 2013).

HFrEF: Medical Devices

Research supports a similar mortality benefit compared with Caucasians for cardiac resynchronization therapy (CRT) in eligible African Americans with NYHA class II–IV HFrEF (Fontaine et al., 2016; Ziaeian et al., 2014). Studies have shown, however, that African American patients who are eligible for CRT may be less likely to receive it compared with Caucasians (Farmer et al., 2009; Piccini et al., 2008). African Americans also have a higher risk for SCD (Fender et al., 2014), and there is underutilization of implantable cardiac defibrillators (ICDs) for primary and secondary prevention in African Americans. The SCD-HeFT trial found no difference in the rate of ICD shocks and similar reductions in mortality between Caucasians and African Americans with ICDs (Mitchell et al., 2008). A study of elderly Medicare beneficiaries admitted after cardiac arrest also found similar mortality reductions although African Americans were less likely to receive an ICD (Groeneveld et al., 2003). However, the MADIT-II trial, which included a fairly small number of African Americans ($n = 102$), found no reduction in sudden cardiac death or mortality in African Americans (Vorobiof et al., 2006), and a prospective cohort study of patients with HFrEF receiving ICDs found African Americans were more likely to die without receiving an appropriate shock (i.e., to not benefit from the ICD) although a substantial number also received appropriate shocks (Zhang et al., 2014). The reason for possibly less response is unclear and warrants further study.

HFrEF: Advanced Circulatory Support and Transplant

There are limited data on outcomes for left ventricular assist devices specifically in African Americans. Small studies show similar outcomes and no difference in survival compared with Caucasians (Tsiouris et al., 2013; Aggarwal et al., 2012), and African American patients should be considered for advanced circulatory support in the same manner as all other patients. However, a study using data from the National Inpatient Sample from 2002 to 2003 found African Americans with heart failure or cardiogenic shock were less likely to undergo LVAD implantation (Joyce et al., 2009). Compared with Caucasians, more African Americans present

with critical heart failure and at higher risk for LVAD (Stulak et al., 2013). African Americans are also more likely to be sicker at the time of listing for transplant and have a higher mortality while awaiting transplant (Morris et al., 2016). Disparities in counseling and timely referral for transplant and advanced circulatory support should be addressed (Morris et al., 2016).

African Americans have higher mortality and risk of graft failure after cardiac transplant. Compared with both Caucasians and Hispanics, the risk of acute and chronic graft failure and mortality after heart transplant is higher (Morris et al., 2015; Kilic et al., 2015). Although African Americans are more likely to be transplanted at worse performing centers, this does not fully explain the effect (Kilic et al., 2015). There is a trend toward increased mortality even at high-performing centers, and African Americans transplanted at low-performing centers have higher than expected mortality (Kilic et al., 2015). While short-term posttransplant survival has improved equally in White, Black, and Hispanic recipients, long-term survival has not improved in Blacks or Hispanics (Morris et al., 2016; Singh et al., 2011). Although social inequities in access to care/high-performance centers likely do play a role, they do not appear to be the only reason for increased mortality. A more recent study demonstrated that disparities in access appeared to have improved after the ACA Medicaid Expansion (Breathett et al., 2017). Immunologic differences likely play an important role in the increased risk of acute and chronic rejection and cardiac allograft vasculopathy. Further study and attention to individualizing immunosuppressive regimens for African Americans may improve outcomes (Morris et al., 2016).

Heart Failure With Preserved Ejection Fraction

The majority of African Americans with heart failure actually have HFpEF, as many as 73% in the ARIC study (Gupta et al., 2013). There is little evidence to support mortality benefits for any particular class of drugs or treatment for HFpEF for the general population or for African Americans. Treatment is largely symptomatic with diuretics and focused on control of hypertension. One small retrospective study of African Americans with diastolic dysfunction did find lower progression of diastolic dysfunction among those prescribed calcium channel blockers (Prendergast et al., 2014). However, further research is needed to better define the optimal hypertension regimens (if one exists) and if any treatments influence mortality or rehospitalization.

End of Life and Palliative Care

Palliative care is underutilized by patients with advanced heart failure in general but even more so for African Americans (Givens et al., 2010). The best way to improve utilization of palliative care and focus on comfort/patient priorities at the end of life is not yet determined. One randomized trial of African American patients with advanced heart failure who had been hospitalized with a heart failure exacerbation found that a palliative care consultation during the hospitalization did not increase the likelihood of choosing comfort care (Hopp et al., 2016).

Future Study and Directions

Heart failure, both HFrEF and HFpEF, represents a major cause of morbidity and mortality for African Americans. More study and work are needed to prevent future cases and improve outcomes for patients with the disease. Improved prevention is needed, through improved treatment and prevention of hypertension, diabetes mellitus II, and obesity. Exercise has been found to offer similar protective effects against heart failure in African Americans compared with Caucasians (Bell et al., 2013). Better understanding of and efforts to address disparities in access and quality of care, socioeconomic status, and differences in medication adherence, self-care, and health literacy are essential. Such inequalities greatly affect both prevention efforts and outcomes for patients with existing heart failure.

Further studies are also needed to better clarify the best ways to prevent and treat HFpEF, both in African Americans and the general population. Some of the best studied pharmacological treatments in African Americans are beta-blockers and ACEi/ARBs, which have been shown to benefit African Americans; yet even for these medications, some studies have found conflicting results or suggested a lesser magnitude of effect. Further, large studies of these and less studied treatments such as aldosterone receptor antagonists, ICDS, and LVADs would help to further clarify treatments. Given the high incidence of heart failure in African Americans, future clinical trials should make an effort to ensure a large number of African Americans are included.

Lastly, further understanding the genetic basis for differential response to treatments in African Americans will help to individualize therapy based on a biological likelihood of response rather than relying on race that is in part a social construct that inevitably groups some patients together who have disparate genetic differences in susceptibility to heart failure and response to treatment.

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Heart Failure in Cancer Patients

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Introduction

Recent advances in the detection and treatment of cancer have led to a vast improvement in cancer survival. In 2012, the estimated number of breast cancer survivors in the United States was 3 million (Moslehi, 2013). Between 2003 and 2012, the mortality due to non-Hodgkin's lymphoma and leukemia decreased by 2.5% and 1% per year respectively (Siegel et al., 2015). However, improved cancer survivorship comes at the cost of adverse cardiovascular effects associated with cancer therapies. Cardiovascular disease was the primary cause of mortality among elderly breast cancer survivors (Patnaik et al., 2011). Modern cancer therapies including radiotherapy, chemotherapy, and targeted therapies have the potential to cause heart failure (HF), hypertension, ischemia, cardiac arrhythmias, and conduction diseases (Bloom et al., 2016). Chemotherapy-induced cardiomyopathy is considered as a separate entity in this broad spectrum of cancer therapy-mediated cardiotoxicity (Mukku et al., 2016). In this article, we discuss the definitions, pathophysiology, mechanisms, imaging, prevention, and treatment of HF in cancer patients.

Definitions

There are no universally accepted definitions of cancer therapy-mediated cardiomyopathy (CTMC). One of the first definitions of cardiotoxicity was outlined in a trial evaluating the impact of doxorubicin on left ventricular ejection fraction (LVEF) (Alexander et al., 1979). In this study, a decline in LVEF by >15% from baseline level to below 45% on serial MUGA scan was defined as moderate cardiotoxicity. More recently, the Food and Drug Administration (FDA) defined anthracycline-mediated cardiotoxicity as >20% reduction in LVEF from normal baseline level or >10% reduction from low baseline LVEF (FDA Drug Label, 2007). The Cardiac Review and Evaluation Committee defined trastuzumab-mediated cardiotoxicity as an asymptomatic decline in LVEF of ≥10% or a symptomatic decline of ≥5% to a value <55% (Seidman et al., 2002). The American Society of Echocardiography and the European Association of Cardiovascular Imaging published a consensus statement which defined cardiotoxicity as a ≥10% reduction of LVEF to a value <53%, verified on subsequent imaging (Plana et al., 2014). The above-mentioned definitions of cardiotoxicity are based on arbitrary cutoffs. A broad definition for the diagnosis of CTMC should incorporate abnormal biomarkers and LV dysfunction detected by imaging techniques.

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Pathophysiology and Mechanisms

There are a number of chemotherapeutic drugs that cause cardiovascular adverse effects without cardiomyopathy or symptomatic HF. In this review, we focus mainly on cancer therapies associated with cardiomyopathy and HF, particularly anthracyclines, HER2-targeted therapies, and VEGF inhibitors.

Ewer and Lippman classified cardiotoxicity based on the type and degree of structural abnormalities, in addition to the likelihood of reversibility (Ewer and Lippman, 2005). Type I cardiotoxicity is referred to typical pathologic changes in cardiac myocyte ultrastructure causing irreversible injury. Type 2 cardiotoxicity does not damage cardiac myocyte ultrastructure and there is an increased chance of recovery after chemotherapy is discontinued (Ewer and Lippman, 2005). Traditionally, anthracycline- and trastuzumab-mediated cardiotoxicity are categorized as type 1 and 2 cardiotoxicity, respectively. However, this oversimplified classification is controversial as it does not take into account the pathophysiologic mechanisms of other chemotherapeutic drugs (Mukku et al., 2016). For instance, sunitinib, a tyrosine kinase inhibitor, causes ultrastructural changes which can be categorized as type 1 cardiac toxicity but these changes have been shown to be reversible (Mukku et al., 2016). Recent studies have demonstrated that the early detection and timely initiation of cardioprotective medications can partially reverse anthracycline-mediated type 1 cardiotoxicity, and prevent progression to symptomatic HF (Cardinale et al., 2015). Conversely, trastuzumab-mediated cardiotoxicity can cause myocardial injury with elevation of troponins, and may not always be reversible (Cardinale et al., 2010).

Anthracyclines

Anthracyclines are a class of chemotherapeutic drugs used for the treatment of hematologic malignancies and solid tumors (Blum and Carter, 1974). In breast cancer patients, doxorubicin- and epirubicin-based chemotherapy regimens are used not only before and after definitive surgery but also in the metastatic setting (Bloom et al., 2016). Anthracyclines enhance the synthesis of reactive oxygen species, impair DNA repair by inhibition of topoisomerase II, and downregulate protein synthesis by binding to nuclear DNA (Chen et al., 2007; Tewey et al., 1984) (**Fig. 1**). While the inhibition of topoisomerase 2-α in rapidly proliferating cancer cells is responsible for its antitumor action, the inhibition of this enzyme in cardiac myocytes causes cardiotoxicity (Lyu et al., 2007). Studies have suggested that the risk of doxorubicin-mediated cardiotoxicity exists even at cumulative doses <250 mg/m^2; thus, a safe dose threshold remains unclear (Pein et al., 2004). However, it is important to note that the risk of cardiotoxicity with anthracyclines is dose dependent and is further amplified with the use of radiotherapy, the presence of traditional cardiovascular risk factors, and concomitant administration of other cardiotoxic agents such as cyclophosphamide and trastuzumab (Volkova and Russell, 2011).

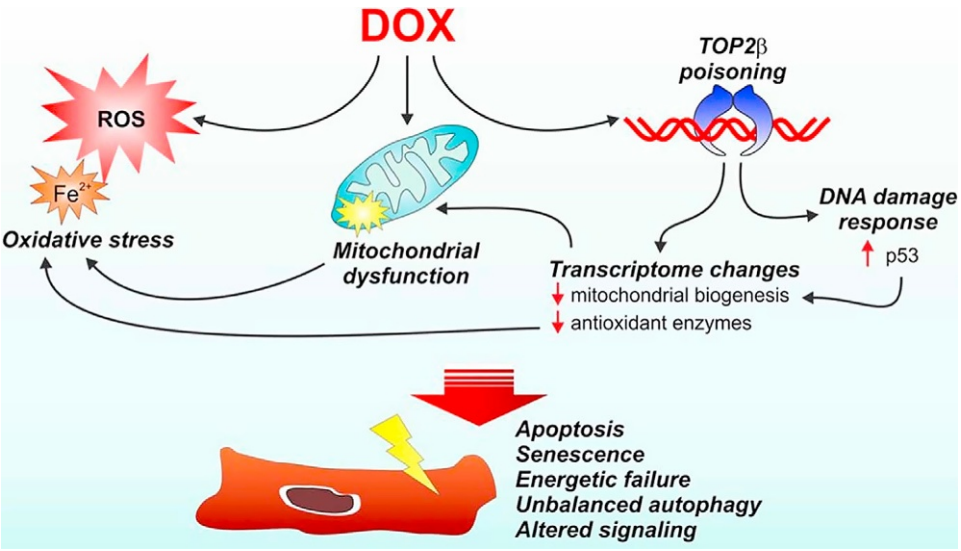

Fig.1 Cardiomyocyte-intrinsic molecular mechanisms underlying anthracycline cardiotoxicity. Anthracyclines, such as doxorubicin (DOX), lead to cardiotoxicity by promoting the production of reactive oxygen species (ROS), via direct (unstable DOX metabolites, such as DOX-semiquinone, react with oxygen and generate hydrogen peroxide and superoxide) and indirect (DOX chelates free iron and modulates the activity/expression of major iron-transporting/binding proteins) mechanisms. Alternatively, DOX can interact with cardiomyocyte topoisomerase 2β (Top2β), an enzyme responsible for managing DNA tangles and supercoils, thus inducing DNA double-strand breaks. DNA damage, in turns, activates the tumor suppressor protein p53, which is responsible for the activation of DNA repair proteins, but also of repression of genes involved in mitochondrial biogenesis/recycling and oxidative phosphorylation pathways. Finally, DOX can accumulate within mitochondria of cardiomyocytes and exacerbate metabolic failure of these organelles. Altogether, these molecular events contribute to cardiomyocyte death and ultimately to cardiac dysfunction. Adapted with permission from Mercurio, V., Pirozzi, F., Lazzarini, E., Marone, G., Rizzo, P., Agnetti, G., Tocchetti, C.G., Ghigo, A. and Ameri, P. (2016). Models of heart failure based on the cardiotoxicity of anticancer drugs. *Journal of Cardiac Failure*. **22**, 449-458.

HER2-Targeted Therapies

Human epidermal growth factor receptor- (HER2-) targeted agents include trastuzumab, lapatinib, and pertuzumab (Sendur et al., 2013). These drugs are used for the treatment of HER2-positive cancers, characterized by overexpression of the HER2 gene leading to an increased production of HER2 protein in malignant cells (Sendur et al., 2013).

Trastuzumab can cause dose-independent, potentially reversible cardiac dysfunction (Ewer and Lippman, 2005). The exact mechanism of trastuzumab-mediated cardiotoxicity is not known. Trastuzumab is a monoclonal antibody against HER2 protein, a receptor tyrosine kinase belonging to the ErbB family. Neuregulin ligand binds to ErbB receptor and promotes cardiac homeostatis (Lemmens et al., 2007). In HF, there is an increased production of neuregulin as this ligand is released by myocardial cells in response to stress (Baliga et al., 1999; Zhao et al., 1998; Ky et al., 2009). It has been proposed that the inhibition of the neuregulin ErbB pathway in cardiac myocytes by trastuzumab leads to myocardial dysfunction (Mercurio et al., 2016).

Patients receiving concurrent anthracycline therapy are at a higher risk of trastuzumab-mediated cardiotoxicity when compared to those on trastuzumab alone (Seidman et al., 2002). Age, diabetes, renal dysfunction, and previous heart disease are also associated with an increased risk of cardiomyopathy (Onitilo et al., 2014). Notably, when compared to trastuzumab, the risk of cardiotoxicity with other HER2-targeted therapies is significantly lower (Sendur et al., 2013).

VEGF Inhibitors

These drugs target vascular endothelial growth factor (VEGF) and include small molecule tyrosine kinase inhibitors (sorafenib, sunitinib) as well as monoclonal antibodies (bevacizumab) (Yang and Papoian, 2012; Choueiri et al., 2011). VEGF not only regulates the cardiac myocyte growth and function but also promotes the integrity and growth of coronary and systemic circulation (Mercurio et al., 2016). Inhibition of VEGF signaling may result in hypertension, thromboembolism, myocardial dysfunction, and HF (Schmidinger et al., 2008) (**Figs. 2–4**).

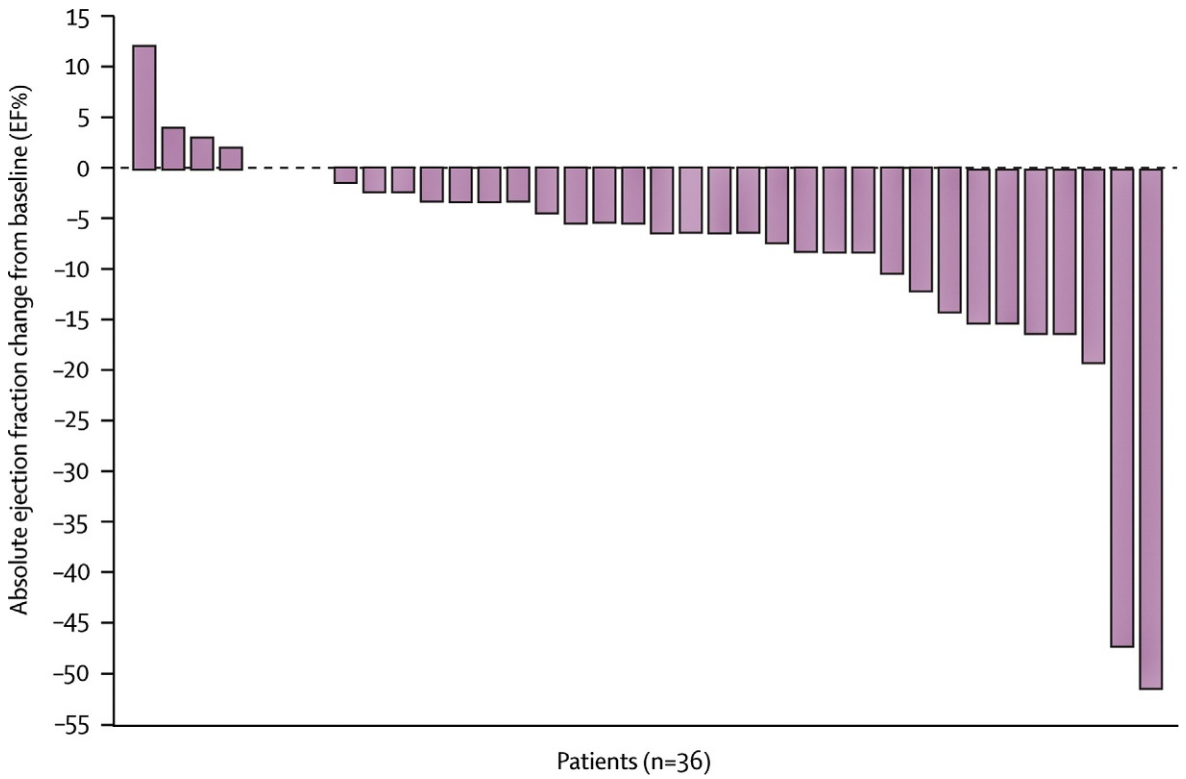

Fig. 2 Absolute maximum change in left ventricular ejection fraction (LVEF) from baseline in individual patients given the approved sunitinib dose. Individual data for absolute maximum change in LVEF from baseline to treatment with sunitinib 50 mg per day, 4 weeks on and 2 weeks off, is shown for all 36 patients. Three patients had no change in LVEF from baseline and are represented without bars. Adapted with permission from Chu, T.F., Rupnick, M.A., Kerkela, R., Dallabrida, S.M., Zurakowski, D., Nguyen, L., Woulfe, K., Pravda, E., Cassiola, F., Desai, J., George, S., Morgan, J.A., Harris, D.M., Ismail, N.S., Chen, J.H., Schoen, F.J., Van den Abbeele, A.D., Demetri, G.D., Force, T., Chen, M.H. (2007). Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. *Lancet.* **370**, 2011–2019.

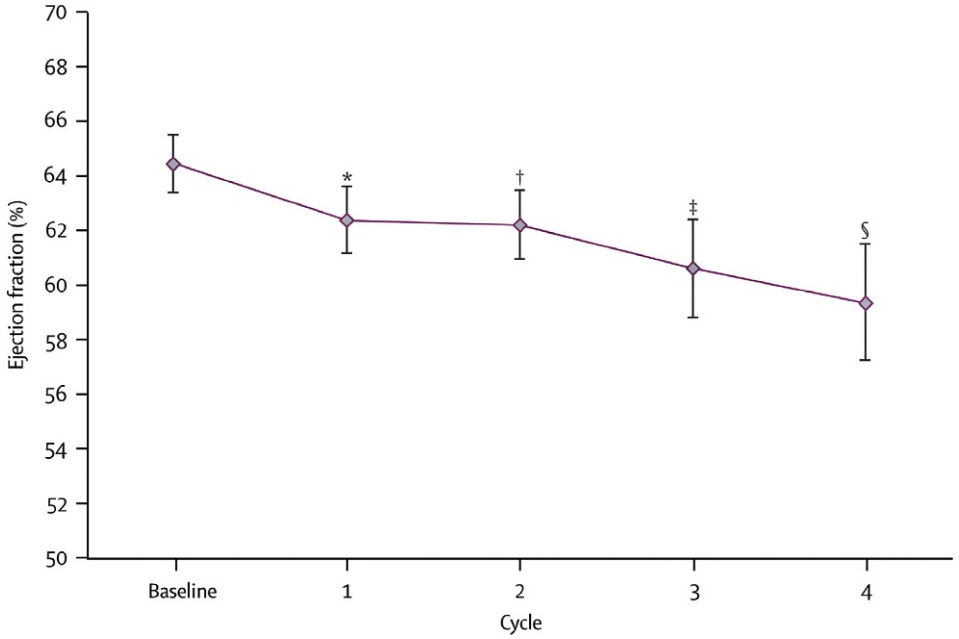

Fig. 3 Model of predicted change in mean left ventricular ejection fraction (LVEF) by treatment cycle. Repeated measures, mixed-model regression analysis of LVEF data derived from 36 patients during first four cycles of sunitinib treatment revealed progressive decrease in LVEF with an increase in number of sunitinib cycles. The model demonstrated an initial 2% decrease from baseline, followed by 1.5% decrease with cycle for every subsequent cycle. Predicted LVEF at baseline was 64.5%; after cycle 1, 62.4%; after cycle 2, 62.3%; after cycle 3, 60.6%; and after cycle 4, 59.4%. $^{*}P=0.048$ (cycle 1); $^{\dagger}P=0.044$ (cycle 2); $^{\ddagger}P=0.013$ (cycle 3); and $^{\S}P=0.007$ (cycle 4). Vertical bars represent SE. Adapted with permission from Chu, T.F., Rupnick, M.A., Kerkela, R., Dallabrida, S.M., Zurakowski, D., Nguyen, L., Woulfe, K., Pravda, E., Cassiola, F., Desai, J., George, S., Morgan, J.A., Harris, D.M., Ismail, N.S., Chen, J.H., Schoen, F.J., Van den Abbeele, A.D., Demetri, G.D., Force, T., Chen, M.H. (2007). Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. *Lancet.* **370**, 2011–2019.

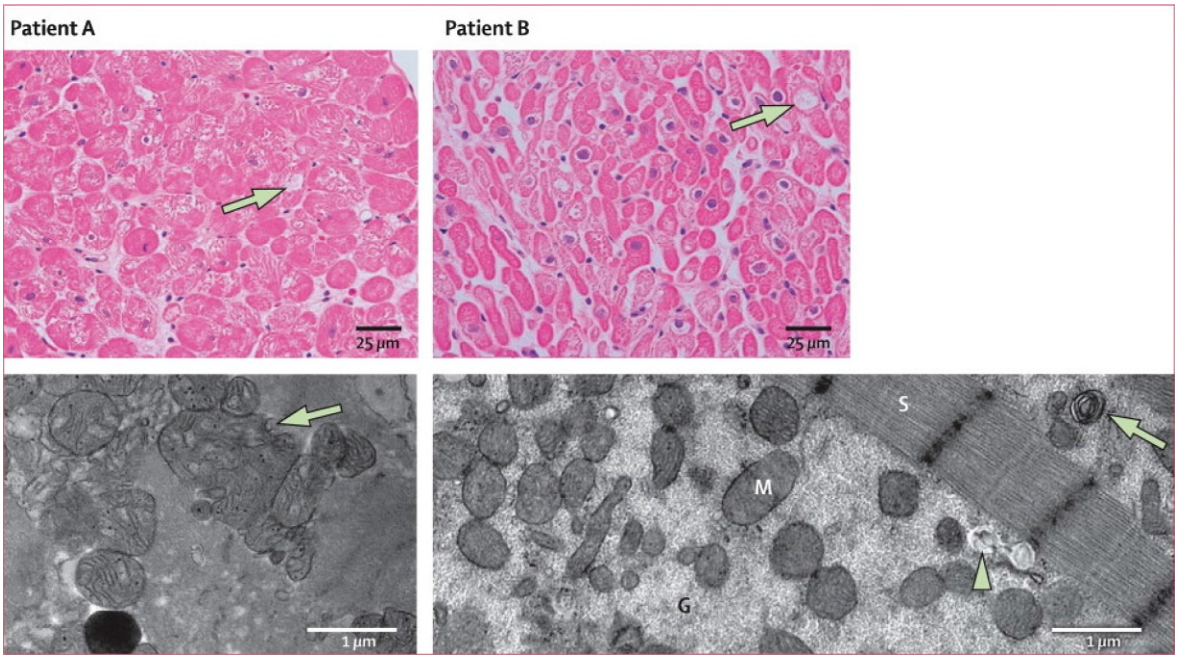

Fig. 4 Endomyocardial biopsy samples from two index patients who developed congestive heart failure on sunitinib. Light photomicrographs from patients A and B revealed myocyte hypertrophy with mild degenerative changes and diffuse, moderate cardiomyocyte vacuolisation (*arrows*). Oedema, interstitial or replacement fibrosis, regional infarct or focal cell necrosis, or myocarditis, or inflammation was not noted. Transmission electronmicrograph from patient A (bottom *left*) showed swollen, abnormal mitochondrial configurations (*arrow*) with effaced cristae. Transmission electronmicrograph from patient B (bottom *right*) showed numerous cytoplasmic granular densities consistent with glycogen accumulation (G), membrane whorl (*arrow*), and lysosomal precipitates (arrowhead). Sarcomeres (S) appeared well organized and structurally intact in patients A and B. (Light microscopy, stained with haematoxylin and eosin, bar = 25 μm; transmission electronmicroscopy, bar = 1 μm.). Adapted with permission from Chu, T.F., Rupnick, M.A., Kerkela, R., Dallabrida, S.M., Zurakowski, D., Nguyen, L., Woulfe, K., Pravda, E., Cassiola, F., Desai, J., George, S., Morgan, J.A., Harris, D.M., Ismail, N.S., Chen, J.H., Schoen, F.J., Van den Abbeele, A.D., Demetri, G.D., Force, T., Chen, M.H. (2007). Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. *Lancet.* **370**, 2011–2019.

Radiotherapy-Induced Cardiotoxicity

Radiotherapy involves exposure to high-energy particles, X-rays, and gamma radiation which breakdown DNA and in turn impair cell proliferation and repair (Groarke et al., 2014). It mainly affects rapidly proliferating tumor cells that have a high metabolic rate. The effect on normal cells is influenced by their inherent susceptibility and the intensity of ionizing radiation (Groarke et al., 2014). Radiotherapy to the chest for breast cancer and Hodgkin's lymphoma can cause damage to the heart and vascular system (Jaworski et al., 2013). This may manifest 20–30 years after initial exposure (Tukenova et al., 2010). Based on current evidence, a safe threshold for radiotherapy is unknown (Tukenova et al., 2010; Darby et al., 2013).

Radiation therapy can damage all layers of the heart (Filopei and Frishman, 2012). Recent improvements in technology have decreased the incidence of acute pericarditis secondary to radiotherapy from 20% to 2.5%(Jaworski et al., 2013). In patients who receive $\geq$1500 centiGy radiation to the chest, the incidence of chronic pericarditis at 30 years is 3% (Mulrooney et al., 2009). Injury to the endocardium and valves can initially cause valve retraction as well as regurgitation and subsequently lead to stenosis (van Leeuwen-Segarceanu et al., 2011). The typical myocardial changes secondary to radiation exposure include diffuse interstitial fibrosis, capillary rarefaction, wall thickening, and luminal narrowing of arterioles and capillaries (Herrmann et al., 2014). Injury to the endothelial layer causing micro-hemorrhage and worsening of radiation-mediated inflammation leads to myocardial dysfunction (Seemann et al., 2012). Additionally, leaking of albumin into myocardial interstitium contributes to restrictive cardiomyopathy (Herrmann et al., 2014).

Risk Factors for Chemotherapy-Mediated Cardiomyopathy

There are a number of patient-related risk factors which contribute to cardiotoxicity. These include traditional cardiovascular risk factors such as hypertension, age, nicotine use, postmenopausal status, as well as preexisting coronary artery disease, LV dysfunction, or HF (Albini et al., 2010). A recent study attributed the development of cardiotoxicity at a relatively small dose of anthracycline to genetic polymorphism. This evidence suggests that some patients may have genetic predisposition to chemotherapy-induced cardiotoxicity (Blanco et al., 2012). Chemotherapy-related risk factors include administration of drugs in bolus form, use of larger doses, prior chest irradiation, prior treatment with anthracyclines, or concurrent use of chemotherapeutic agents (Valachis and Nilsson, 2015).

Biomarkers

Multiple studies have evaluated the utility of biomarkers for early detection and risk stratification of chemotherapy-mediated cardiotoxicity (Witteles, 2016). Two commonly used biomarkers are troponin and natriuretic peptide (Witteles, 2016).

Troponins are thought to be biomarkers of myocardial injury (Bloom et al., 2016). Changes in troponin levels with each cycle of therapy can serve as a marker of risk of cardiotoxicity (Bloom et al., 2016). In a study of over 700 patients undergoing high-dose chemotherapy, immediate elevation in troponin I levels suggested a high risk of future LV dysfunction. On the other hand, lack of troponin elevation predicted a low risk of cardiotoxicity (Cardinale et al., 2004). Troponin elevation after trastuzumab therapy is almost exclusively observed in patients with prior treatment with anthracyclines. Tropinin levels increase in the first 2–3 months of trastuzumab therapy and return to normal levels during the subsequent 3 months in the majority of patients (Cardinale et al., 2010; Ky et al., 2014; Morris et al., 2011; Sawaya et al., 2012). In some studies, troponin elevation after trastuzumab therapy was associated with increased risk of LVEF reduction and major adverse cardiac events (Cardinale et al., 2010). However, other studies suggested that troponin elevation was not predictive of subsequent cardiac events (Morris et al., 2011). Studies evaluating recently approved tyrosine kinase inhibitors did not provide any evidence to support troponin monitoring in these patients (Schmidinger et al., 2008; Hall et al., 2013; Perik et al., 2008).

The utility of natriuretic peptides as a predictor of left ventricular dysfunction is unclear. In one study, elevation of natriuretic peptides after treatment with doxorubicin predicted subsequent LV dysfunction (Lipshultz et al., 2012). However, another study did not show any link between biomarkers and ventricular dysfunction (Sawaya et al., 2012). Natriuretic peptides do not have any role in early detection of cardiac toxicity secondary to trastuzumab (Witteles, 2016). The role of monitoring natriuretic peptides for patients receiving VEGF inhibitors is not well defined. Asymptomatic elevation in natriuretic peptides was observed after the use of VEGF inhibitors but it did not conclusively predict future LV dysfunction (Hall et al., 2013).

Imaging

Echocardiography

Accurate estimation of LVEF is vital in cancer patients due to its potential implications on the chemotherapy regimen (Kongbundansuk and Hundley, 2014). Two-dimensional echocardiography is a commonly used tool for monitoring ventricular function in these patients (Plana et al., 2014; Cheitlin et al., 2003). Measurement of LVEF by this imaging modality may be limited by image quality. Another disadvantage is its inability to detect subtle changes in LVEF (Cheitlin et al., 2003). The American Society of Echocardiography recommends the use of contrast for a clear delineation of endocardial borders in breast implant patients

(Mulvagh et al., 2008). Three-dimensional echocardiography, with a lower inter-observer variability and high reproducibility, is a superior tool for assessment of LV function and to detect small changes in LVEF (Thavendiranathan et al., 2013a). The use of speckle tracking and strain imaging (Baliga and Young, 2011) can lead to the detection of small decline in ventricular function at an early stage (Sawaya et al., 2012). The reduction in global longitudinal strain may be noted even with a preserved LVEF. A decline of >11% in global longitudinal strain was a major predictor of cardiomyopathy in one study (Negishi et al., 2013). However, the use of this technology is limited by the lack of universal availability, absence of standard definitions, and nonuniformity of measurement between vendors (Bloom et al., 2016).

Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance imaging (CMR) is superior to echocardiography for the assessment of ventricular size and function. The use of CMR can lead to a higher estimation of prevalence of chemotherapy-induced cardiomyopathy (Bloom et al., 2016; Armstrong et al., 2012). CMR also gives a noninvasive description of the myocardial tissue in terms of edema, scarring, and inflammation, which can help distinguish early and late cardiotoxicity in cancer patients (Thavendiranathan et al., 2013b). CMR may also have a prognostic role in cancer patients receiving chemotherapy. In one study, indexed LV mass by CMR was the strongest predictor of adverse cardiac events in patients with anthracycline-mediated cardiomyopathy (Neilan et al., 2012). In a different study evaluating the role of CMR, subepicardial late gadolinium enhancement in the lateral wall of left ventricle was observed in all patients in trastuzumab-mediated cardiomyopathy (Fallah-Rad et al., 2008). Although CMR is considered as a gold standard for the assessment of LV function, its routine use is limited by availability, cost, and other patient-specific variables such as claustrophobia and implantable cardiac devices (Bloom et al., 2016).

Radionuclide Imaging

In the past, multigated acquisition scan, due to its high reproducibility and availability, was the primary imaging modality for monitoring LV function (Jiji et al., 2012). Echocardiography has a higher inter-observer variability compared to multigated acquisition scan. But echocardiography is available at a lower cost and provides structural and functional information in addition to LVEF (Plana et al., 2014; Jiji et al., 2012). The main drawback of multigated acquisition scan is radiation exposure. Since the goal of medicine is to limit the radiation to "as low as reasonably achievable," one should evaluate the risk versus benefit of using this imaging modality for each individual patient (Plana et al., 2014).

Prevention Strategies

Prevention strategies can be broadly divided into (a) primary prevention—prophylactic treatment in patients at risk for cardiomyopathy and (b) secondary prevention—initiation of medical therapy in patients showing subclinical signs of cardiomyopathy such as elevated biomarkers (Khouri et al., 2012). A new approach described as primordial prevention is also being proposed. This refers to the institution of medical therapy after the primary cancer diagnosis but before the initiation of chemotherapy (Khouri et al., 2012).

Primary Prevention

Cancer patients with no known structural heart disease, who are receiving chemotherapy, are at risk for developing cardiomyopathy and are therefore considered to have Stage A HF. In the following section, we discuss the role of cardioprotective medications for primary prevention.

β-Blockers

Multiple studies have examined the role of β-blockers for the prevention of chemotherapy-mediated cardiotoxicity. Carvedilol is thought to have antioxidant and iron-chelating properties which prevent histopathologic changes induced by anthracyclines (Oliveira et al., 2004). In a randomized study involving 50 patients receiving anthracyclines, prophylactic administration of carvedilol attenuated cardiac dysfunction (Kalay et al., 2006). Nebivolol, used before planned chemotherapy, led to a greater preservation of LVEF (Kaya et al., 2013). Preliminary results of the recently completed MANTICORE (Multidisciplinary Approach to Novel Therapies in Cardiology Oncology Research) study suggest that the use of bisoprolol attenuates trastuzumab-mediated cardiotoxicity (Pituskin et al., 2015). The other β-blockers do not appear to have the same beneficial effects. While metoprolol is not cardioprotective (Georgakopoulos et al., 2010), propranolol may be potentially harmful for cancer patients (Choe et al., 1978) (**Figure 5**).

Renin–Angiotensin Inhibitors

Preclinical studies reveal that angiotensin II (Toko et al., 2002) and endothelin I (Bien et al., 2007) are involved in anthracycline cardiotoxicity. A study in rats suggested that renin–angiotensin inhibitors may be protective against anthracycline-mediated cardiotoxicity (Abd El-Aziz et al., 2001). Different mechanisms have been proposed to explain the beneficial effects of

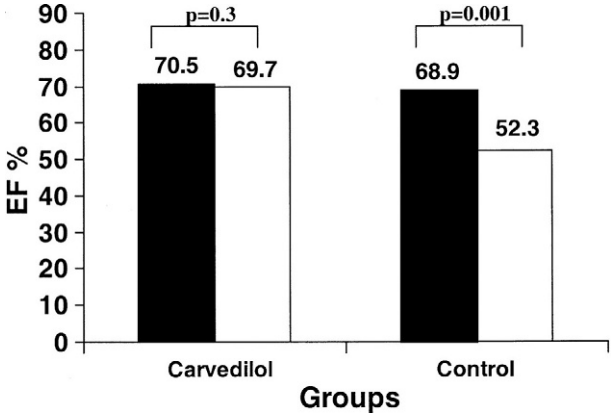

Fig. 5 Carvedilol in anthracycline-induced cardiomyopathy. Baseline LVEF (black bars) and after chemotherapy (white bars) in carvedilol ($N=25$) and control ($N=25$) groups. Data expressed as mean values. Adapted with permission from Kalay, N., Basar, E., Ozdogru, I., Er, O., Cetinkaya, Y., Dogan, A., Inanc, T., Oguzhan, A., Eryol, N. K., Topsakal, R., Ergin, A. (2006). Protective effects of carvedilol against anthracycline-induced cardiomyopathy. *Journal of the American College of Cardiology* **48**, 2258–2262.

renin–angiotensin inhibitors. These drugs have antifibrotic properties, decrease oxidative stress, and reduce the angiotensin-mediated inhibition of neuregulin/Erb system (Valachis and Nilsson, 2015). Evidence from multiple trials studying the efficacy of renin–angiotensin inhibitors in patients receiving chemotherapy has been largely equivocal. In one randomized study involving 125 patients with hematologic malignancies, enalapril did not reduce the incidence of anthracycline-mediated HF (Georgakopoulos et al., 2010). In another study involving patients receiving trastuzumab, the use of perindopril did not prevent a decline in LVEF (Pituskin et al., 2015). However, in the recently published PRADA (Prevention of Cardiac Dysfunction during Adjuvant Breast Cancer Therapy) trial, the use of candesartan, in breast cancer patients receiving anthracyclines and trastuzumab, prevented a significant decline in LVEF (Gulati et al., 2016).

Aldosterone Antagonists

Aldosterone antagonists are thought to protect against cancer therapy-mediated cardiac toxicity by its antifibrotic, antioxidant actions and by preventing apoptosis (Akpek et al., 2015). In a randomized study involving 83 patients with breast cancer, concomitant use of spironolactone and anthracyclines resulted in a greater preservation of LVEF (Akpek et al., 2015).

Combination of β-Blockers and Renin–Angiotensin Inhibitors

In the OVERCOME trial (Prevention of Left Ventricular Dysfunction with Enalapril and Carvedilol in Patients Submitted to Intensive Chemotherapy for the Treatment of Malignant Hemopathies), combined treatment with carvedilol and enalapril, in patients with hematologic malignancies, prevented a decline in LVEF and reduced the incidence of composite endpoint of death or HF (Bosch et al., 2013).

Secondary Prevention

Secondary prevention refers to the treatment of subclinical cardiomyopathy detected by elevated biomarkers or strain imaging. Cardinale et al. showed that the use of enalapril, in patients determined to be high-risk based on troponin I levels, prevented anthracycline-mediated cardiomyopathy (Cardinale et al., 2006). In another study, β-blockers improved LV systolic function in the cancer patients who developed subclinical cardiomyopathy detected by a decline in global longitudinal strain >11%(Negishi et al., 2014).

Dexrazoxane

Dexrazoxane, an intravenous iron chelating agent, is approved by the FDA as a cardioprotective agent in patients receiving anthracyclines (Hensley et al., 2009). This drug also inhibits the production of anthracycline-bound DNA–Top2 complexes (Sawyer et al., 2010; Hahn et al., 2014). The American Society of Clinical Oncology suggests restricting this medication to the adult patients with metastatic breast carcinoma who received >300 mg/m (Siegel et al., 2015) of anthracyclines and may benefit with additional dose of anthracyclines (Hensley et al., 2009). In one study involving adult cancer patients receiving anthracyclines, the use of dexrazoxane reduced the risk of HF and the rate of cardiac events (Kalam and Marwick, 2013; Marty et al., 2006). In another study involving pediatric patients with Hodgkin's disease, dexrazoxane increased the risk of secondary cancer (Tebbi et al., 2007). But this finding could not be confirmed in other studies (Lipshultz et al., 2007; Hellmann, 1996).

Statins

In addition to their lipid lowering action, statins also have cardioprotective effects mediated by their pleiotropic properties (Acar et al., 2011). In a small randomized study involving 40 patients receiving anthracyclines, the use of atorvastatin was associated with a lower rate of LVEF decline <50% (Acar et al., 2011). In a retrospective observational study involving 628 patients, statins significantly reduced the incidence of HF (Seicean et al., 2012). Further studies are needed to study the efficacy of statins for the prevention of CTMC.

Surveillance and Management of CTMC

Current surveillance protocols have been developed by expert consensus. One strategy involves risk stratification of patients based on cancer-related and cardiovascular risk factors (Herrmann et al., 2014). Baseline transthoracic echocardiogram with strain should be obtained before chemotherapy. Although the role of biomarkers is not well defined, monitoring of biomarkers is suggested (Herrmann et al., 2014). The timing and frequency of follow-up and serial imaging is institution-specific but should be individualized based on the patient-related risk factors and chemotherapy regimen (Hamo et al., 2016).

The Canadian Trastuzumab Working group suggests that LV function be assessed pretreatment and every 3 months for the duration of trastuzumab therapy with more frequent and closer monitoring for the patients determined to be high risk (Mackey et al., 2008). The FDA suggests holding trastuzumab for a minimum of 4 weeks if LVEF decreases $\geq$16% from baseline level or if LVEF drops by $\geq$10% from baseline to a value below normal. Trastuzumab can be restarted if the LVEF increases to normal in 4–8 weeks and the overall reduction from pretreatment level is $\leq$15% (FDA Drug Label, 2010).

Guidelines for discontinuation of anthracyclines are not clearly delineated (Herrmann et al., 2014). It is generally suggested that doxorubicin be stopped if the patient develops symptomatic HF (FDA Drug Label for DOXIL-doxorubicin hydrochloride injection s, liposomal, n.d.). In one study, a decline in LVEF >4% after a cumulative dose of 200 mg/m (Siegel et al., 2015) of doxorubicin has been shown to predict future anthracycline-mediated cardiomyopathy (Nousiainen et al., 2002). Surveillance imaging is recommended every 6 months for 1 year after completion of chemotherapy. In patients considered high risk, reevaluation at 3 months after completion of chemotherapy is suggested (Herrmann et al., 2014).

Treatment of patients who develop LV dysfunction and symptomatic CTMC should be according to AHA/ACC HF guidelines (Hamo et al., 2016).

Heart Failure Therapies for End-Stage CTMC

Conventional advanced HF therapies such as cardiac resynchronization therapy, mechanical circulatory devices, and orthotopic heart transplantation may be beneficial for end-stage chemotherapy-mediated cardiomyopathy (Hamo et al., 2016). In a small observational study involving patients with doxorubicin-mediated cardiomyopathy, cardiac resynchronization therapy improved symptoms and echocardiographic parameters to the same degree as seen in other nonischemic cardiomyopathy patients (Rickard et al., 2010). Otherwise, there is limited data regarding the benefit of cardiac resynchronization therapy in cancer patients. The results of the ongoing Multicenter Automated Defibrillator Implantation Trial—Chemotherapy-Induced Cardiomyopathy (MADIT-CHIC; Clinical Trial Registration No. NCT02154721) are eagerly awaited (Multicenter Automatic Defibrillator Implantation Trial, 2016).

Mechanical support devices are available as bridge-to-transplantation as well as destination therapy (Mukku et al., 2016). The patients with CTMC who receive LV assist devices have 1-, 2- and 3-year survival rates of 73%, 63%, and 47%, respectively (Mukku et al., 2016). Evaluation of the right ventricle before LV assist device implantation is extremely important, as these patients have an increased risk of right ventricular dysfunction. Not surprisingly, these patients are more likely to need right ventricular assist devices (Oliveira et al., 2014).

The number of patients with CTMC referred for orthotopic heart transplantation is steadily increasing (Oliveira et al., 2012). Patients with CTMC make up 0.8%–2.5% of all heart transplant recipients (Mukku et al., 2016). When compared to other nonischemic cardiomyopathy patients, CTMC patients had lower rates of rejection but higher rates of infection and malignancy in the first year after transplantation (Oliveira et al., 2012). However, overall survival was similar in both patient cohorts (Oliveira et al., 2012).

Conclusion

With improvements in cancer treatments and better survival, early detection and management of cardiomyopathy has become increasingly important. The first step is the risk stratification of cancer patients with prior cardiovascular conditions or known cardiac risk factors. These patients require regular imaging depending on their individual risk. Patients who develop asymptomatic LV dysfunction or overt HF require adjustment or interruption of chemotherapy. The goal is to detect and treat cardiomyopathy at an early stage so that life-saving cancer therapies can be administered without interruption (Fig. 5).

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Heart Failure in Low- to Middle-Income Countries

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Introduction

A syndrome rather than a distinct diagnosis, with many potential pathways to developing cardiac dysfunction, heart failure (HF) is a diverse condition that is difficult to characterize from the individual to societal level. Consequently, it would be a mistake to assume that the characteristics and burden of HF will be the same from one country and population to the next. This is particularly true when considering the likely differences between countries with diverse socio-demographic, socio-economic, and disease profiles. Currently, HF is largely defined by how it presents in high-income countries, with research gravitating toward relatively younger cohorts with a predominance of affected men with coronary artery disease. For example, in the seminal PARADIGM Trial cohort (mean age 64 years) nearly 80% were men and 60% had an ischemic cardiomyopathy (McMurray et al., 2014). The consequence of this imbalance is a paucity of data to describe the burden and risk factors of HF from a low- to middle-income country (LMIC) perspective. Data from the Global Burden of Disease studies (1990–2000) reported 235 contributors to global death; none of which were HF (Lozano et al., 2012). However, HF was included as a nonfatal health outcome and as a key indicator of population health. Accordingly, the majority of deaths primarily due to HF are documented as such (Goldacre et al., 2005).

Global Burden and Risk Factors of HF

Worldwide, population health is likely to vary markedly due to the wide spectrum of ethnic and cultural communities whose health and welfare are influenced by a broad range of socio-economic factors. As will be described later, the distribution and characteristics of HF reflects this. Overall, however, it is estimated there are approximately 26 million cases of HF globally at any one time (Ambrosy et al., 2014).

There are a number of conditions that confer a high risk for developing cardiac dysfunction and then HF; the two most common conditions being coronary artery disease (most closely linked to ischemic cardiomyopathy and subsequent HFrEF) and uncontrolled hypertension (most closely linked to hypertrophic cardiomyopathy and HFpEF). As the pattern of HF has evolved over the past 20–30 years, numerous reviews have focused on its risk factors/antecedents from high-income countries (Killip, 1985; Mendez and Cowie, 2001). Notably, Khatibzadeh and colleagues published a systematic review focusing on the numerous risk factors for HF from a global perspective (Khatibzadeh et al., 2013). They found that more than 50% of HF patients in Western, Eastern, and Central Europe presented with ischemic heart disease compared to 30%–40% in East Asia, Asia Pacific, Latin America, and the

Caribbean. Alternatively, in sub-Saharan Africa, ischemic heart disease was found in only 10% of HF cases. Significantly, hypertension was the most consistent risk factor for HF among all regions (≥17% crude prevalence). On an age and sex-adjusted basis, hypertension was most common in sub-Saharan Africa (33%, range 30%–36%), and Central and Eastern Europe (35%, range 33%–37%). Rheumatic heart disease was more prevalent in East Asia (34%) and sub-Saharan Africa (14%). Dilated and restrictive cardiomyopathy was most common in sub-Saharan Africa (adjusted prevalence 26%, range 23%–29%), Latin America, and the Caribbean (20%, range 17%–23%), as well as in high-income countries in Asia Pacific (17%, range 13%–21%). Beyond these broad trends, there are relatively less common forms of HF (in the developed world) that are or have been endemic to certain parts of the developing world; including HF linked to endomyocardial fibrosis, Chagas disease, peripartum cardiomyopathy (PPCM), and HIV cardiomyopathy (Keates et al., 2017).

Gaps in HF Research in Low- to Middle-Income Countries

Population health data from many regions of the world are scarce, particularly that focusing on the differences of HF between urban and rural populations and the evolution of risk factors contributing to the syndrome over time. Overall, specific factors contributing to the spectrum of cardiomyopathies leading to HF are underreported. This reflects a shortage of resources and diagnostic capabilities, with many historical reports on HF based on clinical diagnoses in the absence of echocardiography. Paradoxically, surveillance for HF is especially poor in those communities most at risk of developing HF, particularly where communicable causes of cardiomyopathy are common. Generally, right HF/pulmonary hypertension is poorly documented and there is a paucity of detailed description of the subtypes of valvular heart disease (such as the various manifestations of rheumatic heart disease). Additionally, congenital heart disease is usually categorized with "other causes contributing to HF" and represents an under-researched contributor to the syndrome. (Van Der Linde et al., 2011).

HF in Low- to Middle-Income Regions

The regions that predominantly contain LMICs are: sub-Saharan Africa, Latin America and the Caribbean, Middle East and North Africa, Europe and Central Asia, East Asia and Pacific, and South Asia (The World Bank, 2017). However, it is important to note that there are high-income countries nested within these regions, as well as particularly disadvantaged populations nested in the high-income countries (e.g., Indigenous Australians) in whom HF more closely resembles that found in the developing world.

Sub-Saharan Africa

Countries within sub-Saharan Africa (**Fig. 1**), barring Seychelles, are largely defined as low-to-middle income (The World Bank, 2017). In this region, as in other parts of the developing world there is a critical lack of effective interventions, such as gold-standard pharmacotherapy, management programs, and invasive procedures/devices. Consequently, there is a critical need for new research investigating the progression, pathogenesis, and prognosis of HF within Africa (Keates et al., 2017).



Fig. 1 The sub-Saharan Africa Region.

Burden of heart failure

Acute decompensated HF is the most common primary diagnosis for patients admitted to hospital with heart disease in sub-Saharan Africa (Kraus et al., 2015; Sliwa et al., 2008; Damasceno et al., 2012). Recent data from the INTER-CHF Registry prospectively characterized the contemporary sociodemographic, clinical variables, HF risk factors, and treatment in patients with acute HF in several LMICs across different regions of the world (Dokainish et al., 2015); revealing important interregional differences in the process. Notably, HF patients in sub-Saharan Africa were more likely to present as NYHA Class III/IV and least likely to be prescribed a beta-blocker. Moreover, they were markedly younger (mean age of 53 years versus 69–72 years) than their Western counterparts and more likely to be female (Crespo-Leiro et al., 2016; Adams et al., 2005). Typically, therefore, HF strikes Africans in what should be the prime, and most productive years of their lives.

Risk factors of heart failure

In the THESUS-HF registry including 1006 acute HF patients from nine sub-Saharan African countries, the most forms of HF were hypertensive heart disease/cardiomyopathy (45%), dilated cardiomyopathy (18%), and rheumatic heart disease (14%) (Damasceno et al., 2012). Earlier reports published between 1957 and 2005 provide evidence of an evolving transition from communicable (excluding the emergence of HIV cardiomyopathy) to more noncommunicable forms of heart disease and HF (Mayosi, 2007). Accordingly, within the INTER-CHF cohort, the most common causes of HF were hypertensive or ischemic cardiomyopathy, while the contribution of rheumatic heart disease was relatively low. The high incidence of hypertension and increased recognition of ischemic heart disease (still relatively low when compared to other regions of the world) as causes of HF have also been observed in other single-center studies (e.g., the Heart of Soweto Study) (Stewart et al., 2008).

Major forms of HF in sub-Saharan Africa

For patients in sub-Saharan Africa, hypertension is considered to be a distinct biological entity (Drazner, 2011). Not only is hypertension especially prevalent (particularly in urban communities (Sanderson et al., 1995)), it is often more aggressive (Adams et al., 2005) and more resistant to treatment (Addo et al., 2007), typically resulting in HF. In the Heart of Soweto study (Stewart et al., 2011), case presentations were dominantly women (54%) with 42% presenting with a secondary diagnosis of hypertension. Among the latter, hypertensive HF (57%, mean age 60 ± 14 years) was the most common manifestation of underlying hypertension.

Dilated cardiomyopathy (including the idiopathic form) contributed to 20% of HF patients captured by the THESUS-HF registry (Damasceno et al., 2012). Familial dilated cardiomyopathy cases are consistently reported to present at a younger age (mean 26 years old) compared with idiopathic dilated cardiomyopathy (39 years old) (Ntusi et al., 2011). It has long been assumed (based on the volume of reports) that PPCM is particularly prevalent among African women. The recent EROP registry examined the characteristics of PPCM among 500 women from a range of ethnic backgrounds and from all continents (Sliwa et al., 2017). As in previous studies (Gentry et al., 2010; Brar et al., 2007), there were marked differences in socio-economic background and ethnicity of cases. However, timing and mode of presentation remained relatively similar overall. There appeared to be a higher incidence of PPCM in sub-Saharan Africa and Asia compared to Europe. This may, in part, be explained by a higher prevalence of preexisting cardiomyopathy (28% vs. 10%) in African and Asian patients. (Elkayam et al., 2001). Bromocriptine therapy, a prolactin-inhibitor, has emerged as a promising therapeutic strategy for the treatment of PPCM (Elkayam and Goland, 2010; Hilfiker-Kleiner et al., 2017).

The clinical spectrum of HF within sub-Saharan Africa is further broadened by cardiac complications relating to HIV/AIDS and its therapy (Sliwa et al., 2008). A prospective study on 243 asymptomatic HIV patients (Chong et al., 2003) from Zambia reported 29% of patients presenting with major cardiac abnormalities relating to HIV were commonly associated with increased incidence of HF (Soliman et al., 2011). In the Heart of Soweto study, 24% of patients with HIV-associated cardiomyopathy had asymptomatic left ventricular dysfunction (Sliwa et al., 2012). HIV-associated cardiomyopathy leads to a rapid progression of mortality in patients who are not treated with HAART (Asanuma and Kitakaze, 2011). Many HIV-positive patients in sub-Saharan Africa now have access to HAART which, by itself may have adverse cardiovascular effects (Thienemann et al., 2013).

In the Heart of Soweto study, 36% of the cases were diagnosed with rheumatic heart disease (Sliwa et al., 2010), many presenting with late-stage disease and associated valvular HF. As in other African cohorts (Bloomfield et al., 2013), a small proportion of HF cases in Soweto were associated with tuberculosis/pericardial disease compared to a relatively high number of cases with right HF/pulmonary hypertension (Sliwa et al., 2010). In the poorer regions of Central and Eastern parts of Africa in particular, endomyocardial fibrosis occurs in endemic and a major cause of HF (Bloomfield et al., 2013) (**Fig. 2**).

Middle East and North Africa

The entire region of the Middle East and North Africa comprises LMICs: specifically, Algeria, Arab Republic, Djibouti, Egypt, Iran, Iraq, Jordan, Lebanon, Libya, Morocco, Syrian Arab Republic, Tunisia, West Bank and Gaza, and Yemen Republic (**Fig. 3**; The World Bank, 2017).

Burden of heart failure

To our knowledge, there are no reliable data to describe the characteristics and burden of HF in Northern Africa. However, there are a number of tertiary hospitals, outcome studies derived from LMICs in the Middle East (Al-Shamiri, 2013). A study based on a highly equipped tertiary center in Saudi Arabia, found that the in-hospital mortality was 5.3% among those presenting with acute HF,

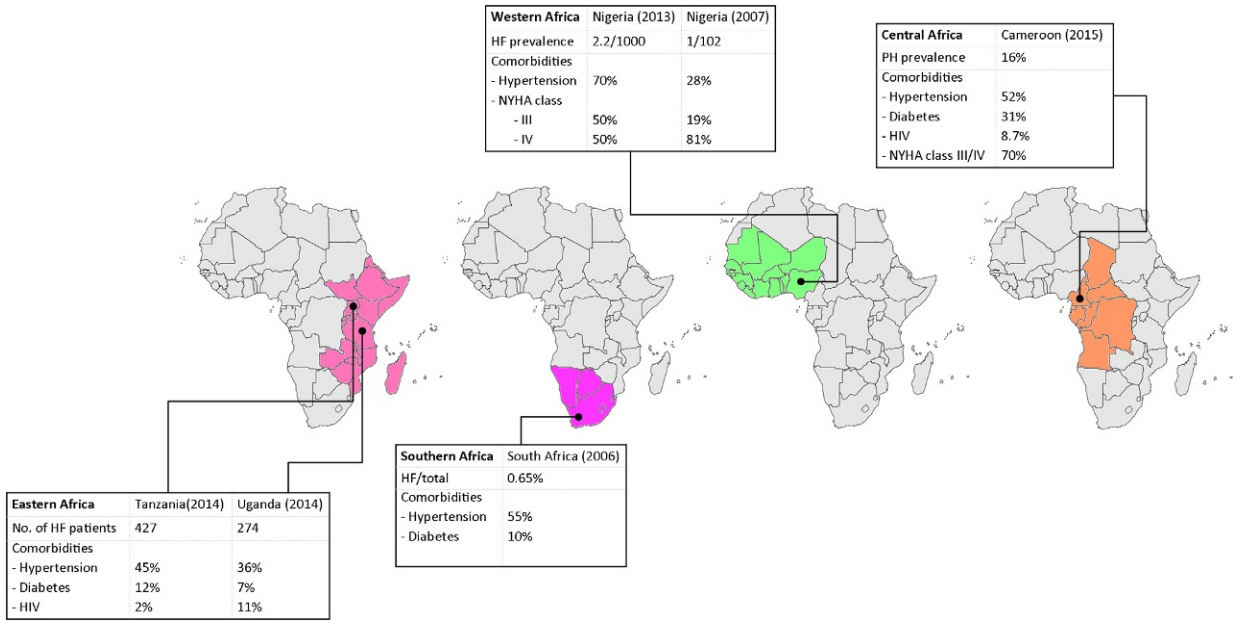

Fig. 2 Characteristics of primary HF hospitalizations across sub-Saharan Africa. *PH*, pulmonary hypertension. *Western Africa* data from PPCM cases. *Central Africa* data based on PH/right HF cases. Data from Tanzania (Makubi et al., 2014), Uganda (Okello et al., 2014), South Africa (Stewart et al., 2008), Nigeria (Akinwusi et al., 2013; Isezuo and Abubakar, 2007), and Cameroon. (Dzekem et al., 2015).



Fig. 3 The Middle East and North Africa Region.

increasing to 7.5% at 30 days (Alhabib et al., 2011). An equivalent study in Yemen showed a higher mortality rate of 9% (Bahaj, 2010). Broadly consistent with these data, a study focusing on in-hospital mortality associated with HF in the Middle East (1991–2010) found a reduction in mortality rates (in both men and women) from 8.4% to 4.8% during this period (Al Suwaidi et al., 2012).

Risk factors of heart failure

Many studies have examined the different risk factors of HF in the Middle East and North Africa region. The INTER-CHF study found the most common risk factor being ischemic cardiomyopathy (50%), followed by idiopathic dilated cardiomyopathy (19%),

hypertensive heart disease (10%), and rheumatic valvular heart disease (Dokainish et al., 2016). However, in Yemen and Oman, hypertensive heart disease was found to be the most common risk factor (25%) with idiopathic dilated cardiomyopathy (11%) being responsible for a relatively small proportion of HF cases. (Ibrahim, 2003) Of note, compared to the Middle East overall, in Egypt the relative contribution of valvular disease to HF is markedly higher (Al-Shamiri, 2013).

Europe and Central Asia

Within Europe and Central Asia, 36% of countries are considered as LMI. As shown in Fig. 4, these countries include: Armenia, Albania, Azerbaijan, Belarus, Bosnia and Herzegovina, Bulgaria, Georgia, Kazakhstan, Kosovo, Kyrgyz Republic, Macedonia, Moldova, Montenegro, Romania, Russia, Serbia, Tajikistan, Turkey, Turkmenistan, Ukraine, and Uzbekistan.

Burden of heart failure

The HAPPY study found that in Turkey, the crude prevalence of HF was 2.9% in 2012; a higher prevalence than many other Western countries despite its relatively younger population (Degertekin et al., 2012). A study of recurrent HF admissions across 20 cities of the Russian Federation found that the rehospitalization rate was 31% during the first 30 days following an acute HF admission of 31% (falling to 10% between 180 and 360 days). In-patient case fatality was 9%; increasing to 13% and 43% at 30-days and 12 months postdischarge, respectively (Arutyunov et al., 2015). As in other LMICs, however, there is a paucity of HF management programs designed to optimize treatment and outcome in affected individuals. However, a prospective observational study of this strategy conducted in Eastern Europe and focusing on patients with HFrEF showed that this strategy not only increased the prescription of beta-blockers (7%–81%) and ACE inhibitors (18%–93%) but reduced emergency department presentations and hospitalizations by 41% and 53%, respectively (Hebert et al., 2011).

Risk factors of heart failure

There is limited data for the risk factors of HF in the LMICs of Europe and Central Asia. However, the PROTECT trial, conducted in 17 countries (including North America (15%), Europe (52%), Israel (16%), and Argentina (3%)), investigated regional differences in acute HF (Mentz et al., 2014). Among European cases, hypertension, ischemic heart disease, and atrial fibrillation (58%–65%) were the most prevalent risk factors for HF; a similar pattern being observed in the RUS-HFR Study in Russia (Sitnikova et al., 2015).

Latin America and the Caribbean

Latin America and the Caribbean are relatively diverse from a socio-economic perspective with a number of high-income countries (38% of the region). LMICs in the region comprise Haiti, Bolivia, El Salvador, Guatemala, Honduras, Nicaragua, Argentina, Belize, Brazil, Colombia, Costa Rica, Cuba, Dominica, Dominican Republic, Ecuador, Grenada, Guyana, Jamaica, Mexico, Panama, Paraguay, Peru, St. Lucia, St. Vincent and the Grenadines, Suriname, and Venezuela (Fig. 5; The World Bank, 2017).

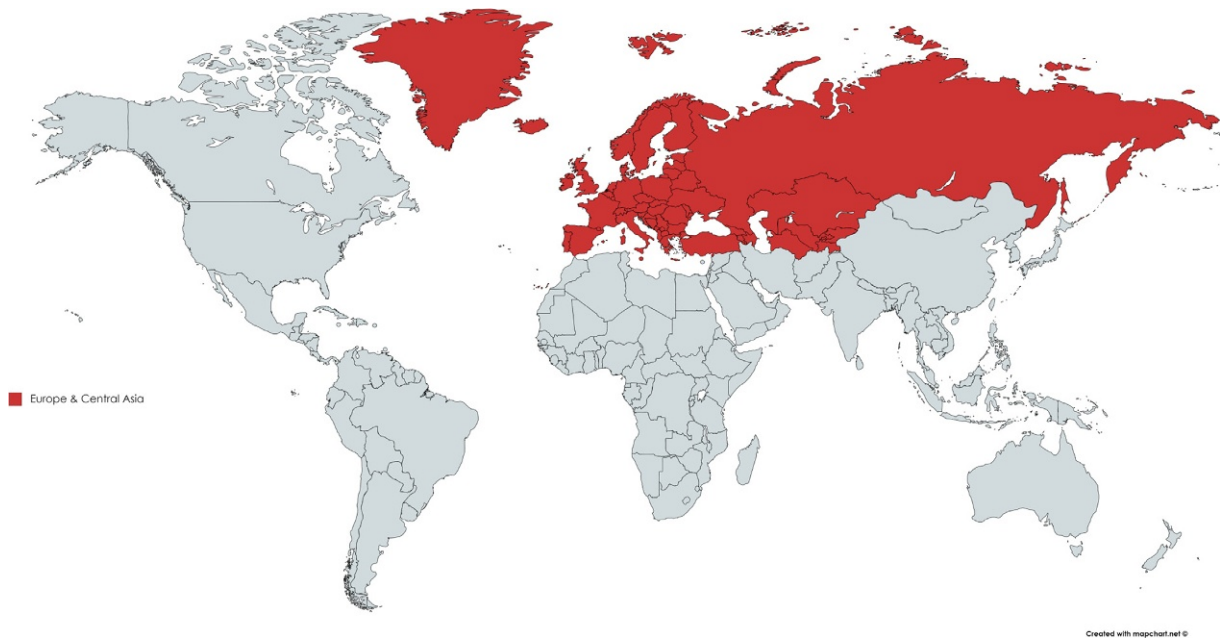

Fig. 4 The Europe and Central Asia Region.



Fig. 5 The Latin America and Caribbean Region.

Burden of heart failure

Although the main cause of hospitalization (based on available data) in the region is reportedly HF (Bocchi, 2013), there is a relative lack of data to describe the burden of disease imposed by the syndrome. However, the SEADE Foundation in São Paulo, Brazil found that HF and its contributory risk factors accounted for just over 6% of all deaths in that country (Chen et al., 2011). As with many parts of the globe, viable epidemiological data for HF are often restricted to inpatients and outpatients receiving ambulatory care in tertiary hospitals (Chen et al., 2011). In Argentina, among a cohort of hospitalized elderly patients, HFpEF was predominant in women (Giorgi et al., 2008). A study conducted in Brazil from 1999 to 2005, found a decreasing pattern in mortality rates due to HF; however, this was not the case for patients aged $\geq$80 years (Gaui et al., 2010). The Brazilian Ministry of Health (DATASUS) reported that from the total number of hospitalizations in 2007 from cardiovascular disease, 39% were due to HF, with a mean stay of 5.8 days and inpatient case fatality 7% (Bocchi, 2013), and a diagnosis of HF being a marker of high inpatient case fatality within the broader Brazilian patients population (Silva et al., 2009). In rural areas of Brazil, HFpEF was diagnosed in almost 50% of patients (being dominant in women) while HFrEF was dominant in men (Balieiro et al., 2009).

Risk factors of heart failure

The EVEREST Study (Gheorghiade et al., 2012) and the Eplerenone Post-AMI Heart Failure Efficacy and Survival Study (Pitt et al., 2003) compared North and South America data on the causes and outcome in patients with acute and chronic HF. The EVEREST study examined the characteristics of HF patients from Eastern Europe ($N=1619$), North America ($N=1251$), South America ($n=699$), and Western Europe ($n=564$). With a mean overall age of 62.2 years, the study determined that HF patients in South America were significantly younger compared to the other regions and had markedly different risk factor profiles (see **Fig. 6**), with less myocardial ischemia in the South American cohort. Differences were also noted in the use of pharmacological therapy with South American patients being prescribed proportionately more ACE-inhibitors (93% vs. 75%), aldosterone blockers (65% vs. 35%), however, North Americans were prescribed more beta-blockers (78% vs. 56%). Some differences (such as cardiovascular interventions, severity of the diseases, and drug use) appear to be associated with low socio-economic status.

Consistent with the above data, as in other parts of the world, there is evidence of epidemiological transition (Yusuf et al., 2001), with a clear rise in coronary artery disease due to an increasing prevalence of its risk factors (such as diabetes, obesity, aging population, and hypertension). Accordingly, ischemic cardiomyopathy is now attributed to be a major cause of HF in Latin America (Cubillos-Garzon et al., 2004). However, infectious diseases (such as rheumatic heart disease and Chagas disease) still remain common and also contribute substantially to the burden of HF in this region (Bocchi, 2013).

In a historic Brazilian report, 903 hospitalized cases (medium age 52 years) with a diagnosis of HF in a tertiary center, found that the most common risk factors were ischemic cardiomyopathy (33%), idiopathic cardiomyopathy (26%), valvular heart disease (22%), hypertensive heart disease (7%), and Chagas disease (6%) (Barretto et al., 1998), the findings of which are broadly supported by other studies in the region (Mangini et al., 2008). On a similar historical basis, a study in Argentina in 1993 showed that there was a slightly higher prevalence of hypertensive heart disease compared to ischemic cardiomyopathy (32% vs. 29%)

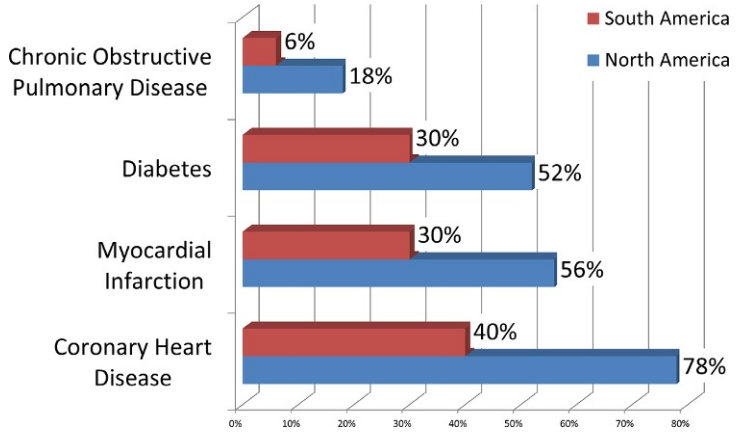

Fig. 6 Comparison of South versus North American HF Trial cohorts (Gheorghiade et al., 2012; Pitt et al., 2003).



Fig. 7 The East Asia and Pacific Region.

(Perna et al., 2006); these findings being supported by findings from the more contemporary Brazilian Evaluation of Levosimendan Infusion Efficacy multicenter study (Bocchi et al., 2003; Bocchi et al., 2008b). Among outpatients in a Brazilian cohort in the 1990s, idiopathic cardiomyopathy was present in 37%, Chagas disease in 20%, ischemic cardiomyopathy in 17%, and hypertensive heart disease, 14% (Freitas et al., 2005). The GESICA Study (Argentina) (Doval et al., 1994), REMADHF Study (Brazil) (Bocchi et al., 2008a), and a study in Mexico (Mendez et al., 2007) reported a prevalence of ischemic cardiomyopathy ranging from 22% to 47% followed by hypertensive and valvular heart disease. The GESICA and RAMADHF studies found that Chagas disease to be a major cause of HF with a prevalence ranging from 10% to 28%. Notably, there has been a significant decline in infected individuals in Latin America from around 16–18 million in the early 1990s to 10–12 million in the early 2000s due to disease control programs (Acquatella, 2007; Celermajer et al., 2012).

East Asia and Pacific

As with other parts of the developing world, it is difficult to report on the burden of HF and its risk factors in this region due a reliable epidemiological data. The LMICs within this region are: American Samoa, Cambodia, China, Federated States of Micronesia, Fiji, Indonesia, Kiribati, Korea, Laos, Malaysia, Marshall Islands, Mongolia, Myanmar, Palau, Papua New Guinea, Philippines, Samoa, Solomon Islands, Thailand, Timor-Leste, Tonga, Tuvalu, Vanuatu, and Vietnam (Fig. 7; The World Bank, 2017).

Burden of heart failure

As indicated, the challenge of reporting the burden of HF in the LMICs of Asia is highlighted by the limited number of reports available for this region. Interestingly, the number of hospitalized, de novo patients diagnosed with HF appears to be higher in Korea compared to other LMICs in this region, whilst Malaysia has reported the highest prevalence of HF (6.7%) in this region (Hung et al., 2000). Overall, a similar median length of hospital stay has been reported in most Asian-based registries examining the characteristics of HF admissions: the KorAHF Registry (Korea) reported 8 days (Lee et al., 2014), and the ADHERE International Study (including Australia, Hong Kong, Indonesia, Malaysia, Philippines, Singapore, Taiwan, and Thailand) reported 6 days (Atherton et al., 2012). However, a study conducted in China reported a median hospital stay of 22 days in the year 2000 (Wang et al., 2014). The KorAHF Registry reported a 30-day and 180-day all-cause mortality rate of 1.2% and 9.2% and an HF readmission rate of 6% and 24%, respectively (Lee et al., 2014).

Risk factors of heart failure

In Malaysia and Taiwan, the proportion of HF cases attributable to ischemic cardiomyopathy ranges from 25% to 47% (Tseng, 2011; Chong et al., 2003; Tsutsui et al., 2007), with hypertensive heart disease being the least common risk factor in Malaysia (19%) (Tseng, 2011). Valvular heart disease has been reported to be a relatively low risk factor of HF (compared to other LMICs) in East Asia (15%–35%) (Chong et al., 2003). Alternatively, atrial fibrillation (21%–42%) and diabetes (12%–36%) have been found to be relatively common in HF patients in the region (Tsutsui et al., 2007; Chong et al., 2003; Tseng, 2011).

South Asia

South Asia is considered to be the most populous and the most densely populated geographical region in the world. The entire region of South Asia is considered to comprise LMICs: Afghanistan, Nepal, Bangladesh, Bhutan, India, Pakistan, Sri Lanka, and Maldives (Fig. 8).

Burden of heart failure

Data describing the burden of HF are inherently unreliable for South Asia. Consequently, many figures are projected from Western Countries. For example, it is known that HF is predominant in the elderly; therefore the burden of HF is likely to increase with the progressing aging of the region with the projected increase in people aged older than 60 years from 133 million in 2011 to 494 million in 2051 (Pillai and Ganapathi, 2013). Pais and colleagues estimated the prevalence of HF in India to be 1.9% based on the rates from the United States (Pais and Xavier, 2011). If these rates are applied to the 2011 population of India and the entire South Asian region, the number of patients with HF is projected to be 22.7 million and 30 million, respectively (Pillai and Ganapathi, 2013). A prospective study was conducted in 2013 to determine the clinical outcomes of patients with HF in Sri Lanka. Of the patients studied, 60% were found to be de novo HF cases with a mean hospital stay of 5.5 days (Matthias and Ekanayaka, 2013).



Fig. 8 The South Asia Region.

Risk factors of heart failure

The Sri Lanka study discussed earlier found the three most common HF risk factors to be acute ischemic cardiomyopathy (37%), anemia (41%), and respiratory disease (10%) (Matthias and Ekanayaka, 2013). The predominance (up to 77% of HFrEF cases) of ischemic cardiomyopathy in Pakistan has been confirmed by a number of other studies (Pillai and Ganapathi, 2013). Ischemic cardiomyopathy is also reported to be the most common contributor to HF in South Asians living in Pakistan (Jafary et al., 2007), Nepal (Dubey et al., 2012), and India (Vora et al., 2017), as well as South Asian migrants in countries outside the region (Galasko et al., 2005; Chong et al., 2003). Emerging infectious diseases have also been found to contribute to the burden of HF. For example, 4% of patients in the leptospirosis outbreak in Sri Lanka were complicated by the syndrome (Agampodi et al., 2011). Additionally, rare cardiomyopathies have also been noted in specific regions such as endomyocardial fibrosis in India (Sivasankaran, 2009).

Indigenous Populations Within High-Income Countries

Any commentary on the burden of HF would be remiss without some consideration of Indigenous populations living in high-income countries. For example, in Australia the life expectancy of Aboriginal people is over a decade less than that of non-Aboriginal people, with 70% of the gap attributable to noncommunicable diseases (Mcgrady et al., 2012). More consistent with HF in LMICs, Indigenous Australians presenting with de novo HF are relatively young (mean age around 50 years), comprise more women, with markedly higher age-adjusted incidence rates compared to the rest of the (high-income) Australian population (Mcgrady et al., 2012). In New Zealand, Sopoago and colleagues (Sopoaga et al., 2010) found that Mäori people were six times more likely to be admitted to the hospital with a cardiomyopathy and four to five times more likely to present with rheumatic heart disease. These disparities were observed in earlier research (data period 1988–98) where HF case fatality was almost nine times higher in Mäori men aged 45–64 years than their non-Mäori counterparts, with similar patterns in women (Atherton et al., 2012; **Fig. 9**).

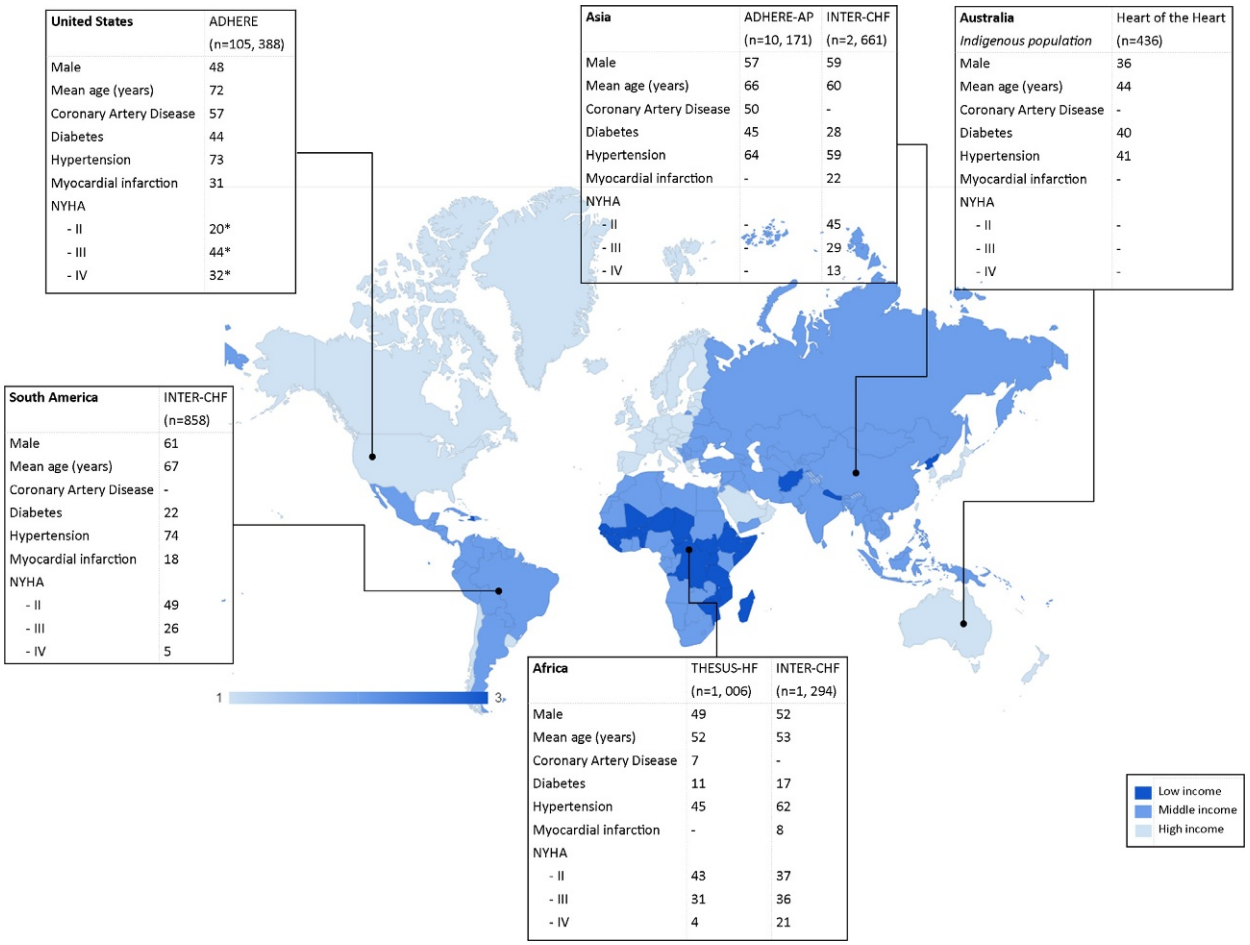

Fig. 9 Regional differences in the pattern of HF: insights from large-scale studies.

Key Considerations

HF poses an increasing problem for global healthcare systems, with current epidemiologic data in the different regions of the world remaining poorly described. An international approach that includes ethnically diverse populations of all ages and genders is urgently needed. Because so much information is missing, current guidelines developed by different societies in high-income countries may not be readily extrapolated to the developing world setting.

Future research investigating risk factors/antecedents for HF in community-based settings, across several world regions, is urgently needed. However, such studies are costly and as a result rarely receive full support. INTER-CHF, a longitudinal case study in LMICs, is one such study and described significant regional variability in sociodemographic factors, clinical characteristics, risk factors, and treatments of HF in several regions of the world. Studies such as these highlight the proportion of patients lacking appropriate care and treatment for HF. Further research of this nature may indicate opportunities for prevention and HF intervention studies that specifically target strategies to reduce the rapidly evolving burden of HF in the developing world.

Key Issues

- There is little to no evidence to describe the prevalence and incidence of HF in developing countries.
- Presentation of HF occurs at a much younger age (mean age 53–67 years) in developing countries. This is in contrast to developed countries where HF is increasingly a condition that affects the elderly (mean age 70–72 years).
- Nonischemic causes of HF (hypertensive heart disease, rheumatic heart disease, and dilated cardiomyopathy) account for around two-thirds of cases in sub-Saharan Africa, but ischemic cardiomyopathy is likely to be an increasing cause of HF in the future.
- Indigenous populations in developed countries have similar HF profiles to those living in developing countries.
- In the developing world, many forms of HF are highly preventable. Future research should address strategies which cost-effectively historically high levels of obesity, diabetes and hypertension.

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Heart Failure in Minorities

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Abbreviations

AA	African Americans
ABC	Aging, and body composition
A-HeFT	AA HF trial
ARIC	Atherosclerosis risk in communities
ARR	Aldosterone-renin ratio
BMI	Body mass index
BNP	B-type natriuretic peptide
BP	Blood pressure
CARDIA	Coronary artery risk development in young adults
CHD	Coronary heart disease
CHF	Congestive heart failure
CVD	Cardiovascular
DM	Diabetes mellitus
eGFR	Estimated glomerular filtration rate
HA	Hispanic Americans
HFPEF	HF with preserved EF
HFREF	HF with reduced EF
HOMA-IR	Homeostasis model assessment of insulin resistance
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
MESA	Multi-Ethnic Study of Atherosclerosis
MI	Myocardial infarction
TTR	Transthyretin gene

In this section, we review the epidemiology, standard and novel risk factors contributing to congestive heart failure (CHF) in African Americans (AA) and Hispanic Americans (HA). We discuss the most common comorbidities and genetic dispositions that play a role in the physiology and management of disease in these groups.

Heart Failure in African Americans

Epidemiology of Heart Failure in African Americans

Heart failure (HF) affects 5.7 million adults in the United States annually (Mozaffarian et al., 2016). The annual incidence of HF events per 1000 in the population for AA men between the age of 65 and 74 years and between the age of 75 and 84 years are 16.9 and 25.5, respectively. For AA women, the rates are 14.2 and 25.5, respectively (Go et al., 2014). In the Atherosclerosis Risk in Communities (ARIC) Study, age-adjusted incidence rates per 1000 person-years were 8.1 in AA women, 6.0 in AA men, 3.4 for white women, and 6.0 in white men (Go et al., 2014). Compared to non-Hispanic whites, AA have a higher burden of CHF incidence, morbidity, and mortality that is incompletely explained by standard cardiovascular disease (CVD) risk factors. In the Multi-Ethnic Study of Atherosclerosis (MESA), AA had the highest proportion of CHF not preceded by a myocardial infarction (MI) (Bahrami et al., 2008). Additionally, CHF appears to affect AA at younger ages. In the ARIC Study, AA had a higher incidence of CHF at age less than 75 and a higher five-year mortality rate than non-Hispanic whites (Loehr et al., 2008). In the Coronary Artery Risk Development in Young Adults (CARDIA) Study, CHF before the age of 50 years was far more common in AA compared to whites (Bibbins-Domingo et al., 2009).

Risk Factors for Heart Failure in African Americans

Understanding the risk factors for CHF in AA is imperative as it gives the insight to aid in prevention. Traditional risk factors (most of which are modifiable) for CHF include coronary heart disease (CHD), cigarette smoking, hypertension, obesity, age, and diabetes (He et al., 2001). In the Health, Aging, and Body Composition (ABC) Heart Study, a higher proportion of CHF was attributable to modifiable risk factors in AA compared to whites (68% vs. 49%; 6 of 8 risk factors had at least a 5% higher attributable risk in AA compared to whites). Such data suggests a unique opportunity for targeting lifestyle and other modifiable risk factors in AA to lower the burden of CHF (Sharma et al., 2014).

Hypertension

Hypertension is of particular importance as a risk factor for CHF in AA. AA have the highest prevalence rates of hypertension globally (Sharma et al., 2014). In the National Health and Nutrition Examination Survey from 1999 to 2000, AA compared to white Americans had a higher prevalence of hypertension at 41.4% and developed hypertension at a younger age (Hertz et al., 2005). AA were also less likely to reach blood pressure goal (Hertz et al., 2005; Randolph et al., 2016). In MESA, AA had a higher risk of incident HF that was thought to be related to the prevalence of risk factors, particularly hypertension (Bahrami et al., 2008). In the AA community-based cohort followed by the Jackson Heart Study (JHS), every 10 mmHg increase in blood pressure was associated with a higher risk of CHF (Randolph et al., 2016). In the Health ABC study, uncontrolled blood pressure (BP) generated the highest population attributable risk (PAR) for the development of HF (30.9%) (Go et al., 2014). Hypertension has been strongly associated with incident HF, and blood pressure treatment strategies have demonstrated efficacy to prevent HF. In the Systolic Hypertension in the Elderly Program of over 4700 participants aged above 60 years or more with systolic hypertension, antihypertensive therapy including a low-dose diuretic exerted a strong protective effect in preventing HF. In the Hypertension in the Very Elderly Trial, treatment of elderly (>80 years of age) participants with indapamide with or without the angiotensin converting enzyme inhibitor perindopril lowered the mean blood pressure by 15/6 mmHg and resulted in a 64% reduction in the rate of HF at 2 years. Based on these data, treatment of hypertension particularly in aged individuals is associated with reduced HF. However, less is known about specific therapies in AA.

The specific mechanisms by which hypertension leads to CHF is not completely understood (Sharma et al., 2014). AA who develop HF tend to have uncontrolled hypertension (Sharma et al., 2014). One theory is that hypertension leads to ventricular remodeling and subsequent changes in structure and left ventricular (LV) systolic function that predisposes this group to higher rates of HF (Sharma et al., 2014). Indeed, the majority of AA with CHF have HF with reduced ejection fraction (EF) HF with preserved ejection fraction (HFPEF) compared to HFPEF (Sharma et al., 2014).

Obesity

Obesity has been associated with both LV dysfunction and incident HF in a number of ethnic groups (Krishnamoorthy et al., 2016; Kenchaiah et al., 2002). Excess weight causes increase in both blood volume (preload) and blood pressure (afterload) that lead to the structural and functional changes preceding clinical HF (Hall et al., 2015; Eaton et al., 2016). Obesity may also lead to HF through coexisting risk factors that commonly accompany it including hypertension, diabetes, ischemic heart disease, inflammation, and renal dysfunction. AA have high rates of obesity which may, in part, contribute to the excess burden of HF in this group. Specifically, obesity may contribute to HFPEF, which is the predominant type of HF observed in AA cohort studies (Gupta et al., 2013). For example, obesity has been implicated as a key player in HFPEF for AA women (Eaton et al., 2016). Approximately 26% of the attributable risk for HFPEF in a multiracial cohort of postmenopausal women was related to obesity. In the study, obesity was

found to be a more potent risk factor for AA women compared to white women. In a follow-up study of National Health and Nutrition Examination Survey 1 from 2001, obesity was associated with 1.3 relative risk of incident HF and a population attributable risk of 9.0% (He et al., 2001). AA represented 14% of the study group. In the JHS, among obese and morbidly obese participants, for every 1 kg/m^2 increase in body mass index (BMI), there was a 5% increase in the risk of prevalent HF (Krishnamoorthy et al., 2016).

Diabetes

Diabetes is also a strong contributor to the development of CHF in AA (Zhao et al., 2014). Every 1% increase in Hemoglobin A1C (HbA1c) was associated with 6% increase in the risk of HF in over 17,000 AA (Zhao et al., 2014). In MESA, 21% of AA participants with CHF had diabetes and further diabetes significantly contributed to the higher incidence of HF in AA (Bahrami et al., 2008). In the I-Preserve trial (Irbesartan in Heart Failure With Preserved Ejection Fraction), patients with HFPEF and diabetes had more signs of congestion, worse quality of life, higher N-terminal pro-B-type natriuretic peptide levels, and overall poorer prognoses (hazard ratio (HR) 1.75 for death or HF hospitalization) compared with those without diabetes. The mechanisms by which diabetes leads to the development of HF are not completely understood but are likely multifactorial. In addition to increasing the risk of ischemic heart disease, metabolic factors appear to play an important role. Altered substrate metabolism, myocardial lipotoxicity, oxidative stress, mitochondrial dysfunction, cardiac fibrosis, and apoptosis leading to impaired cardiac function (diastolic and systolic) are likely contributors to diabetic cardiomyopathy and HF. Given the high rates and overlap of diabetes and obesity in AA, further study of their effects on impaired cardiac function and HF is warranted.

Left ventricular hypertrophy

Left ventricular hypertrophy (LVH) poses a significant risk for CHF in AA. This is concerning because multiple investigations have shown that the prevalence and incidence of LVH are greater in AA compared to white Americans (He et al., 2001), and this disparity does not appear to be completely explained by risk factor burden alone (Drazner et al., 2005; East et al., 2004). In Health ABC, LVH by electrocardiography was a significant risk factor for incident HF in AA and this risk was independent of hypertension. In the JHS, after adjusting for clinical risk factors, an increase in echocardiographic left ventricular mass (LVM) over 8 years was associated with incident CVD defined as CHD, stroke, HF, and intermittent claudication (Fox et al., 2015).

Further, echocardiographic LV geometry appears to contribute to HF type which has prognostic implications. Those individuals with HFPEF tend to have the more concentric form of hypertrophy with thicker ventricular walls and LV diastolic dysfunction (Sharma et al., 2014). Alternatively, those with HFREF more commonly present with the eccentric form of hypertrophy. HFPEF comprise at least 50% of those with CHF in the population. AA and white Americans are equally affected by HFPEF despite the higher burden of risk factors predisposing to the HFPEF phenotype seen in AA (Sharma et al., 2014). In one recent study, survival among those with symptomatic HF improved over time in patients with HFREF but not in those with HFPEF and diastolic dysfunction was shown to be predictive of death among asymptomatic patients (Go et al., 2014).

Coronary heart disease

Although AA have higher rates of nonischemic cardiomyopathies compared to white Americans, CHD still incurs a highly significant risk for CHF in AA (Sharma et al., 2014; Coughlin et al., 1993). In the Health ABC study, AA participants were more likely than white participants to develop HF and CHD (PAR of 29.5% in blacks vs. PAR of 23.9% in whites) had among the highest population attributable risk for HF in both races (Go et al., 2014). CHD events are often followed by cardiac remodeling that result in the development of CHF. First noted in hearts post-MI and later identified in dilated cardiomyopathies including those due to myocarditis, cardiac remodeling is characterized by progressive increase in the LV chamber size, thinning of the ventricular walls, and transformation of the LV geometry to a more eccentric, spherical-shaped heart (Pfeffer et al., 1988; Florea et al., 1999). These changes are associated with a reduction in systolic function and subsequent development of CHF.

Comorbidities Associated With Heart Failure in African Americans

Pulmonary disease

Despite existing data supporting pulmonary disease as a significant comorbidity associated with CHF in predominantly or exclusively non-Hispanic white populations, there is limited data available in large AA cohorts. In MESA, pulmonary hyperinflation was associated with increased LVM on MRI (Smith et al., 2013). In a recent MESA report, subclinical lung disease was associated with reduced LV end-diastolic volume and stroke volume on magnetic resonance imaging (MRI), potentially due to increased LV stiffness and increased LV end-diastolic pressure (as a result of increased pulmonary vascular resistance due to hypoxia, hypercapnia, and endothelial damage) (Aaron et al., 2017).

These findings were further supported by a recent Framingham study where investigators showed the importance of noncardiac major organ system dysfunction in predicting incident CHF events. After adjusting for HF risk factors, cardiac systolic and diastolic function, lower ratio of forced expiratory volume (FEV1) to forced vital capacity (FVC), was associated with a 30% increased risk of CHF. Specifically, FEV1/FVC was associated with HFpEF (Lam et al., 2011). Further research is needed to identify the extent to which pulmonary disease plays a role in CHF in AA.

Chronic kidney disease

AA are disproportionately affected by both chronic kidney disease (CKD) and CHF. In the Health ABC Study, lower estimated glomerular filtration rate (eGFR) and higher cystatin C levels were associated with incident HF in both AA and whites; however, the magnitude was greater for AA compared to whites (HR 1.76 vs. 1.19 respectively) (Bibbins-Domingo et al., 2006). Hypertension, diabetes, and obesity, all higher in AA, are major risk factors for both CKD and CHF. The high prevalence of the apolipoprotein (APOL1) alleles which are present in approximately 13% of AA, significantly increases the rate of renal decline and CKD (Grams et al., 2016). As renal injury and dysfunction worsen, there is augmented renal sodium and water reabsorption and subsequently elevated blood pressure (Hall et al., 2014). This increase in BP further exacerbates renal dysfunction in addition to having detrimental effects on the heart including increased LVM. In the JHS, increased LVM was associated with estimated GFR decline and end-stage renal disease in AA (Hall et al., 2015). In a pooled analysis of three large longitudinal cohort studies, CKD was independently associated with an increased risk of HF (HR 2.3) and to a similar degree as CHD. This risk was 3.5-fold higher in AA compared to whites (Bansal et al., 2017). In patients with chronic HF, every 10 mL/min/1.73 m^2 decrease in eGFR was associated with a 10% increased risk of CVD death or HF hospitalization (Damman et al., 2017). While renal dysfunction has been implicated in the causal pathway for HF, cardiorenal interactions are increasingly recognized to contribute to overall health. Specifically, several cardiorenal syndromes have been defined (Udani and Koyner, 2010). CKD also sometimes confounds the treatment pathway for HF patients. For example, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers have significant mortality benefits in patients with HF with HFrEF (Pfeffer et al., 2003). However, these drugs can sometimes increase serum creatinine and may prevent their administration to patients with the underlying renal disease.

Atrial fibrillation

Atrial fibrillation (AF) and HF are increasingly prevalent, closely interrelated, and share many risk factors (Kotecha and Piccini, 2015). AF incidence is predicted to double over the next 20 years (Colilla et al., 2013). In a global registry of patients with AF, 33% of paroxysmal, 44% of persistent, and 56% of permanent AF patients had HF symptoms (Chiang et al., 2012). Common shared risk factors for AF and HF include hypertension, age, male sex, and obesity. However, AA have 41% lower adjusted risk of AF compared to whites despite a higher prevalence of many traditional AF risk factors (Alonso et al., 2009). The lower risk in AA may be related to less left atrial dilation or genetic differences compared to whites (Dewland et al., 2016; Marcus et al., 2010). In AA, echocardiographic measures including LV diameter, left atrial dimension, LVM index (LVMI), and reduced EF (<50%) were associated with higher AF risk in the ARIC Study. AF can be either a cause or an effect of HF (Bekwelem et al., 2014; Anter et al., 2009). Potential mechanisms by which AF may lead to the development or progression of HF include (1) increased resting heart rate/shorter diastolic filling time, (2) atrioventricular dyssynchrony, and (3) reduced effective atrial contraction. In patients with sinus rhythm, conversion to AF was associated with reduced peak oxygen consumption, increased mitral and tricuspid regurgitation, reduced cardiac index, and worsened NYHA functional class (Pozzoli et al., 1998). Despite a lower burden of AF in AA compared to whites, the risk of incident HF was twice as high in AA in the ARIC Study over a follow-up period of 20 years (Magnani et al., 2016).

Aging

CHF is primarily a disease process of the elderly with 50% of HF diagnoses and 90% of HF deaths occurring in those over 70 years of age (Strait and Lakatta, 2012). Although aging itself may not directly cause HF, diseases associated with aging (ischemic heart disease, valvular dysfunction, etc.) contribute to this increased HF prevalence. As treatment strategies for these diseases have improved (i.e., percutaneous coronary and valve interventions), patients with these predisposing conditions are living longer albeit with some degree of cardiac dysfunction. Some factors associated with aging that exacerbate or contribute to the pathogenesis of HF include diastolic dysfunction, impaired renal function, and arterial stiffness.

APOL1 risk alleles are associated with exaggerated age-related nephron loss which may accelerate normal age-related kidney dysfunction thereby augmenting the renin–angiotensin–aldosterone system, increasing salt-sensitivity, and sodium and water retention—all factors which can lead to or exacerbate HF (Hoy et al., 2015). Similarly to whites, diastolic dysfunction measured by reduced tissue Doppler velocities and diastolic parameters reflecting less LV compliance was significantly associated with future HF in a population of AA with hypertension and CKD (Peterson et al., 2013). Overall, aging is a multifactorial process that is associated with a number of pathogenic factors which contribute to HF.

Arterial stiffness/endothelial function

Arterial stiffness is an early predictor of hypertension, a major risk factor for HF. The underlying mechanism may be related to increased oxidative stress, decreased nitric oxide availability, exaggerated vasoconstrictor response, and attenuated responsiveness to vasodilators and nitric oxide (Sharma et al., 2014). AA race is a significant predictor of impaired microvascular vasodilatory function, greater large arterial wave reflections, and stiffness even after adjusting for traditional CVD risk factors (Morris et al., 2013). In the ARIC Study, arterial elasticity was associated with greater LVMI and higher LV filling pressures (measured by E/e′ ratio) in AA but not in whites suggesting that AA are more sensitive to increased afterload as a stimulus for LV structural and functional remodeling—both precursors to HF (Fernandes-Silva et al., 2017).

Genetics and Heart Failure in African Americans

Existing evidence indicates that disparity in the development of HF and response to some medications (Rotimi et al., 2013) may partly be due to the influence of genetic polymorphisms present at a slightly higher frequency in certain ethnic groups (McNamara, 2004; McNamara et al., 2009). While it is understood that human populations are similar at the DNA level, insights gleaned from advances in high-throughput genomics have shown that genetic differences between ancestral and ethnic groups may have important evolutionary and medical relevance (Rotimi et al., 2013). Our understanding of such differences will facilitate treatments tailored to specific patients according to their genetic features.

Of the genes previously reported to be associated with risk of HF in AA are regarded as potential candidates for targeted therapy (Sharma et al., 2014). G protein and transthyretin gene (*TTR*) have been the most studied. Polymorphism C→T at nucleotide 825 (C825T) in exon 10 of the β3 subunit of pertussis toxin-sensitive G-type protein is more frequent in individuals of African ancestry. Several epidemiologic studies have reported a significant association of the 825 T allele with increased risk of hypertension; obesity, and LVH (Siffert, 2000). Additionally, C825T polymorphism has been proposed as a genetic marker for arteriosclerosis (Hanon et al., 2002) and clinical trials are being conducted. In the AA HF trial (A-HeFT), McNamara and colleagues found that the effectiveness of fixed-dose hydralazine/isosorbide dinitrate was enhanced in AA patients who were *GNB3* 825 T homozygotes compared with 825C allele carriers (McNamara et al., 2014).

The V122I variant in the *TTR* gene has been implicated in cardiac amyloidosis and occurs in 3%–4% of AA. In one autopsy study, more than one-fourth of AA with cardiac amyloidosis were heterozygous for the V122I allele (Buxbaum and Ruberg, 2017). Quarta et al. (2015) reported that the V122I transthyretin variant was associated with increased risk of incident HF and worse systolic and diastolic function among AA in the ARIC study followed over two decades.

In recent genome-wide association studies (GWAS), loci associated with incident HF in AA have been identified. Some of these loci have been replicated while others have not, so their biological significance is less clear. In the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium, Smith et al. (2010) discovered that the *LRIG3* gene (encoding leucine-rich repeats and immunoglobulin-like domains) was associated with increased risk of incident HF in individuals of African ancestry only. Functional work on this gene has yet to be conducted. Other GWAS studies involving B-type natriuretic peptide (BNP), a blood biomarker that serves as a diagnostic tool for HF, led to the identification of *KLKB1* and *NPPB* genes in the general population of AA (Musani et al., 2015). In a separate GWAS, Fox et al. (2013) found in a meta-analysis of four AA cohorts, common genetic variants associated with cardiac structure and function.

Most of these genetic findings for HF in AA are preliminary and further investigation is warranted. Indeed, a move from GWAS (or hypothesis generating studies) to prospective validation may be necessary, particularly for trials such as A-HeFT. While further exploration to identify the contribution of genetics to HF is warranted, as of now, the existing literature suggests that genetic influences are limited relative to traditional and sociocultural risk factors known to contribute substantially impact ethnic differences in disease occurrence.

Relation of Biomarkers to Heart Failure in African Americans

Multiple blood biomarkers have shown to be independently associated with cardiac remodeling and incident CVD (including CHF) in AA substantiating the theory that multiple biological pathways influence LV structure and outcomes in this group. In the JHS, investigators found that there was a significant and positive relation of aldosterone–renin ratio (ARR), BNP pathway, and homeostasis model assessment of insulin resistance (HOMAIR) to LV geometry (unpublished data). In a recent study, MESA investigators found that elevated NT-proBNP and high sensitivity troponin T were predictive of incident HFPEF with no significant interaction between race/ethnicity and these risk factors (Silverman et al., 2016). In other analysis in the JHS, BNP was the only biomarker retained in a prediction model for all CVD after incorporating standard risk factors, biomarkers, and subclinical disease markers in the risk prediction model. BNP relation to all CVD incidence was thought to be most likely due to the use of CHF in the definition of all CVD outcomes (Fox et al., 2016). The natriuretic peptide pathway is involved in a number of aspects of CVD physiology that accounts for its strong predictive value for CHF and CVD. These include (1) salt and fluid balance, (2) cardiac and vascular structure and function, (3) BP control, and (4) inhibition of the renin–angiotensin–aldosterone system (Fox et al., 2016; Martinez-Rumayor et al., 2008; Woodard and Rosado, 2008).

Risk Models for Heart Failure in African Americans

Several risk models have been developed and later validated in minority populations for CHD and stroke by Framingham Heart Study, ARIC Study, and CHS investigators. These models appear to perform well in the AA population (Lumley et al., 2002; Wolf et al., 1991; Wilson et al., 1998; Chambless et al., 2003, 2004). Health ABC Study (Butler et al., 2008) and the ARIC study (Agarwal et al., 2012) investigators have also developed CHF prediction scores using common clinical variables to predict incident HF risk. The ARIC study model included middle-aged white adults and AA adults, whereas in the Health ABC Study, the cohort tested was elderly, and approximately 40% of participants were AA (Fox et al., 2016). In a more recent study conducted in JHS, investigators developed multiple models in a community-based cohort of AA to predict general CVD risk (defined as CHF in combination with CHD, stroke, angina, and claudication) incorporating standard risk factors, blood biomarkers, and subclinical disease markers.

These models were validated in two external AA groups. Investigators found that biomarkers added minimal incremental improvement in discrimination beyond a model with only standard risk factors (Fox et al., 2016). Adding subclinical measures to traditional risk factors (both ankle–brachial index alone and with echo LV ejection fraction and LVM), significantly enhanced the predictive usefulness of the risk model (Fox et al., 2016). In the same investigation, the Framingham Risk Score and American College of Cardiology/American Heart Association risk algorithms worked well for all CVD in the Jackson cohort compared to models generated within the Jackson cohort utilizing all three tiers suggesting that there may not be a need for a unique risk calculator in AA (Fox et al., 2016).

Heart Failure in Hispanics/Latinos

The US Hispanic/Latino population is the single largest and one of the fastest growing ethnic minorities in the United States (US) (The United States Census Bureau, n.d.). The US Census Bureau projects that this subgroup of the population will more than double, from 53.3 million in 2012 to 128.8 million in 2060. Consequently, by the end of that period, nearly one in three US residents would be Hispanic, up from about one in six today. Further, while the non-Hispanic Whites population over the age of 65 years is expected to grow by 83% between 2000 and 2030, Hispanics of this same age group are projected to grow by 328% (The United States Census Bureau, n.d.). Thus, the public health impact of HF among the Hispanic population may be magnified. A sizeable proportion of this population has major CVD risk factors (Rodriguez et al., 2014). Compared to non-Hispanic Whites, Hispanics/Latinos have a higher prevalence of uncontrolled blood pressure, obesity, diabetes, and dyslipidemia (Daviglus et al., 2012; Dominguez et al., 2015).

With respect to HF, few studies that have specifically examined Hispanics/Latinos in the US found that Hispanics/Latinos have a higher incidence of HF compared to non-Hispanic Whites and that Hispanics/Latinos present with HF are younger with more comorbidities and a lower LV ejection fraction (Bahrami et al., 2008; Rodriguez et al., 2014; Vivo et al., 2009). In the Echocardiographic Study of Hispanic/Latinos (ECHO-SOL), an echocardiographic ancillary study of The Hispanic Community Health Study/Study of Latinos (HCHS/SOL), about half of the 1818 middle-aged and older Hispanic/Latinos adults had cardiac dysfunction, yet fewer than 1 in 20 participants knew they had a problem (Mehta et al., 2016). In the ECHO-SOL community-based sample, half were obese; half had high blood pressure; two-third had diabetes; two-third reported low levels of physical activity; and a fifth were current smokers (Mehta et al., 2016). Every unit increase in waist–hip ratio was significantly associated with 0.27% lower ejection fraction (Allison et al., 2015). Prediabetes and diabetes were associated with significantly reduced ejection fraction and a 36%–90% higher odds for diastolic dysfunction among the same community-based cohort Hispanic/Latinos (Demmer et al., 2016).

In MESA, Hispanics/Latinos were at high risk of developing HF, higher than non-Hispanic Whites and Chinese Americans and second only to AA who had the highest risk. Hispanics with HF are more likely to be underinsured and target doses of the angiotensin-converting enzyme inhibitor/angiotensin receptor blocker were reached in a lower percentage of Hispanic persons when compared with AA persons (Hebert et al., 2010). Hispanics/Latinos are least likely to have an assessment of LV ejection fraction and to be discharged from angiotensin-converting enzyme inhibitor treatment compared with non-Hispanic white patients and AA (Cohen et al., 2010). In addition, Hispanics/Latinos have higher rates of HF hospitalizations (Brown et al., 2005) and readmissions for HF (Alexander et al., 1999) but have lower in-hospital (Brown et al., 2005) and short-term mortality (Alexander et al., 1999). Rather than an indicator of advanced disease, HF hospitalization may be a marker of the first encounter with the health care system due to poor access to outpatient care among Hispanics/Latinos. Furthermore, community-based studies have found that Hispanics/Latinos have worse diastolic function and higher LV stiffness compared to estimates in non-Hispanic Whites (Mehta et al., 2016; Russo et al., 2010).

Unfortunately, most of these data, have been drawn from selective populations or subgroup analyses and may not accurately represent the clinical characteristics and outcomes of Hispanics/Latinos with HF. Hispanics/Latinos in the US represent a relatively young demographic such that the future public health burden of clinical HF as this population ages is potentially currently being underestimated. There is a need for further research with an adequate representation of Hispanic/Latino patients in HF registries and trials.

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Heart Failure in the Elderly

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Abbreviations

BMI	Body mass index
BNP	B Natriuretic peptide
CAD	Coronary artery disease
COPD	Chronic obstructive pulmonary disease
CRT	Cardiac resynchronization therapy
CRTD	Cardiac resynchronization with defibrillator
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
ICD	Implantable cardioverter defibrillators
LV	Left ventricular
LVAD	Left ventricular assist device
LVEF	Left ventricular ejection fraction
MI	Myocardial infarction
TGF-β	Transforming growth factor *beta*

Introduction

The prevalence of Heart failure (HF) in United States is 2.2%, and it is expected to rise to almost 3% by 2030 (Writing Group Members et al., 2016; Heidenreich et al., 2013). HF syndrome is diagnosed in 0.7% of persons 45–55 years of age but may be 8.5% in those >70 years (Redfield et al., 2003). Between 1979 and 2004 hospitalizations in United States, HF-related diagnoses tripled and currently HF is considered the most common reason for hospitalization in individuals >65 years of age in high-income countries. Practically, 80% of all HF patients are >65 years of age with an annual likelihood of hospital admission of 35% which may ascend to 75% if 5 comorbidities are present (Braunwald, 2015; Fang et al., 2008). In high-income countries, evidence-based treatments have improved the survival of HF patients, but at the same time national health systems have to spent 2% of the entire health budget for the management of HF (Ziaeian and Fonarow, 2016).

Concurrent data from USA and European registries show that overall age-adjusted prevalence of HF tends to stabilize ore even decrease in women, nevertheless the number of HF patients is paradoxically increasing worldwide (Vos et al., 2012; Ziaeian and Fonarow, 2016; Zhao et al., 2015). A global improvement in standards of nutrition, hygiene, housing availability, medical care, and general living conditions has dramatically increased life expectancy in previously considered low or medium income countries. Meanwhile a western-like way of life in these populations has increased the perseverance of obesity, hypertension, and diabetes (Braunwald, 2015; Zhao et al., 2015). As a result, similarly to what was observed in westernized societies, the previous decays, the cause of death is shifting from infection, inflammatory, and nutrition-related diseases observed in younger ages to cardiovascular

degenerating conditions diagnosed in elderly populations. HF syndrome is currently a global pandemic of the elderly, placing a heavy burden in healthcare providers and affecting the long-term planning of national health systems (Ziaeian and Fonarow, 2016; Vos et al., 2012; Zhao et al., 2015).

Age-Related Changes of the Myocardium and Aorta

Cellular Level

In the elderly population HF syndrome may be regarded as a downstream evolution of the "normal" deterioration of the myocardium. Collagen deposition plays a key role in cardiac aging. Fibroblasts are stimulated by multiple signaling hormones and control the secretion of fibronectin collagen and laminin in the extracellular matrix. The content of the extracellular matrix is also regulated by matrix metalloproteinase and their inhibitors (matrix metalloproteinase-2, −7, −9, tissue inhibitor of metalloproteinases-1, −2, −4). (Fig. 1) Individuals without cardiovascular disease show an age-related increase of metalloproteinase paralleling left ventricular (LV) concentric remodeling and diastolic dysfunction (Bonnema et al., 2007). In the Framingham study tissue inhibitor of metalloproteinases-1 levels increased proportionally with age and was associated with increased ventricular dimensions and wall thickness even after adjustment for age (Sundstrom et al., 2004). Of particular interest in the process of myocardial aging is the Transforming growth factor beta (TGF-β) signaling pathway which mediates phenotypic transformation of fibroblasts to myofibroblasts (Dobaczewski et al., 2011). Brooks and Conrad showed that TGF-b (1) heterozygous mutant mice exhibit less age-related myocardial fibrosis than normal mice (Brooks and Conrad, 2000). TGF-β is also activated with high levels of reactive oxygen species which tend to increase in the aging myocardium (Cucoranu et al., 2005). Titin, a giant protein of the sarcomere, can also modulate passive LV stiffness in the elderly population. Indeed, inadequate phosphorylation of titin and altered isoform composition was found in a model of old dogs with diastolic dysfunction when compared to a normal younger group (Hamdani et al., 2013).

The link of systemic inflammation and fibrosis in the elderly population has also been confirmed in many studies. Age is associated with increased markers of inflammation (e.g., Tumor necrosis factor-α, Interleukin-6, Interleukin-18, Monocyte chemoattractant protein-1, Vascular cell adhesion molecule 1) even after correction for cardiovascular disease (Miles et al., 2008). (Fig. 1) Even in the absence of a specific disease, this phenomenon is attributed to accumulation of multiple comorbidities that lead to a constant systemic inflammation state. Inflammation, in turn, imposes oxidative stress in the microvascular endothelium and leads to a pathophysiologic cascade: reduction of bioavailability of nitric oxide of the inflamed endothelium, decrease in cyclic guanosine monophosphate levels, decrease in protein kinase G activity, acceleration of the inflammation process via the subendothelial migration of leukocytes, hypophosphorylation of titin, increase of collagen deposition and ultimately fibrosis, and hypertrophy via the accelerated action of endothelin 1 and angiotensin II (Paulus and Tschope, 2013) (Fig. 1).

Mitochondrial DNA deletions and mitochondrial protein oxidation also are linked with cardiac aging. Studies have shown that homozygous mitochondrial polymerase γ (Polgm/m) knock-in mice have signs of premature aging such as cardiac enlargement

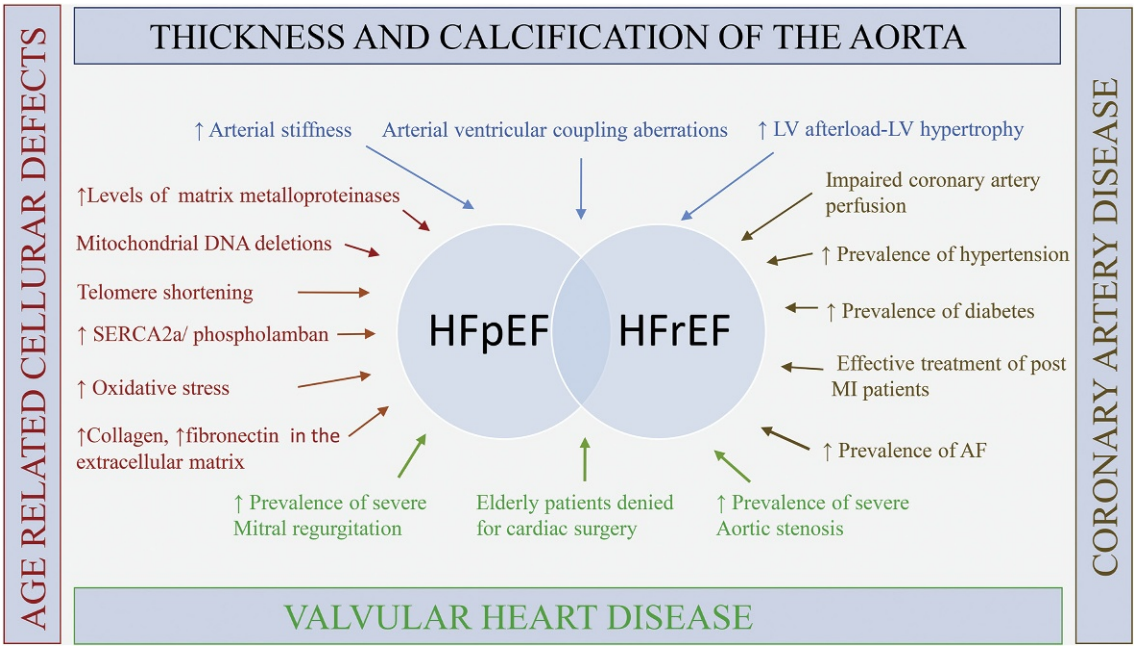

Fig. 1 Multiple parameters are implicated in the pathophysiology of heart failure in the elderly.

(Trifunovic et al., 2004). Additionally, other animal models demonstrated that the natural process of macroautophagy which disposes of dysfunctional mitochondria may decline with age (Taneike et al., 2010). Telomere shortening may further impair mitochondrial function and induce apoptosis (**Fig. 1**).

Various other pathophysiological mechanisms that are implicated in this "normal" aging course have only recently been appraised. Amyloid is a rare underestimated infiltrative disease that causes of HF mainly in older men (70 years) and is associated with a poor 5-year survival of 35.7% (Connors et al., 2016). It is estimated that 25% of the population aged >80 years have amyloid deposits of the cardiac muscle. Contemporary data show that the prevalence of wild-type familial transthyretin (TTR) amyloidosis is 13% among HF patients ≥60 years diagnosed with HF with preserved ejection fraction (HFpEF) (Gonzalez-Lopez et al., 2015).

The role of miRNA in the process of aging is also noteworthy. It was demonstrated that genetic deletion of miR-34a that regulate the expression of numerous gene pathways may ameliorate age-related myocardial apoptosis (Boon et al., 2013). Additionally, the role of protein SMP30 has been recently investigated. There is evidence that levels of SMP30 may fall by 40% with age in animal models, while SMP30 knockout mice have severe cardiac remodeling and dysfunction (Misaka et al., 2013).

Cardiac function and structure

The abovementioned age-related changes of fibrosis and endothelial dysfunction promote cardiac diastolic function through the development of a stiff myocyte. Meanwhile, a different age-related pathophysiological mechanism of impaired active diastolic relaxation has been proposed. In the normal cardiac cell phosphorylation of phospholamban increases the affinity of sarcoplasmic/endoplasmic reticulum Ca2+ ATPase (SERCA) for Ca, augmenting SERCA pump activity and consequently increasing Ca reuptake in the myocardial cell at the phase of active diastolic relaxation (**Fig. 1**). However, progressive age is associated with a decrease of SERCA levels and an increase of SERCA2a/phospholamban ratio (Cain et al., 1998). In support of this concept, animal models have shown that improvement of SERCA2a/phospholamban ratio through the gene transfer of the SERCA2a protein may restore cardiac diastolic function (Schmidt et al., 2000). Furthermore, age-induced elevated oxidative stress may directly damage the SERCA channel. In addition to that, deterioration of responsiveness to beta adrenergic stimulation may also lead to diminished phosporilization of phospholamban (Lancel et al., 2010). Alteration in Ca handling may also prolong the action potential and predispose to arrhythmias.

Undoubtedly, abnormal diastolic function is the hallmark of the aging myocardium. Diastolic dysfunction increases proportionally with age and may be prevalent in 50% of patents >80 years (Dannenberg et al., 1989). Older individuals exhibit an increase in the isovolumic relaxation time and a lower proportion of total diastolic filling. The atrial contraction contributes significantly to LV filling which, given time, results in atrial enlargement. As a result, a decrease in mitral E/A ratio is observed. In fact, elderly normal individuals exhibit similar LV filling patterns with younger patients (40–60 years) with mild diastolic dysfunction (Dalen et al., 2010).

Although diastolic dysfunction in elderly HF group is well documented, there are paradoxically no obvious significant age-related changes in LV structure. In registry of 765 autopsies the mean values for LV wall dimensions did not change significantly with age, with the exception of ventricular septum which was marked thicker in the elderly (Kitzman et al., 1988). According to Laplace's law, isolated septal hypertrophy in the elderly may result from chronic wall tensions developed in this area owing to the large septal diameter of the LV. In line with these results cardiac magnetic resonance studies of 336 healthy adults even showed a small reduction of LV mass with age (Hees et al., 2002). Also, there was no significant increase in LV mass with age in the Framingham Heart study among nonobese, nonhypertensive volunteers without a history of cardiovascular disease (Dannenberg et al., 1989).

Nonetheless, the overall architecture of cardiac muscle in the elderly may be ultimately affected. Healthy volunteers show a decline in the longitudinal strain and a correspondent raise in the circumferential strain in order to compensate for loss of systolic function. Radial strain and LV ejection fraction (LVEF) on the other hand tend to remain steady with progressive age (Sun et al., 2013).

Changes in LV systolic and diastolic function are more obvious in the healthy elderly during exercise and are associated with a reduced rise in stoke volume. A relationship of abnormal early relaxation and LV filling pattern with age was noticed in early studies. Older age is also related to impaired autonomic modulation and chronotropic incompetence that prevents the heart from adapting to demands during exercise, that is increase in heart rate, myocardial contractility and diastolic relaxation. Indeed, the maximum volume of oxygen during exercise declines with advancing age by 50% from age 20 to 80 years (Fleg et al., 2005).

HFpEF Phenotype

The HPpEF phenotype primarily affects the elderly population. In community-based studies HFpEF is reported only in 1% of women and 0% of men 25–49 years of age and rises to 8%–10% in women and 4%–6% in men aged ≥80 years (Brouwers et al., 2013). Although in the past decays HFpEF was considered as a transitory stage to HF with reduced ejection fraction (HFrEF) nowadays it is regarded as a distinct HF disease with distinct LV remodeling, cellular and interstitial characteristics. HFpEF patients exhibit increased myocardial total collagen and collagen I expression, which relates to the severity of diastolic dysfunction (Kasner et al., 2011). Dyspneic symptoms are attributed to inherent age-related changes such as LV diastolic dysfunction, atrial dysfunction, vascular stiffening, and endothelial dysfunction combined with proinflammatory coexisting conditions and noncardiovascular comorbidities. Symptomatic elderly HFpEF patients as compared with elderly healthy volunteers with preserved LVEF exhibit impaired echocardiography markers of diastolic and systolic function (mitral annular velocities and radial and longitudinal systolic strain), at rest which also fail to raise normally during exercise (Tan et al., 2009). Although concomitant comorbidities may

accelerate LV diastolic dysfunction, to date it is not clear what exactly shifts diastolic dysfunction from an asymptomatic state to the development of dyspneic symptoms. Specific echocardiography markers including E/e ratio < 14, Valsalva maneuver decrease in E/A by 50%, pulmonary vein Ar duration > mitral A duration by 30 msec, are indicative of significant diastolic dysfunction independent of age (Yamakado et al., 1997; Nagueh et al., 2016).

HFpEF patients have equally poor long-term mortality as compared with their HFrEF counterparts which age-related comorbidities can partly only explain (Mohammed et al., 2012). The definition of normal or near-normal LVEF among elderly patients presenting with HF symptoms is of paramount importance because the classical medical HFrEF therapy does not have any effect on clinical symptoms or long-term mortality. Patients with LVEF <50% but ≥40% are considered a distinct group with intermediate age values (Farmakis et al., 2016). There is currently an area of uncertainty regarding treatment options in HF patients with intermediate LVEF values.

Arterial Stiffness

Cardiac afterload is increased with aging vasculature. In young normal individuals the proximal aorta is rich in elastin while distal branches are progressively stiffer (**Fig. 1**). The elastic proximal aorta expands to accommodate stoke volume and acts as an aortic reservoir in order to ensure blood flow in diastole. Pulse wave reflections travel backward toward the heart during late systole and early diastole, increasing favorably diastolic pressure and therefore improving coronary perfusion. Aging dramatically increases intima-medial layer thickness calcification and endothelial function of large vessels (Elliott and McGrath, 1994). This is considered a result of chronic repeated local injuries and the ensuing inflammatory process. The formation of irreversible advanced glycation end products is also implicated in this course. The whole process is accelerated in the presence of risk factors including hypertension, chronic kidney disease, and diabetes (Paulus and Tschope, 2013). As a result, the aorta becomes more stiff leading to a pathophysiological cascade. In the elderly the reflected waves reach the ascending aorta much earlier in late systole. There is consequently an increase in systolic pressure and a decrease in diastolic pressure, which in turn impairs coronary perfusion and predisposes to ischemia. Moreover, aortic stiffness imposes an increased afterload which absorbs the mechanical energy developed by LV and accelerates LV hypertrophy. Augmentation index and pulse wave velocity are extensively studied markers of aortic stiffness which increase significantly with advancing age and seem to be independently related to long-term cardiovascular outcome (Mitchell et al., 2004; Najjar et al., 2008).

Coronary Artery Disease and Associated Risk Factors

The prevalence of risk factors predisposing to LV diastolic dysfunction and coronary artery disease (CAD) rises with age paralleling the increase in HF incidence: HF risk rises by twofold in patients with systolic blood pressure >160 mm Hg or diastolic blood pressure >100 mm Hg (Lloyd-Jones et al., 2002). As well as increasing the risk for CAD, hypertension promotes HF syndrome though elevated oxidative stress, microvascular inflammation, and myocardial afterload excess (Paulus and Tschope, 2013). In industrialized countries, elderly patients are also more likely to suffer from diabetes with a peak at age 60–74 (17.6%) and has a slightly lower prevalence (14.9%) in persons >75 years (Sloan et al., 2008). Diabetes may predispose to HF though various pathophysiological mechanisms including microvasculopathy, myocardial hypertrophy, fibrosis, and consequent LV systolic dysfunction, and also through atherosclerotic and thrombotic mechanisms (**Fig. 1**).

The effective invasive and medical treatment of myocardial infarction (MI) has also decreased the mortality of post MI patients and plays a central role in the occurrence of HF in the elderly. In a large cohort of patients >65 years of age hospitalized for MI in Canada there was a 28% reduction mortality between 1994 and 2000. Almost 2/3 of included patients developed HF during this follow-up (Ezekowitz et al., 2009). In the Framingham Heart Study the HF incidence following diagnosis of MI increased from 19.6% between 1970 and 1979 to 21.5% between 1980 and 1989 and to 33.9% between 1990 and 1999 (Velagaleti et al., 2008). In another registry of 2596 post MI patients, survival rates of post MI-related HF improved significantly between 1990 and 2010 (Gerber et al., 2016).

At the same time a different dynamic is observed: awareness of hypertension tents to improve overtime and so does the appropriate medical control of hypertension (Yoon et al., 2010). Also statin use is becoming more common and guideline-recommended LDL targets are more regularly achieved (Mann et al., 2008). Smoking habit has also shown a significant global reduction from 41.2% in men and 10.6% in women in 1980 to 31.1% and 6.2% in 2012 respectively, with a striper decline in the last decay (Ng et al., 2014). This trend may finally prove to delay onset of CAD or even ameliorate the burden of HF in Westernized countries.

Heart Valve Disease

Degenerative valvular heart disease is another underestimated cause of HF in high-income countries. Data from the Framingham study suggest that 7%–8% of HF can be attributed to heart valve disease (Kannel, 2000). Autopsy studies have shown that subendothelial thickening of the aortic valve may begin in early adulthood; however, significant aortic calcification, and loss of extensibility of aortic valve, is a disease of the elderly (Kuusisto et al., 2005). In The Framingham study severe aortic stenosis may rise from 2% in individuals between 70 and 80 years to 3%–9% in those >80 years of age (Singh et al., 1999). Additionally, more than moderate mitral regurgitation is present in 11.2% of men over 80 years of age while it is rarely documented in men or women

younger than 50 years. Similarly, degenerative or rheumatic aortic regurgitation may be present in up to 2.3% of individuals >70 years old but is hardly ever documented in ages <50 years (Singh et al., 1999). Moderate or more tricuspid regurgitation was also found in 1.5% of men and 5.6% of women 70–83 years whereas it was rare in younger age groups. Although in the past tricuspid valvulopathy was considered benign, it has recently shown to be relate to worst survival even in patients with normal ventricular function and without pulmonary hypertension. In a 10-year follow-up study of 353 elderly patients (mean age 70 years), free of structural valve disease or significant comorbidities, severe tricuspid regurgitation conferred a higher mortality risk independently of age (Topilsky et al., 2014).

It should be also pointed out that in clinical practice older age is a common reason why otherwise eligible elderly patients are denied for cardiac surgery (Iung et al., 2005). This in turn may increase further the burden of HF in the elderly population (**Fig. 1**).

Clinical profile

The clinical profile of elderly HF patients is drawn from the comparison of age groups from national and international registries and also from negative medical and interventional studies. Different age cut-offs have been used to define the elderly among various studies. Ages <64 old are commonly considered "young," ages between 65 and 74 "middle old," ages 75–84 "old" and ages >85 years are typically considered "oldest old." In the Acute decompensated heart failure syndromes (ATTEND) registry 26.5% of patents <65 years suffered from dilated cardiomyopathy as opposed to 3.3% in the >85-year cohort (Mizuno et al., 2016). Older patients as opposed to younger patients are also more likely to have a normal LVFE upon presentation and higher rates of valvular disease (Mogensen et al., 2011). They are also more commonly women, hypertensive, nondiabetic with a higher prevalence of atrial fibrillation (Herrero-Puente et al., 2012; Komajda et al., 2009; Metra et al., 2015a,b; Fonarow et al., 2009; Mahjoub et al., 2008; Pulignano et al., 2002; Mizuno et al., 2016) (**Table 1**). The prevalence of CAD and atrial fibrillation shows a relative decline after 80 years of age. Additionally, the proportion of diabetes and smoking may also be considerably lower in the "oldest old" subgroups. On the whole, very elderly HFpEF patients tend to show a marked reduction cardiac comorbidity as compared with their younger counterparts. This is probably a result of the highest mortality associated with these risk factors but may also be attributed to the ill-defined medical history and diagnostic defects in the elderly (Mogensen et al., 2011; Stein et al., 2012). Interestingly, atrial fibrillation is less prevalent in very elderly patients with HFrEF but is still prominent in the "old" HFpEF subgroup. Renal dysfunction is also pronounced in the HFrEF "oldest old" group but to a much lesser extent than in the HFpEF "oldest old" group (Stein et al., 2012).

It has been shown that 40% of patients >65 years hospitalized with HF have ≥5 noncardiac comorbidities (Braunstein et al., 2003). Noncardiac comorbidities including stoke, chronic pulmonary disease, and peripheral vascular disease may show a decline >85 years of age although there are discrepancies between studies. In contrast, anemia, myxedema, dementia rheumatic disease, and cancer are more pronounced in the "oldest old" cohorts (Mogensen et al., 2011; Stein et al., 2012; Metra et al., 2015b; Fonarow et al., 2009; Komajda et al., 2009).

Table 1 Clinical profile of elderly as opposed to younger heart failure patients in high-income countries

	(OPTIMIZE-HF)		*Euro heart failure survey II*		*ATTEND*	
Region	259 hospitals/United States		133 hospitals/30 European countries		53 hospitals/Japan	
Date	2003–4		2004–5		2007–11	
Patients *N*	48,612		3577		4791	
Age cut-off	<75	≥75	<80	≥80	<80	≥80
%	47	53	79	21	64	36
Male	56	41[a]	66	44[a]	66	44[b]
BMI or weight	90.2 k	71.2 k[a]	26.9	26[a]	24	21.7[b]
AF	22	39[a]	36	48[a]	34	37[b]
Hypertension	70	72[a]	61	67[a]	64	77[b]
Diabetes	27	23[a]	34	29[a]	39	25[b]
COPD	7	4[a]	19	22[a]	10	16[b]
Creatinine	1.6	1.9	1.2	1.3	1.5	1.4[b]
HFpEF	41	55[a]	28	39[a]	39	58[b]
LVEF cut-off	≥40%		≥45%		40%	
ACE/ARBS	84	79[a]	81	76[a]	45	51[b]
Beta-blockers	85	80[a]	63	53[a]	37	31[b]
MRA	24	17[a]	50	38[a]	21	19[b]
In hospital mortality	2.4	4.9	5.6	10.7[a]	0.5	3[b]

[a]Older vs. younger group.

[b]Comparisons between <65, 65–74, 75–84, ≥85 years of age groups.

ACE, Angiotensin converting enzyme inhibitor; *AF*, Atrial fibrillation; *ARBs*, Angiotensin II receptor blockers; *BMI*, body mass index (kg/m^2); *COPD*, chronic obstructive pulmonary disease; *HFpEF*, heart failure with preserved ejection fraction; *LVEF*, left ventricular ejection fraction; *MRA*, mineralocorticoid receptor antagonist.

It should be emphasized that in multinational registries patients presenting to outpatients' clinics tend to be older (77–81 years) as compared to those presenting to hospital emergency settings (70–73 years) (Mebazaa et al., 2015). Accordingly, the profile of HF patients may differ in keeping with the studied population (hospital vs. outpatient setting, acute HF vs. chronic HF patients, HFpEF vs. HFrEF patients).

Initial Diagnosis and Management

Common precipitating reasons for exacerbation of HF in the elderly include infection (40%) ischemia (17%), excessive water and salt intake (9.2%), hypertension, tachyarrhythmia, noncompliance with medication, and intravenous fluid intake (Taylor et al., 2012). Elderly HF patients are most likely to be admitted to the hospital after an initial evaluation in the emergency department; however this decision is usually influenced by multiple cumulative conditions that may not be directly related to HF (Ezekowitz et al., 2008). There is commonly an overlap of HF signs and symptoms with other common diseases in the elderly. For example, dyspnea may be commonly related to pneumonia or chronic obstructive pulmonary disease (COPD), asthma, and anemia. Also peripheral edema may be a result of chronic venous insufficiency, lymphedema. Moreover, cognitive impairment, confusion, loss of appetite, or delirium may be attributed to concomitant infection or acute neurological conditions. Elderly patients may also deliberately lower the levels of their daily physical activity and mask HF symptoms or, on the other hand, they may attribute dyspnea to normal aging.

Usually, a more dramatic initial clinical picture with tachypnea and lower oxygen saturation is reported in the elderly (Herrero-Puente et al., 2012; Metra et al., 2015a,b). Rales and elevated jugular venous pressure on initial presentation may be more common in elderly versus younger patients (Fonarow et al., 2009). However frequent evaluated signs and symptoms including orthopnea, paroxysmal nocturnal dyspnea, and jugular venous pressure may not be sufficiently sensitive and specific in the elderly (Ekundayo et al., 2009) (**Table 2**). Also, rales may not be present on auscultation and classic signs of pulmonary congestion may not be found in chest radiography (Collins et al., 2006). Nonspecific age-related changes are also frequent in chest radiography (e.g., emphysema) and the cardiothoracic ratio may be difficult to assess.

Atypical presentation in emergency ward entails a comprehensive biomarker workup. Laboratory findings namely creatinine and troponin are commonly higher in elderly patients when compared to younger AHF patient groups (Mahjoub et al., 2008; Metra et al., 2015a; Gustafsson et al., 2004; Herrero-Puente et al., 2012; Komajda et al., 2009). In lack of specific electrocardiography findings and symptoms, minor troponin elevation may reflect impaired renal function, elevated cardiac pressures, impaired oxygen

Table 2 Profile and practical considerations in elderly acute heart failure patients

Demographics	Women (60%), Hypertensive (40%), Nonobese, Nondiabetic (80%), Atrial fibrillation (40%), Coronary artery disease (45%)	Anticipate hypertensive HF and "vascular-type" HF, Anticipate de novo heart failure on the acute setting
Clinical presentation	↑Rales, ↑Jugular venous pressure, ↓Oxygen saturation, ↓Peripheral edema, Infection, ↑bleeding risk	Exclude alternative diagnosis, e.g., COPD pneumonia, anemia, cognitive dysfunction Use BNP and/or NTproBNP to confirm diagnosis, consider measuring, procalcitonin Acknowledge increased bleeding risk with anticoagulation therapy (and NOACs)
Laboratory findings	↑Creatinine, ↑Troponin, ↑Natriuretic Peptides	Calculate glomerular filtration rate Monitor electrolytes (hyperkalemia) Consider ST2 measurement for prognosis
Echocardiography	Preserved systolic function, Increased left atrial dimensions	Evaluate less age-dependent variables including E/e′ >14, Valsalva maneuver decrease E/A $\geq 50\%$, vein Ar duration $>$ mitral A duration by 30 msec. Consider using contrast agents
Comorbidities	Stoke (20%), Peripheral vascular disease (15%), Anemia (20%), Frailty (50%)	Consider HF diagnosis in internal medicine wards, ICU, and geriatric departments—evaluate by a specialist cardiologist Reduce polypharmacy—Consider stopping medication without effect in quality of life (e.g., statins) Screen for depression Evaluate frailty Acknowledge highest risk of surgery than predicted
Highest risk	Very elderly, Confusion, Shock, ↑Troponin, Infection, Diabetes, Renal dysfunction, Frail	Discuss do not resuscitate order Consider palliative care and hospice Talk openly about end of life matters
Medical Treatment	Lower doses of ACE/ARBS beta-blockers and MRAs	Be alert for digoxin toxicity signs Dissemination of HF treatment guidelines in ICU/internal medicine doctors
Device-Based Therapy	Questionable efficacy of ICD	Make individualized decisions for ICD and left ventricular assist devices

ACE, Angiotensin converting enzyme inhibitor; *ARBs*, Angiotensin II receptor blockers; *BNP*, Brain natriuretic peptide; *COPD*, chronic obstructive pulmonary disease; *MRA*, mineralocorticoid receptor antagonist.

supply endothelial dysfunction, and myocardial apoptosis. Natriuretic peptide levels are also commonly reported higher in acute HF elderly patients and the associated "gray zone" values for diagnosis of HF in the emergency department may have to be modified (Metra et al., 2015a; Fonarow et al., 2009) (**Table 2**). Particularly for B Natriuretic peptide (BNP) the exclusion cut-off for HF diagnosis may be adjusted to 250 pg/mL and the confirmatory cut-off to 600 pg/mL. For NTproBNP the gray zone values may be 1750–6000 pg/ML. Soluble ST2 values are less age dependent but their diagnostic efficiency for HF is limited. However, a value >35 ng/mL is clinically relevant and provides independent critical prognostic information (Parikh et al., 2016). Measurement of procalcitonin may also aid in the triage of elderly dyspnoeic elderly patients. In a recent study of patients presenting with dyspnea in the emergency department a high (≥0.10 ng/mL) versus low (<10 ng/mL) value of procalcitonin was more common in elderly patents (72.7 vs. 62.7 years, respectively) and could adequately discriminate pneumonia from heart failure even in the subgroup with the lowest clinical probability of microbial infection. (Alba et al., 2016) (**Table 2**).

Noninvasive ventilation in the emergency department is less frequent in the elderly (Metra et al., 2015a,b; Herrero-Puente et al., 2012). Studies also report a lower rate of cardiac catheterization interventions in elderly acute HF patients (Komajda et al., 2009; Pulignano et al., 2002; Biagi et al., 2011). Elderly as opposed to younger acute HF patients are also considerably less likely to undergo an echocardiogram during hospitalization (Herrero-Puente et al., 2012; Komajda et al., 2009).

Echocardiography workup in the elderly is additionally limited owing to poor acoustic window; therefore contrast agents may be needed for the definition of the endocardial LV border and the diagnosis of apical thrombus. Computed tomography coronary angiography may not be an ideal choice for the elderly with chest pain owing to the severe coronary artery calcification and associated blooming artifacts (de Agustin et al., 2013).

Regarding their hospital stay, elderly HF patients are less likely to be hospitalized in the cardiac units and are commonly referred to internal medicine and geriatric wards (Komajda et al., 2009; Metra et al., 2015b; Mahjoub et al., 2008). Patients admitted in internal medicine departments are less likely to be treated with evidence-based medication and undergo fewer invasive and noninvasive procedures (Di Lenarda et al., 2003; Biagi et al., 2011).

Outcomes and Prognosis

Hospital length of stay usually depends on country-specific policies and economic factors and data regarding the elderly HF population are diverge. For example, in the Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure (OPTIMIZE HF) registry in United States there was no significant difference in the length of hospitalization between elderly (≥75 years) and younger (<75 years) patients (5.6 vs. 5.8 days, respectively) (Fonarow et al., 2009). In contrast, in the Value of Endothelin Receptor Inhibition with Tezosentan in Acute heart failure Study (VERITAS) multinational trial elderly patients ≥72 years had a mean 1.15 longer in hospital stay (Metra et al., 2015a).

Progressing age corresponds within hospital and long-term mortality in all registries and studies. Older (≥75 years) as compared to younger HF patients have a twofold increase in hospital mortality (4.9% vs. 2.4%) (Fonarow et al., 2010) (**Table 1**). Accordingly, the Euro Heart Failure Survey I reported 13% inhospitable mortality in participants ≥80 years versus 5% in patients <80 years (Komajda et al., 2007). Accumulating data from well-designed trials also show a median survival of 20 months for ≥85 years AHF patens versus 50 months for those <85 years (Mogensen et al., 2011). After a long 6-year follow-up of the prophylaxis for thromboembolism in critical care trial (PROTECT) there was a 17% increase of mortality per 5 year increase in age. In all studies age itself confers a significantly higher mortality and morbidity risk both in the older and younger AHF subgroups.

The mortality of elderly HF patients is largely driven by concomitant cardiac and noncardiac comorbidities. Diabetes, tricuspid regurgitation, and renal dysfunction are associated with a poor survival in all HF patients but may confer a worse impact in the younger groups (Mogensen et al., 2011; Stein et al., 2012; Metra et al., 2015a). On the contrary atrial fibrillation, COPD, and mitral regurgitation are more elderly-specific prognosticators. Another interesting finding is that although elderly patients tend to have a lower body mass index (BMI), the mortality benefit of a higher BMI is confined to patients >75 years (Shah et al., 2014).

Many age-specific differences can be explained by the predominance of the "vascular-type," HF in the elderly where, primarily, severe diastolic dysfunction aortic stiffness and noncardiac comorbidities affect outcome. In younger HF patients high systolic blood pressure on evaluation is related with a favorable outcome; nonetheless this effect is attenuated in the elderly. Anemia may have a better prognostic impact for younger patients, in whom it probably indicates a state of higher inflammation and fluid retention (Metra et al., 2015a; van der Meer et al., 2013; Komajda et al., 2009). Natriuretic peptides and troponin elevation are predictors of morality in both age groups; however albumin levels are determinants of mortality mostly in the elderly (Metra et al., 2015a).

Self-care problems and confinement to bed may also account for the high mortality rates in the elderly (Komajda et al., 2009). Poor health literacy, that is the poor capacity to read and understand basic health vocabulary and medical services (including follow-up appointments, medication use, etc.), is another suggested explanation of the ominous prognosis of this frail group (Wu et al., 2016). Another range of less-studied socioeconomic parameters such as ignorance of HF symptoms, housing stability, transportation, distance to hospital, and social support may be responsible for the poor outlook of the elderly in the developing world (Hawkins et al., 2012).

Medical Treatment

Large-scale registries in United States, Europe, and Japan and a large number of smaller studies unanimously agree that life prolonging agents such as Angiotensin converting enzyme inhibitors, (ACEI) Angiotensin II receptor blockers (ARBs), beta-blockers,

and mineralocorticoid receptor antagonists (MRAs) are commonly under-prescribed and under-dosed in elderly as opposed to younger patients (Mogensen et al., 2011; Stein et al., 2012; Metra et al., 2015b; Fonarow et al., 2009; Komajda et al., 2009) (**Table 1**). This may be explained by the high prevalence of associated side effects of neurohormone blocking agents like orthostatic hypotension bradyarrhythmias and renal dysfunction that are common in the elderly. Another proposed explanation is the ineffective dissemination of HF guidelines among medical specialties treating the HF patient, especially in internal medicine and geriatric wards. However, the principal reason for the under prescription of these agents is the skepticism regarding their effectiveness in the elderly. Metabolism and pharmacokinetics of these medications may vary in very elderly populations with lower body mass, different water content, multiple noncardiac comorbidities (e.g., renal, hepatic dysfunction), polypharmacy, and subsequent drug–drug interactions. In fact, early large-scale randomized trials commonly avoided including very elderly patients. For example, in the "effect of enalapril on survival in patients with reduced LV ejection fractions and congestive heart failure" (SOLVED) trial only 8 patients were older than 80 years of age (1991). Also in the "Randomized ALdactone Evaluation Study" (RALES) trial only 9% of included population was older than 80 years of age (Pitt et al., 1999). Likewise, in the "metoprolol CR/XL randomized intervention trial in congestive heart failure" (MERIT-HF) trial beta-blockers improved the outcome of HF patients but none of the participants was older than 81 years (1999). However, in successive studies metoprolol proved to retain its effectives even in HFrEF patients >75 year of age (Deedwania et al., 2004). Also in the OPTIMIZE HF registry administration of beta-blockers proved clinically effective with regard to mortality and rehospitalization outcome in the elderly (Hernandez et al., 2009). In line with these findings, prescription of neurohormonal blocking agents was associated with a better mid-term outcome in the EHFS II registry (Komajda et al., 2009).

Studies and registries demonstrate that digitalis is also more commonly prescribed in elderly compared to younger HF patients (Komajda et al., 2007; Fonarow et al., 2009; Mogensen et al., 2011). However in the "Digitalis Investigation Group" (DIG) trial digoxin demonstrated only a minor readmission benefit and only 5% of included HF patients were >80 years (1997). Caution is recommended with the use of digoxin because of its narrow therapeutic window. Physicians have to be alert for signs of toxicity including nausea, dizziness, and blurred vision (**Table 2**). Also bradycardia or ventricular tachycardia may occur especially in cases with concomitant renal dysfunction and electrolyte imbalance which are not uncommon in the elderly.

Sacubitril Valsartan is currently considered the biggest breakthrough in the treatment of HFrEF after decays of futile research with other agents. It is recommended for NYHA III–IV patients with LVEF ≤35% that are already treated with ACEI or ARBs. The drug combines the neuroblocking benefits of valsartan with the encashment of the positive effects of the endogenous natriuretic peptide system. In the "Prospective comparison of ARNI with ACEI to Determine Impact on Global Mortality and morbidity in Heart Failure" (PRADIGM-HF) clinical trial the benefits of the agent were consistent among all age groups and there was no more significant hypotension, renal impairment, or hyperkalemia in the elderly. Ongoing studies are testing its potential use for HFpEF patents, where currently medical treatment remains symptomatic and empirical.

Advanced Invasive Therapies in the Elderly

CRT and ICD

Although almost 30% of all Cardiac resynchronization (CRT) with defibrillators (CRT-D) CRTs in Europe are implanted in patients >75 years of age, elderly patients were not commonly included in large randomized CRT trials. A recent analysis from a single center showed that LV remodeling and overall mortality benefits from CRT were consistent among 70, 70–79, and ≥80 age groups at 20-months follow-up (Verbrugge et al., 2013). At a longer term follow-up of 7 years of "The Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy" (MADIT-CRT) trial mortality benefits were consistent in all (<60, 60–74, and ≥75 years) age groups (Thomas et al., 2016). In addition to this, more studies have shown that cardiac resynchronization therapy reduces the need for hospitalization and outpatients care but becomes cost-effective only after approximately 4 years from implantation (Linde et al., 2011). The cost and effectiveness OF ICD should be carefully balanced. Currently, CRT guidelines require CRT candidates with a life expectancy of at least 1 year.

Implantable cardioverter defibrillators (ICD) are recommended in elderly patients that meet appropriate criteria. Elderly patients receive equally appropriate device shocks as compared with younger age groups (Yung et al., 2013). In a study of 84 octogenarians ICD implantation proved safe and effective at 5-year follow-up; however, in other studies the effectiveness was not clear after adjustment for comorbidities (Mezu et al., 2011; Strimel et al., 2011). It should be also acknowledged that ICD therapy may be cost-effective if the patient lives more than 7 years, that is almost the life of one battery; nonetheless, median survival in elderly >75 years HF patients hardly ever surpasses 5 years (Hohnloser et al., 2004). In addition to this, in a large Danish nationwide registry 72% of elderly patients treated with IDC for primary prevention did not have any utilization of their device prior to death (Ruwald et al., 2016). Moreover, in a trial of 1116 patients with nonischemic HF randomized to ICD or medical treatment, prophylactic implantation of ICD showed a survival benefit only in younger ages (Kober et al., 2016). There are also concerns that patients with HF and nondialysis-dependent severe chronic kidney disease may not benefit from ICD impanation (Singh et al., 2014).

Pump failure, renal failure, and severe noncardiac comorbidities rather than malignant arrhythmias seem to be the main drivers in mortality in the elderly. This raises several ethical and practical concerns. For example, there is no clear recommendation for replacing the ICD generator in elderly patients that do not any more meet the criteria for ICD implantation, that is patients who did not experience appropriate shocks and/or their LVEF has drastically improved >35%. This subgroup may account for 26% of

patients where the device needs replacement (Kini et al., 2014). Also it is reasonable that all elderly ICD candidates understand the function of the device and anticipate shocks. As a matter of practicality being able to provide informed consent is a minimum prerequisite for ICD implantation in many centers, nonetheless witnessed informed consent is also practiced in cases with cognitive dysfunction.

Mechanical Circulatory Support

It is estimated that among 300 million USA residents there are 200,000–250,000 Class IIIb–IV HF patients. Taking under account that the average age of HF hospitalized patient is 75 years and that many centers are reluctant to consider patients >70 years for heart transplantation (only 1% of transplant patients are >70 years of age) we can acknowledge a rapidly escalating number of potential Left ventricular assist device (LVAD) candidates among the elderly, mainly for a destination therapy (Miller and Guglin, 2013; Mancini and Colombo, 2015).

In addition to that, exclusion criteria for LVAD are less strict than transplantation criteria and do not apply to a large pool of HF patients with advanced age: end-organ dysfunction, intolerance to anticoagulation, increased pulmonary vascular resistance, significant right ventricular dysfunction, and comorbidities including malignancy, significant pulmonary, renal disease, significant obesity, mental illness or alcohol and drug dependences (Mancini and Colombo, 2015). Some studies have linked older age with worse short-term prognosis, however other studies have shown that the survival of patients >70 years was comparable with 65–70 and only modestly inferior to patients 50–64 years (Kilic et al., 2013; Adamson et al., 2011). Apparently poorer clinical status, right ventricular dysfunction, comorbidities including renal failure and diabetes, and a history of cardiac surgery are related to worst outcome (Kirklin et al., 2013). Younger age is however paradoxically associated with higher incidence of pump thrombosis during follow-up which is a finding that needs further confirmation (Kirklin et al., 2015). Psychosocial evaluation of elderly patients before implantation is crucial. Elderly patients with LVAD can suffer severe cognitive impairment or even, given time, become ineligible candidates for heart transplantation (Kirklin et al., 2015).

Transcatheter Interventions

Elderly patients with valvular disease who would be considered nonoperable in previous decays may undergo a plethora of valve repair and valve replacement procedures that do not require the aid of cardiopulmonary bypass. The option of transcatheter aortic valve implantation for elderly patients suffering from symptomatic severe aortic stenosis and with a high risk for conventional surgery is very well documented. Transcatheter valve impanation is also an option for otherwise nonoperable high-risk HF patients with failing bioprosthesic valves in the aorta, mitral, and tricuspid positions (Bapat and Asrress, 2014).

For elderly HF patients with severe mitral regurgitation who are deemed at excessive risk for conventional surgery the role of self-expanding bioprostheses through trans—apical access is coming to front. Such therapeutic options are the Tiara™ TAMI system (Neovasc Inc., Richmond, BC, Canada) and the and CardiAQ™ transcatheter mitral valve implantation system (CardiAQ Valve Technologies, Inc., Irvine, CA, United States) that preclude the need for cardiopulmonary bypass. Initial experience with these systems is very promising (Cheung and Banai, 2016). Mitraclip (Abbott Vascular, part of Abbott Laboratories; Santa Clara, California) is also a therapeutic option for high-risk patients with severe primary and secondary mitral regurgitation which improves LV remodeling and functional status in >75 and ≤75 years old patients (Scandura et al., 2016). Initial experience of Mitralign percutaneous tricuspid valve annuloplasty system for severe functional mitral regurgitation is also promising (Nickenig et al., 2016). For patients with significant functional tricuspid regurgitation and high predicted surgical risk the catheter-guided Trialign device (Mitralign, Inc., Boston, MA, United States) may reduce tricuspid annulus dimensions and improve HF symptoms. Moreover, the transcatheter interatrial shunt device (IASD, Corvia Medical, Tewkesbury, MA, United States) creates an iatrogenic left-to-right atrial shunt device that attenuates excessive exercise-induced left atrial pressures. In an initial single arm trial of HFpEF patients (mean age 69 ± 8 years) the device improved functional capacity and quality of life at 6-month follow-up (Hasenfuss et al., 2016). This is of particularly importance for old HFpEF patients where medical treatment options are scarce.

Frailty

Frailty is documented in up to 50% of HF patients in large registries and influences outcome of HF patients independently of concomitant comorbidities (Afilalo et al., 2014). Advance HF and frailty are therefore distinct entities, although they may share common pathways including increased inflammatory state (C Reactive Protein, Interleukin 1, Tumor necrosis factor a), impaired cortisone regulation, decreased testosterone levels, insulin resistance, skeletal muscle atrophy, and hypercatabolic state. According to the contemporary school of thought the frail phenotype HF patient is more vulnerable to stressors which may trigger decompensation at a lower threshold (Afilalo et al., 2014). Moreover, frail patients exhibit higher functional decline and may require a longer recovery time.

It is clear that frailty sets a therapeutic limit to contemporary costly invasive treatment of HF. A study that used Fried and Walston's definition of frailty among CRT recipients showed that the frail phenotype was four times more likely to have HF decompensation at 1-year follow-up (Dominguez-Rodriguez et al., 2015). Another single center study used a 31-component deficit index and defined 62% of LVAD candidates as frail. At 2-year follow-up those in the highest tertile had almost 40% higher mortality rates compared to 16% at the lowest tertile. Frailty was associated with mortality even after correction for other risk confounders

Table 3 Fried frailty scale

1. Weight loss	Unintentional weight loss of $\geq$4.5 kg (10 lbs) or $\geq$5% of the previous year
2. Weakness	Grip strength of the dominant hand with a Jamar hand-held handgrip strength dynamometer (maximum value of three attempts) Positive at lowest quartile based in gender/body/mass index
3. Self-reported exhaustion	How often in the past week: 1. did you feel that everything you did was an effort? 2. you could not get going? Positive if at least one condition positive $\geq$3 days
4. Slowness	Time to walk 4.57 m (15 ft) at usual pace Positive according to height/gender cut-offs
5. Low activity	Evaluation of 18 activities from the Minnesota Leisure Time Physical Activity Questionnaire that links calorie expenditure to activity. Positive if kilocalorie expenditure in men $<$383 kcal/wk and in women $<$270 kcal/wk.

Nonfrail (score 0), prefrail (score 1–2), frail (score 3–5).

Adapted from Fried, L. P., Tangen, C. M., Walston, J., Newman, A. B., Hirsch, C., Gottdiener, J., Seeman, T., Tracy, R., Kop, W. J., Burke, G., Mcburnie, M. A. and Cardiovascular Health Study Collaborative Research Group (2001). Frailty in older adults: Evidence for a phenotype. *The Journals of Gerontology Series A, Biological Sciences and Medical Science*, **56,** M146–M156.

(Dunlay et al., 2014). In a similar study, weak grip strength weight was predictive of length of stay after LVAD implantation. Surprisingly, handgrip strength increased significantly post-LVAD (Yost and Bhat, 2017).

It is frequently unclear if symptoms fatigue and end-organ dysfunction of elderly HF patients are a result of an "instinct" frailty or a consequence of pump failure and therefore can be partially reversed after improvement of stroke volume and pulmonary capillary wedge pressure with invasive treatments. Specific comorbidities including chronic lung disease, dementia, disabling stoke, peripheral vascular disease, cirrhosis, osteoporosis, severe arthritis, and cancer may be linked more to an "irreversible" frailty phenotype. The fried scale is the most citied method in the bibliography to define frailty (Fried et al., 2001) (**Table 3**). Alternatively, single-component measurements such as 5-m gait speed or handgrip are used because of their simplicity (Pulignano et al., 2016). Given the increasingly aging global population and the economic constrains in most health systems there is currently an unmet need for simple, validated frailty indices in elderly CRT and LVAD candidates. So far, however, there are no specific recommendations for screening of frailty by guideline committee providers.

Palliative Care

For patients who are excluded from LVAD and heart transplant there comes a time where the focus of care shifts from prolonging their life to improving the quality of their remaining life with emotional, psychosocial, and spiritual support. Dyspnea, pain, and other distressing symptoms may require high doses of diuretics, benzodiazepines, and opioids. On the other hand, satins, aspirin, or even first-line drugs may be stopped. At this phase, patients may also wish to discuss "do-not-resuscitate" order or disable their ICD.

However, prognosis of end-stage HF patients is not always easy and reliable. Physicians treating the terminal stage HF patient are also reluctant to talk about palliative care and patients tend to overestimate their life expectancy. Health care providers point out the need for awareness availability and accessibility of palliative care in HF. This option cannot be reserved for patients facing imminent death but at an earlier phase where patients are able to participate in decision-making. Currently, in United Kingdom only a 7% proportion of HF patients were registered for palliative care as opposed to a 48% of cancer patients. Also 72% of registered HF palliative care patients were $\geq$80 years of age whereas only 3% were younger than 60 years (Gadoud et al., 2014). Similarly on the other side of the Atlantic, only 13% of patients receiving hospice care had a primary HF diagnosis; however an increasing trend is observed.

Cost for medical systems

In high-income countries more than 80% of patients are $>$ than 60 years. In United States the number of patients with HF is expected to rise from 5.8 to 8.5 million by 2030. The total HF financial cost in 2012 was estimated 31$ billion and may rise as 70$ billion in 2030 (Ziaeian and Fonarow, 2016; Heidenreich et al., 2013). HF-related hospitalizations are also projected to rise by 50% by the year 2030. Almost 2% total budgets are spent for HF in high-income countries including United States, Germany, and Sweden and more than half of the medical cost concerns interventions and repeated hospitalizations at the time nearing death.

Age per se may not be related with high HF expenditures in high-income countries. Instead hospitalization in urban and teaching hospitals and advanced invasive procedures increase expenditures (Ziaeian et al., 2015). However, advanced disease managed programs and Natriuretic Peptide guided therapy are less effective in the elderly especially when comorbidities are prevalent (Sanders-van Wijk et al., 2013).

At present almost 86% of universal HF spending concerns high-income countries and regards only 16% of global HF patients, while there is few information about the HF financial cost in developing countries (Ziaeian and Fonarow, 2016).

Limitations of Our Global Perspective

Unfortunately, although several studies have compared elderly versus younger population in high-income countries to date there are no such direct comparisons for middle and low-income countries. Inevitably assumptions are made based on registries carried out in westernized communities. This is partly justified because a global shift toward a westernized lifestyle is observed. Indeed, in earlier decades data from acute HF registries in Africa and Asia had shown a high prevalence of hypertension (45%) and rheumatic heart disease (14%) with ischemic heart disease consistently reported less than 10% (Damasceno et al., 2012). However in the "INTERnational Congestive Heart Failure" (INTER-CHF) study ischemic heart disease was a leading cause (20%) of HF in Africa (Dokainish et al., 2016). Similarly, a shift toward ischemic etiology of HF over hypertension and valvular etiology is noted in Asian countries as compared with registries in previous decays (Ziaeian and Fonarow, 2016) (**Table 4**).

However, an actual direct extrapolation of data from high-income countries may not be entirely appropriate. Patients in registries from developing countries are considerably younger (**Tables 4** and **5**). There are significant regional dissimilarities with regard to HF etiology as for example patients from Middle East and South America tend to be more obese and suffer more commonly from diabetes compared to African and Asian patients. Also rheumatic fever is almost extinct from developed countries; however almost 470,000 new cases are expected worldwide, the majority of which occur in low income African countries (Essop and Nkomo, 2005). Chagas disease accounts for 3.8% of heart failure cases in South America and is associated with high mortality in younger age groups. There are also dissimilarities with respect to clinical presentation as in the "Asian Sudden Cardiac Death in

Table 4 Clinical presentation according to the region in the INTER-CHF study (Dokainish et al., 2016)

Years 2012–14	*INTER-CHF Africa*	*INTER-CHF Middle East*	*INTER-CHF South America*	*INTER-CHF Asia*
Mean age	53.4	56.4	67.1	60[a]
Number	1294	1000	858	2661
BMI	25.5	30	28.5	24.4[a]
Male	51.8	72.3	61.2	59.1[a]
Hypertension	61.6	68.4	73.6	59[a]
Diabetes	17.1	56	21.9	27.9[a]
MI	8.2	19.1	18.3	22.3[a]
HFpEF	28.8	11	29.0	40.7[a]
NYHA II–IV	56.4	37.4	31.1	41.7[a]
ACE/ARBs	77.1	81.6	75.4	67.9[a]
B-blockers	48.3	85.9	73.3	60.8[a]
MRAs	59.4	45.8	55.1	44[a]
Diuretics	93.7	87.8	77.6	62.1[a]

[a]$P < 0.001$.

ACE, Angiotensin converting enzyme inhibitors; *ARBs*, Angiotensin II receptor blockers; *BMI*, body mass index (kg/m^2); *CAD*, coronary artery disease pulmonary disease; *HFpEF*, heart failure with preserved ejection fraction; *MI*, myocardial infarction; *MRA*, mineralocorticoid receptor antagonist.

Table 5 Profile of HF patients with LVEF $\leq$40% in the Asian sudden death heart failure registry (Lam et al., 2016)

Years 2012–15	*High income*	*Middle income*	*Low income*
Mean age	62.6	57.7	57.3[a]
Number	2247	1212	1817
BMI	24.8	25.1	24.9
Male	78.3	80.4	76.5[b]
Hypertension	59.7	54.9	40.2[a,b]
Diabetes	46.2	36	36.1[a]
CAD	58.8	49.5	48.5
LVEF	27	28	28[a]
NYHA II–IV	27	40	41[a,b]
ACE/ARBs	76.6	66.9	79.4[a,b]
B-blocker	86.1	83	66.5[a,b]
MRAs	54.5	70	58.8[a,b]
Diuretics	82.3	83.2	79.3[a,b]

[a]$P < 0.005$ vs. high income.

[b]$P < 0.005$ vs. middle income.

Income level was defined according to World Health Organization as lower in Indonesia, Philippines, India; middle in China, Thailand, Malaysia and higher in Singapore, Hong Kong, Taiwan, South Korea, and Japan.

ACE, Angiotensin converting enzyme inhibitors; *ARBs*, Angiotensin II receptor blockers; *BMI*, body mass index (kg/m^2); *CAD*, coronary artery disease; *LVEF*, left ventricular ejection fraction; *MRA*, mineralocorticoid receptor antagonist.

Heart Failure" (ASIAN-HF) registry 27% patients from high-income countries were in NYHA III or IV compared to 40% and 41% in middle and low income countries, respectively (**Table 4**). Also an initial dramatic clinical presentation with orthopnea, pulmonary rales, and S3 is more common in lower income countries (Lam et al., 2016). It is also a fact that patients in low-income countries commonly have no health or medication insurance (e.g., >70% in Africa) and are subsequently derived from any costly treatment. Indeed, only 6.1% of HF patients from low income countries receive a ICD, pacemaker, or CRT compared to 14.6% and 20.8% of middle and high-income countries, respectively (Lam et al., 2016).

Another point of concern is that in the developing world registries are carried in centers located in urban-elite territories and results may not be generalizable in the broader population. Patients are usually included in primary care and diagnostic criteria of HF are commonly unspecified. Even well-designed HFs are registries executed in low income countries acknowledge several weaknesses: Paradoxically more patients are lost at follow-up in urban areas as compared to rural areas. At the same time a lower rate of major cardiovascular events is documented in urban as opposed to rural areas (Yusuf et al., 2014). Ongoing world HF registries are expected to show a global perspective of this complex issue and indicate county-specific measures to improve the survival and quality of life of elderly HF patients (Filippatos et al., 2015).

Areas of Uncertainty—Future Directions

There are still open issues regarding the diagnosis and management of this frail group. The role of novel biomarkers (ST2, adrenomedullin) for diagnosis and prognosis remains uncertain. Also specific cut-offs of natriuretic peptides in the elderly are also lacking. A multimarker approach could be more appropriate in older HF patients; however its cost and effectiveness need to be validated. There is also currently a gap of evidence regarding specific cut-offs in echocardiography to define severe diastolic dysfunction in the octogenarians and nonagenarians. Measurements of arterial stiffness can also be integrated in the diagnostic workup; however exact recommendations are lacking.

Accurate prognosis is critical in the elderly owing to financial limitations of advanced treatments but so far validated prognostic scores have included only a low proportion of very elderly patients. For example the mean age was 63.3 years in "The N-terminal ProBNP Investigation of Dyspnea in the Emergency Department" (PRIDE) score and 65 years in the "Seattle Heart Failure Model" (SHFM) (Mozaffarian et al., 2007; Baggish et al., 2006). To date there are also few studies and registries with long (>2 years) follow-up of elderly HF patients.

There is also an unmet need to represent more elderly patients in large-scale trials of new therapeutic agents. For instance, a prespecified percentage (e.g., 30%) of included patients could be older than 75 years of age with also a predetermined minimum number of comorbidities. Treatment goals may also be modified, emphasizing on improvement of quality of life. To date there is also no universally accepted frailty scores that can overcome socioeconomic barriers among the evaluated populations.

Another high priority unsolved issue is the lack of evidence-based medical treatment in the prevalent HFrEF phenotype in the elderly. The heterogeneous pathophysiology of HFrEF is currently in the center of intensive research in a genetic, cellular, and clinical level. So far a plethora of pharmaceutical agents have failed to improve mortality in this group. Sacubitril valsartan and serelaxin, a natural vasodilator, are promising new agents but large-scale trials are still pending (Filippatos et al., 2014).

HF in the elderly is a health concern that transcends national borders. There is a clear need for a better global perspective that will aid country and region-specific interventions regarding prevention, diagnosis, and treatment in the elderly, especially in socioeconomically disadvantaged countries (Teo et al., 2013). Long-term medical planning can accordingly be tailored in keeping with realistic expectations. Global and country-specific dissemination of HF guidelines is also crucial to assert evidence-based treatments and quality of care.

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Heart Failure Monitoring

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Introduction

Heart failure (HF) is a syndrome caused by many etiologies which share the common phenotype of volume overload and poor end-organ perfusion. It affects an estimated 23 million people worldwide (Roger, 2013) and its prevalence and incidence are expected to rise due to longer life expectancy, especially increased longevity in those patients who have risk factors for the development of HF, e.g., coronary artery disease, hypertension, diabetes mellitus, and obesity (Roger, 2013). In the United States, there are an estimated 6 million people with the diagnosis of HF. It is projected that by 2030, there will be 8 million people with HF and that the cost of caring for HF patients will rise from $31 billion to $80 billion (Heidenreich et al., 2013).

Goals of HF management are to improve survival, prevent worsening symptoms, and reduce risk for hospital admission. To accomplish these goals, many centers now have developed disease management programs specific to HF. Although the monitoring technologies used might be very different among programs, the basic structure is the same—a system by which changes in the patient's clinical status are directly communicated to a dedicated provider team that respond to these changes with the intent of preventing hospital admission and maintaining quality of life (QOL) for these patients.

Selecting the most clinically effective and cost-effective means of optimizing management remains a challenge for clinicians. Many monitoring systems have been studied, ranging from the creation of specialty care teams to implantation of invasive devices purely for the purpose of HF monitoring. The purpose of this review is to better understand the evidence (or in some cases lack of evidence) behind the modalities that have been created and studied for monitoring the HF patient. It is hoped that after reviewing the pros and cons of each approach, the provider can then decide which approaches are best suited for their programs and patients.

Goals of Heart Failure Monitoring

Based on the Seattle HF Model, combined use of ACE inhibitors, β-blockers, mineralocorticoid receptor antagonists, and defibrillators, was predicted to decrease morbidity and mortality for systolic heart failure (HFrEF) patients by an absolute 25% over the past 2 decades (Levy et al., 2006). However, although programs targeted at improving compliance with guideline directed medical therapy (GDMT) for HF, such as "Get with the Guidelines—Heart Failure"(AHA) and the tracking of HF Discharge Instructions by CMS, have resulted in the majority of HF patients being treated appropriately with evidence-based, goal-directed medical therapy, recent studies show that this has resulted in not only marginal decreases in mortality (Barker et al., 2006; Curtis et al., 2008), but also no change and possible worse readmission rates (Heidenreich et al., 2010; Dharmarajan et al., 2013; Kociol et al., 2012). For diastolic HF (HFpEF) patients, readmission and mortality rates remain unchanged and comparable to HFrEF (Meta-analysis Global Group in Chronic Heart Failure (MAGGIC), 2012). Annually there are 1 million hospitalizations for HF in the United States with 30-day readmission rates in the 25% range. Cost for HF care remains the highest of all medical conditions (Roger, 2013).

Regardless of cause and acuity, HF admissions are heralded by volume overload (Gheorghiade et al., 2013). Hence, the universal goal of HF monitoring is to detect subclinical volume overload and intervene as early as possible to prevent readmission. Clearly reducing the number of hospitalized days improves QOL for the HF patient. However, decongestion brings other benefits (besides preventing readmission) to the HF patient. There is evidence that suggests achieving and maintaining normovolemia improve QOL and survival and prevent damage to other organ systems.

Achieving normovolemia correlates with further improved QOL in HF patients. A study of ambulatory HF patients who were listed for transplantation and who underwent afterload reduction and diuresis to achieve a pulmonary capillary wedge pressure of <15 demonstrated significant improvements in all aspects of exercise functional capacity as measured by cardiopulmonary exercise stress test (Stevenson et al., 1995). In another study patients with elevated filling pressures but no clinical findings of congestive HF were hospitalized, had a pulmonary artery catheter (PAC) placed, and were diuresed to normovolemia. Patients reported significant subjective improvement in symptoms after diuresis (Androne et al., 2004; Chomsky et al., 1997) which correlated with significant improvement in objective measurements in exercise duration, minute ventilation, lactate levels, and Borg dyspnea scores (Chomsky et al., 1997). Secondary end points of the ESCAPE trial, a randomized, controlled trial comparing HF therapy guided by clinical assessment alone versus with a PAC, included changes in functional and HF quality of life measures: 6-min walk test, cardiopulmonary stress test, Minnesota Living with HF questionnaire (validated measurement of QOL), and time trade-off score (quantification of how many months of life out of 24 months patient would trade to feel better). Results showed a trend for more improvement in the group randomized to PAC at 1 month in all of the measures with time trade-off showing the greatest improvement, a significant result that was sustained for 6 months (Binanay et al., 2005). These findings were attributed to greater reduction in filling pressures in the PAC study group. Validating these findings is a post hoc analysis of the ESCAPE trial subjects who received a PAC and had reduction of wedge pressure to <16 mmHg and right atrial pressure to <8 mmHg. Percent reduction in wedge pressure after therapy correlated with improvement in measurements of QOL and functional capacity which included dyspnea during activity, global assessment, worst symptom score, 6-min walk test, and Minnesota Living with HF questionnaire (Rogers et al., 2006).

Volume status may predict mortality in HF patients. Stevenson et al. (1990) showed that the inability to achieve pulmonary capillary wedge pressure of <16 mmHg without causing hemodynamic compromise during treatment of acute decompensated systolic HF is associated with worse prognosis (38% 1-year survival versus 83%, $p = 0.0001$). Another *post hoc* analysis of patients in the ESCAPE trial who received a PAC found elevated filling pressures (wedge and right atrial pressures) after PAC-directed diuresis to be the only hemodynamic parameters significantly associated with adverse outcomes (Cooper et al., 2016). In a prospective, observational study of nonedematous ambulatory HF patients, hypervolemia as measured by blood volume analysis was found in 65% of the study group. Mortality, defined as either death or urgent transplantation, was significantly higher in the hypervolemia group than the normovolemia/hypovolemia group (39% 1-year event rate versus 0%, $p < 0.01$) (Androne et al., 2004).

Another important negative consequence of chronically elevated filling pressures in the HF patient is the downstream development of significant pathology in the liver, pulmonary vasculature, and right ventricle, each of which can increase the mortality of HF patients and also render them ineligible for advanced therapies such as left ventricular assist device implantation and/or cardiac transplantation.

Cardiac hepatopathy can be both acute and chronic. "Acute cardiogenic liver injury" is seen in the context of cardiogenic shock. Patients have elevated alkaline phosphatase and significantly elevated transaminases which correlate histologically with ischemic injury—necrosis surrounding central veins due to hypoxia. Studies of patients with HF and acute cardiogenic liver injury found that elevated right-sided or venous filling pressures highly correlated with ischemic injury but hypotension and low cardiac output did not (Seeto et al., 2000; Henrion et al., 1994). Thirty day and 180 day mortalities for HF patients with acute liver injury are high at 17.6% and 31.6%, respectively (Nikolaou et al., 2013). "Congestive hepatopathy" is an often subclinical process secondary to chronic hepatic congestion, decreased hepatic blood flow, and decreased arterial oxygen saturation (Giallourakis et al., 2002). Histopathology can show various stages from sinusoidal fenestrae enlargement early in the course of the disease to necrosis, fibrosis, and cirrhosis (Samsky et al., 2013). The association of both acute cardiogenic liver injury and congestive hepatopathy with venous congestion suggests that treatment of the venous congestion may be preventative. Indeed there are histological reports of disease regression, hepatic regeneration, and even fibrotic resolution in patients with cardiac hepatopathy after HF has been successfully treated (Rosenberg, 2004).

Secondary pulmonary hypertension is also a consequence of chronic congestion. Left untreated, left-sided heart failure results in elevated filling pressures, chronic pulmonary edema, and subsequent remodeling of the pulmonary vasculature, eventually resulting in fixed pulmonary arterial hypertension (Delgado et al., 2005). Chronically elevated pulmonary arterial pressures result in right ventricular failure. Hence the common saying, "the most common cause of right heart failure is left heart failure." Fixed pulmonary hypertension is a contraindication to heart transplant because the donor right ventricle is unable to meet the sudden increased workload (Mehra et al., 2006; Stadnick and Ignaszewski, 2010). In advanced heart failure, low right ventricular ejection fraction independently predicts functional capacity and mortality (Di Salvo et al., 1995). Severe right ventricular failure is a major cause of morbidity and mortality following implantation of a left ventricular assist device and therefore considered a contraindication (Khazanie and Rogers, 2011). In the absence of significant right ventricular failure, chronic decongestion after implantation of a left ventricular assist device has been reported to result in reversal of pulmonary hypertension such that patients later become eligible for heart transplantation (Mikus et al., 2011; Etz et al., 2007; Zimpfer et al., 2007). This suggests that both secondary pulmonary hypertension and right heart failure are avoidable secondary consequences of chronic congestion and again emphasize the importance of HF monitoring.

Noninvasive Monitoring

Team-Based Care

Both the American Heart Association and European Society of Cardiology guidelines recommend a team-based approach in the care of heart failure patients (Yancy et al., 2013a; McMurray et al., 2012). There have been many small randomized-controlled

studies comparing usual care to team-based care and the majority of the studies demonstrate significant decrease in both heart failure and all-cause readmissions (McAlister et al., 2001; Sochalski et al., 2009). One limitation of these studies is considerable variability among the studies in how team-based care is defined. A meta-analysis and systematic review of these randomized-controlled trials both came to the same conclusion—that the most effective team-based care approach utilizes a multidisciplinary team with enhanced access to in-person visits either at home or in the clinic (McAlister et al., 2001; Sochalski et al., 2009). Multidisciplinary teams reduced mortality by 25%, HF hospitalizations by 26%, and all-cause hospitalizations by 19%(McAlister et al., 2001).

Monitoring the HF patient at the office or at a home visit has significant limitations. In the office the provider determines volume status based on the patient's history and physical examination findings. Accurate assessment is limited by a number of factors shown in various studies, including lack of sensitivity of physical signs for estimating hemodynamics in HF (Stevenson and Perloff, 1989), lack of symptoms in HF patients who are volume overloaded by objective measurements (Androne et al., 2004; Chomsky et al., 1997), and inability by many clinicians to accurately estimate central venous pressures on exam (Davison and Cannon, 1974; Brennan et al., 2007; Sinisalo et al., 2007). Home visits also require resources that are limited, especially so in the current economic climate.

Currently the vast majority of HF patients are being monitored by what is probably the most noninvasive, and affordable monitoring technology—the bathroom scale (Glasgow et al., 2002). Daily weights are either routinely called to the team by the patient or called in when changes in weight meet certain parameters defined by the provider team so that the team can provide timely intervention (Glasgow et al., 2002). This type of monitoring is known as structured telephone support. Weight-based monitoring has been highly criticized. One study shows that only 14% of patients with HF weight themselves daily (Moser et al., 2005). The Fluid Accumulation Status Trial (FAST) showed a sensitivity of only 22.5% for daily weight monitoring in predicting a HF hospitalization (Abraham et al., 2011a) and this finding is supported by two other studies that showed changes in weights do not reliably predict changes in filling pressures (Abraham et al., 2011a; Yu et al., 2005; Bourge et al., 2008). Several metaanalyses of randomized, controlled trials performed by the same research team over the past decade repeatedly demonstrate reduced rates of rehospitalizations for HF patients followed by multidisciplinary teams with structured telephone support (Clark et al., 2007; Inglis et al., 2011; Inglis et al., 2015), suggesting that the scale, when used in certain settings, can be beneficial.

With the advent of technology and the need to remotely monitor large numbers of HF patients, telemonitoring has recently evolved from the structured telephone support to use of electronic equipment provided to the patient (i.e., scale, automated blood pressure cuff, etc.) which collects daily symptoms as well as weight, heart rate, and blood pressure and transmits this data to the care team via digital/broadband/satellite/wireless/blue-tooth. Whereas earlier meta-analyses and randomized-controlled trials concluded that telemonitoring was effective at reducing both HF and all-cause admissions (Dharmarajan et al., 2013; Inglis et al., 2011), two larger randomized, controlled studies later demonstrated a neutral effect (Chaudhry et al., 2010; Mortara et al., 2009) with resultant controversy in the HF community regarding the benefits of telemonitoring. More recently the results of several metaanalyses regarded as highly comprehensive (Inglis et al., 2015; Klersy et al., 2009; Polisena et al., 2010) suggest that telemonitoring is effective, reducing HF related hospitalizations by 7%–48% and all-cause mortality by 17%–47%.

Serum Biomarkers

Numerous serum biomarkers have been validated for the prognostication of HF, and include those involved in myocyte stretch (e.g., natriuretic peptides, ST2, GDF-15), myocyte necrosis (e.g., troponins, CKMB), systemic inflammation (e.g., CRP, TNF-alpha), oxidative stress (e.g., oxidized LDLs), extracellular matrix turnover (e.g., MMPs, galectin-3), and neurohormones (e.g., renin, angiotensin II, aldosterone, norepinephrine). However, data regarding their serial use in HF monitoring to prevent events remains weak. Pertinent criteria for a useful biomarker in the monitoring of HF patients include: (a) be easily measured with high analytical precision (Januzzi et al., 2015); (b) be measurable at a reasonable cost on short notice (Morrow and de Lemos, 2007); (c) have low biological variation (Januzzi et al., 2015); (d) should reflect important pathophysiologic processes involved in HF presence and progression without recapitulating clinical information that is already available (Januzzi et al., 2015); and (e) respond to a therapeutic modification that results in an improvement of clinical outcomes (Bohm et al., 2011). The most promising category of biomarkers for use in serial HF monitoring, which will be discussed in this review, are those upregulated during myocyte stress—BNP, NT-proBNP, MR-proANP, sST2, and GDF-15.

Brain natriuretic peptide (BNP) is a 32-amino acid protein secreted by the cardiac ventricles in response to volume overload and ventricular stretch. Its inactive metabolite, NT-proBNP, has been validated as a surrogate marker that accurately reflects BNP activity (Wu, 2006; Costello-Boerrigter et al., 2006; Goetze et al., 2006). Henceforth, for simplicity, BNP and NT-proBNP will both be referred to simply as BNP in the rest of this review. BNP assays became almost universally available at hospitals and outpatient laboratories after studies showed that normal BNP levels reliably exclude the diagnosis of heart failure (Chomsky et al., 1997; Goetze et al., 2006; Morrison et al., 2002). Elevated BNP levels are associated with worse outcomes and are useful for risk stratification. For every 100 ng/L increase in BNP level, there is a corresponding 35% relative increase in the risk of death (Doust et al., 2005). Treatment with each of the GDMT medications for heart failure (ACE inhibitors, ARBs, MRAs, β-blockers, and decongestion with diuretics) has been shown to result in a decrease in BNP level (Yoshimura et al., 2002; Ferreira et al., 2001; Murdoch et al., 1999; Latini et al., 2002; Tsutamoto et al., 2001). The use of BNP to guide uptitration of GDMT has been studied extensively in randomized trials and the vast majority of the studies report results of patients being on higher doses of GDMT with

significantly reduced mortality (Berger et al., 2010; Troughton et al., 2000; Pfisterer et al., 2009; Jourdain et al., 2007; Januzzi et al., 2011). The results of one study showed no difference in outcomes between patients randomized to BNP-guided therapy versus intensive clinical management where providers were instructed to aggressively uptitrate medical therapy based on current guidelines, suggesting that it was the instructions provided to uptitrate GDMT that resulted in the benefit and that measuring BNP levels is unnecessary (Lainchbury et al., 2009).

The results of the same randomized trials cited previously (Berger et al., 2010; Troughton et al., 2000; Pfisterer et al., 2009; Jourdain et al., 2007; Januzzi et al., 2011; Lainchbury et al., 2009) inconsistently reported specifically on the outcome of decreasing HF hospitalizations because HF hospitalization in the studies usually constituted part of the primary outcome and was not necessarily analyzed separately. Four of the randomized studies did analyze HF hospitalizations as a separate endpoint and found that the BNP-guided groups had significantly fewer HF hospitalizations (Berger et al., 2010; Troughton et al., 2000; Pfisterer et al., 2009; Lainchbury et al., 2009). The findings of these studies are supported by a recent metaanalysis showing that BNP-guided treatment significantly decreased the risk of HF hospitalizations (Xin et al., 2015). At the time of this writing the utility of serial BNP measurements to monitor HF patients with the purpose of reducing hospitalization is thought to be beneficial but not definitely established, rendering it a Class IIb in the 2013 ACC/AHA guideline for the management of HF (Yancy et al., 2013b).

Discordant clinical findings may be explained by the biological characteristics of BNP. Baseline BNP and NT-proBNP levels are known to be higher in the elderly, higher in females, and lower in the morbidly obese and in patients with HFpEF. Two studies show that BNP-guided therapy has been shown to be not beneficial in patients over the age of 75(Pfisterer et al., 2009; Lainchbury et al., 2009). BNP has also been shown to have a high biological variation of 92%, meaning that in the same healthy subjects there was 92% change from baseline in the absence of significant clinical instability (Wu et al., 2013). These highly variable biological characteristics of BNP challenge its reliability as a biomarker for monitoring HF.

Atrial natriuretic peptide (ANP) was the first natriuretic peptide described in heart failure but was not able to be assayed due to analytical instability. ANP also has an inactive metabolite called midregional (MR) proANP, which is much more stable and can be assayed. A large prospective study demonstrated MR-proANP is noninferior to BNP for the diagnosis of HF in patients presenting to the emergency room with dyspnea (Maisel et al., 2010). Checking MR-proANP when BNP levels are nondiagnostic appeared to be useful in obese patients (whose BNP levels might be falsely low) (Maisel et al., 2010). However, MR-proANP levels were not able to predict rehospitalization or visits to the emergency room (Maisel et al., 2010). A small, prospective, cohort study looking at the value of following MR-proANP serially showed that changes in levels of >30% were significantly and proportionally associated with increased risk for poor outcome. The risk for poor outcome was further significantly increased when MR-proANP was combined with cardiac troponin T levels (Miller et al., 2012). More studies are needed to determine the utility of MR-proANP in the monitoring and management of HF.

ST2 is a member of the interleukin (IL)-1 receptor family whose production is stimulated by myocardial strain. There are two forms of ST2—membrane bound (ST2L) and soluble (sST2). sST2 has low biological variability of only 30% making it a desirable biomarker to study. The activating ligand for ST2 is IL-33 which promotes antihypertrophic, antifibrotic, and antiapoptotic activity through ST2L which is believed to result in antiremodeling of the myocardium (Seki et al., 2009; Kakkar and Lee, 2008). *In vitro* sST2 is believed to block the effects of circulating IL-33 thereby promoting hypertrophy, fibrosis, and apoptosis, i.e., cardiac remodeling (Kakkar and Lee, 2008). In accordance with these findings, studies of HF patients show a significant relationship between sST2 concentrations and the presence of left ventricular adverse remodeling, diastolic dysfunction, and poor right ventricular function (Daniels et al., 2010; Shah et al., 2009). Elevated sST2 levels should predict worse outcomes and this was demonstrated in three large clinical studies (Broch et al., 2012; Gaggin et al., 2014; Anand et al., 2014). sST2 levels appear to decrease in response to treatment with β-blockers and valsartan (Gaggin et al., 2014; Anand et al., 2014) and combination treatment with ACE inhibitors, ARBs, MRAs, and β-blockers appear to correlate with lower sST2 levels (Januzzi et al., 2015). Patients with high baseline sST2 levels who were titrated to higher β-blocker doses were at lower risk for cardiovascular events than those with the same sST2 levels on lower β-blocker doses and uptitrating β-blockers in patients with low baseline sST2 levels resulted in much lower reduction in risk (Gaggin et al., 2014), suggesting sST2 levels might be useful in guiding GDMT. However, the results of one study suggest that decreased sST2 levels are not associated with a reduced risk of events, challenging its usefulness as a biomarker for HF monitoring (Anand et al., 2014). A randomized, controlled study is needed to test the hypothesis that sST2 monitoring is helpful in the management of HF.

Growth differentiation factor (GDF)-15 is a member of the transforming growth factor-β superfamily which plays critical roles in cardiovascular development and repair. *In vitro* and *in vivo* studies show that GDF-15 is produced by myocytes in response to mechanical stretch, neurohormonal stimulation, ischemia, and proinflammatory cytokines (Kempf et al., 2006; Frank et al., 2008; Xu et al., 2006). Like many other biomarkers studied in HF, higher GDF-15 levels are associated with worse prognosis and higher mortality (Gaggin et al., 2014; Kempf et al., 2007; Wang et al., 2010; Lok et al., 2013). Research about the role of GDF-15 in HF monitoring is still in the preliminary stages as only two studies have evaluated the role of serial GDF-15 levels. Anand et al. (2010) analyzed serial measurements of GDF-15 in heart failure patients and their results suggested that increases in GDF-15 levels were independently associated with mortality and morbidity. Interestingly, treatment with valsartan did not result in a significant change in GDF-15 levels in this study, challenging its usefulness as a HF biomarker (Anand et al., 2010). Likewise Cotter et al. (2015) reported that changes in GDF-15 levels but not baseline GDF-15 levels, predicted adverse outcomes, suggesting that serial monitoring of GDF-15 could be helpful in HF. However like Anand et al. (2010), they also reported findings suggesting GDF-15 levels may not change in response to therapy. The degree of dyspnea or dyspnea relief was not associated with baseline GDF-15 levels nor with changes in GDF-15 levels (Cotter et al., 2015).

Although validated for use in diagnosis and prognosis, appropriate use of biomarkers in monitoring and treating HF remains undefined. The use of biomarkers is certainly desirable due to its minimally invasive and cost-effective nature. It is unlikely that a single biomarker will be identified that can be used in the diagnosis, prognosis, monitoring, and treatment of all HF patients. More likely studies of patterns of biomarkers either via proteomics or genomics will generate more personalized diagnosis and treatment algorithms.

Minimally Invasive Monitoring

Blood Volume Analysis

Blood volume analysis (BVA) is a minimally invasive means of measuring intravascular volume status. The test is available commercially, and qualifies for both Medicare and third party payer reimbursement. A large bore IV is placed in the patient and albumin radiolabelled with Iodine-131 is injected into the patient by a trained technician. This is followed by a twelve minute waiting period to allow the radiolabeled albumin to redistribute, then five blood samples are drawn, one every six minutes. The patient's height, weight, and gender are used to calculate ideal blood volume. Total blood volume, red cell volume, and plasma volume are determined from the redistribution of the radiolabelled albumin as measured from the five blood samples. The final report provides both the number of milliliters and percent volume difference from ideal plasma volume. The accuracy of BVA has been validated against pulmonary arterial catheter measurements with good correlation (Androne et al., 2004). Hypervolemia in HF patients as determined by BVA is associated with high mortality and morbidity (39% event rate at 1 year) (Androne et al., 2004). The converse has also been demonstrated: normo- or hypo-volemia in HF patients as determined by BVA is associated with excellent survival of 100% up to 1000 days of follow up (Androne et al., 2004). This method has also been studied in HF patients with anemia and can reliably separate the truly anemic patient from a patient appearing to have anemia, but in fact has low hemoglobin due to hemodilution from being hypervolemic (Androne et al., 2004). BVA has not been studied in the context of longitudinal monitoring nor whether intervention based on its results reduces mortality or morbidity. Because BVA is minimally invasive and can be performed on both inpatients and outpatients with results available in 90 min, further studies validating its utility in HF monitoring are warranted.

Intrathoracic Impedance

Although technically considered invasive, monitoring of intrathoracic impedance utilizes the equipment of defibrillator or pacemaker devices which are implanted primarily for benefits unrelated to HF hospitalization and therefore this modality adds no additional "invasive" risk to the patient. Therefore I have included review of these devices under the category of being minimally invasive.

Intrathoracic impedance is measured by the amount of time it takes for the right ventricular lead to send an electrical current to the implanted pulse generator. Pulmonary edema results in decreased intrathoracic impedance, the measurement of which can be included as a part of certain permanent pacemakers (PPM), implantable cardioverter-defibrillators (ICD) and cardiac resynchronization therapy devices (CRT) and the devices can be programmed to report and alarm for abnormal parameters as frequently as daily if desired by the clinician. An inverse correlation between intrathoracic impedance and pulmonary capillary wedge pressure and between intrathoracic impedance and net fluid loss during hospitalization for acute decompensated HF has been established (Yu et al., 2005), suggesting that measuring and monitoring thoracic impedance might be helpful in the management of HF.

Many small prospective, observational studies have been conducted to determine how best to use the information provided by these devices, especially intrathoracic impedance, to predict a HF hospitalization. Results from these studies are highly variable with sensitivities ranging between 20% and 92% for intrathoracic impedance in predicting HF events (Abraham et al., 2011a; Yu et al., 2005; Vollmann et al., 2007; Heist et al., 2014; Forleo et al., 2013; Maines et al., 2011; Conraads et al., 2011; Soga et al., 2011). Two prospective, observational studies investigated alternative impedance vectors and algorithms. DEFEAT-PE (Detect Fluid Early from Intrathoracic Impedance Monitoring) showed low sensitivities for all the different impedance algorithms ranging 20%–30% for HF events (Heist et al., 2014). The other study by Forleo et al. looking at only biventricular defibrillators with multivector impedance monitoring capabilities reported a sensitivity of 61.5% which was similar to the sensitivities reported by many other studies which used the standard right ventricular lead to pulse generator vector (Forleo et al., 2013). In one prospective, observational trial with an unmatched cohort control group, rate of HF hospitalizations or emergency room admissions was 56% lower in the group whose intrathoracic impedance was being measured and followed, again suggesting that monitoring thoracic impedance should be beneficial (Molon et al., 2014).

In contrast, the randomized, controlled trials of intrathoracic impedance have not shown improved outcomes with respect to mortality and rehospitalization. In the LIMIT-CHF (Lung Impedance Monitoring in Treatment of Chronic Heart Failure) study, eighty patients were randomized to either intrathoracic impedance alarm turned on with instructions to increase diuretics for 1 week following an alarm or control (usual care with alarm turned off) (Domenichini et al., 2016). There was no significant difference in HF hospitalization between the two groups. The study found only nonsignificant reductions in time until event diagnosis and time from event to clinical decision. The MORE-CARE (Monitoring Resynchronization Devices and Cardiac Patients) study randomized 8965 patients receiving CRT-D devices to remote device checks which included thoracic impedance versus standard care. Again there was no difference between the two groups in the primary endpoint which included death,

cardiovascular, and device-related hospitalizations (Boriani et al., 2016). The CONNECT (Clinical Evaluation of Remote Notification to Reduce Time to Clinical Decision) study randomized almost 2000 patients after device implantation to either standard care or remote monitoring for 15 months (Cleland et al., 2010). Lead impedance was only one of many data points transmitted. There was no difference in rate of hospitalizations, but did show decrease in median time from event to clinical decision, fewer clinic visits per patient per year, and reduction in the average duration of hospitalizations in the remote monitoring group, all of which were statistically significant.

Much interest remains in determining how best to use these devices in HF monitoring. The PARTNERS-HF (Program to Access and Review Trending Information and Evaluate Correlation to Symptoms in Patients with Heart Failure) study is another prospective, observational study which combined intrathoracic impedance with other measurements—atrial fibrillation duration, rapid ventricular rate during atrial fibrillation, high fluid index, low patient activity, heart rate variability, low CRT pacing, or implantable cardioverter-defibrillator shocks. Having two or more abnormal measurements had a 5.5-fold increased risk of HF hospitalization with pulmonary signs or symptoms within the next month (hazard ratio: 5.5, 95% confidence interval: 3.4–8.8, $p < 0.0001$) (Whellan et al., 2010). PARTNERS-HF is provocative in that it suggests that all device-related parameters, rather than just intrathoracic impedance, should be studied in a randomized, controlled trial. However, CONNECT was a large randomized, controlled trial looking at many of the same measurements and did not demonstrate a benefit in the intervention group with respect to decreasing HF hospitalization.

Several study design and clinical factors were mentioned or alluded to in the aforementioned studies on intrathoracic impedance that may have confounded the results: (1) studying change in intrathoracic impedance from baseline as opposed to an absolute impedance threshold for all patients; (2) studying the same device and leads; (3) obtaining measurements at the same specified time interval after device implantation for all patients; (4) understanding and selecting a specified time interval after device implantation when impedance and lead are considered stabilized; and (5) establishing a standard "euvolemic" impedance at the start of the study when the patient has been objectively determined to be euvolemic.

In patients who qualify for implantation of these devices based on ACC/AHA guidelines, inclusion of this capability does not add risk to the procedure and so expert consensus endorses use of device monitoring in these patients (Wilkoff et al., 2008).

Invasive Monitoring

The latest monitoring technology involves implantation of devices solely for the purpose of hemodynamic monitoring some of which are accompanied by medication management protocols determined by the provider team. In recent years three devices have been developed and tested. Only one of the three devices received FDA approval. All three devices are reviewed in the following, because the trials, tribulations, and processes of each trial illustrate the challenges of studying the applicability of devices in complex diseases such as HF.

Right Ventricular Pressure Monitor

In the normal heart the right ventricular end-diastolic pressure is equal to the pulmonary artery end-diastolic pressure, which is also equal to the pulmonary capillary wedge pressure and the left atrial pressure. Utilizing this concept, an implantable right ventricular pressure monitoring lead called the Chronicle device was developed to continuously measure right ventricular pressures, heart rate, and body temperature. Similar to a pacemaker the device has a generator and a modified unipolar pacemaker lead. The initial pilot study of this device was observational and demonstrated that RVSP increased 4 ± 2 days prior to HF hospitalizations. During the second phase of the pilot study, data was made available to clinical providers for clinical decision making. Hospitalizations during this phase were decreased by 57% ($p < 0.01$) (Adamson et al., 2003). COMPASS-HF (Chronicle Offers Management to Patients with Advanced Signs and Symptoms of Heart Failure), a prospective, randomized clinical trial, compared using hemodynamic data from the Chronicle device to usual care in 274 patients (Bourge et al., 2008). The study showed a nonsignificant 21% reduction in the Chronicle group compared to the usual care control group in the primary efficacy endpoint of reduction in HF events, which included hospitalizations, emergency room, or urgent care visits requiring intravenous diuretic therapy. Weaknesses of the trial included being underpowered for the primary endpoint, having an event rate that was lower than anticipated in the control group, and failure by clinicians to adequately lower filling pressures or titrate medications. Usefulness of the Chronicle device in reducing filling pressures in order to prevent HF events was considered inadequately tested and its utility at this time remains uncertain.

Left Atrial Pressure Monitor

HeartPOD is a device developed to directly measure left atrial pressure. The device consists of a subcutaneously implanted case containing a coil antenna and a sensor lead that is placed in the left atrium through a transseptal puncture. Unique to this device is a handheld "patient advisor module" which when held over the implanted antenna, powers the sensor, receives and transmits left atrial pressure, body temperature, and intracardiac electrocardiogram to the care team. The patient advisor module displays the left atrial pressure to the patient with reminders about physician-directed, individualized medication strategies based on the

readings which encouraged independent patient self-management. HeartPOD was evaluated in the HOMEOSTASIS (hemodynamically Guided Home Self-Therapy in Severe Heart Failure Patients) study in which all 40 patients who had either NYHA class III or IV HF received the device (Ritzema et al., 2010). For the first 3 months both providers and patients were blinded to the hemodynamic and continued with usual care. After 3 months hemodynamic data became available to both the providers and the patients and individualized therapy instructions guided by these pressures were provided to the patients. Results showed significantly improved hemodynamics, survival, and event-free survival. The positive findings warranted further evaluation in a randomized, controlled trial called LAPTOP-HF (Left Atrial Pressure Monitoring to Optimize HF Therapy). Unfortunately LAPTOP-HF was terminated early by the Data and Safety Monitoring Board in June 2014 due to a high number of implant-related complications related to transseptal catheterization. In failure there is always opportunity, and LAPTOP-HF was the first randomized, controlled HF device trial to provide defined treatment guidelines which likely contributed to the success of the pilot trial. More importantly the study highlighted the importance of balancing the risk of invasive devices with the benefits of the information.

Pulmonary Arterial Pressure Monitor

Recently a wireless pulmonary arterial pressure monitoring system called CardioMEMS has been developed and tested. The CardioMEMS device is a small 15×2 mm capacitor that is coil and pressure sensitive without leads and batteries (**Fig. 1**). It is placed in the pulmonary artery by right heart catheterization. The device is powered by electromagnetic coupling from external antennae placed on the back of the patient during readings. When powered, pressure data is collected and transferred wirelessly to a secure website managed by the care team. The CardioMEMS device was validated against the PAC and echocardiogram in a pilot study of 12 HF patients and found to be accurate (Verdejo et al., 2007). Its usefulness was studied in the CardioMEMS Heart Sensor Allows Monitoring of Pressure to Improve Outcomes in NYHA class III Heart Failure Patients (CHAMPION) trial (Abraham et al., 2011b). Five-hundred and fifty patients with NYHA class III HF received the CardioMEMS device. Half were managed using data from the device and the other half randomized to routine usual care. Results of the study showed significantly fewer HF hospitalizations in the CardioMEMS group when compared to the routine care control group (28% risk reduction, $p = 0.0002$). A post hoc analysis of CHAMPION study subjects with HFpEF (LVEF $\geq$ 40%) showed a 46% lower rate of HF hospitalization in the CardioMEMS group when compared with the control group (Adamson et al., 2014). There was a very low complication rate in the CHAMPION trial which was comparable to the reported complication rate of a right heart catheterization. The main differences (and strengths) of this study when compared with prior studies of implanted hemodynamic monitors were the mandated pressure targets and treatment protocols, which were lacking in COMPASS-HF but may have been the main contributing factor to the success of HOMEOSTASIS. A major weakness of the trial design is its inability to separate the contribution of the device data from the contribution of the strict hemodynamic targets and management protocol.

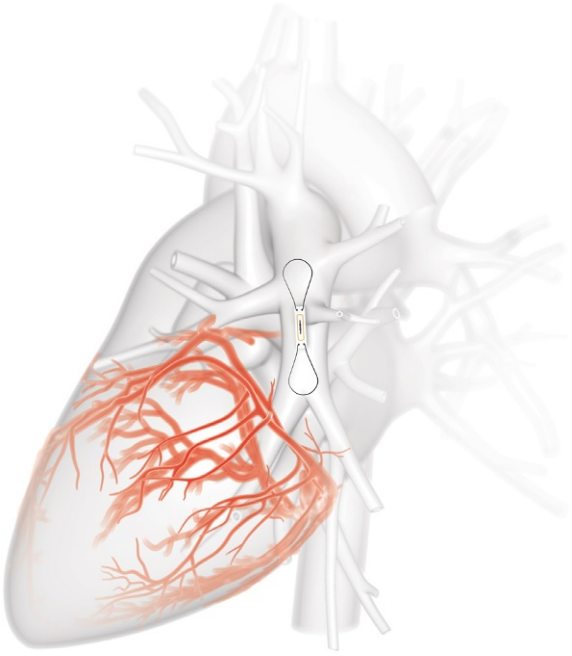

Fig. 1 CardioMEMS device placed in the pulmonary artery. Courtesy of St. Jude Medical, LLC.

Conclusions

Goals of HF monitoring are to reduce hospitalizations, improve QOL, decrease mortality, and prevent secondary end-organ damage. Management of HF is challenging due to geographical barriers, socioeconomic factors, and available resources. Current research evidence identifies the following four interventions as the most effective at reducing HF hospitalization—the multidisciplinary care team, structured telephone support, telemonitoring, and the CardioMEMS device. The metaanalyses and research trials of these four interventions included HFpEF patients and therefore it is reasonable to assume that the results are applicable to all HF patients. The vast majority of the studies published on BVA, biomarkers, and intrathoracic impedance were studies of HFrEF patients. More investigation is needed to establish their roles (if any) in HF monitoring, especially in the HFpEF population.

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Heart Failure With Preserved Ejection Fraction

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Introduction

Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous phenotype that is the product of multiple physiologic and anatomic factors that result in symptoms of heart failure in the setting of normal or near normal systolic function. Exercise intolerance is a nearly universal hallmark feature. It is estimated that nearly 50% of heart failure patients have preserved ejection fractions and that survival is similar to heart failure with reduced ejection fraction (HFrEF) with a fifty percent survival rate at 5 years (Borlaug and Paulus, 2012). Although somewhat lower, the mortality of patients with HFpEF at 3 years approaches that of patients with HFrEF (23.4% vs. 26.3% respectively) (MAGGIC, 2012). In the MAGGIC analysis, patients with HFpEF as compared to those with HFrEF were older (mean age 71 years vs. 66 years), were more often female (50% vs. 28%), more often had a history of hypertension (51% vs. 41%) and atrial fibrillation (27% vs. 18%). They less often had ischemic disease (43% vs. 59%) (MAGGIC, 2012). Risk factors include hypertension with I-PRESERVE and TOPCAT trials reporting a prevalence of 88% and 91%, respectively (Shah et al., 2013; McMurray et al., 2008). Female gender and age greater than 65 years constitute further risk factors (Lekavich et al., 2015). Diabetes and obesity, components of the metabolic syndrome, also seem to be emerging as potential risk factors (Le Winter and Meyer, 2013). In general, HFpEF patients more often die of noncardiovascular and nonheart failure causes than HFrEF (Campbell and McMurray, 2014). Again from MAGGIC, patients with HFpEF had lower risk of cardiovascular death than those with HFrEF with an adjusted HR 0.55 (MAGGIC, 2012). With prevalence estimates projecting an increase of 1% per year (Borlaug and Paulus, 2012), the proportion of patients with heart failure who have preserved ejection fractions will continue to increase.

Pathophysiology

This is a multifactorial process with many proposed pathways and areas of emerging interest and research. The various pathophysiologic pathways that are postulated to promote HFpEF will be discussed.

One area of thought is that there is abnormal systemic vasorelaxation and increased ventricular stiffening which leads to increased filling pressures despite a preserved EF.

Often these patients show concentric LV remodeling, reduced LV passive compliance, and slowed or incomplete relaxation (Borlaug and Paulus, 2012). This contributing factor is highlighted by the fact that diastolic dysfunction is a criteria for diagnosis according to the European diagnostic guidelines. Another potential area of pathology is inflammation. Risk factors such as diabetes and obesity lead to a proinflammatory state which can influence the myocardium to become fibrotic (Le Winter and Meyer, 2013). A recent study that evaluated biomarker levels of HFpEF and HFrEF patients revealed that HFpEF patients have serum markers that are more consistent with inflammation and fibrosis compared to that of HFrEF patients that had upregulation of myocardial stress and injury pathways (Sanders-van Wijk et al., 2015).

At a cellular level, there is hypophosphorylation of protein kinase A and G on cardiac titin which help to modulate stiffness and relaxation, as well as titin isoform modulations leading to isoforms with higher passive stiffness. Additionally, there has been shown to be abnormal calcium handling in the sarcoplasmic reticulum. This is particularly evident during low levels of myocyte stimulation at rates of 100–110 per minute, which would equate to heart rates of 100–110 per minute (Le Winter and Meyer, 2013). This observation may help explain some of the profound exercise intolerance that is observed in this patient population. This is likely secondary to the reduced myocardial relaxation rates resulting in decreased LV stroke volume especially at higher heart rates. This is the opposite of normal myocardium which increases relaxation at higher stimulation rates to allow for active ventricular relaxation and low pressure filling (Borlaug et al., 2006).

Regarding the extracellular matrix, there is an excess of collagen Type 1 which is due to down regulation of matrix metalloproteinases (MMP) which are responsible for collagen degradation. This is in contrast to dilated cardiomyopathy patients who have been found to have an upregulation of MMP leading to increased collagen matrix degradation (Borlaug et al., 2006) and decreased

ventricular thickness. For these reasons, there is an inherent increase in ventricular and vascular stiffening in HFpEF patients. Interestingly, in the HFpEF myocardium, the Starling curve is shifted up and right because of the decrease in LV filling volume and increase in LV pressure. This leads to very large blood pressure fluctuations based on changes in preload and afterload (Borlaug et al., 2006). The high blood pressure that results during exercise further impairs LV filling leading to decreased cardiac output and exercise intolerance. There is also evidence that myocardial energy reserve may be a factor. Myocardial phosphorus MR spectroscopy shows lower creatine phosphate/adenosine triphosphate ratio compared to controls indicating a lower energy reserve (Borlaug et al., 2006). As LV relaxation is an active process, an energy starved tissue may not be able to effectively relax during times of higher demand.

Presenting Symptoms and Exercise Dysfunction

Exercise time, peak workload, peak VO_2, ventilatory threshold VO_2, and percent predicted peak VO_2 were impaired in HFpEF compared to controls. Effort and dyspnea scores were higher in HFpEF at matched submaximal workloads indicating greater perceived difficulty with exercise. Borlaug et al. found limitations in chronotropic, contractile, endothelial, and vascular reserve resulting in impaired ventricular–arterial coupling during exercise. Interestingly, despite a "preserved EF," contractile reserve is impaired in HFpEF patients compared to matched controls. Because of the abnormal vasorelaxation and blunted contractile reserve, ventricular–arterial coupling is impaired with exercise (Borlaug et al., 2010). Also worth noting is the reduction in cardiac index (1.25 in HFpEF vs. 2.02 in healthy controls) due to impaired chronotropy even when controlling for beta blocker therapy. Inappropriate heart rate recovery following exercise and inappropriate heart rate decrease in response to baroreflex stimulation was also demonstrated in this study, which implies impaired autonomic tone (Borlaug et al., 2010). Interestingly, compared to matched controls, there was no difference in pulmonary blood volumes with exercise, thus calling into question the theory that impaired diastology and resultant necessary high filling pressures leads to increased pulmonary volume which leads to dyspnea. Surprisingly, change in end diastolic volume with exercise was similar to age matched controls. In a study which evaluated response to nitroprusside, an exaggerated blood pressure drop was noted in HFpEF patients. Conversely, HFrEF patients experienced a significant increase in stroke volume and cardiac output suggesting that a decrease in afterload is most beneficial to HFrEF patients and that HFpEF patients rely more on ventricular filling for stroke volume, as decreased preload also had an effect here (Schwartzenberg et al., 2012). In HFpEF patients, increased contractility results in a higher end systolic elastance (Ees) compared to controls resulting an exaggerated blood pressure response to exercise as mentioned earlier with the Starling curve shift. Additionally, it has been shown that HFpEF Ea/Ees ratios are generally nearly 0.5 indicating near maximal efficiency along the pressure volume loop (Ea = effective arterial elastance) (Schwartzenberg et al., 2012). This fact is demonstrated by the observation that even slight variations in either preload or afterload will lead to exaggerated variations in systemic arterial pressures. Arterial pressure variations, increased myocardial energy demands and impaired myocardial energy reserve likely all contribute to impaired exercise capacity in patients with HFpEF (Kawaguchi et al., 2003).

Diagnosis

Universally accepted diagnostic criteria for HFpEF remain undefined. However, there are currently four proposed diagnostic frameworks that are used for clinical identification of HFpEF. Initially, in 1998 the European Work Group proposed a framework, including signs or symptoms of heart failure, normal to mildly reduced systolic function, and evidence of diastolic dysfunction which included both echocardiogram and invasive cardiac catheterization measurements (European Study Group on Diastolic Heart Failure, 1998). Vasan and Levy (2000) described a tiered diagnostic approach using possible, probable, and definite categories based on signs or symptoms of heart failure, presence of normal >50% EF within 72 h of heart failure episode, and cardiac catheterization for definite diagnosis of LV diastolic dysfunction (Vasan and Levy, 2000). After much debate about the utility of measuring diastolic dysfunction, a third set of diagnostic criteria appeared making clinical evidence of heart failure and normal LVEF and chamber size essential, and LV hypertrophy, LA enlargement, and echocardiogram, or cardiac catheterization evidence of diastolic dysfunction optional/confirmatory (Yturralde and Gaasch, 2005). The European Society of Cardiology has perhaps the most comprehensive set of diagnostic guidelines, and states that in addition to clinical symptoms of heart failure one must have LVEF $\geq$50%, evidence of diastolic dysfunction as defined by: elevated left ventricular end diastolic pressure (LVEDP) >16 mmHg, pulmonary capillary wedge pressure >12 mmHg, $E/e' > 15$, mitral flow velocity Doppler signal showing E/A ratio <0.5 + deceleration time (DT) >280 ms, LA size >40 mL/m^2 or LV mass >149 g/m^2 for men or >122 g/m^2 for women (Paulus et al., 2007). Of note, the presence of LV mass criteria which places focus on the role of hypertension in pathophysiology. ACCF/ACC defines HFpEF with 3 criteria: clinical signs or symptoms of HF, evidence of preserved or normal LVEF $\geq$50%, and evidence of abnormal LV diastolic dysfunction that can be determined by Doppler echocardiography or cardiac catheterization (Yancy et al., 2013). While there is a proposed definition, there are no sanctioned diagnostic criteria from the ACC. This highlights the difficulty in clinically diagnosing these patients as evidence by the multiple competing diagnoses that must be excluded before arriving at an HFpEF diagnosis (Fig. 1).

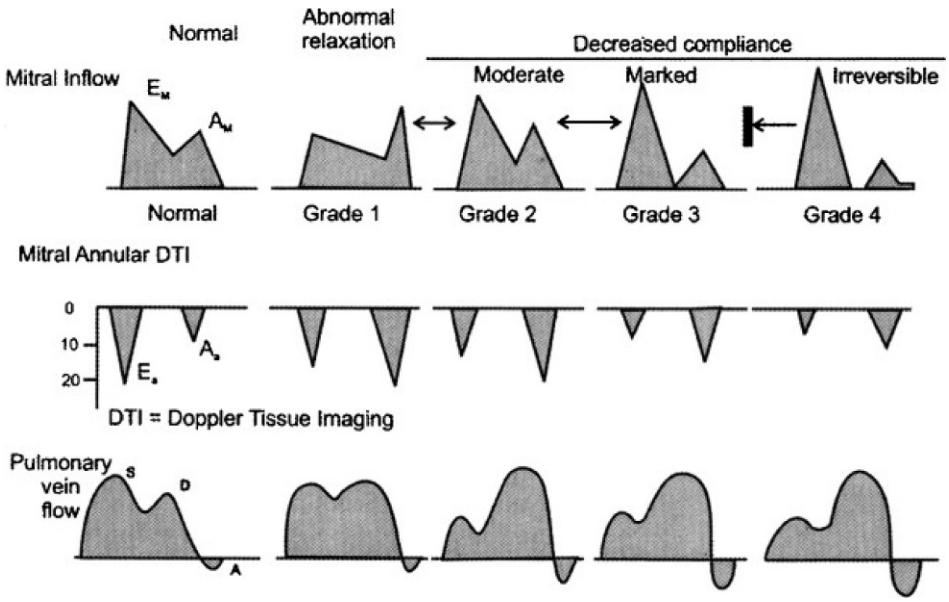

Fig. 1 Schematic representation of mitral inflow, mitral annular Doppler tissue imaging, and pulmonary vein flow in various stages of diastolic dysfunction. Reprinted from Shammas, R.L.; Khan, N.U.; Nekkanti, R.; Movahed A. (2007). Diastolic heart failure and left ventricular diastolic dysfunction: What we know, and what we don't know! *International Journal of Cardiology* **115**(3), 284–292.

Management

The lack of universally accepted diagnostic criteria has confounded the design of clinical trials. The heterogonous nature of patients with HFpEF implies different pathophysiologic mechanisms, risk factors, and natural disease history among this broad group of patients. Often, EF cutoffs of 40%–55% are used although the American Society of Echocardiography (ASE) defines a normal EF as 55% or greater (Lang et al., 2015). This makes universal management guidelines based on randomized trials impossible. This is demonstrated in a recent analysis of the TOPCAT trial patients where evaluation based on EF subgroups in increments of 5% was undertaken. Within each subset, there were differences amongst sex, with males having a lower EF than females even among this "preserved ejection fraction" group. Additionally, the presence of coronary artery disease was lower in patients with higher EF which suggests that patients in EF groups <50% may more frequently have ischemic heart disease. With specific regard to TOPCAT, ultimately with subgroup analysis, patients with an EF ≥ 60% derived minimal benefit from spironolactone therapy and patients toward the lower end of the EF scale (cutoff 45%) derived some benefit from spironolactone (Solomon et al., 2016). This suggests that there are likely different pathophysiologic mechanisms at play with even just minor changes in ejection fraction. Again pointing out perhaps why large-scale trials have been ineffective in identifying potential therapeutic targets.

There are three notable large-scale trials directed toward HFpEF management:

CHARM-Preserved–In the CHARM-preserved trial, the primary outcome of cardiovascular (CV) mortality or admission for worsening congestive heart failure (CHF) was analyzed. Patients were NYHA Class II–IV, had had CV admission for any reason, and LVEF >40%. They were randomized to placebo or candesartan (uptitrated dose to 32 mg/day). There was a boarderline significant trend ($P=0.051$) toward favorable outcomes in candesartan groups for both CV mortality and CHF hospitalizations after adjustment for covariates. There was a significant reduction in CHF hospitalizations in the candesartan group. Note however the LVEF cutoff for this study is 40% which many would argue is too low to meet the accepted definition of HfpEF (Yusuf et al., 2003).

NEAT-HFpEF—evaluated patients with an EF of ≥ 50% and objective heart failure evidence, previous hospitalization for heart failure with radiographic evidence of pulmonary congestion, elevated left ventricular end diastolic pressure at rest (≥ 15 mmHg) or elevated pulmonary capillary wedge pressure at rest (≥ 20 mmHg) or with exercise (≥ 25 mmHg), an elevated level of N-terminal pro-brain natriuretic peptide (NT-proBNP) (>400 pg per milliliter) or brain natriuretic peptide (BNP) (>200 pg per milliliter) or Doppler echocardiographic evidence of diastolic dysfunction. The target dose was 120 mg isosorbide mononitrate. Interestingly, patients were less active on nitrates than placebo (all doses), had lower 6 min walk scores, and had lower quality of life scores along with unfavorable trends in proBNP levels (Redfield et al., 2015). As we have just discussed the mechanism by which slight alterations in preload affect have drastic effects on both stroke volume and blood pressure, this result is not surprising.

TOPCAT was a multicenter trial evaluating the use of spironolactone in patients ≥ 50 years of age with LVEF ≥ 45% and a heart failure hospitalization within 12 months defined according to predefined signs and symptoms. Interestingly, there was no requirement for evidence for diastolic dysfunction in these patients. There was no difference found in primary outcomes of death from CV causes, aborted cardiac arrest, or hospitalization from heart failure. However, the rate of hospitalization for any cause,

including repeat hospitalizations, was reduced in spironolactone patients (394 vs. 475, 6.8 vs. 8.3 per 100 person-years) (Pitt et al., 2014).

Recently, the ACC/AHA/HFSA updated their recommendations regarding HFpEF. It remains a Class I indication to manage hypertension to a systolic target of <130 along with use of diuretics for symptom relief. There is a new Class IIb recommendation to use aldosterone antagonists in patients with an EF ≥ 45% if they meet certain criteria (elevated BNP or HF admission within 1 year, estimated glomerular filtration rate >30 and creatinine <2.5 mg/dL, potassium <5.0 mEq/L) to reduce hospitalizations. There is now a Class III recommendation for routine use of nitrates or phosphodiesterase-5 inhibitors in this patient population (Yancy et al., 2017). To date, there are no randomized controlled trials to show that there are any agents which reduce mortality in HFpEF; thus most therapies are directed at symptoms and risk factor modifications. With regard to atrial fibrillation, a common comorbidity, it is suggested that in HFpEF patients, nondihydropyridine calcium channel blockers may be used with better effect for rate control than beta blockers (Yancy et al., 2013).

Besides the fact that limited to absent diagnostic criteria exists, there are no reliable prognostic or diagnostic biomarkers as such as BNP or NT-proBNP are for HFrEF patients. A recent evaluation did find that significant prognostic markers in HFpEF patients among this particular cohort were: high sensitivity troponin, soluble interleukin-like receptor 1, blood urea nitrogen, soluble fms-like tyrosine kinase, and N-terminal pro-brain natriuretic peptide in regard to both overall and hospital free 18-month survival. These biomarkers were also found to be expressed in HFrEF without any difference in prognostic significance between the two presentations with the exception for Cystatin-C which was less of prognostic value compared to HFrEF. Interestingly, NT proBNP was only significantly useful if practitioners guided their therapy based on symptoms, not NT proBNP levels. A different set of prognostic factors was significantly expressed more in HFrEF patients implying that there are likely several overlapping pathways in both forms of heart failure though some pathways are expressed more than others among the two classifications. In the future these biomarkers may have important therapeutic implications as well as risk stratification possibilities (Sanders-van Wijk et al., 2015).

Conclusion

HFpEF is a growing phenotype of heart failure patients and we are learning more about its pathophysiology. Diagnostic criteria that are reliable and validated are still lacking making therapeutic targets difficult to attain. Until that time, management of risk factors remains the best hope for treating these patients.

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Heart Regeneration with Stem Cell Therapies

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Introduction

Cardiovascular disease remains the leading cause of death worldwide despite current advancements in therapy (Mozaffarian et al., 2016). The morbidity and mortality of ischemic heart disease, the most common type of heart disease, have been greatly reduced by advances in pharmacological and surgical interventions aimed at salvaging viable myocardium. However, these approaches do not prevent the deleterious ventricular remodeling that occurs after myocardial infarction (MI), which can eventually lead to heart failure (HF). The only available option to cure end-stage HF is heart transplantation, which is hampered by low donor availability and issues with long-term immunosuppression. Given the limited options to treat HF, there is a great need for novel therapeutics to slow or even prevent cardiac remodeling and HF.

The paucity of therapies for cardiac disease is in part due to the fact that the heart was considered a terminally differentiated organ without regenerative potential. The landmark discovery of multipotent cardiac stem cells (CSCs), expressing c-kit (CD117), that can give rise to myocytes, smooth muscle, and endothelial cells within the heart, reframed this vital organ as one with self-regenerative capacity (Beltrami et al., 2003). This observation spurred enthusiasm for therapeutic approaches to enhance the innate regenerative capacity of the damaged heart. Stem cells, which are defined by their ability to proliferate and self-renew as well as differentiate into lineages of specialized cell types, have emerged as an exciting new therapy for regenerating the billions of cells that are lost or damaged in cardiac disease.

The first study to evaluate the regenerative capacity of transplanted stem cells in damaged myocardium was performed in 1992 (Marelli et al., 1992). Satellite cells (skeletal muscle stem cells) were implanted into canine hearts following cryoinjury of the myocardium in hopes that these cells would directly differentiate into new cardiomyocytes. Unfortunately, these cells did not produce any clear indication of regeneration. Over the past 20 years, preclinical, translational, and clinical studies have provided strong evidence that stem cells can prevent cardiac remodeling and stimulate repair and replacement of damaged myocardium. Because not all cell types have produced positive results, the search for the most beneficial cell-based therapy continues. The ideal stem cell for the treatment of cardiac disease is one that is readily available, can home (localize) to and survive in damaged myocardium, and can either directly or indirectly repair damaged myocardium. Numerous candidate stem cell types have been examined for their potential benefit, however, given the vast differences between animal models of cardiac disease and actual patients, the ultimate test of stem cell efficacy must be carried out in clinical trials. In this chapter, we will discuss several types of cells utilized in clinical trials and provide future perspectives for stem cell therapy in cardiac disease (**Table 1**).

Table 1 Cell types utilized for cardiac regeneration therapy

Cell type	*Source*	*Common markers*	*Advantages*	*Disadvantages*	*Clinical trials*
Skeletal myoblasts	Muscle biopsy	CD56+	Autologous resistance to ischemia	Do not differentiate into cardiomyocytes ventricular arrhythmias	Phase II
Bone marrow mononuclear cells	Bone marrow	CD117+, CD34+	Extensive clinical background paracrine effects safety profile	Minimal direct myocardial regeneration low engraftment	Phase III
Mesenchymal stem cells	Bone marrow adipose tissue Wharton's jelly and cord blood	CD105+, CD117+, CD45−	Autologous and allogeneic paracrine effects antiinflammatory effects safety profile	Weak cardiomyocyte differentiation low engraftment	Phase III
Endothelial progenitor cells	Bone marrow blood	CD34+, CD133+	Vasculogenesis	Weak cardiomyocyte differentiation low engraftment	Phase III
Cardiac stem cells	Cardiac biopsy	c-kit+, CD45−,	Differentiate into all cardiac lineages	Questionable cardiomyocyte commitment	Phase II
Embryonic stem cells	Embryo	Oct4+, Nanong+, SSEA4+	Pluripotent	Immunosuppression ethical and legal issues teratoma formation	Phase I
Induced pluripotent stem cells	Somatic cells	Oct4+, Nanong +, SSEA4 +	Pluripotent autologous readily obtained	Teratoma formation low maturity	Preclinical stage

Therapeutic Stem Cells

Mechanism of Action

The search for the ideal cell-based therapy has focused on harnessing two major mechanisms for cardiac repair: direct stem cell differentiation into new cardiomyocytes and stem cell stimulation of endogenous cardiac repair mechanisms. Multipotent stem cells such as mesenchymal stem cells (MSCs) can engraft and differentiate into new cardiomyocytes (Quevedo et al., 2009), although there are conflicting results as to degree to which these cells actually differentiate and the maturity of the new cardiomyocytes, and this mechanism may play only a limited role in the therapeutic capacity of these cells in vivo (Silva et al., 2005). MSCs also secrete numerous factors that act in a paracrine manner to promote survival and proliferation of resident cardiomyocytes, stimulate endogenous CSCs, induce angiogenesis, and moderate the damage caused by inflammatory cells and fibroblasts (Golpanian et al., 2016). Finally, MSCs have also been shown to recruit endogenous c-kit + CSCs via cell–cell interactions and enhance their differentiation into cardiomyocytes (Hatzistergos et al., 2010). Indeed, the mechanism by which stem cells prevent and even reverse cardiac remodeling is complex and multifaceted, involving both direct differentiation and modulation of endogenous repair mechanisms including stimulation of endogenous stem cell populations (**Fig. 1**).

Cell Types

Endogenous stem cells are found in virtually every tissue, and initial research in stem cell therapy for cardiac disease focused on multipotent stem cells derived from adult tissues. Given the vast medical experience in harvesting and expanding cells from bone marrow, many studies have examined bone marrow-derived stem cells. Bone marrow contains a heterogeneous population of progenitor cells that includes MSCs, endothelial progenitor cells (EPCs), and hematopoietic stem cells. Clinical trials have examined both fractionated bone marrow, where each stem cell type is isolated and tested individually, or unfractionated bone marrow, where all bone marrow-derived mononuclear cells (BMMNCs) are administered together (Bianco et al., 2001; Lambers and Kume, 2016). The abundant source of diverse stem cells and their ease of isolation have made bone marrow an attractive cell source, and bone marrow-derived stem cells have been utilized for more cardiac regenerative research than any other stem cells to date (Nguyen et al., 2016). Stem cells derived from other adult tissues including peripheral blood and adipose (fat) tissue have also been applied to clinical trials for cardiac disease (Houtgraaf et al., 2012).

Other cells such as induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) are attractive as therapeutics because of their greater capacity to differentiate into new cell types, including cardiomyocytes. Compared to multipotent bone marrow-derived stem cells identified above, ESCs and iPSCs are pluripotent (i.e., they can develop into any type of cell of the body except for extra-embryonic tissue). Pluripotent cells possess a vast potential for differentiation and proliferation. However, the therapeutic application of these cells is hampered by the risk of tumor formation, such as teratomas and teratocarcinomas, and the observation in preclinical studies that these cells fail to fully mature in vivo (Sun and Nunes, 2017; Hamel et al., 2017). Ethical concerns are also associated with the use of human ESCs since these cells are isolated from preimplantation embryos. Together, these issues have limited the therapeutic use of ESCs.

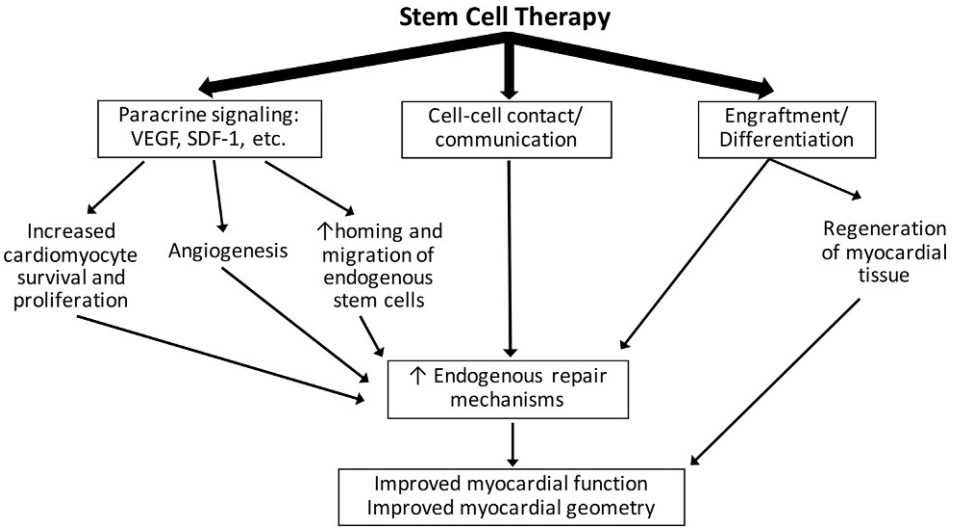

Fig. 1 Mechanisms of action of stem cell therapy for cardiac disease. Stem cells lead to improved cardiac structure and function by reversing myocardial remodeling. This occurs through a multifaceted process involving both direct repair via engraftment differentiation of the transplanted stem cells and indirect stimulation of endogenous repair mechanisms through paracrine signaling and cell–cell interactions. MSCs, in particular, can stimulate neoangiogenesis, migration of endogenous cells, and cardiomyocyte survival via paracrine signaling. They secrete secreting cytokines and growth factors including vascular endothelial growth factor (VEGF), and stromal cell-derived factor-1 (SDF-1) among others. Adapted from Karantalis, V., Balkan, W., Schulman, I. H., Hatzistergos, K. E. and Hare, J. M. (2012). Cell-based therapy for prevention and reversal of myocardial remodeling. *American Journal of Physiology. Heart and Circulatory Physiology* **303**, H256–H270, with permission from the publisher.

Another type of pluripotent cell was introduced in 2006 when Dr. Shinya Yamanaka demonstrated that adding genes that encode four specific transcription factors converts terminally differentiated adult cells into iPSCs (Takahashi and Yamanaka, 2006). This technique holds great promise for the field because iPSCs can be propagated indefinitely and can be induced to differentiate into a wide variety of cell types, including cardiomyocytes. For this momentous discovery, Dr. Yamanaka and Sir John Gurdon were awarded the 2012 Nobel Prize. iPSCs appear to offer many of the same advantages of ESCs without the ethical issues. However, similar to ESCs, iPSCs have the potential for tumor formation and immune incompatibility between donors and recipients (Medvedev et al., 2010). Despite these issues, iPSCs remain promising and exciting therapeutic candidates for regenerating the damaged heart and are being studied extensively in preparation for future clinical use.

Autologous Versus Allogeneic Stem Cells

One area of controversy in stem cell therapy is the source of the cells, either autologous or allogeneic. Some studies have used stem cells isolated from the patient's own tissue (autologous cells), while others advocate the use of stem cells from healthy donors (allogeneic cells). Despite the potential risk of cell rejection by the recipient, allogeneic cells offer the possibility of an effective, off-the-shelf therapeutic. Autologous cells must be harvested from the donor and expanded in culture, and the number and quality of the cells obtained are often dependent on the health status of the donor. In contrast, allogeneic cells, particularly if obtained from young, healthy donors, produce better outcomes than autologous cells (Cogle et al., 2014; Poglajen and Vrtovec, 2015). Clinical trials comparing autologous and allogeneic stem cells in cardiac disease suggest that allogeneic cells provide greater benefit to patients (Hare et al., 2012, 2017; Premer et al., 2015a).

Methods of Delivery

In addition to the cell type, the dosage, the timing (either in the acute phase or chronic phase of cardiac disease), and delivery method are all important practical considerations in determining the ideal cell-based therapy. Currently, four main cell delivery has been employed, two that require catheter access, intracoronary (arterial and venous) and transendocardial delivery, and two that do not require catheterization, epicardial and intravenous delivery (**Fig. 2**). Intracoronary delivery involves the injection of the cells into the coronary vessels that perfuse the heart tissue, but requires the stem cells to transmigrate across the blood vessel wall to reach the myocardium. Transendocardial delivery employs a small needle at the end of the catheter to directly inject the cells into the ventricular myocardium and serves to concentrate the cells into specific areas surrounding the infarcted area (scar) following MI. Epicardial injections are delivered to the outside of the heart accessed via surgery, while intravenous injection can involve either stem cell infusion into the coronary veins or the peripheral vasculature. While there are advantages and disadvantages to each strategy, there remains no definitive evidence of the superiority of one method over another; however, a recent meta-analysis suggests that for MSCs, transendocardial delivery provided the most favorable results (Kanelidis et al., 2017).

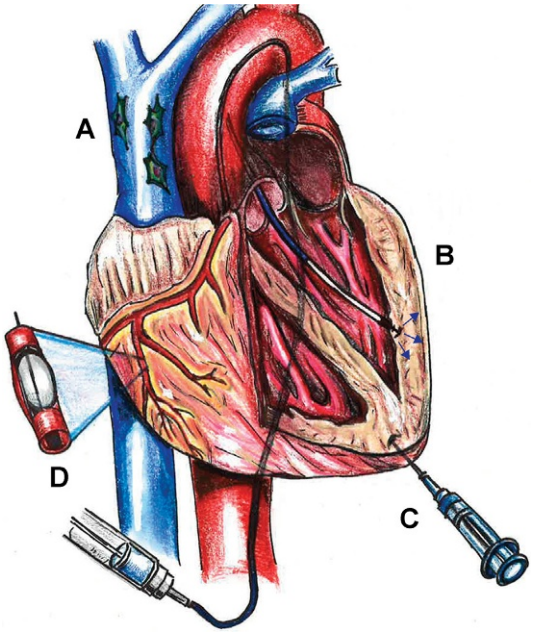

Fig. 2 Methods of stem cell delivery. (A) Intravenous delivery (peripheral veins not shown). (B) Transendocardial injection via catheter. (C) Epicardial injection. (D) Intracoronary infusion via catheter. Reproduced from Golpanian, S., Wolf, A., Hatzistergos, K. E. and Hare, J. M. (2016). Rebuilding the damaged heart: Mesenchymal stem cells, cell-based therapy, and engineered heart tissue. *Physiological Reviews* **96**, 1127–1168, with permission.

Bone Marrow-Derived Mononuclear Cells

Supported by the vast clinical experience in bone marrow transplant medicine, BMMNCs were the first stem cells to be incorporated into treatment for heart disease. BMMNCs are comprised of multiple progenitor cells and are isolated from bone marrow aspirates from the pelvis. The cells are then expanded in culture and delivered to the recipient patient, usually as autologous transplants. In 2002, the first randomized placebo-controlled trial using BMMNCs for heart disease was performed to evaluate their efficacy after intracoronary infusion in patients who had suffered an acute myocardial infarction (AMI) (Assmus et al., 2002). The initial beneficial effects of these cells were hypothesized to be from modulation of postinfarction left ventricle (LV) remodeling, and the 5-year follow-up results demonstrated, modest but sustained improvement of left ventricular ejection fraction (LVEF) (Leistner et al., 2011). This early, small trial highlighted the potential therapeutic benefits of the cells, and several subsequent trials have been done in both acute and chronic ischemic cardiac disease. Unfortunately, BMMNC treatment has failed to show consistent results across multiple studies.

BMMNCs were first tested as therapeutics after AMI in hopes that cell delivery in the early stage of ischemic cardiac disease would attenuate cell death, scar formation, and the development of ischemic HF. Strauer et al. were the first to show that intracoronary infusion of BMMNCs for AMI improves cardiac perfusion and function (Strauer et al., 2002). Additional benefits, including improved LVEF, regional wall motion, and reduced end systolic volume (ESV) have also been seen (Assmus et al., 2002; Fernandez-Aviles et al., 2004). The "Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction" (REGENT, NCT00316381) trial compared fractionated BMMNCs to CD34+/CXCR4+ (fractionated) bone marrow-derived cells and placebo in patients with AMI and impaired LVEF. Treatment was delivered by intracoronary injection. Neither cell treatment produced improvements compared to controls (Tendera et al., 2009). In the "Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration" (BOOST trial, NCT00224536) intracoronary delivery of BMMNCs after AMI resulted in a significant improvement in LVEF at 6 months compared to placebo (Wollert et al., 2004); however, there were no long-term benefits noted at the 18-months follow-up (Meyer et al., 2006). While these cells have consistently proven to be safe, multiple studies have failed to show a conclusive benefit in both short and long-term follow-up. In fact, a recent meta-analysis investigating 2732 patients found no differences in mortality or major adverse cardiac events (MACE) associated with BMMNC therapy compared to controls and no differences in LVEF or New York Heart Association (NYHA) class in short-term follow-up. The authors concluded that there was inadequate evidence of a positive effect of cell therapy in the acute setting. A significant limitation of this meta-analysis was its lack of power, given the small sample size of each trial (Fisher et al., 2015). The absence of a consistent benefit of BMMNCs may be secondary to the proinflammatory environment of an acute insult, but such an environment is hostile to all cell types, including BMMNCs. Given the small size and inconsistent results of these phase I and II trials, it remains unclear if BMMNCs truly provide a benefit for acute MI.

Two clinical trials have been conducted to determine the optimal timing of delivery of BMMNCs following MI. The "Transplantation in Myocardial Infarction Evaluation" (TIME, NCT00684021) study was published in 2013 and compared the

effects of intracoronary cell delivery versus placebo at 3 and 7 days after ST-segment elevation MI in 120 patients (Traverse et al., 2012). The Late TIME trial (NCT00684060) randomized 87 patients to receive autologous BMMNCs versus placebo 2–3 weeks after PCI for MI (Traverse et al., 2011). In both studies, there were no significant improvements at 6 months follow-up in LVEF or regional wall motion compared to placebo. Taken together, these negative studies provide further evidence that BMMNCs therapy may not be efficacious in the setting of AMI. The "Effect of Intracoronary Reinfusion of Bone Marrow-derived Mononuclear Cells on All Cause Mortality in Acute Myocardial Infarction" (BAMI, NCT01569178), a Phase III European multicenter placebo-controlled trial with a projected enrollment of 3000 patients, will be appropriately powered and could provide definitive conclusions as to the therapeutic efficacy of BMMNCs for AMI (Mathur et al., 2017).

Similar to the AMI setting, autologous BMMNCs have produced mixed results in chronic ischemic HF (Taylor et al., 2016). The safety of transendocardial injection of BMMNCs was first established by Perin et al. who observed no periprocedural events upon injection of cells in patients with chronic ischemic cardiomyopathy (ICM). The cell-treated patients also experienced significant improvements in perfusion and contractility in viable areas of the myocardium (Perin et al., 2003, 2004). Similarly, subjects in a trial with chronic ischemia experienced enhanced myocardial perfusion and cardiac function 3 months after transendocardial BMMNC injection (Tse et al., 2003). The "Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction" TOPCARE-CHD (NCT00289822) study reported reductions in probrain natriuretic peptide and proatrial natriuretic peptide (both of which are elevated in HF) at 3 months following intracoronary injection of BMMNCs in subjects with ischemic HF (Assmus et al., 2007). However, others have reported conflicting results. While BMMNC treatment in patients with ischemic HF did improve angina symptoms and quality of life scores, cell treatment failed to significantly increase myocardial perfusion and functional capacity as assessed by myocardial oxygen consumption (MVO_2) and LVEF (Perin et al., 2011). Interestingly, upon secondary analysis, subjects <60 years of age had higher levels of mesenchymal progenitor cells in their BMMNC population and experienced improved MVO_2 as compared to controls (Perin et al., 2011). The larger "Effectiveness of Stem Cell Treatment for Adults with Ischemic Cardiomyopathy" (FOCUS-Study, NCT00824005) trial did not meet its functional end points by failing to show improvements in LVEF, MVO_2, or perfusion 6 months after transendocardial stem cell injection. However, on posthoc analysis, an increase was observed in LVEF, left ventricular end systolic volume (LVESV), and MVO_2 max in patients with higher levels of $CD19^+$ B cells, $CD11b^+$ monocytes, $CD31^{dim}$ subsets, CXCR4+ migratory cells and lower levels of $CD31^{bright}$ lymphocytes in their bone-marrow profile at baseline (Perin et al., 2012; Taylor et al., 2016). The differences in patient response based on age and the types of progenitor cells present in their harvested bone marrow highlight the greater efficacy specific cells types and cells harvested from younger patients. Finally, the recent "Retrograde Delivery of Autologous Bone Marrow in Patients With Heart Failure" (REVIVE, NCT01299324) trial evaluated the effects of BMMNCs delivered via retrograde coronary sinus injection, in ischemic and nonischemic HF subjects. This trial showed that cell delivery was safe and although not all secondary endpoints were met, LVEF was improved in both groups and the end systolic diameter was decreased in the nonischemic HF group (Patel et al., 2015).

Given the discrepancy between conditions, delivery method and follow-up in these studies, there are a number of variables that could be modified in an effort to enhance the efficacy of future BMMNC trials. In the AMI setting, cell therapy has overall produced little or no lasting improvements in cardiac structure and function, possibly due to poor stem cell survival in the inflammatory milieu of the AMI tissue. Cell therapy may be more efficacious if injected once the acute inflammatory response has waned but before deleterious post-MI remodeling has become irreversible. Once the untoward effects of HF have set in, secondary analysis of trials in the chronic setting has shown us that patient age may influence the functional efficacy of autologous BMMNCs. The reduced effectiveness of cells from older individuals suggests that allogeneic cells may be more beneficial than their autologous counterparts, although potential issues related to immune tolerance may need to be addressed.

Further research on BMMNC therapy has focused on identifying the most effective, fractionated subpopulation of progenitor cells. Murray et al. found that one constituent of BMMNCs, hematopoietic stem cells, do not readily acquire a cardiac phenotype following MI (Murry et al., 2004), suggesting their weak regenerative capacity. In contrast, specific nonhematopoietic subtypes of BMMNCs such as MSCs and EPCs provide significant contributions to regeneration. MSCs have been extensively studied based on their relative ease of isolation, immunomodulatory properties, potential as an allogeneic cell therapeutic, secretion of paracrine factors, and the ability, albeit at low frequency, to engraft and differentiate into cardiomyocytes and vascular components (Hatzistergos et al., 2010). EPCs produce endothelial cells that line the inner surface of vessels and EPC function correlates with cardiac function (Munzel et al., 2008). In clinical trials, EPCs improve myocardial perfusion (Quyyumi et al., 2011) and symptoms related to refractory angina (Henry et al., 2016).

Mesenchymal Stem Cells

Multiple trials utilizing BMMNCs have not produced consistent positive findings, leading to a search for a more therapeutically potent cell. Bone marrow-derived mesenchymal stem cells (BMMSCs) comprise approximately 0.1%–0.05% of BMMNCs and were first reported in 1970 (Friedenstein et al., 1970). MSCs are defined by their ability to adhere to plastic, expression of the cell surface markers CD105, CD73, and CD90, the absence or very low levels of several other specific markers including CD34 and CD45, and the ability to differentiate into osteogenic, adipogenic, and chondrogenic lineages in vitro (Dominici et al., 2006). MSCs are found in multiple tissue types, including bone marrow, heart, umbilical cord, and adipose tissue (Golpanian et al., 2016). These cells are ideal candidates for cardiac therapy because they are easy to isolate from these various tissues, are true multipotent stem cells, and are immunotolerant and immunosuppressive, eliciting virtually no rejection response from the recipient when delivered as an

allogeneic product (Hare et al., 2012, 2017). As a cell therapy for the cardiac disease, MSCs display greater clinical efficacy compared to BMMNCs (Heldman et al., 2014).

This improved benefit of MSCs in cardiac regeneration is likely related to the factors they secrete, which act locally on nearby cells (paracrine effect). These paracrine factors promote immunomodulation, neovascularization, and remodeling of the extracellular matrix leading to reduced scar size (Karantalis et al., 2012; Sanina and Hare, 2015; Williams and Hare, 2011). MSCs secrete numerous factors including vascular endothelial growth factor (VEGF) (Wang et al., 2014), angiopoetin-1 (Kalinina et al., 2015), fibroblast growth factor (FGF) (Wang et al., 2014), interleukin (IL)-1 and IL-6 (Jaimes et al., 2017), stromal cell-derived factor 1 (SDF1) (Ghadge et al., 2011), and insulin-like growth factors I and II (Cheng et al., 1998). Other effects are mediated by cell-to-cell contact and direct differentiation. MSCs have been evaluated in several clinical trials for their safety and feasibility in acute and chronic heart diseases.

Bone marrow-derived mesenchymal stem cells

In 2004, Chen et al. investigated the effect of autologous BMMSCs delivered by intracoronary injection in patients with AMI. Cells were delivered approximately 18 days after the initial percutaneous stent procedure. This study showed improvement in LVEF, systolic and diastolic LV volumes, and perfusion defects compared to placebo. These improvements were maintained at 6 months follow-up (Chen et al., 2004). Hare et al. demonstrated the safety and efficacy of allogeneic MSC therapy for AMI in a phase I clinical trial. This double blinded, placebo-controlled study showed that MSCs from young, healthy donors significantly improved forced expiratory volumes (FEV1), LV end diastolic volume, LV function, and reduced ventricular arrhythmias at 12 months compared to placebo (Hare et al., 2009). In a phase I/II clinical trial, Chullikana et al. confirmed that allogeneic MSCs are safe (Chullikana et al., 2015). They assessed markers of inflammation and rejection of the allogeneic cells by evaluating lymphocyte markers (CD3, CD4, CD8) and serum proinflammatory cytokines levels such as IL-1, TNF-α, and interferon-γ, but observed no evidence of immunologic response, further supporting the safety of allogeneic MSC therapy.

The promising results seen in the acute phase of ischemic heart disease encouraged the application of MSC therapy to chronic disease. In 2006, Chen et al. evaluated the effect of autologous BMMSCs in patients with chronic ICM. The results of this trial demonstrated a significant decrease in perfusion defect from baseline to 12 months and patient functional capacity, as indicated by the 6-minute walk test, Minnesota Living with Heart Failure Questionnaire (MLHFQ) score, and NYHA classification improved after 3 months compared to placebo (Chen et al., 2006). The double blinded, randomized "Bone Marrow-Derived Mesenchymal Stromal Cell Treatment in Patients with Severe Ischaemic Heart Failure" (MSC-HFNCT00644410) trial compared intramyocardial injection of placebo versus autologous BMMSCs in patients with severe ischemic HF. In this trial, cell-treated patients exhibited significantly improved LVESV, stroke volume, myocardial mass, and LVEF compared to placebo. Despite these changes in cardiac volumes and pump function, overall patient functional parameters did not show a significant improvement (Mathiasen et al., 2015). The "Transendocardial Autologous Cells (hMSC or hBMC) in Ischemic Heart Failure Trial" (TAC-HFT, NCT00768066) was a randomized, double-blind, placebo-controlled trial that evaluated the safety and efficacy of autologous BMMSCs as compared to BMMNCs and placebo (Heldman et al., 2014). This clinical trial was the first to directly compare two different cell types delivered via transendocardial injection into patients with chronic ICM. Patients treated with both cell types showed a significant improvement in the MLHFQ score; however, 6-minute walk distance, regional cardiac contractility, cardiac strain, and scar size were improved only in the BMMSC group (**Fig. 3**). This study was the first to provide evidence that BMMSCs produce greater effects on cardiac remodeling, scar size reduction, and functional parameters when compared to BMMNCs. The PROMETHEUS trial (NCT00587990) examined the efficacy of autologous BMMSCs delivered via epicardial injection in ICM patients undergoing coronary artery bypass graft (CABG) surgery. This trial showed that areas of the heart receiving BMMSCs exhibited significant improvements in functional and structural parameters that in turn drive improvement in global LV function (Karantalis et al., 2014).

MSCs are immunotolerant (do not promote immune rejection) because they lack major histocompatibility complex class II antigens and are also immunosuppressive. These properties make them favorable candidates for allogeneic transplantation, as they are less likely to be identified and destroyed by the recipient's immune system (Karantalis et al., 2015a). The Comparison of Allogeneic versus Autologous Bone Marrow-Derived Mesenchymal Stem Cells Delivered by Transendocardial Injection in Patients with Ischemic Cardiomyopathy (The POSEIDON Randomized Trial: NCT01087996) was the first to compare three different doses (20 million, 100 million, and 200 million) of both autologous and allogeneic MSCs injected in a transendocardial fashion in patients with ICM. This trial demonstrated that both cell types were equally safe. Allogeneic and autologous cells alike decreased scar size and improved the sphericity index (a measure of LV dilation, which is increased in HF). The allogeneic group improved the LV end diastolic volume while the autologous group did not. Conversely, the autologous group improved in the 6-minute walk test and the MLHFQ, while the allogeneic group remained unchanged. Interestingly, the 20 million group produced the greatest improvement in EF and LV volume as compared to the others, which demonstrates an inverse dose response, a rather common finding in cardiac cell-based therapy (Hare et al., 2012; Suncion et al., 2014; Tompkins et al., 2017). A subanalysis from this study showed that unlike autologous BMMSCs, allogeneic cells improved EPC proliferation and flow-mediated vasodilation, two parameters that are impaired in HF; indicating improved functioning of endothelial cells (Premer et al., 2015b).

While the vast majority of studies examining the benefits of stem cell therapy for cardiac disease have focused on acute and chronic ischemic HF, nonischemic dilated cardiomyopathy (NIDCM), remains a challenging and widespread disease with no cure except heart transplant. Recent studies have begun to assess the effect of stem cell therapy on patients with NIDCM. A case report published in 2011 documented that the intracoronary infusion of autologous MSCs in an 11-year-old boy with progressive dilated cardiomyopathy was safe and improved the patient's clinical condition including improvements in NYHA functional class, paroxysmal nocturnal dyspnea, and reduced the hospitalization rate (Zeinaloo et al., 2011).

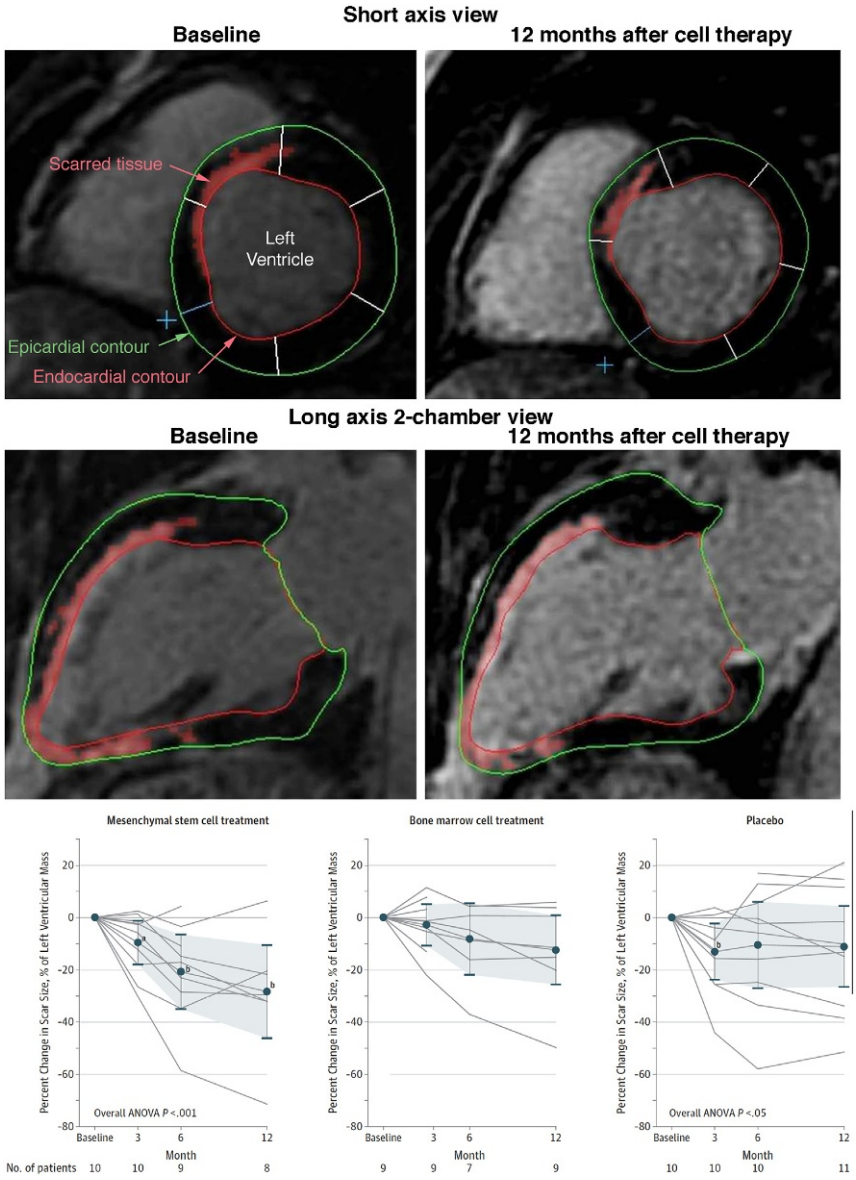

Fig. 3 Chronological change of scar evaluated by cardiac magnetic resonance imaging. Short-axis and long-axis two-chamber views of the heart, with delayed tissue enhancement depicting scar tissue (*pink*). Twelve months after injection of mesenchymal stem cells, scar mass was reduced in a patient from the TAC-HFT trial. Scar mass as a percentage of left ventricular mass was reduced in the MSC-treated and placebo groups. Reproduced from Heldman, A. W., Difede, D. L., Fishman, J. E., Zambrano, J. P., Trachtenberg, B. H., Karantalis, V., Mushtaq, M., Williams, A. R., Suncion, V. Y., Mcniece, I. K., Ghersin, E., Soto, V., Lopera, G., Miki, R., Willens, H., Hendel, R., Mitrani, R., Pattany, P., Feigenbaum, G., Oskouei, B., Byrnes, J., Lowery, M. H., Sierra, J., Pujol, M. V., Delgado, C., Gonzalez, P. J., Rodriguez, J. E., Bagno, L. L., Rouy, D., Altman, P., Foo, C. W., Da Silva, J., Anderson, E., Schwarz, R., Mendizabal, A. and Hare, J. M. (2014). Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: The TAC-HFT randomized trial. *JAMA* **311**, 62–73, with permission of the publisher.

The recent "Percutaneous Stem Cell Injection Delivery Effects On Neomyogenesis in Dilated Cardiomyopathy Trial" (POSEIDON-DCM trial, #NCT01392625), a counterpart to the POSEIDON trial in ICM patients, compared transendocardial delivery of allogeneic and autologous BMMSCs in an NIDCM population and demonstrated the safety of both cell types. Allogeneic BMMSCs produced greater improvements in cardiac function, performance (6-minute walk distance, MLHFQ), endothelial function, and FEV1 than autologous MSCs (Hare et al., 2017) (**Fig. 4**). These changes were accompanied by reductions in the proinflammatory serum immune marker TNF–α. Interestingly, allogeneic cell treatment also led to a systemic decrease in exhausted (poorly functional) T-cells (important mediators of adaptive immunity) as well as an increase in %Switch Memory B-cells (a predictive biomarker of antibody response to pathogens) (Hare et al., 2017). Butler et al. evaluated the benefits of allogeneic "ischemia tolerant" MCSs (grown in hypoxic conditions) delivered intravenously to NIDCM patients. This single-blinded, controlled, crossover study showed that cell treatment produced improvements in the 6-minute walk distance, Kansas City Cardiomyopathy Questionnaire scores and serum immune cells (CD3+, CD4+, and natural killer cells). In addition, there was no difference in rehospitalization or adverse effects up to 90-days posttreatment compared to the control group (Butler et al., 2016).

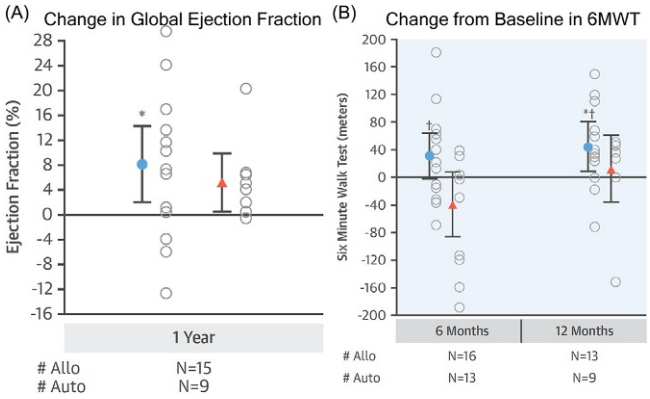

Fig. 4 Allogeneic MSCs demonstrate superior efficacy for treating NIDCM. Twelve months after treatment with MSCS, NIDCM patients from the POSEIDON-DCM trial showed improved (A) EF and (B) 6MWD. These improvements were superior in the allogeneic MSC-treated (*blue circles*) compared to autologous MSC treated (*red triangles*) patients. Adapted from Hare, J. M., Difede, D. L., Rieger, A. C., Florea, V., Landin, A. M., El-Khorazaty, J., Khan, A., Mushtaq, M., Lowery, M. H., Byrnes, J. J., Hendel, R. C., Cohen, M. G., Alfonso, C. E., Valasaki, K., Pujol, M. V., Golpanian, S., Ghersin, E., Fishman, J. E., Pattany, P., Gomes, S. A., Delgado, C., Miki, R., Abuzeid, F., Vidro-Casiano, M., Premer, C., Medina, A., Porras, V., Hatzistergos, K. E., Anderson, E., Mendizabal, A., Mitrani, R. and Heldman, A. W. (2017). Randomized comparison of allogeneic versus autologous mesenchymal stem cells for nonischemic dilated cardiomyopathy: POSEIDON-DCM trial. *Journal of the American College of Cardiology* **69**, 526–537, with permission from the publisher.

Adipose-derived mesenchymal stem cells

While BMMSC isolation requires aspiration of bone marrow from the pelvis, adipose-derived mesenchymal stem cells (AdMSCs) can be isolated from biopsies of fat tissue. The safety and efficacy of AdMSCs for ICM were first assessed in "A Randomized Clinical Trial of AdiPOse-derived Stem ceLLs in the Treatment of Patients With ST-elevation myOcardial Infarction-the APOLLO trial" (NCT00442806) in which AdMSCs were delivered via an intracoronary route to patients with ICM. This randomized, double-blind, placebo-controlled, phase I/IIa trial established the safety of AdMSCs, as well as their ability to induce significant reductions in scar size and improve myocardial perfusion (Houtgraaf et al., 2012). Additional phase II clinical studies, including ADVANCE (NCT01216995) and AMICI (NCT01781390), where AdMSCs were used to treat AMI, have not reported their results.

Endothelial Progenitor Cells

Another stem cell constituent of BMMNCs, EPCs have also garnered interest as a potential therapeutic for cardiovascular disease. EPCs are CD34+ mononuclear cells that can be isolated from bone marrow or peripheral blood. CD34 is a cell surface marker primarily expressed on the surface of endothelial, hematopoietic, and vascular cells (Asahara et al., 1997). Cell populations enriched for CD34+ promote neovascularization and restore microcirculation in ischemic limb and ischemic myocardial models more effectively than unselected mononuclear cells (Kawamoto et al., 2006). EPCs, because of their ability to induce angiogenesis and modify endothelial function, were first tested in the setting of angina (chest pain secondary to inadequate blood flow to the myocardium) and their use was expanded to both ICM and NIDCM.

Bone marrow-derived EPCs

Initial studies utilizing EPCs as a therapy for heart disease demonstrated that peripheral blood or bone marrow-derived CD34+ cells can improve clinical outcomes in patients with refractory angina, including the incidence of MI, the frequency of angina episodes, and exercise tolerance. Losordo et al. demonstrated that intramyocardial injection of autologous EPCs is safe, feasible, significantly decreased weekly angina frequency, and improved exercise tolerance in patients with Canadian Cardiovascular Society (CCS) class III–IV angina at enrollment compared to placebo (Losordo et al., 2007). EPC therapy also increased myocardial perfusion, but notably, these improvements were more pronounced with lower doses of EPCs (Losordo et al., 2011). A similar study showed that autologous bone marrow-derived EPCs reduced angina frequency and nitroglycerin use per week, and increased exercise tolerance time and CCS angina class in patients with intractable angina (Wang et al., 2010). The larger phase III RENEW trial (NCT01508910) examined intramyocardial EPC delivery for angina, but was terminated prior to completion due to financial issues; however, the midterm results were consistent with observations from previous trials (Povsic et al., 2016).

While EPCs have produced beneficial effects in patients with refractory angina, they have also shown promise as therapeutics for both ischemic and nonischemic cardiomyopathies. The potential benefits of EPC therapy in HF were investigated by transplanting autologous CD34+ cells into patients with ICM and an LVEF $\leq$ 35%, during CABG surgery. Cell treatment elicited significant improvement in LVEF at 1, 3, and 6 months compared to the CABG only group (Patel et al., 2005). The multicenter, randomized placebo-controlled REGENT trial (NCT00316381) compared nonselected BMMNCs with selected CD34+/CXCR4+ EPCs (Tendera et al., 2009). As described above, the cell therapy provided no benefit to patients with AMI compared to placebo after 6 months.

EPCs induce modest improvements in LVEF when administered to patients with ICM and their effects on patients with NIDCM have been even more impressive. In a study by Vrtovec et al., 110 patients were randomized to receive autologous EPC treatment or

placebo control (Vrtovec et al., 2013). In this study, EPCs were mobilized by granulocyte colony-stimulating factor and injected into the coronary artery supplying cardiac segments with the greatest perfusion defect (limited blood supply). After 1 and 5 years, the cell treated group showed a significant improvement in LVEF, 6-minute walk distance, and long-term survival (Vrtovec et al., 2013). In a later study, this group also demonstrated that transendocardial CD34+ cell therapy is ineffective for NIDCM patients with diabetes mellitus (Vrtovec et al., 2016). Treatment increased LVEF and decreased N-terminal probrain natriuretic peptide levels in the insulin-resistant and non-insulin-resistant groups, but not in the diabetes mellitus group at 6-months posttreatment (Vrtovec et al., 2016).

Circulating EPCs

EPCs travel through the circulatory system, and endogenous bone marrow-derived CD34+ EPCs are actually mobilized to the peripheral circulation during the natural history of AMI. Moreover, the degree of mobilization has been correlated with improved outcomes after MI, which spurred the hypothesis that circulating EPCs may be beneficial in the acute phase of the ischemic cardiac disease (Quyyumi et al., 2016). Therefore, circulating progenitor cells have been studied as a treatment for cardiac repair in several trials. In the "Transplantation Of Progenitor Cells And Regeneration Enhancement in Acute Myocardial Infarction" (TOPCARE-AMI) trial (Schachinger et al., 2004), intracoronary injection of peripherally-derived circulating progenitor cells improved global LVEF and LVESV after 4 months. At 1-year follow-up, imaging analysis showed improved LVEF, scar size, and LV hypertrophy in the cell-treated group. In a randomized, placebo-controlled study of intracoronary EPC delivery in patients after recanalization of chronic coronary occlusion, EPC treatment produced increased coronary flow reserve, reduced infarct size, and increased LVEF in the treatment group at 3 months posttreatment (Erbs et al., 2005). Despite these initial promising results, a study comparing circulating progenitor cells to BMMNCs in patients with stable ischemic heart disease showed that bone marrow-derived cells were superior to circulating progenitor cells (Assmus et al., 2006). Global LVEF and regional contractility assessed at 3 months posttreatment were significantly increased in the bone marrow group only, but not in the circulating progenitor cell group (Assmus et al., 2006).

Bone marrow-derived CD133+ EPCs

In addition to CD34, CD133, a prominin 5 transmembrane glycoprotein 1, is coexpressed in a substantial number of hematopoietic cells with potent hematopoietic and angiogenic capacities (Quirici et al., 2001; Reyes et al., 2002). Injection of bone marrow-derived CD133+ cells into the infarct zone during CABG resulted in significant improvements in LV end diastolic volume, LVEF, and local perfusion in the stem cell-treated infarct area at 12 days posttreatment relative to baseline (Stamm et al., 2004). Another study investigating the safety and efficacy of intracoronary administered CD133+ cells into a patient with AMI (Bartunek et al., 2005) demonstrated feasibility but was associated with increased incidence of coronary events (Bartunek et al., 2005). In the "Safety and Efficacy of Autologous Endothelial Progenitor Cell CD133 for Therapeutic Angiogenesis" trial (PROGENITOR, NCT00694642) (Jimenez-Quevedo et al., 2014), a phase I/II, multicenter, single-blinded and randomized trial, patients with refractory angina were treated with granulocyte-colony-stimulating factor and randomized to receive CD133+ cells or no treatment. Although the findings supported the feasibility and safety of transendocardial injection of CD133+ cells into patients with refractory angina, the trial did not meet its primary endpoints of reducing cardiovascular death, nonfatal MI, ischemic stroke, need for revascularization, and procedure-related complications. However, there was a significant improvement in the number of angina episodes per month and angina functional class in the cell treatment group, but not in the controls.

Cardiac Stem Cells

Until recently, the heart was considered a postmitotic organ, incapable of regeneration, thus limiting the available options for therapeutics for cardiac disease. The discovery of a CSC population within the adult heart in spurred interest in using these stem cells as a novel cell-based therapeutic for regenerating damaged myocardium (Beltrami et al., 2003). CSCs are crucial components of the heart's endogenous repair machinery and are characterized by the expression of the cell surface marker, c-kit (CD117).

The regenerative ability of endogenous adult CSCs has been questioned and a murine study suggests that adult c-kit+ cells play little if any role in cardiomyocyte regeneration (van Berlo et al., 2014). Other murine studies indicate that c-kit+ cells can play an important role in cardiomyogenesis (Hatzistergos et al., 2015, 2016). Administration of c-kit+ CSCs following ICM in mice (Fransioli et al., 2008) and swine (Bolli et al., 2013; Kulandavelu et al., 2016; Tang et al., 2016; Williams et al., 2013) show that these cells promote significant improvements in cardiac function and structure. The therapeutic efficacy of c-kit+ CSCs in humans was first assessed in the setting of chronic ICM. In phase I "Cardiac Stem Cell Infusion in Patients with Ischemic Cardiomyopathy" (SCIPIO, NCT00474461) trial, autologous CSCs were harvested from the right atrial appendage of patients with severe HF undergoing CABG. The cells were expanded and readministered via intracoronary infusion 3–4 months later. Compared to placebo, the CSC-treated group had significant increases in LVEF at 4-months posttherapy. Moreover, the CSC group exhibited improvements in scar size, regional contractility, and HF symptoms (Bolli et al., 2011). The "Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction" (CADUCEUS, NCT00893360) trial evaluated the effectiveness of intracoronary delivery of cardiosphere-derived autologous stem cells in patients with ICM. Cardiospheres are a small population of cells isolated from biopsied heart tissue that spontaneously forms clusters comprised of heterogeneous cells, and are characterized by multiple markers, including CD105+, c-kit+, CD90+, CD34+, and CD31+. Despite the lack of improvements in LVEF in the cell-treated group, there was a significant reduction in scar mass, as well as an increase in viable myocardium and contractility at the 6-months follow-up (Makkar et al., 2012). Trials to enhance the potency of CSCs are ongoing. The "Combination of Mesenchymal and C-kit+ Cardiac Stem Cells as Regenerative Therapy for Heart Failure" trial (CONCERT-HF, NCT02501811) is designed to

test if the potency of CSCs is enhanced by combining them with MSCs. This landmark multicenter trial plans to enrol more than 100 patients and is based on results of preclinical studies in swine models of MI where the combination of autologous MSCs and CSCs produced greater effects than single cell types alone (Karantalis et al., 2015b; Williams et al., 2013).

Skeletal Myoblasts

Skeletal myoblasts were one of the first generation stem cell therapies to be studied, and the first to be utilized in a dog model of cardiac injury (Marelli et al., 1992). These cells have high proliferative capacity and resistance to ischemia and it was thought that their ability to promote recovery from muscle injury would translate to the heart. The 2008 MAGIC trial was the first randomized study of myoblast transplantation in ICM patients. Cells were administered via intramyocardial injection during open heart surgery, but they failed to produce significant increases of LVEF compared to placebo. Furthermore, the myoblasts did not integrate into the myocardium and in some cases, actually caused ventricular arrhythmias (Menasche et al., 2008). In contrast, the smaller (23 patients) "Study to Assess the Efficacy and Safety of Transplanting Autologous Skeletal Myoblasts, Into Infarcted Heart, Using a Catheter Delivery System" (CAuSMIC, NCT00626314) trial showed that transendocardial injection of skeletal myoblasts was safe, improved functional class and reversed ventricular remodeling (Dib et al., 2009). However, the skeletal lineage commitment of myoblasts appears to prevent these cells from becoming cardiomyocytes. This, coupled with their potential to induce arrhythmias, has limited their clinical applicability.

Pluripotent Stem Cells

Pluripotent stem cells (ESCs, iPSCs) are described in more detail in another chapter, but will be discussed briefly here. These cells can transform into virtually any adult cell and have superior proliferation capacities compared to MSCs and EPCs. There are some caveats to their use in therapeutic applications, as they have the potential to form teratomas and can instigate immune reactions, which hinders their advancement to clinical use. Differentiating pluripotent stem cells into cardiomyocytes prior to infusion should limit the generation of teratomas and expand their clinical applicability; however, there is a risk for arrhythmia because these cells can remain immature following engraftment (Behfar et al., 2007). Riegler et al. showed that human ESC-derived cardiomyocytes survive long-term in a rat model of ICM without teratoma formation. However, this long-term survival was not accompanied by cardiac functional improvements (Riegler et al., 2015). Functional improvements were observed when human ESC-derived cardiomyocytes were introduced into a macaque model of AMI (Chong et al., 2014) (**Fig. 5**), again with no teratoma generation. These findings paved the way for a phase I clinical trial investigating ESC-derived cardiac progenitor cells embedded into a fibrin scaffold and surgically delivered onto the infarct area for patients with ICM. A case report of a single patient who received this treatment reported LVEF recovery and improved symptoms without adverse events (Menasche et al., 2015).

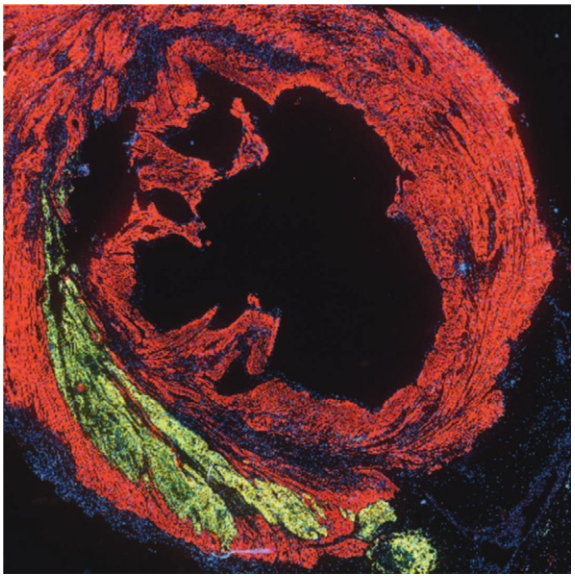

Fig. 5 hESC induced cardiomyocytes transplanted into infarcted macaque heart. Confocal immunofluorescence of a macaque heart subjected to myocardial infarct and transplantation of hESC-CMs. This image was taken 14 days after stem cell injection. hESC-CMs coexpressing GFP can be seen embedded in the *dark* infarcted region. Alpha-actin, staining in cardiomyocytes, is shown in *red*. Reproduced from Chong, J. J., Yang, X., Don, C. W., Minami, E., Liu, Y. W., Weyers, J. J., Mahoney, W. M., Van Biber, B., Cook, S. M., Palpant, N. J., Gantz, J. A., Fugate, J. A., Muskheli, V., Gough, G. M., Vogel, K. W., Astley, C. A., Hotchkiss, C. E., Baldessari, A., Pabon, L., Reinecke, H., Gill, E. A., Nelson, V., Kiem, H. P., Laflamme, M. A. and Murry, C. E. (2014). Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. *Nature* **510**, 273–277, with permission from the publisher.

Similar to ESCs, iPSCs require effective positive selection (cardiomyocyte differentiation and purification) and negative selection (to avoid teratoma formation) for clinical application. While this approach may prove effective, the immature nature of iPSC-derived cardiomyocytes (iPSC-CMs), like that for ESC-derived cardiomyocytes, currently presents a hurdle. iPSC-CMs are mechanically and electrophysiologically immature, resembling fetal cardiac tissue and techniques to further mature them for clinical use are needed (Csobonyeiova et al., 2015). Nevertheless, extensive research and an increasing number of preclinical and clinical studies should improve the safety and efficacy of iPSCs and iPSC-derived cells in cardiac cell-based therapies. In fact, one group in Japan has evaluated surgical engraftment of bioengineered sheets of myocardium derived from autologous iPSCs into damaged hearts in animal models, and this method is currently undergoing investigation in phase II clinical trial in Japan (Sawa, 2017).

Stem Cell Modification and Other Approaches

An alternative approach to augment stem cell therapy is the pretreatment of cells to enhance their therapeutic potency. There are several strategies, such as pretreatment of cells with growth factors (Bartunek et al., 2013), in vitro exposure to hypoxia (Bellio et al., 2016), genetic modulation (Samse et al., 2016; Kulandavelu et al., 2016; Quijada et al., 2012), and specific cell phenotype selection (Tendera et al., 2009). Because CSCs must be isolated through surgery or cardiac biopsy, both of which are invasive and carry some risk, several preclinical studies have sought to modify MSCs to make them more like CSCs. Treatment with a "cardiac cocktail" of several growth factors and other bioactive agents can successfully trigger MSCs toward a lineage-specified cardiac phenotype, or so-called cardiopoietic stem cells and these cells, and these cells demonstrated beneficial effects on damaged myocardium in an animal model (Behfar et al., 2010). Cardiopoietic stem cells have since been translated into clinical trials. One such study is the "Cardiopoietic stem Cell therapy in heart failure" (C-CURE, NCT00810238) multicenter, randomized trial that the safety and efficacy of transendocardial injection of cardiopoietic stem cells for IHF (Bartunek et al., 2013).There were no adverse events or systemic toxicity associated with these cardiopoietic stem cells. Moreover, at 6 months significant improvements in LVEF, ESV, and 6-minute walk test were seen in the cell pretreated group compared to standard of care alone. These promising results led to a phase III, sham-controlled multicenter trial, the Safety and Efficacy of Autologous Cardiopoietic Cell for Treatment of Ischemic Heart Failure trial (CHART-1) (NCT01768702), which examined the efficacy of cardiopoietic stem cells from autologous BMMSCs in IHF. At 39 weeks, compared to placebo, cell therapy failed to show improvements in the primary efficacy endpoint, which was a composite of mortality, worsening HF, MLHFQ, LVESV, and LVEF (Bartunek et al., 2017). Further analysis revealed a subpopulation of patients with severe heart enlargement (LVEDV 200–370) that did significantly improve in the primary endpoint.

Another interesting alternative approach to next generation stem cell therapy is the direct reprogramming of somatic cells into cardiomyocytes using a cocktail of transcription factors in vivo. By circumventing the pluripotent state, there is a decreased risk of teratoma formation and immune rejection. Although this method has shown promising results in murine studies, it has not been successfully replicated in human somatic cells. Transduction efficiencies are low, and generated cardiomyocytes are immature. The safety and efficacy of these manipulated cells have yet to be established in a large animal preclinical models (Cambria et al., 2016).

Conclusions and Future Perspectives for Stem Cell Therapeutics

Over the last 10–15 years of clinical trials involving stem cell therapies for cardiac disease, there have been both failures and remarkable breakthroughs that move the field forward. While not every study has proven successful, multiple studies have demonstrated that that stem cell therapy is remarkably safe. Preclinical and clinical trials to date have addressed many questions, but there is still uncertainty as to which stem cell type, source, combination, modification, delivery, etc. is the most effective for which type of cardiac regeneration, and which symptoms will respond to which treatment. Of the stem cells extensively tested, MSCs appear to be superior for short-term efficacy, and are the ideal cell type for allogeneic transplant because of their immunotolerant status. Despite promising preclinical data, the newer trials assessing the efficacy of MSCs modified to achieve a cardiac phenotype have shown the little benefit of these cells, highlighting the importance of clinical trials in this field. The next phase of studies will likely focus on enhancing the recipient tissue environment, improving cell delivery and engraftment, and optimizing the timing of cell administration. Studies to date indicate that it is highly unlikely that the injection of a single, unmodified cell type can reverse the entirety of the cardiac damage. Cell combinations, genetic manipulation, preconditioning, embedding cells within matrices have all produced improvements but it is unlikely that these approaches alone will replace the injured myocardium; treatment must also activate endogenous cells within the myocardium to promote healing.

Development of the "optimal treatment" is ongoing, but novel technologies continue to raise and answer questions. Phase III clinical trials including "Efficacy and Safety of Allogeneic Mesenchymal Precursor Cells (Rexlemestrocel-L) for the Treatment of Heart Failure" (DREAM HF-1, NCT02032004) and BAMI, (NCT01569178) are starting to recruit large populations of patients. Results of these large trials will hopefully answer critical questions about the efficacy of allogeneic MSCs and BMMNCs to further shape the direction of cell-based therapies in the near future.

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Heart–Lung Transplantation

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Introduction

The age of thoracic organ transplant began in the 1960s, as years of research led to the first transplants. In 1967, the first heart was successfully transplanted in South Africa by Christiaan Barnard (Barnard, 1967). Prior to that, in 1963, the first lung transplant was performed at the University of Mississippi (Hardy et al., 1963). After these two landmark events, another important transplantation happened—the first heart–lung transplant. In 1981, the team at Stanford successfully transplanted a 45-year old female with the first heart–lung bloc. The patient had the primary diagnosis of primary pulmonary hypertension (Reitz et al., 1982).

Heart–lung transplants provide an opportunity to treat patients with end-stage cardiac and end-stage pulmonary disease. According to the International Society of Heart and Lung Transplant, nearly 3400 adult heart–lung transplants have been completed to date, and currently, about 65 are completed per year. Estimated median survival for heart–lung transplant patients is 3.3 years, with a condition survival, meaning survival if they reached the 1 year mark, of 10.3 years, based on data obtained in 2014 (Yusen et al., 2016). The top diagnosis leading to heart–lung transplant has remained congenital heart disease, with roughly 35% of patients receiving heart–lung transplants having this diagnosis. The other top indications to transplant include pulmonary artery hypertension (27%) and cystic fibrosis (13%). Other diagnoses compromise 5% or fewer transplants (Yusen et al., 2016). As results have improved, the total number of transplants has increased; however, total volume remains limited by donor availability and the improved success of double lung transplants.

Patient Selection

With the improvement and success of a double-lung transplant, the growth of heart–lung transplant has peaked; however, there still exists a role in a select patient population. The process for evaluation of heart–lung transplant begins with the diagnosis of end-stage heart and end-stage lung disease. Typically a right heart catheterization will be the final determinant, demonstrating right atrial pressures >15, indicating severe right heart failure. At that point, the patient would not be a candidate for a double lung transplant, but rather for a heart–lung bloc.

As with all operations, determining good surgical candidacy is an important factor. Evaluating laboratory values including kidney function, liver function, coagulation, lipid panels, hemoglobin A1c, and thyroid function all factor into the decision of whether the potential recipient can undergo an operation. Another value checked is the panel reactive antibody (PRA). This number is a value score associated with the number of antibodies a person has. The higher the PRA, the higher the number of antibodies a patient possesses. A patient with a high PRA requires a more specific donor in order to prevent acute rejection. If a patient's PRA is greater than 25%, a prospective crossmatch is obtained in order to prevent hyperacute rejection.

The final decision to list a patient is determined by the local transplant committee. In the case of heart–lung transplants, patients are listed according to their lung allocation score (LAS), the scoring system used in determining placement for lung transplants. The higher a patient's LAS, the higher the rank the patient occupies on the transplant list.

Contraindications to transplantation do exist. Relative contraindications include age >65, diabetes, HIV, and active hepatitis B or C. Absolute contraindications include active malignancy, active infection, active drug or alcohol use, and active cigarette smoking.

Donor Preservation

Prior to going to the operating room, the donor's organ systems are maintained in order to preserve end organ function. In situations involving a donor heart, attention is paid to reducing cardiac workload and oxygen consumption. Often this goal

can be difficult due to neurogenic shock and the requirement for pressor agents to maintain a mean arterial pressure above 60. Additionally, if the lungs are being matched, volume status is critical to prevent pulmonary edema in both the donor as well as ultimately in the recipient. Tidal volumes are kept at 6–8 cc/kg and FiO_2 is maintained as low as possible to prevent oxygen toxicity. Often times, atelectasis can occur and recruitment breaths will be necessary in the operating room.

Donor Operation

Upon arrival to the donor hospital, the operating team should review all necessary paperwork, including history and physical examination, brain death summary, and necessary laboratory work. In addition, for heart–lung transplant, the echocardiogram, chest X-ray, and if applicable, the chest CT should be reviewed. In the operating room, a bronchoscope with suction should be available to visually evaluate the lungs.

The patient is brought to the operating room by the home hospital's anesthesia team and organ donation nurses. Once positioned, the bronchoscopy can be performed, ensuring no major anatomical abnormalities that might prevent transplantation, inspecting the airways for signs of aspiration, and providing any necessary pulmonary toilet.

The patient is then prepped with chlorhexidine from the chin through the groins and draped in order to perform a full sternotomy and laparotomy. Following median sternotomy, the sternal retractor is placed and the pleural spaces are opened in order to visually inspect the lungs. At this time, some advocate for ligating the pulmonary ligament bilaterally to facilitate mobilization. Each lung is evaluated for pulmonary contusion or other abnormalities. Recruitment breaths are then given, in order to recruit all atelectatic segments of the lung.

Upon completing the lung evaluation, the heart can be evaluated in the standard fashion. The pericardium is opened and incised as superiorly as possible, and inferiorly to the diaphragm. Visual inspection of the heart is performed, after which, the surgeon should manually trace the coronary arteries to ensure no significant disease.

Once confirmed that the heart also is acceptable, additional dissection is performed to prepare the heart–lung bloc. The superior vena cava (SVC) is separated from the right pulmonary artery and the inferior vena cava is released from the posterior pericardium. The trachea is mobilized as high as possible, to the larynx if able, and released posteriorly from esophageal attachments. Limited dissection is performed on the lateral trachea to preserve blood supply to what will be the new anastomosis and surrounding mediastinal tissue is left en bloc.

Once all transplant teams are ready, the patient is prepared for crossclamp and explant of organs. Typically at this time, 30,000 units of heparin are administered. Prostaglandins are then injected either intravenously or directly into the pulmonary artery to promote pulmonary vasculature vasodilation. It is not uncommon for this step to also lower systemic blood pressure. Pulmonary artery cannulation is then performed proximal to the bifurcation, and a cardioplegia needle is inserted into the ascending aorta. Additionally, the left atrial appendage is snared using a Satinsky clamp and the tip is ligated. The appendage will later be used to vent the left side of the heart. After 3 minutes following heparinization, crossclamps are then applied. The SVC is snared or tied off, the inferior vena cava is cut, and the left atrial appendage is vented. Ice and cold slush are placed on the heart and in both plural spaces, and pool tip suction is used to remove blood from the field. At this time, the cardioplegia and pulmoplegia lines should be opened and running freely. Once all fluid is given, anesthesia is asked to give one final breath and to hold it. The endotracheal tube is retracted and the trachea is stapled with the lungs moderately inflated. A 15-blade knife is used to make a clean cut across the trachea. The inferior vena cava is fully divided, and then the SVC and ascending aorta are divided as high as possible. If the pulmonary ligaments were not previously ligated, they must be now in order to fully mobilize and then remove the heart–lung bloc.

Once removed, the heart–lung bloc is inspected on the back table for any intracardiac abnormities that would require repair. They are packaged in cold fluid, either saline or University of Wisconsin (UW), a series of two sterile bags, and then a plastic container. The container is placed in one final bag and then a cooler for transport.

Recipient Operation

When a probable donor is found, the recipient will be called in from home. The standard preoperative routine of shaving, chlorhexidine wipes, etc. will be performed in the hospital prior to arrival in the operative room. Once transplant is confirmed, the patient will receive initial immunosuppressive therapy with mycophenolate mofetil (MMF) and/or a calcineurin inhibitor.

The patient then proceeds to the operating room. The patient is positioned supinely. Anesthesia is induced and the patient is intubated. The decision of single or double-lumen endotracheal tube is institution dependent. Standard monitoring lines are present, including arterial line, central venous line with Swan–Ganz catheter. Ultimately, the Swan will need to be retracted during the implantation.

Surgical approaches for the heart–lung transplant include the preferred median sternotomy or bilateral anterolateral thoracotomies, or the clamshell incision. The surgical approach is typically dependent on the extent of adhesions to be encountered in the pleural spaces. Patients with prior lung surgeries or severe inflammatory lung processes would benefit from a clamshell incision in order to control bleeding from adhesions.

After entering the chest, the operation begins by isolating and snaring the hilar vasculature outside the pericardium. As this area is dissected, special care is taken to preserve the phrenic nerves. Pedicles are created on each side roughly 3 cm in width. Posterior to the pedicle, the pericardium is excised to facilitate placement of the donor organs.

Following hilar dissections, the vena cava and the ascending aorta are dissected and encircled with snares. The patient is placed on cardiopulmonary bypass using a high aortic cannulation stitch and bicaval venous cannulation. The cardiopulmonary bypass circuit typically has a white blood cell filter to prevent host cells from entering the soon-to-be implanted graft.

The trachea, typically approached between the SVC and the innominate artery, is encircled roughly five rings above the carina. While dissecting the posterior aspect, the vagus nerves, traveling along the esophagus, must be preserved.

The recipient organs are then sequentially removed, starting with the heart. The lungs are then removed, first by stapling the vasculature and finally stapling across each bronchus. Special care is given to the left pulmonary artery, where a segment is left in the recipient near the ligamentum. This remnant preserves the left recurrent laryngeal nerve.

After preparation on the back table, the heart–lung block is ready to be implanted. The block is first inserted by placing the right lung under the phrenic pedicle, and then the left lung under the left pedicle. While there is no standard procedure to sew the anastomoses, the preferred order begins with the trachea. The endotracheal tube is retracted back, and using a 15-blade, the trachea is cut. Once the anastomosis, sewn with 4–0 nonabsorbable monofilament, is complete, the lungs can be ventilated in order to recruit alveoli and prevent on-going atelectasis. This area will be difficult to inspect for hemostasis once all remaining structures are sewn in place. It is therefore imperative to ensure this area will not bleed once perfusion is resumed.

The remaining structures are then sewn—the inferior vena cava, then SVC, and finally the aorta. Special care must be taken while sewing the cava in place in order to prevent strictures, which can impair cardiac filling. By using a donor superior vena cuff, the AV node should be preserved. Prior to sewing the aortic anastomosis, the heart can begin to fill and deair. Following its completion, cardioplegia is reattached and suction is placed on the aortic root to deair and prevent embolic complications.

Once all anastomoses are complete and the patient is ready to be weaned from cardiopulmonary bypass, 500–1000 mg of methylprednisone is given. It is imperative that the patient received the full dose prior to reperfusion of the grafts, that is—release of the aortic cross clamp. Atrial and ventricular pacing wires are placed, the heart is defibrillated if needed, and isoproterenol is started to keep the heart rate between 90 and 110. Depending on pulmonary arterial pressures and right heart function, inhaled epoprostenol or nitrous oxide may be needed. The chest is closed in standard fashion once hemostasis in ensured, and the patient is transported to the intensive care unit intubated.

Acute Postoperative Management

The postoperative management of a heart–lung transplant patient incorporates aspects of both a heart transplant and lung transplant. Key management points in the acute setting involve fluid management, ventilator weaning, and maintenance of cardiac output. These three tenants are intricately involved and after a heart–lung transplant can be opposing forces, as the support for heart transplant patient and lung transplant patient can be opposites regarding fluid resuscitation. The patient should return to the intensive care unit intubated, which he or she will remain for roughly 24 hours. The pacing wires are plugged in and set on a backup rate of 90. Chest tubes are placed to suction, and a full set of postoperative labs are sent.

The fluid management of a heart–lung transplant patient is a delicate balance. Heart transplants are aggressively resuscitated to maintain a central venous pressure, or CVP, around 8–10. Often multiple liters of fluid are required as the time spent on cardiopulmonary bypass increases the body's inflammatory response. Conversely, lung transplants are often time kept "dry," indicating minimal fluid resuscitation in order to prevent pulmonary edema. Thus, in postheart–lung transplants, fluid is judiciously managed. Vasoactive medications, specifically norepinephrine and vasopressin, will be used to support blood pressure while maintaining a CVP around 8. Phenylephrine, due to is alpha-agonist activity, has a great effect on pulmonary vascular bed constriction, and therefore is not the first line after a lung or heart–lung transplant.

Over the first 24 hours, the ventilator is weaned to minimal settings first, and then toward extubation after the stability of the patient is verified. While oxygen is essential to life, higher than normal levels can be detrimental to postoperative transplanted lung. Daily chest X-rays are used to track the status of pulmonary edema and monitor for some of the first signs of graft dysfunction. Additionally, bronchoscopies are performed to assist in airway clearance immediately post operation and to evaluate suture lines for dehiscence and stricture. At least one bronchoscopy is performed prior to extubation. Once the patient is extubated, the aggressive pulmonary toilet is employed to encourage airway clearance and lung expansion.

Essential to any postcardiac surgery, patient is the maintenance of cardiac output, usually though inotropes and fluid. Due to the stiffness of the transplanted heart, increased heart rate is the primary method used to increase output. Posttransplant patients are kept between 90 and 110 in the initial postoperative period until the new heart can relax and its diastolic function improves. Additionally, inotropes are used to support contractility as the new heart acclimates to its new anatomic and physiologic environment.

Immunosuppression is a key component of acute posttransplant management. Treatment begins concurrently with the patient coming off cardiopulmonary bypass, as the patient is dosed with 500–1000 mg of methylprednisone. Methylprednisone is redosed on the day of surgery at 125 mg every 8 hours for three doses. Following the three doses, methylprednisone, or oral prednisone, is dosed at 1 mg/kg/day, and then tapered depending on the transplant center's protocol and patient specific needs. MMF therapy also begins at this time, with 1500 mg dosed every 12 hours.

At this point, further immunosuppressive management depends on the patient's renal function, the presence of allosensitivity, and/or primary graft dysfunction. If the patient is not experiencing any acute kidney injury or failure, a calcineurin inhibitor is started at a low dose, 1–2 mg twice a day, with monitoring of daily trough levels. If the patient is having renal dysfunction, induction therapy with an antithymocyte globulin is started. Typically, it is dosed at 1.5 mg/kg. Center-specific monitoring of white blood cells, platelets, and/or CD3 counts are used to determine continued dosing strategy. Ultimately, the plan would be to resume using a calcineurin inhibitor within 7–14 days posttransplant.

The first posttransplant milestone is the initial biopsy to assess for rejection. Depending on institutional schedules, the first biopsy is typically done between postop days 5 and 7. Research has shown that a biopsy sample from the bronchial graft is more predictive of rejection than a sample from the endo- and myocardium of the right ventricle (Sibley et al., 1993). Thus biopsies are typically just taken from the lung. Ultimately, this lowers the risk of tricuspid disease in the transplanted heart, as the valve is not disturbed and the cords and papillary muscles are left intact. Pacing wires are routinely kept in place until after this first biopsy in the event of rejection and graft dysfunction. Provided no signs of rejection, the wires can then be removed.

Acute Complications

The acute postoperative time period is crucial in the heart–lung transplant patient as different types of complications and setbacks can occur, including technical, medical, rejection, and primary dysfunction. Technical or surgical complications are similar to those experienced in other heart and or lung operations. Bleeding, tamponade, hemothorax, etc. that result in reoperation or exploration of the transplant patient can negatively affect outcomes and can prolong hospital courses. As mentioned above in the operation section, there are particular areas to examine carefully during the implant due to their limited accessibility once the heart–lung block is in place. Examination of all sutures lines and areas of adhesions ensuring hemostasis will help prevent bleeding complications.

Acute medical complications postheart–lung transplantation typically center around fluid management. The delicate balance of fluid typically places the patient on a continuum from renal failure (too little volume) to severe pulmonary edema (too much volume). Most patients will be managed with a "dry" mentality, providing just enough fluid to maintain adequate cardiac output, tolerating a minor acute kidney injury in the process. Even if that injury progresses to acute kidney failure, given recovery time and a period of dialysis, renal function can return, provided baseline function was normal. Additionally, if a patient gets too "wet," or fluid overloaded, diuresis can help relieve the volume burden while positive pressure ventilation can maintain oxygenation in the interim.

The most feared acute complications are acute rejection and graft dysfunction. Prevention of rejection begins as the patient arrives at the hospital. As detailed earlier, patients receive initial therapy prior to the operating room. Patients also receive corticosteroids prior to reperfusion of the transplanted grafts. These treatments are done to reduce leukocyte activity reducing the possibility of a hyperacute rejection. The first assessment for rejection typically happens between posttransplant day 5 and 7, and involves biopsies of the transplanted lungs. Further antirejection medication adjustments occur following the biopsy results.

Despite these treatments, optimal preservation techniques, and short ischemic times, patients can still experience primary graft dysfunction. Studies have reported primary graft dysfunction at 10%–15% (Date et al., 1996). For transplanted hearts, this process can manifest as right heart failure or global heart failure. Supportive inotropes, or even extracorporeal membrane oxygenation (ECMO), might be necessary during the acute postoperative period. For transplanted lungs, graft dysfunction manifests as impaired oxygenation and ventilation. The patient will develop high peak pressures, indicating stiff, noncompliant, and poorly functioning lungs. Treatment typically involves delayed extubation, keeping the patient as dry as possible, and limiting ventilator trauma through oxygen and pressure. If severe enough, ECMO must be considered to support the patient in the acute postoperative period. In the case of a heart–lung transplant, graft dysfunction can manifest in one or both grafts. Mortality for primary graft failure can be as high as 60% (Christie et al., 1998). Invasive monitoring and frequent review of laboratory values, every 4–6 hours immediate to posttransplant, help to detect early signs of dysfunction.

Posttransplant Care

The major tenants of posttransplant care focus on management of immunosuppressive medication, infection prevents, and monitoring for rejection or failure of the transplanted grafts. Each institution will have specific drugs and tailored regimens, but the main principles of balancing too much suppression, leading to infections and possible malignancy, and too little suppression, leading to graft rejection, are balanced and monitored. Living near the transplant center and having regular clinic visits help in close monitoring and good outcomes. As detailed earlier, current median survival is 3.3 years, but if a patient survives the first year, the median survival jumps to 10.3 years (Yusen et al., 2016).

Infection prevention is essential to posttransplant care, and as soon as a patient is stable after surgery prophylaxis is initiated. Depending on the cytomegalovirus, or CMV, the status of the recipient and donor, ganciclovir will be prescribed. Systemic antifungal prophylaxis, such as voriconazole, may be given while in the hospital. Typically, antifungal mouthwash is prescribed to prevent oral thrush or a *Candida* infection. Another important agent to prevent is *Pneumocystis* species, for which Bactrim, or trimethoprim-sulfamethoxazole, is given.

Each institution will develop a schedule, or routine for biopsies and monitoring for rejection. Additionally, other studies such as echocardiograms, heart catheterizations, and pulmonary function tests help to track the function of the transplanted grafts. Chronic rejection takes the form of accelerated coronary artery disease in the heart and bronchiolitis obliterans in the transplanted lungs. Immunosuppression regimens can be adjusted at the first signs of rejection. Various interventions of medication may temporize the disease progression; however, the only true curative treatment is retransplant.

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Hemodynamic Changes of Pregnancy

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Introduction

Pregnancy produces a unique physiologic state that adapts to meet the hemodynamic and metabolic needs of the growing fetal-placental circulation. In this context, the maternal cardiovascular system undergoes a series of dynamic changes, culminating in an increased plasma volume, increased cardiac output (CO), and decreased systemic vascular resistance (SVR). These changes serve to ensure adequate delivery of oxygenated blood to peripheral tissues and the fetus. Maternal physiologic adaptations, coordinated by a complex neurohormonal cascade, drive structural and functional changes. Failure of the maternal cardiovascular system to appropriately adapt to the metabolic demands of pregnancy can unmask preexisting heart disease, resulting in maternal and fetal morbidity and mortality. Given that cardiovascular diseases are the number one cause of maternal mortality in the United States (Pregnancy Mortality Surveillance System | Pregnancy | Reproductive Health | CDC, 2017), a thorough understanding of the normal cardiovascular changes induced by pregnancy is necessary to diagnose and manage cardiovascular disease, or, stated simply, "normal must be known before abnormalities can be detected" (Dieckmann et al., 1934). This module will examine the hemodynamic changes in pregnancy during the growth of the fetus (antepartum), through labor (peripartum), and after delivery (postpartum).

Antepartum

The hemodynamic changes of pregnancy, characterized by an early decrease in SVR and mean arterial pressure (MAP), coupled with an increase in CO and glomerular filtration rate (GFR), begin in the embryonic period, before they are functionally necessary and prior to maturation of the utero-placental unit (Duvekot et al., 1993; Clapp et al., 1988; Robson et al., 1989). Indeed, the same changes can be seen after ovulation in nonpregnant women; during the mid-luteal phase of the menstrual cycle, women experience a decrease in SVR, with a commensurate increase in left ventricular chamber size, stroke volume, and CO (Chapman et al., 1997; Veille et al., 1986). Early initiation of these changes are mediated by several circulating hormones that increase in concentration during pregnancy, including chorionic gonadotropin, estradiol, and progesterone, all of which bind to receptors located in vascular endothelium and smooth muscle (Veille et al., 1986; Clapp and Capeless, 1997; McGill, 1989). Furthermore, animal studies suggest a role for increased nitric oxide (NO) synthesis in systemic vasodilation and reduction in vascular resistance (Deng et al., 1996).

Preload

Venous compliance and blood volume are principal determinants of preload and can be altered without direct modification of the myocardium. Dating to the early 19th century, obstetricians observed a substantial increase in circulating volume as a normal physiologic process, driven primarily through increased plasma volume and erythrocyte mass (Miller et al., 1915). These changes serve to increase the available supply of hemoglobin and other vital nutrients to the growing fetus. In fact, successful fetal growth appears to rely, in part, on appropriate expansion of plasma volume, as evidenced by the positive correlation between plasma volume and birth weight (Pirani et al., 1973).

Plasma volume increases progressively (**Fig. 1**) (Peck and Arias, 1979) from early pregnancy, peaks between 28 and 34 weeks, and then plateaus as the pregnancy approaches term (Salas et al., 2006; Metcalfe et al., 1981). The initial phase, starting in the first trimester, occurs rapidly, with only gradual volume expansion in the third trimester. A larger plasma volume is necessary to support a larger fetus or multigravida state; consequently, inadequate volume expansion is related to vascular pregnancy complications,

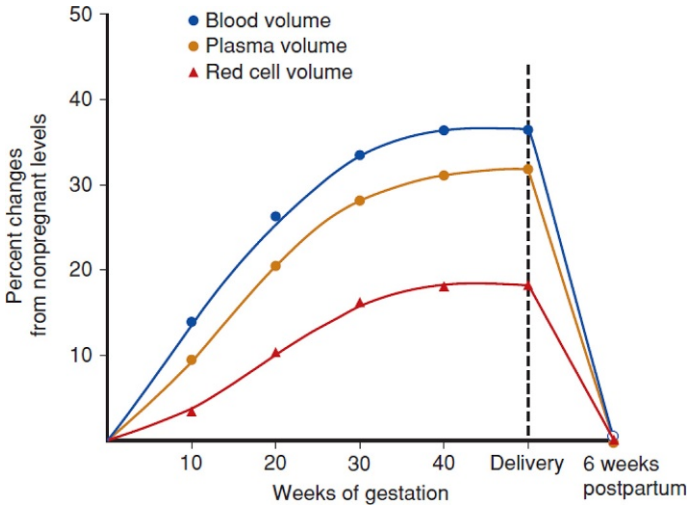

Fig. 1 Blood, plasma, and red cell volume during pregnancy. Reproduced with permission from Peck, T. M. and Arias, F. (1979). Hematologic changes associated with pregnancy. *Clinical Obstetrics and Gynecology* **22**, 785.

including intrauterine growth restriction, preeclampsia, and fetal demise (Hytten, 1986; Gallery et al., 1979). Plasma volume increases 30%–50% above nonpregnant values, with a range from 20% to 100% in individual women (Pritchard, 1965; Sanghavi and Rutherford, 2014; Paidas and Hossain, 2010). To this end, changes begin as early as week 6 of gestation, prior to full development of the fetal-placental circulation (Chapman et al., 1998), an effect related to systemic vasodilation and increased renal perfusion caused by persistence of the corpus luteum (Chapman et al., 1997; Van Beek et al., 1996). The luteal body produces the hormone relaxin at high concentrations during early gestation. Relaxin mediates increased renal perfusion, peripheral vasodilation, and plasma volume expansion via activation of the NO pathway (Deng et al., 1996; Conrad and Novak, 2004). Further, plasma volume expansion occurs between weeks 7 and 12 of the first trimester through the placental production of progesterone and estrogen, both of which have vasodilatory effects (Kristiansson and Wang, 2001; Bernstein et al., 2001). Stimulation of estrogen receptors present in the vascular endothelium exerts a direct vasodilatory effect via prostacyclin and NO synthesis (Tostes et al., 2003). The decreased effective arterial blood volume, in turn, leads to compensatory sodium retention and volume expansion through activation of the renin-angiotensin-aldosterone system (RAAS) (Salas et al., 2006; Longo, 1983). Additionally, the presence of the utero-placental circulation, which acts as an arteriovenous shunt, prompts further volume expansion. The rate of expansion peaks during the second trimester, with a slower, but continued increase until approximately week 32, after which the volume typically stabilizes until term. This increase in circulating volume causes, as would be expected, an increase in the atrial natriuretic peptide (ANP) via concomitant atrial enlargement (Chapman et al., 1998).

Along with expansion in plasma volume, erythrocyte mass (i.e., the number of circulating red blood cells) increases by 20%–30% during pregnancy (Paidas and Hossain, 2010; Longo, 1983; Means, 2013). Erythrocyte production is driven primarily by erythropoietin, which binds selectively to associated receptors on red-cell progenitors in the bone marrow. In the nonpregnant adult, erythropoietin production is promoted via sensed hypoxemia and red cell hemoglobin concentration. In order to increase red blood cell mass during pregnancy despite normoxic conditions, human placental lactogen (hPL) serves as a potent hematopoietic stimulant to drive erythrocyte production. hPL augments erythrocytosis via enhanced sensitivity to erythropoietin, increased iron incorporation, and direct effects on red-cell progenitors (Jepson and Lowenstein, 1968; Berczi and Nagy, 1991). Pregnancy-related erythrocytosis is further potentiated by progesterone, uteroferrin, and prolactin via the JAK/STAT pathways, which act in concert to prompt the differentiation, proliferation, and survival of nascent red blood cells (Watowich, 2011; Soares et al., 2007; Bazer et al., 1991). This increase in red cell mass, however, can be inhibited by conditions that limit the resources required for erythrocyte production, such as iron or copper deficiency (Rodak et al., 2007; Langston, 2015). Notably, plasma volume increases to a greater degree than red cell mass, resulting in a relative, physiologic anemia of pregnancy. This dilutional anemia typically produces a 1–2 g/dL drop in hemoglobin concentration, with hemoglobin concentration as low as 11 mg/dL considered normal (Cunningham et al., 2013). Hemoglobin levels reach a nadir in the late second trimester and subsequently stabilize until delivery.

In sum, a primary decrease in SVR, in concert with the early hormonal changes described above, prompt a 30%–50% increase in plasma volume, accompanied by a 20%–30% increase in red blood cell mass to meet the increased maternal metabolic needs of pregnancy. Despite the increased circulating volume, pulmonary capillary pressure and central venous pressure remain within the normal range (Clark et al., 1989).

Afterload

A hallmark of the hemodynamic changes of pregnancy is a decrease in SVR through vasodilation, with a commensurate decrease in blood pressure. The drop in SVR begins at approximately week 5 of pregnancy, under the influence of a persistent luteal body. This

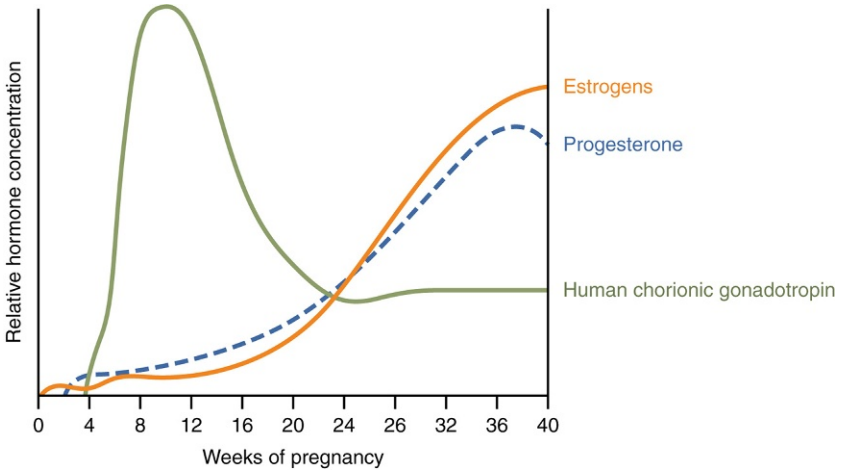

Fig. 2 Concentrations of various sex hormones throughout pregnancy. Reproduced under the Creative Commons License from OpenStax, Maternal Changes During Pregnancy, Labor, and Birth. OpenStax CNX. Jun 4, 2013 http://cnx.org/contents/f068080a-4376-4d88-a150-33e105a4cad8@3.

process continues until week 32 of pregnancy, at which time the vascular resistance normally plateaus until term. SVR decreases, in part, as a result of several circulating hormones (**Fig. 2**) (Cheung and Lafayette, 2013), including relaxin, prostacyclin, estrogen, and progesterone, which serve at various levels to increase the activity of the NO, a common final pathway in the vasodilation of pregnancy (Sladek et al., 1997).

Relaxin, produced in high levels by the corpus luteum, enacts both rapid and persistent vasodilation. The rapid, more pronounced vasodilatory response to relaxin begins with G-protein-dependent phosphorylation of nitric oxide synthase, prompting NO production and subsequent relaxation of vascular smooth muscle (Conrad and Emerging, 2011). NO stimulates cyclic guanosine monophosphate (cGMP) production, which reduces intracellular calcium concentration, thereby inhibiting smooth muscle contraction. The persistent, though less dramatic, sustained vasodilatory response to relaxin occurs by increased production of arterial gelatinases, which hydrolyze endothelin to an active form that further activates the NO pathway (Conrad, 2016). Serelaxin, a recombinant form of human relaxin-2, is currently being investigated as a potential therapeutic agent in acute heart failure, though recent phase III trial data suggests that it does not reduce cardiovascular death at six months when added to standard therapy (Tietjens and Teerlink, 2015; Novartis provides update on Phase III study of RLX030 (serelaxin) in patients with acute heart failure | Novartis, 2017).

Early studies of vascular tone in pregnancy described the role of eicosanoids in this process, including prostacyclin (PGI2), a potent vasodilator and inhibitor of platelet aggregation, and thromboxane (TXA2), a vasoconstrictor which can potentiate platelet aggregation (Myatt and Elder, 1977; Mitchell et al., 1978; Walsh, 2004). Arachidonic acid is converted to prostaglandin and thromboxane via the constitutive cyclooxygenase (COX-1) and inducible COX-2 enzymes (Fitzpatrick, 2004). Prostacyclin synthase in the vascular endothelium produces PGI2, which can then act in a paracrine manner to exert its vasodilatory effect. In the pregnant female, PGI2 production is augmented by the placenta, uterine vessels, amnion, chorion, and decidua (Walsh, 2004). As a result of the increased synthesis of PGI2 in comparison to its counterpart, TXA2, systemic vasodilation is potentiated throughout pregnancy. Alterations in the prostacyclin-thromboxane balance, particularly with decreased placental PGI2 production, have thus been implicated in the pathogenesis of preeclampsia (Weiner et al., 1989; Ylikorkala et al., 1981). Clinical trials of low-dose aspirin, an inhibitor of TXA2 synthesis, aimed at preventing preeclampsia in at risk women have yielded promising results (CLASP Collaborative Group, 1994; Sibai et al., 1993; Schiff et al., 1989), prompting the current recommendation to prescribe aspirin 81 mg for women considered to be at high risk for preeclampsia (LeFevre and U.S. Preventive Services Task Force, 2014).

Estrogen acts through several different mechanisms to cause vasodilation and subsequent volume expansion. Dehydroepiandrosterone sulfate (DHEA-S), produced by the maternal and fetal adrenal glands, is converted to estrogen in the placenta, accounting for the progressive increase in the concentration of this sex hormone during pregnancy (Manabe et al., 1999). Activation of the traditional isoforms of the estrogen receptor (ERα, ERβ), in addition to the G protein-coupled estrogen receptor (GPER), increased NO synthase activity, cGMP production, and caused vasodilation of the systemic and uterine circulation (Maliqueo et al., 2016; Tropea et al., 2015; Rosenfeld et al., 1996). Estrogen further causes vasodilation by stimulation of PGI2 synthesis via upregulation of COX-1 expression (Maliqueo et al., 2016). In a similar manner to estrogen, progesterone increases NOS activity, PGI2 production as mechanisms to promote vasodilation. Progesterone also directly decreases intracellular calcium concentrations to achieve the same effect (Maliqueo et al., 2016).

Plasma renin activity also rises considerably in pregnancy (Wilson et al., 1980). The RAAS is primarily thought to exert vasoconstrictive effects. However, during pregnancy, estrogen alters the dynamics of the RAAS, favoring its vasodilatory effects, sodium retention, and volume expansion (O'Donnell et al., 2014). Activation of angiotensin converting enzyme isoform 2 (ACE2) yields angiotensin-(1–7) (ang-(1–7)), a component of vasodilatory arm of the RAAS (Valdes et al., 2009). Ang-(1–7), via the Mas receptor, once again increases nitric oxide synthase activity and thus vasodilation (Sampaio et al., 2007). Additionally, angiotensin II acts via the angiotensin II type 2 receptor (AT2R) to promote vasodilation (Widdop et al., 2002).

The sum of these pathways is a primary reduction in SVR, which, in turn, prompts nonosmotic release of arginine vasopressin and further activation of RAAS due to a sensed decrease in effective arterial blood volume. These processes result in increased sodium and water retention, and thus, volume expansion (Schrier, 1988). In animal models, failure of these pathways is hypothesized as a potential cause of poor maternal/fetal outcomes, due to sustained systemic hypertension, preeclampsia, prevention of the normal renal vasodilation, and proteinuria (Deng et al., 1996).

Blood Pressure

The result of these changes in volume and vascular tone results in decrease in systemic blood pressure, though to a lesser degree than the significant decrease in peripheral vascular resistance (**Table 1**) (Ouzounian, 2012). This is because the effect of systemic vasodilation on blood pressure is partly ameliorated by an increase in CO. Diastolic pressure typically decreases to a greater extent (5–10 mmHg), than systolic blood pressure, (2–3 mmHg) (Head et al., 2009). Measureable mean arterial blood pressure changes begin at week 6 of pregnancy, dropping to a nadir between weeks 16 and 20. Blood pressure subsequently begins to rise again, approaching prepregnancy levels at term (Hall et al., 2011; Moutquin et al., 1985).

Heart rate

With the decreased SVR and increased circulating volume, heart rate increases throughout pregnancy in order to maintain adequate CO, reaching its peak near term. Heart rate increases up to 25% (approximately 10–20 beats per minute) above prepregnancy values, starting as early as week 5 of gestation and continuing into the late third trimester (Sanghavi and Rutherford, 2014; Mahendru et al., 2014; Rubler et al., 1977).

Stroke volume

Increases in stroke volume also begin as early as week 6 of pregnancy, reaching a peak near week 32, and remaining stable until delivery (Clapp and Capeless, 1997). The increased preload, in addition to the increased ventricular wall muscle mass, result in augmented myocardial contractility.

Cardiac Output

As early as 1932, Grollman and colleagues concluded that CO increased during pregnancy, approximately 50% above baseline levels (Grollman, 1932; Gilson et al., 1997). CO rises to a peak of greater than 6 L/min at 32 weeks, then declines slightly until term, though still to a value greater than the prepregnancy baseline (Mabie et al., 1994; Hennessy et al., 1996). The initial increase in total CO results from augmented stroke volume, with the later increase in CO occurring secondary to elevation in heart rate (**Fig. 3**) (Liu and Arany, 2014), combined with the aforementioned decrease in SVR. Notably, the difference in arterial-venous oxygen content narrows during early pregnancy, suggesting that the increase in supply, during the normal pregnancy, exceeds demand (Conrad and Novak, 2004).

CO is also position dependent, given the mass effect of the gravid uterus on the inferior vena cava (IVC) and aorta. When supine, the uterus compresses the IVC, decreasing venous return to the heart and thus reducing CO. In fact, near term (38–40 weeks), there can be an increase in CO of 25% when moving from the supine to left lateral decubitus position, with associated increase in left-ventricular end-diastolic pressure (Gilson et al., 1997; Secher et al., 1979; Kundra et al., 2012; Armstrong et al., 2011; Lange et al., 1988). Recent analysis on cardiac parameters with left lateral tilt, as assessed by magnetic resonance imaging, suggests an increase in CO, stroke volume, and end-diastolic diameters of both ventricles (Rossi et al., 2011). Additionally, there is a differential distribution of blood flow and CO during pregnancy (**Table 2**) (Ouzounian, 2012); the gravid uterus can receive 17% of total output, compared to 2% in nonpregnant states (Thaler et al., 1990; Konje et al., 2001). This may result in reduced relative output to skeletal muscle and the splanchnic bed (Palmer et al., 1992). Renal blood flow increases enough to up to 70%, with a resultant

Table 1 Circulatory changes during normal pregnancy

	Changes at various times (wk)					
Parameter	5	12	20	24	32	38
HR	↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑↑	↑↑↑↑
Systolic blood pressure	↔	↓	↓	↔	↑	↑↑
Diastolic blood pressure	↔	↓	↓↓	↓	↔	↑↑
Stroke volume	↑	↑↑↑↑↑	↑↑↑↑↑↑	↑↑↑↑↑↑	↑↑↑↑↑	↑↑↑↑↑
CO	↑↑	↑↑↑↑↑↑	↑↑↑↑↑↑↑	↑↑↑↑↑↑↑	↑↑↑↑↑↑↑	↑↑↑↑↑↑↑
Systemic vascular residence	↓↓	↓↓↓↓↓	↓↓↓↓↓↓	↓↓↓↓↓↓	↓↓↓↓↓↓	↓↓↓↓↓
Left ventricular ejection fraction	↑	↑↑	↑↑	↑↑	↑	↑

↑, ≤5%; ↑↑, 6%–10%; ↑↑↑, 11%–15%; ↑↑↑↑, 16%–20%; ↑↑↑↑↑, 21%–30%; ↑↑↑↑↑↑, >30%; ↑↑↑↑↑↑↑, >40%.

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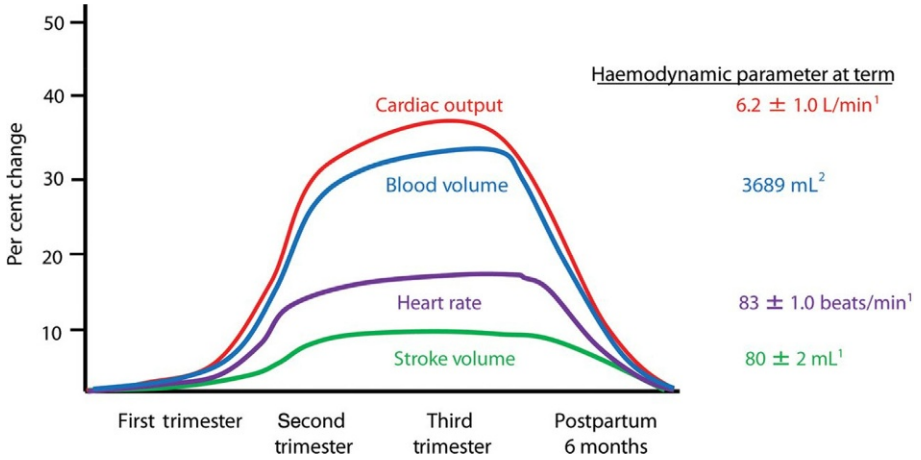

Fig. 3 Percent change in stroke volume, heart rate, blood volume, and CO during pregnancy. Reproduced with permission from Liu, L. X. and Arany, Z. (2014). Maternal cardiac metabolism in pregnancy. *Cardiovascular Research* **101**, 545–553.

Table 2 Changes in regional blood flow during normal pregnancy

Organ	*Change*	*Comments*
Uterus	Increased	Increase from 50 mL/min at 10 wk to 1200 mL/min at term
Kidneys	Increased	30%–80% increase with 50% increase in GFR. Returns to nonpregnant state at term
Extremities	Increased	Flow to hands greater than flow to feet
Skin	Increased	Results in warm skin, clammy hands, nasal congestion
Liver	Unchanged	—
Brain	Unchanged	—
Breast	Increased	May cause flow murmurs

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increase in the GFR by 50% (Thornburg et al., 2000). In fact, the positional variation in CO due to aortocaval compression makes it such that maternal resuscitation is more effective if the mother is in the left lateral position (Tan and Tan, 2013).

Anatomic Changes and Echocardiography

The noted changes in plasma volume, vascular resistance, and CO result in distinctive cardiac remodeling during normal pregnancy. As volume expands during pregnancy, all the four cardiac chambers increase in size to accommodate this physiologic state (Liu and Arany, 2014). The relative impact of this volume is greater on the right, with an up to 20% increase in right atrial and ventricular sizes, compared to an approximate increase of 10% in the left-sided chambers (Ain et al., 2012). This chamber dilation also results in dilation of the valve annulus, causing clinically insignificant tricuspid or mitral valve regurgitation in up to a third of pregnant women. Ventricular remodeling may occur, along with other anatomic changes to the heart as a result of the loading conditions. As a result of increased wall stress, eccentric hypertrophy occurs, resulting in progressive increase in left ventricular mass of, peaking at 15%–25% above prepregnancy levels at term (Robson et al., 1989; Geva et al., 1997; Simmons et al., 2002). Left ventricular end-diastolic dimension also increases, without a significant change in ventricular filling pressure, pulmonary artery pressure, or pulmonary capillary wedge pressure, as noted by both echocardiography and cardiac catheterization (Clark et al., 1989; Bader et al., 1955; Estensen et al., 2013). Notably, cardiac remodeling during pregnancy appears similar to the changes seen in athletes (Simmons et al., 2002). These physiologic changes to the structure of the heart are typically well tolerated, without significant morbidity, mortality, or long-term negative effects on cardiac function (Li et al., 2012).

Echocardiography reveals no significant change in ejection fraction during normal pregnancy (Katz et al., 1978), though the elevated CO can be noted via Doppler echocardiography (Vered et al., 1991). Additionally, as pregnancy progresses, functional multivalvular regurgitation may be noted in a majority of women due to the aforementioned annular dilatation. Reflecting relatively greater increase in right-sided chamber size, physiologic valvular regurgitation of the tricuspid and pulmonic valves is noted more frequently than mitral regurgitation (Campos et al., 1993). Aortic regurgitation is not a normal finding in pregnancy (Campos et al., 1993; Dennis, 2011). Finally, a trace to small pericardial effusion may be noted in up to 40% of pregnant women, typically resolving after delivery (Haiat and Halphen, 1984; Ristic et al., 2003). In twin pregnancies, these changes are more pronounced, with a particular increase in heart rate, CO, and atrial size (Robson et al., 1987a). New research with speckle-tracking will provide further insights regarding the noninvasive evaluation of maternal cardiac function during pregnancy (Cong et al., 2015).

Electrocardiogram

These anatomic changes, in combination with the expansion of the gravid uterus and elevation of the diaphragm, result in anterior and leftward displacement of the heart, which prompts changes on the electrocardiogram (Vårtun et al., 2015). The culmination of the various cardiovascular changes of pregnancy can yield an increased sinus rate, left axis deviation, nonspecific ST-T changes, T-wave inversion, and increased atrial/ventricular ectopy (Wenger et al., 1964; Schwartz and Schamroth, 1979; Carruth et al., 1981). Additionally, there is an increase in the prevalence of supraventricular tachyarrhythmias, potentially driven by physiologic atrial enlargement (Adamson and Nelson-Piercy, 2007).

Physical Exam

The physical examination in pregnancy reflects the physiologic state of hypervolemia, with its associated changes in CO, chamber size, and blood pressure. Resulting from the increased circulating volume, we see increases in the level of jugular venous pulsation and a systolic flow murmur (often along the left sternal border). Pedal edema is often present, caused by elevated hydrostatic pressure in the venous system and a relative decrease in albumin concentration. Changes in the cardiac chambers can result in a loud first heart sound (S1), a split second heart sound (S2), and even a third heart sound (S3), in addition to a laterally displaced point of maximal impulse. Furthermore, with the aforementioned increase in annular diameter, systolic murmurs associated with valvular regurgitation are common. Coincident with this hyperdynamic state, tachycardia and bounding peripheral pulses can also be noted (Goodlin et al., 1983). With increased blood flow to peripheral tissues, nasal congestion may be noted and the extremities can be warm and erythematous. Consequently, mammary blood flow also increases, causing superficial vasodilation and a continuous murmur known as the mammary souffle (Tabatznik et al., 1960).

Delivery/Postpartum

The hemodynamic changes of pregnancy serve to provide adequate supply of oxygen and nutrients for the mother and maturing fetus. At delivery, these changes are further augmented by the uterine contractions, driven by increased sympathetic tone, and mobilization of extravascular tissue. These events result in elevated heart rate, higher stroke volume, and thus increased CO, both at baseline and during contractions. In the first stage, CO can increase up to 15%, with an additional 50% increase in the second stage of labor. Consequently, blood pressure also rises transiently during this time (Robson et al., 1987b). CO peaks immediately postdelivery, due to autotransfusion from the placenta and relief of aortocaval compression, resulting in a significantly increased venous return. Within 1 h of delivery, however, CO returns to prelabor levels via reduction in heart rate, stroke volume, and blood pressure (Soma-Pillay et al., 2016). The return to prepregnancy hemodynamics, however, takes more time (Ruys et al., 2013). CO begins to drop further in the first 24 h, returning to baseline at 6–8 weeks postdelivery. Notably, ANP and brain natriuretic peptide (BNP), both elevated as a result of the elevated circulating volume of pregnancy, help mediate postpartum diuresis and a return to prepregnancy volume status by 6 weeks postpartum (Peck and Arias, 1979; Ouzounian, 2012). Vascular resistance and blood pressure require up to 12 weeks to return to normal, and cardiac chamber size by echo can be enlarged for up to 24 weeks. Notably, our recent research suggests that these changes in heart structure and function may not revert completely to prepregnancy homeostasis, may accumulate with each successive pregnancy, and be detectable in midlife on cardiac MRI (Parikh et al., 2012).

However, these changes may persist beyond a year and are potentiated by subsequent pregnancies (Clapp and Capeless, 1997). Given the complex hemodynamic and anatomic effects of pregnancy on the cardiovascular system, clinical history, physical examination, and diagnostic testing must be considered with these changes in mind.

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Hemodynamics of the Right Heart in Health and Disease

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Introduction

Historically relegated the "underdog" ventricle, a status perhaps bolstered by seminal work in the 1940s and 1950s supporting cardiac output preservation even in the face of complete right ventricular cauterization (Kagan, 1952; Starr et al., 1943), the right ventricle (RV), and right heart, has leapt in repute and respect as it has humbled us with its importance in functional capacity and prognosis in congenital heart disease, pulmonary arterial hypertension, myocardial infarction, and heart failure (D'Alonzo et al., 1991; Di Salvo et al., 1995; Gavazzi et al., 1997; Ghio et al., 2001). In this article, we summarize salient features of RV anatomy and physiology, review important hemodynamic concepts of the right heart in health and apply these concepts in major disease states, and review right heart hemodynamic assessment by both invasive and noninvasive approaches.

RV Anatomy

Located immediately behind the sternum and most anteriorly situated, the RV is demarcated by the tricuspid valve and pulmonary valves. The RV can be divided into tripartite components: (1) inlet (tricuspid valve, chordae tendineae, and papillary muscles); (2) apical trabeculated myocardium; and (3) smooth outlet region. This classification approach lends itself clinically relevant particularly in congenital heart disease where one or more of the components may be absent (Goor and Lillehei, 1975; Ho and Nihoyannopoulos, 2006). Hallmark differences between the left ventricle (LV) and RV are shown in **Table 1**. Importantly, the RV myocardial two-layer fiber orientation dictates its contraction pattern; the outer circumferential fibers contribute toward inward movement compared to the longitudinal fibers at the endocardial level promoting longitudinal motion. This longitudinal shortening of the RV myocytes at the endocardial level lends insight into why longitudinal measures of RV systolic function, particularly via echocardiography, are robust in RV function assessment.

Normal Right Heart Hemodynamic Concepts

RV Preload

The primary function of the right heart, specifically RV, is to receive all the blood returning from the periphery (venous return) and to pump into the pulmonary circulation with the rate of venous return dictated by peripheral resistance. Due to the low-pressure, highly

Table 1 Key differentiating features of RV compared to LV

Embryologic	Second heart field (RV, portions of atria, outflow tract, interatrial septum, cardiac conduction system) First heart field (LV, atrioventricular canal, portions of atria)
Myocyte fiber orientation	RV—2 layers (outer – oblique/circumferential, inner – longitudinal) LV—3 layers (outer – oblique, middle – circumferential, inner – longitudinal)
Morphologic differences	(1) Hinge point of septal leaflet is more apical in relation to anterior mitral valve leaflet (2) Greater than 2 papillary muscles (3) Coarse apical trabeculations (4) Presence of moderator band in RV (5) Trileaflet configuration of tricuspid valve with septal papillary attachments
Shape	Triangular when viewed from side; crescent-shaped in cross-section
Size	RV mass is 1/6th of LV; slightly larger end-diastolic volume than LV; thinner than LV
Perfusion	Infundibulum and anterior RV receive more constant arterial supply (conal artery) from right coronary artery; coronary artery dominance impacts vascularization of inferior/diaphragmatic wall and inferior septum; left coronary artery supplies the free wall of the RV adjacent to the anterior portion of septum and apical RV

compliant pulmonary circuit the RV pumps the same stroke volume of the LV but at one-fourth of the stroke work (Anastasiadis, 2015). Hemodynamic studies by Pinky and colleagues (Pinsky, 2014) evaluating the effect of positive end-expiratory pressure on RV function showed that RA transmural pressure (or RV filling pressure) has no relationship on RV end-diastolic volume, supporting the concept that the RV operates under its unstressed volume; thus RA pressure should not be equated to RV preload (Pinsky, 2014; Tyberg et al., 1986). A clinically important fact, RA pressure should be not used to predict volume responsiveness (Shippy et al., 1984) but can help predict impending RV failure when ongoing fluid resuscitation results in no cardiac output increase and an abrupt increase in RA pressure is seen (Jardin et al., 1985). A corollary finding is if RV volume does impact RA pressure it is in the setting of an abnormal RV (RV hypertrophy, RV diastolic dysfunction, or RV over-distension) (Pinsky, 2014).

RV Afterload

While the right and left ventricles circulate the same blood volume, the pulmonary circulation is notably one of low-resistance, only one-sixth of the systemic circulation, and utilizes one-fifth of energy of the left ventricle (Lammers et al., 2012). This high-flow, low-pressure system of the right heart requires efficient ventricular-vascular coupling. In its purest sense, RV afterload is the RV wall stress that occurs during RV ejection. With its small ventricular radius and constant wall thickness during ejection, the largest contributor to RV wall stress is the RV pressure during ejection. However, the RV pressure during ejection is complex and composed of four main components: (1) resistance to blood flow during steady state; (2) compliance (the blood storage capacity of the pulmonary system); (3) arterial wave reflections that occur as a result of pulsatile blood flow; and (4) blood inertance during RV ejection (Tedford, 2014). The most comprehensive method for RV vascular load assessment, taking into account all four components, is pulmonary artery input impedance (with application of Fourier transformation which separated pressure and flow signals into their respective sums of harmonic oscillations) classically described in 1965 (Milnor et al., 1969). However, pulmonary impedance is both difficult to measure and interpret; simpler models, such as the 3-element Windkessel models, (extending original work in a two-element model by Frank (1899)) have been applied to the arterial circulation. The three-element model, resistance, capacitance, and impedance, is a lumped model of the entire systemic arterial tree to define the total load on the heart (Westerhof et al., 2010). In physiologic terms, resistance (due to dependence on vessel diameter) is quantified by small arteries, arterioles, and capillaries as the pulmonary vascular resistance (PVR)—a static, steady-state resistance. Compliance, representing the storage capacity of all arteries and arterioles with the largest contribution usually from the proximal elastic conduit arteries, takes into consideration the pulsatile load in the system. However, in the pulmonary tree, the total compliance is distributed throughout the arterial tree with the main, right, and left PA only contributing to 15%–20% of the pulmonary compliance (Saouti et al., 2009). Characteristic impedance (Z_c) of the proximal pulmonary artery accounts for the fact that blood must be accelerated in early ejection and into a compliant artery; thus blood mass, pulmonary artery diameter, and wave speed components are utilized (Tedford, 2014; Westerhof et al., 2010; Saouti et al., 2010).

Relationship of RV to Load

The heart pumps at near maximal efficiency and maximal power. Whether it maintains at this operational level depends on both its cardiac and arterial state, that is, ventriculo-arterial coupling. The RV adapts to increased vascular load by enhancing "coupling" to maintain flow (Vonk Noordegraaf et al., 2017). This coupling concept is best depicted by constructing RV pressure-volume (P-V) loops for a single cardiac cycle with the four sides of the loop denoting: (1) isovolumetric contraction; (2) ejection; (3) isovolumetric relaxation; (4) filling. The loop demarcates boundaries on the end-systolic pressure-volume relationship (ESPVR) and end-diastolic pressure–volume relationship (EDPVR). Relatively linear, ESPVR slope is *RV* end-systolic elastance (E_{es}) which represents RV contractility (muscular properties and muscle hypertrophy) with the intercept volume-axis V_o. The *pulmonary* arterial elastance (E_a), generated at the end-diastolic volume axis extending to the intersection of the ESPVR at the end-systolic pressure–volume point

of the RV P-V loop, represents RV afterload (often simplistically, PVR). EDPVR, a nonlinear parameter, represents diastolic properties and can characterize two characteristics: (1) diastolic stiffness (represented by the change in pressure divided by the change in volume on any given point on the EDPVR curve); (2) ventricular capacitance (the ventricular volume at any given pressure) (Vonk Noordegraaf et al., 2017; Burkhoff et al., 2015). Ventriculo-arterial coupling can be defined as the ratio of RV elastance to pulmonary elastance (E_{es}/E_a). RV mechanical work and oxygen consumption is most optimal when this ratio is between 0.5 and 2.0 (Burkhoff and Sagawa, 1986; Sunagawa et al., 1985; Kuehne et al., 2004; Sagawa, 1988). **Fig. 1** depicts a normal RV P-V loop with changes in E_a and E_{es} with changes in afterload, preload, and RV contractility.

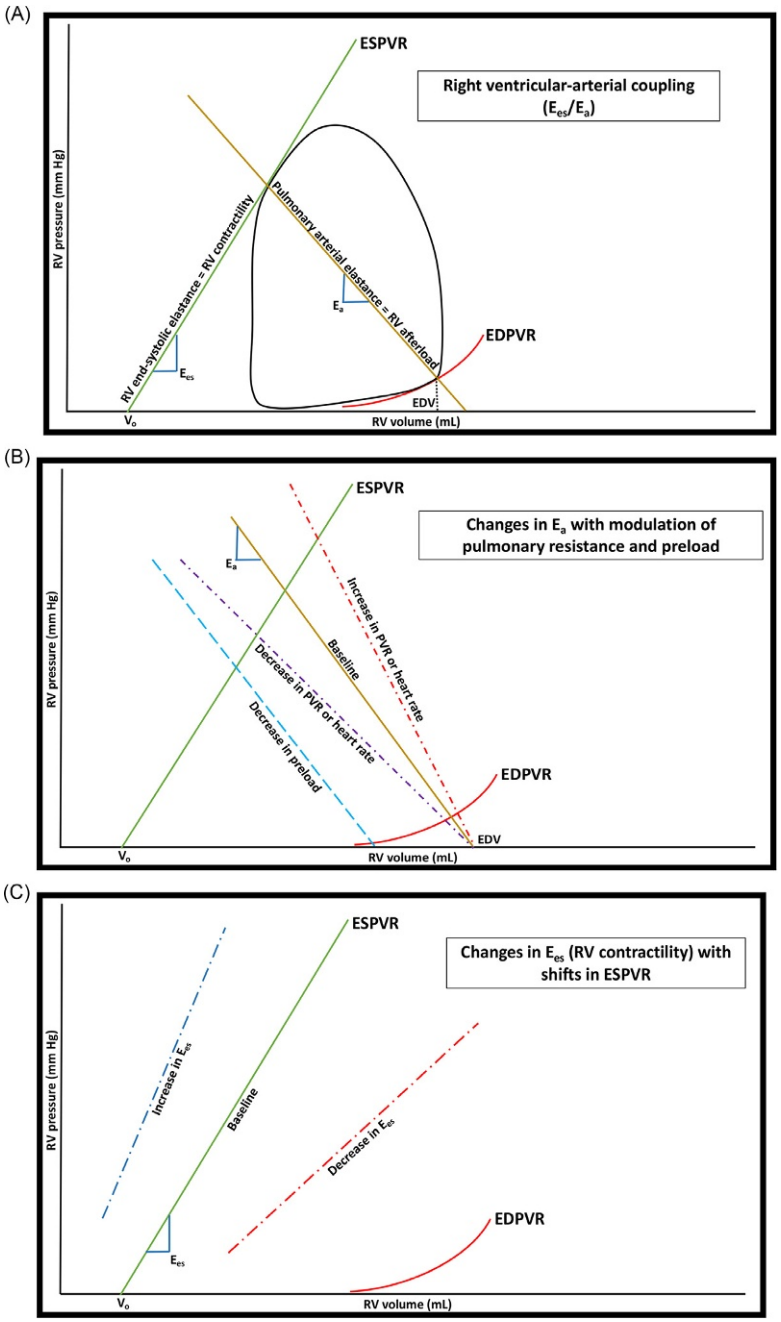

Fig. 1 Overview of pressure-volume loops and RV-arterial coupling. (A) Normal pressure-volume loop depicting ESPVR, EDPVR, E_a, and E_{es}. Right ventricular-arterial coupling relationship is E_{es}/E_a. (B) E_a slope is altered with changes in resistance and volume; ESPVR and EDPVR are static assuming no other changes are occurring with the heart. (C) Changes in contractility of RV are reflected with ESPVR shifts; leftward-upward with increase in contractility; downward-rightward with decrease in contractility. Modified from Burkhoff, D., Sayer, G., Doshi, D., Uriel, N. (2015). Hemodynamics of mechanical circulatory support. *Journal of the American College of Cardiology* 66, 2663–2674, with permission from Elsevier.

Pressure Proportionality in the Right Heart

Compared to the 50% increase in systemic vascular resistance in hypertension, PVR may increase by a factor of 4 or more in the pulmonary circuit (Handoko et al., 2016; London et al., 1987). Accordingly, the pulmonary arterial pressure will increase and interestingly (and likely underappreciated), the pulmonary arterial (PA) systolic and diastolic pressures are proportional to the mean pulmonary pressures (Chemla et al., 2004; Syyed et al., 2008) even across a wide range of increased PVRs with and without elevated pulmonary capillary wedge pressure (PCWP) (Handoko et al., 2016). Reliable calculations of mean and diastolic pressures estimated from invasive or echocardiography-derived PA systolic pressures (sPAP) are as follows: diastolic pulmonary pressure (dPAP) = 0.36 × sPAP; mean PAP (mPAP) = 0.61 × sPAP; pulmonary pulse pressure (PP) = 0.6 × sPAP (Handoko et al., 2016).

Resistance and Compliance: An Inverse Relationship in the Pulmonary Circuit

The source of compliance is a major difference between the pulmonary and systemic arterial systems. Compliance in the pulmonary system is distributed over the entire arterial system (which is dependent on the number of peripheral vessels which is ~8-10 times more compared to the systemic arterial system where 80% of compliance is located in the thoracic-abdominal aorta) (Kind et al., 2011). Due to this difference, pulmonary resistance and compliance pulmonary resistance and compliance are inextricably related in the pulmonary system. Originally described in 1971 (Reuben, 1971), the hyperbolic relationship between PVR and pulmonary compliance has been further studied in pulmonary hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH) (Saouti et al., 2009; Lankhaar et al., 2008; Lankhaar et al., 2006). In addition, the impact of elevated PCWP shifts this hyperbolic relationship downward-leftward; thus, elevated filling pressures may augment pulmonary pulsatile load leading to lower compliance for any given PVR (Tedford et al., 2012). **Fig. 2** depicts this hyperbolic relationship between PVR and pulmonary compliance.

Ventricular Interdependence

The impact of size, shape, and compliance of one ventricle on the performance of the other ventricle via mechanical interactions is called interventricular dependence (Feneley et al., 1985), a critical concept in the pathophysiology of right heart dysfunction. Three main components contribute to ventricular interdependence: (1) the interventricular septum; (2) the pericardium; (3) the muscular fiber continuity between the RV and LV; the contribution of each of this components varies by phase in cardiac cycle. Unquestionably, diastolic ventricular interaction is paramount; seminal hemodynamic studies have shown that increasing RV volume shifts the LV EDPVR upward and leftward (i.e., less compliance) and reciprocally, as LV volume increases, a similar upward-leftward of the EDPVR curve is seen in the RV. This relationship was similarly shown with independent loading of a ventricle (with a fixed volume) resulting in similar upward-leftward shift of the EDPVR of the contralateral ventricle (Bemis et al., 1974; Elzinga et al., 1974;

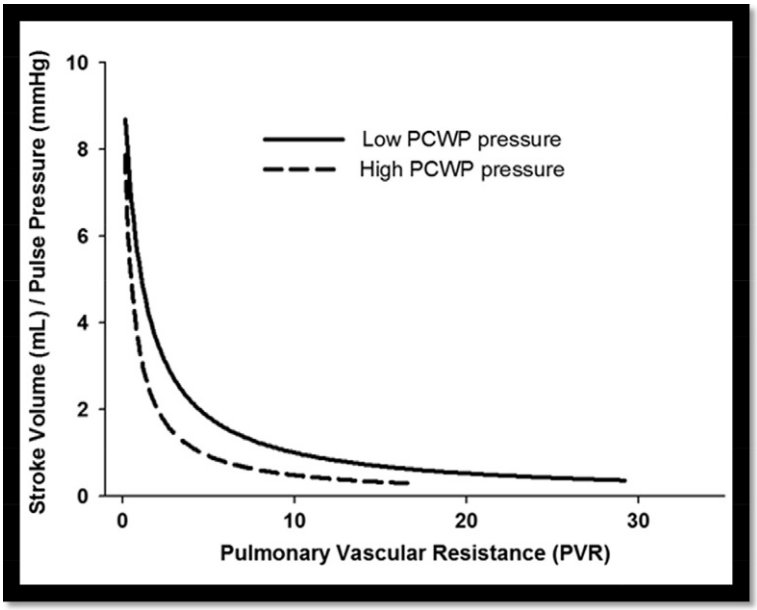

Fig. 2 Hyperbolic relationship between pulmonary vascular resistance and pulmonary compliance (represented by stroke volume/pulse pressure) with interaction with left ventricular filling pressures (represented by PCWP). *PCWP*, pulmonary capillary wedge pressure. Adopted from Tedford, R. J. (2014). Determinants of right ventricular afterload (2013 Grover Conference series). *Pulmonary Circulation* 4, 211–219.

Laks et al., 1967; Santamore et al., 1976; Taylor et al., 1967). These have significant clinical consequences: decreased distensibility (with the shift of the EDPVR upward-leftward), LV preload decline, increase in LV end-diastolic pressure, and lowering of cardiac output (Haddad et al., 2008a). Impaired LV filling can be related to: (1) parallel interaction—leftward bowing of the septum into the LV or (2) series interaction—decreased LV filling due to lower RV stroke volume (Gan et al., 2006). The septum and pericardium are both important in diastolic ventricular interdependence and the presence of the pericardium enhances the proportion of mechanical contribution of the septum to ventricular interdependence (Slinker and Glantz, 1986). However, the pericardium may be less vital in systolic interdependence (Feneley et al., 1985; Santamore and Dell'Italia, 1998). The interventricular septum mediates the major contribution to systolic interdependence; animal studies show LV contraction contributes to 20%–40% of the RV systolic pressure and volume outflow (Santamore and Dell'Italia, 1998; Damiano et al., 1991) and without RV dilation, the septum can maintain circulatory stability even in the setting of an inert RV patch or RV scarring (Hoffman et al., 1994).

Invasive Right Heart Hemodynamic Assessment

Invasive measurements via Swan-Ganz catheterization continue to be the mainstay in assessment of right heart hemodynamics in clinical practice. Variables of interest with normal ranges are displayed in **Table 2**.

Definitions of Pulmonary Hypertension

Pulmonary hypertension (PH) is defined as a mPAP $\geq$ 25 mmHg at rest (Hoeper et al., 2013). PH can be divided into two main subtypes: (1) precapillary PH (mPAP $\geq$ 25 mmHg and PCWP $\leq$ 15 mmHg); and (2) postcapillary PH (mPAP $\geq$ 25 mmHg and PCWP $\geq$ 15 mmHg (Hoeper et al., 2013; Galie et al., 2016). Precapillary PH includes the clinical groups pulmonary arterial hypertension (PAH) (precapillary PH criteria in addition to PVR > 3 WU in the absence of other etiologies), PH due to lung disease, chronic thromboembolic PH, and PH due to other/multifactorial mechanisms. Postcapillary PH includes PH secondary to left heart disease and PH due to other/multifactorial mechanisms (Galie et al., 2016). Pulmonary vascular resistance (PVR) is calculated taking mPAP-PCWP/cardiac output. PVR > 3 WU is part of the hemodynamic definition for PAH (McLaughlin et al., 2009). Importantly, patients with left heart disease can have additional classification by evaluating the trans-pulmonary gradient (TPG) or diastolic pressure gradient (DPG). TPG, the difference between mPAP-PCWP, has been used to define "passive" PH (TPG < 12 mmHg) vs. "reactive" PH (TPG $\geq$ 12 mmHg) (Galie et al., 2009). Some studies have proposed that TPG is influenced by mechanisms that impact mPAP including resistance, cardiac output, compliance, and left heart filling pressures (Naeije et al., 2013; Provencher et al., 2008; Vachiery et al., 2013) and advise the use of the DPG (end-diastolic pressure (dPAP)—PCWP) to determine pulmonary vascular disease (Naeije et al., 2013; Provencher et al., 2008). In most patients, the DPG lies between 1 and 3 mmHg, and even with patients with left heart disease (excluding shunts) the DPG $\leq$ 5 mmHg (Naeije et al., 2013; Vachiery et al., 2013). A DPG < 7 mmHg (and/or PVR $\leq$ 3 WU) can be classified as isolated postcapillary PH compared to combined post- and precapillary PH is defined with a DPG $\geq$ 7 mmHg (and/or PVR $\geq$ 3 WU) (Galie et al., 2016). Recently, Handoko and colleagues (Handoko et al., 2016) conducted an elegant hemodynamic study with 1054 patients comprised of patients without PH, patients with elevated mPAP and low PCWP, and patients with elevated mPAP and elevated PCWP. The conclusions of this study implied that dPAP and mPAP depend equally on PCWP (with PCWP between 1 and 31 mmHg) and both depend similarly on CO, and

Table 2 Normal right heart catheterization hemodynamics

Right atrium (RAP)	
Mean pressure	0–6 mmHg
Right ventricle (RV)	
Peak-systolic pressure	18–24 mmHg
End-diastolic pressure	0–6 mmHg
Pulmonary artery	
Mean pressure (mPAP)	11–20 mmHg
Peak-systolic pressure (sPAP)	18–28 mmHg
End-diastolic pressure (dPAP)	6–15 mmHg
Pulmonary capillary wedge pressure (PCWP)	
Mean pressure	8–12 mmHg
Resistance [(mPAP-PCWP)/cardiac output)]	
Pulmonary vascular resistance	<2 WU (40–160 dynes*s/cm^5)
Cardiac index (cardiac output/BSA)	2.8–4.2 L/min/m^2
Trans-pulmonary gradient (mPAP-PCWP)	<12 mmHg
Diastolic pressure gradient (DPG)	1–3 mmHg
RV stroke work index (RVSWI)	580–735 mmHg $\times$ mL/m^2
Pulmonary compliance (PAC)	3.8–12 mL/mmHg
Pulmonary artery pulsatility index (PAPi)	>1.85

there were no significant advantages to using DPG vs TPG (Handoko et al., 2016). At this time, however, there is still insufficient data in determining the best variable to predict outcomes in PH with left heart disease.

Hemodynamic Approach for Assessment of RV Performance

The utilization of several hemodynamic parameters is helpful in assessing right heart function and performance. Noninvasive cardiac imaging, particularly echocardiography and cardiac magnetic resonance imaging, have a critical role in assessment of RV performance via structural information of right heart volumes, contraction and motion, myocardial disease, in addition to hemodynamic assessments that can be applied in a more frequent manner for clinical monitoring. However, the gold standard assessment for measuring absolute intracavitary pressures still lies in the invasive assessment, particularly when guiding more advanced therapies in right heart management. It must be acknowledged that noninvasive hemodynamic evaluations that can target the hemodynamic parameters listed below are either currently available or being developed and while may prognosticate, are beyond the scope of this review. We will reserve the majority of our description of the RV performance assessment to an invasive assessment but will include essential noninvasive measurements that aid in this evaluation. Much of our evaluation of RV performance has been gleaned from the mechanical support literature, particularly with studies evaluating RV failure post left ventricular assist device (LVAD) implantation and determining risk factors/predictors for poor RV outcomes. No single parameter has been absolutely consistent across studies, but important parameters are highlighted below.

RA Pressure

An elevated right atrial pressure can herald significant RV dysfunction. RA pressures >15 mmHg are predictive of RV failure in the mechanical circulatory support (MCS) literature (Atluri et al., 2013; Dang et al., 2006). An elevated RAP/PCWP ratio (greater than 0.63) has been shown to be predictive of RV failure in patients receiving LVAD (Kormos et al., 2010).

RV Stroke Work Index

RV stroke work index (RVSWI) provides a quantitative measure of the ability of the RV to generate pressure and flow incorporating both hemodynamics and right ventricular function and serves as a measure of RV contractility. The calculation for the formula is as follows:

$$\text{RVSWI}\ \left(\text{mmHg}\times\text{mL/m}^2\right)=\left[\text{mPAP}\ \left(\text{mmHg}\right)-\text{RAP}\ \left(\text{mmHg}\right)\right]\times\text{SV}\ \left(\text{mL}\right)/\text{BSA}\ \left(\text{m}^2\right)$$

To convert to g/m^2/beat multiply by the density of mercury factor of 0.0136 to the equation. *SV*, stroke volume; *BSA*, body surface area.

Several studies have shown that RVSWI can predict mortality and length of hospitalization following lung transplantation (Armstrong et al., 2013), predict right ventricular failure prompting RV assist device post-LVAD implantation (Fitzpatrick et al., 2008), and predict duration of inotropic therapy for RV failure post-MCS therapy (Schenk et al., 2006). RVSWI < 300–600 mmHg × mL/m^2 (Starr et al., 1943) generally portends worse RV performance (Ochiai et al., 2002).

Pulmonary Compliance

Pulmonary compliance (PAC) is an attribute in the pulmonary system that, in simple terms, can be defined as a change in lumen area for a given change in pressure. Compliance calculation allows the characterization of all the pulmonary vasculature to accommodate blood in systole and release in diastole and is a measure of arterial distensibility. Calculation of pulmonary compliance via hemodynamic measurements using stroke volume and pulmonary pulse pressure has been validated (Lankhaar et al., 2006; Stergiopulos et al., 1999; Stergiopulos et al., 1995) with prognosis in HF (Dupont et al., 2012). Calculation of pulmonary compliance allows calculation of the pulsatile load (in addition to resistive load) contribution to afterload in the pulmonary circulation (Ghio et al., 2015). Pulmonary compliance, thus, can be defined with the following formula:

$$\text{PAC}\ \left(\text{mL/mmHg}\right)=\text{SV}\ \left(\text{mL}\right)/\text{pulmonary artery pulse pressure}\ \left(\text{mmHg}\right)$$

Pulmonary artery pulse pressure = sPAP (mmHg) − dPAP (mmHg)

Pulmonary Vascular Resistance

PVR, a major component of RV afterload, contributes significantly to RV dysfunction and has been shown to be a powerful predictor of RV failure early postoperative following LVAD implantation with increasing weight with increasing quartile of PVR (Drakos et al., 2010). Calculation of PVR is as follows:

$$\text{PVR}\ \left(\text{Wood's unit}\right)=\left[\text{mPAP}\ \left(\text{mmHg}\right)-\text{PCWP}\ \left(\text{mmHg}\right)\right]/\text{CO}\ \left(\text{L/min}\right)$$

To convert to dynes*s/cm^5, multiple above equation by 80.

Pulmonary Artery and Ventricular Elastance (Right Heart Ventriculo-Arterial Coupling)

Ventriculo-arterial coupling quantification usually requires complex invasive measurements with specialized, conductance catheters and varying loading conditions which often precludes its application in clinical practice (Sunagawa et al., 1985; Chantler et al., 2008; Sanz et al., 2012). Single-beat methods have been described and validated by multiple studies with the following derived equations (Sunagawa et al., 1985; Chantler et al., 2008; Sanz et al., 2012).

$$\text{RV elastance} = E_{es}\ (\text{mmHg/mL/m}^2) = \text{mPAP}\ (\text{mmHg})/\text{end} - \text{systolic volume}\ (\text{mL})^*$$
$$\text{Pulmonary arterial elastance}\ (\text{mmHg/mL/m}^2) = E_a = [\text{mPAP}\ (\text{mmHg}) - \text{PCWP}\ (\text{mmHg})]/\text{stroke volume}\ (\text{mL})^*$$
$$\text{Ventriculo} - \text{arterial coupling} = E_{es}/E_a$$

With borderline pulmonary hypertension, end-systolic pressure (numerator in elastance equation) is best estimated using sPAP instead of mPAP (Vanderpool et al., 2015).

Pulmonary Artery Pulsatility Index

Pulmonary artery pulsatility index (PAPi) is a novel hemodynamic index described by Kapur et al. (Korabathina et al., 2012) as a tool to predict RV dysfunction and need for RV support following acute myocardial infarction. PAPi also predicted RV failure and need for RV support after LVAD implantation (Kang et al., 2016; Morine et al., 2016). PAPi has not been studied in other populations of RV failure and requires additional study for generalizability.

$$\text{PAPi} = [\text{sPAP}\ (\text{mmHg}) - \text{dPAP}\ (\text{mmHg})]/\text{RAP}\ (\text{mmHg})$$

Structural/Functional Considerations by Noninvasive Evaluation

From an imaging perspective, a comprehensive RV assessment for structure and function should include the following parameters: (1) RV shape and architecture; (2) RV volume; (3) RV hypertrophy and mass; (4) RV contractility; (5) ventricular interdependence. Echocardiography remains the first-line imaging modality for RV assessment, particularly due to its versatility and accessibility. Cardiac magnetic resonance imaging (CMR) has been becoming more utilized as an informative and vital tool in RV evaluation. RV volume is most accurately characterized by CMR. Ideally, a contractility index would be a parameter independent of loading conditions, sensitive to inotropic conditions, independent of cardiac size and mass, and with clinical utility (Carabello, 2002). Several indices of RV contractility have been applied widely; however, many do not meet these ideal conditions and may be imperfect in reflecting contractility. Lastly, assessment of ventricular interdependence can be evaluated by the flattening of the interventricular septum (D-shaped left ventricle) during the cardiac cycle. RV volume overload is present if the septal flattening is evident only during diastole; flattening throughout the cardiac cycle with prominence in end systole heralds RV pressure overload. Selected parameters of RV structure, volume, contractility, and interventricular dependence with normal values are shown in **Table 3**.

Right Heart Hemodynamics in Disease States

RV adaptation to disease and pathology is complex and is dependent on a multitude of factors. The most three most vital factors include: (1) the type and severity of right heart injury or stress; (2) time course (acute vs. chronic); (3) time of onset of the disease (newborn, pediatric, or adult years) (Haddad et al., 2008b). RV ventricular failure has been defined as a complex clinical syndrome (with cardinal symptoms including exercise limitations and fluid retention) as a consequence of the RV's inability to maintain flow output to match metabolic needs and consequent increase in right heart filling pressures (Naeije and Manes, 2014; Vonk-Noordegraaf et al., 2013). Most pathophysiologic states that contribute to RV failure include pressure overload, volume overload, combination of pressure-volume overload, and intrinsic RV failure in the absence of pulmonary hypertension (e.g., myocardial infarction). **Table 4** reviews mechanisms and specific causes of right heart failure.

Pressure Overload

When the RV is faced with a sudden increase in afterload, cardiac output is preserved via two proposed mechanisms: (1) increase in RV end-diastolic volume via Starling's law of the heart can be applied to beat-to-beat changes in preload and afterload; and (2) a homeometric contractile adaptation described by Anrep's law of the heart (de Vroomen et al., 2000; von Anrep, 1912). In the setting of chronic pulmonary hypertension, the highest wall stress initially is believed to develop in the pulmonary infundibulum, the RV region that develops the earliest evidence of hypertrophy (Naeije and Manes, 2014). Over time, infundibular hypertrophy with normal RA pressures extends to generalized RV hypertrophy with sustained elevated RA pressures with eventual progression to RV dilation similar to end-stage LV failure (Pinsky, 2016). The transition point from adaptive compensatory remodeling (concentric hypertrophy with preservation of systolic and diastolic function, preserved E_{es}/E_a coupling) to maladaptive remodeling resulting in decrease in contractility, eccentric hypertrophy, with dilation is not completely understood but likely the result of mismatch in

Table 3 Selected parameters of RV structure, volume, interventricular dependence, and contractility

Characteristic	*Criteria*	*Significance*
RV volume	>101 mL/m^2 (CMR)	Volume overloaded
RV mass	>32 g/m^2 in females (CMR)	Pressure overloaded—concentric hypertrophy
	>36 g/m^2 in males (Maceira et al., 2006) (CMR)	Volume overloaded—eccentric hypertrophy
	RV wall thickness >5 mm (Lang et al., 2015) (TTE)	
Ventricular interdependence-D-shaped LV	Eccentricity index >1 (Ryan et al., 1985)	RV pressure or volume overload
		Diastolic D-shaped LV supports volume overload; systolic D-shaped LV supports pressure overload
TAPSE	<1.7 cm (Lang et al., 2015)	Decreased contractility/systolic function
		Prognostic value
RVEF	$<61\pm7\%$ (48%–74%) (Maceira et al., 2006) (CMR)	Decreased contractility
RV myocardial performance index	>0.54 (via tissue Doppler) (Lang et al., 2015) (TTE)	Decreased RV global function
		Prognostic value
	>0.43 (via pulse-wave) (TTE)	

CMR, cardiac magnetic resonance; *TTE*, transthoracic echocardiography.

Table 4 Mechanisms and specific causes of right heart failure

Pressure overload
Left-sided heart failure (most common cause)
Pulmonary embolism (common)
Other causes of PH
RV outflow tract obstruction
Peripheral pulmonary stenosis
Double-chambered RV
Systemic RV
Volume overload
Tricuspid regurgitation
Pulmonary regurgitation
Atrial septal defect
Anomalous pulmonary venous return
Sinus of Valsalva rupture into RA
Coronary artery fistula to RA or RV
Carcinoid syndrome
Rheumatic valvulitis
Ischemia and infarction
RV myocardial infarction
Ischemia contributing to RV dysfunction in congenital heart disease and RV overload states (particularly pressure overload)
Intrinsic myocardial process
Cardiomyopathy and heart failure
Arrhythmogenic RV dysplasia
Sepsis
Inflow limitation
Tricuspid stenosis
Superior vena cava stenosis
Complex congenital defect
Ebstein's anomaly
Tetralogy of Fallot
Transposition of the great arteries
Double-outlet RV with mitral atresia
Pericardial disease
Constrictive pericarditis

RA, right atrium; *RV*, right ventricle; *PH*, pulmonary hypertension.

Reproduced from Haddad, F., Doyle, R., Murphy, D. J., Hunt, S. A. (2008). Right ventricular function in cardiovascular disease, part II: Pathophysiology, clinical importance, and management of right ventricular failure. Circulation 117, 1717–1731. With permission from Wolters Kluwer Health, Inc.

myocardial oxygen demand and perfusion, oxidative stress, inflammation, apoptosis, mitochondrial dysfunction, and pathologic neurohormonal signaling (Naeije and Manes, 2014; Vonk-Noordegraaf et al., 2013). How the RV adapts is impacted by the type of original insult (e.g., Eisenmenger syndrome often exhibits adaptive remodeling with RVF only very late in disease course vs. PAH with connective tissue or idiopathic PAH with maladaptive remodeling often early and progressive) (Campo et al., 2010; Chung et al., 2010). Many studies have evaluated RV-arterial coupling in a variety of PH models and RV failure is evident once RV function and afterload lose equilibrium.

Volume Overload

Congenital heart disease and valvular disease (congenital/acquired) often have substantial volume loads on the RV; the three most common lesions associated with RV volume overload are tricuspid regurgitation, atrial septal defects, and pulmonic regurgitation. The RV is known to have better adaptation mechanisms to volume overload than pressure overload. Eccentric hypertrophy is the initial adaptive response of the RV to chronic volume overload; cardiomyocytes lengthen by increasing sarcomeres in series and increasing myofibrils, capillary supply, and mitochondria (Apostolakis and Konstantinides, 2012). Initially, the hypertrophic response maintains coupling, but over time and with progressive insults, ventriculo-arterial uncoupling is seen. In addition, progressive RV hypertrophy outstrips the underlying capillary bed capacity, tricuspid annular dilation with secondary tricuspid regurgitation creates a vicious cycle of worsening volume overload, and RV dilation creates leftward septal shift leading to impairment in LV filling. Over many decades, cardiac dysfunction and heart failure can ensue (Apostolakis and Konstantinides, 2012).

Conclusion

Right heart health, previously undervalued, has been recognized as an important predictor of survival and functional capacity. Deepening our foundation of the basic physiology and hemodynamics of this unique system aids us in our efforts to target and understand RV disease while developing effective therapeutics in right heart failure.

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Hormonal Therapy in the Treatment of Chronic Heart Failure

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Introduction

Chronic heart failure (CHF) is a clinical syndrome in which structural and functional cardiac abnormalities induce an impairment of cardiac output or an increase of intracardiac pressures at rest or during stress, resulting in typical symptoms (e.g., ankle swelling, fatigue, dyspnea) or signs (peripheral edema, pulmonary crackles, or elevated jugular venous pressure) (Ponikowski et al., 2016; Yancy et al., 2016). In the last few decades, cardiovascular disease (CVD) mortality rates have dropped dramatically by about two-third in high-income countries (Nabel and Braunwald, 2012). Nevertheless, among the CVDs, CHF represents an unfortunate exception to such encouraging trend, showing a 5-year mortality that is still approximately 50%, even worse than many cancers (Askoxylakis et al., 2010; Braunwald, 2013). In the last few years, the so-called model of neurohormonal activation has been used to provide mechanistic basis of the natural progression of CHF. According to such a model, the overactivity of neurohormone pathways, that is, the renin–angiotensin–aldosterone or the adrenergic system, in the first stages of the disease, plays a compensatory role, but soon becomes in itself responsible for the worsening and progression of CHF, triggering a vicious circle leading to further and following deleterious increase of neurohormonal action (Braunwald, 2013; Mann, 1999). Based on this model, to counteract the neurohormonal activation, a therapeutic approach aimed at blocking the activated pathways has been postulated and implemented through the current guidelines (beta-blockers, ACE-I/ARBs, aldosterone receptor blockers) (Cole et al., 2014). However, since apparently CHF progress at a certain point independent of neurohormonal status, some limitations on the full effectiveness of the proposed therapy have been postulated. In particular, the obstacle to fully antagonize the overactivated hormonal pathways with the available drugs and the presence of alternative escaping metabolic pathways, such as the action of myocardial chimases for the conversion of angiotensin I in angiotensin II, have partially nullified the neurohormonal model. Thus, the uncertainty regarding the comprehension of all the mechanisms behind the pathogenesis of CHF has lead the way to the search of alternative approaches, capable of improving patient survival and relieving his/her symptoms. Increasing evidences suggest that, rather than the increase of the pathways regulated by the neurohormonal overactivity in itself, might be the loss of balance between the activation of these catabolic pathways and the impairment of a set of anabolic hormonal stimuli to characterize the progression of the disease. Reduction in the activity of anabolic hormones can be due to either reduction of their circulating plasma levels (e.g., growth hormone (GH) and its tissue effector insulin-like growth factor-1 (IGF-1), thyroid hormone (TH), anabolic steroids) or inhibition of their intracellular signaling pathways (i.e., GH resistance, insulin signaling impairment, resulting in insulin resistance (IR)) (Broglio et al., 2000; Suskin et al., 2000; Kontoleon et al., 2003; Pingitore et al., 2005; Doehner et al., 2005; Jankowska et al., 2006). Such a novel approach, rooted in the downregulation of many hormonal pathways, can be defined the hormonal-metabolic model and considers the CHF as a multiple hormonal deficiency syndrome (MHDS) (Saccà, 2009; Arcopinto et al., 2015a,b; Salzano et al., 2016). The deficiency of the action of a given hormone can be associated with reduced functional capacity of the heart and is an independent risk factor for poor clinical outcome (Niebauer et al., 1998; Iervasi et al., 2003; Doehner et al., 2005; Marra et al., 2012, 2016; Arcopinto et al., 2015a, 2017). Strikingly, the deficiency in the anabolic pathway sustained by the impairment of multiple hormones has been associated with even worst impaired cardiovascular performance and poor prognosis in CHF (Jankowska et al., 2006).

GH/IGF-1 Axis

GH and IGF-1 have an indispensable role in preserving cardiac performance and morphology in adulthood (Saccà and Fazio, 1996; Saccà, 1999). Activation of GH/IGF-1 pathway promotes a myocardial profile characterized by preserved capillary density, absence of fibrosis, and changes of calcium regulatory proteins, all factors playing a role in the protection against ischemia–reperfusion and mechanical stretching (Cittadini et al., 1996; Stromer et al., 2006; Bruel and Oxlund, 1999). In addition, GH/IGF-1 stimulation induces reduction of apoptosis and release of endothelial nitric oxide, resulting in vasodilation (Cittadini et al., 1997; Saetrum

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Opgaard and Wang, 2005; Boger, 1998; Merola et al., 1996; Napoli et al., 2002, 2003). In particular, IGF-1 but not GH, through calcium sensitization of the myofilaments, increases myocardial contractility, a downstream mechanism not associated with arrhythmias (Cittadini et al., 1998). Moreover, GH increases skeletal muscle protein content suggesting an additional role in improving CHF by contrasting the consequences of skeletal muscle cachexia (Napoli et al., 1995). IGF-1 administration in preclinical studies induces an increase in the size of cardiomyocytes and in the expression of mRNAs for actin, myosin light chain 2, and troponin associated with the reduction in apoptosis (Ito et al., 1993; Ren, 2002).

GH deficiency (GHD) is present in more than one-third of patients with CHF (Cittadini et al., 2009; Broglio et al., 2000). Many mechanisms might be involved in the reduced activity of GH/IGF-1 axis in CHF: reduced GH secretion or peripheral resistance to its action, hypothalamic somatostatin hyperactivity, drug interference, poor nutritional status, or increased circulating and tissue levels of inflammatory cytokines (Broglio et al., 1999, 2000; Cicoira et al., 2003; Climent et al., 2007; Saccà et al., 2003; Mann, 1999; Colao, 2008).

In the general population, GHD is associated with increased cardiovascular mortality and the coexistence of many factors might trigger this increase (Rosen and Bengtsson, 1990). GHD patients show impaired cardiac performance, increased peripheral vascular resistance, and reduced exercise capacity (Saccà, 1997) and the degree of severity of the deficiency appears to be a major determinant of cardiac impairment (Colao et al., 2004). Recently, we have shown that the presence of GHD in patients with CHF identifies a subgroup with worse clinical status and increased all-cause mortality, higher depression scores, impaired quality of life, presence of left ventricular (LV) remodeling, lower physical performance, and increased NT-proBNP levels (Arcopinto et al., 2017). Larger LV volumes with elevated wall stress, as well as higher filling pressures, and impairment of right ventricle function characterize patients with CHF and GHD. In addition, at similar values at anaerobic threshold, these patients demonstrated a worse cardiopulmonary performance with significantly lower peak VO_2 and reduced ventilatory efficiency.

The data regarding the effects of perturbations in circulating plasma levels of IGF-1 are less consistent. In the general population, a low level of IGF-1 is predictive of cardiovascular mortality, ischemic heart disease, and CHF (Juul et al., 2002; Vasan et al., 2003; Laughlin et al., 2004). In the population of patients with CHF, IGF-1 levels have been found decreased, normal, or even increased when compared with healthy controls (Anker et al., 2001; Al-Obaidi et al., 2001; Anwar et al., 2002; Andreassen et al., 2009). Several studies show that low levels of IGF-1 associate with poor outcome, greater neurohormonal activation and cytokine activation, reduced skeletal muscle performance, and endothelial dysfunction (Niebauer et al., 1998; Napoli et al., 2002; Petretta et al., 2007). Recently, our group demonstrated that IGF-1 circulating plasma levels are predictors of all-cause mortality in CHF patients (Arcopinto et al., 2015a,b). Given the inconsistency of the data about circulating levels of IGF-1 in the population with CHF, there is no agreement on which one can be the best marker of GH/IGF-1 axis activity. Rather than isolated IGF-1 levels, the IGF-1/GH ratio relates to survival in CHF patients (Petretta et al., 2007). Watanabe showed that the IGF-1/IGF-binding protein-3 ratio is associated with increased rate of all-cause mortality, cardiac death, and a composite outcome of cardiac death and rehospitalization in a population of CHF patients (Watanabe et al., 2010).

Experimental studies in animal models of postischemic heart failure have reported beneficial effects of treatments with GH, or GH-releasing peptide, on cardiac function, peripheral vascular resistance, and survival (Yang et al., 1995; Duerr et al., 1996; Ryoke et al., 1999). Early treatment of large myocardial infarction with GH attenuates pathologic LV remodeling and improves LV function (Cittadini et al., 2009). Since the nineties, many studies have focused on the effect of GH treatment in patients with CHF: the results have not been always consistent (Fazio et al., 1996; Frustaci et al., 1996; Isgaard et al., 1998; Osterziel et al., 1998; Genth-Zotz et al., 1999; Jose et al., 1999; Spallarossa et al., 1999; Perrot et al., 2001; Smit et al., 2001; Napoli et al., 2002; Acevedo et al., 2003; Adamopoulos et al., 2003; Cittadini et al., 2003).

Le Corvoisier and colleagues, in an elegant meta-analysis summarizing the findings, show that GH treatment improves several relevant cardiovascular parameters in patients with CHF; in particular, they reported an increase in LV ejection fraction (LVEF) and a reduction in systemic vascular resistance (Le Corvoisier et al., 2007). Moreover, a long-term modification in cardiac morphology (reduction of LV diastolic diameter and an increase in LV wall thickness) was demonstrated. Tritos et al., in another meta-analysis, illustrated that treatment with GH resulted in an increase of exercise duration, maximum oxygen uptake, LVEF, cardiac output, and improvement in systemic vascular resistance and NYHA class level (Tritos and Danias, 2008).

The inconsistency of the results obtained from different trials on GH treatment of patients with CHF might be explained by differences in duration of treatment, target, or dosage. Recently, the concept has emerged that the GH/IGF-1 patient profile might prejudice the response to the treatment with GH. Therefore, patient selection is extremely relevant to obtain the hoped-for results. In particular, GHD appears to be the condition needed to get the best response in CHF patients. In a randomized, single-blind, controlled trial, we studied the relevance of GHD on the effects of GH replacement therapy in patients with CHF. After 6 months of treatment, GH replacement therapy increased LVEF, peak oxygen uptake, exercise duration and flow-mediated vasodilation, and improved the quality of life score. Furthermore, GH administration decreased circulating N-terminal pro-brain natriuretic peptide levels (Cittadini et al., 2009). On these premises, we extended the study with a 4-year follow up. After 4 years, the GH replacement therapy induced the significant reductions of both LV end-diastolic and end-systolic volume indexes, circumferential wall stress, and an increase in LVEF, suggesting the activation of the reverse remodeling of the LV. Although the result did not reach statistical significance, GH treatment improved remarkably the peak VO_2. Finally, the combination of death from any cause and hospitalization due to CHF worsening was sensibly reduced by the treatment with GH in this group of patients with CHF, although hard clinical endpoints were not the purpose of the study (Cittadini et al., 2013).

TNF-α, interleukin-6, and other proinflammatory cytokines increased levels have also been associated with poorer prognosis in patients with CHF. GH therapy has shown to normalize the circulating levels of TNF-α andinterleukin-6 (Adamopoulos

et al., 2002). Consistently, the levels of soluble adhesion molecules, granulocyte-macrophage colony-stimulating factor, and macrophage chemoattractant protein-1 were also affected by GH administration (Acevedo et al., 2003), suggesting an immunomodulatory role for GH therapy in CHF.

Androgens

The role of anabolic steroids on cardiovascular system and skeletal muscle is well recognized. Testosterone is the primary androgen in the adulthood and some evidences have linked its levels to the risk of CVDs (Khaw et al., 2007). Consistently, male gender is an independent risk factor of atherosclerosis (Criqui, 1986). Furthermore, several evidences, in vivo and in vitro, suggested that androgens exert adverse effects on the cardiovascular risk factor profile and induce the development of atherosclerosis (Muller et al., 2003; Dubey et al., 2002; Adams et al., 1995; McCrohon et al., 1999; Ng et al., 2003). Several clinical studies have shown that low levels of androgens associates with increased carotid intima-media thickness in middle-aged and elderly men (Mäkinen et al., 2005; Muller et al., 2003) and risk of CVDs and all-cause mortality (Haring et al., 2010; Laughlin et al., 2008; Malkin et al., 2010a).

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEA-S), produced mainly by the adrenal glands, are the most abundant androgens in humans. Although DHEA-S derives mainly from the conversion to testosterone in peripheral tissues, direct actions on the cardiovascular system (Komesaroff, 2008) and association with cardiovascular variables have also been shown (Tivesten et al., 2014).

Testosterone exerts its effects through genomic and nongenomic actions and testosterone receptors (TR) are present in cardiomyocytes, where their stimulation induces protein synthesis and cell hypertrophy (Marsh et al., 1998) and regulates myocardial contractility, through a mechanism involving L-type Ca^{2+} channel, the Na^{2+}/Ca^{2+} exchanger, b1-adrenoceptors, and myosin heavy chain subunits (Golden et al., 2003, 2004). TR are present also in endothelial and vascular smooth muscle cells, where testosterone decreases the intracellular Ca^{2+} flux through interaction with voltage-operated calcium and potassium channels (Liu et al., 2003). Moreover, the endogenous testosterone/TR system exerts a protective action on the angiotensin II-induced vascular remodeling by inhibiting the gene expression for the components of NADPH oxidase, resulting in control of superoxide production and reduction in the activation of JNK and TGF-beta-Smad signaling pathways (Ikeda et al., 2009).

In healthy males, the decline in androgens levels with aging contributes to sarcopenia, visceral adiposity deposition, and osteopenia. The physiologic phenomenon of andropause is characterized by a decrease in serum testosterone level of about 3.5 ng/dL per year starting from the forth decade of life (Lapauw et al., 2008). Even circulating DHEA-S levels shows an age-dependent decline starting at the age of about 25 years (Kaufman and Vermeulen, 2005). However, it must be said that the criterion for testosterone deficiency is not widely accepted, although is often considered when morning total testosterone level drops below 300 mg/dL in two or more occasions in presence of consistent symptoms and signs (Bhasin et al., 2010). However, signs and symptoms of testosterone deficiency blend with those of common comorbidities making uncertain the identification of reliable testosterone deficiency in elderly. Nowadays, the annual incidence of hypogonadal levels of testosterone in men is 20% in people 60 or older and 30% in 70 or older (Harman et al., 2001).

Epidemiological studies demonstrated that testosterone deficiency in old men associates with increased risk of death (Haring et al., 2010; Laughlin et al., 2008) and low serum testosterone levels might be used as a marker of poor survival (Haring et al., 2010).

In patients with CHF syndrome, 25% of patients have low plasma levels of testosterone (Anker et al., 1997; Moriyama et al., 2000; Kontoleon et al., 2003; Malkin et al., 2009) and such reduction might well be involved in the impairment of skeletal muscle function and exercise tolerance (Iellamo et al., 2010a) and contributes to the poor survival (Pugh et al., 2004; Malkin et al., 2010b; Volterrani et al., 2012). In addition, reduced serum levels of DHEA-S predict all-cause and cardiovascular mortality in elderly (Ohlsson et al., 2010).

The concept that testosterone deficiency affects cardiac function encouraged studies on the effects of testosterone replacement in CHF. In an experimental model of CHF, testosterone treatment induced physiological cardiac growth without increase in markers of hypertrophy or accumulation of collagen (Nahrendorf et al., 2003). In animal model of ischemia–reperfusion injury, testosterone administration preserved cardiomyocytes by activating ATP-sensitive K channels and upregulating cardiac alpha(1)-adrenoceptors (Tsang et al., 2008). Compared with placebo, in 12 male patients with CHF, a single dose of testosterone (60 mg orally) induced a decrease in peripheral vascular resistance and after-load, associated with higher cardiac output (Pugh et al., 2002).

In a following double-blind, placebo-controlled study, the same group showed that intramuscular testosterone therapy (100 mg every 2 weeks for 12 weeks) in 20 male patients with CHF increased the 6-min walking distance and improved the Minnesota Living with Heart Failure Questionnaire (Pugh et al., 2004). These promising results prompted a robust randomized, double-blind, placebo-controlled trial on the effects of testosterone replacement therapy in unselected CHF patients (Malkin et al., 2006). In such a study, 76 men with CHF were randomly assigned to receive either testosterone (5 mg/day administered by an adhesive skin patch) or placebo for 12 months. In the group treated with the hormone, an increase in serum testosterone level by 40% was observed. Despite testosterone did not modify LV morphology or function, a significant increase in shuttle walk distance and dominant handgrip strength was observed. Moreover, the clinical severity of CHF improved as demonstrated by the shift of at least one New York Heart Association class for 35% of patients treated with testosterone versus 8% of patients receiving placebo. Since the functional improvement recorded during testosterone treatment was not associated with any relevant change in heart function or morphology, an hypothetical positive effect of testosterone on skeletal muscle could be invoked to justify the improvement in

patient performance. On the other hand, the involvement of peripheral mechanisms in the maintenance and progression of CHF is so relevant that the possibility that testosterone may act through such mechanisms does not undermine the potential importance of its use.

A few years ago, in a double-blind, randomized, placebo-controlled study, 70 elderly patients with CHF were treated for 12 weeks with very long acting intramuscular testosterone. The data from this trial showed that peak VO_2 and ventilatory efficiency, as assessed by the VE/VCO_2 slope, improved significantly. In addition, the beneficial effects of testosterone therapy were confirmed by improvement in IR and the slowdown of skeletal muscle deterioration, in particular in large, weight-bearing muscles, as demonstrated by the increase in quadriceps maximal voluntary contraction. The deterioration of skeletal muscle strength in elderly patients with CHF is a relevant phenomenon capable of bringing about early fatigue and limiting exercise tolerance (Caminiti et al., 2009). More recently, 50 male CHF patients recruited in a RCT receiving intramuscular long-acting androgen injection once every 4 weeks for 12 weeks confirmed the previous finding (Mirdamadi et al., 2014). The effects of testosterone (intramuscular testosterone 6×100 mg in 12 weeks) have been studied also during a rehabilitation program in male patients with CHF and low testosterone status. After 24 supervised sessions of mixed exercise, an improvement in peak oxygen consumption, leg strength, and response to quality of life questionnaire was demonstrated in the patients treated with testosterone (Stout et al., 2012).

The decline of androgen levels with aging is a phenomenon detectable also in women. Such consideration prompted a study involving 36 female patients with CHF, treated with either transdermal testosterone (300 mg patch, applied twice/week) or placebo for 6 months (Iellamo et al., 2010b). At the end of the study, treated patients displayed an improvement in 6-min walking distance and peak oxygen consumption and a reduction of IR.

Taken together, the studies looking at the effects of testosterone administration did not show changes in the size or function of heart ventricles. However, they invariably exhibited an improvement in cardiovascular performance and muscular strength, supporting the hypothesis that the action of testosterone might be mediated through peripheral mechanisms involved in the pathogenesis of CHF, that is, improvement in skeletal muscle or vascular function, rather than on the heart function in itself.

Insulin Action

The existence of an intimate link between diabetes and CHF has been apparent for many years. Since the first data from the Framingham heart study were published, the fact that among diabetic patients there is a large prevalence of patients with CHF and that CHF patients have an increased risk of being diabetic appeared clear. In addition to the increased risk of CVDs, diabetic patients show abnormalities in the metabolism of the heart able to induce a condition of cardiomyopathy independent of atherosclerosis, hypertension, and coronary artery disease: the so-called diabetic cardiomyopathy. In the last few years, the concept emerged that the mechanisms behind the development of diabetic cardiomyopathy are quite complex. In particular, among the defects critically present in diabetes and potentially responsible for heart damage, IR has emerged forcefully. On the other hand, IR can be present in patients with CHF independent from diabetes and recent data prove that, roughly, one-third of patients with CHF are insulin resistant and almost half have diabetes or IR (Suskin et al., 2000). Furthermore, IR does not appear to be a simple bystander, but plays an active role in the development and prognosis of the disease. In particular, in a population of elderly people, the presence of IR, as measured by hyperinsulinemic euglycemic clamp study, independently of obesity, showed to be a powerful predictor of CHF (Ingelsson et al., 2005). IR correlates with the severity of CHF when categorized by the 6-min walking distance or VO_{2max} (Swan et al., 1997; Suskin et al., 2000). Finally, and more importantly, the presence of IR is strictly associated with poor prognosis in patients with CHF (Doehner et al., 2005). On the other hand, it has been experimentally demonstrated in animals that once CHF is established, IR can rapidly develop or worsen in the heart and skeletal muscle (Nikolaidis et al., 2004). Many different mechanism can potentially be evoked to explain the onset or worsening of IR in patients with CHF, such as endothelial dysfunction, skeletal muscle loss, cytokines surge, sympathetic nervous system overactivity, FFA elevation, physical inactivity, etc., likely induced by the impaired hemodynamic. With regard to the mechanisms dangerously triggered by IR and responsible of the worsening of CHF performance and prognosis, a few hypothesis can be proposed. Under normal circumstances, heart metabolism is very flexible and myocardiocytes are capable of switching from FFA to glucose utilization according to the metabolic and energetic needs and the different age (Witteless and Fowler, 2008; Iozzo et al., 2010). When CHF develops, heart metabolism switches from a preferential FFA oxidation to the more convenient glucose uptake (Huss and Kelly, 2005; Witteless and Fowler, 2008). Preferential glucose oxidation under the circumstances supports a favorable energetic profile, allowing the production of ATP and other high-energy substrates with lower oxygen consumption (Huss and Kelly, 2005). Unfortunately, when IR is present, heart metabolism becomes less agile and capable of utilizing glucose, and cardiomyocytes are compelled to an unfavorable inflexibility with the preferential use of FFA to produce energy (Witteless and Fowler, 2008). On the assumption that IR represents a relevant player in the maintenance and progression of CHF, the aim of the therapy of the disease should be to counteract IR. Life style interventions, a pharmacologic approach, or both can oppose IR. The life style interventions, pillars of the treatment of IR in the general population, are weight control and physical activity. Unfortunately, in patients with CHF both approaches have pitfalls. Weight control or reduction must be weighed against the risk of cachexia, a frequent and serious complication of CHF. On the other hand, physical activity is of limited use, given the difficulty that CHF patients have in this regard. Several different drugs, used for the treatment of diabetes, are capable of improving IR. First, the insulin sensitizers thiazolidinediones are drugs directly addressing IR. Although the treatment with glitazones has shown some interesting results (Masoudi et al., 2005), they are contraindicated in patients with CHF and diabetes for the increased paradoxical risk of inducing or worsening CHF (Gerstein et al., 2006). Metformin, first choice drug for the

treatment of diabetes, has been for long time contraindicated in patients with CHF for the potential risk of lactic acidosis enhanced by the tissue ischemia present in CHF. However, in an experimental model of CHF, metformin, but not rosiglitazone, improves cardiac function and performance very powerfully (Cittadini et al., 2012). Recently, a critical analysis of the data available in the literature has clearly indicated that the use of metformin was not associated with an increased risk of lactic acidosis (Masoudi et al., 2005) and associated with the improvement of many functional parameters (Wong et al., 2012). Therefore, metformin has become the drug of choice in patients with diabetes and reduced EF (Ponikowski et al., 2016). Recently, the field of diabetes has been flooded with a series of new and promising drugs. A series of drugs act by increasing the plasma activity of glucagon-like peptide-1 (GLP-1). Native GLP-1 cannot be routinely used for treatment because of its short half-life (less than 2 min) and the need of parenteral administration. However, GLP-1 administration under experimental circumstances has provided promising results in the field of CHF. In dogs with CHF, GLP-1 administration improved LV function (Nikolaidis et al., 2004). In 12 patients with severe CHF, with or without diabetes, continuous subcutaneous administration of GLP-1 for 5 weeks improved LVEF, VO_{2max}, 6-min walking distance, and the response to a QoL questionnaire (Sokos et al., 2006). The mechanisms responsible for the striking improvement in these patients are unclear. However, it has been hypothesized that improvement of IR might have played a role. Given the short half-life of native GLP-1, increase in the activity of GLP-1 can be achieved through two different approaches. Dipeptidyl-peptidase IV inhibitors (DPP-IVi) or GLP-1 agonists act by increasing the GLP-1 activity, by either inhibiting the enzyme DPP-IV, responsible of the degradation of endogenous GLP-1 and in so doing increasing the level of endogenous GLP-1, or providing a GLP-1 analog resistant to the action of the DPP-IV, respectively. Three recent randomized clinical trials of cardiovascular safety, involving more than 36,000 patients with diabetes, half of them treated with DPP-IVi, have been completed (Scirica et al., 2013; White et al., 2013; Green et al., 2015). Although CHF was not the focus of the three trials, surprising observations have been made. In the SAVOR-TIMI trial (Scirica et al., 2013), an unexpected statistically significant increase in the number of patients hospitalized for heart failure was reported for the group treated with saxagliptin. In the EXAMINE trial (White et al., 2013), the treatment with alogliptin showed a 19%, although nonsignificant, increase in the incidence of hospitalization for CHF in the 2700 patients treated with the drug. Finally, in the TECOS trial (Green et al., 2015), no signal of increase in CHF hospitalization was observed treating the patients with sitagliptin. However, the safety data collected in the real world practice suggest that this family of drug is generally safe to use, but certainly, the hopes of improving the burden of CHF have been neglected. With regard to the data relative to the GLP-1 agonists, three RCT have been published so far (Pfeffer et al., 2015; Marso et al., 2016a,b)). In these trials, the diabetic patients did not show any change in CHF hospitalization due to the treatment with lixisenatide, liraglutide, or semaglutide, respectively. However, the effects of liraglutide, a long-acting analog of GLP-1 administered once a day, on heart performance and patient safety in patients with CHF have been investigated (Jorsal et al., 2017). In this study, 241 CHF patients, with (74 patients) or without diabetes, were treated with either liraglutide (122 patients) or placebo in a randomized, double-blinded, placebo-controlled, multicentre trial. Primary end-point of the study was changed in LVEF. At the end of the 24 weeks of treatment, LVEF improved more in the placebo group than in the liraglutide arm, although the difference did not reach statistical significance (LVEF: −0%–8% active vs. placebo group). Unfortunately, serious cardiac adverse events were more frequent with liraglutide than placebo ($P=0.04$). In a similar, but smaller and open label, trial, albiglutide was tested versus placebo (Lepore et al., 2016). In this study also, GLP-1 analog failed to improve ventricular function in patients with CHF. Extremely interesting data have been recently provided by the diabetic patients treated with empagliflozin in the EMPA-REG OUTCOME trial were (Zinman et al., 2015). Empagliflozin is the first drug of the family of the inhibitors of sodium glucose cotransporter 2 (SGLT2i) to complete the cardiovascular safety trial required by the FDA to approve the drug in the United States. Other drugs with the same mechanism of action, dapagliflozin and canagliflozin, are currently being studied in similar CV safety trials. The administration of gliflozins induces the reduction of blood glucose concentration by increasing the loss of glucose with the urine. Such reduction removes the impact of glucose mass effect on the cells, improving IR and glucose transport (Rossetti et al., 1987; Napoli et al., 1995). During the EMPA-REG OUTCOME trial, diabetic patients treated with the empagliflozin, in addition to reduction in all-cause and CVD mortality, showed a significant reduction in hospitalization for CHF. Similarly, in a recent large real world study recruiting 300,000 diabetic patients, dapagliflozin, empagliflozin, and canagliflozin induced a reduction in death or hospitalization for HF (Kosibord et al., 2017). The relevant mechanisms behind such reduction are unclear and many of them have been invoked: changes in substrate (i.e., butyrate, glucose), hormones (i.e., glucagon), changes in the intracellular metabolism, or reduction in IR might be implicated. However, the diuretic effect of the gliflozins must be kept in mind when data on hospitalization are analyzed, since diuresis per se may improve the hemodynamic in patients with CHF.

Thyroid Hormone

The importance of the TH role in the regulation of cardiovascular function has been clear for many years. TH perturbations, as in hypo- or hyperthyroidism, have a potent impact on the cardiovascular apparatus, involving both the regulation of heart rhythm and function. TH exerts its powerful intracellular effects through genomic and nongenomic mechanisms. The action of TH on heart function might be divided in a direct action on cardiac structure and an indirect action mediated by the very potent effects the TH have, both acutely (Napoli et al., 2007) and chronically (Napoli et al., 2001; Napoli et al., 2010) on the vessel wall. The effects on cardiac structure are mediated through the expression of proteins involved in many different ways in the contractile response of the ventricles (Iervasi et al., 2003). On the other hand, the effects observed on the peripheral circulation have a potent effects on hemodynamic and, consequently, on cardiac function. The implications of TH action on heart function can be easily evaluated by

looking at the effects that hypo- or hyperthyroidism have on hemodynamic. In particular, thyroid diseases are associated with changes in cardiac output, contractility, and vascular resistance. On the other hand, the treatment of these diseases, if quickly started, before irreversible changes have taken place, is rapidly followed by the restoration of normal cardiovascular functions (Klein and Danzi, 2007; Napoli et al., 2010). The levels of circulating TH must be maintained in a pretty narrow normal range, as suggested by the fact that either elevation or decrease of TH is followed by increased cardiovascular mortality (Biondi et al., 1999; Iervasi et al., 2007). Treatment with low dose of oral (0.1 mg/day of T4) of CHF patients has led to an increase in LVEF and improvement in exercise tolerance (Moruzzi et al., 1996). T3 administration induces an increase in cardiac output in few hours (Malik et al., 1999; Hamilton et al., 1998; Pingitore et al., 2008). On the other hand, T3 has potent and rapid activity on the vessel wall and is capable of improving endothelial function when infused intra-arterially in healthy volunteers (Napoli et al., 2007). In addition to the obvious hemodynamic effect of heart function and structure, the improvement of endothelial function with T3 administration has important implication in CHF, since a better perfusion of peripheral tissue, including skeletal muscle, might potentially improve exercise tolerance.

Conclusions

In the last few years, the hypothesis that CHF is a clinical syndrome characterized by an imbalance between catabolic and anabolic pathways has prepotently emerged. Such imbalance is sustained by the neurohormonal activation from one side and MHDS on the other side. Reduction in the activity of GH/IGF-1 axis, androgens, insulin, and TH is associated with the clinical worsening of CHF. More importantly, the correction of these defects is followed by significant clinical improvements. On the basis of these observations, new trials focusing on the identification and treatment of multiple hormonal deficiencies in CHF patients should be supported. Meanwhile the MHDS should be routinely searched in the patients with CHF and a consistent treatment evaluated.

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Further Reading

Mann DL and Bristow MR (2005) Mechanisms and models in heart failure. The biomechanical model and beyond. *Circulation* 111: 2837–2849.

Hypertensive Heart Disease

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Introduction

Hypertensive heart disease (HHD) can be defined as the response of the heart to the afterload imposed on the left ventricle by the progressively increasing arterial pressure and total peripheral resistance that entails hypertension (Frohlich et al., 1992). HHD is characterized by increased left ventricular (LV) mass leading to left ventricular hypertrophy (LVH). In fact, hypertension-induced LVH consists of a constellation of genetic, structural and functional alterations of cardiac tissue that allow its differentiation as a separate entity (Lip et al., 2000). LVH is associated with alterations of cardiac contractility and relaxation, abnormalities of myocardial perfusion, and disturbances of cardiac rhythm in hypertensive patients. In addition, LVH is an independent risk factor related to cardiovascular complications in these patients.

Diagnostic methods to detect LVH in the routine clinical setting include electrocardiography and echocardiography, although novel biochemical and imaging tools are being developed to help diagnose and treat patients in a personalized manner. A number of studies have established that reduction of LV mass is attained with effective antihypertensive treatment and that reversal of LVH and its underlying mechanisms decreases the occurrence of adverse cardiovascular events in hypertensive patients. However, there is still room for further improvement because it is now appreciated that treated hypertensive patients in which LVH regresses still have considerable cardiovascular risk (Struthers, 2013). On the other hand, due to the aging of the population and the increase in the prevalence of hypertension and related comorbidities, HHD remains a major socioeconomic problem. Therefore, there is an important need for physicians to recognize this entity, understand its pathophysiology, and be aware of the available treatment options.

Pathophysiological Aspects

Physically, in conditions of pressure overload due to systemic hypertension, the left ventricle undergoes hypertrophic growth, which is characterized by the thickening of the LV wall and the augmentation of LV mass, thus resulting in concentric LVH. This is as predicted by the law of Laplace. Functionally, this early LVH is a compensatory process that helps the heart in sustaining cardiac output despite increased afterload imposed by systemic hypertension (Frohlich et al., 2011). However, this process is only an initial "adaptive" response to biomechanical stress associated with hemodynamic load; chronic exposure to the stress eventually leads to impaired inotropic (contraction)/lusitropic (relaxation) function which, in many cases, progresses to congestive heart failure (HF). This "maladaptive" evolution is intimately linked to the underlying histological changes of the cardiomyocyte and noncardiomyocyte components of the myocardium that result in its structural remodeling (**Fig. 1**) (Knöll et al., 2011).

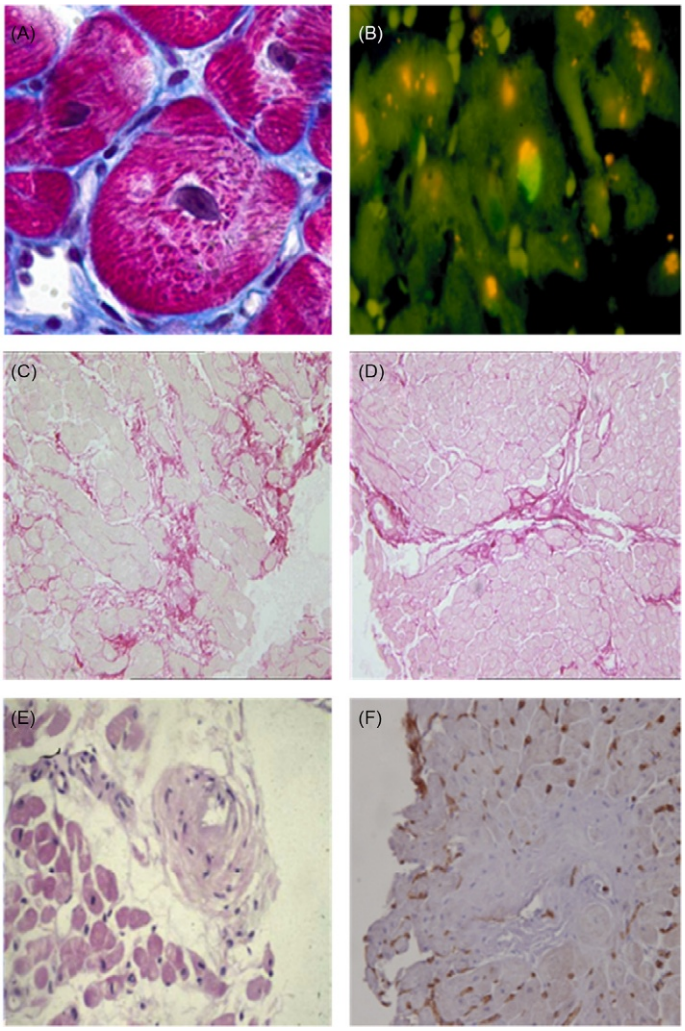

Fig. 1 Main microscopic lesions found in the hypertensive myocardium: (A) view of a transversely enlarged cardiomyocyte (Masson's trichrome staining, magnification 100 ×); (B) a cardiomyocyte exhibiting apoptotic appearance (TUNEL, magnification 100 ×); (C) excessive interstitial collagen deposition (picro-sirius red staining, magnification 20 ×); (D) perivascular fibrosis (picro-sirius red staining, magnification 20 ×); (E) an intramyocardial artery presenting wall thickening and lumen narrowing (Masson's trichrome staining, magnification 20 ×); (F) capillary rarefaction (von Willebrand staining, magnification 20 ×).

Cardiomyocyte Lesions

As mentioned before, the response of the cardiomyocyte to pressure overload must not be considered simply as an adaptive compensation, as it has critical detrimental consequences (Frohlich et al., 2011).

Hypertrophy

The primary mechanism by which the heart reduces the stress on the LV wall imposed by pressure overload is the hypertrophic growth of cardiomyocytes. This entails the stimulation of intracellular signaling cascades that activate gene expression and promotes protein synthesis, protein stability, or both, with consequent increases in protein content and in the size and organization of force-generating units (sarcomeres). This, in turn, leads to increased size of individual cardiomyocytes with resulting augmentation in LV mass and thus LVH (Sugden and Clerk, 1998). Although it is unclear how the mechanical stretch of cardiomyocytes is transduced across the cell membrane, it probably involves stretch-sensitive ion channels, a Na^+/H^+ exchanger, integrins and integrin-interacting molecules as well as other internal and membrane-bound stretch sensors, in a complex network that links the extracellular matrix, the cytoskeleton, the sarcomere, calcium (Ca^{2+})-handling proteins, and the nucleus (Clerk et al., 2007).

The changes in genetic expression characteristic of the cardiomyocyte hypertrophic response involve reexpression of a fetal gene program, as well as repression of postdevelopmental genes (**Table 1**) (Barry et al., 2008; Kuwahara et al., 2012). The long-held views are that, in response to pressure overload, these morphological and genetic changes are positive and serve to restore cardiac

Table 1 Genes whose expression is altered during cardiomyocyte hypertrophy

Activated genes
β-Myosin heavy chain
Fetal isoforms of contractile proteins (skeletal α-actin and β-myosin heavy chain)
Fetal type cardiac ion channels (hyperpolarization activated cyclic nucleotide-gated channel and T-type Ca^{2+} channel)
IVS3A form of calcium channel
α3-Subunit of Na^+, K^+-ATPase
B subunit of creatine kinase
Atrial and brain natriuretic peptides
Switch from fatty acid oxidation to glycolysis genes
Lactate dehydrogenase M subunits
Genes directing cardiomyocyte lengthening
Smooth muscle genes (smooth muscle α-actin and SM22α)
Inhibited genes
α-Myosin heavy chain
Calcium ATPase of sarcoplasmic reticulum (SERCA2)
β_1-Adrenergic receptors
M_2 muscarinic receptors
Early transient K^+ current, I_{to}
Myoglobin
N2BA titin isoform

muscle economy and counteract myocardial dysfunction. However, blunting of cardiomyocyte hypertrophy and attenuation of the fetal gene reexpression do not necessarily result in immediate LV dysfunction or HF despite the pressure overload. Therefore, genetic reprogramming associated with cardiomyocyte hypertrophy may no longer be considered as an adaptive process (Meijs et al., 2007). Moreover, the detailed analysis of the genetic changes that accompany cardiomyocyte hypertrophy indicates that they translate into derangements in energy metabolism, contractile cycle and excitation–contraction coupling, cytoskeleton and membrane properties. These changes determine mechanical dysfunction which, in turn, provides the basis for cardiomyocyte malfunction, which is associated with LVH and predisposes the ventricle to diastolic and/or systolic dysfunction (Dhalla et al., 2009).

Death

The death of cardiomyocytes by apoptosis is abnormally increased in patients with HHD with HF (González et al., 2006). Cardiomyocyte apoptosis has been proposed to occur as a result of an imbalance between the factors that induce or block apoptosis. In this sense, inducers of cardiomyocyte apoptosis (e.g., mechanical stretching and angiotensin II) predominate over suppressors (e.g., agonists of the gp130/LIFR survival pathway) in arterial hypertension (Fortuño et al., 2001).

Apoptosis of cardiomyocytes may contribute to the development of LV dysfunction/failure through three different pathways. First, apoptosis may be one mechanism involved in the loss of contractile mass and function of the hypertensive myocardium, as association of increased cardiomyocyte apoptosis with diminished cardiomyocyte number has been found in hypertensive patients (González et al., 2006). Second, some mechanisms that are activated during the apoptotic process may also interfere with the function of viable cardiomyocytes before death (Narula et al., 2001). For instance, caspase-3 cleaves cardiac myofibrillar proteins, resulting in an impaired force/Ca^{2+} relationship and myofibrillar ATPase activity. Also, the release of cytochrome *C* from mitochondria during apoptosis may impair oxidative phosphorylation and ATP production, thus leading to energetic compromise and functional impairment. Third, in addition to contributing to histological remodeling of the myocardium, cardiomyocyte apoptosis may also contribute to geometric remodeling of the LV chamber. In fact, severe cardiomyocyte apoptosis may lead to side-to-side slippage of cells, mural thinning, and chamber dilatation. This wall restructuring secondary to severe cardiomyocyte apoptosis may create an irreversible state of the myocardium, facilitating progressive dilatation and the continuous deterioration of LV hemodynamics and performance with time (Chandrashekhar, 2005).

It is important to point out that, although apoptosis is a hallmark of HHD, in the response to any given cardiac injury various modalities of cell death are stimulated, as they are interconnected by common cellular pathways at multiple points (Whelan et al., 2010). For instance, autophagy is activated during hypertensive LVH, serving to maintain cellular homeostasis. Excessive autophagy eliminates, however, essential cellular elements and possibly provokes cardiomyocyte death, which contributes to myocardial remodeling (Wang et al., 2010).

Noncardiomyocyte Lesions

It has become evident in the last 25 years that in addition to lesions involving the cardiomyocytes, changes in the extracellular matrix and the microvasculature are also key components of the structural remodeling of the myocardium and have a profound detrimental impact on the overall cardiac function, thus contributing to the development of HHD.

Myocardial fibrosis

Myocardial fibrosis is the exaggerated accumulation of extracellular matrix, namely collagen type I fibers, within the interstitium and surrounding intramural coronary arteries and arterioles in the myocardium. This excess of myocardial collagen present in hypertensive LVH is the result of the combination of several alterations (Berk et al., 2007): (i) increased procollagen synthesis by fibroblasts and phenotypically transformed fibroblast-like cells or myofibroblasts; (ii) increased extracellular conversion of procollagen into microfibril-forming collagen by specific proteinases; (iii) increased spontaneous microfibril assembly to form fibrils; (iv) enhanced cross-linking of fibrils to form fibers by the action of lysyl oxidases (LOX); and (v) unchanged or even decreased fiber degradation by matrix metalloproteinases (MMPs).

Fibrosis might contribute to the structural and clinical alterations of HHD through diverse mechanisms. First, there is a connection between fibrosis and LV dysfunction (Brower et al., 2006). The accumulation of collagen fibers compromises the rate of relaxation, diastolic suction, and passive stiffness, thereby contributing to impaired diastolic function. In addition, continued accumulation of collagen fibers, together with changes in their spatial orientation, further impairs diastolic filling. These changes further compromise cardiomyocyte contraction and myocardial force development thus impairing systolic performance. Second, the impaired coronary flow reserve associated with LVH might be related to, among others, perivascular fibrosis (Schwartzkopff et al., 1993). In fact, the amount of perivascular collagen has been correlated inversely with coronary flow reserve in patients with LVH (Dai et al., 2012). Third, the increased deposition of fibrotic tissue which occurs in association with hypertension and LVH, results in diffusion problems in a situation in which oxygen and nutritional demands are increased (Frohlich, 2001). Finally, interstitial fibrosis may also contribute to ventricular arrhythmias in hypertension (McLenachan and Dargie, 1990). Thus, hypertensive patients with dysrhythmias exhibit higher values of LV mass and myocardial collagen than patients without arrhythmias, despite the finding that the ejection fraction and the frequency of coronary vessels with significant stenosis may be similar in the two groups of patients. Fibrosis induces conduction abnormalities thereby promoting local reentry arrhythmias. Moreover, whereas the key role of ectopic foci in pulmonary veins, which may trigger atrial fibrillation, has been recognized, atrial fibrosis has been identified as the main mechanism for atrial fibrillation (Boldt et al., 2004). This suggests that atrial fibrosis in hypertensive patients (namely those with chronic HF) may promote more widespread changes in the myocardial collagen matrix.

It is worth mentioning that the alterations in the quantity (namely amount of type I collagen) and quality (namely the degree of cross-linking of the fibers) of the myocardial collagen matrix also may influence adversely the clinical outcome in patients with HHD. For instance, it has been shown that whereas increased collagen type I cross-linking is associated per se with HF hospitalization in patients with HHD and HF (López et al., 2016), the coincidence of increased collagen type I cross-linking with severe collagen type I deposition is associated with HF hospitalization and mortality (cardiovascular and all-cause) (Ravassa et al., 2017).

Microvascular alterations

The hypertensive myocardium is characterized by multiple structural alterations in the small intramyocardial vessels (Feihl et al., 2008). On the one hand, hyperplasia or hypertrophy, and altered vascular smooth muscle cellular alignment may promote encroachment of the tunica media into the lumen, thereby causing both increased medial thickness/lumen ratio and reduced maximal cross-sectional area of intramyocardial arteries. On the other hand, vascular density in LVH becomes relatively decreased. This seems to result from capillary rarefaction or inadequate vascular growth in response to increasing muscle mass. These microcirculatory alterations, together with perivascular fibrosis, contribute to decreased coronary flow reserve (CFR) of patients with HHD (Kelm and Strauer, 2004). In fact, there is an association between decreased CFR and LV systolic and diastolic dysfunction in HHD during stress maneuvers (Galderisi et al., 2002; Ikonomidis et al., 2012; Kozàkovà et al., 2003).

Recently, the role of coronary microvascular endothelial inflammation in HF with preserved ejection fraction (HFpEF), in the context of systemic conditions such as hypertension, has been highlighted (Paulus and Tschöpe, 2013). Coronary microvascular endothelial inflammation would decrease the bioavailability of nitric oxide and cyclic guanosine monophosphate content, resulting in decreasing cardiomyocyte protein kinase G (PKG) activity. Thus low PKG activity may promote cardiomyocyte hypertrophy, hypophosphorylation of the cytoskeletal protein titin, stimulation of fibroblast differentiation into myofibroblasts with high fibrogenic activity, and increased myocardial stiffness. This sequence of events suggests that coronary microvascular endothelial inflammation may be responsible for myocardial remodeling in HFpEF of hypertensive etiology. This idea was recently supported by the demonstration that CFR was abnormally reduced in hypertensive patients with LVH and patients with HFpEF of hypertensive etiology (Kato et al., 2016). Of interest, reduced CFR was found to be associated with microvascular endothelial activation in HFpEF patients (Franssen et al., 2016).

Clinical Aspects

HHD evolves progressively along two consecutive phases, one subclinical in which elements of myocardial remodeling may be present but do not result in altered clinical parameters, and one clinically overt in which remodeling causes signs, symptoms, and a clinical profile of HF (Drazner, 2011). HHD is a multifactorial disease: whereas several factors linked to genetics, gender and lifestyle will determine the initial hemodynamic and nonhemodynamic damage of the left ventricle in the hypertensive patient, other factors related to ageing and comorbidities (e.g., diabetes, obesity, and chronic kidney disease) will influence the subclinical evolution of HHD (**Fig. 2**).

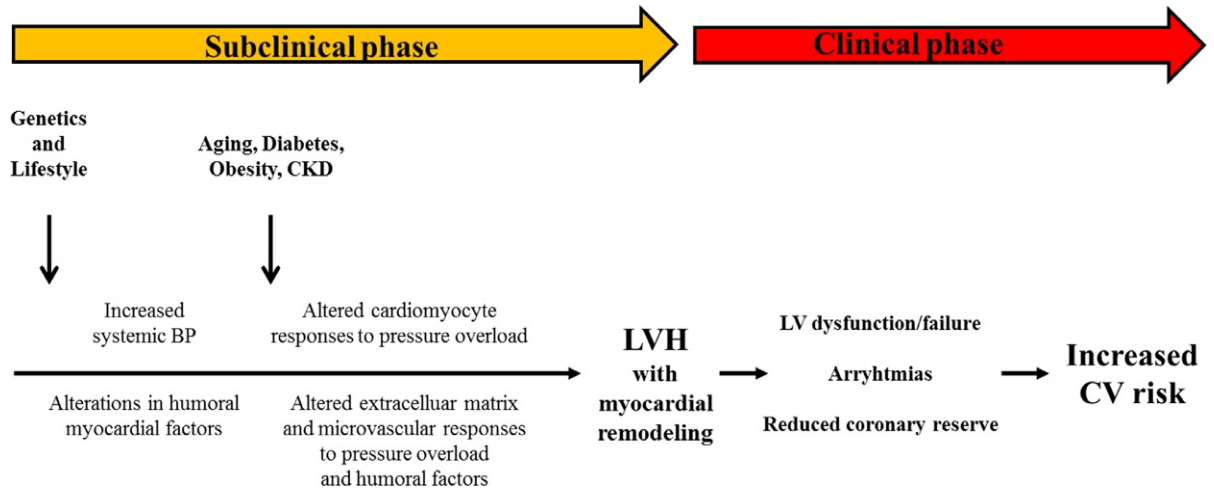

Fig. 2 Phases of the hypertensive heart disease continuum. *BP*, Blood pressure; *CKD*, chronic kidney disease; *LVH*, left ventricular hypertrophy; *CV*, cardiovascular.

Clinical Manifestations

Most patients with HHD, although at risk, are asymptomatic. However, dyspnea, edema, angina, palpitations, syncope, and sudden death can be present when complications occur. In fact, epidemiological studies have suggested that hypertension is the most common etiological factor for chronic congestive HF, being present in 50% of cases. Besides alterations of LV myocardium characteristic of HHD, hypertension may itself result in HF as a consequence of underlying coronary artery disease and arrhythmias, such as atrial fibrillation.

Unlike HF caused by coronary artery disease, in which HF progresses after a discrete event (myocardial ischemia or infarction) that damages the heart muscle, LV dysfunction/failure in the patient with HHD may progress gradually from the stage of compensated LVH with subclinical LV dysfunction to clinically overt HF (Drazner, 2011). It is worth mentioning that it has been estimated that among 30%–45% of hypertensive patients with HF present diastolic dysfunction, but normal systolic function (i.e., HFpEF). In addition, hypertensive patients with LVH may have symptoms and electrocardiographic signs of myocardial ischemia in the absence of atherosclerosis of epicardial vessels due to a combination of microvascular disease and diminished CFR (Preik et al., 2003). On the other hand, ventricular arrhythmias, atrial fibrillation, and sudden cardiac death are more prevalent in patients with hypertension and LVH than in those hypertensive patients without hypertrophy or in normotensive individuals (Yiu and Tse, 2008).

Diagnosis, Prevention, and Treatment

The classical physical findings in patients with HHD are related to the presence of LVH and include a sustained, enlarged, and displaced outside the midclavicular line apical impulse and an S_4 gallop best heard in the left lateral decubitus position.

The two most common tools used to confirm the diagnosis of LVH are the electrocardiogram (Schillaci et al., 2012) and the echocardiogram (Marwick et al., 2015) (**Table 2**). Beyond the diagnosis of LVH, the electrocardiogram provides unique information on rhythm disturbances, PR and QT intervals, whereas the echocardiogram supplies precise information about LV wall thickness (and thus on the geometric pattern of LVH), LV function, wall motion abnormalities, and left atrial size. Each provides different performance for diagnosis of LVH, with the electrocardiogram being more specific (95% vs. 84%) but much less sensitive (50% vs. 88%) than the echocardiogram. The echocardiographic determination of the cardiac architecture with the use of LV mass and relative wall thickness data allows for the identification of three LV geometry patterns (concentric hypertrophy, eccentric hypertrophy, concentric remodeling) with clear implications regarding prognosis (Marwick et al., 2015). In fact, concentric LVH carries the highest risk and eccentric LVH an intermediate risk, whereas concentric remodeling is associated with a smaller, albeit important risk (Koren et al., 1991).

The first step in HHD prevention is the control of blood pressure by the control of predisposing factors and, of course, the use of antihypertensive therapies (Pfeffer, 2017). Clinical studies have consistently shown that blood pressure reduction is a fundamental strategy to prevent LVH in patients with arterial hypertension (Georgiopoulou et al., 2010). However, there may be differences on risk-reduction effectiveness among the available antihypertensive therapies (Georgiopoulou et al., 2010; Sciarretta et al., 2011), which may be linked to the different ability of the drugs to interfere with myocardial remodeling.

Antihypertensive drugs are, in fact, effective in reducing LV mass. One meta-analysis including 80 double-blind, randomized controlled trials with 146 active treatment arms and 17 placebo arms showed that after adjustment for treatment duration and change in diastolic pressure, there was a significant difference among medication classes in decreasing LV mass (Klingbeil et al., 2003). The decreased LV mass indexed by body surface area or LV mass index induced by the different classes was as follows: AT_1

Table 2 Electrocardiographic and echocardiographic diagnostic criteria for left ventricular hypertrophy

Electrocardiogram[a]
Sokolow-Lyon index
$S_{V1} + (R_{V5}$ or $R_{V6}) > 3.5$ mV
$R_{aVL} > 1.1$ mV
Cornell voltage criteria
$S_{V3} + R_{aVl} \geq 2.8$ mV (for men)
$S_{V3} + R_{aVl} \geq 2.0$ mV (for women)
Cornell voltage-duration product
QRS duration × Cornell voltage >2436 mm × ms
QRS duration × sum of voltages in all 12 leads >17,472 mm × ms
Romhilt-Estes point score system (LVH is diagnosed if 5 points are computed)
Any limb lead R wave or S wave ≥2.0 mV: 3 points
S_{V1} or $S_{V2} \geq 3.0$ mV: 3 points
R_{V5} to $R_{V6} \geq 3.0$ mV: 3 points
ST-T wave abnormality (no digitalis therapy): 3 points
ST-T wave abnormality (digitalis therapy): 1 point
P terminal force in V_1 >40 mV-ms: 3 points
Left axis deviation: 2 points
Intrinsicoid deflection in V_5 or $V_6 \geq 50$ ms: 1 point
Echocardiogram[b]
Indexed left ventricular mass (left ventricular mass:body surface area)
Men >115 g/m^2
Women >95 g/m^2
Indexed left ventricular mass (left ventricular mass: [height])$^{2.7}$
Men >48 g/m$^{2.7}$
Women >44 g/m$^{2.7}$
Relative wall thickness (2 × posterior left ventricular wall thickness): left ventricular diameter in diastole) ≥0.42

[a]Schillaci et al. (2012).
[b]Marwick et al. (2015).

receptor blockers>calcium antagonists>ACE inhibitors>diuretics>beta-blockers. In paired comparisons, AT_1 receptor blockers, ACE inhibitors, and calcium antagonists were more effective at reducing LV mass index than diuretics and beta-blockers.

Additional strategies, such as selective denervation of the renal sympathetic nerves to lower blood pressure, have also been shown to significantly reduce LV mass and improve diastolic function in patients with resistant hypertension (Brandt et al., 2012). It is worth to note that patients who did not respond hemodynamically to renal denervation did show LVH regression, supporting the notion of renal denervation effects on LVH independent of blood pressure.

Impact on Public Health

The impact of HHD on public health is undeniable (Ambrosy et al., 2014; Pfeffer, 2017). First, LVH is highly prevalent in hypertensive patients, as assessed either by electrocardiogram (Cuspidi et al., 2012a) or by echocardiography (Cuspidi et al., 2012b), with an average of 40%–45%. Second, considered as a categorical variable, LVH significantly and independently increases the risk of HF and other cardiac (e.g., coronary artery disease, arrhythmias, sudden death) and noncardiac (e.g., stroke) complications, as shown by data from the Framingham study (Levy et al., 1990). Third, recent evidence challenge the notion that blood pressure control by antihypertensive treatment completely prevents HHD-associated risk. It is classically admitted that cardiovascular risk decreases significantly in hypertensive patients in whom LVH regresses with antihypertensive treatment compared to patients in whom LVH persists, in spite of similar hemodynamic effectiveness of the treatment (Devereux et al., 2004). However, a meta-regression analysis of 14 studies in 12,809 hypertensive patients failed to demonstrate a continuous relationship between treatment-induced LVH changes and the occurrence of cardiovascular events (namely, myocardial infarction and new onset HF) (Constanzo et al., 2013). Moreover, a study conducted in 763 hypertensive patients followed up for an average of 12 years showed that the progression of LV mass with respect to the baseline value had a predictive value for cardiovascular events independent of the initial measurement and course of the office blood pressure (Gosse et al., 2012).

Current Challenges

Although current management of HHD is still focused on controlling blood pressure and reducing the increased LV mass (Schiattarella and Hill, 2015), it must be remarked that the impact of currently available antihypertensive agents on myocardial remodeling may not be optimal (**Table 3**). This may in part be due to the lack of personalized diagnosis and therapy in HHD.

Table 3 Clinical evidence-based hemodynamic and cardiac effects of antihypertensive agents

Pharmacological class	*Decrease of blood pressure*	*Reduction of LV mass*	*Repair of remodeling lesions*[a]
Alpha-blockers	Yes	Mild	Unknown
Beta-blockers	Yes	Mild–moderate	Apparently not
Calcium antagonists	Yes	Moderate	Apparently not
Diuretics	Yes	Mild	Proven for torasemide[b]
Aldosterone antagonists	Yes	Mild–moderate	Proven for spironolactone[c]
Direct renin inhibitors	Yes	Marked	Unknown
Angiotensin converting enzyme inhibitors	Yes	Marked	Proven for lisinopril[d]
Angiotensin receptor blockers	Yes	Marked	Proven for losartan[e]
Angiotensin receptor blocker and neprilysin inhibitor	Yes	Unknown	Unknown

LV, left ventricular.

[a]Refers to some of the lesions (namely, fibrosis).

[b]López et al. (2004).

[c]Izawa et al. (2005).

[d]Brilla et al. (2000).

[e]Díez et al. (2002).

Therefore, additional strategies aimed at specific diagnosis by noninvasive biochemical (González et al., 2009) or imaging (Hoey et al., 2014) techniques, as well as individually tailored therapeutic repair (González et al., 2011) of the microscopic changes responsible for hypertensive myocardial remodeling are needed.

Search for Patients Predisposed to LVH and LV Dysfunction

Emerging evidence indicates that identification of subsets of patients with a common denominator may help select a therapeutic approach. For instance, 24 h monitoring of blood pressure may help identify those hypertensive patients highly predisposed to develop LVH (i.e., patients with early morning pressure rise or with a nondipping profile) and therefore more prone to benefit from the ability of some antihypertensive drugs to prevent it (Kawano et al., 2008).

LV mass is a highly complex phenotype influenced by interacting effects of multiple hemodynamic and nonhemodynamic factors, including genetic factors. Identification of genes influencing LV mass may enhance the detection of those patients requiring early treatment. In this regard, a meta-analysis of case–control and association studies has shown that the D allele of the insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme (ACE) gene behaved as a marker of future LVH in untreated hypertensive patients (Kuznetsova et al., 2000).

Finally, novel diagnostic tools, such as quantification of the myocardial strain by speckle-tracking echocardiography, may help shed light in the cardiac dysfunction of patients with HFpEF (Saito et al., 2016), providing clues for specific preventive and therapeutic approaches of this growing clinical entity.

Noninvasive Diagnosis of Myocardial Remodeling

The noninvasive assessment of the histological alterations that underlie HHD is key to personalized diagnosis and treatment. On the one hand, novel imaging tools are key contributors of diagnosis of myocardial remodeling. Cardiac magnetic resonance imaging (CMR) not only can measure LV mass and dimensions more accurately than conventional echocardiographic techniques (Hoey et al., 2014; Janardhanan and Kramer, 2011), but it is also a promising technique for characterizing the myocardial composition, particularly with the analysis of T1 mapping and late gadolinium enhancement areas in the myocardium may represent fibrotic regions (Janardhanan and Kramer, 2011). In addition, CMR is rapidly evolving with the introduction of an increasing number of high-affinity molecular probes potentially useful to monitoring specific alterations in the myocardium such as apoptosis (i.e., using ^{99m}Tc-labeled annexin A5 that binds to apoptotic cardiomyocytes) and collagen synthesis (i.e., using ^{99m}Tc-labeled peptides that bind to activated fibroblasts) (Chun et al., 2008).

On the other hand, the identification of biochemical markers of potential usefulness for clinical management of cardiac diseases is emerging as a very active field (González et al., 2009). Regarding biochemical markers related to cardiomyocyte alterations, plasma concentration of CT-1 has been correlated with LV mass in untreated hypertensive patients (González et al., 2009), suggesting that plasma CT-1 may be a potential marker for assessment of cardiomyocyte hypertrophy and LVH in hypertensive patients. In addition, the decrease of plasma CT-1 induced by antihypertensive treatment is associated with the reduction of LV mass in patients with LVH (González et al., 2009), suggesting that this cytokine may be useful to assess ability of antihypertensive drugs to reduce cardiomyocyte growth and decrease LV mass. Moreover, CT-1 is associated with myocardial systolic dysfunction in asymptomatic hypertensive patients (Ravassa et al., 2013), indicating that CT-1 may also provide information regarding the clinical impact of myocardial remodeling. Regarding biochemical markers related to myocardial fibrosis, diverse studies indicate that they are promising tools to assess this aspect of myocardial remodeling in hypertensive patients (González et al., 2009; López et al., 2015). Clinical data indicate that the serum levels of the carboxy-terminal propeptide of procollagen type I (PICP), excised from the

procollagen molecule in its maturation process, is a marker of the amount of collagen in the myocardium (González et al., 2009; López et al., 2015). In addition, a recent work indicates that the circulating levels of the carboxyl-terminal telopeptide of collagen type I, normalized by circulating MMP-1 levels, may be indicative of the degree of cross-linking of myocardial collagen (López et al., 2016; Ravassa et al., 2017). As mentioned before, not only do these biomarkers help diagnose by giving evidence of the remodeling that takes place in the myocardium, but they may also provide information about hospitalization or death risk (López et al., 2016; Ravassa et al., 2017) and may even influence therapeutic approaches (Díez et al., 2002; López et al., 2007, 2015).

Another aspect of myocardial remodeling that may benefit from noninvasive diagnosis is the coronary microcirculation (Paulus and Tschöpe, 2013). Although research so far has focused mainly in coronary artery disease, novel imaging techniques may clarify the role of altered cardiac microcirculation in HHD (Camici et al., 2015).

Antiremodeling Strategies

Available experimental and clinical data suggest that the goal of repairing myocardial remodeling is also achievable in HHD using available antihypertensive agents (**Table 3**). For instance, in patients with HHD, ACE inhibition reduced myocardial fibrosis, irrespective of blood pressure and LV mass reduction, and was accompanied by improved LV diastolic function (Brilla et al., 2000). In addition, despite similar antihypertensive and antihypertrophic efficacy, the AT_1 receptor blocker losartan, but not the calcium channel blocker amlodipine, reduced cardiomyocyte apoptosis (González et al., 2002) and myocardial fibrosis (Díez et al., 2002) in patients with HHD. Reduction of myocardial fibrosis by the AT_1 blocker was associated with decreased LV chamber stiffness and the improvement of diastolic filling in hypertensive patients (Díez et al., 2002).

Since the action of most of the reviewed antihypertensive therapies on myocardial remodeling is limited, the attention has been recently turned to the potential of targeting specific intracellular events (González et al., 2011). Hypothetically, these novel therapeutic interventions could be directed to the following aspects: first, to block the detrimental intracellular mechanisms activated in mechanical stress-triggered cardiomyocyte hypertrophic response (e.g., kinases such as Rho-kinase, and phosphatases such as calcineurin). Second, to prevent inhibition of "negative" signaling modulators and interacting proteins that are repressed in such conditions (e.g., increasing the availability and actions of cyclic GMP). Third, to take advantage of beneficial mechanisms inherent in the mechanical stress-triggered cardiomyocyte hypertrophic response (e.g., via enhancement of important aspects of the IGF1/PI3K/Akt pathway like angiogenesis). Fourth, to block the deleterious modulation that pathological stresses impose on protein synthesis (e.g., through regulation of HDACs or sets of microRNAs). Fifth, to prevent mitochondrial oxidative stress (e.g., through mitochondrial targeted small molecules and peptides). Sixth, to preserve functioning cardiomyocytes (e.g., through the inhibition of the process of apoptosis and/or preservation of cell survival mechanisms). Seventh, to regenerate lost cardiomyocytes (e.g., using stem cell therapy). Eighth, to restore the normal turnover of collagen network (e.g., inhibiting exaggerated collagen synthesis and deposition at nonphysiological localizations).

Two examples may illustrate the mentioned possibilities. First, experimental data in mice show that the systemic administration of the mitochondrial targeted antioxidant peptide SS-31 ameliorated angiotensin II-induced LVH, diastolic dysfunction, myocardial apoptosis, and fibrosis in these animals, despite the absence of blood pressure-lowering effect (Dai et al., 2011). SS-31 reduced also angiotensin II-induced mitochondrial reactive oxygen species and oxidative damage, as well as mitochondrial biogenesis in cardiac cells. Second, circulating miRNA-21 is increased in hypertensive patients, as well as in the experimental model of HHD of the spontaneously hypertensive rat (SHR). MiR-21 targeting in SHR normalized mitochondrial cytochrome-*b* expression and oxidative stress, which resulted in attenuated blood pressure, cardiomyocyte hypertrophy, and myocardial fibrosis (Li et al., 2016).

Conclusion

HHD begins with the hypertrophic response of cardiomyocytes to increased hemodynamic load. The increase in LV wall thickness normalizes increased wall stress and, therefore, LVH is initially beneficial. However, sustained and progressive load, that is accompanied by the activation of humoral factors, is associated with additional responses of the cardiomyocyte and noncardiomyocyte compartments of the myocardium that result in its structural remodeling and the deleterious long-term consequences on myocardial function, electrical activity, and perfusion that significantly increase the morbidity and mortality in hypertensive patients with LVH. A detailed understanding of the complexities underlying these responses will aid in the development of novel diagnostic and therapeutic approaches aimed to prevent or minimize their deleterious impact on the heart of the hypertensive patient in a tailored, effective, and safe manner. This knowledge is also critical due to the increasing prevalence of HHD and its risk factors, and taking on account its social and economic burden (Ambrosy et al., 2014; Pfeffer, 2017).

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Hypertrophic Cardiomyopathy

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Introduction

Hypertrophic cardiomyopathy (HCM) is a common genetic cardiovascular disorder affecting 1 in 500 of the general population. While historically it was viewed as a rare condition associated with excess mortality, in the current era with contemporary management strategies the majority of patients will achieve normal or near-normal life expectancy (Maron et al., 2015). Despite this, HCM remains one of the most common causes of sudden cardiac death (SCD) in young people and athletes.

An increase in left ventricular wall thickness or hypertrophy (LVH), in the absence of coexistent cardiac or systemic conditions which could result in LVH (such as hypertension and aortic valve stenosis), is central to the diagnosis. This increase in wall thickness can involve virtually any segment of the left or less frequently the right ventricular myocardium. On a microscopic level there is evidence of myocyte disarray, patchy myocardial fibrosis or scarring, and abnormalities of the small intramural blood vessels.

Genetic Basis of the Disease

HCM is typically inherited in an autosomal dominant pattern, whereby each offspring of an affected individual has a 50% chance of inheriting a pathogenic mutation. Most cases are caused by a mutation in the genes coding for the sarcomere proteins, with over 80% of cases arising from a mutation in either the MYH7 (myosin heavy chain) or MYBPC3 (myosin binding protein C) genes. However, in some cases the disease can result from a sporadic mutation in one of these genes.

Pathophysiology

Diastolic and systolic function

The presence of LVH, myocardial disarray, and fibrosis results in an increase in left ventricular stiffness. This adversely affects myocardial relaxation and diastolic function, leading to an increase in intracavitary pressures in both the left ventricle (LV) and left atrium (LA). In a small subset of patients (<15%) the presence of significant myocardial fibrosis can result in a progression to systolic dysfunction (burnt-out HCM), characterized by progressing wall thinning and chamber dilatation.

Obstruction

Intracavitary and left ventricular outflow tract obstruction (LVOTO) is seen in up to 70% of patients, either at rest or with provocation such as exercise. The pattern of hypertrophy of the LV often determines whether obstruction will arise and is typically seen in patients with classic asymmetric LVH affecting the basal interventricular septum.

Myocardial ischaemia

Myocardial ischaemia in patients with HCM can arise from several causes, including disease of the large epicardial coronary arteries as well as a supply–demand mismatch due to significant LVH. However, even in the absence of epicardial coronary artery disease, abnormalities of the small intramural vessels are noted, resulting in microvascular dysfunction.

Arrhythmias

Chronic increases in LV and LA pressure secondary to diastolic dysfunction and LVOTO result in progressive LA dilatation, and increase the risk of supraventricular arrhythmia. Atrial fibrillation occurs in up to 20% of patients with HCM, and carries with it an increased risk of stroke and thromboembolic events. Myocardial fibrosis and disarray serve as a substrate for ventricular arrhythmia, which carries with it an increased risk of SCD. While nonsustained ventricular tachycardia (NSVT) is seen in 15%–20% of patients on ambulatory ECG monitoring, sustained VT is less common.

Autonomic dysfunction

Autonomic dysfunction can be demonstrated in up to a third of patients with HCM, and typically manifests as an abnormal blood pressure response to exercise (i.e., an inability to augment systolic blood pressure appropriately). The underlying mechanism is an inappropriate firing of stretch-sensitive mechanoreceptors in the LV myocardium, resulting in exaggerated drops in systemic vascular resistance. In addition, these abnormalities of vascular control can result in episodes of presyncope or syncope.

Clinical Presentation

Patients with HCM may present with symptoms, or may be identified after the incidental finding of abnormalities on an ECG or echocardiogram performed for other reasons. In the current era of family screening a significant number of cases are diagnosed in asymptomatic family members of an affected individual.

Up to 50% of patients with a confirmed diagnosis of HCM are either asymptomatic or present with minimal symptomatic limitations. Shortness of breath and chest discomfort are common, predominantly as a result of underlying diastolic dysfunction and LVOTO. Chest pain may present as typical angina or may be atypical. In patients with LVOTO, symptoms are typically worse after a heavy meal (postprandial exacerbation). Dizziness and syncope may occur at rest or on exertion, and arrhythmia and dynamic LVOTO are important causes. Palpitations are not uncommon, and may signify underlying episodes of atrial fibrillation of ventricular arrhythmia.

Investigations and Diagnosis

The diagnosis of HCM centers around the identification of unexplained LVH.

Clinical examination

The classic findings on clinical examination are shown in **Fig. 1**.

Electrocardiography (ECG)

A 12-lead ECG is often used as a screening tool for HCM, and is abnormal in up to 95% of patients. Classic ECG abnormalities include the presence of LVH by voltage criteria, widespread Q waves, and repolarization abnormalities. Diffuse and giant ($>$10 mm) T-wave inversion is a distinctive pattern seen in patients with apical hypertrophy. Ambulatory ECG monitoring plays a vital role not only in the investigation of symptoms of palpitations but also for the identification of patients at risk for SCD (nonsustained ventricular tachycardia) and stroke (paroxysmal atrial fibrillation) (**Fig. 2**).

Imaging

Transthoracic echocardiography (TTE) is the mainstay of diagnosis, allowing direct assessment not only of LV wall thickness but also assessment of LVOTO, concomitant mitral valve disease and dysfunction, and the assessment of diastolic and systolic function. The diagnosis hinges on the presence of a wall thickness of $>$15 mm in any myocardial segment, or in the paediatric population $\geq$2 standard deviations above the mean (Z-score) after correction for age and body surface area (Nagueh et al., 2011). While the classic description is of asymmetric hypertrophy involving the interventricular septum, hypertrophy can be concentric or involve the apical segments of the ventricle preferentially. LVOTO is present in over two-thirds of patients, occurring under resting conditions in 30% of cases, and provocable in a further 40% (Maron et al., 2006). Mitral regurgitation is seen frequently in patients with obstruction due to systolic anterior motion of the mitral valve, with a typically posteriorly directed jet into the LA (**Fig. 3**). However, intrinsic structural abnormalities of the mitral valve and subvalvular apparatus frequently coexist in patients with HCM, and further contribute to the degree of mitral regurgitation. Diastolic dysfunction, LVOTO, and mitral regurgitation all result in progressive LA dilation.

Cardiac magnetic resonance imaging (CMR) adds valuable additional information to the diagnostic work-up of patients with presumed or confirmed HCM (Nagueh et al., 2011). It is particularly helpful in patients with suboptimal TTE image quality, and in patients in whom the ECG is strikingly abnormal and the diagnosis is suspected but TTE is equivocal. CMR provides superior

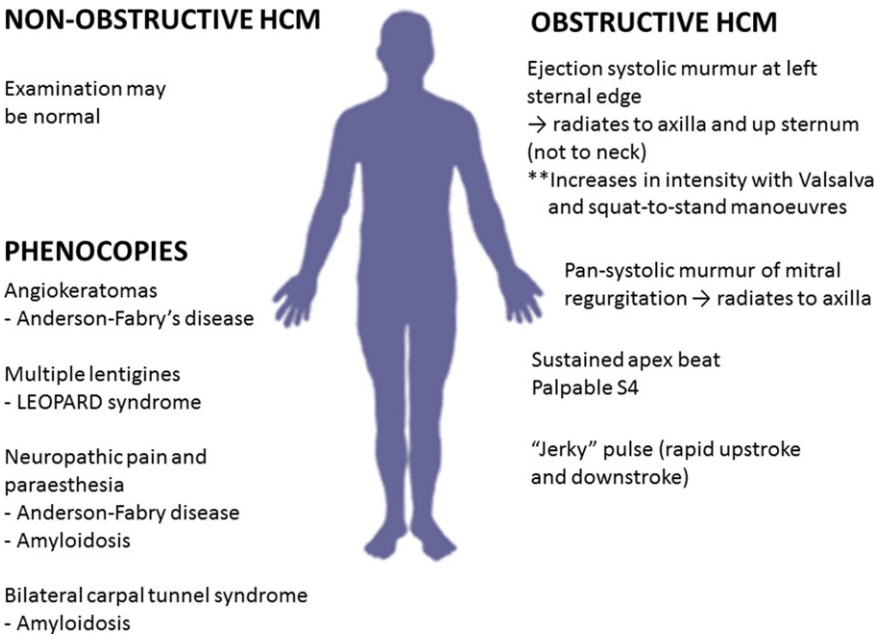

Fig. 1 The classic findings on clinical examination in patients with hypertrophic cardiomyopathy. Most abnormalities on examination are related to the presence of left ventricular outflow tract obstruction and associated mitral regurgitation. Some of the "red flags" which may point to an alternative diagnosis or disease "phenocopy" are shown as well, and highlight the importance of a complete clinical examination.

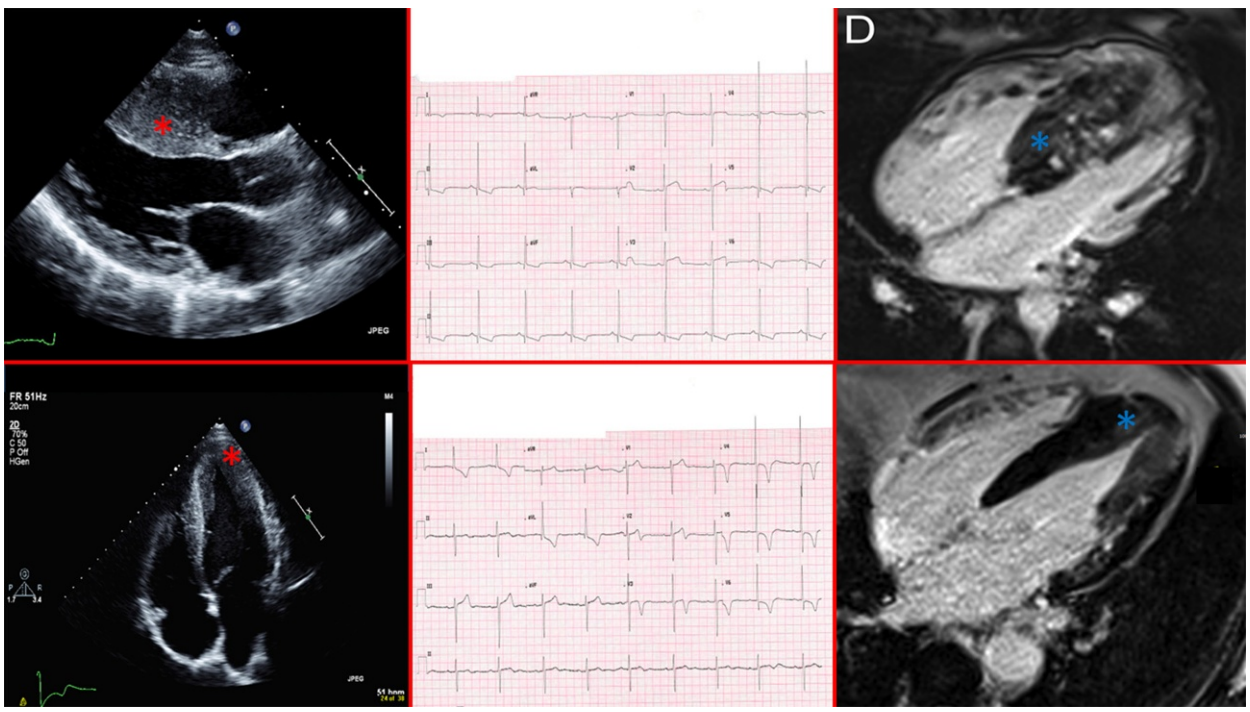

Fig. 2 The classic imaging findings on ECG and imaging are shown in a patient with asymmetric septal hypertrophy (*upper panel*) and apical hypertrophy (*lower panel*). *Upper panel*—the marked septal hypertrophy is noted (*red asterisk*), along with LVH and repolarization abnormalities on the ECG (ST segment and T-wave changes). The CMR image demonstrates the presence of extensive myocardial fibrosis in the hypertrophied segments (*blue asterisk*). *Lower panel*—hypertrophy is confined to the apical segments of the left ventricle (*red asterisk*), with the classic giant negative T-waves seen on ECG. In this patient, myocardial fibrosis is again seen in the hypertrophied segments of the LV apex (*blue asterisk*).

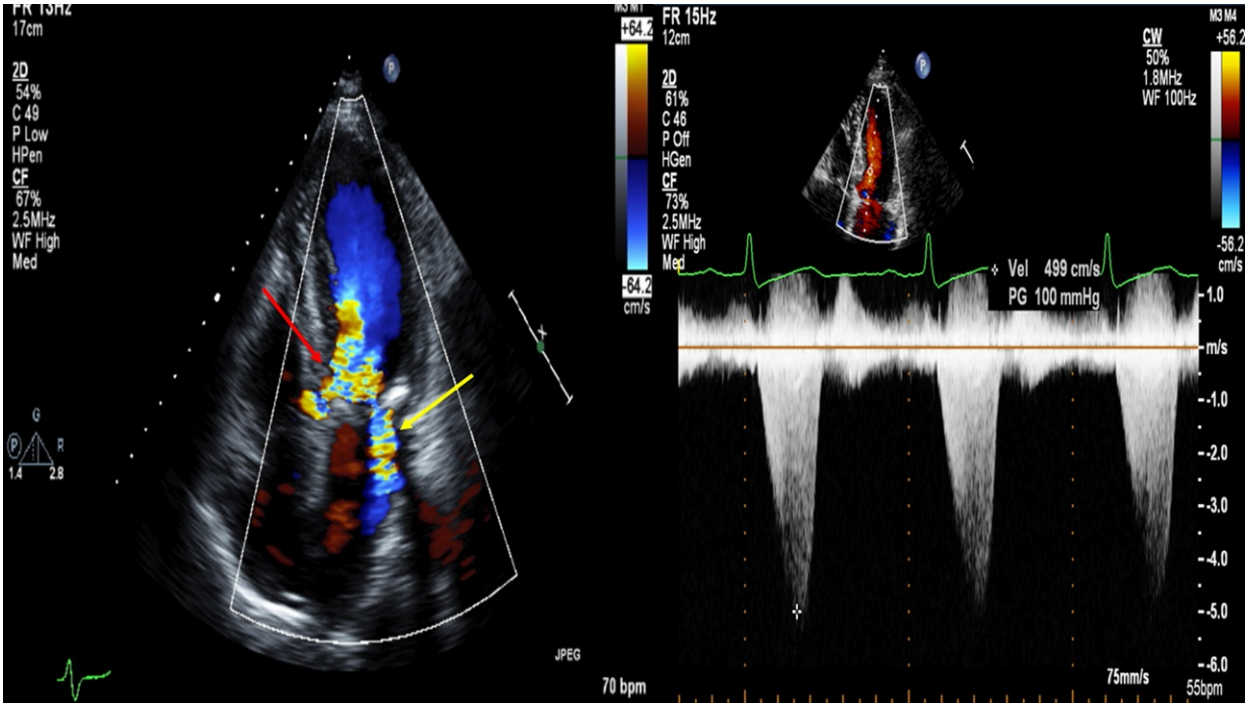

Fig. 3 On color flow imaging on transthoracic echocardiography there is marked turbulence of blood flow in the left ventricular outflow tract (*red arrow*), along with a posteriorly directed jet of mitral regurgitation (*yellow arrow*). A cursor is placed across the outflow tract and using continuous wave Doppler an outflow tract gradient of 100 mmHg can be recorded, indicating severe outflow tract obstruction under resting conditions.

delineation of apical hypertrophy and allows identification of apical infarction and aneurysm formation. Gadolinium contrast agents allow identification of underlying myocardial scar and fibrosis, the pattern and degree of which may often alert the clinician to the possible presence of a "mimic" or phenocopy of HCM such as cardiac amyloidosis or Anderson-Fabry disease. The degree of myocardial fibrosis may relate to risk of development of heart failure, arrhythmia, and SCD (Fig. 2).

Exercise testing

Exercise testing allows objective assessment of exercise capacity, blood pressure response to exercise, and exercise-induced arrhythmia. When combined with TTE, it can identify patients with provocable LVOTO.

Ischaemic testing

Exercise ECG and functional testing such as myocardial perfusion scanning and stress echocardiography are not sensitive markers of underlying epicardial coronary artery disease, given that inducible ischaemia and ECG changes may occur in the absence of significant stenosis of an epicardial coronary artery. CT coronary angiography is most useful in those cases in whom a low likelihood of coronary artery disease (CAD) exists, but in the presence of traditional risk factors for CAD and an intermediate to high likelihood of CAD coronary angiography is preferred.

Differential Diagnosis

The presence of LVH does not always represent a pathological response, and can represent a physiological or compensatory response to high levels of athletic training. However, the presence of LVH represents a pathologic response to hemodynamic loading of the ventricle in cases such as systemic hypertension and aortic stenosis. Unexplained LVH can also occur in a range of systemic and genetic disorders, most notably lysosomal and glycogen storage disorders such as Anderson-Fabry disease and Danon disease. Infiltrative disorders such as cardiac amyloidosis, mitochondrial disorders, and genetic syndromes such as Noonan syndrome account for a small percentage of cases. Accurate identification of the underlying cause of unexplained LVH is important to guide appropriate therapy.

In patients in whom a "mimic" or phenocopy of HCM is suspected, a routine panel of blood tests to assess renal and liver function, full blood count, plasma BNP, and troponin should be performed. Impaired renal function and proteinuria may be seen in systemic amyloidosis and Anderson-Fabry disease, whereas liver tests and serum creatine phosphokinase may be abnormal in mitochondrial and glycogen storage disorders. In cases of suspected amyloidosis, serum and urine protein electrophoresis are mandatory. Assessment of plasma/leucocyte alpha galactosidase A activity should be performed in all men aged >30 years, and in

any case where Anderson-Fabry disease is clinically suspected. Enzyme levels are often within the normal range in affected females and genetic testing is necessary to confirm the diagnosis (Elliott et al., 2014).

Disease Course and Progression

While the majority of patients have minimal or no symptoms, and follow a benign and stable clinical course, in some cases progressive congestive heart failure or sudden cardiac death may occur. Progressive symptoms, secondary to diastolic dysfunction, LVOT obstruction, mitral regurgitation, or development of left ventricular systolic dysfunction, may occur and significantly impact quality of life (Fig. 4).

Three distinctive modes of death have been demonstrated in patients with HCM, with an overall annual mortality of <1%/year: (1) sudden and unexpected, (2) progressive heart failure, and (3) ischemic stroke secondary to atrial fibrillation (Maron et al., 2000).

Clinical Management

The clinical management of patients with HCM focuses on the treatment of symptoms, assessment and reduction of risk of SCD, and screening of at-risk family members either clinically or with cascade genetic testing.

Pharmacologic

The treatment of symptoms is determined by the presence or absence of LVOTO (Fig. 5), and beta-blocker and calcium-channel blockers remain the mainstay of first-line therapy, titrating up to a maximum tolerated dose. Fig. 5 demonstrates a schematic for the management of patients. In patients with obstruction who remain symptomatic despite the use of first-line agents, Disopyramide can be added as second-line therapy. Given its potential to result in QT prolongation, initiation should be performed under close cardiac and ECG monitoring. Attention should also focus on optimizing antihypertensive therapy in patients with concomitant systemic hypertension, as vasodilator agents such as angiotensin receptor inhibitors and alpha-blockers can aggravate outflow tract obstruction.

In the absence of LVOTO, oral diuretics can be added after a calcium-channel blocker or beta-blocker to control symptoms of dyspnoea.

Intervention

When symptoms related to ongoing LVOTO persist despite pharmacological therapy, as is the case in up to one-third of patients, interventional strategies may become necessary to improve quality of life. This may take the form of surgical septal myectomy, with resection of muscle from the basal and mid-interventricular septum, with or without concomitant coronary artery bypass grafting and mitral valve surgery. Septal myectomy can safely be performed in expert centers with extremely low rates of morbidity and mortality. Alternatively, alcohol septal ablation, performed through a percutaneous approach, involves injection of ethanol into a septal perforator vessel identified as supplying the myocardium of the hypertrophied basal interventricular septum. This creates an area of infarction with subsequent healing and thinning of the myocardium over a 6-12-month period. Dual-chamber pacing is an alternative for those patients who have an unacceptably high risk for either of the above interventional procedures. However, the benefit from pacing is usually modest compared with myectomy and alcohol ablation (Fig. 5).

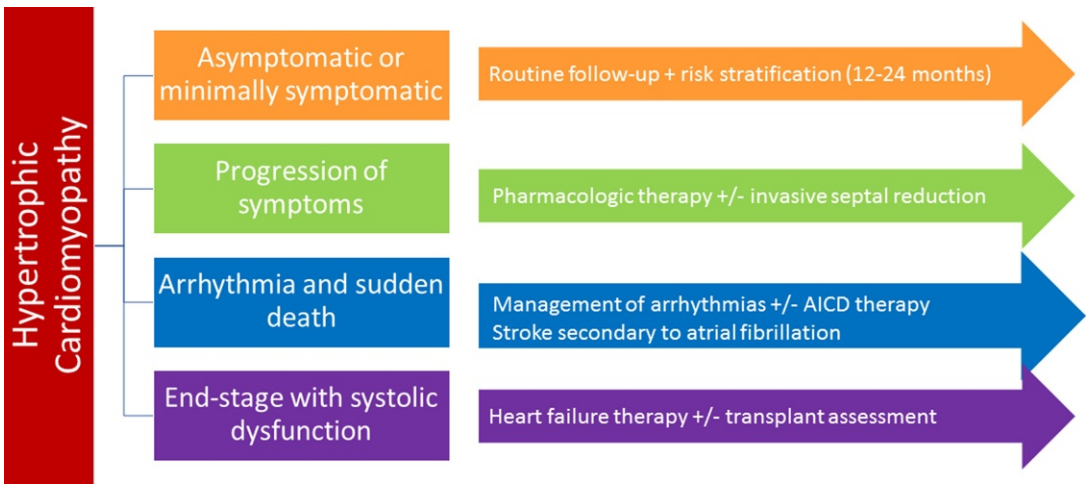

Fig. 4 Patients may progress along one of several disease courses, and may in fact be affected by various complications of the disease (such as progressive heart failure and arrhythmia).

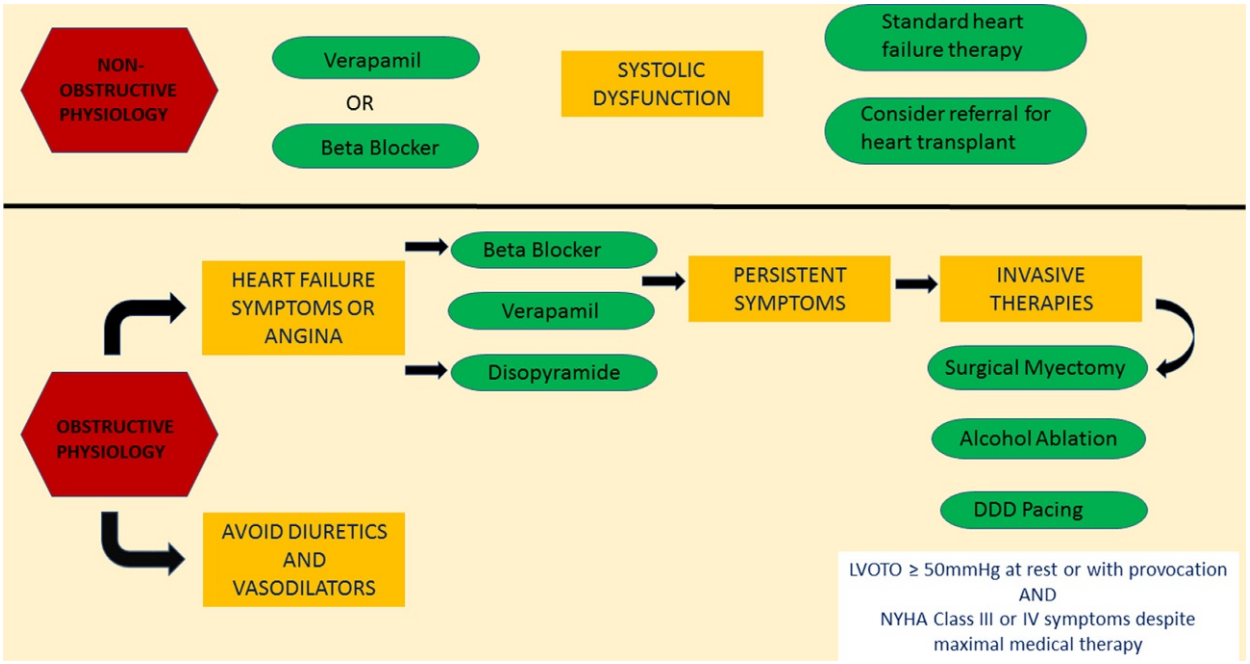

Fig. 5 An algorithm for the symptomatic treatment of HCM, stratified by the presence or absence of left ventricular outflow tract obstruction. The mainstay of therapy remains beta-blocker and calcium-channel blocker therapy. Invasive septal reduction therapies are reserved for those patients with medically refractory symptoms and significant outflow tract gradients either at rest or with provocation.

Risk stratification

SCD remains the most devastating complication of the disease, and is often the first presentation. Implantation of an automated implantable cardioverter defibrillator (AICD) can offer protection from SCD in patients deemed to be at higher risk.

An AICD should be implanted in any patient who has survived an aborted SCD or experienced a high-grade ventricular arrhythmia due to the increased risk of recurrence (up to 10%/year).

The challenge remains identifying patients at high risk of SCD and reassuring those at low risk, while also factoring in the risk–benefit of lifelong AICD therapy with its associated complications.

Established risk factors include: (1) a family history of sudden death, (2) a maximum wall thickness $\geq$30 mm, (3) one or more recent unexplained syncopal episodes, and (4) the presence of NSVT (especially in patients younger <30 years). Other SCD risk factors or modifiers of risk include: (1) an abnormal blood pressure response to exercise, (2) LVOTO, (3) the presence of significant myocardial fibrosis on CMR, and (4) the presence of an apical aneurysm (Gersh et al., 2011). The decision to implant an AICD cannot be solely determined by a checklist of risk factors, which can both over- or underestimate the risk in an individual patient, but integrated with the clinical assessment of the physician and the wishes of the patient.

Family screening

Family screening, either clinical (ECG and echocardiography) or genetic (if a pathogenic mutation has been identified in the family), should be offered to all first-degree relatives after appropriate counseling about the potential implications of a positive test.

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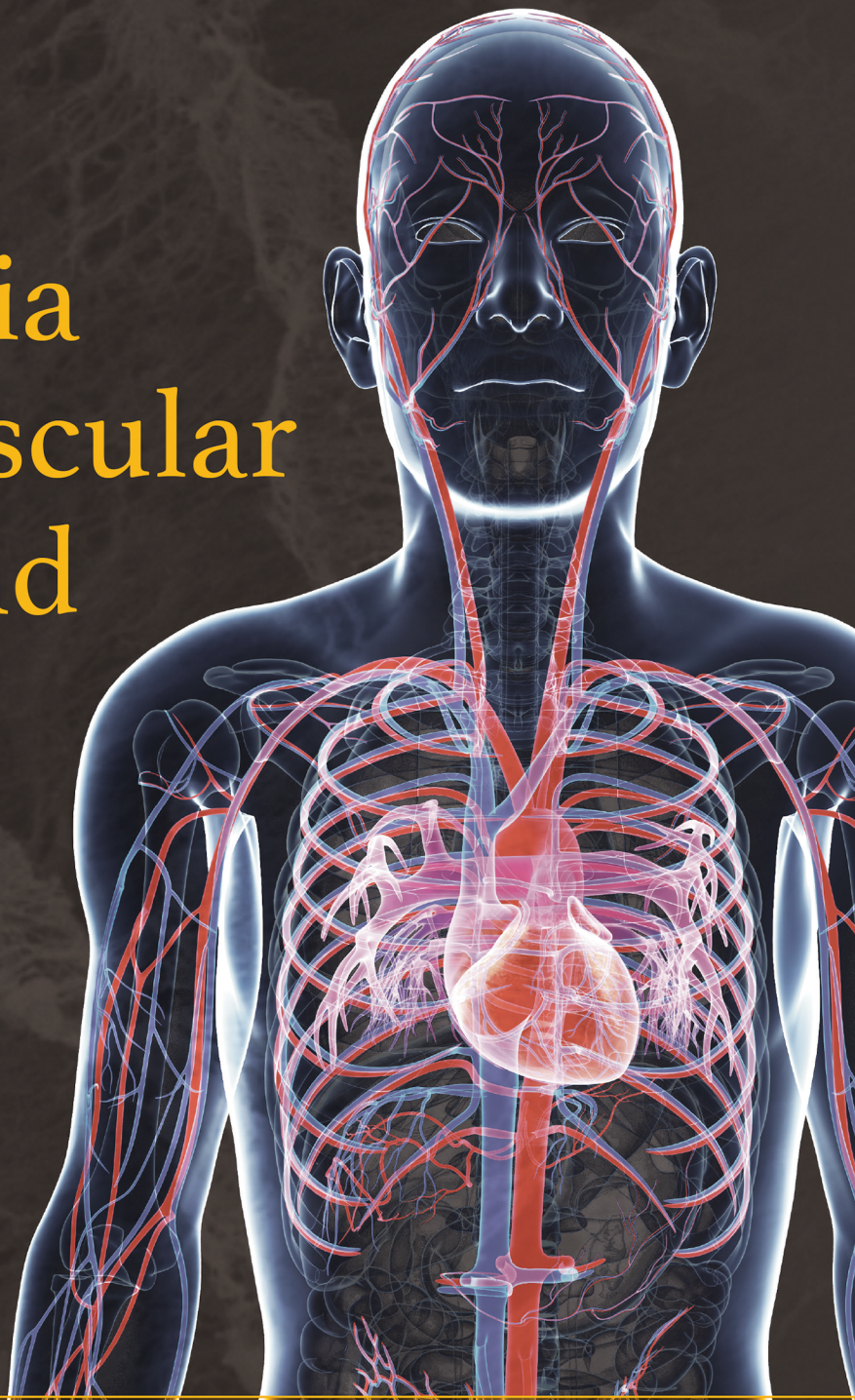

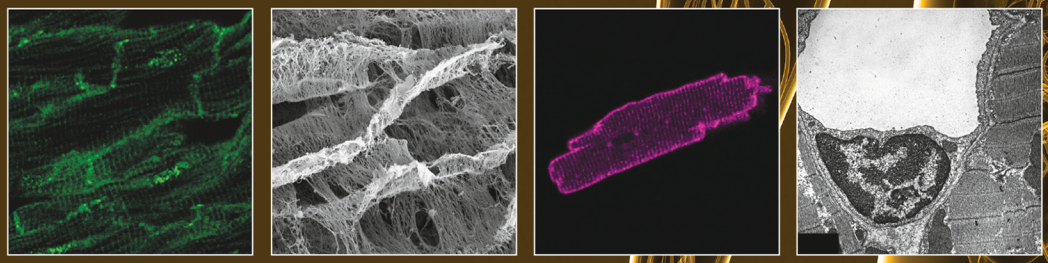

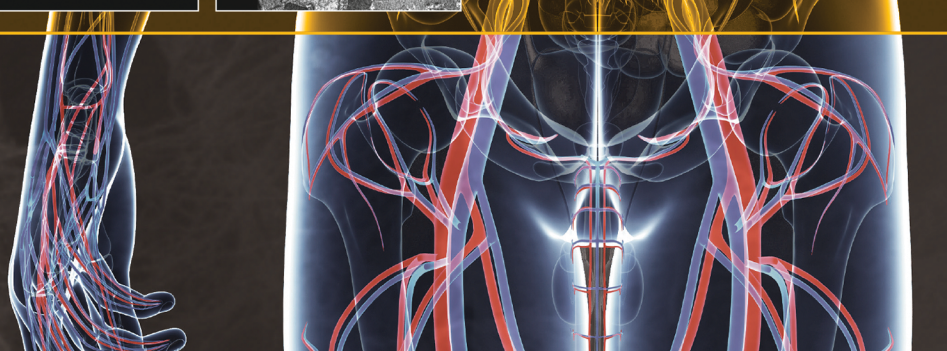

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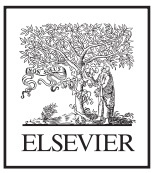

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PREFACE

The burden of cardiovascular disease is growing across the globe as the leading noncommunicable cause of morbidity and mortality. This is despite outstanding research that has improved our understanding of cardiovascular biology, the determinants of disease, and development of new therapies. We are optimistic that the next generation of scientists and clinicians will further these efforts and ultimately bring about changes that will reduce risk and improve outcomes for patients worldwide. We have assembled this encyclopedia with the explicit intent to provide this generation with easy access to past and current wisdom, with the hope that this helps to accelerate innovation in cardiovascular medicine.

This encyclopedia represents both a dream and a labor of love. The dream is to create a scientific compendium that recognizes that the methods and tools used to access and study the scientific literature have changed, with a burgeoning impact of digital technology—the so-called pocket book encyclopedia that is accessible on a smart phone fitting into medical overalls, and that can also be read as a textbook at a desk! These changes in the access of publications have been accompanied by the evolving need for studying different aspects of biology and disease as stand-alone chapters not necessarily read sequentially. Furthermore, as editors we recognize the fundamental importance of conveying knowledge through brilliantly illustrated texts that are profusely embedded with links to key references. The critical importance to use multimedia tools and to provide downloadable slides for educational purposes is reflected in this encyclopedia. Additionally, we want to ensure the feasibility of updating text, figures, and other reading material iteratively even after a publication date, recognizing the rapid advent of information and huge strides made in cardiovascular medicine every day. Thus, this work represents the end of the beginning of a live text that will metamorphose over time, being guided by the rapid strides and advances in both cardiovascular medicine and publication science.

Overall, we conceptualized this work as providing broad coverage of science and also serving the expansive needs of a readership that is quite diverse, that is, encompassing medical students, residents, fellows, and postdoctoral scientists interested in cardiovascular medicine. We hope the labor of love of an outstanding team of editors and a stellar group of authors is readily evident to the broad readership we have targeted.

Assembling this compendium had many challenges. Early on we made the choice to cover the broad range of cardiovascular biology, pathophysiology, epidemiology, and treatment of disease. We sought to assure equal coverage to foundational cardiovascular cellular and molecular biology as well as state-of-the-art treatments. Covering the breadth inevitably led to some overlap between some chapters, and yet we certainly have some residual gaps. We hope to have the opportunity to fill these gaps in future editions. There is also inevitable variation in the style and scope of articles in this multiauthor publication. We and the section editors felt it was more important to allow our expert authors freedom in how they chose to present their topic.

We invite feedback on how this encyclopedia can be improved as a resource for trainees worldwide. Undoubtedly, as noted above, there are gaps in content areas that need to be bridged in future iterations of this encyclopedia. We look forward to hearing from and listening to our readership about how the text can be better organized or better compiled to serve their scholarship better in future years.

This project was made possible through the hard work of many people from across the globe. We were pleased to have section editors and authors from around the world. Drs. Kessler and Schunkert, renowned cardiologists with an outstanding record of accomplishment in genetic research, have compiled a series of chapters on genetics and genomics. Dr. Baliga, a distinguished cardiologist and educator, has conceptualized a tour de force of chapters that capture all aspects of the heart failure syndrome, whereas Dr. DiSalvo, a master clinician and educator, has synthesized a fine tapestry of chapters focusing on diagnostic testing with key sections on cardiac imaging, interventions, and device therapy. Dr. Raj, an outstanding clinical investigator and

electrophysiologist, brought together an exemplary collection of chapters that offer coverage of a broad spectrum of topics in cardiac electrophysiology. Drs. Major and Liaw, both highly accomplished basic scientists and educators, were instrumental in identifying topics of fundamental importance to the field of cardiovascular biology and medicine. Dr. Lenihan, a pioneering clinical scientist and outstanding cardiologist, brought together a special set of articles that represent interactions between cardiovascular and other fields of medicine. Dr. Gale, a celebrated cardiologist with expertise in comparative effectiveness research and clinical cardiology, offers a series of stellar chapters underscoring various aspects of the pathogenesis and management of atherosclerotic cardiovascular disease. Dr. Kokubo, a leading preventive cardiologist with specialization in cardiovascular epidemiology, has orchestrated state-of-the-art chapters on cardiovascular epidemiology.

We would like to thank our colleagues at Elsevier who provided steadfast support of an exemplary nature throughout this long journey. Last but not the least, the editors would like to thank their families, their mentors, and their respective institutions for the constant encouragement, advice, and support over the years.

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Imaging of Mitral Regurgitation

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Introduction

While the rate of significant valvular heart disease is rising worldwide as the population ages, moderate to severe mitral regurgitation continues to be the most prevalent form of severe valvular disease in developed countries (Nkomo et al., 2006; Coffey et al., 2016; Iung et al., 2003; Shu et al., 2016). The exact prevalence of this disease is difficult to identify due to regional and temporal heterogeneity in the definition of severe valvular regurgitation as well as referral bias for echocardiography in clinical studies. In the Framingham Offspring Study, mild or greater mitral regurgitation was present in 19% of participants receiving screening echocardiograms (Coffey et al., 2016). Pooled prospective cohort studies of community-dwelling individuals assessed with echocardiography suggest that mitral regurgitation is present in 1.7% of the population, including 9.3% of individuals $\geq$75-years old (Nkomo et al., 2006). The prevalence of moderate or greater mitral regurgitation rises with age and appears to have a male predominance in 60%–75% of cases in surgical series (Nkomo et al., 2006). In an African-American cohort older than age 50, 0.7% of individuals had moderate or more severe mitral regurgitation (Coffey et al., 2016). Mitral regurgitation is associated with larger left ventricular diastolic and left atrial diameters compared to individuals without valvular heart disease (Nkomo et al., 2006). Given that mitral regurgitation is a heterogeneous entity, occurring either due to primary abnormalities with the valve or valvular apparatus or secondary abnormalities due to dysfunction in the left atrium or ventricle, the exact prevalence of mitral regurgitation depends on its etiology and definition of severity. For example, while mitral valve prolapse has a population prevalence of 2.4% (Freed et al., 1999), the majority of these individuals have trivial to mild regurgitation. In the setting of cardiomyopathy, up to 49% have mild to moderate mitral regurgitation and 24% have severe mitral regurgitation (Coffey et al., 2016).

Etiologies of Mitral Valve Disease

Mitral regurgitation can result from a variety of derangements affecting any portion of the valvular apparatus. Mitral regurgitation is generally classified as either primary (i.e., organic) due to an abnormality with the valvular leaflets or secondary (i.e. functional), which is typically caused by an abnormality of the atrium or ventricle. Though the terminology has changed over time, ischemic mitral regurgitation is often inappropriately used to refer to functional mitral regurgitation due to ischemic remodeling of the left ventricle. Properly described, ischemic mitral regurgitation represents a type of functional mitral regurgitation due to tethering and apical displacement of the posteromedial papillary muscle in the setting of myocardial infarction. This prevents full excursion of the inferolateral left ventricular wall and annular dilation from progressive volume overload.

While mitral regurgitation may be classified by the presence of valvular leaflet involvement, it may also be classified by functional derangement. A commonly used functional classification is that produced by Alain Carpentier, stratifying mitral

pathologies into Type I denoting normal leaflet motion, Type II denoting increased leaflet motion, and Type III denoting restricted leaflet motion (Carpentier, 1983).

Primary Mitral Regurgitation

Primary mitral regurgitation is due to dysfunction of the valvular tissue and is associated with a number of pathologies that can affect different parts of the valvular apparatus (Table 1).

Table 1 Causes of primary mitral regurgitation

Mitral annulus
Mitral annular calcification
Idiopathic
Rheumatic fever
Chronic kidney disease
Hyperparathyroidism
Infective endocarditis (abscess)
Trauma (valve surgery)
Mitral leaflet
Myxomatous valve disease (mitral valve prolapse)
Rheumatic heart disease
Infective endocarditis
Systemic inflammatory disorders
Systemic lupus erythematosus (Libman-Sacks lesion)
Scleroderma
Connective tissues disorders
Marfan syndrome
Ehlers-Danlos syndrome
Osteogenesis imperfecta
Congenital
Mitral valve cleft
Parachute mitral valve
Fenestrated mitral valve
Trauma
Penetrating chest injury
Percutaneous balloon valvuloplasty
Hypertrophic cardiomyopathy (systolic anterior movement of mitral valve leaflet)
Drug-related (Fen-Phen)
Chordae tendineae
Myxomatous valve disease (mitral valve prolapse)
Rheumatic heart disease
Infective endocarditis
Connective tissues disorders
Marfan syndrome
Ehlers–Danlos syndrome
Osteogenesis imperfecta
Acute myocardial infarction
Trauma
Blunt chest trauma
Percutaneous Balloon valvuloplasty
Idiopathic spontaneous rupture
Papillary muscle
Myocardial infarction or ischemia
Infiltrative diseases
Amyloidosis
Sarcoidosis
Trauma
Prosthetic valve
Infective endocarditis
Pannus or thrombus formation
Bioprosthetic valve degeneration
Immobilized ball/disc in mechanical prosthesis
Paravalvular Leak
Ring or strut fracture

Mitral Valve Prolapse

The most common cause of primary mitral regurgitation in developed countries is mitral valve prolapse, also known as degenerative valvular disease. Mitral valve prolapse is common, with an estimated prevalence of 2.4% in the general population (Nkomo et al., 2006; Coffey et al., 2016; Iung et al., 2003; Freed et al., 1999). More recently, recognition of the mitral valve's saddle-shaped three-dimensional structure has led to improved specificity of diagnosis, and therefore current estimates of disease prevalence are lower (Freed et al., 1999; Levine et al., 1988). Current criteria for diagnosis requires superior displacement of the mitral valve leaflets more than 2 mm from the annular plane during systole in the parasternal long-axis or the apical three chamber views on echocardiography (Nishimura et al., 2017). Mitral valve prolapse may be further delineated into classic disease with maximal thickening of the leaflets greater than 5 mm during diastasis and nonclassical with maximal thickness less than 5 mm (Freed et al., 1999). The majority of these individuals have mild mitral regurgitation; one series demonstrated that only 7% of patients with classic prolapse and no patients with nonclassic prolapse had severe mitral regurgitation (Freed et al., 1999). Nevertheless, mitral valve prolapse is the leading cause of isolated mitral regurgitation as well as regurgitation requiring surgery (Delling and Vasan, 2014). This is due in part to the propensity for papillary muscle or chordal rupture from the structural weakening by myxomatous degeneration seen in this disease, which can lead to sudden and severe mitral regurgitation due to a flail leaflet (**Figs. 1** and **2**). Patients with mitral valve prolapse have high rates of reported chest pain and dyspnea approximating 11%–15%, and while subjects with this disease are

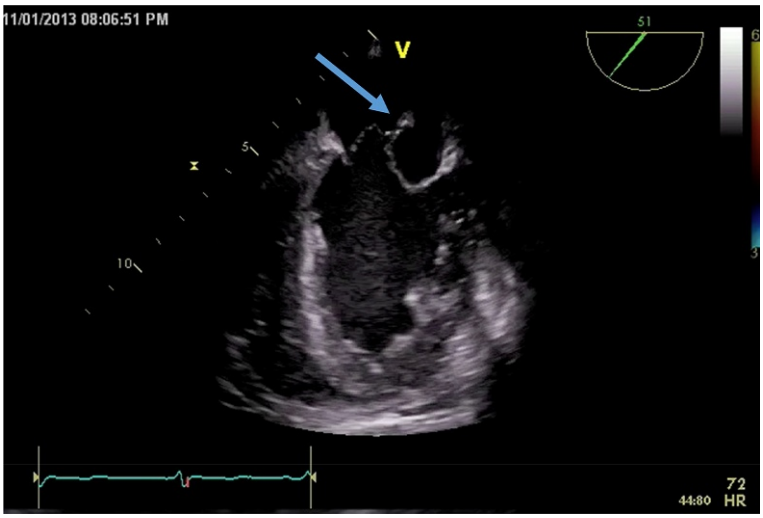

Fig. 1 Transesophageal echocardiogram of flail leaflet. This transesophageal echocardiogram in the mid-esophageal position demonstrates a portion of a torn papillary muscle head (*arrow*), which prolapses into the left atrium during systole, in a patient with mitral valve prolapse. The anterior mitral valve leaflet bows into the left atrium, indicating a flail leaflet. The patient had severe mitral regurgitation as a result.

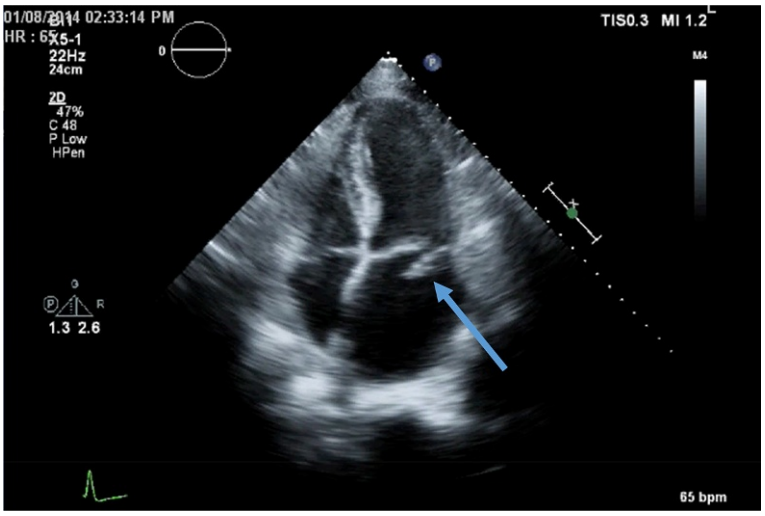

Fig. 2 Transthoracic echocardiogram of flail mitral valve leaflet. This transthoracic echocardiogram image of the mitral valve in the apical four chamber view, demonstrates a flail posterior leaflet (*arrow*), bowing into the left atrium in a patient with mitral valve prolapse and chordal rupture. Both anterior and posterior mitral valve leaflets are thickened consistent with the patient's known diagnosis of Barlow's syndrome. Of note, the left atrium appears enlarged consistent with increased left atrial pressure from mitral regurgitation. The patient had severe mitral regurgitation and required valvular repair.

overall leaner than those without prolapse, their rates of having heart failure, atrial fibrillation, cerebrovascular disease, and syncope are similar to the general population (Freed et al., 1999). Furthermore, while there has been concern that mitral valve prolapse may be associated with ventricular arrhythmias and sudden death, this risk is isolated to those with prolapse and severe mitral regurgitation with left ventricular dysfunction (Freed et al., 1999). Mitral valve prolapse is associated with two distinct valvular phenotypes, Barlow's syndrome representing extensive bileaflet involvement, and fibroelastic deficiency, representing isolated disease and prolapse of the P2 segment of the mitral valve (Delling and Vasan, 2014).

Rheumatic Heart Disease

Other notable etiologies of primary mitral regurgitation are rheumatic heart disease and endocarditis, which respectively, represent 14.2% and 3.5%of all mitral regurgitation cases in one study on population prevalence (Iung et al., 2003). Worldwide, rheumatic heart disease is the second most common etiology of mitral regurgitation (Iung et al., 2003). With recurrent streptococcal infection, autoimmune destruction with resultant thickening and calcification of the valvular commissures leading to mitral stenosis and regurgitation can occur. Involvement of the subvalvular apparatus with chordal thickening and shortening is seen with progressive rheumatic involvement, and this is primarily associated with mitral regurgitation rather than stenosis (**Fig. 3**) (Guilherme et al., 2004). Rheumatic heart disease may affect the aortic and tricuspid valves in addition to the mitral valve and rarely can affect the pulmonic valve (Guilherme et al., 2004).

Endocarditis

Vegetations on the mitral valve may be due to infectious or noninfectious causes and can lead to regurgitation by causing poor coaptation of leaflets, or perforation of paravalvular tissue leading to chordal rupture (**Fig. 4**). Fistulization caused by abscess formation can also lead to paravalvular regurgitation. Mitral valve endocarditis is increasingly common in elderly patients and those with indwelling lines and hemodialysis catheters, though the increasing prevalence of injecting drug use may alter these epidemiologic trends (Wurcel et al., 2016). Endocarditis may also affect prosthetic valves in the mitral position leading to valvular dysfunction and regurgitation. Mitral regurgitation is commonly seen with mechanical mitral valve prostheses but generally is mild in severity with centrally located regurgitant flow. Any paravalvular or more significant regurgitation seen with mitral valve prostheses should be considered pathologic and prompt work-up for endocarditis in the appropriate clinical setting.

Secondary Mitral Regurgitation

Secondary mitral regurgitation, also known as functional mitral regurgitation, may be caused by any abnormality leading to atrial or ventricular dilation as well as disease of the subvalvular apparatus (**Table 2**). Coronary artery disease and myocardial ischemia and/or infarction commonly contribute to secondary mitral regurgitation. With regional dysfunction of the inferolateral wall, particularly in the setting of ischemia, tethering of the posteromedial papillary muscle leads to insufficient anterior excursion of the posterior mitral valve leaflet, malcoaptation, and significant mitral regurgitation. While transient ischemia may cause myocardial stunning with failure of inferolateral wall thickening and subsequent intermittent mitral regurgitation, this typically resolves with

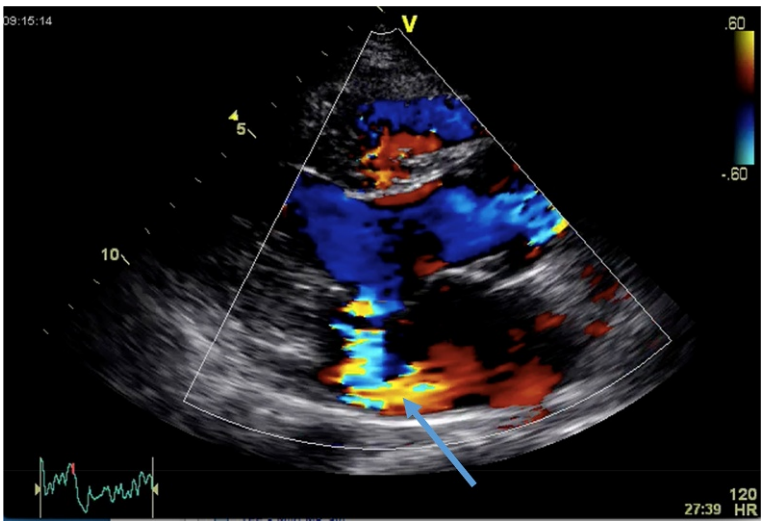

Fig. 3 Rheumatic mitral valve disease causing mitral regurgitaiton. This transthoracic echocardiographic image of the parasternal long axis-view demonstrates a patient with rheumatic mitral valve disease with shortening of the subvalvular apparatus, causing retraction of the posterior leaflet and a posteriorly directed jet of moderate mitral regurgitation (*arrow*).

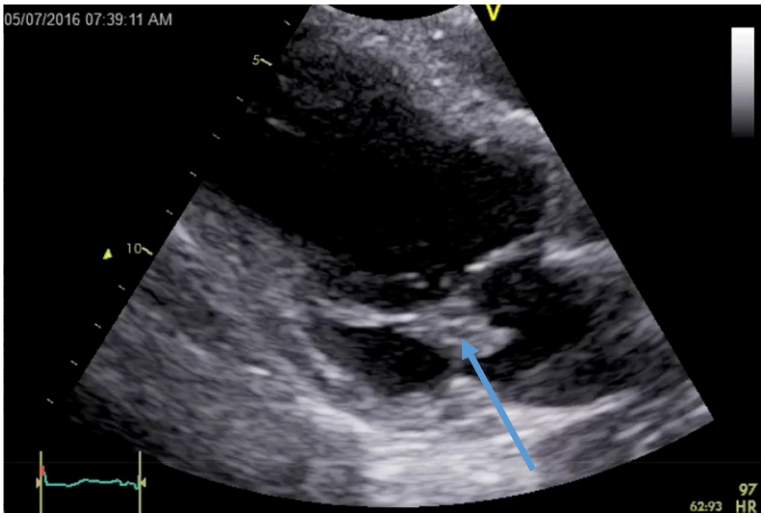

Fig. 4 Transthoracic echocardiogram of patient with infective endocarditis. This zoomed parasternal long-axis view of a transthoracic echocardiogram in a patient with suspected infective endocarditis demonstrates a large vegetation attached to the posterior mitral valve (*arrow*). Consistent with the behavior of valvular vegetations, the apparent echodense mass is present on the left atrial side of the valve.

Table 2 Causes of secondary mitral regurgitation

Mitral annulus dilatation
Myxomatous mitral valve disease
Dilated cardiomyopathy
Connective tissue disorders
Left atrial enlargement
Atrial fibrillation
Congestive heart failure (systolic and diastolic)
Left ventricular abnormalities
Dilated cardiomyopathy (left ventricular dilatation)
Coronary artery disease (focal wall motional abnormalities or papillary muscle dysfunction)
Left ventricular aneurysm
Right ventricular pacing (left ventricular dysychrony)

improvement in myocardial perfusion. In contrast, infarction of inferolateral wall leads to persistent mitral regurgitation. Additionally, ischemia, infarction, or infiltration may lead to papillary muscle rupture causing a flail mitral valve leaflet and significant mitral regurgitation.

Ventricular dilation from either ischemic or nonischemic cardiomyopathies frequently results in secondary mitral regurgitation. In one series, any mitral regurgitation was present in 73% of those with cardiomyopathy with up to 24% having severe secondary mitral regurgitation (Di Salvo et al., 2010). In secondary mitral regurgitation due to ventricular dilation, resulting apical and lateral papillary muscle displacement can lead to tenting and poor coaptation of the mitral leaflets (Fig. 5). Additionally, the increased volume loading of the left atrium in this setting may lead to further ventricular remodeling with annular enlargement, thus worsening coaptation and resulting in more regurgitation over time. Mitral regurgitation may also be caused by atrial dilation in the setting of atrial disease or long-standing atrial arrhythmias with remodeling.

Outcomes of individuals with secondary mitral regurgitation are difficult to distinguish from their underlying comorbidities, but evidence suggests a worse prognosis compared to individuals without mitral regurgitation (Di Salvo et al., 2010). While chordal-sparing mitral valve replacement is typically preferred to mitral valve repair in individuals with severe symptomatic mitral regurgitation, the overall benefit of surgery in this setting is unclear. The emergence of transcatheter therapies may improve outcomes for patients with severe mitral regurgitation who are not candidates for cardiac surgery, but further study is needed to determine the optimal population who would derive the most benefit from these technologies (Nishimura et al., 2017).

Natural History and Outcomes Associated With Mitral Regurgitation

Individuals with significant mitral regurgitation due to primary mitral valve disease are at risk for adverse outcomes in the presence of symptoms (David et al., 2013; Lamas et al., 1997a; Ling et al., 1996; Suri et al., 2008; Grigioni et al., 2001), atrial fibrillation (Suri et al., 2008), pulmonary hypertension (Marechaux et al., 2011; Patel et al., 2014), or depressed left ventricular systolic function

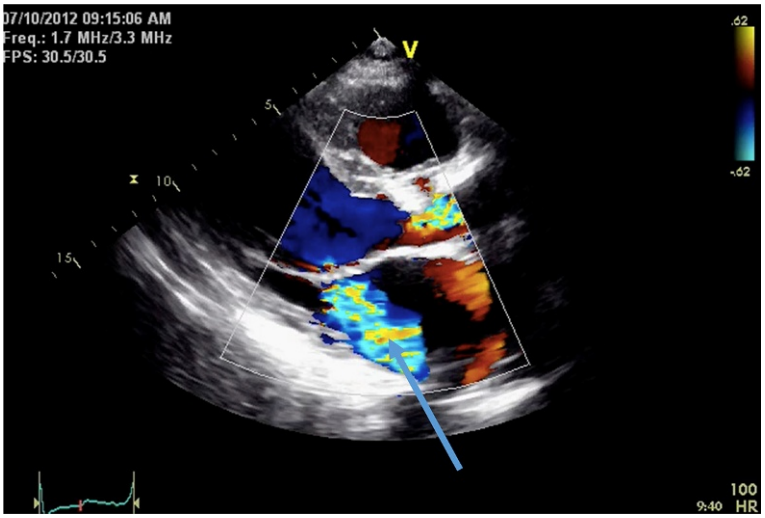

Fig. 5 Transthoracic echocardiogram of severe secondary mitral regurgitation. This parasternal long-axis view of a transthoracic echocardiogram in a patient with a dilated cardiomyopathy demonstrates apical displacement of the mitral valve leaflets (*tenting*) and poor coaptation leading to a centrally directed jet of mitral regurgitation (*arrow*).

(Enriquez-Sarano et al., 1994). However, data on outcomes of individuals with asymptomatic severe mitral regurgitation are more heterogeneous. Sarano et al. demonstrated that asymptomatic individuals with primary mitral regurgitation and an effective orifice area (EOA) of at least 40 mm^2 had a five-year survival less than expected by census estimates and a nearly five-fold increased risk of death from cardiac causes (Enriquez-Sarano et al., 2005). In the cohort of 232 patients who underwent cardiac surgery in this study, there was a 72% reduction in mortality suggesting a role for early surgery in severe asymptomatic patients with primary mitral valve disease.

Secondary mitral regurgitation following myocardial infarction is also associated with poor outcomes. In the Survival and Ventricular Enlargement study, individuals with any degree of mitral regurgitation shortly after myocardial infarction were at a two-fold risk of cardiovascular death at 3.5 years of follow-up (Lamas et al., 1997b). The degree of mitral regurgitation has been shown to be associated with long-term mortality and cardiac mortality risk at 5 years after myocardial infarction (Grigioni et al., 2001). The role of surgery in secondary mitral regurgitation is less clear. Retrospective data suggest that mitral valve surgery for individuals with advanced heart failure is both safe and efficacious with significant improvements in left ventricular ejection fraction, left ventricular volumes, and improvement in functional class and quality of life (Di Salvo et al., 2010). However, limited clinical trial data from the Cardiothoracic Surgical Trials Network do not clearly suggest a benefit to the addition of mitral valve repair in individuals with moderate or greater mitral regurgitation undergoing concomitant coronary artery bypass grafting at one year (Smith et al., 2014). Specifically, though the presence of moderate to severe mitral regurgitation was less common in the mitral repair cohort, there were no differences in adverse cardiac or cerebrovascular events, deaths, readmissions, functional status, or quality of life at one year (Smith et al., 2014). Whether or not subgroups of individuals may benefit from surgical or percutaneous treatment of mitral valve disease is a subject of ongoing research. Additionally, therapy directed toward management of heart failure such as cardiac resynchronization therapy may impact the natural history of functional or ischemic mitral regurgitation in this setting (Di Salvo et al., 2010; Auricchio et al., 2011). The optimal criteria for defining severe secondary mitral regurgitation has also been controversial, with some data suggesting worsened outcomes at a lower EOA compared with primary mitral regurgitation (Nishimura et al., 2017; Uretsky et al., 2015; Grayburn et al., 2014).

Imaging of Mitral Valvular Disease

While computed tomography, cardiac magnetic resonance imaging, and myocardial perfusion imaging have increasingly important roles in the evaluation and management of patients with significant mitral regurgitation, echocardiography remains the most pervasive modality for identifying the presence and severity of mitral regurgitation, as well as providing guidance for subsequent interventions.

Trans-Thoracic Echocardiography

The most common imaging modality used to identify mitral regurgitation is trans-thoracic echocardiography (TTE). Two-dimensional imaging or M-mode may identify abnormalities in left ventricular global or regional systolic function, atrial size

and function, and abnormalities with the mitral valve or valvular apparatus, including valvular vegetations, congenital abnormalities, and commissural thickening, which may indicate the likely etiology for regurgitation. Furthermore, color Doppler echocardiography permits detection of the mitral regurgitation jet, assessment of jet direction, and evaluation of the severity of disease including jet area and vena contracta (**Fig. 6**). Continuous and pulse wave Doppler may be used to calculate several markers of degree of regurgitation such as EOA, regurgitant fraction, and regurgitant volume. Additionally, examination of flow patterns may reveal alterations consistent with severe disease such as blunting or reversal of systolic flow in the pulmonary veins or significant pulmonary hypertension (**Fig. 7**). Stress echocardiography using TTE can additionally identify patients with inducible pulmonary hypertension with exercise, stress-induced ischemic mitral regurgitation, and latent left ventricular systolic dysfunction (Nishimura et al., 2017). Echocardiography can suggest concomitant valvular lesions, mitral stenosis, and underlying problems with left ventricular filling that may contribute to regurgitant severity. While evaluation of regional or global changes in left ventricular deformation using tissue Doppler or speckle-tracking strain imaging holds promise for better defining subclinical left ventricular dysfunction, lack of intervendor standardization on normal values for strain has impaired more widespread adoption of this technology (Collier et al., 2017). Furthermore, while 3D echocardiography has been shown to more accurately define left

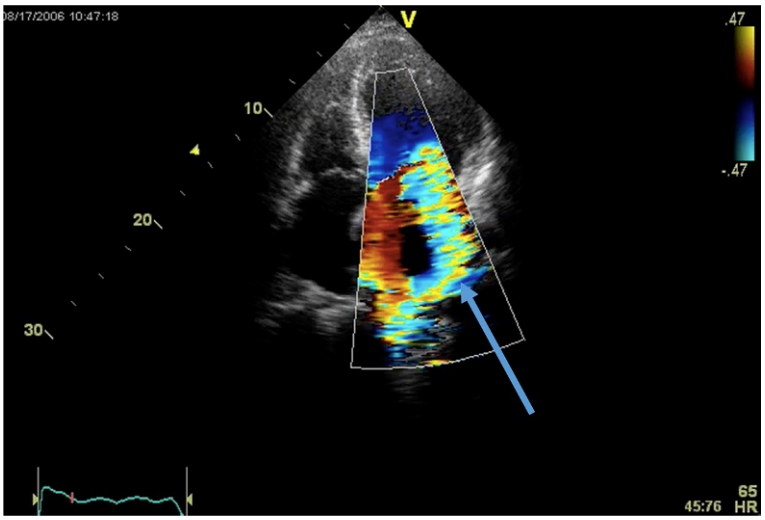

Fig. 6 Color flow doppler representation of severe mitral regurgitation. This zoomed-out apical four chamber view of a transthoracic echocardiogram in a patient with an underlying cardiomyopathy demonstrates severe functional mitral regurgitation (*arrow*) directed into the right pulmonary veins. The jet of mitral regurgitation has a large area on color flow Doppler and extends to the back wall of the left atrium, suggesting a large degree of regurgitant volume.

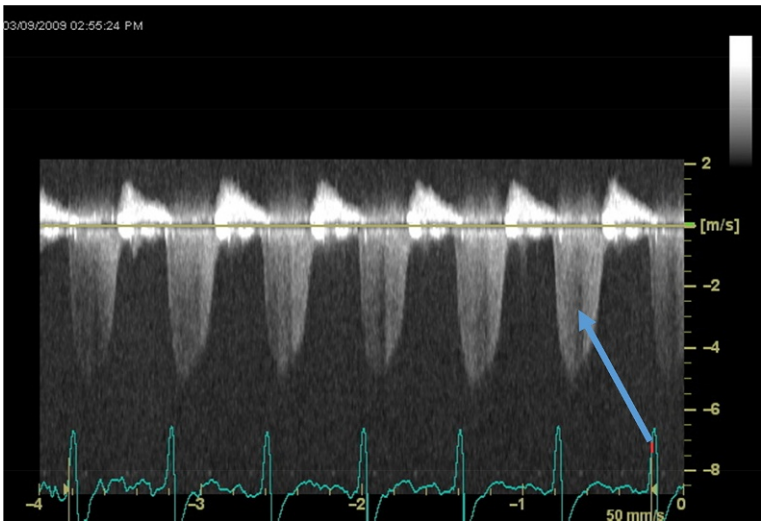

Fig. 7 Continuous wave Doppler of mitral regurgitation. This continuous wave spectral Doppler image of a patient with mitral regurgitation demonstrates high velocity flow during systole consistent with mitral regurgitation (*arrow*). Both the density and shape of the continuous wave jet can be used to help quantify the severity of mitral regurgitation. Moreover, the peak velocity and integral of the observed velocities is used in the calculation of the effective regurgitant orifice using the PISA method.

ventricular ejection fraction compared to 2D echocardiography and thus may predict impairment in left ventricular ejection at an early disease stage, there has not been widespread adoption of this technology for TTE use at the current time (Zoghbi et al., 2017).

Quantitation of Mitral Regurgitation Severity

TTE may also be used to define the severity of mitral regurgitation through several complimentary methods (**Table 3**) (Zoghbi et al., 2017). Both morphologic characteristics of the valve and chambers and quantitative methods are used to define disease severity, as no single metric is perfectly sensitive and specific for defining significant mitral regurgitation (Zoghbi et al., 2017). Assessment of the volumetric degree of regurgitation is possible by approximating the effective regurgitant orifice size using the vena contracta, the smallest diameter of the regurgitant jet as visualized by color Doppler. The vena contracta is best measured in zoomed views perpendicular to the axis of regurgitation in order to maximize axial resolution and is independent of flow rate and driving pressures. It can be used to accurately quantify eccentric jets that may be miscategorized as less severe through other methods. However, this estimate may underestimate regurgitant severity in the presence of multiple jets or regurgitation that does not extend the duration of systole. Furthermore, using the vena contracta requires adequate imaging of flow convergence region, which may be impossible in patients with suboptimal images. 3D measures of the vena contracta are rapidly emerging as an alternative metric for defining MR severity and may identify severe functional MR in cases where the flow convergence method may underestimate the ERO area. Yet, currently utilization and analysis of 3D echo images are cumbersome and subject to similar limitations as 2D measurements. Another tool to quantify MR, the color jet area, is measured in apical zoomed views using color Doppler, either in isolation or as a ratio of jet area to left atrial area. The jet area is easy to measure but is dependent on hemodynamics and other technical variables such as the Nyquist limit. For these reasons it has been shown to be imprecise in multiple studies, particularly in the presence of nonholosystolic MR or eccentric jets.

Quantitation of the regurgitant volume is possible by calculation of the difference between stroke volume through the mitral valve and stroke volume through the left ventricular outflow tract (i.e., forward stroke volume). This technique is valid in the presence of multiple and eccentric jets and has been validated against cardiovascular magnetic resonance for quantitative definition of lesion severity; however, it is lengthy for routine use in clinical practice and involves multiple measurements with potential for compounding measurement error. Additionally, it is inaccurate in the setting of combined mitral and aortic regurgitation. Owing to these difficulties, echocardiographic calculation of regurgitant volume is not routinely used in practice to define lesion severity. The flow convergence method is more commonly utilized to quantitate MR severity. This technique relies on the principle of conservation of mass, namely that the volume of blood entering the regurgitant orifice is the same as the regurgitant volume. In principle, the regurgitant flow can be quantified by taking the product of the aliasing velocity and the volume of a hemisphere whose radius is defined by the proximal isovelocity surface area (PISA). This is determined by lowering the color Doppler baseline in the direction of the regurgitant jet and measuring the radius as the vertical distance from the point of flow convergence to the edge of the hemispheric isovelocity shell (**Fig. 8**). The ERO area is then further defined as the ratio of the regurgitant flow rate and peak systolic velocity of the mitral regurgitant jet as measured by continuous wave Doppler at the same time in the cardiac cycle as the PISA. This method has been shown to be accurate and reproducible at defining ERO area and regurgitant volume and is strongly associated with adverse outcomes in degenerative and functional mitral regurgitation. While this method is commonly used in

Table 3 Assessing the severity of mitral regurgitation[a]

Parameter	*Mild*	*Moderate*	*Severe*
Structural parameters			
Left atrial size	Normal	Normal or dilated	Usually Dilated
Left ventricular size	Normal	Normal or dilated	Usually Dilated
Mitral leaflets/support apparatus	Normal or abnormal	Normal or abnormal	Abnormal structure/flail leaflet/ruptured papillary muscle
Doppler parameters			
Color flow jet area	Small, central jet (<4 cm^2 or $<20\%$ of left atrial area)		Large central jet (>10 cm^2 or $>40\%$ of left atrial area)
Mitral inflow (pulsed wave)	Dominant A wave	Variable	Dominant E wave (usually ≥ 1.2 m/s)
Jet density and contour (continuous wave)	Incomplete or faint; parabolic	Dense; usually parabolic	Dense; early-peaking, triangular
Pulmonary vein flow	Systolic dominance	Systolic blunting	Systolic flow reversal
Quantitative parameters			
Vena contracta width (cm)	<0.3	0.3–0.69	≥ 0.7
Regurgitant volume (mL/beat)	<30	30–59	≥ 60
Regurgitant fraction (%)	<30	30–49	≥ 50
Effective regurgitant orifice area (cm^2)	<0.20	0.20–0.39	≥ 0.40

[a]Adapted from Zoghbi WA, Enriquez-sarano M, Foster E, et al. Recommendations for evaluation of the severity of native valvular regurgitation with two-dimensional and Doppler echocardiography. Journal of the American Society of Echocardiography 2003;16(7):777–802.

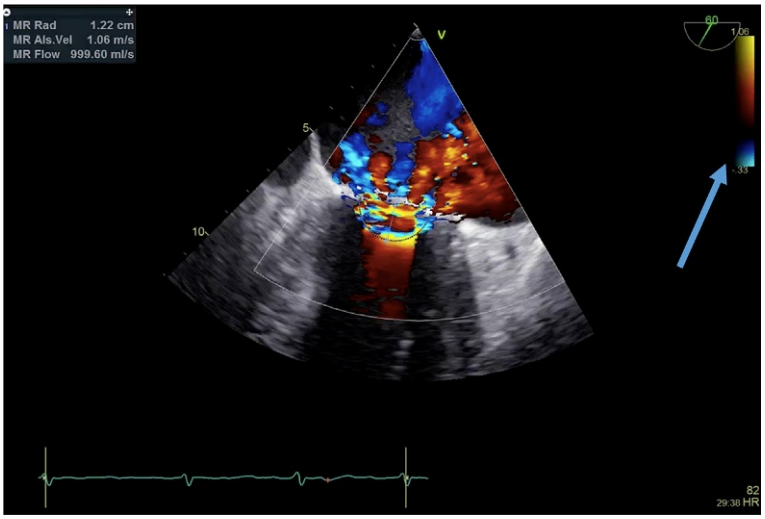

Fig. 8 PISA calculation of mitral regurgitation severity on transesophageal echocardiogram. This transesophageal echocardiographic image in the mid-esophageal position demonstrates calculation of the radius of the PISA shell to determine mitral regurgitation severity. Note that the aliasing velocity has been altered (*arrow*) to exclude low velocity flows from the calculation of the PISA radius.

research applications for defining MR severity, it may not be accurate in the setting of multiple or eccentric jets. Due to errors in measuring the PISA radius, it may also misclassify the severity of MR in patients with very large or small PISA radii.

Other ancillary findings on echocardiography may support a diagnosis of significant mitral regurgitation. For example, using pulsed wave Doppler at the mitral valve leaflet tips, one can define the velocity of blood flow during myocardial filling. A velocity of blood flow during early diastole (E-wave velocity) ≥ 1.2 m/s is sensitive for the diagnosis of severe mitral regurgitation and a pattern of filling that predominates in late diastole virtually excludes it. However, an elevated E-wave velocity is not specific for severe mitral regurgitation and depends on other physiologic parameters including left ventricular relaxation and filling pressures. Severe mitral regurgitation on continuous wave Doppler generally has a triangular contour with an early peak velocity denoting a large regurgitant pressure wave and has a density higher than mild mitral regurgitation due to the increased number of red blood cells scattering the ultrasound signal, though these findings are not quantitative and may be influenced by other technical factors such as gain settings and jet direction. Finally, evaluation of blunting or reversal of flow in the pulmonary veins using a pulse wave Doppler sample volume placed 1 cm distal to the ostium of the pulmonary vein, may identify significant mitral regurgitation due to the elevation in left atrial pressures and blunting or reversal of left atrial filling during systole. Additionally, demonstration of flow reversal in multiple pulmonary veins is specific for severe mitral regurgitation and a normal pulmonary vein pattern excludes severe mitral regurgitation. However, regurgitant jet flow directly into the pulmonary vein may alter the ability to discern increased left atrial pressure and systolic blunting may be seen in multiple other etiologies in which left atrial pressure is elevated.

Owing to the difficulty of any single modality for defining significant mitral regurgitation, current guidelines by the American Society of Echocardiography suggest that regurgitant lesions should satisfy 4 or more specific criteria for either mild or severe mitral regurgitation to fall into those respective classification groups (Zoghbi et al., 2017). For cases in which 2–3 criteria are met, assessment of the ERO area is recommended to better define mitral regurgitation grade. When TTE quality is poor or quantitative parameters are indeterminate or discordant with the clinical presentation, either transesophageal echocardiography or cardiovascular magnetic resonance is reasonable to obtain to better evaluate regurgitant severity.

Transesophageal Echocardiography

Transesophageal echocardiography (TEE) may be used to define the presence, severity, and etiology of mitral regurgitation. Additionally, TEE may have particular utility in those with poor transthoracic echocardiographic windows to better define the mechanism of mitral regurgitation and for presurgical planning prior to intervention on the mitral valve. The mitral valve is best visualized at the mid-esophageal level with the ability to identify specific scallops at particular scan angles acquired. Since TEE allows for adjustment of the scan angle of the piezoelectric crystals, the mitral valve and surrounding structures may be interrogated from multiple vantage points, thus providing a comprehensive assessment of valvular and perivalvular pathology. The left pulmonary veins may be identified by turning the transducer laterally and angulating it superiorly from zero degrees. The right pulmonary veins are best identified in the 90 degrees imaging plane turning the tranducer toward the patient's right side. Visualization of the pulmonary veins allows for pulse wave Doppler interrogation of the pulmonary venous flow to evaluate for blunting or systolic reversal. Additionally, the mitral valve may be viewed in short axis through retroflexion of the TEE probe in the transgastric position to assist in identifying the particular scallops involved. The subvalvular structures of the mitral valve are well visualized in the transgastric position at 90 degrees rotation. All of the techniques previously mentioned for evaluation of mitral

regurgitation severity are available with TEE. Moreover, owing to the higher frequency of the TEE probes and the shallower depth of imaging, axial resolution is improved compared with trans-thoracic imaging and thus the anatomy and etiology of mitral regurgitation is better defined. Furthermore, while mitral annular calcification and other abnormalities may cause acoustic shadowing on transthoracic imaging and lead to inaccurate conclusions on the presence or severity of mitral regurgitation, the ability to acquire near-field images of the left atrium with TEE allows for more accurate identification of the presence of mitral regurgitation.

Role of 3D Imaging in Mitral Regurgitation

Another advantage of TEE relative to other imaging techniques is the ability to perform real-time 3D imaging. In 3D imaging, a three-dimensional matrix array of piezoelectric crystals acquires a pyramidal sector that permits evaluation of depth along the scan plane. Full 3D left ventricular volumes may be acquired with TEE using multibeat acquisition and respiratory gating, allowing accurate quantification of the left ventricular chamber sizes and ejection fraction. Full volume acquisition may also be used to define the right ventricular ejection fraction and function, which is suboptimally evaluated using 2D imaging due to the complex geometric assumptions of the right ventricle. Furthermore, 3D "zoom mode" is now routinely used to visualize the mitral valve from the top-down (i.e., "surgeon's" perspective), which may assist in the identification of valvular pathology, affected scallops, and presence/sizing of masses (Fig. 9). This view may be used in preoperative planning to assess the likelihood of successful mitral valve repair in the setting of severe degenerative mitral regurgitation. "Zoom mode" 3D imaging improves temporal resolution at the cost of spatial resolution and sector size, and thus the evaluation of regurgitant jets using 3D color Doppler is currently limited by frame rate. However, further advancements in 3D technology may improve the accuracy of MR quantification in the future. Simultaneous biplane and multi-plane images may be acquired to evaluate a structure of interest in multiple orthogonal planes, assisting with procedural guidance and improving with accurate quantitation. Additionally, 3D imaging, particularly "zoom mode" and "multiplane" imaging, can be used for intraoperative and intraprocedural guidance of mitral valve interventions. Intraoperative TEE can be used to verify the preoperative diagnosis, guide placement of cardioplegia and bypass cannulae as well as pulmonary artery catheters, and to assess for immediate complications of the procedure or any residual mitral regurgitation after valve repair or replacement. With the advent of the edge-to-edge percutaneous mitral valve repair technique (e.g., MitraClip) and the development of transcatheter mitral valve replacement, use of TEE and especially 3D TEE are likely to play a larger role in the management and outcomes of patients undergoing structural procedures on the mitral valve. As with transthoracic and 2D imaging, 3D TEE requires adequate imaging windows and lack of acoustic shadowing to optimally visualize structures of interest and thus must be interpreted within the context of the imaging study.

Conclusions

With the aging of the population, rates of significant valvular heart disease including mitral regurgitation are likely to increase. Noninvasive cardiac imaging, particularly echocardiography, provides an essential tool for clinicians to diagnose and manage patients with mitral regurgitation. The role of echocardiography is likely to expand even further with improvements in 3D and strain imaging as well as the increasing role of structural interventions in treating mitral regurgitation.

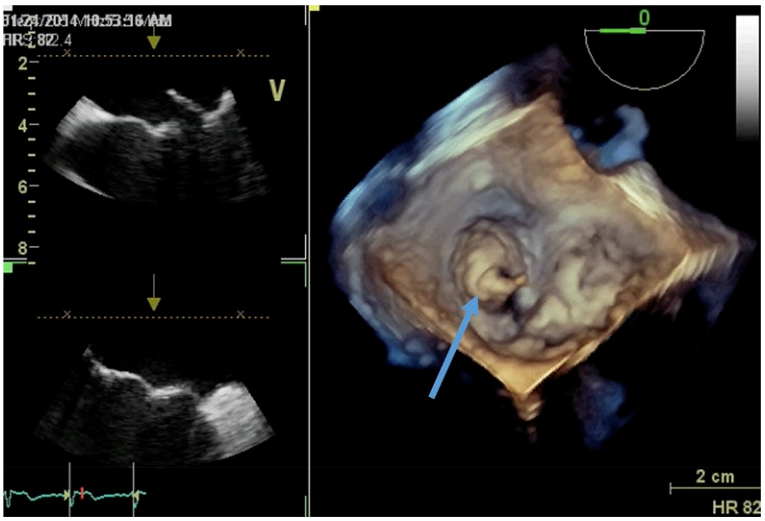

Fig. 9 Three-dimensional echocardiography of mitral valve. This transesophageal image demonstrates biplane (*left*) and live 3D volume rendered images of the mitral valve in a patient with mitral valve prolapse and a flail P3 scallop (*arrow*). The patient was found to have severe mitral regurgitation.

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Imaging: CT Scanning of the Heart and Great Vessels

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Introduction

Cardiovascular disease is the primary cause of overall mortality and morbidity in the western population (Nabel and Braunwald, 2012; Fuster and Mearns, 2009; Heidenreich et al., 2011). As such, there is a constant effort to establish biomarkers and develop tools in order to improve disease prevention and management.

Owing to new technological advancements in computed tomography (CT) technology leading to the introduction of multi-detector row computed tomography (MDCT) and improved temporal and spatial resolution, imaging of the beating heart became feasible in clinical practice. As a result, coronary CT angiography (CCTA) and MDCT of the aorta became the noninvasive modalities of choice for the assessment of cardiovascular disease (De Cecco et al., 2014a; Agarwal et al., 2009; Wadgaonkar et al., 2015; Shaw et al., 2012).

However, CCTA is primarily a tool for the anatomical assessment of coronary artery disease (CAD), rather than functional consequences of the disease. CT technology is improving and it is now possible to assess myocardial perfusion using technologies such as static single-energy CT myocardial perfusion imaging (CTMPI) as well as the hemodynamic significance of a coronary artery stenosis using technologies such as CCTA-derived fractional flow reserve (CT-FFR) and transluminal attenuation gradient (TAG). As a result of these advancements, CCTA could transition from a robust tool for the morphological assessment of CAD into a comprehensive modality providing additional functional information and thus better guiding patient management.

This article will present an overview of the current state-of-the-art cardiovascular CT techniques and applications and a discussion of new technology and future possibilities in cardiovascular CT imaging.

CT of the Heart

Technical Considerations

Spatial and temporal resolution

CT technology requires a very high level of spatial resolution in order to visualize the coronary arteries, which measure only a few millimeters in diameter, and even smaller features, such as plaque and stenosis. Since spatial resolution depends on both the number of detectors and the slice width, 64 or higher slice scanners using collimated slice width of 0.5, 0.6, or 0.625 mm were used to achieve appropriate isotropic resolution to visualize the coronary arteries. In routine clinical practice, the resolution for cardiac imaging is $0.5 \times 0.5 \times 0.5$ mm^3, which allows for smooth, low-noise images adequate enough to evaluate the heart's anatomy, bypass grafts, and coronary arteries. However, this spatial resolution is still not optimal for coronary artery assessment in the context of heavy calcifications where calcium blooming prevents a reliable assessment of vessel lumen due to the overestimation of stenosis (Vanhecke et al., 2011). Another challenge associated with this resolution is stent patency and in-stent restenosis assessment (Maintz et al., 2009), which seems to improve with the use of third-generation dual-source scanners (Gassenmaier et al., 2014; Mangold et al., 2016a) and iterative reconstruction (Geyer et al., 2015).

Further improvement in spatial resolution is crucial, but a decrease in image noise and an increase in image quality usually go hand in hand with higher radiation dose levels. However, this problem might be solved with the use of novel reconstruction algorithms, such as iterative reconstruction, which have been shown to substantially increase image sharpness and counteract a noise increase while reducing radiation dose (Mangold et al., 2016b).

Because the heart is in constant motion, a high temporal resolution (i.e. acquisition time per image) is needed in order to achieve high image quality; the faster the scan time, the better the visualization of cardiac and coronary anatomy. Modern 64- and 128-slice CT scanners can achieve gantry rotation times as low as 0.27 s resulting in temporal resolution as low as 135 milliseconds. This is sufficient to attain high image quality in patients with a regular heartbeat and normal to low heart rate. It becomes more challenging, however, when patients have elevated heart rates or sustained arrhythmia (Mangold et al., 2016b). Algorithmic approaches to correct motion artifacts have also been proposed (Apfaltrer et al., 2013; Fuchs et al., 2014).

Nevertheless, a temporal resolution of 50 milliseconds is required for optimal heart scans with high image quality, particularly in patients with irregular heart rates (Leschka et al., 2006). The introduction of the dual-source CT (DSCT) system featuring two X-ray tubes coupled with two detectors positioned at a 90-degree angle has led to great improvements in temporal resolution (Flohr et al., 2006). As a result, gantry rotation times were reduced to 83, 75, and 66 milliseconds with first-, second-, and third-generation DSCT systems, respectively (McCollough et al., 2008). Several clinical studies have, in fact, shown that reliable CCTA could be achieved using DSCT, even without heart rate control (Scheffel et al., 2006; Matt et al., 2007; Ropers et al., 2007; Weustink et al., 2009; Li et al., 2014; Yang et al., 2015; Nance et al., 2011). While most recent DSCT scanners have 192 slices, other vendors pursue the strategy of enhancing temporal resolution by building CT hardware with up to 640 slices. Thus, full cardiac coverage can be achieved within one heartbeat.

Electrocardiogram (ECG)-gating

An important consideration in heart imaging is phase-consistent acquisitions in order to capture the heart in both the systolic and diastolic phase. In order to achieve that goal, ECG-gated acquisitions must be performed. There are two techniques for ECG- gating: retrospective and prospective gating.

Generally, retrospective gating has been the more popular technique. Using this technique, the heart is continuously scanned throughout the entire cardiac cycle while the scanner table is moving and the patient's ECG is simultaneously recorded. Once the acquisition is completed, the data is analyzed retrospectively using the patient's ECG as a guide to select the data within the corresponding heart phase. This data is then used for phase-consistent image reconstructions of the heart. The main advantages of this technique are the ability to retrospectively perform reconstruction in any cardiac phase, minimizing imaging artifacts, and the ability to evaluate cardiac function given the multiphase acquisition. The main drawback remains the associated high radiation dose due to continuous irradiation during the cardiac cycle.

With the advent of newer CT systems, prospective ECG-gating has been proposed as an alternative to retrospective gating. With this technique, the patient's ECG is used to trigger the start of the acquisition, without table movement, at a user-determined distance from an R wave. Only the region of the heart aligned with the detectors will be scanned. Once the acquisition is completed, the scanner moves the table to the next position in order to scan the next part of the heart, and the process repeats. Because the acquisition only occurs in the selected phase, as opposed to continuously throughout the cardiac cycle, radiation dose is minimized (Stolzmann et al., 2008; Earls et al., 2008; Arnoldi et al., 2009). Consequently, functional evaluation and changing the reconstruction phase in order to reduce artifacts are not possible with this technique. Moreover, acquisition becomes more challenging in patients with irregular rhythms or elevated heart rates.

Radiation dose

Radiation dose in CT is an important source of concern for the medical community and the general public (Brenner and Hall, 2007; Einstein et al., 2007). A multicenter study demonstrated that radiation dose from cardiac CT varied from 5 to 30 mSv with an average dose of 12 mSv (Hausleiter et al., 2009). With the increased use of cardiac imaging, there have been efforts to establish radiation dose reduction techniques.

While there are many methods to reduce radiation, the choice of ECG-gating method is most important in determining radiation dose reduction. As mentioned above, prospective ECG-gating only requires irradiation during a specific interval of the cardiac cycle; thus, radiation dose can be reduced to 1–4 mSv using this technique (Ohnesorge et al., 2000). In a meta-analysis of 20 studies comparing prospective and retrospective ECG-triggering, the pooled effective dose was 3.5 mSv with prospectively triggered scans compared to 12 mSv using retrospective scanning (Menke et al., 2013). These results were confirmed by the PROTECTION III trial in which a radiation level of 3.5 mSv was achieved with prospective scanning as opposed to 11.2 mSv with retrospective ECG-gating. Consequently, prospective ECG-triggering is preferred in the reduction of radiation dose, particularly in patients with low, steady heart rates. Studies also demonstrated the ability to reach radiation doses lower than 1 mSv using ECG-triggered high-pitch scanning with DSCT (Neefjes et al., 2011; Yin et al., 2013). Again, however, this technique is particularly useful in patients with low to moderate, regular heartbeats.

Another approach to reducing radiation is low kV imaging. Because iodine contrast is increased at low kV levels, it is possible to maintain a good contrast-to-noise ratio (CNR) while lowering radiation dose. Studies have shown the ability to reduce radiation dose with 100 and 80 kV scanning protocols while maintaining image quality (Hausleiter et al., 2010; Cao et al., 2014). These techniques are particularly useful in small- to moderate-sized patients, as low kV scanning is associated with high noise levels in obese patients. Novel scanning systems are capable of providing diagnostic scans with reduced tube voltages (70 and 80 kV), resulting in a reduction of radiation dose by 49%–68% without compromising image quality, even in obese patients. In a recent study, the combination of high pitch with low kV scanning was able to reduce radiation dose to as low as 0.44 mSv (Meyer et al., 2014).

Radiation dose can be further decreased by using iterative reconstruction. With iterative reconstruction, a correction loop is introduced to the reconstruction process resulting in improved resolution in high contrast regions and noise reduction in low contrast areas, which are prerequisites for radiation dose reduction (Thibault et al., 2007). This becomes particularly useful when combined with other dose reduction techniques to achieve considerable dose reductions (Yin et al., 2013; Stehli et al., 2014; Layritz et al., 2014; Chen et al., 2013). In one study, the combination of low kV, high pitch scanning with iterative reconstruction resulted in a radiation dose as low as 0.06 mSv (Schuhbaeck et al., 2013). In addition to achieving lower radiation dose levels, the use of iterative reconstruction was found to improve image quality and diagnostic accuracy (Spears et al., 2014).

Calcium Scoring

Background

Risk prediction for CAD is based on the Framingham Risk Score (FRS), which takes into account classic cardiovascular risk factors such as age, sex, hypertension, hyperlipidemia, diabetes mellitus, and tobacco use (Wilson et al., 1998). Although FRS was found to be accurate (Wilson et al., 1998), the tool did not account for some CAD risk factors (Greenland et al., 2010). Consequently, new techniques to better stratify risk were sought out, particularly for patients in the intermediate (10%–20% 10-year risk) and low risk groups.

By improving image quality and reducing motion artifacts, MDCT made it possible to capture high quality images of the beating heart and coronary arteries in fine detail, including small calcium deposits in the artery walls. Because calcium deposits are readily detectable by X-ray-based imaging and are a direct sign of subclinical atherosclerotic disease (Rumberger et al., 1995), this tool quickly became of interest to clinicians as a new means of predicting CAD risk.

Technique

Coronary Artery Calcium Scoring (CACS) is a noncontrast CT study usually performed on a MDCT scanner. Calcium deposits in coronary artery walls can then be visualized (**Fig. 1**). The overall calcium burden is calculated and a calcium score called the Agatston score is generated based on the Agatston formula (Agatston et al., 1990). A growing body of literature has found this score to be an independent predictor of CAD and major adverse cardiac events (MACE) (Kondos et al., 2003), allowing clinicians to better stratify patients into appropriate risk groups based on their scores, (**Tables 1** and **2**) and thus better guide management.

Clinical relevance

Several studies have established the effectiveness of CACS in predicting the risk of future cardiac events, particularly in asymptomatic patients with intermediate risk based on FRS (Detrano et al., 2008; Greenland et al., 2004; van Kempen et al., 2011; Vogler et al., 2013; Becker et al., 2005). As a result, CACS was incorporated into the guidelines for risk assessment of asymptomatic patients with an intermediate 10-year risk (based on the FRS), effectively improving risk stratification and helping guide patient management (Greenland et al., 2007; Taylor et al., 2010). A CAC score of 0 was also associated with a very low risk (<1%) of cardiac event in patients presenting with atypical chest pain (Haberl et al., 2001; Georgiou et al., 2001). As a result, CACS was proposed as a tool to exclude significant stenosis in low-risk patients presenting with atypical chest pain (Oudkerk et al., 2008).

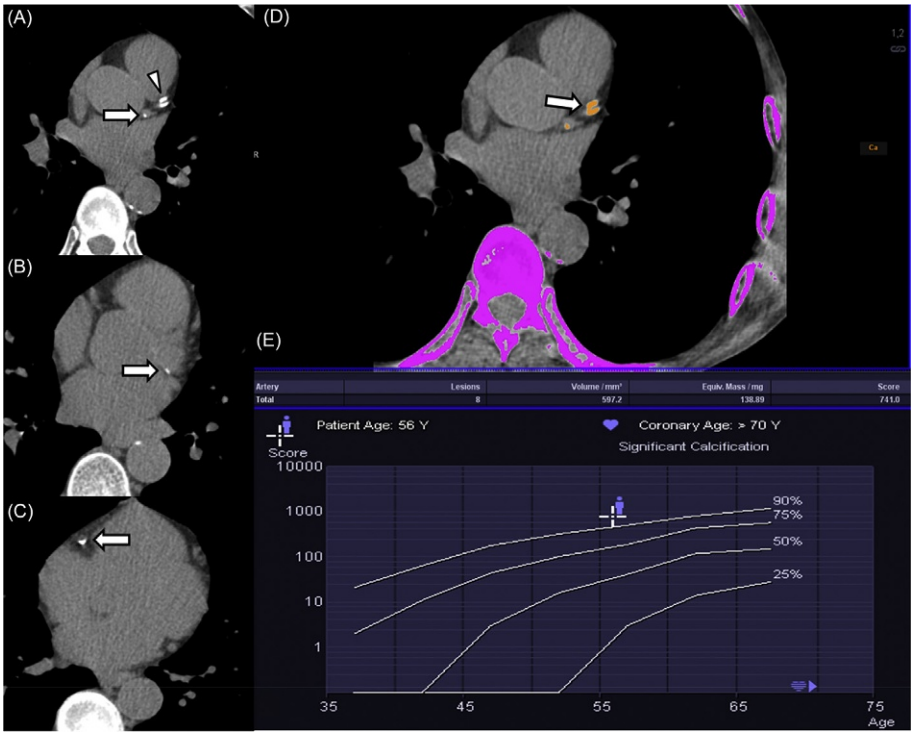

Fig. 1 A 59-year-old male with dyslipidemia and hypertension received a Coronary Artery Calcium Scoring (CACS) scan. (A) Axial noncontrast image of the heart showing a calcified plaque in the left main (*arrow*) and left anterior descending (*arrowhead*) coronary arteries. (B) Axial noncontrast image of the heart showing a small calcified plaque of the circumflex coronary artery (*arrow*). (C) Axial noncontrast image of the heart showing a calcified plaque of the right coronary artery (*arrow*). (D) The Agatston method was used to calculate a calcium score of 741. (E) Age-specific percentile curves show that the patient's CACS is in the 95th percentile for his age group.

Table 1 Relative risk ratio of coronary heart disease (CHD) based on calcium score

Calcium score	*Relative risk ratio*
1–112	1.9
100–400	4.3
400–1000	7.2
>1000	10.8

Table 2 Annual coronary heart disease (CHD) and myocardial infarction (MI) rate based on calcium score

Calcium score	*Annual CHD/MI rate*
0	0.40%
300–399	2.80%
400–1000	4.60%
>1000	7.10%

Limitations

The most important concern regarding CACS scanning is radiation exposure. Studies show variable radiation exposure from CACS scans ranging from 0.8 to 10.5 mSv with a mean dose of 3.1 mSv (Kim et al., 2009). While these values are relatively low, they still raise questions of a possible increased risk of radiation-induced cancer. While there is no direct evidence of radiation-induced cancer, there is still controversy regarding this topic (Huda et al., 2010). One study estimated an increased risk of radiation-induced cancer, particularly breast and lung cancer, as well as leukemia; estimated risk was higher for women and decreased with increasing age at screening (Kim et al., 2009). However, risk of death related to radiation-induced cancer is still relatively low compared to everyday risks and should not prevent patients from receiving necessary tests, especially since heart disease remains the primary cause of death overall (Meinel et al., 2014a). In addition, as CT technology continues to improve, radiation dose is further reduced

(Marwan et al., 2013; McQuiston et al., 2016). Innovative techniques, such as tin prefiltration of the X-ray beam at 100 kV instead of the standard 120 kV, have been demonstrated as effective in reducing radiation dose to the submillisievert level for CACS (Tesche et al., 2017).

Coronary CT Angiography

Background

CAD and its complications, such as MI, heart failure and stroke, are the leading cause of mortality and morbidity worldwide (Go et al., 2013). There are continual efforts to improve the management of this disease and develop effective preventive strategies to decrease its prevalence in the general population and avoid its potentially fatal complications.

Over the past decades, invasive coronary angiography (ICA) has been the gold standard for visualization of the coronary arteries. However, this is an invasive procedure and can result in complications, albeit rarely (Zanzonico et al., 2006). Most patients receive ICA for diagnostic rather than therapeutic purposes (Maier et al., 2006). MDCT, owing to its higher spatial and temporal resolution, can capture images of the beating heart. The higher temporal resolution compensates for the heartbeat and respiratory motion while higher spatial resolution makes way for higher quality acquisitions. As a result, MDCT was quickly considered a possible alternative to ICA for noninvasive CAD diagnosis (De Cecco et al., 2014a; Achenbach and Daniel, 2001).

Technique

Capturing the heart with the fewest possible motion artifacts and highest possible image quality was not possible until the introduction of 64-slice MDCT systems, which are now a prerequisite for CCTA in clinical practice (Leschka et al., 2006).

CCTA is an ECG-gated, contrast-enhanced scan allowing the visualization of the coronary artery lumen and arterial wall. This tool has many clinical applications, the most important of which are myocardial ischemia and infarction detection, stenosis visualization, and atherosclerotic plaque detection and characterization (**Figs. 2–4**). Other applications are also now feasible: preinterventional planning for cardiac surgery or interventional cardiology, stent patency evaluation, heart failure evaluation, follow-up after heart transplant and coronary artery bypass graft surgery, and imaging of congenital heart disease in pediatric patients (Baumann et al., 2015a; Meinel et al., 2015).

Clinical relevance

Several studies comparing CCTA to gold standard ICA have shown the former's reliability and accuracy in detecting significant coronary artery stenosis (>50% narrowing of the vessel lumen) (Li et al., 2013, 2014; Menke et al., 2013; von Ballmoos et al., 2011; Salavati et al., 2012). The results for sensitivity and specificity ranged from 93% to 99% and 86% to 89.6%, respectively, translating to a negative predictive value (NPV) of 95%–100%. Furthermore, CCTA excludes CAD with a high degree of certainty.

Moreover, studies have already demonstrated the short-term prognostic value of this technique; a meta-analysis demonstrated that the presence of significant stenosis on CCTA was associated with a 10-fold increased risk of MACE, whereas any CAD increased

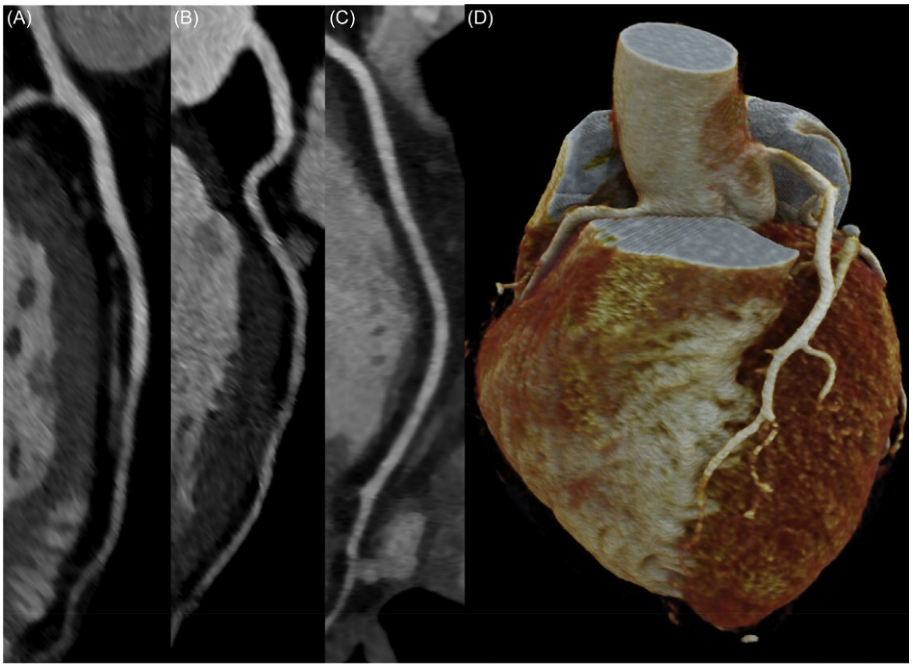

Fig. 2 Coronary CT angiography in a 48-year-old male presenting with chest pain. Curved multiplanar views of the (A) left anterior descending, (B) circumflex, and (C) right coronary artery. (D) Cinematic Rendering (Siemens Healthineers, not intended for clinical use) reconstruction of the heart.

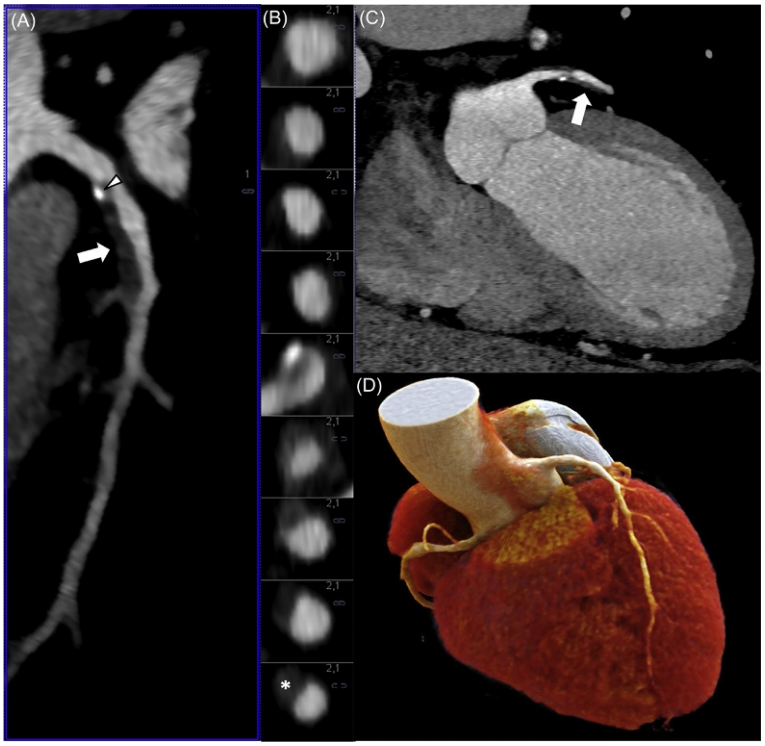

Fig. 3 A 65-year-old male with hypertension, diabetes mellitus, and a history of smoking presenting with exertional chest pain. A triple rule-out study was performed. (A) Curved multiplanar reconstruction of the left anterior descending (LAD) coronary artery shows a mixed calcified (*arrowhead*) and noncalcified (*arrow*) plaque. (B) Successive cross-sectional images of the LAD show the plaque causing moderate stenosis of the lumen (*asterisk*). (C) Axial image of the heart displaying the mixed atherosclerotic plaque (*arrow*). (D) Three-dimensional reconstruction of the heart with the new Cinematic Rendering algorithm.

the risk of MACE 4.5-fold (Bamberg et al., 2011a). More recently, long-term studies have shown an increased risk of MACE when CAD is detected on CCTA. In a cohort of 1584 patients followed for a median of 5.6 years, Hadamitzky et al. showed a 1.1% risk of MACE with obstructive CAD (Hadamitzky et al., 2013). Another study following 218 patients for a median of 6.9 years reported annual MACE rates of 2.7% and 6.0% for nonobstructive and obstructive CAD, respectively (Dougoud et al., 2014). Based on this evidence, CCTA has become an appropriate initial test for the evaluation of patients with stable angina and an intermediate to high risk of CAD, particularly in the setting of equivocal ECG and blood test results (Wolk et al., 2014).

Based on several clinical trials proving that CCTA can safely exclude CAD in the acute chest pain setting (Litt et al., 2012; Hoffmann et al., 2012), this modality is an important test in the workup of patients with chest pain in the emergency setting, particularly if they have low to intermediate risk of CAD, as it allows for quick and safe triage of patients (Taylor et al., 2010). Furthermore, triple rule-out studies, which consist of ECG-gated contrast-enhanced scans of the coronary arteries, pulmonary arteries, and aorta are now commonly performed in the acute chest pain setting to look for three common and potentially fatal causes of chest pain (CAD, pulmonary embolism, and aortic dissection) in one comprehensive test (White et al., 2005).

Left ventricular function assessment is a crucial part in managing patients with CAD (White et al., 1987). Some studies have demonstrated the superiority of CT over echocardiography, scintigraphy, and left ventriculography for this assessment (Yamamuro et al., 2005; Dewey et al., 2006; Sugeng et al., 2006). Furthermore, it has been shown that most novel CT scanners have approached the accuracy of cardiac MR (CMR) in assessing left ventricular function (Busch et al., 2008; van der Vleuten et al., 2009; Bastarrika et al., 2008a). However, echocardiography and CMR remain more popular for the primary assessment of cardiac function (Busch et al., 2008; van der Vleuten et al., 2009; Bastarrika et al., 2008b). Left ventricle function analysis with CT is currently performed in clinical practice when a patient is scanned with the retrospective ECG-gating technique since, as mentioned earlier, this technique contains data acquired throughout the cardiac cycle, which can be reconstructed to derive global parameters of ventricular function (**Fig. 5**). Moreover, multiphasic CT acquisitions have also been shown to detect wall motion abnormalities, an important consequence of CAD (Dirksen et al., 2005; Fischbach et al., 2007; Wu et al., 2008).

CCTA is now considered an important tool in the evaluation and management of patients with ischemic heart disease (De Cecco et al., 2014a). However, there remains controversy over the appropriate use of this technique; specific indications and guidelines are still being developed.

Limitations

It is necessary to acknowledge the minor concerns that are considered risks associated with CCTA: contraindications for iodinated contrast agent in cases of known allergy, hyperthyroidism, or renal impairment, and the inherent burden of ionizing radiation from CT. Effective dose values vary greatly depending on the acquisition technique. An average of 12 mSv has been reported for



Fig. 4 A 58-year-old male presenting with chest pain; he had an occluded left anterior descending artery. A stent was placed and a post-stent coronary computed tomography angiography (CCTA) was performed. (A) Axial section of the heart showing an area of hypodensity (*arrow*) in the apex consistent with myocardial infarction. (B) Sagittal section of the heart displaying the infarct (*arrowhead*) as well as myocardial thinning (*arrow*). (C) Multiplanar reconstruction of the left anterior descending artery showing the patent stent (*arrow*) and a noncalcified plaque (*arrowhead*).

retrospective ECG-gating, whereas the average falls to 3.5 mSv with prospective gating (De Cecco et al., 2014a; Menke et al., 2013). Novel scanning techniques such as dual-energy high-pitch acquisitions have reduced radiation doses to 1.5 mSv for CCTA (Yin et al., 2013), and a recent study managed to reduce radiation dose by 90% using a non-ECG-gated high pitch acquisition CT angiography (CTA) of the whole aorta (Zhang et al., 2016).

While techniques to further reduce radiation are continually investigated, it is worth mentioning that data regarding radiation-induced cancer is scarce (Meinel et al., 2014a). It is estimated that for a radiation dose of 10 mSv, the lifetime risk of developing a fatal cancer due to radiation is 1 in 2000, with an increased risk in younger, healthier patients and women, and a lower risk in older patients and men (Meinel et al., 2014a). Additionally, image quality of CCTA can be greatly hampered by artifacts, reducing diagnostic accuracy.

CTA of the Great Vessels

Carotid arteries

Atherosclerosis is a systemic inflammatory and multivessel disease (Libby et al., 2011). Consequently, assessment of the carotid arteries for the presence of atherosclerotic plaques beyond the coronary arteries is critical. Indeed, carotid artery atherosclerosis is a major cause of cerebrovascular events, which are themselves a major cause of morbidity and mortality (Barnett et al., 2000), highlighting the importance of evaluation of the carotids for efficient disease prevention.

Following the improved image resolution and faster acquisition times, CTA became a robust and reliable test for imaging of the carotid arteries (**Fig. 6**). Using this technique, Koelemay et al. found a sensitivity and specificity for carotid stenosis of 97% and 99%, respectively (Koelemay et al., 2004). Additionally, some studies reported CTA as accurate in detecting signs of plaque vulnerability such as ulcerations (Saba et al., 2007), large lipid cores, and plaque hemorrhage (Wintermark et al., 2008).

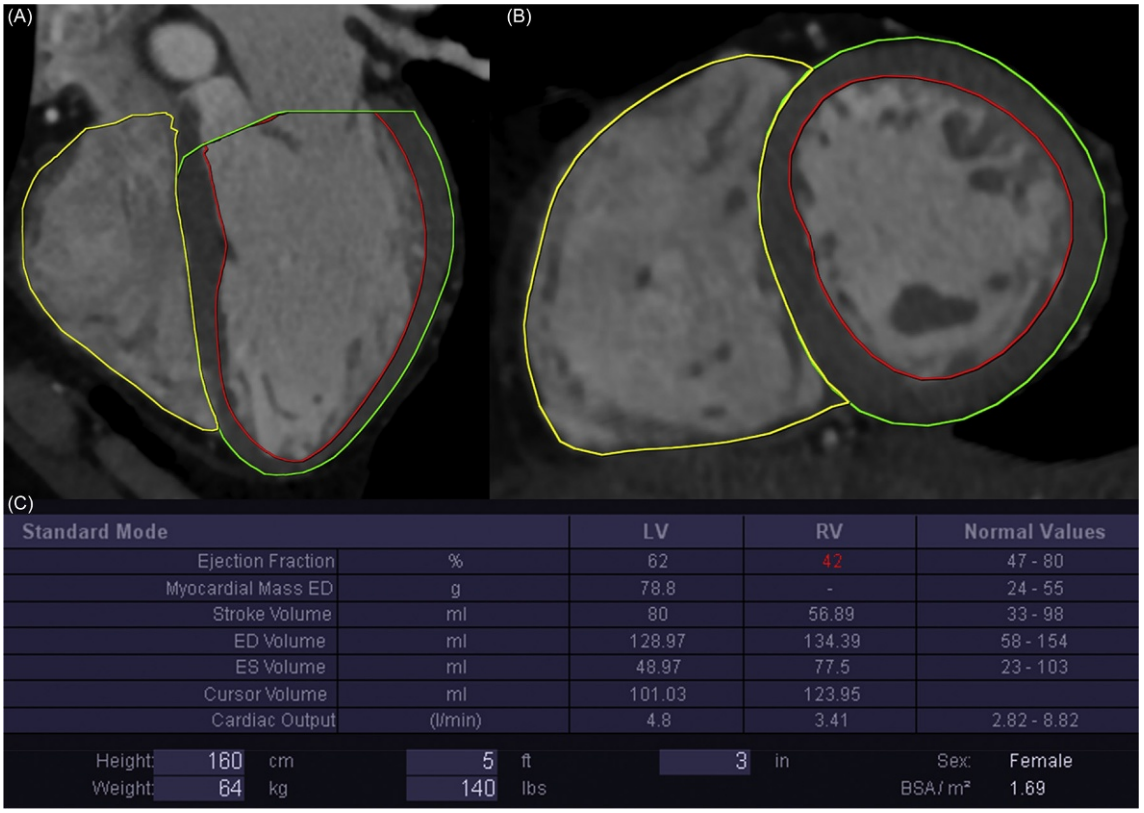

Fig. 5 A 65-year-old female presenting with chest pain and shortness of breath. (A) Two-chamber and (B) short-axis views of the heart displaying the segmentation of the right and left ventricles. (C) A table showing quantitative values of heart function.

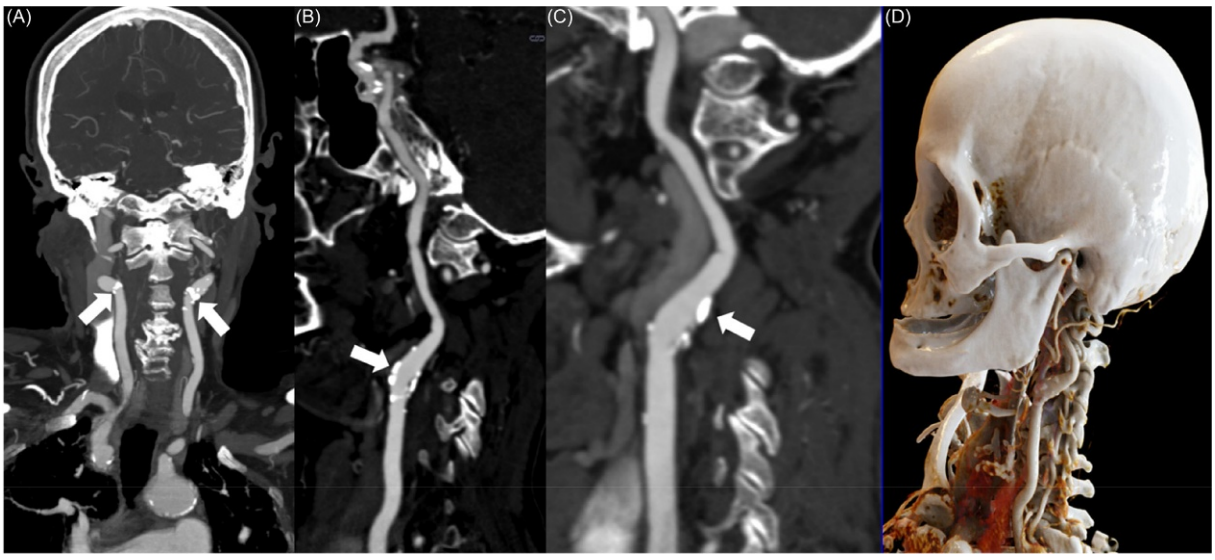

Fig. 6 CT angiography of the carotids in a 75-year-old male presenting with vertigo and slurred speech. (A) Coronal view of the carotid arteries showing calcifications of the right and left carotids (*arrows*). Curved multiplanar view of the (B) right and (C) left carotid arteries, which show calcifications (*arrows*). (D) Cinematic Rendering reconstruction of the head and neck vessels displaying the three-dimensional anatomy of the neck vessels.

Aorta

The ability to scan the whole aorta and branches from the carotid to the iliac arteries in a single breath hold has made CTA the better tool to evaluate the aorta and its runoffs, replacing invasive angiography as the gold standard (Prokop, 2000; Hayter et al., 2006).

CTA is very accurate in detecting many aortic anomalies such as aneurysms (Hartnell, 2001) and dissections (Shiga et al., 2006), which are important causes of morbidity and mortality (Figs. 7 and 8). Atherosclerosis and calcifications, which were found to be

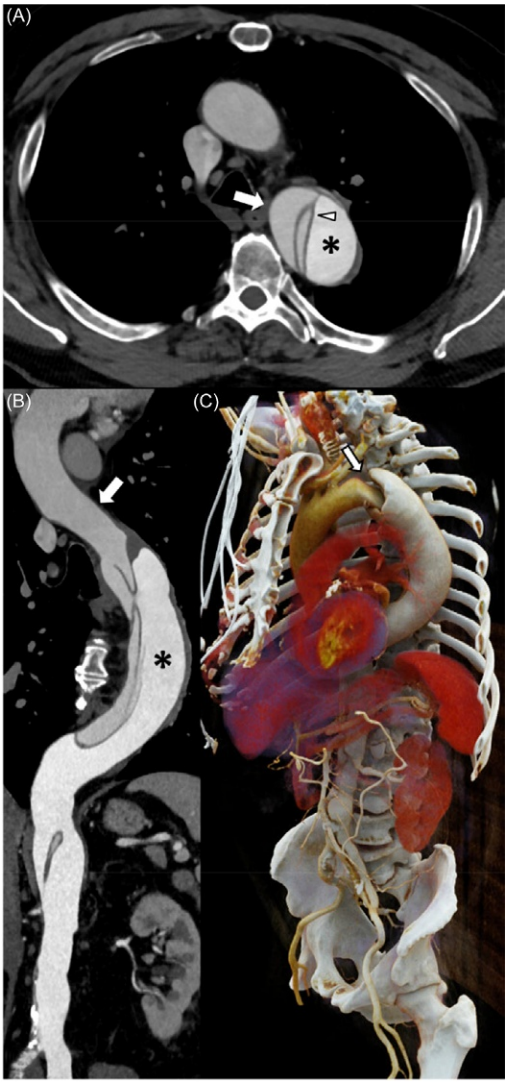

Fig. 7 CT angiography of the aorta in a 70-year-old male presenting with chest and back pain. (A) Axial view of the aorta showing an aortic dissection with the creation of a dissection flap (*arrowhead*), a false lumen (*arrow*), and a true lumen (*asterisk*). (B) Curved multiplanar reconstruction of the aorta displaying the false lumen (*arrow*) and the true lumen (*asterisk*). (C) Cinematic Rendering reconstruction of the aorta displaying the three-dimensional anatomy of the dissection (*arrow*).

predictors of stroke and CAD (Song et al., 2016; Amarenco et al., 1994; Rohani et al., 2005), stenosis, congenital anomalies, and wall thickening, as seen in inflammatory diseases, are also visualized by CTA (**Fig. 9**). Furthermore, CTA is used to assess posttreatment progression and complications such as restenosis, endoleak, and thrombosis (**Fig. 10**).

Future Directions

Plaque characterization

In the natural evolution of atherosclerosis, plaques can become vulnerable, that is more prone to rupture, when they have a thin fibrous cap and a large lipid-rich core (Little, 1990; Renker et al., 2015; Kolodgie et al., 2004). It has been shown that the compositional characteristics of atherosclerotic plaques can be more predictive of MACE than the degree of stenosis (Bittencourt et al., 2014). As a result, plaque composition analysis has become an increasingly important aspect of better risk stratification in cardiac imaging. The introduction of 64-slice scanners has allowed accurate plaque characterization; plaques are usually classified as calcified (density higher than 400 Hounsfield units (HU)) or noncalcified (density between 0 and 150 HU) (Rasouli et al., 2006). Noncalcified plaques can be further subcategorized into fibrous and lipid-rich plaques with a density of less than 60 HU (Nance et al., 2012; Becker et al., 2003). Several studies have shown the accuracy of CCTA in detecting plaque components with invasive vascular ultrasound (IVUS) as the reference standard (Voros et al., 2011; Gao et al., 2011). Particularly, Obaid et al. found that there was favorable diagnostic accuracy in the detection of calcified, necrotic, and fibro-lipidic components using plaque maps derived from plaque attenuation (Obaid et al., 2013) (**Fig. 11**).

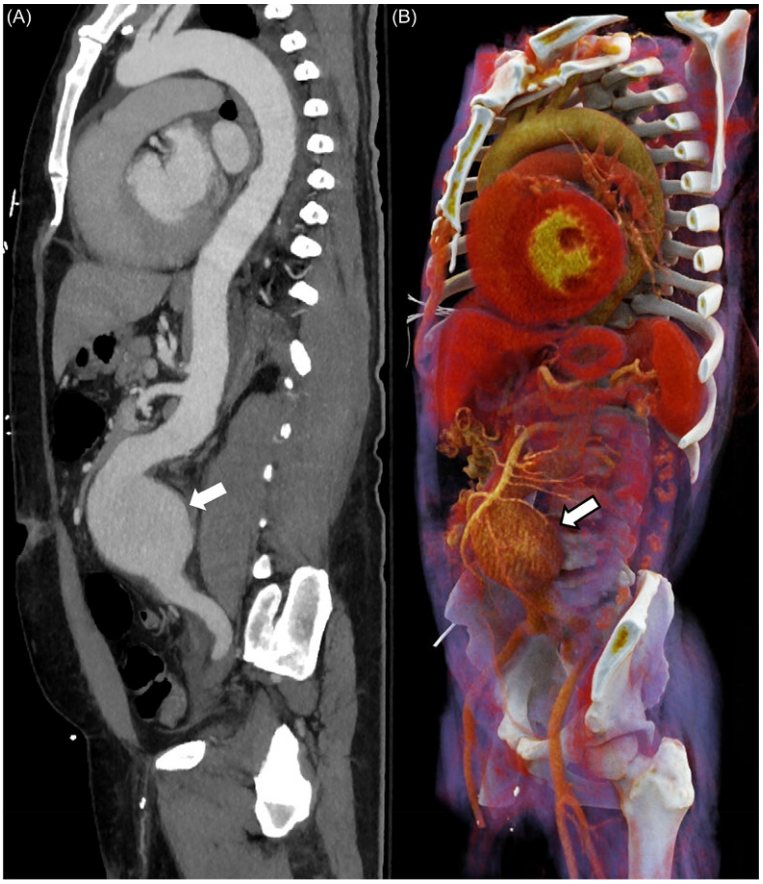

Fig. 8 CT angiography in a 62-year-old male presenting with abdominal pain. (A) Sagittal and (B) Cinematic Rendering reconstructions of the aorta showing a massive infrarenal aneurysm (*arrow*).

Novel CT systems have the potential to further characterize plaque composition (Ohnesorge et al., 2005). Several vulnerable plaque features can be detected by CCTA, most notably the napkin-ring sign. The napkin-ring sign is identified as a region of low attenuation surrounded by a ring of high attenuation, which is thought to represent a necrotic core surrounded by a fibrous cap (Maurovich-Horvat et al., 2010) (**Fig. 12**). CCTA also detects other important signs of vulnerable plaque: spotty calcifications (⊠3 mm calcifications surrounded by noncalcified plaque) (van Velzen et al., 2011), low-attenuation plaque (density = 30–60 HU), and positive remodeling, defined as an enlargement of both the vessel lumen and arterial wall seen in early atherosclerosis. These features were detected by CCTA with good correlation when compared to IVUS ($r = 0.94$, $P < 0.001$), as reported by Nakazato et al. (Nakazato et al., 2013). Moreover, these signs were independently associated with cardiac events (Puchner et al., 2014). Specifically, spotty calcifications detected on CCTA were associated with plaque progression and culprit lesions (Kataoka et al., 2012). Low-density plaques, positive remodeling, and spotty calcifications were all signs present in culprit lesions when compared with nonculprit lesions (Benedek et al., 2013). Additionally, Otsuka et al. found that the presence of the napkin-ring sign on CCTA had a high predictive value for MACE that was independent of other risk factors (Otsuka et al., 2013). More recently, Tesche et al. found that CCTA-derived markers such as the remodeling index and the napkin-ring sign were predictive of MACE, achieving the highest predictive values when combined with FRS (Tesche et al., 2016a). However, limited spatial and temporal resolution poses a challenge to identifying thin-capped fibroatheromas and lipid-rich necrotic cores, both important markers of plaque vulnerability, which could potentially cause high-risk lesions to go unnoticed (Obaid et al., 2013). Concomitantly to software and hardware technology improvements, plaque characterization approaches are evolving, which could play a more important role in stratifying patients and lead to better treatment management.

Dual-energy CT (DECT)

In DECT image acquisition, patients are scanned at two different kilovoltage (kV) levels: usually a low level of 80–90 kV and a high level of 140–150 kV (Ruzsics et al., 2008). The underlying consideration is that different materials show specific attenuation characteristics depending on the applied tube voltage. Whereas traditional single-energy CT depends only on the measured density (HU) of materials that are penetrated by the CT X-ray beam, DECT allows for the identification and differentiation of tissues and molecules such as calcium, iodine, and uric acid. Depending on vendor-specific systems, this can be achieved either with a dual-source dual-detector setup (Kang et al., 2010) or with a single-source rapidly switching between low and high kV (Matsumoto et al., 2011). While there remain concerns about the radiation dose associated with CCTA, DECT allows for the subtraction of iodine from



Fig. 9 CT angiography in a 26-year-old female presenting with joint pain and fever. (A, B) Axial and (C) curved multiplanar reconstructions of the aorta showing thickening of the aortic wall (*arrows* and *arrowhead*) consistent with Takayasu's disease.

contrast-enhanced images, resulting in so-called virtual noncontrast images (**Fig. 13**). To date, these reconstructions cannot fully replace true unenhanced images because of low image quality (De Cecco et al., 2013); although it has been shown that they have comparable diagnostic accuracy in clinical scenarios such as active bleeding detection (Sun et al., 2015). Despite the relatively low image quality, these reconstructions hold great promise as an ancillary diagnostic image set, reducing radiation dose by almost 50% (Sun et al., 2013). Due to its ability for material decomposition, DECT could also somewhat improve plaque characterization even though preliminary results have been inconsistent (Barreto et al., 2008; Tanami et al., 2010; Henzler et al., 2011). Another promising DECT approach in angiography consists of the reconstruction of the so-called monoenergetic images (Albrecht et al., 2016a,b), sometimes referred to as monochromatic images (**Fig. 14**). Since image noise usually increases with lower kV levels, it is not possible to take full advantage of the increased contrast in the image. Monoenergetic DECT reconstructions combine the benefits of the simultaneous datasets: the high contrast of the low energy series with the low noise of high energy series (Grant et al., 2014). Thus, various authors demonstrated tremendous increases in quantitative image quality indices such as CNR and signal to noise ratio (SNR) in virtual monoenergetic CTA (Albrecht et al., 2016a,b; Beeres et al., 2016). Notably, the highest objective image quality of these monoenergetic images at 40 keV did not coincide with the highest qualitative, sometimes referred to as "subjective," image quality at keV levels ranging from 50 to 70 keV (Albrecht et al., 2016a,b; Beeres et al., 2016). Several authors suggested that virtual monoenergetic imaging could allow for distinct reductions in contrast media volume (Chung et al., 2015; Clark et al., 2015). More importantly, DECT has the ability to assess myocardial perfusion (Vliegenthart et al., 2012a), an important element in CAD risk assessment as discussed later in the review.

CT-myocardial perfusion

While CCTA has been accepted as a modality to assess CAD in patients with intermediate risk (Taylor et al., 2010), it is mainly used as a tool for anatomical and morphological assessments as it is not accurate in predicting ischemia (Stolzmann et al., 2010; Mouden and Ottervanger, 2009). However, determining which lesions reduce myocardial perfusion is important for a comprehensive anatomical and functional evaluation of CAD with CCTA and helps to better guide coronary intervention. CTMPI allows for the assessment of functional and morphological parameters in a single scan.

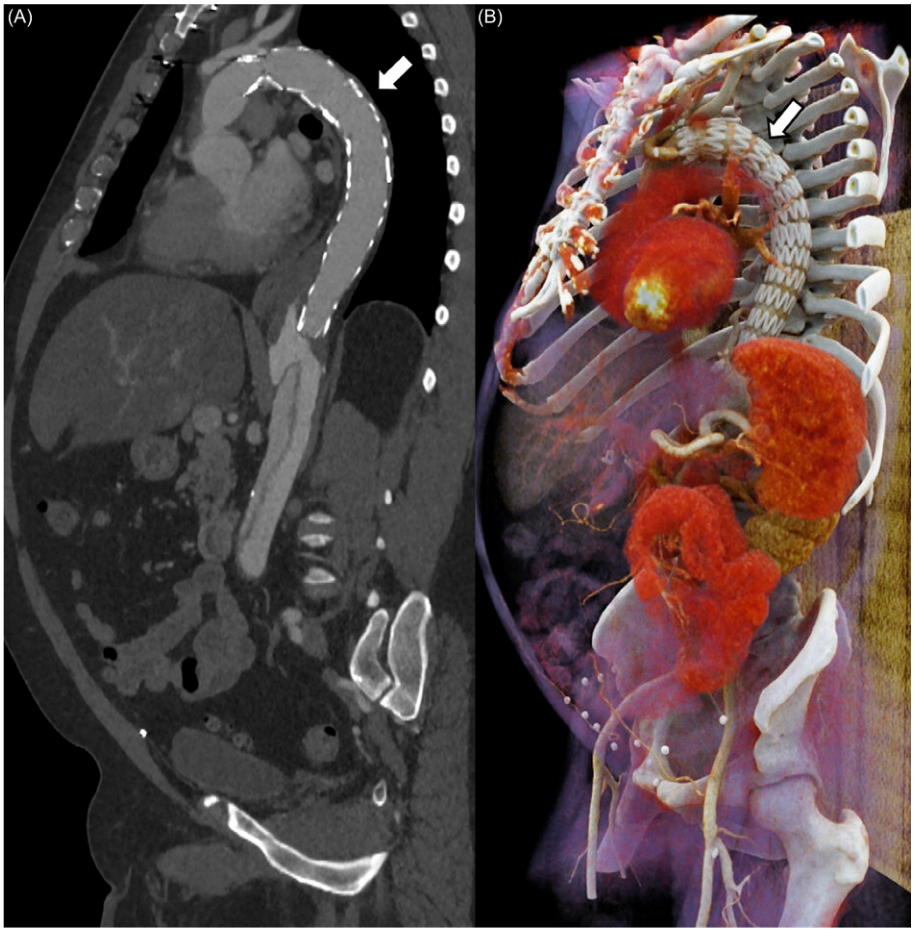

Fig. 10 CT angiography in a 68-year-old female post thoracic aorta dissection repair. (A) Sagittal and (B) Cinematic Rendering reconstructions of the aorta showing a stent in the thoracic aorta (*arrow*).

Myocardial perfusion is based on the distribution of iodinated contrast material during its first pass through the myocardium. Since the distribution of the contrast material is determined by the arterial blood supply, myocardial perfusion defects can be identified as hypo-attenuating areas containing reduced amounts of contrast material (George et al., 2006). There are different approaches for CT evaluation of myocardial perfusion: static myocardial perfusion, which can be further categorized into single-energy-CTMPI (SE-CTMPI) and dual-energy-CTMPI (DE-CTMPI), and dynamic CTMPI (dCTMPI).

Static CT perfusion allows for a visual, qualitative assessment of a single snapshot of contrast attenuation (**Fig. 15**). The snapshot encompasses the entire left ventricle and is acquired during early first-pass enhancement. In order to detect early differences in contrast uptake, it is crucial that the acquisition is performed during peak enhancement of the coronary arteries (Bischoff et al., 2013). Because the same parameters and datasets are used for static myocardial perfusion as for the morphological assessment of the coronary arteries, theoretically, any scanner capable of CCTA can be used and no additional acquisitions are needed for the perfusion evaluation at rest (Vliegenthart et al., 2012b; Nagao et al., 2008). Myocardial perfusion is evaluated by visual assessment of contrast enhancement, preferably in multiphase reconstructions, to better discriminate between true defects, which persist throughout the cardiac cycle, and artifacts only visible during a single cardiac phase (Vliegenthart et al., 2012b). SE-CTMPI has been widely investigated and most studies have shown high sensitivity and specificity for the detection of hemodynamically significant lesions compared with single photon emission computed tomography (SPECT) or SPECT with ICA (Cury et al., 2010, 2011; George et al., 2009, 2012; Kachenoura et al., 2009; Blankstein et al., 2009; Rocha-Filho et al., 2010; Ko et al., 2012a; Nasis et al., 2013; Osawa et al., 2014; Rochitte et al., 2014; Feuchtner et al., 2011). Sensitivity ranged from 50% to 96% and specificity from 68% to 98%, translating to a NPV and PPV of 79%–98% and 55%–94%, respectively. However, the main drawback is that the timing of the acquisition is crucial; missing the peak attenuation could greatly reduce diagnostic accuracy (Bischoff et al., 2013). Performing data acquisition in consecutive regions of the heart during different cardiac cycles results in heterogeneous contrast uptake, which hinders perfusion assessment.

Additionally, DE-CTMPI is based on a dual-simultaneous image acquisition at only one time point. DE-CTMPI merges datasets from both energy spectra allowing for the generation of a color-coded iodine distribution map where iodine concentration is



Fig. 11 Coronary CT angiography in a 58-year-old male presenting with chest pain. (A) Curved multiplanar and (B) axial images of the heart showing the plaque segmentations for plaque analysis. (C) A graph is generated showing the volume, size, and ratio of each plaque component.

normalized to areas of normal myocardial perfusion (**Fig. 16**). This iodine concentration is an indicator of myocardial perfusion and thus, myocardial blood pool can be quantified based on the amount of iodine per voxel (Koonce et al., 2014). Several studies have already demonstrated the good accuracy of DE-CTMPI in detecting hemodynamically significant CAD at rest and stress (Kim et al., 2014; Ko et al., 2011, 2012b, 2014a,b; Ruzsics et al., 2009; Wang et al., 2011; Weininger et al., 2012; Delgado et al., 2013; Delgado Sanchez-Gracian et al., 2016). De Cecco and colleagues showed a significant increase in specificity from 33% to 67% when DE-CTMPI was added to CCTA, as compared with ICA (De Cecco et al., 2014b). Moreover, studies have reported the reclassification of perfusion defects using DE-CTMPI; almost half of the defects classified as reversible with SPECT were reclassified as fixed with DE-CTMPI (Vliegenthart et al., 2012b; Wang et al., 2011; Meinel et al., 2014b). In addition to the limitations of SE-CTMPI, an additional drawback of DE-CTMPI is the nonstandardized iodine distribution maps, which make perfusion assessment highly user-dependent.

dCTMPI requires whole heart coverage and great temporal resolution to sample the heart multiple times during the first pass of the contrast agent. Currently, only MDCT scanners with 256 or 320 detector rows or second-generation DSCT scanners with shuttle mode are capable of dCTMPI (Weininger et al., 2012; Hsiao et al., 2010; Bastarrika et al., 2010a,b). The main advantage of the dynamic technique is the ability to perform an objective quantitative evaluation of myocardial perfusion through quantification of myocardial blood flow and myocardial blood volume (So et al., 2012; Rossi et al., 2014) (**Fig. 17**). Several studies have already demonstrated the good accuracy of dCTMPI compared with cardiac MRI (Weininger et al., 2012; Bastarrika et al., 2010a; Bamberg et al., 2014), SPECT (Weininger et al., 2012), SPECT plus ICA (Ho et al., 2010; Wang et al., 2012), and invasive fractional flow reserve (FFR) (Rossi et al., 2014; Ho et al., 2010; Bamberg et al., 2011b; Greif et al., 2013; Huber et al., 2013). The respective sensitivity and specificity ranged from 76% to 100% and 74% to 100%, which translates to a NPV and PPV of 82%–100% and 48%–100%, respectively. Since dCTMPI requires multiple acquisitions in shuttle mode, this technique is inherently associated with substantially increased radiation dose levels in comparison with traditional CCTA. This distinctly increased dose of ionizing radiation is the primary drawback of this technique, limiting its adoption into clinical routine. Presently, the use of dCTMPI is limited to research studies and academic centers.

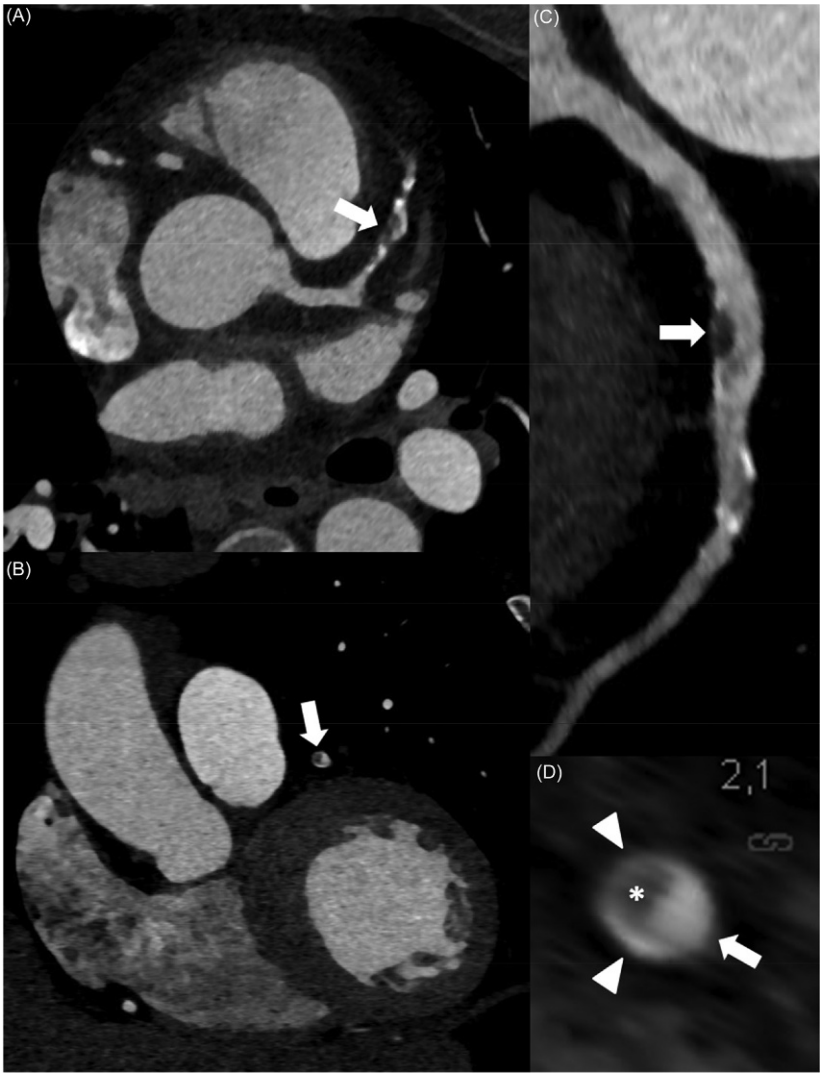

Fig. 12 Coronary CT angiography in a 67-year-old female presenting with chest pain. (A) Axial, (B) coronal, and (C) curved multiplanar views of the heart displaying an atherosclerotic plaque of the left anterior descending (LAD) artery (*arrow*). (D) Cross-sectional view of the LAD showing a napkin-ring sign consisting of the lumen (*arrow*), the lipid core (*asterisk*), and fibrotic cap (*arrowheads*).

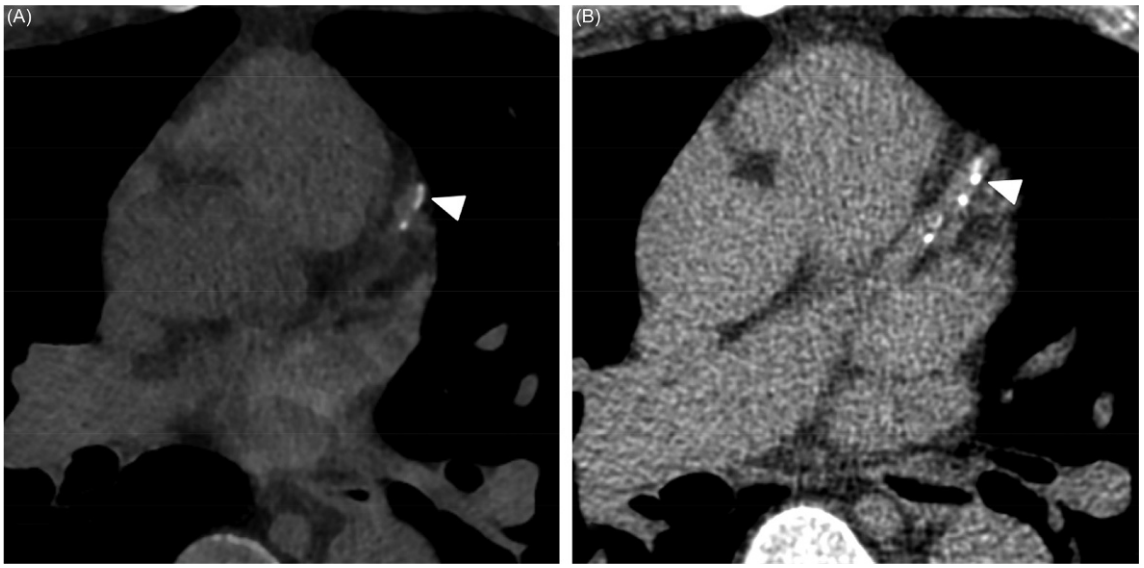

Fig. 13 Dual-energy coronary CT angiography in a 59-year-old female presenting with chest pain. (A) Virtual noncontrast reconstruction based on the standard contrast-enhanced scan showing calcifications in the left anterior descending (LAD) artery (arrowhead). (B) Standard noncontrast calcium scoring scan of the same patient displaying calcifications in the LAD.

CT-FFR

The evaluation of FFR with ICA provides important information regarding the hemodynamic significance of CAD (Patel et al., 2012). However, even though this technique is currently the reference standard, its invasive nature makes it an impractical choice for the general evaluation of CAD (Pijls and Sels, 2012). Third-generation DSCT scanners have vastly improved the temporal and spatial resolution of standard CCTA. These improvements have resulted in improved lumen definition and plaque characterization throughout the coronary tree. These advancements in CT technology allow the calculation of noninvasive CT-derived FFR values through the application of computational fluid dynamics to standard CCTA datasets (Kim et al., 2010). While CCTA has been shown to have high sensitivity and NPV in excluding significant CAD, its specificity and PPV for detecting ischemia-causing disease are low (Min et al., 2013). As a result, the ability to add physiologic information through CT-FFR to the anatomical datasets of CCTA has high clinical relevance.

The feasibility of CT-FFR with standard CCTA has been demonstrated (Leipsic et al., 2014) (**Fig. 18**). Recent studies have shown CT-FFR to be a highly accurate technique for the diagnosis of hemodynamically significant stenosis when compared with invasive FFR (Norgaard et al., 2014; Tesche et al., 2016b; Wang et al., 2015; Baumann et al., 2015b). Moreover, adding CT-FFR to CCTA was found to significantly improve diagnostic accuracy for hemodynamically significant CAD (Renker et al., 2014). A recent meta-analysis showed that CT-FFR improved specificity when combined with CCTA, without altering sensitivity for the detection of hemodynamically significant lesions (Baumann et al., 2016). One benefit of CT-FFR is that it is based on the standard CCTA data, meaning it does not require additional acquisitions or radiation exposure. However, CT-FFR still requires time-consuming, off-site computation in super-computer core laboratories, a problem that could be solved with more novel computational algorithms, which seem feasible in an on-site manner (Baumann et al., 2015b; Renker et al., 2014).

Transluminal attenuation gradient (TAG)

TAG is a novel postprocessing technique that provides functional information in the context of CAD. TAG is defined as the gradient of contrast opacification along the coronary artery (Stuijfzand et al., 2014). It has already been shown that TAG correlates well with ICA and adding TAG to CCTA provides incremental value for the detection of hemodynamically significant CAD (Wong et al., 2013;

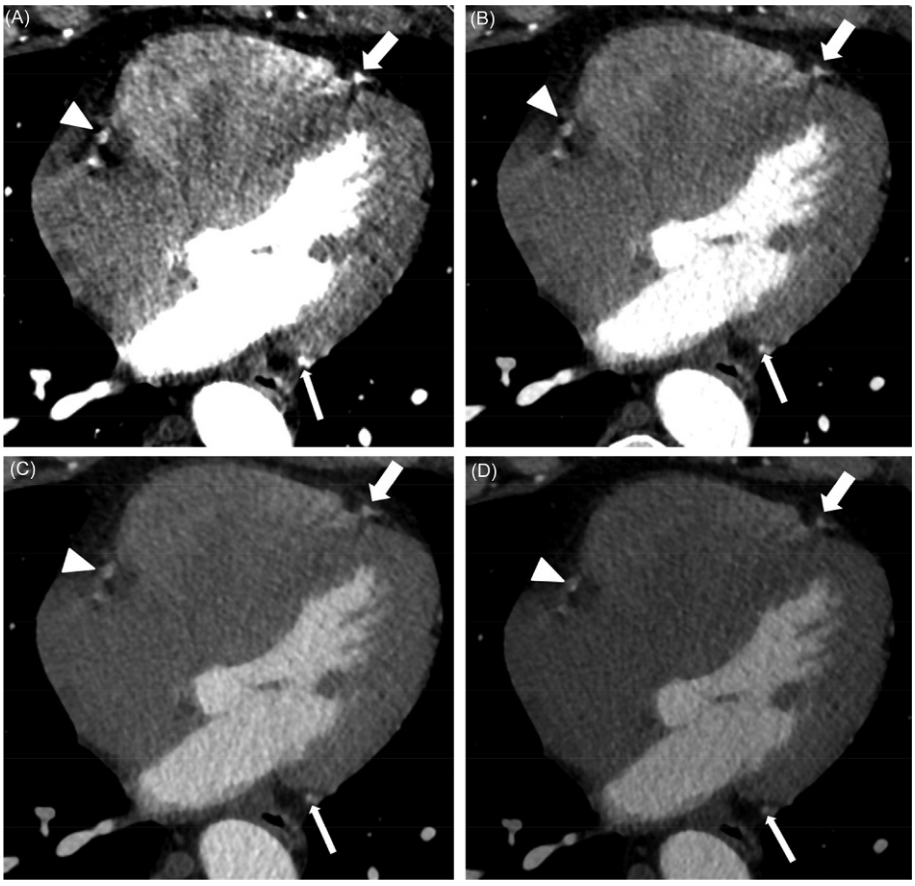

Fig. 14 Dual-energy coronary CT angiography in a 64-year-old male presenting with chest pain. VMI + reconstructions at (A) 40, (B) 55, and (C) 70 keV and (D) standard linearly blended M_0.6 reconstruction displaying the superior contrast and delineation of the right coronary artery (*arrowhead*), left anterior descending (*thick arrow*) and circumflex (*thin arrow*) at lower keV levels.

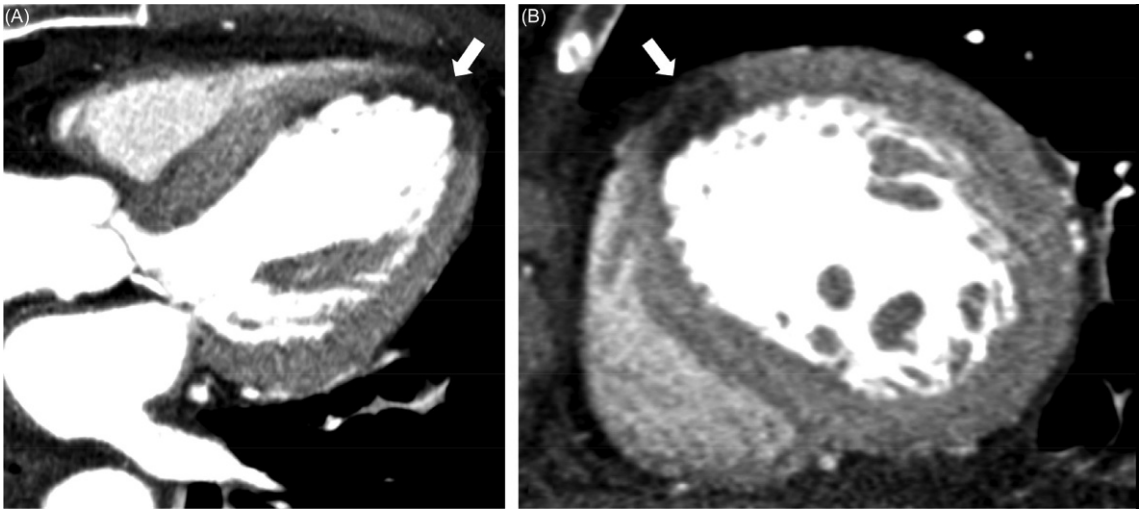

Fig. 15 Static single energy CT myocardial perfusion in a 73-year-old male presenting with chest pain. (A) Axial and (B) short axis views of the heart showing an apical area of hypodensity corresponding with myocardial infarction (*arrow*).

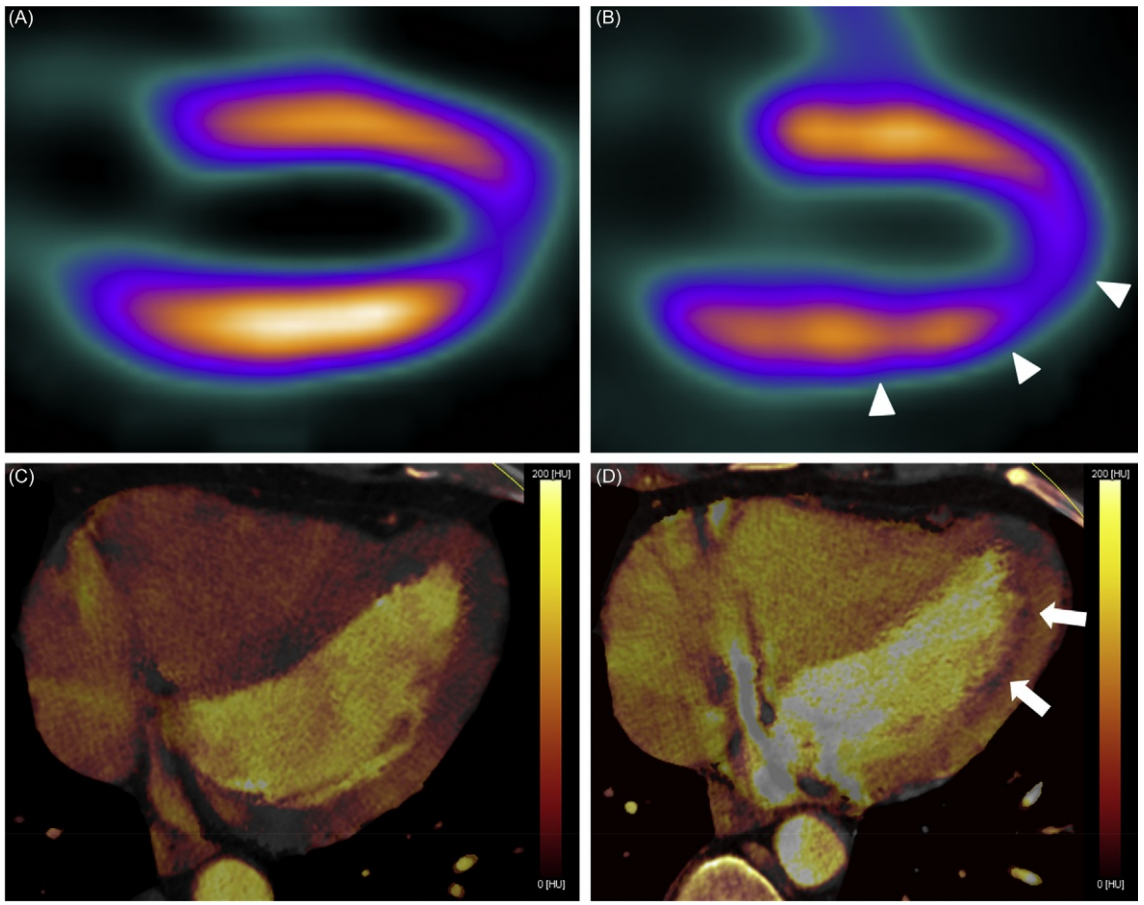

Fig. 16 Dual energy CT myocardial perfusion in a 68-year-old female with previous abnormal stress study. (A) Single-photon emission CT in rest phase showing normal myocardial perfusion. (B) Single-photon emission CT in stress phase showing reduced myocardial perfusion in the apical wall extending to the lateral wall (*arrowheads*). (C) Dual energy CT myocardial perfusion in rest phase showing a normal myocardial perfusion. (D) Dual energy CT myocardial perfusion in stress phase showing reduced perfusion in the apical and lateral walls (*arrows*).

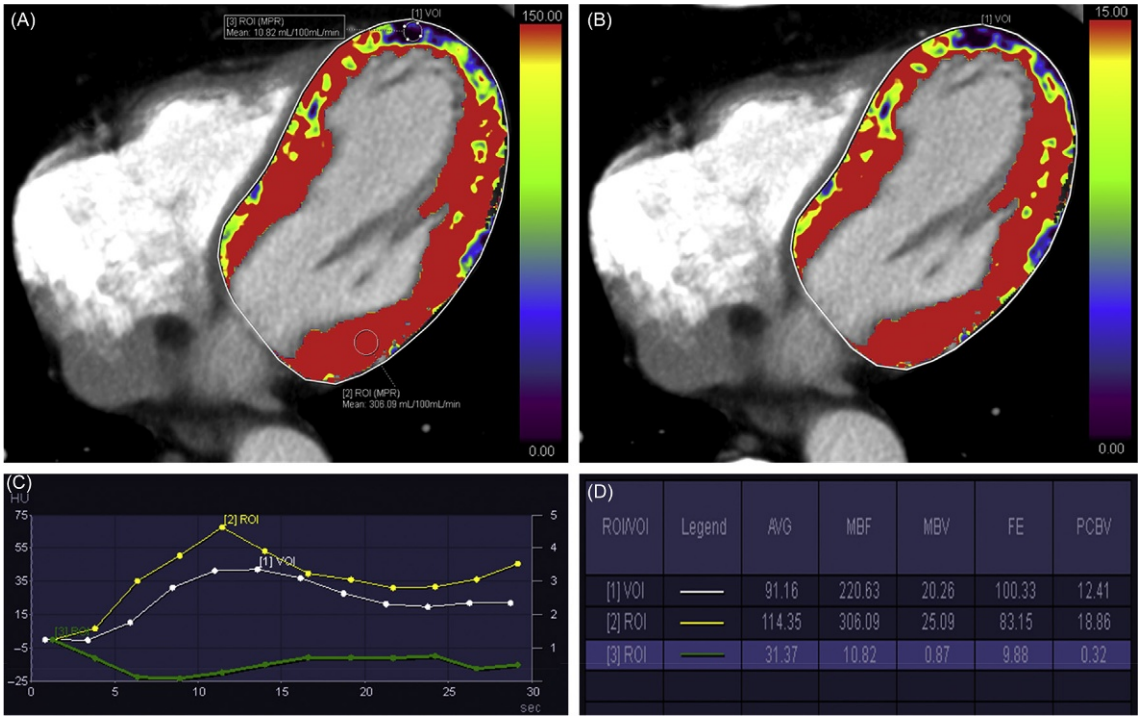

Fig. 17 Dynamic CT myocardial perfusion in a 72-year-old male presenting with chest pain. (A) Myocardial blood flow and (B) myocardial blood volume showing a diminished perfusion in the apical wall. (C) Time attenuation curves and (D) quantitative perfusion values can also be generated.

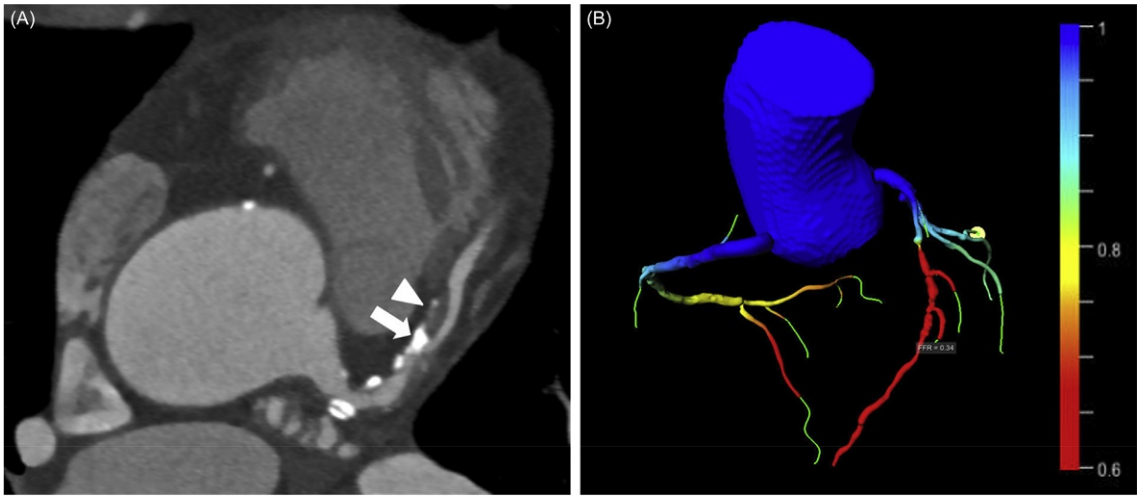

Fig. 18 Coronary computed tomography angiography (CCTA)-derived fractional flow reserve (CT-FFR) in a 58-year-old male presenting with chest pain and shortness of breath. (A) Axial view of the heart showing extensive coronary artery disease of the left anterior descending (LAD) with both calcified (*arrow*) and noncalcified (*arrowhead*) plaques resulting in severe stenosis of the vessel. (B) CT-FFR of the same patient showing markedly reduced blood flow in the LAD with an FFR value of 0.34.

Ko et al., 2016). As with CT-FFR, TAG can be achieved with standard CCTA acquisitions without requiring additional scans or increasing radiation exposure. Further studies are needed to confirm the utility of TAG in routine clinical practice.

Conclusion

Cardiovascular imaging plays an increasingly important role in the clinical diagnosis and management of cardiovascular diseases. As scientific evidence continues to build, guidelines for appropriate indications will evolve, ensuring appropriate use and cost-effectiveness.

As CT technology improves, new applications for cardiovascular CT are being explored, widening the scope of this modality from simple morphologic assessment to comprehensive evaluation of cardiac anatomy, function, and perfusion.

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Imaging: Echocardiology—Assessment of Cardiac Structure and Function

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Introduction

Assessment of cardiac structure and function is the foundation of cardiac imaging, and the echocardiographic examination specifically has this role at its core. Echocardiography is a noninvasive modality and is the most commonly used method of cardiac imaging. It has the distinctive capability of providing real-time images of the heart, is low risk, does not confer radiation to the patient, is portable, and is widely available. As a result, echocardiography is a valuable diagnostic test to evaluate the cardiovascular system and assess physiologic function in a variety of diseases. It provides reliable structural, functional, and hemodynamic information, making an impact in clinical cardiology and is a valuable tool for cardiovascular research.

Left Ventricle

General

Since the inception of echocardiography, this technology has been used in the evaluation of the left ventricle (LV). Assessment of the left ventricle is an integral part of every echocardiogram and defining its structure and function is often the primary indication for the examination. Evaluation of ventricular function offers important prognostic information and guidance for clinical management of the patient. Echocardiography conveys both qualitative and quantitative measures of left ventricular function, and there are a variety of parameters which provide insight into cardiovascular physiology and disease pathology. Assessment of the LV has improved as the technology has evolved to include functions such as strain imaging and three-dimensional (3D) echocardiography; however two-dimensional (2D) echocardiography remains the primary tool for quantification and evaluation of the left ventricular structure and function.

Shape

The shape of the left ventricle is normally comprised of a relatively symmetric short axes and a long axis taken from the base at the mitral annulus to the apex. The basal portion of the ventricle is shaped similar to a cylinder making the minor-axis views appear circular. The apical portion in the long-axis views appears somewhat rounded, giving it the shape of a hemiellipse. Various assumptions about the shape of the left ventricle are made in order to calculate the size and volumes, most of which require geometric simplifications, introducing some degree of error. As technology has progressed from single-plane imaging, to 2D echocardiography and later 3D techniques, the accuracy and reproducibility in determination of the left ventricular shape, size, and function has improved as the number of assumptions is reduced. One method to quantify LV shape is the sphericity index although it is not commonly used in a clinical setting. This ratio is expressed as the left ventricular long-axis length divided by the diameter and a ratio of less than 1.5:1 typically implies pathologic remodeling (Tischler et al., 1993; Douglas et al., 1989).

Size

Linear dimensions

Left ventricular size is most commonly measured as linear dimensions and volumes of the LV cavity (**Table 1**). These are typically gained from measurements of the endocardium at end-diastole and end-systole, allowing wall thickness and global LV function to be calculated using these parameters. Historically, M-mode measurements in the parasternal long-axis view of the heart have been used to obtain these linear dimensions. The aim is to identify the endocardium through the short (minor) axis of the left ventricle at the tips of the mitral leaflets. This angle-dependent practice is significantly limited, however, by off-axis orientations of the ultrasound beam relative to the left ventricular surface, introducing error into the calculations. Further, M-mode, which measures the LV in only one region, has a limited ability to recognize segmental wall motion abnormalities which may lead to over or underestimation of the overall left ventricular size and function. The strength of M-mode is its high-quality temporal resolution which is valuable in timing of cardiac events.

The pursuit of more consistent and accurate measurements of cardiac size and function led to the development of 2D echocardiography (Picard et al., 2008). This technology allows a more comprehensive view of the cardiac structures compared with the single-dimension measurements made by M-mode. 2D echocardiography can be utilized to guide the M-mode to assure a more perpendicular orientation of the ultrasound beam to the long axis of the LV for measurement, or to directly measure the left ventricular endocardium on still-frames at end-diastole and end-systole of the 2D image (Lang et al., 2015) (**Fig. 1**). The recommended technique is to perform this measurement in the parasternal short-axis view at the level of the chordae, using the blood–tissue interface to delineate the points of measurement (Lang et al., 2006).

Volumes

Left ventricular volumes can be measured using 2D or 3D echocardiography. These techniques reduce inherent errors from the geometrical assumptions that are employed for volume calculations derived from linear dimensions. 2D echocardiographic (2DE)-derived volumes rely on endocardial border tracing during end-systole and end-diastole in multiple tomographic planes. The recommended transthoracic echo (TTE) planes for these volumetric tracings are the apical four-chamber and apical two-chamber views of the left ventricle. In order to obtain more accurate volumetric measurements, care must be taken to maximize the LV area obtained by the echo while avoiding foreshortening in order to include the apex of the left ventricle for tracing. Another prerequisite to minimizing errors in volume measurements includes ensuring adequate endocardial border

Table 1 Normal values for echocardiographic parameter of LV size and function

	Male	*Female*
Parameter	*Mean ± SD*	*Mean ± SD*
LV internal dimension		
Diastolic dimension (mm)	50.2 ± 4.1	45.0 ± 3.6
Systolic dimension (mm)	32.4 ± 3.7	28.2 ± 3.3
LV volumes (biplane)		
LV EDV (mL)	106 ± 22	76 ± 15
LV ESV (mL)	41 ± 10	28 ± 7
LV EF biplane (%)	62 ± 5	64 ± 5

EDV, End-diastolic volume; *EF*, Ejection fraction; *ESV*, End-systolic volume; *LV*, Left ventricle; *SD*, Standard deviation. Adapted from Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., Flachskampf, F. A., Foster, E., Goldstein, S. A., Kuznetsova, T., Lancellotti, P., Muraru, D., Picard, M. H., Rietzschel, E. R., Rudski, L., Spencer, K. T., Tsang, W., Voigt, J. U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography* **28**, 1–39 e14.

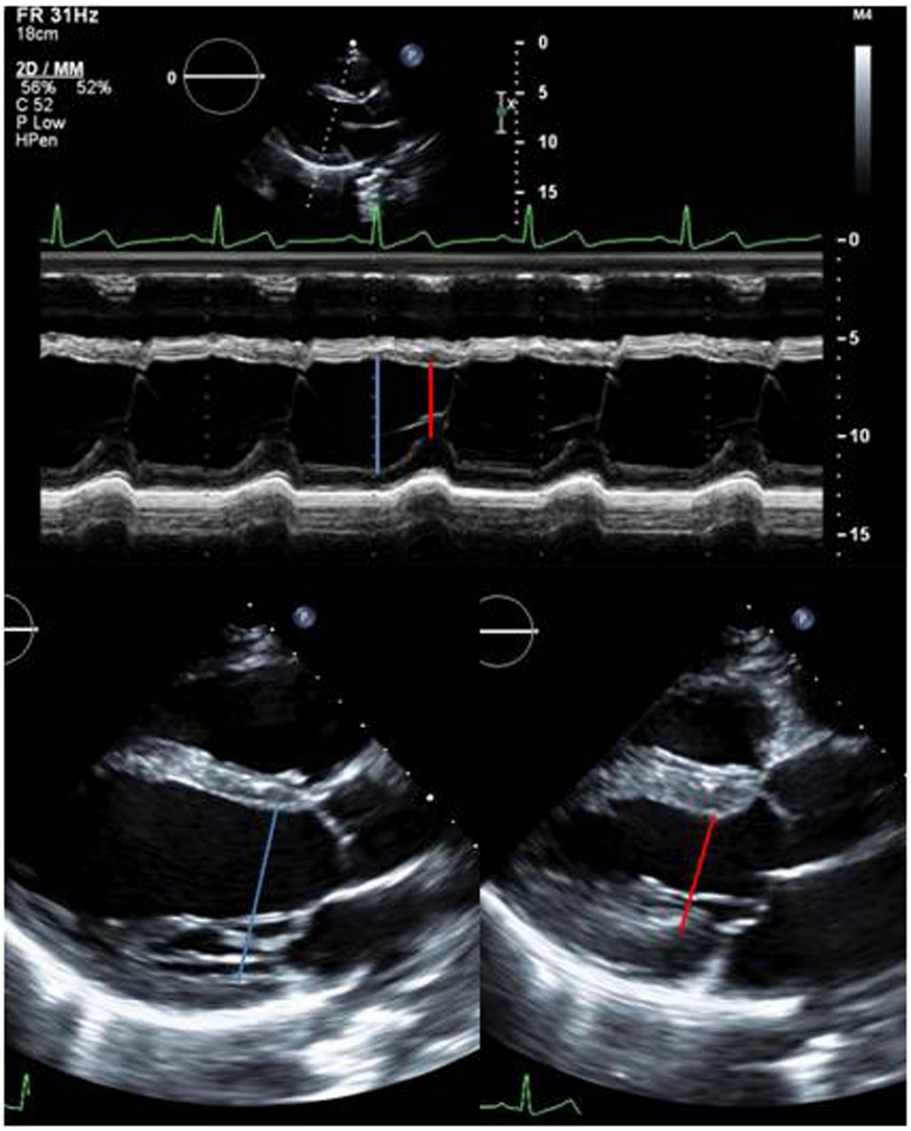

Fig. 1 LV internal linear dimensions. Acquired in the parasternal long-axis view, LV internal dimensions must be obtained perpendicular to the long axis and measured at the level of the mitral valve leaflet tips. Top panel shows 2D-guided M-mode measurements at end-diastole and end-systole. Bottom panel shows corresponding measurements made on still-frame 2D-echo.

definition within the LV cavity. When there is a need to improve image quality to enhance endocardial demarcation, echo contrast agents should be used. This is especially important when two or more adjacent endocardial segments are not well visualized (Mulvagh et al., 2008; Lang et al., 2015).

The most robust and commonly used practical method for 2DE volume calculation is the biplane method of disks. This method applies Simpson's rule, which states that the "volume of a large figure can be calculated from the sum of the volumes of a series of smaller, similar figures." (Vuille and Weyman, 1994) This method is currently recommended by a consensus of experts as it corrects for shape distortions and reduces geometric assumptions of the LV shape compared with alternative 2DE methods (**Table 1**). First, it requires tracing of the left ventricular cavity in the apical four-chamber and apical two-chamber views at end-systole and end-diastole (**Fig. 2**). This area is subsequently rendered into a series of stacked disks of equal height, whose volumes are added together from base to apex, producing LV volumes at end-systole and end-diastole using the formula volume $= (\pi/4)\sum_{i=1}^{20}(a_i b_i)(L/20)$. Another option for determining volume is the area–length method, which assumes the base of the LV is shaped as a cylinder and the apex is an ellipsoid, making the LV a "bullet shape." This is performed by measuring the length of the left ventricle from base to apex in the apical four-chamber view and cross-sectional area of the mid-LV by planimetry in the parasternal short-axis view (**Fig. 3**) and applying the formula volume $= 5/6(AL)$ (Lang et al., 2005, 2015).

Because 2D echocardiography remains a tomographic technique, volume calculations necessitate geometric assumptions regarding the left ventricle. In contrast, 3D echocardiography does not require geometric assumptions, is unaffected by

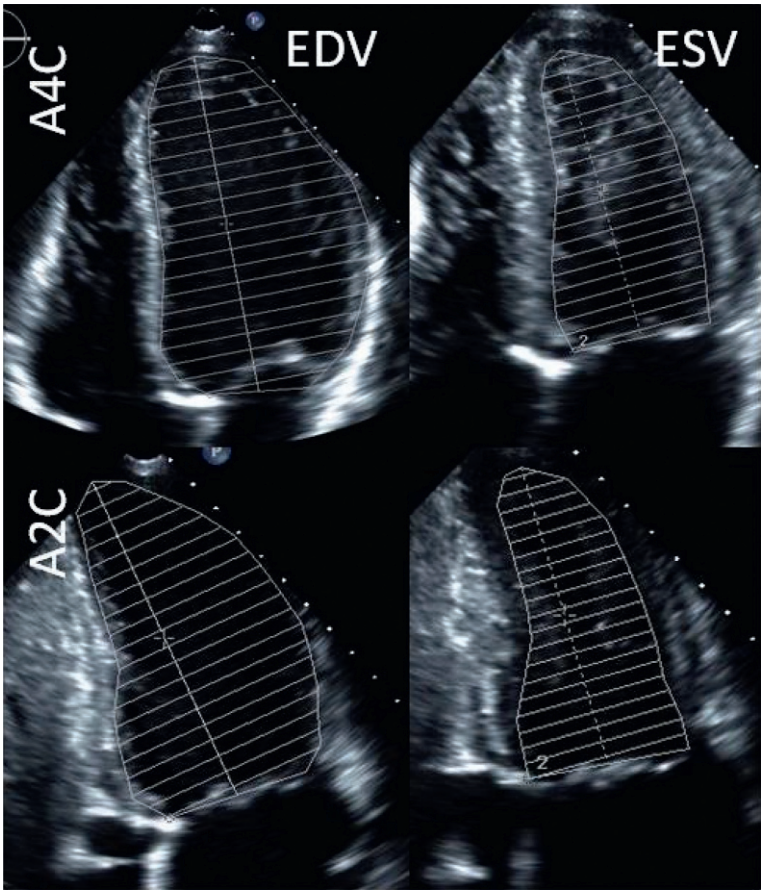

Fig. 2 LV method of disk summation. Volume measurements are made by tracing the blood–tissue interface in the apical four and apical two-chamber views, at end-diastole and end-systole. The shape is closed by a virtual line made across the level of the mitral valve annulus. *A4C*, apical four-chamber; *A2C*, apical two-chamber; *EDV,* end-diastolic volume; *ESV*, end-systolic volume.

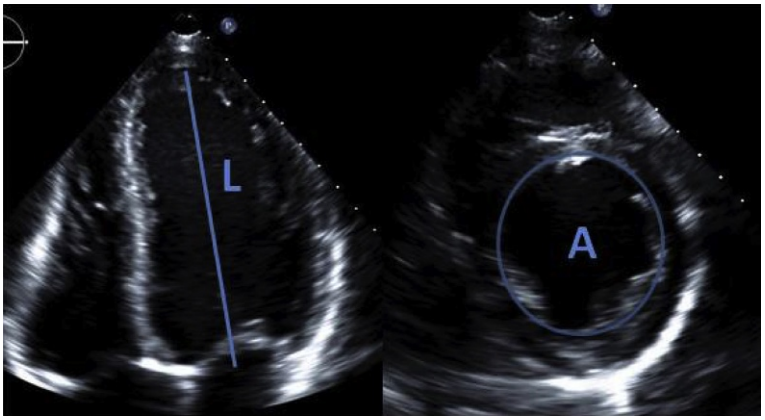

Fig. 3 LV area–length volumetric method. Volume is determined by measuring the length of the left ventricle in the apical four-chamber view and the area of the mid-level of the left ventricle in the parasternal short-axis view, using specific assumptions regarding LV shape. *L*, length; *A*, area.

foreshortening, and has demonstrated improved accuracy and precision compared with alternate imaging modalities in assessing the LV volumes (Dorosz et al., 2012). 3D echocardiographic data is transferred to a workstation, allowing the volume to be calculated offline (Fig. 4). One method directly adds the volumes of the voxels, or 3D volume elements, that are enclosed by the LV endocardial borders. Another technique utilizes the disc summation method, adding component 3D volumes after identifying the LV borders. The major drawbacks of 3D-derived volumes include a dependency on high-quality 2D echo acquisition and its lower temporal resolution, which may limit accurate identification of end-systole and end-diastole (Lang et al., 2015).

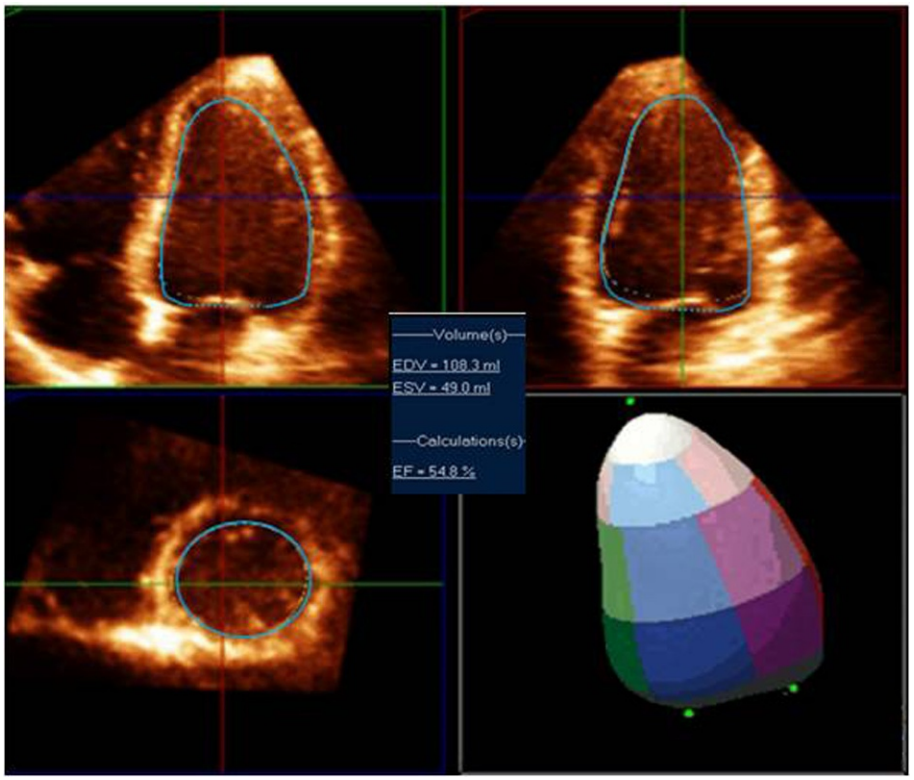

Fig. 4 Three-dimensional Echocardiography. From a 3-dimensional echo data set of the left ventricle (LV) the endocardium can be traced (*top left*, *top right*, and *bottom left panels*) to obtain the LV volume throughout the cardiac cycle and derive the EF. The LV endocardium can be divided according to the 17-segment model (*bottom right panel*). *EDV*, end-diastolic volume; *ESV*, end-systolic volume; *EF*, ejection fraction.

Systolic Function

Fractional shortening

In addition to assessing wall thickness and determination of LV internal dimensions during systole and diastole, linear dimensions supply the information necessary to compute endocardial fractional shortening of the left ventricle. Fractional shortening is a simple measurement for determining the global LV function and can be derived from linear measurements made on 2D imaging or 2D-guided M-mode. As previously mentioned, using linear measurements to derive global LV function can be problematic in the presence of regional wall motion abnormalities. If these abnormalities are located in the area where the measurements are performed they may lead to underestimation of the global LV function because the rest of the ventricle may be normal. In contrast, if the measurements are made in a normal region, for example, the base of the LV, and the mid or apical LV is hypokinetic or akinetic, it could lead to overestimation of the global function. In the absence of regional abnormalities, fractional shortening may provide simple and useful information to the clinician and in research studies. However, in patients with wall motion abnormalities or diseases that distort the LV shape, volumetric measurements provide a more accurate assessment of the global function. The merits of the fractional shortening method include its simplicity, reproducibility, and ease of use to gather data in large populations.

Ejection fraction

Ejection fraction (EF) is the percentage of blood volume ejected in each cardiac cycle and is a representation of LV systolic performance. It is calculated from the end-diastolic and end-systolic volumes of the left ventricle. The formula for calculating EF is:

$$\mathrm{EF} = \frac{(\mathrm{EDV} - \mathrm{ESV})}{\mathrm{EDV}}$$

where EF is ejection fraction, EDV is end-diastolic volume, and ESV is end-systolic volume. Note that the difference between the end-diastolic volume and end-systolic volume is the stroke volume. See **Table 2** for the normal and abnormal ranges of EF.

The left ventricular volume measurements may be obtained from 2D echo or 3D echo, as discussed previously. The current recommendation for assessment of the EF is to use the biplane method of disk summation. In echocardiograms with sufficiently high-quality imaging, 3D echo-based volume can be used to calculate the EF. Studies of 3D echo have shown that it is more accurate and reproducible than 2D echo measures of EF when compared with reference standards. This most likely reflects the fact that no geometric assumptions about the LV shape are required.

Table 2 LV EF reference ranges and severity partitions using 2D echocardiography

	Male				*Female*			
	Normal	*Mildly abnormal*	*Moderately abnormal*	*Severely abnormal*	*Normal*	*Mildly abnormal*	*Moderately abnormal*	*Severely abnormal*
LV EF (%)	52–72	41–51	30–40	<30	54–74	41–53	30–40	<30

LV EF, Left ventricular ejection fraction.

Adapted from Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., Flachskampf, F. A., Foster, E., Goldstein, S. A., Kuznetsova, T., Lancellotti, P., Muraru, D., Picard, M. H., Rietzschel, E. R., Rudski, L., Spencer, K. T., Tsang, W., Voigt, J. U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography* **28**, 1–39 e14.

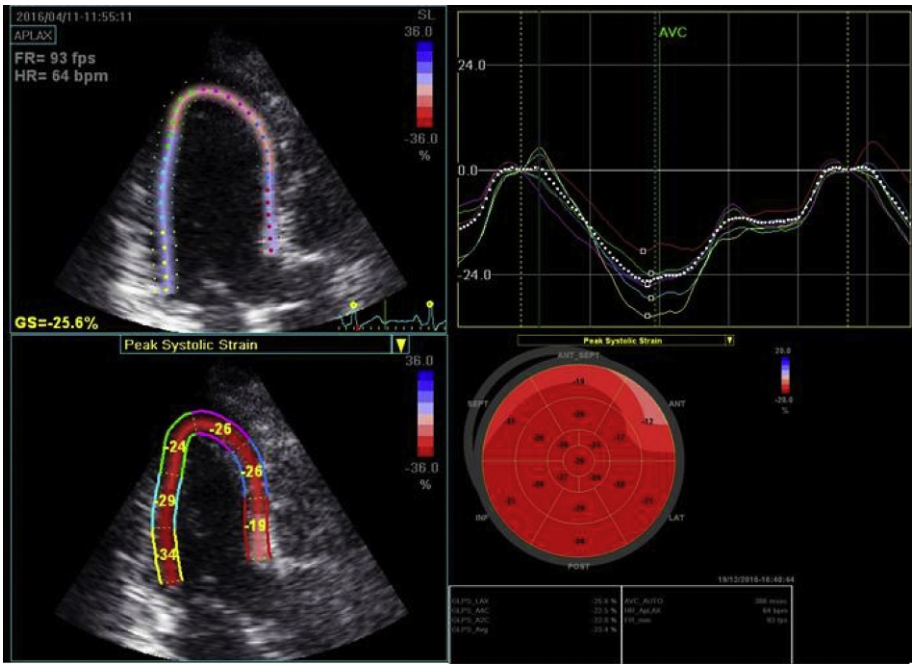

Fig. 5 Strain Imaging. The left ventricle is traced (*top left panel*) and then processed by the strain software algorithm, assigning values (*bottom left panel*). Each endocardial segment is tracked throughout the cardiac cycle and the percent strain is plotted over time (*top right panel*). Once all 17 segments are traced and processed, an average global longitudinal strain can be calculated and a bull's eye plot containing values of all the segments can be displayed (*bottom right panel*).

Strain

Myocardial strain can be determined by echocardiography (Fig. 5). This measure of myocardial deformation can be assessed in longitudinal, radial, or circumferential axes. Lagrangian strain refers to the change in length of an object compared with its baseline length. Longitudinal strain (LS) is the most commonly used strain-based assessment of the left ventricular global function. With echocardiography, this is often performed using "speckle-tracking," software that allows the ultrasound system to track the motion of features in the heart by using the naturally occurring speckle pattern in the myocardium when imaged by ultrasound. These specular reflectors are unique patterns (analogous to a fingerprint) that remain relatively stable from frame to frame during echocardiography, allowing their position to be tracked throughout the cardiac cycle thus allowing measurement of relative lengths of myocardial segments. The change in length between these myocardial features is quantified from end-diastole and end-systole. LS is then calculated as:

$$\text{LS}\ (\%) = \frac{(\text{MLs} - \text{MLd})}{\text{MLd}},$$

where LS is longitudinal strain, MLs is myocardial length at end-systole, and MLd is at end-diastole. Myocardial length in the longitudinal axis in systole is smaller than in diastole, making LS a negative number. More negative LS (a higher number in absolute terms) represents more robust ventricular function. This is consistent since a smaller end-systolic measurement and a larger end-diastolic measurement will produce a higher absolute difference.

Global longitudinal strain (GLS) is an average of the strain calculations across the left ventricle. 2D echo-derived GLS has been shown to have some incremental benefits over LV EF in various conditions because of its reproducible nature and ability to identify subclinical myocardial dysfunction (in the setting of normal EF). Quantitation of circumferential strain at the base and apex of the LV can be used to measure the twist or torsion of the ventricle. Assessment of the timing of peak radial strain can be used to evaluate the synchrony of myocardial thickening and is often used to identify candidates for Cardiac Resynchronization Therapy in heart failure or to assess response to such therapy. Current applications of strain echocardiography include monitoring of patients receiving chemotherapy with potentially cardiotoxic effects and in cardiac amyloid. Currently one of the limitations of this technique is the difference in normal values among different echocardiography machines marketed by different vendors and different software versions on a particular echocardiography machine; however a peak GLS in the range of −20% or higher (in absolute numbers) is considered to be normal (Mor-Avi et al., 2011; Lang et al., 2015).

Regional LV Function

For purposes of assessment of regional LV function, the ventricle is divided into segments. For all cardiac imaging modalities a 17-segment model has been developed. The 17th segment is a cap at the LV apex. This segment is used to describe a perfusion pattern, but since it does not extend from epicardium to endocardium, it is not used to describe wall motion. Thus for echocardiographic indications a 16-segment model without the apical cap is often used (Fig. 6). The typical pattern of myocardial perfusion for each coronary artery can be overlaid on this segmentation (Fig. 7).

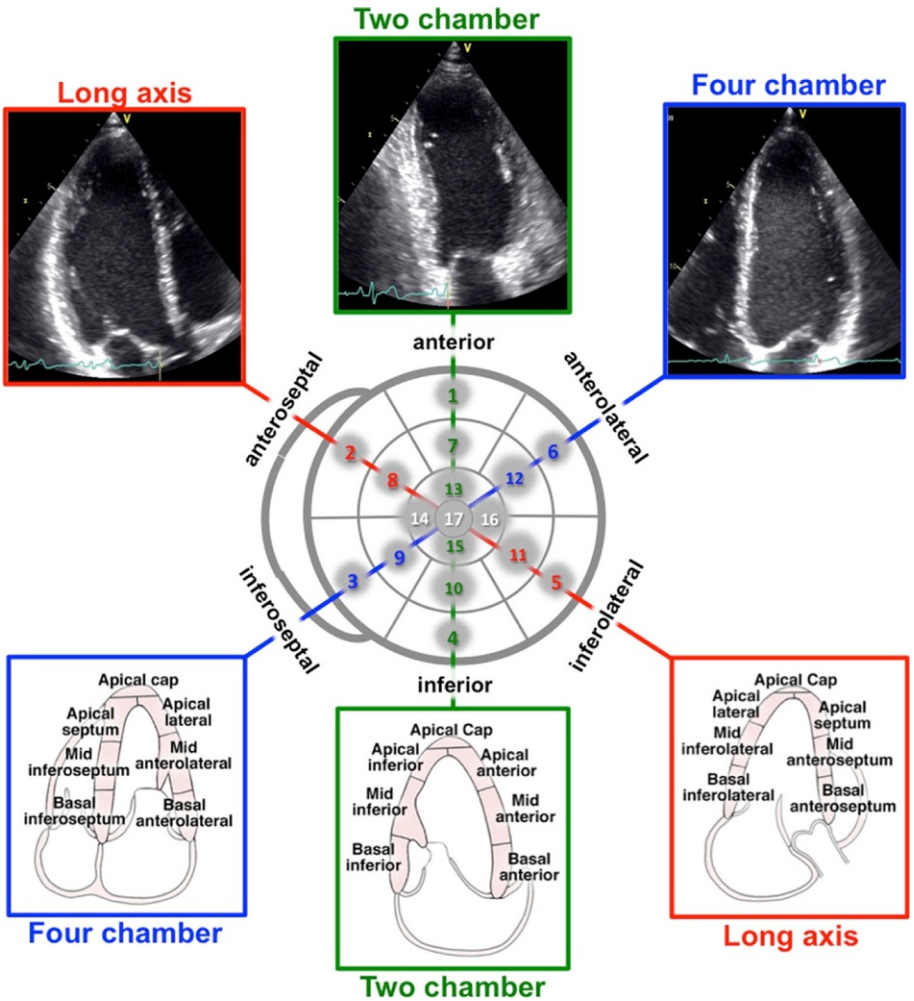

Fig. 6 17-segment model and its correlated echocardiographic territory. Orientation of apical four-chamber (A4C), apical two-chamber (A2C), and apical long-axis (ALX) views in relation to the bull's-eye display of the left ventricular segments (*center*). Top panels show actual images, and bottom panels schematically depict the LV wall segments in each view. Reproduced with permission from Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., Flachskampf, F. A., Foster, E., Goldstein, S. A., Kuznetsova, T., Lancellotti, P., Muraru, D., Picard, M. H., Rietzschel, E. R., Rudski, L., Spencer, K. T., Tsang, W., Voigt, J. U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography* **28**, 1–39 e14.

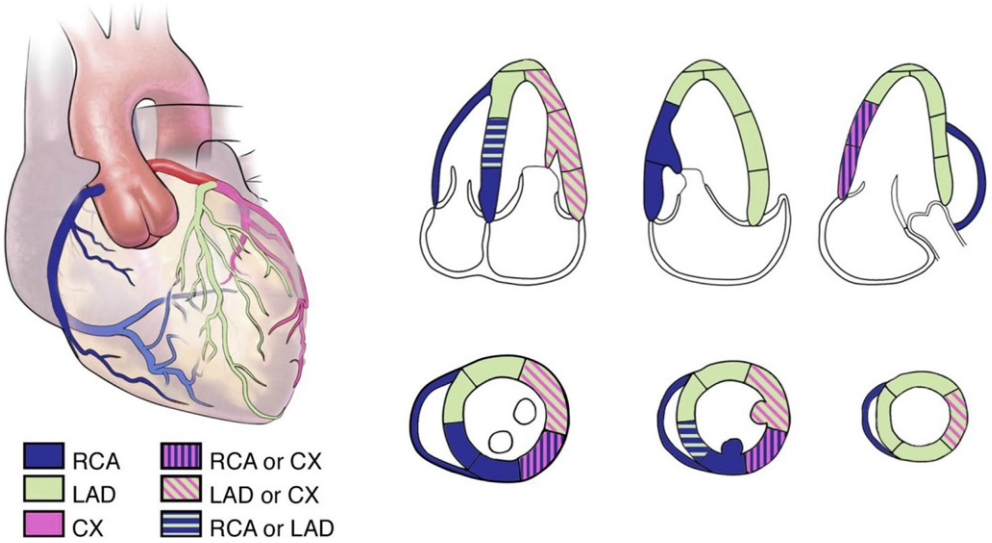

Fig. 7 Coronary artery supply by echocardiography. Typical distributions of the right coronary artery (RCA), the left anterior descending coronary artery (LAD), and the circumflex coronary artery (CX) and their associated left ventricular myocardial territories in standard echocardiographic views. Reproduced with permission from Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., Flachskampf, F. A., Foster, E., Goldstein, S. A., Kuznetsova, T., Lancellotti, P., Muraru, D., Picard, M. H., Rietzschel, E. R., Rudski, L., Spencer, K. T., Tsang, W., Voigt, J. U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography* **28**, 1–39 e14.

Visual assessment of the left ventricular regional wall motion is typically a semiquantitative method. This method utilizes an estimation of endocardial motion and wall thickening of each segment of the left ventricle. Each segment should be analyzed individually, in multiple views and a score assigned based on this analysis. The recommended scoring system is: normal or hyperkinetic = 1, hypokinetic = 2, akinetic = 3, dyskinetic or aneurysmal = 4. The LV wall motion score index is the average score for each segment, calculated as the total summed score divided by the number of segments, with a higher value indicating more diseased segments in terms of quantity and/or severity.

Most commonly, regional wall motion abnormalities represented by these segments are due to coronary artery disease and are notable for decreased systolic thickening. However, conditions such as sarcoidosis, myocarditis, and stress-induced (Takotsubo) cardiomyopathy may cause regional dysfunction in the absence of flow-limiting epicardial coronary disease. Wall motion abnormalities of the interventricular septum with normal wall thickening intact may be caused by cardiac pacing of the right ventricle, left bundle branch block, following cardiac surgery, or right ventricular volume overload.

LV Mass

LV mass can be calculated using linear dimensions (from M-mode or 2D echo), 2D-based formulas using area, or 3D echo. In each calculation, measurements are performed at end-diastole. Additionally, all of these methods are designed to calculate the volume of the LV myocardium and are then converted to mass by multiplying it by 1.04 g/mL or 1.05 g/mL, the density of the myocardium. Each method has its advantages and limitations. Studies show that calculating LV mass using linear dimensions is simple and quick and has the most established prognostic benefit (Armstrong et al., 2012; Lang et al., 2015). The linear method formula, also known as the "cube formula," is:

$$\text{LV mass} = 0.8 \times 1.04 \times \left[(\text{IVS} + \text{LVID} + \text{PWT})^3 - \text{LVID}^3\right] + 0.6\,\text{g}$$

where IVS is the interventricular septum thickness, LVID is LV internal diameter, and PWT is the posterior wall thickness, all taken from the parasternal imaging location (Fig. 8).

However, similar to utilizing linear dimensions in measuring LV size, this method comes with important limitations. The linear formula assumes the LV thickness is the same throughout the LV. If the LV is measured by M-mode and the ultrasound beam is obliquely directed then this exaggerates small errors because overestimation of the LV wall thickness will be cubed. Thus if this method is used it is best to use 2D-directed M-mode to ensure on-axis, accurate wall thickness and cavity measurements.

There are two commonly used 2DE-based methods of determining LV mass; the truncated ellipsoid method and the area-length method. Both derive the myocardial volume by subtraction of the internal LV volume from the total epicardial volume. This remainder (the volume of the LV walls without the cavity) is then multiplied by the density of the myocardium, providing the LV mass (Fig. 8). Both of these methods are subject to less geometric assumptions than the linear method and can correct for some distortions in the shape of the LV; however they are more time-consuming and rely on higher-quality imaging. The two formulas are shown here:

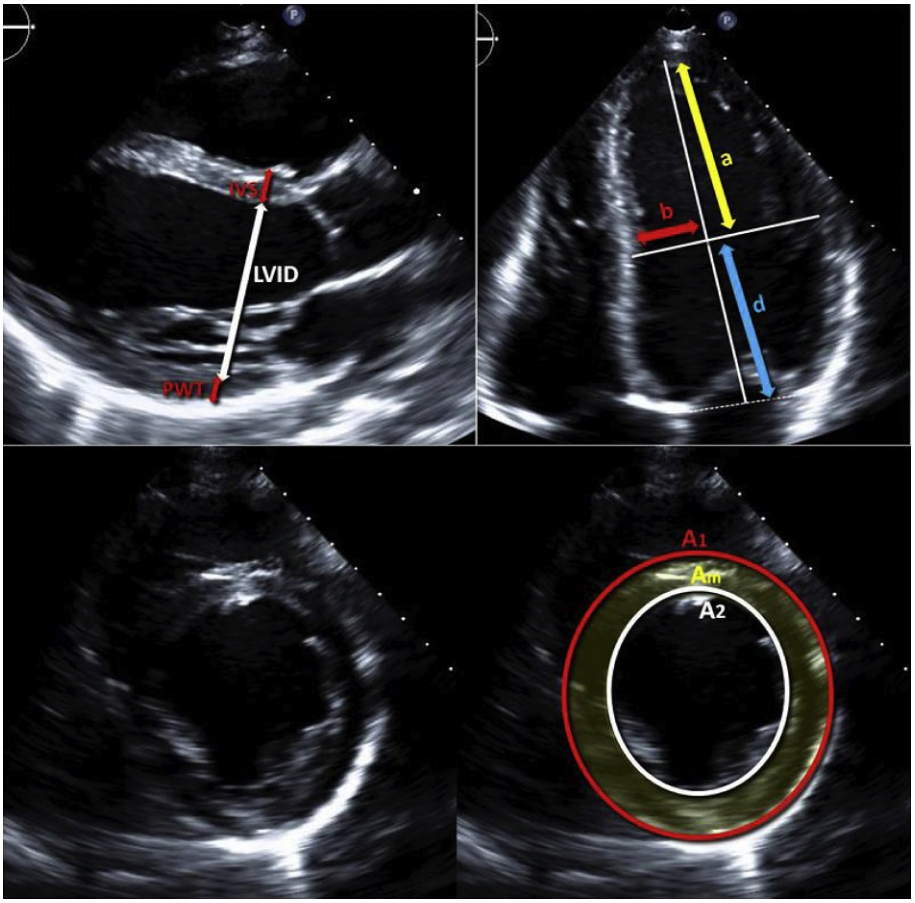

Fig. 8 LV mass assessment by 2D echocardiography. *Top left:* Linear dimensions used in the cube formula; *IVS*, interventricular septum; *LVID*, left ventricular internal dimension (diastole); *PWT*, posterior wall thickness. *Top right:* Dimensions needed for 2D echo-based formulas (see text). *Bottom row:* Parasternal short-axis view at the mid-LV level, needed for the area-length method. Bottom right depicts the areas at this level: *A*1, epicardial area; *A*2, endocardial area; *Am*, myocardial area as defined by subtracting *A*2 from *A*1.

Truncated ellipsoid:

$$\text{LV mass} = 1.05\pi\left\{(b+t)^2 \times \left[{}^2\!/_3(a+t) + d - d^3/3(a+t)^2\right] - b^2 \times \left[2a/3 + d - d^3/3a^2\right]\right\}$$

where a is the distance from the minor axis to the endocardium at the LV apex, b is the LV minor radius, d is the distance from the minor axis to the mitral valve plane, and t is the mean wall thickness.

Area-length:

$$\text{LV mass} = 1.05\pi\left\{\left[{}^5\!/_6 A1(a+d+t)\right] - \left[{}^5\!/_6 A2(a+d)\right]\right\}$$

where $A1$ is the epicardial cross-sectional area and $A2$ is the endocardial cross-sectional area. These are complex calculations that can be performed by the software of the ultrasound machine once the variables have been entered.

3D echocardiography provides a direct measurement without geometrical assumptions and is more accurate and reproducible than other methods. However, this is a newer modality, is not as widely available, and relies on high-quality imaging. Once the LV epicardial and endocardial surfaces are defined the myocardial volume is calculated similar to the method described earlier for 3D LV volume by quantitating the voxels within the prescribed region.

Regardless of the technique employed to determine LV mass, it is important to consider that there is variability between them and thus the same measurement approach should be used to assess a patient over time. Current guidelines have established the normal values for LV mass, when indexed to body surface area, as less than 95 g/m^2 for women and less than 115 g/m^2 for men.

Once LV mass has been determined, relative wall thickness (RWT) is computed with the formula:

$$\text{RWT} = (2 \times \text{PWT})/(\text{LVIDd})$$

where PWT is posterior wall thickness and LVID is LV internal dimension at end-diastole. LV mass together with relative wall thickness allows the classification of various patterns of LV hypertrophy and remodeling. These include concentric hypertrophy

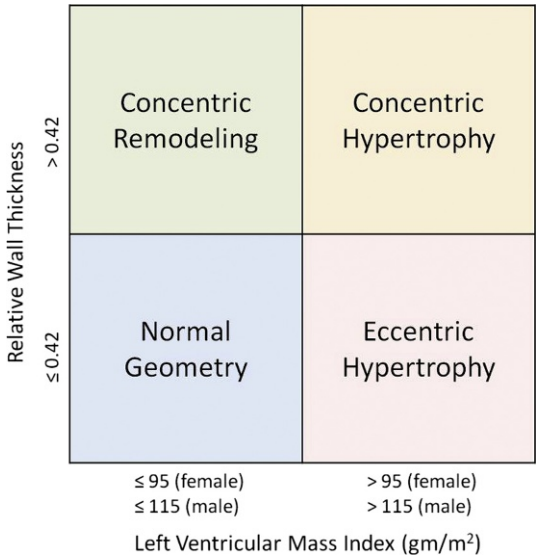

Fig. 9 Relative wall thickness (RWT) versus LV mass index. Patients with normal LV mass and normal RWT have normal geometry (bottom left panel). Patients with normal LV mass and increased RWT have concentric remodeling (top left panel). Patients with increased LV mass can have either eccentric hypertrophy (normal RWT, bottom right panel) or concentric hypertrophy (increased RWT, top right panel).

(RWT > 0.42) as you may see in patients with hypertension; eccentric hypertrophy (RWT < 0.42), commonly seen in cases of dilated cardiomyopathy; or concentric remodeling in the case of athletes with a normal LV mass and a RWT > 0.42. Fig. 9 illustrates these classifications determined by LV mass and RWT.

LV diastolic function

LV diastolic function can be assessed by echocardiography in several ways. Normal LV diastolic function is noted by normal LV diastolic pressure and relaxation indices. It is determined by both passive elastic properties of the heart and active relaxation properties. Disorders of LV filling are associated with increased LV diastolic pressure, and this can occur as a disorder of filling, relaxation, or a reduction in compliance (increased stiffness) of the LV.

There are many indirect measures of LV diastolic function by echocardiography. For example, an increased LA dimension or volume can occur in the setting of increased LV diastolic pressure. This measure has prognostic value. Increased LV wall thickness is often associated with a reduced rate of LV relaxation or increased stiffness both of which can lead to increased LV diastolic pressure. Doppler measures of pulmonary venous blood flow velocity can also be utilized to infer relative LA pressure and thus in the absence of mitral valve disease, the LV diastolic pressure. With increasing LA pressure the pulmonary venous systolic forward flow velocities are reduced and the normal duration of the reversed flow during atrial contraction is increased. Pulmonary venous Doppler is not specific for elevated LA pressure as there are other factors that can influence its pattern. In fact, healthy young adults without elevated LA pressure may demonstrate this reversal in the normal systolic to diastolic velocity ratio. Thus the pulmonary venous Doppler measure is used as a supporting index but not in isolation to assess diastolic function.

Doppler of the mitral valve, both the transmitral inflow velocity patterns and the mitral annular velocities, are the key echocardiographic measures to assess diastolic function. The transmitral Doppler filling pattern reflects differences in the LA and LV pressure during diastole. The peak velocity of the passive filling portion of transmitral flow (E wave) and its velocity decay (deceleration time) can be used to identify elevations in LA pressure and whether such are due to a delay in LV relaxation (long deceleration time) or a decrease in LV compliance (short deceleration time). The ratio of the peak velocity of the passive filling phase (E wave) to the early diastolic annular peak velocity (é) correlates with mean LA pressure in some conditions.

Algorithms have been developed to classify grades of diastolic function and dysfunction (Redfield et al., 2003; Nagueh et al., 2009; Nagueh et al., 2016). In addition, the assessment of diastolic function by echocardiography in unique populations such as in the presence of atrial fibrillation, hypertrophic cardiomyopathy, reduced LV systolic function, and heart block can be challenging, but various cutoff values for echocardiographic parameters have been established (Nagueh et al., 2016). Elastic and relaxation properties of the myocardium change with aging, and this can make the discrimination of normal from abnormal findings challenging in the elderly population. Diastolic myocardial strain assessment by echocardiography is now being investigated as a method for quantitation of LV diastolic function.

Right Ventricle

General

Much of the attention in echocardiography is focused on the assessment of the left ventricle; however the right ventricle often plays a key role in the well-being of the patient. In patients presenting with signs and symptoms of cardiopulmonary disease, a thorough evaluation of the right ventricle (RV) is crucial as it is strongly associated with clinical outcomes in several diverse diseases and after some cardiac procedures. This comes with unique obstacles and challenges as many of the standard echocardiographic views are oriented with respect to the left ventricle. An assessment of the right ventricle focuses on many of the same goals as its counterpart on the left, including an evaluation of its shape, size, function, and mass.

Shape

The crescent shape of the right ventricle makes it a complex chamber to study. It is composed of three regions: the trabecular apex, the outflow tract including the subpulmonary infundibulum, and a smooth muscular inflow region which serves as the body of the ventricle. One cannot image all of these regions in a single, 2D image plane. This makes quantification of the RV size and function especially challenging because of the difficulty obtaining reliable and consistent imaging planes for analysis and the many assumptions used to estimate function from the images. Advances in 3D echocardiography, however, have led to improved accuracy in assessing the right ventricle.

Size

Linear

The size of the right ventricle is a marker of prognosis in many patients. Dilation of the right ventricle occurs in response to volume and/or pressure overload or in cases of RV failure. Studies have shown that increased RV diameter and increased RV to LV end-diastolic ratios predict survival in patients with chronic lung disease and acute pulmonary embolism, respectively (Burgess et al., 2002; Fremont et al., 2008; Rudski et al., 2010; Mendes et al., 1994).

Visual assessment of the right ventricle alone has been shown to have significant limitations in accuracy and interobserver variability (Ling et al., 2012). Therefore, it is essential to quantify the RV dimensions to accurately interpret the size and function of this chamber. Due to the complexities in RV anatomy and its variable appearance in the standard left ventricular-centric echo imaging view, it can be challenging to perform accurate and consistent assessments of this structure. Right ventricular measurements are best approached from an apical four-chamber view, focused on the RV (**Fig. 10**). The LV apex should be seen in the view to avoid foreshortening, with the largest RV basal diameter displayed for calculations (**Fig. 11**). According to the current guidelines, the normal linear dimensions for the RV are a basal diameter of 25–41 mm, a mid-ventricular diameter of 19–35 mm, and a longitudinal diameter of 59–83 mm.(Lang et al., 2015).

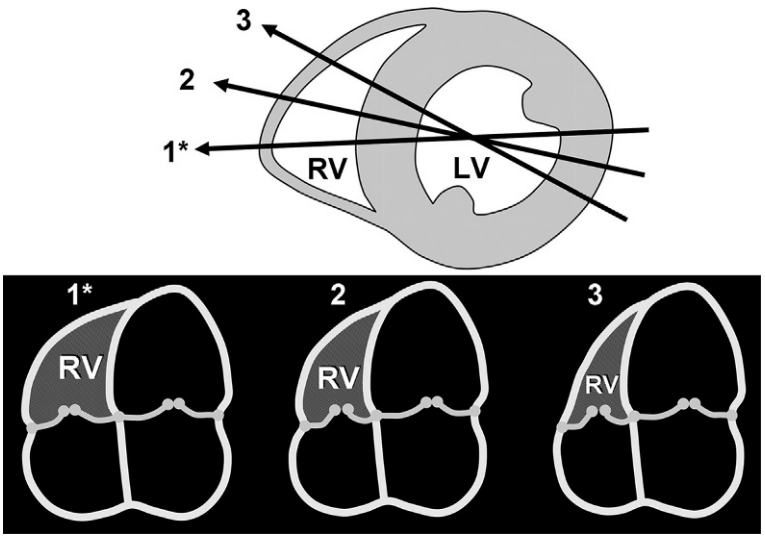

Fig. 10 RV-focused view. Diagram of the apical four-chamber view focusing on the right ventricle (RV). *Top image* shows how the plane of the ultrasound beam can be directed in multiple ways through the RV resulting in different dimensions. *Bottom panel* depicts corresponding images using the same ultrasound imaging planes. The illustration labeled *1** represents the recommended RV-focused view. Reproduced with permission from Rudski, L. G., Lai, W. W., Afilalo, J., Hua, L., Handschumacher, M. D., Chandrasekaran, K., Solomon, S. D., Louie, E. K., Schiller, N. B. (2010). Guidelines for the echocardiographic assessment of the right heart in adults: A report from the American Society of Echocardiography endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography. *Journal of the American Society of Echocardiography* **23**, 685–713; quiz 786–788.

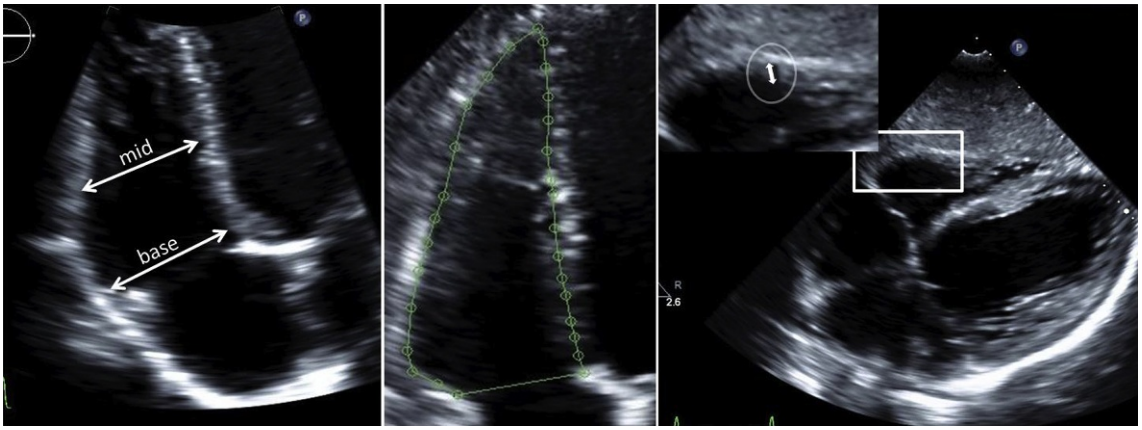

Fig. 11 Right ventricular size and thickness. *Left panel:* RV linear dimensions in the apical four-chamber RV-focused view at end-diastole. *Base*, maximal dimension in the basal third of the ventricle; *mid*, mid-cavity dimension in the middle third of the ventricle. *Middle panel:* Manual tracing of RV endocardial border to determine area measurement. *Right panel:* measurement of RV free wall thickness in subcostal view at end-diastole. Measurement of wall thickness should be made at the point that the tricuspid valve tip would be if it were maximally open.

Volume and Volumetric Estimations of RV Function

2DE estimations of RV volume include area-length methods, disk summation methods, and other methods. However, due to the complexity of estimating RV volumes in 2D echocardiography, recent guidelines recommend against these methods. RV EFs are calculated using the formula (end-diastolic volume–end-systolic volume)/end-diastolic volume. When used in the past, the normal value was reported as an RV EF above 44% (Lang et al., 2015; Rudski et al., 2010).

3D echocardiography provides significant advantages over 2D echo in regard to RV volume estimation. Although it is limited by image quality or incomplete visualization of the entire chamber in severely enlarged right ventricles, it delivers more accurate assessments of the end-diastolic and end-systolic volumes of the right ventricle. 3D echo supplies improved test-retest variability when compared to 2DE measurements of the right ventricle. Both 2D and 3D methods tend to underestimate these volumes compared with cardiac MRI because echo methods tend to exclude the RV endocardial trabeculations from the enclosed volume but MRI includes these structures in the enclosed volume (Jenkins et al., 2007; Gopal et al., 2007). Current guidelines suggest that in laboratories with 3D echocardiography experience, disk summation by 3D echo may be used to report EFs, and it may further be used for serial volume and EF determinations. Using this method, the median reference value for RV end-diastolic volume is 91 mL with a range of 61–150 mL representing the 5th-95th percentile. The median RV end-systolic volume is 35 mL with an equivalent range of 16–62 mL. The median reference value for RV EF using this method is 62%, with a range of 47%–77% representing the 5th-95th percentile (Lang et al., 2015; Rudski et al., 2010).

Nonvolumetric Assessments of RV Function

The assessment of RV systolic function is in some ways more challenging than the left ventricle. There are methods to judge global function, including RV EF (see above), fractional area change (FAC), RV dP/dt, and the myocardial performance index (MPI). As with the left ventricle, there are also methods for interpreting regional RV function, including strain imaging, Doppler-derived systolic annular velocities (S′), and tricuspid annular plane systolic excursion (TAPSE). Not all of these indices of RV function require visualization of the RV endocardial border. Each method has limitations and must be interpreted within the context of the overall study including the other indices measured. At least one quantitative measure of RV function should be incorporated into routine interpretations of echocardiograms. Normal reference values for and images portraying these methods are seen in **Table 3** and **Fig. 12**, respectively.

The RV fractional area change represents the difference in the RV area between diastole and systole, normalized to diastole and expressed as a percentage. The formula is:

$$\text{RV FAC} = (\text{end} - \text{diastolic area} - \text{end systolic area})/\text{end} - \text{diastolic area} \times 100.$$

At both end-diastole and end-systole the RV endocardial border is traced in an apical 4-chamber view, with the trabeculations, papillary muscles, and moderator band included within this area. The lower limit of normal RV function by FAC is 35% (Lang et al., 2015; Rudski et al., 2010).

RV dP/dt refers to the rate of pressure rise in the ventricles (change in pressure/change in time) and has been validated as an index of ventricular systolic function. Using the tricuspid regurgitant signal by continuous wave Doppler, one calculates the time it takes as the pressure changes, which is measured via the simplified Bernoulli equation ($4\ V^2$) at velocities in the ascending limb of the TR

Table 3 Reference values for RV function parameters

Parameter	*Normal threshold*
Fractional area change (%)	≥35
Three3-dimensional EF (%)	≥45
TAPSE (mm)	≥17
Global longitudinal strain (%)	≥−20 (larger absolute number)
Pulsed doppler Sé (cm/s)	≥9.5
RV dP/dt (mm Hg/s)	≥400
Pulsed doppler MPI	<0.43
Tissue doppler MPI	<0.54

RV, Right ventricle; *EF*, Ejection fraction; *MPI*, Myocardial performance index.
Adapted from Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., Flachskampf, F. A., Foster, E., Goldstein, S. A., Kuznetsova, T., Lancellotti, P., Muraru, D., Picard, M. H., Rietzschel, E. R., Rudski, L., Spencer, K. T., Tsang, W., Voigt, J. U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography* **28**, 1–39 e14.

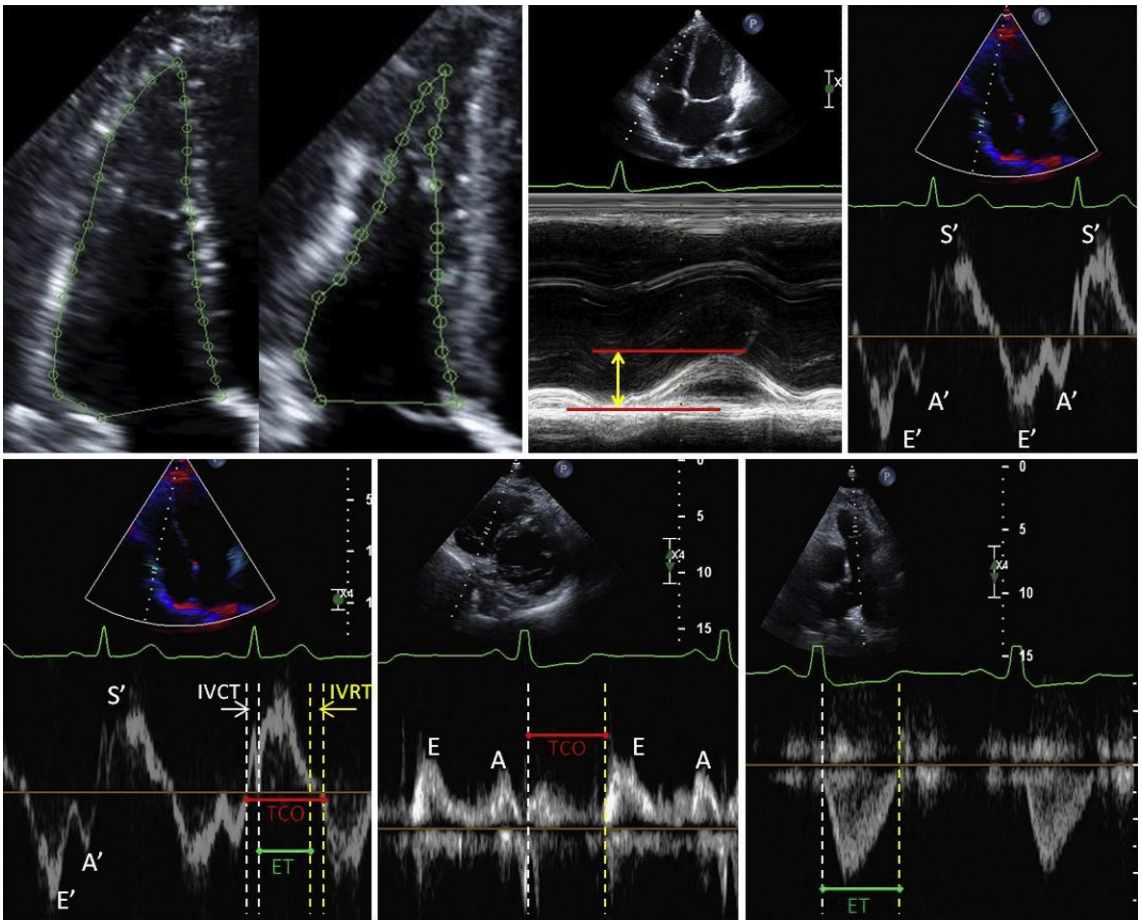

Fig. 12 Nonvolumetric measures of right ventricular function: *Top left panels:* Right ventricular (RV) area during end-diastole and end-systole are used for fractional area change (FAC). *Top middle panel:* M-mode measure of tricuspid annular longitudinal excursion (TAPSE) between end-diastole and peak systole. *Top right panel:* Pulsed tissue Doppler imaging of tricuspid annulus depicting peak Sé wave. *Bottom left panel:* Tissue Doppler measurements for RIMP (RV index of myocardial performance). *Bottom middle and right panels:* Pulsed wave Doppler measurements for RIMP. *TCO*, tricuspid valve closure to open time; *ET*, ejection time (through the pulmonic valve); *IVCT*, isovolumic contraction time; *IVRT*, isovolumic relaxation time.

signal. Commonly, this is measured by determining the time it takes for the TR signal to increase in velocity from 1 to 2 m/s. This is a difference of 12 mm Hg of pressure (16–4 mm Hg), and this is divided by time (in seconds) between the two velocities to calculate mean dP/dt. Abnormal RV dP/dt is considered less than 400 mm Hg/s (Rudski et al., 2010). This echo measure differs from invasively derived dP/dt obtained in the cardiac catheterization laboratory. Invasive hemodynamics provide an instantaneous, peak dP/dt, as opposed to a mean dP/dt derived via echocardiography.

The MPI, also called the RV Index of Myocardial Performance (RIMP) or Tei index, is a calculation of the ratio of time that the ventricle spends in ejection of blood. The formula is:

$$\text{RIMP} = (\text{IVRT} + \text{IVCT})/\text{ET}$$

where IVRT is the isovolumetric relaxation time, IVCT is the isovolumetric contraction time, and ET is the ejection time. Another way to calculate it is:

$$\text{RIMP} = (\text{TCO} - \text{ET})/\text{ET}$$

where *TCO* is the duration of time the tricuspid valve is closed (which encompasses IVRT, IVCT, and ET). The time measurements of these variables can be done using pulsed wave Doppler or tissue Doppler. One must be sure that the heartbeats used for time measurements have similar R-R intervals. More time spent in ejection relative to isovolumetric phases signifies better RV function, thus lower values for the RIMP are normal. RIMP < 0.43 by PW Doppler and < 0.54 by tissue Doppler imply normal RV function. This measurement is load dependent and is not reliable in conditions associated with elevated RA pressure (Lang et al., 2015; Rudski et al., 2010).

The TAPSE method is a relatively simple technique used to assess RV longitudinal performance. To perform this measurement, an M-mode cursor is aligned through the lateral tricuspid annulus in the apical four-chamber view and one measures the amount of longitudinal motion of the annular plane in systole. TAPSE < 17 mm suggests RV dysfunction, and it is correlated with parameters such as radionuclide angiography, RV EF, and FAC (Kaul et al., 1984; Miller et al., 2004). It has limitations including the assumption that the basal segment is representative of global RV function. It is angle dependent, as the base to apex motion of the RV must be along the path of the M-mode line. Thus any translational motion of the heart in another direction will reduce the accuracy of this measurement.

Doppler tissue imaging, which assesses the velocity of the annulus as it moves during the cardiac cycle, can provide a reliable and reproducible measurement, similar in methodology to the TAPSE. Here, the pulsed Doppler sample volume is placed in the lateral tricuspid annulus in the apical four-chamber view. The Sé wave is observed moving toward the transducer in systole, and its peak velocity is easily measured. The limitations are similar to that of TAPSE, and it also has been correlated with methods such as radionuclide angiography. A free wall-derived Sé velocity >9.5 cm/s suggests normal RV function (Lang et al., 2015; Rudski et al., 2010).

RV strain and strain rate can be used to assess global and regional RV systolic function. It can be measured by Doppler or tissue tracking. Measurement of Doppler strain is angle dependent, which is not present with speckle-tracking echocardiography (STE). Since it may be difficult to orient the heart in the echocardiographic image such that the RV free wall motion is parallel to the Doppler interrogation line STE is preferred. RV GLS, an average of the three segments of the RV free wall and septum or the free wall segments alone, is typically used to describe function. As with the LV strain, limitations of this technique include the variation in values among vendors and software versions, thus requiring different reference points for normal values for different echo machines or software versions. However a peak GLS in the range of -20% or higher (in absolute value) is likely normal regardless of vendor or software version. Because of the need for further studies to provide additional data, no definitive reference ranges for strain-derived normal function are recommended in current guidelines (Lang et al., 2015).

Right Ventricular and Pulmonary Pressures

Right ventricular systolic pressure (RVSP), often used as a surrogate for pulmonary arterial systolic pressure (PASP), can be estimated relatively easily utilizing Doppler profiles in echocardiography. Using this for estimation of PASP, however, only applies if there is no obstruction between the right ventricle and the pulmonary artery. The peak RV–RA pressure gradient is derived by applying the simplified Bernoulli equation to the peak tricuspid regurgitant jet velocity. The RA pressure is then added to this value to determine RVSP. The right atrial pressure is estimated from the inferior vena cava (IVC) diameter and its response to respiration as described later in the section dedicated to the right atrium. Because the Doppler velocity measurement is angle dependent, however, one could underestimate the true peak TR velocity and RVSP. Therefore, it is recommended that this tricuspid regurgitant (TR) Doppler velocity be measured from several echocardiographic windows and to use the highest velocity for measurement. This measurement cannot be used in some patients with an inadequate TR jet for measuring the velocity, seen as an incomplete, or cut off, Doppler profile. Injection of agitated saline often improves the Doppler profile in these cases (Himelman et al., 1989). In patients with very severe tricuspid regurgitation (TR), the Doppler profile may be shortened as well due to early equalization of the RV–RA gradient and again should not be used in these cases as it may underestimate the true RVSP. This is also true when there is a large tricuspid valve regurgitant orifice as the regurgitant flow may be laminar rather than turbulent, in which case the simplified Bernoulli equation may not apply.

Pulmonary arterial diastolic pressure (PADP) can be similarly estimated using the velocity of the end-diastolic pulmonary regurgitant jet. Using the modified Bernoulli equation $\text{PADP} = 4(\text{EDPR})^2 + \text{RA pressure}$, where EDPR is the end-diastolic pulmonary regurgitant velocity allows this estimation to be performed. The mean PA pressure (MPAP) can be calculated using the PASP and PADP via the formula $\text{MPAP} = 1/3(\text{PASP}) + 2/3(\text{PADP})$. Alternatively, the MPAP can be calculated using the pulmonary acceleration time (AT) using the equation:

$$\text{MPAP} = 79 - (0.45 \times \text{AT})$$

where AT is the time in milliseconds from the onset of ejection to peak systolic velocity, measured using pulsed wave Doppler of the pulmonary artery in systole. Generally, a shorter AT represents a higher pulmonary vascular resistance and therefore higher PA pressure. This formula is especially useful when the Doppler signal from the TR jet is inadequate or cannot be obtained. Its use is limited to cases of normal heart rates, however, because it depends on the timing of pulmonary ejection and can be erroneous at high or low heart rates.

Mass

Right ventricular wall thickness is determined by measuring the width of the RV free wall by M-mode or 2D echo. RV hypertrophy, often the result of increased right ventricular systolic pressure, can be due to various infiltrative and hypertrophic cardiomyopathies, as well as pulmonic valve stenosis or elevated pulmonary pressure. The current guidelines recommend that RV free wall thickness be measured at end-diastole from the subcostal or left parasternal view of the heart, about where the tip of the anterior tricuspid leaflet would be when fully open and parallel to the RV wall (**Fig. 11**). A normal RV wall thickness should be 5 mm or less. Care should be taken to exclude trabeculae, papillary muscles, and epicardial fat, which would erroneously increase the measurement (Rudski et al., 2010; Lang et al., 2015).

Left Atrium

The left atrium (LA) has three principal roles in the function of the heart. It acts as a contractile pump during atrial contraction, it is a reservoir for pulmonary venous blood during ventricular systole, and it is a conduit for blood to pass into the left ventricle during diastole (Rosca et al., 2011). In the absence of mitral stenosis, the left atrium dilates in response to increased pressure, ultimately causing LA dysfunction. This may lead to atrial fibrillation, thrombus formation, and stroke. LA enlargement confers higher risks in many situations including postmyocardial infarction, in diabetics, and in patients with dilated cardiomyopathy (Rosca et al., 2011; Lonborg et al., 2013; Kim et al., 2008; Modena et al., 1997; Poulsen et al., 2013).

Anatomy and Shape

From the perspective of blood flow, the left atrium begins at the pulmonary veno-atrial junction of the four pulmonary veins and ends at the mitral annulus where the blood passes through to the left ventricle. It is a relatively smooth-walled structure with a finger-like compartment known as the left atrial appendage attached to the anterolateral aspect of the left atrial body. This appendage plays an important role, as it is the area most likely to develop thrombus in LA mechanical dysfunction such as in atrial arrhythmias. For this reason it is often amputated or ligated during cardiac surgery or via percutaneously delivered left atrial appendage closure devices to decrease the risk of thromboembolism and stroke in patients who cannot tolerate anticoagulation. On the septal wall of the left atrium is the foramen ovale, a fetal remnant of the area where blood flow was able to pass through between the left and right atrium before birth when the fetus received oxygenated blood from the mother instead of its own pulmonary circulation. In approximately 25% of the population, there is a remaining connection between the left and right atria, called a patent foramen ovale. Atrial septal defects due to congenital anomalies in other areas of the septum may also occur, although less commonly.

Size

Left atrial size can be determined using linear dimensions, area, and volume measurements. By convention LA size is determined at ventricular end-systole, before the mitral valve opens, when the atrium is at its largest. The anteroposterior (AP) dimension is the most commonly used linear determination of LA size because it is the LA dimension with the lowest intrameasurement variability. It is performed in the parasternal long-axis view, using 2D echocardiography or 2D-guided M-mode (**Fig. 13**). It is easy to perform and is reproducible; however the single dimension measurement may not accurately represent left atrial size. The upper reference values for this method are 40 mm in males and 38 mm in females, as seen in **Table 4** (Lang et al., 2015).

The area of the left atrium can be obtained via planimetry in the apical four and apical two-chamber views. This tracing should be along the inner border of the LA, excluding the inlet of the pulmonary veins and the area between the mitral valve annulus and the mitral valve leaflets. This is more representative of LA size than the AP diameter; however this method makes the assumption of a symmetric atrial shape.

Current guidelines recommend assessment of left atrial size using volumetric measurements, which require fewer geometric assumptions. Left atrial volumes reflect both the extent and the chronic nature of elevated left-sided pressures. Specifically, the biplane disk summation method is recommended, using the apical four-chamber and apical two-chamber views of the left atrium. Similar to the modified Simpson's method used in measuring LV volume, here the LA volume is calculated by adding the volume of a stack of cylinders of equal height (**Fig. 13**). The area–length method of volumetric calculation can also be used; however it is not the preferred method. The upper reference limit for 2D echo-derived LA volume is 34 mL/m^2 as seen in **Table 4** (Lang et al., 2015). In experienced echo labs, newer techniques using 3D data sets may be used. They are more accurate than 2D methods as they include no geometric assumptions. However, limited data on reference values are currently available.

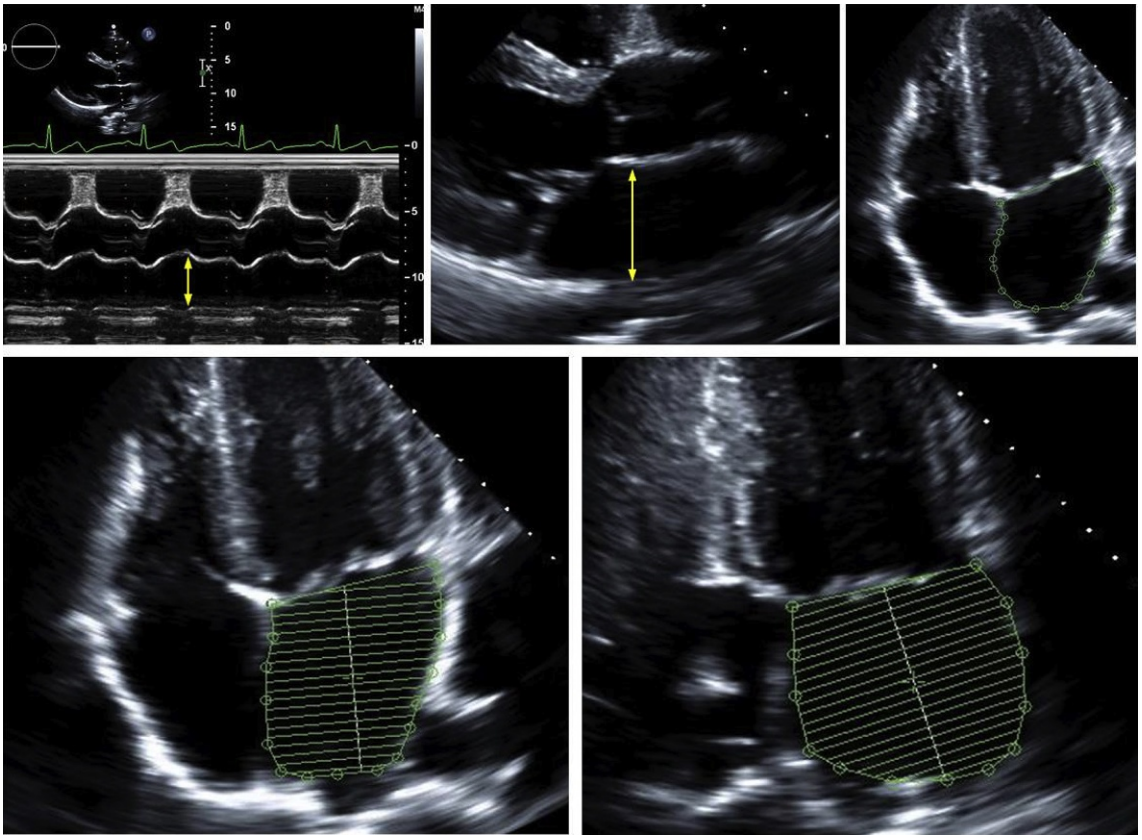

Fig. 13 LA size assessment by 2D echocardiography. *Top left and middle panels:* 2D-guided M-mode and 2D still frame with arrow measuring anterior-posterior LA dimension at ventricular end-systole in parasternal long-axis view. *Top right panel:* Apical four-chamber view with tracing of left atrium, measuring 2-dimensional area. *Bottom panels:* Measurement of LA volume using disk summation method.

Table 4 Normal LA size from 2D echocardiography

Parameter	*Male*	*Female*
LA AP dimension (cm)	3.0–4.0	2.7–3.8
Indexed AP to BSA (cm/m^2)	1.5–2.3	1.5–2.3
LA volume/BSA (mL/m^2)	16–34	16–34

LA, Left atrium; *AP*, Anteroposterior; *BSA*, Body surface area.
Adapted from Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., Flachskampf, F. A., Foster, E., Goldstein, S. A., Kuznetsova, T., Lancellotti, P., Muraru, D., Picard, M. H., Rietzschel, E. R., Rudski, L., Spencer, K. T., Tsang, W., Voigt, J. U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography* **28**, 1–39 e14.

Right Atrium

The right atrium (RA) has the same three primary functions as the LA: acting as a reservoir for systemic venous return (analogous to pulmonary venous return to the LA), acting as a conduit for blood to flow through the tricuspid valve in early diastole, and active RV filling via atrial contraction during late ventricular diastole. The RA is primarily imaged in the apical four-chamber view in transthoracic imaging, and thus 2D assessments are limited by lack of standard orthogonal imaging planes. Compared with the left atrium, there are significantly less data regarding right atrial size, normative reference values, and its importance to patient outcomes (Lang et al., 2015; Rudski et al., 2010).

Size

The right atrial size can be measured by linear dimensions, area, or volume. These are performed at ventricular end-systole, when the right atrium is at its largest. Conventionally, the right atrium should be measured from an apical four-chamber view in which both

Table 5 Normal RA size from 2D echocardiography

Parameter	*Male*	*Female*
RA minor axis/BSA (cm/m^2)	1.9 ± 0.3	1.9 ± 0.3
RA major axis/BSA (cm/m^2)	2.4 ± 0.3	2.5 ± 0.3
RA volume/BSA (mL/m^2)	25 ± 7	21 ± 6

RA, Right atrium; *BSA*, Body surface area.
Data displayed as mean ± standard deviation.
Adapted from Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., Flachskampf, F. A., Foster, E., Goldstein, S. A., Kuznetsova, T., Lancellotti, P., Muraru, D., Picard, M. H., Rietzschel, E. R., Rudski, L., Spencer, K. T., Tsang, W., Voigt, J. U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography* **28**, 1–39 e14.

the mitral and tricuspid valves and largest medial–lateral annular dimensions are visualized. Linear dimensions include major (superior RA wall to bisector of tricuspid annular dimension) and minor (lateral wall to interatrial septum) axes. Mean and standard deviation for RA major and minor axes are listed in **Table 5**.

Current guidelines recommend using volume to calculate RA size, either by area–length or disk summation methods. Similar to calculating area, the RA is traced in the four-chamber view. A single-plane area–length measurement of the RA can be performed using this formula:

$$8/3\pi\left[(A)^2/L\right]$$

where A is the area defined by the tracing and L is the superior–inferior length of the atrium. The disk summation method is similar to that mentioned in previous sections; however in this case only uses a single plane with the assumption of a symmetrical cavity shape (**Fig. 14**). The mean and standard deviations for 2D echo-derived RA volume are listed in **Table 5** (Lang et al., 2015). 3D data sets may be used to calculate more accurate RA volume; however, this is not routinely performed in clinical echo labs.

RA Pressure

Right atrial pressure is commonly estimated in echocardiography using the IVC diameter and its response to respiration. Combining these two parameters allows a simplified method for estimating a reasonable RA pressure in many patients. The IVC diameter should be measured about 1–2 cm from the caval–atrial junction (**Fig. 15**). An IVC diameter of ≤2.1 cm that collapses >50% with a sniff supports an estimated RA pressure of 3 mm Hg (range, 0–5 mm Hg). An IVC diameter of >2.1 cm that collapses <50% with a sniff supports an estimated RA pressure of 15 mm Hg (range, 10–20 mm Hg). Cases that are indeterminate due to discrepant findings between IVC size and its response to a sniff suggest an estimated RA pressure of 8 mm Hg (range, 5–10 mm Hg). This method allows individualized echo-specific factors to be taken into account in assigning an RA pressure, which is preferable to assuming a fixed RA pressure across all patients regardless of what the imaging signals (Rudski et al., 2010).

Aortic Root

The aortic root is well visualized in the parasternal long-axis TTE view. This structure includes the aortic valve annulus, aortic leaflets, the sinuses of Valsalva, and the sinotubular junction (STJ), which lead to the tubular portions of the ascending aorta. The aortic annulus is a virtual ring (not a true anatomic structure) defined by joining the basal attachments of the three aortic valve leaflets. This measurement is important in sizing aortic valve replacements, especially in transcatheter aortic valve replacements (TAVR). This measurement may be challenging by transthoracic echocardiography and often requires measurements by transesophageal echocardiography or computed tomography allowing cross-sectional dimensions, perimeter, and area measurements. By TTE a linear anteroposterior measurement should be made in mid-systole when the aortic leaflets are maximally opened and the annulus is at its largest. It is measured in the parasternal long-axis view from inner edge to inner edge (I–I) between the hinge point of the right coronary cusp anteriorly and the fibrous trigone between the left and noncoronary cusps, seen posteriorly (**Fig. 16**).

The other aortic root measurements, including the sinuses of Valsalva, the STJ, and the proximal ascending aorta, should be made at end-diastole, perpendicular to the long axis of the aorta (**Fig. 16**). By 2D echocardiography, these measurements are made from leading edge to leading edge (L–L); however it is important to note that other modalities such as CT and MRI use other conventions. Echocardiography has not adopted these other conventions because the normative values are reported using the L–L standard. Some institutions utilize an inner edge to inner edge measurement convention, especially if this is being used to calculate flow such as stroke volume. See **Table 6** and **Fig. 17** for aortic root sizing reference values.

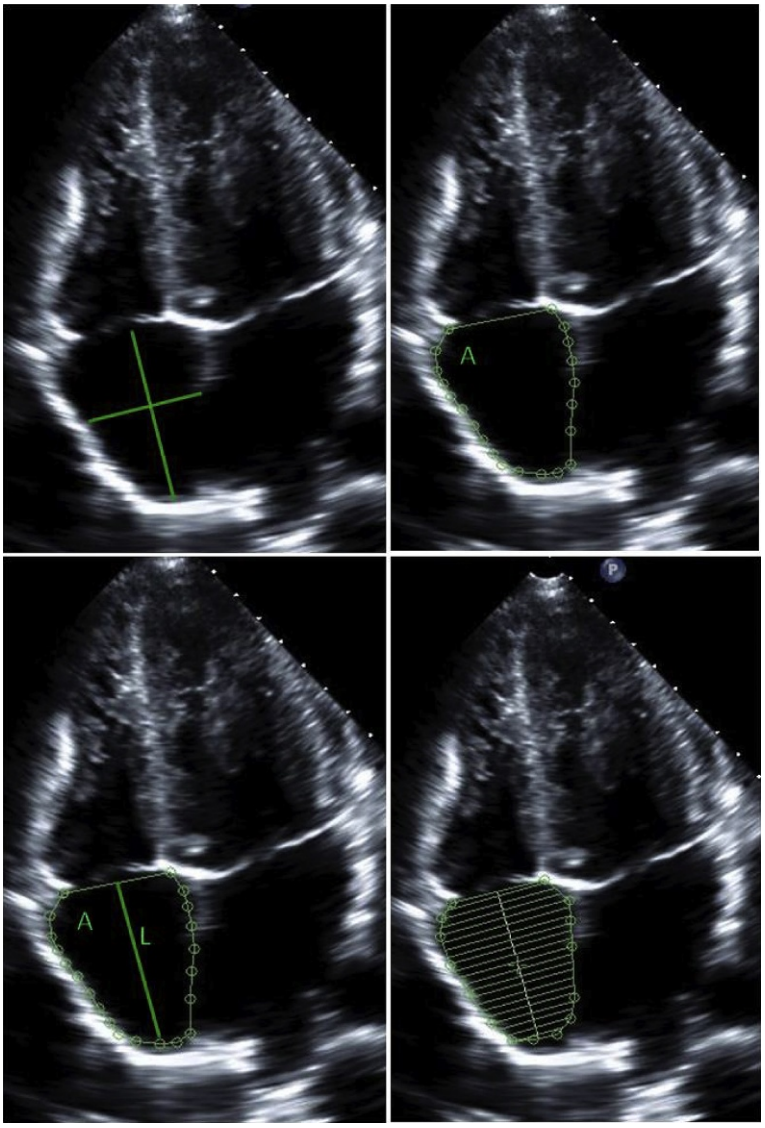

Fig. 14 Right atrial size assessment by 2D echocardiography. *Top left panel:* RA linear dimensions depicting the major (superior–inferior) and minor (lateral–septal) dimensions. *Top right panel:* 2D-derived area *(A)* using tracing of the blood–tissue interface within the right atrium. *Bottom row:* 2D volumetric methods, including the (bottom left) area–length method and (bottom right) disk summation method. *L*, length of atrium.

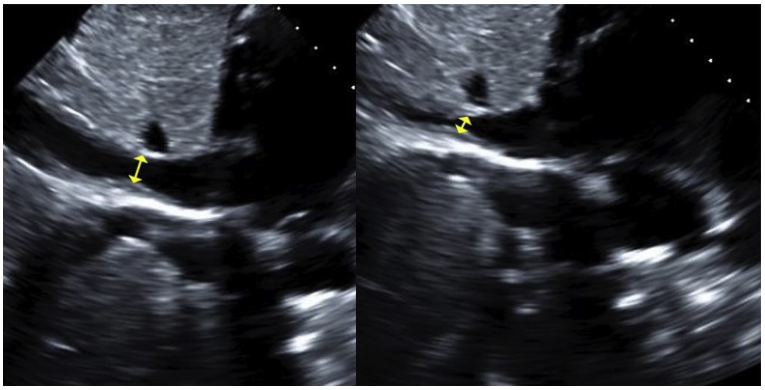

Fig. 15 Inferior vena cava measurements for estimation of RA pressure. The maximal size of the IVC and its response to respiration can be used to estimate RA pressure (see text). *Left panel:* maximum IVC diameter during inspiration and (*right panel*) its collapse during expiration.

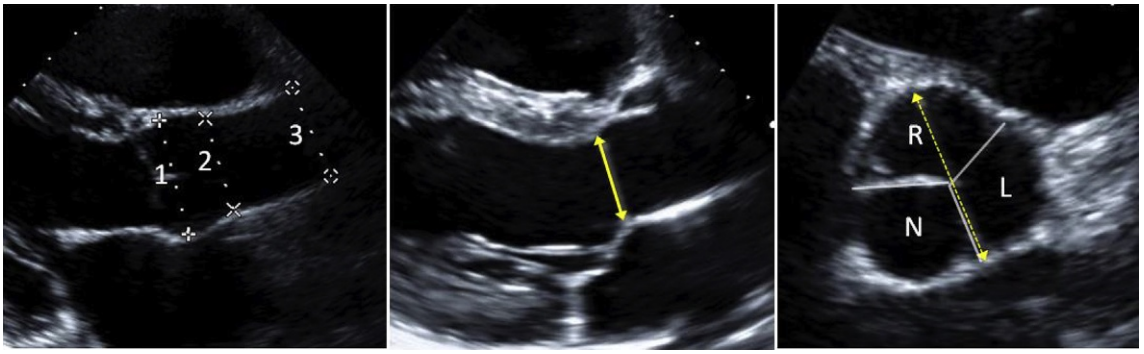

Fig. 16 Aortic root sizing by echocardiography. *Left panel:* Sinuses of Valsalva (1), sinotubular junction (2), and proximal ascending aorta (3) are measured here at end-diastole. *Middle and left panels:* Measurement at the aortic annular level, which should be measured in mid-systole. The arrow depicts parasternal long-axis (*middle panel*) measurement and corresponding parasternal short axis (*right panel*) with measurement from base of the right coronary cusp (*R*) to the fibrous trigone between the left (*L*) and noncoronary (*N*) *cusps.*

Table 6 Normal aortic room dimensions in adults

	Men		*Women*	
	Absolute (cm)	*Indexed (cm/m^2)*	*Absolute (cm)*	*Indexed (cm/m^2)*
Annulus	2.6 ± 0.3	1.3 ± 0.1	2.3 ± 0.2	1.3 ± 0.1
Sinuses of Valsalva	3.4 ± 0.3	1.7 ± 0.2	3.0 ± 0.3	1.8 ± 0.2
Sinotubular junction	2.9 ± 0.3	1.5 ± 0.2	2.6 ± 0.3	1.5 ± 0.2
Proximal ascending aorta	3.0 ± 0.4	1.5 ± 0.2	2.7 ± 0.4	1.6 ± 0.3

Adapted from Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., Flachskampf, F. A., Foster, E., Goldstein, S. A., Kuznetsova, T., Lancellotti, P., Muraru, D., Picard, M. H., Rietzschel, E. R., Rudski, L., Spencer, K. T., Tsang, W., Voigt, J. U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography* **28**, 1–39 e14.

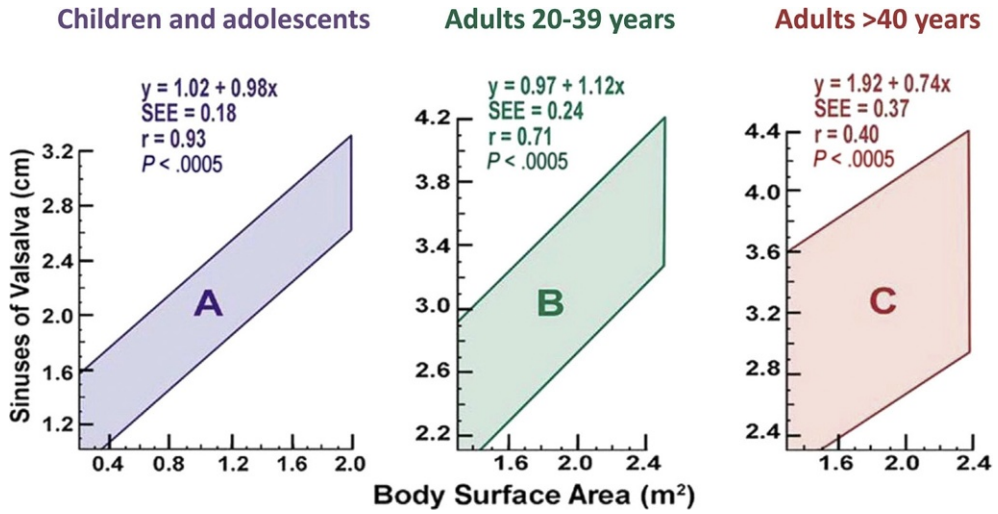

Fig. 17 Normal aortic root measurements indexed to BSA by age. The 95% confidence intervals for aortic root diameter at sinuses of Valsalva on the basis of body surface area (BSA) in children and adolescents (A), adults aged 20–39 years (B), and adults aged ≥40 years (C). Reproduced with permission from Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., Flachskampf, F. A., Foster, E., Goldstein, S. A., Kuznetsova, T., Lancellotti, P., Muraru, D., Picard, M. H., Rietzschel, E. R., Rudski, L., Spencer, K. T., Tsang, W., Voigt, J. U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography* **28**, 1–39 e14.

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Immune Mechanisms in Cardiac Physiology

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Introduction

The immune system fulfils an important sensory function in the detection of circumstances requiring immune-mediated response, repair, or regeneration. The importance of immune cell activation in the contexts of infection, inflammation, and injury repair is well established. Prominent features of an inflammatory response typically include increased production and mobilization of white blood cells. Less is known, however, about the extent to which the immune system contributes to tissue health at homeostasis.

Immune cells govern tissue homeostasis and debris management during physiologic cell turnover, but these roles are subclinical and thus may remain undetected by clinically relevant outcome measures such as elevation in white blood cell counts in the peripheral blood stream.

Homeostasis in the Adult Heart

At homeostasis, resident tissue macrophages are present within the myocardium and facilitate the clearance of apoptotic cardiomyocytes during normal cell turnover. While there is heterogeneity in macrophage subsets, basic functions of macrophages include sampling of the environment by phagocytosis and endocytosis, receptor and antigen presentation, and production of secretory factors that can modulate the immune response. Macrophages also contribute to immune quiescence at steady state by depleting chemotactic proteins, such as integrins and selectins, which mediate leukocyte infiltration. Macrophages contribute to angiogenic balance at homeostasis by interacting with endothelial cells both by direct contact and by secretion of pro- or anti-angiogenic paracrine factors. In vitro study of cultured macrophages has led to characterization of a proinflammatory phenotype, defined by the production of proinflammatory cytokines, and an antiinflammatory phenotype, defined by production of antiinflammatory factors. In vivo macrophage phenotype classification is more complex, however, and is best understood as a spectrum of macrophage phenotype and function determined by multiple environmental factors. Of note, resident cardiac tissue macrophages can also contribute to fibrosis at steady state by activation of fibroblasts, a process that increases with age. In general, macrophages proliferate locally during steady state to replenish the resident cardiac macrophage population. While resident cardiac macrophages are the predominant immune cell subset observed in the adult heart at homeostasis, small numbers of resident mast cells, dendritic cells, and lymphocytes are also present.

Innate Immunity in the Heart at Homeostasis

In addition to the role of tissue macrophages in efficiently clearing cell debris caused by cell turnover, pattern-recognition receptors (PRRs) expressed on innate immune cells also contribute to maintenance of homeostasis. PRRs, including toll-like receptors (TLRs), recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), and they also play a role in mediation of sterile inflammation, as in the case of ischemia–reperfusion injury. PRRs can lead to activation of inflammatory signaling pathways, such as those mediated by nuclear factor-κB (NFκB). Subsets of PRRs, including nucleotide-binding oligomerization domain (NOD)-like receptors, can also initiate assembly of inflammasomes, which are cytosolic complexes that trigger production of proinflammatory cytokines interleukin (IL)-1β and IL-18.

Adaptive Immunity in the Heart at Homeostasis

Regulatory T (T_{Reg}) cells, characterized by $CD4^+CD25^+FOXP3^+$ cell surface marker expression, are a subset of helper T cells that contribute to maintenance of homeostasis and tolerance in the heart. T_{Reg} cells suppress effector T cells by contact-dependent and contact-independent mechanisms. Loss or disruption of T_{Reg} function is associated with the development of chronic inflammation and autoimmunity. T_{Reg} cells also produce the inhibitory cytokines transforming growth factor β (TGFβ) and IL-10, which contribute to their immunosuppressive profile. While modulation of T_{Reg} cell number or function may theoretically be an attractive therapeutic approach to diseases characterized by an excessive immune response, T_{Reg} cell therapies are only in the very early stages of development.

Immune Contributions During Injury and Repair

Ischemic injury is a predominant cause of myocardial injury, resulting in necrosis-dependent release of intracellular components and other DAMPs. Ischemia and ischemia–reperfusion injury cause mast cell degranulation and activation of endothelial cells, both of which contribute to a proinflammatory amplification loop mediated by histamine, tumor necrosis factor alpha (TNFα), complement activation, reactive oxygen species (ROS), and IL-1β. Increased production of proinflammatory cytokines and chemokines leads to the recruitment of neutrophils and exogenous monocytes and macrophages (Frangogiannis, 2014). The temporal course of CD45 positive immune cell infiltration into the heart following injury by surgically induced myocardial infarction in mice is demonstrated in Fig. 1. Most experimental knowledge about the time course of immune cell contribution following infarction is derived from mouse models due to the limited resources available to study these time-dependent changes in large animal models.

Neutrophil infiltration peaks at 24 hours after myocardial infarction in mice, resulting in widespread phagocytosis of tissue debris, neutrophil degranulation, and release of proinflammatory cytokines and factors. Subsequent monocyte and macrophage recruitment is largely dependent on neutrophil-derived chemokine (C-C motif) ligand 2 (CCL2, also known as monocyte chemotactic protein 1 (MCP-1)), and typically peaks at 3–5 days after injury, with a proinflammatory subset predominating at day 3 and an antiinflammatory subset predominating later, at 5–7 days following injury (Nahrendorf et al., 2007). Despite limitations of macrophage subset classification, resident cardiac tissue macrophages are generally considered to more closely

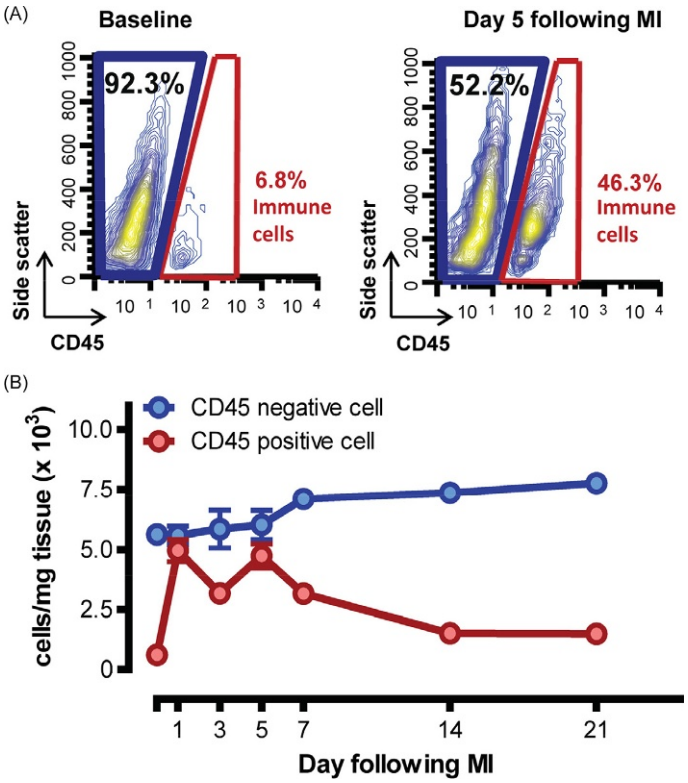

Fig. 1 Infiltration of CD45 positive immune cells into the heart following myocardial infarction (MI). (A) Flow cytometric analysis of cell suspensions derived from whole heart tissue prior to (baseline) and at day 5 following myocardial infarction induced by ligation of the left coronary artery. CD45 was used as a pan-leukocyte marker. (B) Temporal course of changes in CD45 positive and negative cell counts following myocardial infarction as demonstrated by cells per mg of tissue.

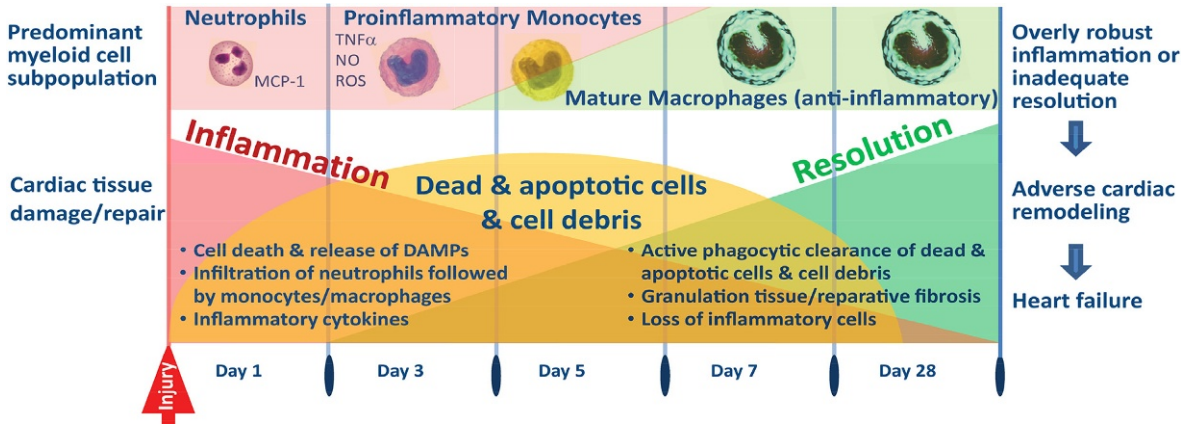

Fig. 2 Schematic overview of the temporal course of acute cardiac tissue damage and repair following myocardial injury. There are two major phases following myocardial injury due to infarction, an early inflammation phase and a subsequent resolution phase. An appropriate balance between these two phases is required for optimal repair. In the inflammation phase, high cytokine production leads to rapid neutrophil influx, followed by proinflammatory monocytes, and the level of dead and apoptotic cells is high. During the resolution phase, maturation of antiinflammatory macrophages corresponds to phagocytic clearance and reparative fibrosis. Over time, excessive inflammation or inability to adequately resolve it during the resolution phase can both contribute to adverse cardiac remodeling and structural changes in the heart that can lead to heart failure. *MCP-1*, monocyte chemotactic protein 1; *TNFα*, tumor necrosis factor alpha; *NO*, nitric oxide; *ROS*, reactive oxygen species.

resemble the antiinflammatory phenotype, whereas blood-derived infiltrating monocytes are considered to be the predominant source of proinflammatory macrophages in the first few days after injury and antiinflammatory macrophages by day 7 (Pinto et al., 2014). Synthesis of highly vascularized connective tissue, referred to as granulation tissue, then gives rise to the final stage of repair characterized by fibroblast activation and reparative myocardial fibrosis. Following resolution of injury, the macrophage population is replenished by local proliferation. A summary of this process is shown in Fig. 2.

Although neutrophils and monocytes/macrophages predominate in the two major phases of myocardial remodeling, other immune cells also play a role in cardiac remodeling after injury. Local perivascular mast cell degranulation is an important source of inflammatory mediators. Mast cells also contribute to fibroblast and vascular cell activation during injury repair. After injury caused by myocardial infarction, both B and T cells are recruited to the injured myocardium. Mouse models have demonstrated that B lymphocytes contribute to myocardial recruitment of proinflammatory monocytes, and that depletion of B cells attenuates the extent of myocardial damage induced by proinflammatory monocytes (Zouggari et al., 2013). B cell depletion is thus a promising potential therapeutic approach for further exploration. T cell contribution to myocardial remodeling has been studied more extensively and is covered in the following sections.

T Cell Role in Repair Following Injury

T cell programming in the thymus assists in creating a population of T cells that can distinguish between self and nonself, a process critical for ensuring that foreign pathogens are appropriately targeted but host tissue is not. Despite this tightly controlled process, antigenic cross-reactivity and other pitfalls of this process can result in T cells that inappropriately attack host tissue, also known as autoimmunity. In the context of host tissue damage, such as myocardial infarction, there is widespread release and exposure of intracellular antigens that can result in T cell sensing of host tissue damage and subsequent T cell activation by costimulatory signals mediated by other cell surface receptors. There are many T cell subsets, typically defined by cell surface marker expression and associated with different stages of T cell development and activation. This section will focus on CD4+ T helper cells and CD8+ cytotoxic T cells. During differentiation, T helper cells are generally shunted toward one of the two major subsets of effector T cells, proinflammatory Th1, or antiinflammatory Th2 cells, as determined by the cytokine milieu. A third subset of T helper cells, $CD4^+CD25^+FOXP3^+$ T_{Reg} cells, are also commonly considered to be antiinflammatory, in part by inhibiting the proinflammatory Th1 response. One limitation of current knowledge is that analysis of T cell subpopulations in the peripheral blood, the most common source for human T cell subset analysis, does not accurately reflect T cell subpopulations in tissue. A related limitation is that while human tissue biopsies of myocardium or draining lymph nodes would be ideal, such invasive samples are difficult to obtain.

Contribution of T cell subsets to cardiac repair

Following myocardial infarction, there is T helper cell activation and proliferation in lymph nodes draining from the heart. The process of activation and infiltration of expanded T helper cells into the myocardium can take a few days and may not peak until day 7 following myocardial infarction in mouse models. T helper cell infiltration is characterized by a predominance of proinflammatory Th1 cells along with a smaller subset of T_{Reg} cells. Once present, proinflammatory Th1 cells appear to contribute to the differentiation of infiltrating monocytes. Absence of $CD4^+$ T cells is associated with a poor outcome following myocardial infarction, suggesting a beneficial role in this context. In contrast, experimental models have established that $CD4^+$ T cells have a detrimental effect in the setting of myocardial ischemia–reperfusion injury, which is mediated mainly through production of interferon gamma (IFNγ) (Yang et al., 2006). The role of $CD8^+$ T cytotoxic cells following myocardial infarction has not yet been clearly established and depletion of $CD8^+$ cells does not yield a significant difference in infarct size in mouse models of myocardial infarction. However, a small subset of angiotensin 2 receptor positive CD8+ T cells present in the peri-infarct zone can produce the antiinflammatory cytokine IL-10. Intramyocardial transplantation of these cells decreases infarct size, suggesting a cardioprotective role following injury (Curato et al., 2010). One common mechanism involved in switching T cells from deleterious to beneficial activation is related to adenosinergic signaling. Myocardial hypoxia raises the extracellular concentration of adenosine from submicromolar to several tens of micromolar, which induces activation of A2 adenosine receptors on T cells. Stimulation of A2 adenosine receptors results in the inhibition of Th1 cytokines, including TNFα and IFNγ, ROS and cytotoxic T cell activity. In addition, adenosine, acting via A2A receptors, elicits production of brain-derived neurotrophic factor, a growth factor with cardioprotective properties, from both $CD4^+$ and $CD8^+$ lymphocytes. $CD8^+$ T cells are also presumably implicated in the activation of T helper cells by presentation of autoantigens on major histocompatibility complex (MHC)-II molecules following myocardial damage, but further work is needed to determine the identity of these autoantigens. Of note, age-related decreases in T cell diversity and expansion of clonal $CD8^+$ memory T cells may contribute to immunosenescence, characterized by decreased adaptive immunity, increased frequency and severity of disease, and presence of chronic inflammation in the elderly (Hoffmann and Spyridopoulos, 2015).

T_{Reg} contribution to cardiac repair

T_{Reg} cells are generally considered to be beneficial following myocardial infarction by inhibiting excessive inflammation and to be protective in heart failure by limiting fibrosis. Molecular mechanisms involved in T_{Reg} cell-dependent inhibition of inflammation include secretion of antiinflammatory factors such as TGFβ and IL-10. T_{Reg} cells constantly express cytotoxic T lymphocyte-associated molecule-4 (CTLA-4) which is a powerful negative regulator of T cell activation. Inhibition of the Th1 response by T_{Reg} cells prevents lymphocyte-induced secondary myocardial tissue damage. The coordinated action of two ectonucleotidases, CD39 and CD73, expressed on T_{Reg} results in the generation of adenosine from adenosine triphosphate (ATP), released from apoptotic and dying cardiomyocytes. The endogenous purine nucleoside adenosine is a potent immunosuppressive and proangiogenic agent, which also contributes to T_{Reg} cell-mediated cardiac repair after myocardial ischemic injury.

Dendritic Cell Role in Repair Following Injury

Dendritic cells are professional antigen-presenting cells that reside in lymphatic tissue and in the heart. Dendritic cells are markedly increased in the infarcted and border zone myocardium after ischemic injury, and they are activated by endogenous toxins such as molecules released from necrotic cells. Upon activation, dendritic cells migrate to secondary lymphoid organs, where they stimulate the clonal expansion and differentiation of effector T cells. Homing of activated T cells into the injured tissue may further amplify local inflammation. The increase in the proinflammatory Th1/Th2 balance is documented in patients with acute myocardial infarction, and it is associated with left ventricular dilation and systolic dysfunction. The presence of autoimmunity to cardiac myosin, actin, and troponin is associated with adverse clinical outcomes after myocardial infarction. Lymphocytes obtained from rats following myocardial infarction can injure normal cardiomyocytes in vitro, and adoptive transfer of splenic lymphocytes from animals after myocardial infarction results in induction of heart failure. Experiments in animal models have also demonstrated that activated and self-antigen loaded dendritic cells induce myocarditis and heart failure in susceptible mice. The exact mechanisms for the induction of autoimmunity and its contribution to myocardial injury require further investigation, but growing evidence indicates that dendritic cells may have a significant role in the excessive inflammatory response after myocardial infarction. However, treatment of dendritic cells with troponin I and IL-37, an inhibitor of innate and adaptive immunity, can induce a semimature tolerogenic phenotype which alleviates adverse cardiac remodeling following myocardial infarction (Zhu et al., 2016). Tolerogenic dendritic cells are characterized by lower levels of MHC-II and CD40 expression, decreased proinflammatory cytokine production, and increased antiinflammatory IL-10 production. Following myocardial infarction, adoptive transfer of tolerogenic dendritic cells increases T_{Reg} cell number, decreases myocardial immune cell infiltration, decreases fibrosis, and improves cardiac functional outcomes.

Inflammatory Cardiomyopathies

Inflammatory cardiomyopathy refers to heart muscle inflammation characterized by the influx of immune cells and the production of cytokines which can cause pain, alterations in blood flow, functional changes, and tissue destruction. This section focuses primarily on the pathophysiology and clinical manifestations of inflammatory cardiomyopathies in humans. For a summary of small animal models of cardiomyopathies, we refer readers to a recent review by Camacho et al. (2016).

Myocarditis

Myocarditis is a heterogeneous group of conditions encompassing infectious and noninfectious etiologies as well as unknown etiologies. There are many known causes of myocarditis, and the most common of these will be discussed here.

Viral myocarditis

Common viruses reported to cause myocarditis include enterovirus, adenovirus, influenza viruses, human herpes virus-6 (HHV-6), Epstein-Barr-virus, cytomegalovirus, hepatitis C virus, and parvovirus B19. For a comprehensive listing of all viruses known to cause myocarditis, we refer readers to a 2013 Position Statement on myocarditis prepared by the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases (Caforio et al., 2013). Diagnosis is typically confirmed by positive Polymerase chain reaction (PCR) amplification of viral genetic material.

The natural progression of viral myocarditis typically involves an initial period where viral replication is occurring intracellularly. Infection may be subclinical at this stage. This is followed, 2–4 weeks later, by an immune response with contributions from both antibody and cell-mediated immune mechanisms. The antibody response may have autoimmune features, including cross-reactivity of antibodies against viral antigens with host cardiac tissue or host cardiac tissue damage leading to exposure of self-antigens leading to an immune response. The next phase in the progression of viral myocarditis involves either (1) resolution and functional improvement or (2) chronic inflammation and/or impairment leading to dilated cardiomyopathy.

Nonviral infectious myocarditis

Nonviral infectious myocarditis can occur in the setting of a number of different bacterial (including rickettsial and spirochetal), protazoal, mycotic, or helminthic infections. While an exhaustive list of causative infectious agents is beyond the scope of this article, it is important to note that myocarditis is a heterogeneous clinical entity that encompasses inflammation caused by a wide variety of triggers, many of which have distinct impacts on disease progression and prognosis despite shared myocardial inflammatory pathway endpoints such as arrhythmias or heart failure.

Lymphocytic and giant cell granulomatous myocarditis

Both lymphocytic and giant cell granulomatous myocarditis involve significant infiltration of immune cells into the myocardium, with subsequent inflammation-mediated myocardial damage. Both entities are believed to be triggered by infectious myocarditis.

Lymphocytic myocarditis

Lymphocytic myocarditis is typically characterized by new onset of heart failure in the setting of systemic viral infection. Biopsy reveals predominant lymphocytic infiltrates into the myocardium. Notably, granulomas are not typically present, which can aid in distinguishing it histologically from other types of inflammatory myocarditis.

Giant cell granulomatous myocarditis

Giant cell granulomatous myocarditis is a rare and often fatal form of myocarditis characterized by the presence of infiltrating immune cells, multinucleated giant cells, granulomas, myocyte hypertrophy, necrosis, and interstitial fibrosis on histopathological analysis. Treatment may require immunosuppressive agents, such as cyclosporine, cardiac device implantation, and heart transplantation. Common complications, which may be due in part to treatment, include renal failure, ventricular arrhythmias, and heart failure.

Drug-induced myocarditis

Drug-induced myocarditis is a form of hypersensitivity reaction that can occur in response to a wide variety of different medications. This category includes drug rash with eosinophilia and systemic symptoms (DRESS)-associated myocarditis. Two common classes of instigating drugs include aromatic anticonvulsants and sulfonamides. Several other drugs implicated in the development of DRESS-associated myocarditis include allopurinol, calcium channel blockers, nonsteroidal antiinflammatory drugs (NSAIDs), antiretrovirals, angiotensin-converting enzyme inhibitors, diuretics, inotropes, and other types of antibiotics and antifungal agents. Additional clinical features might include fever, swelling, lymphadenopathy, rash, chest pain, hemodynamic instability, hepatitis, nephritis, interstitial pneumonitis, and atypical lymphocytosis. Myocarditis can occur early or late in the clinical course, and is characterized by infiltration of eosinophils and lymphocytes, which can be patchy and may not be adequately captured by endomyocardial biopsy. In severe cases, progression to acute necrotizing eosinophilic myocarditis results in cardiomyocyte necrosis and extensive tissue damage. Prompt cessation of the offending drug and immunosuppressive therapy are typically required for optimal treatment outcome, although the reaction can sometimes progress to multiorgan failure and death, even after discontinuation of the inciting medication. Standard heart failure medications, including diuretics and angiotensin-converting enzyme inhibitors (provided that neither was implicated as the cause of the drug-induced myocarditis), and beta-blockers can be helpful in some cases. In fulminant myocarditis with heart failure refractive to therapy, ventricular assist devices (VAD) can be life-saving. While eosinophils are a prominent feature of drug-induced myocarditis, it is important to note that there are several other nondrug-related etiologies of hypereosinophilic syndromes, such as neoplasm, familial, and idiopathic, which can also result in eosinophilic myocarditis.

Idiopathic inflammatory cardiomyopathy

Idiopathic inflammatory cardiomyopathy is primarily a diagnosis of exclusion, in which all established causes of inflammatory cardiomyopathy have been ruled out. This is also referred to as virus-negative inflammatory cardiomyopathy, even though the etiology may still be due to a virus that was not specifically tested for. Furthermore, the ability of viruses to mutate and recombine indicates that diagnostic capabilities may never be fully at pace with viral evolution.

Myocarditis diagnosis and therapy

Suspected myocarditis may be evaluated by detailed clinical history, electrocardiography, echocardiography, and cardiac MRI (magnetic resonance imaging). While histopathological analysis of an invasive endomyocardial biopsy has historically been required to definitively confirm the diagnosis, this is falling out of favor with advancements in cardiac MRI. The clinical presentation of myocarditis might include chest pain, heart failure, or symptoms relating to arrhythmia, but it is important to emphasize that the clinical spectrum of myocarditis spans from subclinical disease to fulminant disease with associated heart failure and life-threatening ventricular arrhythmias. In some cases, sudden cardiac death may be the first overt clinical manifestation of the disease, as determined on postmortem analysis. When endomyocardial biopsy is performed, the specimens should be analyzed for evidence of infectious agents, which may include PCR analysis for the detection of cardiotropic viruses. Blood specimens may also be screened for evidence of autoantibodies, such as those targeting membrane proteins, mitochondrial proteins, or structural proteins. Therapy options are dependent on the determined etiology of the inflammation and may include immunosuppressive therapy, appropriate antimicrobial therapy as applicable, and the placement of implantable cardioverter defibrillators (ICD) or VAD, in the case of severe systolic failure, as indicated. Spontaneous functional improvement or even full recovery may be observed in the weeks following diagnosis, so this should be factored into decision-making about treatment, and some therapeutic interventions may be delayed for 3–6 months when appropriate to do so in order to gauge the extent of the predicted recovery. Given the clinical heterogeneity of this disease, it has been proposed that patients be classified according to risk level as low, intermediate, or high risk, and that these designations might help guide diagnostic decisions as well as therapeutic management (Sinagra et al., 2016). Due to the complexity of the disease, however, these decisions must often be made on a case-by-case level.

Sepsis-Induced Myocardial Dysfunction

Sepsis is a clinical syndrome comprised of a dysregulated systemic inflammatory response that can quickly progress to multiple organ dysfunction syndrome and death. Septic patients may be at high risk for myocardial dysfunction due to the primary cause of

sepsis, such as the direct effect of an infectious agent, or due to the secondary effects of the systemic inflammatory response. Postulated contributing factors include circulating toxins, cytokines, complement activation, apoptosis, mitochondrial damage, catecholamine storm, and metabolic derangements, such as severe acidosis. Myocardial dysfunction in this setting is typically characterized by global myocardial systolic and diastolic dysfunction, or Takotsubo cardiomyopathy with apical akinesis and ballooning being the most classic manifestation.

Cardiac Sarcoidosis

Cardiac sarcoidosis is characterized by infiltrating noncaseating granulomas with associated inflammation and scar formation, leading to ventricular dysrhythmias, sudden cardiac death, atrioventricular conduction block, and development of heart failure. Cardiac MRI and PET–CT (positron emission tomography–computed tomography) are the current preferred modalities for the diagnosis of cardiac sarcoidosis, in part because the sensitivity of endomyocardial biopsy is relatively low due to the patchy nature of the disease. There is no curative treatment, as the cause is poorly understood and the disease process is quite unpredictable, but systemic immunosuppressive therapy has been observed to produce some benefit in limiting active disease and improving left ventricular function, which fits with the contemporary belief that there is a significant immune-mediated component to the disease. Sarcoidosis is typically a systemic illness with the potential to affect nearly any organ system, with mortality highest from pulmonary or cardiac involvement.

Dilated Cardiomyopathies

The clinical categorization of dilated cardiomyopathies includes a polymorphic spectrum of disorders characterized by ventricular dilation and functional impairment in the absence of hypertensive, ischemic, valvular, or congenital heart disease. In essence, patients with inflammatory cardiomyopathy may develop dilated cardiomyopathy, but not all patients with dilated cardiomyopathy have inflammatory cardiomyopathy. The term nonischemic cardiomyopathy encompasses the range of dilated cardiomyopathies due to several different nonischemic causes, such as doxorubicin, alcohol, familial, or viral, among others. Nonischemic cardiomyopathy is the underlying diagnosis leading to heart transplant in 64% of patients in the 18–39 age group, according to the Registry of the International Society for Heart and Lung Transplantation (Lund et al., 2016).

Diagnosis of Cardiomyopathies

Given the relatively recent and rapidly evolving interdisciplinary field of immune mechanisms in cardiac physiology, it is not surprising that there is a lack of consensus on the classification or management of immune-mediated cardiomyopathies. Much of the progress in this field has been forged by nonevidence-based expert opinion on best practices for the diagnosis, classification, and management of this heterogenous group of disorders. One major hindrance to progress in this area is the limited availability of human tissue from endomyocardial biopsies which might allow for more patient-tailored therapies.

Current Therapeutics for Inflammatory Cardiomyopathy

Specific treatment of inflammatory cardiomyopathy is partially dependent on the etiology, but management may also include general therapies common to all causes. These include therapy for heart failure, primary and secondary prophylaxis for arrhythmias, and anticoagulation when indicated. In cases refractory to optimal medical therapy, mechanical support and ultimately transplantation may be indicated. NSAIDs have no demonstrated benefit in the treatment of inflammatory cardiomyopathy and often increase mortality risk. Alcohol and smoking should generally be avoided, and exercise should also be avoided during acute myocarditis. Given the high prevalence of autoantibodies present in certain types of myocarditis, apheresis has been proposed as a possible therapy. Apheresis involves an extracorporeal circuit to separate whole blood into cells and plasma, with removal of antibodies from the plasma and reintroduction of treated plasma and cells into the body. Small initial clinical studies have been very promising, demonstrating improvement in left ventricular ejection fraction, but large randomized controlled studies have not yet been completed to determine if there is a clear benefit to this form of therapy.

Autoimmune Mechanisms

While a moderate amount of inflammation in an acute setting is beneficial for wound healing, a dysregulated inflammatory response can produce an array of chronic inflammatory or autoimmune diseases. Many autoimmune diseases are associated with greatly increased risk of adverse cardiovascular events. The etiology of autoimmune disorders is broad and may include genetic and epigenetic factors, environmental, infectious, and other as yet undiscovered factors.

Determination of Autoimmune Etiology of Myocarditis

In autoimmune myocarditis, autoantibodies may or may not be present and they may or may not be pathogenic. Autoantibodies may be present as a biomarker of disease, or they may be absent, as in cell-mediated autoimmune diseases. In some cases,

myocarditis may be presumed to be of autoimmune etiology when all other known causes are ruled out. In addition, evidence from mouse models suggests that some individuals may be genetically predisposed to autoimmune myocarditis based on MHC genes or other non-MHC genes (Caforio et al., 2013).

Autoimmune Myocarditis

The connection between infection and autoimmune disease processes is well established. Viral myocarditis, for example, is considered to be a common triggering factor for the development of autoimmune myocarditis. Several theories exist for the mechanism by which infection leads to autoimmunity. Molecular mimicry describes the phenomenon by which there is accidental cross-reactivity between foreign antigens and self-antigens. The cryptic antigen theory posits that hidden antigens are exposed or released by tissue damage. The adjuvant effect describes an immune-boosting effect, which may result in activation of preexisting autoreactive immune cells. The dual T cell receptors theory suggests that two different T cell receptors on a single T cell cause dual reactivity to both foreign and self-antigens, leading to autoimmunity in the setting of infection. Of note, existing theories do not provide a good explanation for observed sex differences in the prevalence of many autoimmune conditions. Unlike most autoimmune diseases that more commonly affect women, autoimmune myocarditis rates are higher in males than in females, and males typically experience a higher rate of progression to dilated cardiomyopathy. While the mechanism for progression from viral myocarditis or autoimmune myocarditis to dilated cardiomyopathy is not well understood, there are some characteristic and overlapping features of immune mechanisms leading to resolution or pathology that are summarized in **Fig. 3**.

Secondary Cardiac Manifestations of Systemic Autoimmune Diseases

Atherosclerosis is a systemic inflammatory condition characterized by lipid and immune cell deposition in vessel walls. There are overlapping features of atherosclerosis and autoimmune disease, and patients with autoimmune diseases often have accelerated atherosclerotic progression. Adverse cardiac outcomes in patients with systemic autoimmune disease are likely due to a combination of traditional and nontraditional risk factors, such as the presence of autoantibodies, genetic polymorphisms, and chronic inflammatory status. The systemic autoimmune disease category includes patients with spondyloarthropathies such as psoriatic arthritis and ankylosing spondylitis, who have accelerated rates of atherosclerotic cardiovascular disease and higher rates of cardiovascular mortality. Patients with systemic sclerotic diseases, which can result in vascular and myocardial fibrosis, also experience accelerated cardiovascular disease. Common adverse manifestations of cardiac fibrosis associated with systemic sclerotic diseases include coronary artery disease, arrhythmias, and left ventricular diastolic dysfunction. Cardiac manifestations of antiphospholipid syndrome, a known prothrombotic state, include increased thrombotic events, coronary artery disease, and valvular sclerosis due to immune complex deposition.

Systemic lupus erythematosus is a common systemic autoimmune disease that most commonly affects young women and is associated with substantially increased rates of cardiovascular mortality. Cardiac manifestations of the disease may include coronary artery disease and increased myocardial infarction rates. Rheumatoid arthritis, an autoimmune joint disease with systemic manifestations, is also associated with increased cardiovascular mortality rates, most often due to inflammation promoting coronary artery disease. Of note, sudden cardiac death is more common in these patients than in the general population due to subclinical ischemic heart disease.

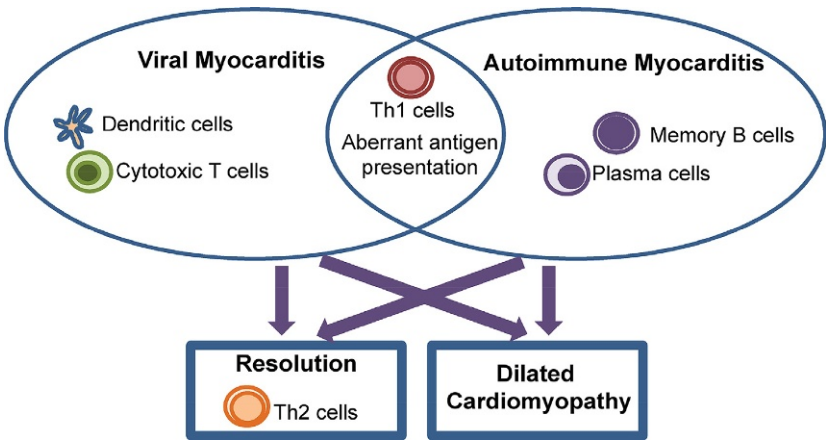

Fig. 3 Schematic overview of predominant immune cell types and overlapping immune mechanisms leading from viral myocarditis or autoimmune myocarditis to resolution or dilated cardiomyopathy. In viral myocarditis, antigen-presenting dendritic cells activate cytotoxic T cells favoring a proinflammatory Th1 response. In contrast, autoimmune myocarditis is primarily B cell-mediated, with contributions from both memory B cells and antibody-secreting plasma cells which also favor a proinflammatory Th1 response. Common immune mechanisms of both viral and autoimmune myocarditis include a proinflammatory Th1 shift and aberrant antigen presentation mediating host tissue destruction and progression to dilated cardiomyopathy. Antiinflammatory Th2 cells, however, can assist in driving the balance back toward resolution of inflammation.

Postpericardiotomy Syndrome

Open heart surgery, device implantation, or other forms of cardiac manipulation or trauma can lead to postpericardiotomy syndrome, an aseptic inflammatory response characterized by fever, pericarditis, and the potential for pleural effusions. It can occur in the weeks or months following cardiac injury, and is generally considered to be an autoimmune phenomenon triggered by exposure of cardiac antigens. Evidence for an autoimmune etiology includes the presence of autoantibodies and immune complexes and the absence of alternative known causes for the fever or other clinical manifestations of the syndrome, which might include leukocytosis, elevated erythrocyte sedimentation rate and C-reactive protein (CRP), new or worsening chest pain, or pleuritic chest pain. As with some other types of autoimmune syndromes, there is evidence that a common viral infection could be a trigger for development of autoimmunity in the postcardiac injury setting. Treatment includes NSAIDs, steroid, and colchicine. Progression to constrictive pericarditis or tamponade may require pericardiocentesis. Of interest, the neutrophil to lymphocyte ratio is reportedly higher in postoperative patients with postpericardiotomy syndrome than in postoperative patients without it, suggesting that it may be useful as a predictive biomarker in this setting (Sevuk et al., 2016). The neutrophil to lymphocyte ratio is a systemic inflammatory marker that correlates with poor prognosis in several cardiovascular conditions. It is also associated with other types of autoimmune conditions.

Cardiac Transplant Rejection

Following heart transplantation, immunosuppresive therapy greatly prolongs graft survival by reducing the incidence and severity of cellular and antibody-mediated rejection. As cardiac transplant and the pathophysiology of transplant rejection will be covered elsewhere in this encyclopedia, this topic will not be covered here. For a review of immune mechanisms in solid organ transplantation, we recommend one by Wood et al. (2012).

Immune Mechanisms in Pediatric Cardiovascular Disease

In contrast to adult patients, heart disease in the pediatric population is more likely to be due to nonischemic causes, including structural, infectious, and inflammatory etiologies.

Kawasaki Disease

Kawasaki Disease is an acute febrile systemic vasculitis of unclear etiology that can occur in early childhood. Epidemiological evidence of epidemics, community outbreaks, and seasonality suggests an infectious cause yet there is no evidence of person-to-person transmission. Genetic factors are believed to contribute as the incidence is highest in East Asia and among family members, but twin studies suggest that environmental factors are weighted more heavily than genetic predisposition. Many microbes or microbe-derived PAMPs are believed to cause Kawasaki disease by causing production and release of DAMPs from host cells, which then activate PRRs on endothelial cells and immune cells. PRR activation subsequently leads to inflammasome activation, as suggested by the characteristic increase in IL-1β levels, and production of chemokines, which attract neutrophils and monocytes/macrophages, with neutrophils predominating early in the blood and macrophages subsequently comprising the majority of infiltrated immune cells. Kawasaki disease is thus felt to be primarily mediated by the innate immune system. While the acquired immune system may play a minor role in disease pathogenesis, animal models suggest that the innate immune system can cause vasculitis even in experimental models lacking an acquired immune system. Kawasaki disease primarily affects the coronary arteries and can lead to coronary artery abnormalities in up to one third of patients if untreated. Other potential cardiac complications include cardiomyopathy, myocardial infarction, heart failure, and arrhythmias. The main treatment is intravenous immunoglobulin (IVIG) therapy and aspirin, and early diagnosis and treatment are both critical for achieving optimal therapeutic outcome. Although the disease is typically self-limited and treatment significantly lowers the risk, persistent coronary artery abnormalities such as aneurysms can also lead to increased risk of myocardial infarction, thrombus, and sudden cardiac death much later in life. Despite therapy, Kawasaki disease is the most common cause of acquired heart disease in children.

ABO-Incompatible Heart Transplantation

Children under 2 years of age are able to tolerate heart transplant from a donor with a different ABO blood type, a phenomenon mediated by the immaturity of the immune system in this young age group. ABO-incompatible transplantation takes advantage of the impaired ability to develop antibodies to polysaccharide antigens in the first 24 months of life. This is also what makes these young children particularly susceptible to severe infections caused by certain polysaccharide-encapsulated bacteria, such as Streptococcus pneumoniae, Neisseria meningitidis, and Haemophilus influenzae type B. At this early stage, the immune system is also able to develop long-term tolerance of the foreign polysaccharide antigens resulting from an incompatible transplant thus yielding outcomes that are equivalent to ABO-compatible transplants.

Immune Mechanisms in Heart Failure

Heart failure, characterized by impairment of pump function, is a common end pathway for various causes of myocardial injury. Our discussion will focus specifically on immune contributions to heart failure. While the role of the immune system in several types of cardiac injury has been well described, less is known about ongoing immune and inflammatory contributions in chronic heart failure trajectory. A summary of factors contributing to progression to heart failure is shown in Fig. 4.

Proinflammatory Cytokines

Several circulating proinflammatory cytokines tend to be elevated in chronic heart failure patients, regardless of the etiology. These include TNFα, IL-1β, and IL-6, galectin-3, TNF receptor 1, and TNF receptor 2 (Dick and Epelman, 2016). Despite strong evidence of systemic inflammation in chronic heart failure, clinical trials of steroid-based therapies targeting systemic inflammation have been largely unsuccessful. Animal studies of therapies targeting TNFα were initially very promising and demonstrated both worsening of heart failure with exogenously administered TNFα and improvement with TNFα-targeting antibodies. More specifically, TNFα is critically involved in the development of cardiac hypertrophy, fibrosis, and apoptosis of cardiomyocytes, all of which contribute to the progression of heart failure. These studies led to the initiation of several large clinical trials which, taken together, failed to demonstrate clinically significant improvement with TNFα blockade and instead revealed adverse cardiac outcomes associated with high dose therapy. In brief, the Randomized Etanercept North American Strategy to Study Antagonism of Cytokines (RENAISSANCE) and the Research into Etanercept Cytokine Antagonism in Ventricular Dysfunction (RECOVER) studies both involved treatment with the soluble TNFα receptor fusion protein etanercept and showed no benefit in heart failure (Coletta et al., 2002). The Randomized Etanercept Worldwide Evaluation (RENEWAL) trial combined and analyzed a large subset of patients from the RENAISSANCE and RECOVER trials and came to similar conclusions about the lack of effectiveness of TNFα blockade in chronic heart failure (Mann et al., 2004). The anti-TNFα in congestive heart failure (ATTACH) trial, involving treatment with the chimeric TNFα neutralizing antibody infliximab, also failed to demonstrate improvement in clinical status and adversely affected the clinical status of moderate-to-severe chronic heart failure patients at high doses (Chung et al., 2003). More recently, the TNFα pathway has been linked to cardioprotection in ischemia–reperfusion injury by facilitating mitochondrial autophagy, a conserved lysosomal degradative pathway for damaged mitochondria which might otherwise trigger cell death. Mitochondrial autophagy is mediated by TNF receptor-associated factor-2 (TRAF2) a scaffolding protein and downstream effector of TNF (Yang et al., 2015).

Autoantibodies and Heart Failure

The observance of autoantibodies in heart failure is common, even in patients with a nonimmune-mediated primary etiology for heart failure, such as ischemia, hypertension, structural, or mechanical causes. Autoantibodies associated with heart failure and cardiac dysfunction include antibodies against the β1 receptor, the M2 muscarinic receptor, mitochondria, myosin, cardiac troponin I, cardiac actin, NA/K ATPase, and several other proteins and intracellular components. Not surprisingly, these same types of autoantibodies can also be observed in the absence of heart failure, and it is as yet unclear whether autoantibodies play a causative

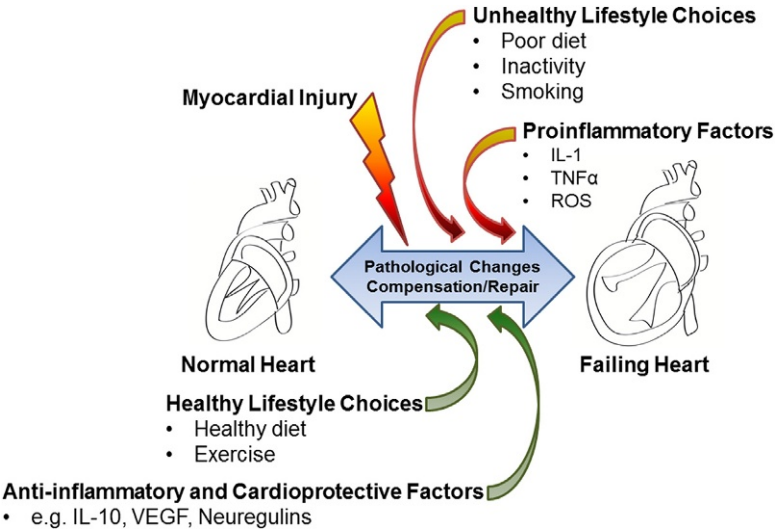

Fig. 4 Schematic overview of factors contributing to progression to heart failure. Following myocardial injury, unhealthy lifestyle choices and proinflammatory factors can expedite the progression to heart failure. In contrast, healthy lifestyle choices, antiinflammatory and cardioprotective factors can slow or reverse heart failure progression.

role in the progression of heart failure. Potential therapies for autoantibody removal include administration of IVIG and immunoadsorption with subsequent immunoglobulin substitution, although the mechanistic evidence supporting both of these therapeutic options is still very limited (Ohlow et al., 2017).

Systemic Inflammation and Cardiovascular Disease

Cardiac inflammation can be categorized as primary, in which the heart is the main organ involved, but it can also be categorized as secondary, in which heart involvement is a feature of more extensive involvement in one or more other organs. This section will address common causes of systemic inflammation leading to secondary cardiac involvement.

Atherosclerosis and Systemic Inflammation

Chronic inflammation can contribute to endothelial damage, which is often described as an initiating event in the development of atherosclerosis. Endothelial damage can be caused by an array of factors including hypertension, smoking, hypercholesterolemia, hyperglycemia, and aging. Once damaged, there is upregulation of endothelial adhesion molecules, recruitment and infiltration of leukocytes, low-density lipoprotein oxidation, inflammatory cell activation, and vascular smooth muscle cell proliferation and migration. Immune-mediated mechanisms of atherosclerosis have been extensively reviewed in the literature and are thus not a focus of this article. For a comprehensive review of this topic, including an update on clinical trials with currently available therapeutics, we recommend a review by Nus and Mallat (2016).

Circadian Contributions to Systemic Inflammatory Profile

Circadian rhythms are set by a combination of endogenous factors and external cues processed by the brain's suprachiasmatic nucleus. The vasculature also demonstrates evidence of circadian gene expression that can be maintained even in the absence of light and dark cues in experimental mouse models. Evidence from animal models demonstrates that disruption of circadian rhythms can contribute to progression of atherosclerosis, metabolic impairments, and systemic inflammation. Evidence from human studies has established higher rates of myocardial infarction, obesity, diabetes, and stroke in shift workers with disrupted circadian rhythms. It has thus been suggested that lack of sleep and/or dysregulation of circadian rhythms be considered as a cardiovascular risk factor. There are currently few mechanistic studies addressing molecular basis for circadian disruption on cardiometabolic profile, and this is an interesting area for future research.

Infection, Microbiota, and Systemic Inflammation

In addition to the previously described increases in cardiovascular mortality due to classic noninfectious systemic inflammatory conditions, such as rheumatoid arthritis, systemic lupus erythematosus, psoriasis, spondyloarthropathies, and systemic sclerotic diseases, several infectious causes of systemic inflammation have also been connected with increased cardiovascular mortality.

Human immunodeficiency virus

HIV infection can lead to chronic systemic inflammation with associated increases in adverse cardiovascular events. HIV patients have higher levels of lipopolysaccharide-induced endotoxemia, increased monocyte activation, and accelerated progression of atherosclerosis such that coronary artery disease development is earlier and more severe in this population. Evidence from clinical studies in HIV patients indicates that this population is at higher risk for subclinical or asymptomatic coronary artery disease. Plaques in HIV-infected patients are also more likely to be unstable, leading to increased incidence of rupture. With the success of antiretroviral therapies transforming HIV infection from a fatal illness to a manageable chronic disease, effective overall disease management requires increasing attention devoted to comorbidities such as cardiovascular disease. Given the increased risk for subclinical coronary artery disease, further research is needed about screening and management of cardiovascular risk in HIV positive patients.

CRP and systemic inflammation

CRP is a nonspecific marker of inflammation that is associated with increased cardiovascular mortality. CRP is elevated in many inflammatory conditions ranging from infection to atherosclerosis to autoimmune diseases. While it is well established that inflammation is associated with cardiovascular disease, further research is needed to determine causality and molecular mechanisms. Several ongoing clinical trials have been designed to address these important questions.

Emerging theory on gut and oral microbiome, inflammation, and cardiovascular risk

There is emerging acceptance that the gut microbiome contributes to overall cardiovascular health. Gut microbiota aid in digestion, contribute to the development of the immune system, and produce metabolites that regulate lipid profiles, glucose management, and adipose tissue storage. Recent estimates suggest that the human colon contains 3.9×10^{13} bacteria in an approximate 1:1 ratio

with the total number of cells in the human body (Sender et al., 2016). While bacteria reside throughout the body, the majority are contained in the colon. Strong evidence supports the interconnectedness of the gut microbiome with obesity, metabolic syndrome, hypertension, inflammatory status, and cardiovascular risk. Animal model development and human clinical studies are ongoing to determine mechanisms by which alterations in the gut microbiome can decrease cardiovascular risk. For example, vaccination with bacterial outer membrane proteins from common gut bacteria enhances adaptive immunity, alters intestinal flora composition, decreases atherosclerotic plaque immune cell infiltration, and promotes an antiinflammatory macrophage phenotype in an apolipoprotein E-null mouse model (Saita et al., 2016). Therapeutic interventions to alter the human gut microbiome are still in development and include prebiotics or probiotics, dietary alterations, targeted metabolic drug therapy, and fecal microbiota transplantation (Koopen et al., 2016). The oral microbiome is also likely to contribute to cardiovascular health. In humans, meta-analysis of clinical studies thus far indicates that periodontitis is strongly associated with hypertension, with the highest odds ratio observed with severe periodontitis (Martin-Cabezas et al., 2016). While the association between the gut and oral microbiome and cardiovascular risk is well established, further research is needed to elucidate biological mechanisms, confirm the intermediary role of systemic inflammation, and prove causality.

Conclusion

The topic of immune mechanisms in cardiac physiology is best understood in the wider context of immune mechanisms contributing to homeostasis. This perspective accommodates the vast field of emerging research on host microbiome contributions to cardiovascular risk factors such as obesity, metabolic syndrome, high blood pressure, and chronic inflammatory conditions. It highlights the benefit of expansion of interdisciplinary inquiry into the mechanisms by which the immune system and cardiometabolism are interconnected. Acceptance of the broader role of the immune system in homeostasis also encourages new advances in the mechanistic understanding of the development of autoimmunity following injury or infection.

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Immune-Mediated Mechanisms of Atherosclerosis

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Introduction

Atherosclerosis is a chronic process triggered by accumulation of cholesterol-containing low density lipoprotein (LDL) particles in the arterial wall (Hansson, 2005; Libby et al., 2011). The notion of atherosclerosis as an inflammatory disease has emerged based on observations of immune activation and inflammatory signaling in human atherosclerotic lesions, inflammatory biomarkers as independent risk factors for cardiovascular events, and an LDL-induced immune activation. The understanding of immune-mediated mechanisms of atherosclerosis may be crucial for the development of antiinflammatory therapies for atherosclerosis (Bäck and Hansson, 2015).

The initiating vascular inflammation may be promoted by several of the classical cardiovascular risk factors, such as hyperlipidemia, hypertension, diabetes, and cigarette smoking. Biomechanical factors, including the switch from a laminar to a turbulent flow at for example arterial caliber changes and bifurcations, may determine the predilection sites for atherosclerosis development (Bäck et al., 2013). At those locations, a combinatorial expression of endothelial adhesion molecules and leukocyte integrins and selectins mediate the recruitment of myeloid or lymphoid cell to initiate atherosclerosis development. The presence of retained LDL together with activated leukocytes marks that the atherosclerotic lesion is emerging, which is depicted in **Fig. 1** and further discussed in the following sections.

As the atherosclerotic plaque develops, smooth muscle cells (SMCs) and collagen form a cap to protect the lipid and inflammatory content from contact with the blood. Degradation of this fibrous cap surrounding the core of a coronary atherosclerotic lesion induces plaque rupture provoking acute thrombosis (Hansson et al., 2015) as depicted in **Fig. 2**. Destabilization of the atherosclerotic lesion and plaque rupture are stimulated by a number of inflammatory mediators and proteolytic enzymes (Ketelhuth and Bäck, 2011), which are released by the inflammatory cells within the lesion (**Fig. 2**).

Innate Immunity: Recognizing the Danger

Monocytes represent the most numerous white blood cells recruited into atherosclerotic plaques. Once resident in the arterial wall, they differentiate into tissue macrophages under the influence of mediators locally formed in the forming atherosclerotic lesions (**Fig. 1**). The retained and modified LDL (**Fig. 1**), for example oxidized forms of LDL (oxLDL), may serve as an initiating stimulus for inflammatory reactions, by being recognized as a danger-associated-molecular-pattern (DAMP) by specific pattern recognition, such as toll-like receptors (TLRs) (Edfeldt et al., 2002; Lundberg et al., 2013). Today at least 10 TLRs have been identified, some localizing to the cell surface (e.g., TLR4, TLR2) others found intracellularly (e.g., TLR3, TLR7) across the endosome membrane. Whereas TLR4 and TLR2 activation is detrimental in atherosclerosis, TLR7 has recently been assigned antiinflammatory properties by suppressing proinflammatory responses in macrophages (Koltsida et al., 2013; Salagianni et al., 2012). The translational implications of those findings are reinforced by the observation that TLR7 transcript levels in atherosclerotic lesions are associated with an improved outcome after vascular surgery (Karadimou et al., 2017), which will be further discussed below.

In addition to LDL, endogenous alarmins, also named as warning signals, are released into extracellular milieu during inflammation and tissue damage. Extracellular heat shock proteins, high mobility group box protein-1 and S100 proteins (Wick et al., 2014; Oesterle and Bowman, 2015; Harris et al., 2012), along with LDL particles and other alarmins, represent an important element of danger signals that promote inflammation associated with atherosclerosis.

Macrophages referred to as classically activated or M1 macrophages will sustain the inflammatory response and induce tissue damage. In contrast, the alternatively activated, or M2, macrophages secrete antiinflammatory mediators and may promote tissue repair and healing (Bäck et al., 2015) by means of a resolution of inflammation (*cf.* infra).

The uptake of lipoprotein particles will induce the conversion of macrophages into foam cells, a pathogenic process that results in the microscopic appearance of lipid-laden macrophages, which is a characteristic of the atherosclerotic lesion. The oxLDL uptake

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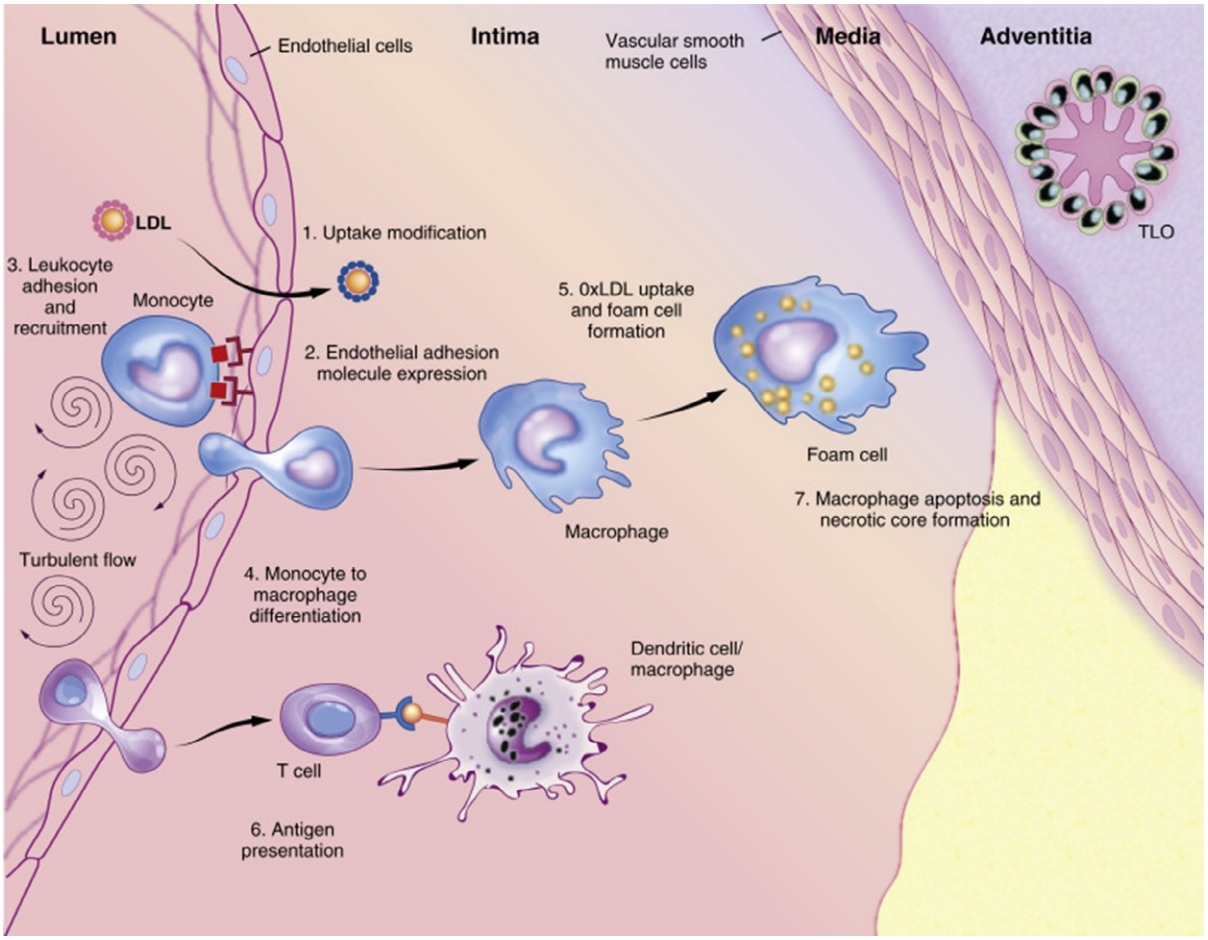

Fig. 1 Immune-mediated mechanisms of atherosclerosis. (1) Low density lipoprotein (LDL) is retained in the vascular wall, where it is modified by oxidation. (2) Oxidized LDL (oxLDL) stimulates endothelial cells to express adhesion molecules, which (3) induces leukocyte adhesion and recruitment. (4) Infiltrating monocytes differentiate into macrophages that (5) take up oxLDL and become foam cells. (6) Dendritic cells and macrophages present antigens to lymphocytes. (7) Macrophage death, for example by apoptosis, creates a lipid-filled necrotic core. Note also the presence of mast cells within the lesion. Tertiary lymphoid organs (TLO) in the adventitia are also depicted (see text for details). Reprinted from Chronic Coronary Artery Disease edited by Lemos and Omland. Bäck and Hansson, Basic Mechanisms of Atherosclerosis, pp. 45–54, 2018 with permission from Elsevier.

initiates several pathways of inflammation in the macrophage. Recently it was shown that oxLDL bound within immune complexes has even more potent effect, leading to activation of the inflammasome (Rhoads et al., 2017). In addition, the internalization of oxLDL by macrophages can lead also to antigen presentation and the consequential activation of the adaptive immune system (Nicoletti et al., 1999).

Macrophage death, for example by apoptosis, creates a lipid-filled necrotic core within the atherosclerotic lesion (**Fig. 1**). Under normal conditions, apoptotic cells are cleared by a specific phagocytosis process, termed efferocytosis, which is an essential response for normal steady state (Serhan, 2014). The defective clearance of lipid-laden apoptotic macrophages in the atherosclerotic lesion further enhances the necrotic core formation.

Also mast cells are present in human atherosclerotic lesions (**Fig. 1**), and may derive from mast cell precursors recruited to the lesions either by means of adventitial vasa vasorum or migration from the lumen toward the arterial intima (Shi et al., 2015). Activation of lesion mast cells by for example immune complexes, LPS, and complement (Lappalainen et al., 2011) results in the release of chemokines, cytokines, and lipid mediators (Dichlberger et al., 2014), which may participate in the atherosclerosis progression, as well as several proteases involved in plaque instability (Kovanen, 2007).

Adaptive Immunity: Fine Tuning the Immune Reaction

T- and B-cells are the major cells involved in adaptive immunity. In conjunction with the innate immune system, the responses of T- and B-cells are essential for the elimination of infectious and toxic agents. The adaptive immune system has the ability to recognize and recall specific responses against foreign and self-antigens. Maladaptive immune responses to self-molecules can lead to the

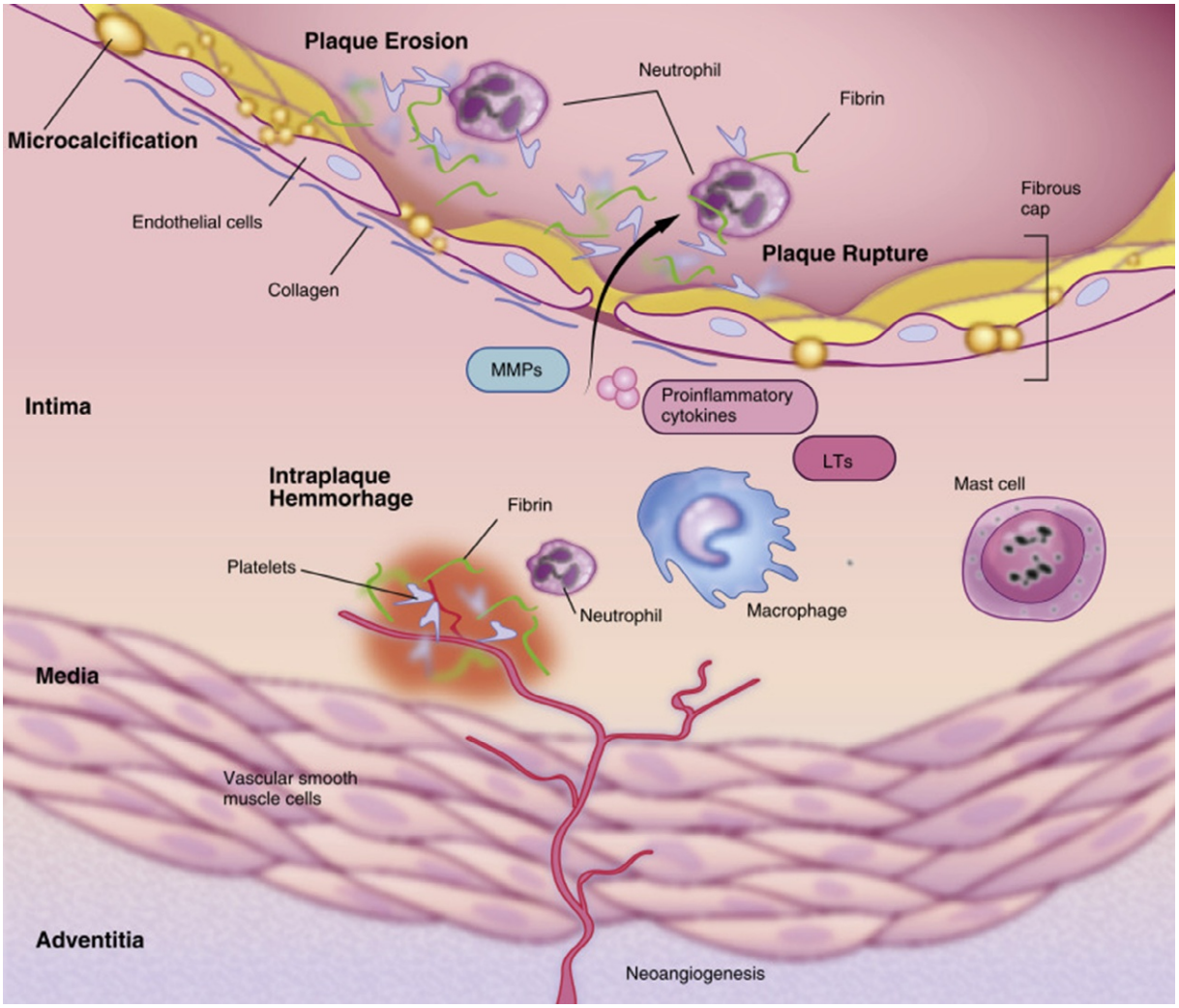

Fig. 2 The vulnerable plaque. The fibrous cap, containing mainly smooth muscle cells and collagen, protects the lipid- and inflammatory content of the atherosclerotic lesion from contact with the blood. In plaque erosion, endothelial cells detach and the exposure of the subendothelial matrix will lead to neutrophil activation and thrombus formation. Degradation of this fibrous cap surrounding the core of a coronary atherosclerotic lesion induces plaque rupture provoking acute thrombosis and vessel occlusion. Hypoxia and growth factors contributes to neo-angiogenesis within the atherosclerotic lesions, which is the source of intraplaque hemorrhage, another important characteristic of vulnerable plaques. *LTs*, *leukotrienes*; *MMPs*, *matrix metalloproteinases*. Reprinted from Chronic Coronary Artery Disease edited by Lemos and Omland. Bäck and Hansson, Basic Mechanisms of Atherosclerosis, pp. 45–54, 2018 with permission from Elsevier.

development of autoimmune diseases. Indeed, the chronic inflammation in atherosclerosis shares many features with autoimmune disorders. For instance, mice that carry global defects in adaptive immunity due to recombinase-activating gene or SCID (severe combined immunodeficiency) defects are much less prone to develop atherosclerotic lesions, even on a hypercholesterolemic background while animals defective in specific components of adaptive immunity display characteristic changes in disease development and lesion formation (Dansky et al., 1997; Zhou et al., 2000; Hansson and Hermansson, 2011). Adoptive transfer of CD4+ T-cells from the autoimmune mouse model for SLE can actually accelerate atherosclerosis in LDLr−/− mice (Wilhelm et al., 2015). However, atherosclerosis differs in two important aspects: atherosclerosis is much more abundant than any of the autoimmune diseases, and it does not show linkage to any specific HLA haplotype. Instead, several different HLA types appear to be permissive. In atherosclerosis, autoimmune responses have been identified that recognize LDL particles and their molecular components (Stemme et al., 1995; Binder et al., 2003; Hermansson et al., 2010). Such T- and B-cell responses to LDL have been shown to be pathogenically important and may also offer new opportunities for cardiovascular therapy (Bäck and Hansson, 2015).

T- and B-cells have been identified in human atherosclerotic lesions as well as in animal models of disease. In experimental models, $CD4^+$ T-cells interact with antigen-presenting cells in the vessel wall, leading to their activation, proliferation, and secretion of cytokines such as IFNγ and TNF (Koltsova et al., 2012). While most B-cells are usually found in the adventitial layer of the arterial wall, in tertiary lymphoid organs (TLOs; **Fig. 1**), T-cells can account for up to 10% of all cells in human plaques. T helper 1 $CD4^+$ (Th1) cells are the most abundant T-cell population in the plaque (Jonasson et al., 1986). However, other T helper cells, for example

Th2 and Th17, as well as regulatory T-cells (Tregs), TCR$\gamma\delta^+$ T-cells, and NKT cells have been described in plaques (Ketelhuth and Hansson, 2011).

Most T-cell subtypes have been implicated in the modulation of experimental atherosclerosis. Indeed, Th1 and Tregs play major opposite roles in the disease, being pro- and antiatherogenic, respectively (Ketelhuth and Hansson, 2016; Klingenberg et al., 2013). In addition to influencing plaque inflammatory burden, pro- and antiatherogenic T-cells can influence plaque stability by affecting the differentiation of SMCs and collagen production, and the release of proteases (Ketelhuth and Bäck, 2011; Gisterå et al., 2013). For example, Th17 and IL-17A have been recently implicated in the regulation of collagen production and thickening of fibrous cap (Gisterå et al., 2013). However, the role of Th17 cells in atherosclerosis remains unclear since they have been implicated in acceleration (van Es et al., 2009; Butcher et al., 2012; Erbel et al., 2009; Gao et al., 2010; Smith et al., 2010) and deceleration (Taleb et al., 2009; Danzaki et al., 2012) of disease.

The natural killer T (NKT) cell type that recognizes lipid antigen rather than protein-derived peptides has been implicated as a proatherogenic cell population. Thus, defects in lipid antigen presentation to NKT cells lead to reduced atherosclerosis, whereas NKT cell activation with exogenous ligands accelerates disease development (Tupin et al., 2004; Nakai et al., 2004; Major et al., 2004; van Puijvelde et al., 2009; Kyriakakis et al., 2010). These effects likely depend on NKT cell populations in the liver as well as in the artery wall.

Antibodies to LDL components and other disease-associated antigens such as heat shock protein-60 show correlations with disease development. Therefore, it was a surprise when two groups reported that B cells play a protective role in atherosclerosis (Caligiuri et al., 2002; Major et al., 2002). Although several additional studies have confirmed that B-cells have atheroprotective properties, their role in disease remains controversial. While the elimination of the majority of B-cells accelerates atherosclerosis, the depletion of certain B2 cell types, for example $CD20^+$ (Ait-Oufella et al., 2010) or $BAFFR^+$ (B cell-activating factor receptor)(Sage et al., 2012), is atheroprotective. These results suggest that similar to T-cells, different B-cell subtypes can convey a protective or deleterious role in atherosclerosis (Ketelhuth and Hansson, 2016).

Considering the pathophysiological roles, the specificity, and the long-lasting characteristics of their responses, the modulation of T- and B-cell responses is an attractive target for the development of novel therapies for CVD (Ketelhuth et al., 2013; Ketelhuth and Hansson, 2016; Bäck and Hansson, 2015). This offers the possibility to trigger long-lasting T- and B-cells, including Tregs, regulatory B-cells (Bregs), and B-1 cells, that can provide antiatherogenic immunity while leaving the general host defense untouched. There are several experimental studies supporting this notion, which needs to be further investigated and validated in humans. One particularly attractive option would be to increase the number of IL-10 producing B cells that reside within the aortic lymph nodes. It is possible that this population may respond directly to atherosclerotic plaque formation (Strom et al., 2015). Conversely, reducing other B cell populations that exacerbate atherosclerosis, such as those that produce GMCSF (Hilgendorf et al., 2014), could also provide a therapeutic avenue. It may appear to be a crude approach to generally target immune populations outside of their disease specific function. However, it should be noted that broad anti-B cell therapy has achieved substantial clinical success with regard to autoimmune disease.

Immunomodulatory therapies against atherosclerosis are under investigation by several groups worldwide (Bäck and Hansson, 2015). In animal models, different vaccination protocols against disease-related antigens, for example oxLDL, Apolipoprotein B-100, HSP65, have shown atheroprotective effects (Palinski et al., 1995; Ameli et al., 1996; George et al., 1998; Zhou et al., 2001). ApoB100 epitopes recognized by circulating autoantibodies in patients and healthy individuals (Fredrikson et al., 2003a) have been shown to reduce vascular inflammation and protect against atherosclerosis (Schiopu et al., 2004, 2007; Strom et al., 2007; Fredrikson et al., 2003b; Klingenberg et al., 2010; Fredrikson et al., 2008; Herbin et al., 2012; Wigren et al., 2011). Atheroprotection has been demonstrated using murine ApoB100-derived T-cell epitopes (Tse et al., 2013), and recently human ApoB100-derived atheroprotective T-cell epitopes have also been identified (Gistera et al., 2017). Notably, the latter study showed that ApoB100 epitopes are predicted to bind several HLA haplotypes, suggesting a large proportion of the world population has the potential to mount T-cell responses against these when used in a vaccine.

Different mechanisms have been proposed for explaining the efficacy of vaccines in mitigating vascular inflammation and plaque development, including the induction of antigen-specific Tregs and antibody-mediated effects, for example, neutralization of oxLDL and the diminishment of its deleterious effects on macrophages and vascular cells, inhibition of scavenger receptor-mediated uptake of oxLDL by foam cells, and Fc-mediated inhibition of macrophage activation (Ketelhuth and Hansson, 2016; Gistera et al., 2017).

Humoral Immunity: The Remote Control

Antibodies are capable of recognizing a broad range of potentially dangerous molecules, including reactivity against modified versions of self. This recognition of altered-self is believed to have important functions in protecting mammals from atherosclerosis formation. The first line of humoral defense is provided by something termed “natural immunity” in this case provided by germline encoded antibodies that are produced in the absence of antigen, but can also be increased in titers following antigenic stimulation. IgM Antibodies that recognize phosphocholine when present in oxLDL are thought to help in the clearance of both oxLDL and apoptotic cell bodies (Shaw et al., 2000), which can accumulate in the atherosclerotic plaque. Antibodies also recognize many other pro-atherosclerotic antigens, especially the neo-epitopes produced during covalent bond formation between the lipid and proteins present in LDL, and also potential danger sequences present in the apolipoproteins themselves (Ketelhuth et al., 2011).

Direct evidence that antibodies can comprehensively reduce or even prevent plaque formation has not been forthcoming, but a number of observations suggest that humoral immunity may provide a pathway to ameliorating atherosclerosis. First, either transfer of total IgM or intravenous injection of immunoglobulins (ivIG) can reduce atherosclerosis in animal models (Cesena et al., 2012; Nicoletti et al., 1998). Second, increasing the concentrations of antibodies with the T15 idiotype, which recognizes phosphocholine, can also reduce the extent of atherosclerosis plaque formation under certain defined conditions (Binder et al., 2003; Faria-Neto et al., 2006), although targeted deletion of an immunoglobulin gene segment that is critical for antiphosphocholine responses following acute infection or immunization does not affect atherosclerosis development (Centa et al., 2016). Similarly, immunization of atherosclerosis susceptible mammals with either phosphocholine or oxLDL reduces the subsequent extent of the disease (Steinberg and Witztum, 2010). This has led to more refined searches to allow for the cloning of antibodies that recognized oxLDL, with some limited success in hindering atherosclerosis development (Schiopu et al., 2007; Tsimikas et al., 2011). It is highly likely that full "neutralization" of oxLDL, recognized as a dangerous moiety by the immune system, will require multiple antibody specificities acting in concert and perhaps through multiple effector systems, including complement and FC receptor-mediated responses. As a final note of caution, antibodies have also been suspected of having pathogenic functions in atherosclerosis, and inhibition of this putative promoting effect may also be a fruitful approach in future therapeutics.

Neural Immune Programming: The Spleen as a Router

Recent discoveries in immunology and neuroscience show that the immune system in addition is regulated by neural reflexes (Olofsson et al., 2012). These insights have spawned clinical trials that use nerve stimulators to treat inflammatory disease. The human study populations to date are small, but the reports are promising and show reduced inflammatory disease activity after activation of an implanted vagus nerve stimulator (Koopman et al., 2016; Bonaz et al., 2016). If rigorous, large-scale clinical trials confirm the efficacy of implanted vagus nerve stimulators, it is conceivable that nerve stimulation could be approved for routine clinical use in treatment of select conditions characterized by excessive inflammation (Tracey, 2015).

The inflammatory reflex, in which the vagus nerve plays a key role, is one of many reflex circuits that control cytokine release and immune cell activity (Andersson and Tracey, 2012). The detailed mapping of this circuit has been important for the emerging understanding of reflex control of inflammation and as a basis for clinical trials of vagus nerve stimulation for treatment of inflammatory diseases. In this reflex circuit, proinflammatory cytokines such as IL-1β trigger sensory activity in the vagus nerve (Watkins et al., 1995; Niijima, 1996). Efferent signals travel through the vagus nerve to the celiac ganglion where the splenic nerve arises. Activation of the vagus nerve gives rise to a cascade of events involving release of norepinephrine by the splenic nerve. Norepinephrine promotes a subset of splenic T-cells, denoted T_{ChAT}, to release acetylcholine, which acts on macrophage cholinergic receptors to reduce release of proinflammatory cytokines, including TNF (Olofsson et al., 2012). Recent reports link components of this pathway to the regulation of blood pressure and potentially to development of hypertension (Olofsson et al., 2016; Carnevale et al., 2016), and suggest that the neural control of the immune system needs to be considered in the development of cardiovascular disease where inflammation is involved, including hypertension and atherosclerosis (Harrison et al., 2011; Libby et al., 2009).

Lipid Mediators: Accelerating and Resolving Inflammation

Activated immune cells are an important source for the production of bioactive lipid mediators from polyunsaturated fatty acids (PUFAs) at all stages of atherosclerosis (**Figs. 1** and **2**). For example, prostaglandins and thromboxanes, which originate from cyclooxygenase (COX) metabolism of phospholipid-derived arachidonic acid, play well-recognized roles in the atherosclerosis process (Gabrielsen et al., 2010; Capra et al., 2014). The use of low-dose aspirin in cardiovascular prevention relies on its irreversible inhibition of COX-1 in platelets, which lack the ability to resynthesize COX enzymes, leading to a selective inhibition of platelet pro-aggregatory TXA_2 formation (Capra et al., 2014). In contrast, the use of COX-2 selective inhibitors has been associated with an increased cardiovascular risk (see Bäck et al. (2012) and references therein).

Arachidonic acid also serves as a substrate for the lipoxygenase (LO) enzymes leading to the formation of lipid mediators that transduce specific signaling patterns in atherosclerotic lesions. Leukotrienes (LTs) act on specific receptors, BLT and CysLT receptors (Bäck et al., 2011), respectively, to transduce several proinflammatory effects with implications for atherosclerosis development, such as leukocyte recruitment and activation, SMC proliferation, endothelial dysfunction (Bäck and Hansson, 2006), and plaque rupture (Bäck, 2009). Interestingly, anti-LTs that are in clinical use for the treatment of asthma and allergic rhinitis have been associated with a reduced risk of recurrent cardiovascular events in retrospective analysis (Ingelsson et al., 2012), further underlining the therapeutic potential for targeting immune-mediated mechanisms in atherosclerosis (Bäck and Hansson, 2015).

In addition to the formation of proinflammatory LTs, LO metabolism of both the omega-6 PUFA arachidonic acid and omega-3 PUFAs participate in the formation of antiinflammatory lipid mediators, such as lipoxins och resolvins (Serhan, 2014). In addition to dampening the immune response, these lipid mediators also actively promote the resolution of inflammation (Serhan, 2014). For example, lipoxins and resolvins stimulate efferocytosis (Serhan, 2014; Petri et al., 2015a), which is an important mechanism for the resolution of inflammation and may serve to decrease the necrotic core formation in atherosclerosis (*cf.* supra and **Fig. 1**).

The opposing effects of the lipid mediators, being either proinflammatory or pro-resolving, taken together with their potent effects in experimental atherosclerosis (Ketelhuth et al., 2015; Petri et al., 2017), indicate that the balance between the two classes of

mediators may be crucial for either stimulating or repressing the immune response in atherosclerosis. Indeed, the expression of their respective receptors is differentially regulated in monocytes (Petri et al., 2015b), suggesting that the composition of the proinflammatory milieu in the atherosclerotic lesion may be decisive to determine if lipid mediator signaling is either proinflammatory or pro-resolving. Furthermore a disturbed lipid mediator balance with prevailing leukotriene over resolvin production can be used as a biomarker of nonresolving inflammation in the context of subclinical atherosclerosis (Thul et al., 2017).

Carotid Endarterectomy Studies: Observing Immune Responses in Human Atherosclerosis

As mentioned above, inflammatory pathways are involved throughout the different stages of atherosclerosis, from early onset (**Fig. 1**) to final ischemic events (**Fig. 2**). However, it is not clear how local immune responses may lead to the life threatening condition of plaque rupture in patients with severe atherosclerosis. Mouse models are very useful to study progression and early phases of atherosclerosis have thereby helped identifying several key factors and immune cells. However, regarding the later stages in human disease we still lack really robust animal models.

Larger cohorts of patients or healthy controls can be used to investigate genetic markers and other measurable parameters using blood samples that are easy to collect. To get a more detailed picture of disease processes one needs to analyze the affected tissue, not only a blood sample. Vascular tissue samples, even very minute, from patient undergoing operations have proved to be very useful not only for immunohistochemistry analysis but also in the growing field of molecular biology analysis. The ability to do large-scale analysis on RNA, DNA, and protein levels have opened new possibilities.

Functional genomic studies have been used in medicine research since the beginning of 2000. The methodologies are developing fast and today these studies use large-scale analysis to get molecular data on pathological processes all the way from gene to RNA to protein. One of the earliest methods introduced was transcript profiling. This type of methods can on a chip analyze mRNA from 20,000 genes in one sample, giving information on which genes are activated or silenced—generating a transcript profile, also called transcriptome, for a given sample.

To be able to study mechanisms within the atherosclerotic plaque on a molecular level, bio-banks have been built of plaques removed under carotid endarterectomy (CEA), also including from each patient. In the prospective BiKE—Biobank of Karolinska Carotid Endarterectomies, the atherosclerotic plaque and circulating leukocytes are collected for mRNA isolation and transcript profiling, thereby building a database of present transcripts in diseased tissue and in circulating leukocytes in relation to the clinical data of the patients (Folkersen et al., 2009, 2012; Perisic et al., 2016; Gabrielsen et al., 2010). The composition and biology of the carotid plaque is assumed to be representative of other plaques found in other parts of the vascular tree, such as coronary arteries. By means of such analysis, specific pathways of both innate and adaptive immunity have been identified. For example receptors and cytokines controlling response in T lymphocytes (Gisterå et al., 2013) and lipid-carrying proteins boosting macrophage-driven inflammation (Agardh et al., 2011), respectively, have been identified. Finally, postsurgical follow-up after CEA can be monitored in order to determine the prognostic valve of a specific transcript profile in the removed atherosclerotic plaque on future cardiovascular events. Using the latter approach, TLR7 was identified as a positive prognostic marker in patients with severe atherosclerosis (Karadimou et al., 2017). Taken together, atherosclerotic plaque biobanks allow a unique translational tool for the search for immune controlling treatments of atherosclerosis in the future (Bäck and Hansson, 2015).

Summary and Conclusions

The immune response in atherosclerosis can be triggered by several known cardiovascular risk factors that initiate the vascular inflammation. The retention and modification of LDL and its recognition as danger signal and antigen triggers both an innate and adaptive immune activation, which is regulated by a number of mediators and also by neural reflexes. As with all immune-mediated mechanisms, the context must also be taken into consideration. For example, how immune reactions in secondary lymphoid organs impact atherosclerotic plaque progression in relation to the local immune responses in the form of tertiary lymphoid organs located in the adventitia and juxtaposed to fulminant atherosclerotic lesions.

The study of the immune-mediated mechanisms of atherosclerosis requires adequate experimental in vitro and in vivo models, as well as observational studies in atherosclerotic patients and high-throughput molecular analysis of human atherosclerotic lesions to determine key pathways. In terms of antiinflammatory treatments in atherosclerosis, we emphasize targeting pattern recognition and/or T- and B- cell responses, as well as immunomodulation by pro- and antiinflammatory lipid mediators, vagus nerve stimulation, antibodies, and vaccines.

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Implanted Pacemakers—Indications and Novel Programming

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Pathophysiology

Pacemakers are the only available ambulatory therapy to treat patients with a slow heart rhythm. The origin of the slow heart rhythm can be either situated on the level of the sinus node or the AV node. It is clinically important to make this distinction because bradycardia caused by AV-node problems could be associated with an increased risk in sudden cardiac death, while sinus node disease is a symptomatic problem without an increased mortality (Kastor, 1975a,b; Shaw et al., 1980, 1985). As such pacing for AV-nodal problems can be life saving, while pacing for sinus node disease is a symptomatic treatment. Bradycardia at sinus node level can manifest itself as a problem of the sinus node, with intermittent sinus arrest or sinus bradycardia. It can also be due to a problem in the junction between the atrium and the sinus node: sino atrial block. Problems of the sinus node are often a sign of generalized atrial myopathy presenting with on the one side bradycardia from the sinus node and the other side atrial tachyarrhythmia (atrial fibrillation, atrial flutter, and ectopic atrial tachycardia). This mixed presentation with slow and fast heart rhythm problems is called brady-tachy syndrome (John and Kumar, 2016). In assessing bradycardia originating from the AV node, it is important to distinguish nodal from infranodal problems. Infranodal conduction disease, situated below the bundle of his, is associated with an increased risk of sudden cardiac death. A second-degree AV block type II, a third-degree AV block, or the presence of severe conduction disturbances with a broad QRS are suspect for an infranodal origin of the problem. This suspicion can be proven by the documentation of a prolonged HV interval on an invasive diagnostic electrophysiological study (Kastor, 1975a,b).

In the majority of patients, the underlying problem is aging or ischemia-dependent fibrosis. This results in the fact that pacemakers are used mainly in the elderly population with implantation at the age of 75–80 years (Greenspon et al., 2012). Due to the fact that the underlying pathophysiological process is identical there is a large overlap between AV-nodal and sinus node problems in 30 up to 50% of the patients in this age group. If patients present with bradycardia at a younger age a more thorough diagnostic approach is warranted. In that case structural or genetic arrhythmogenic heart disease (in particular A/VSD and SCN5a or HCN mutations) or systemic disease (in particular sarcoidosis, amyloidosis, hypothyroidism, HLAB27 associated spondylitis) or extrinsic problems (as increased vagal tone due to endurance sports, infectious disease (in particular Lyme and peri/myocarditis) and medication (ab)use) should be ruled out.

Indications for Pacing

Although there is a clear difference in the approach between the European and North American guidelines in this paragraph, the 2012 ACC/AHA/HRS guidelines and the 2013 ESC guidelines are compared (Epstein et al., 2013; Brignole et al., 2013). The ACC/AHA/HRS guidelines use a traditional approach dividing the indications according to the pathophysiological mechanism (sinus node disease vs. AV block), while the ESC considers the indications by their clinical presentation, distinguishing permanent bradycardia from intermittent bradycardia. While the North-American guidelines provide detailed indications for every possible clinical diagnosis, the European Society of Cardiology gives broader advice, resulting in clearly less statements. For both documents it is clear that most of the guidelines are based on expert consensus. In view of the (patho)physiological obvious effect of the treatment and the lack of pharmacological alternative large randomized clinical trials were historically never undertaken and seem in most recognized indications unethical to set-up (**Table 1**).

Table 1 International guidelines ESC vs. AHA/ACC/HRS

	AHA/ACC/HRS	*Level*	*ESC*	*Level*
Indications for pacing				
Sinus node disease	SND with documented symptomatic bradycardia, including frequent sinus pauses that produce symptoms	IC	When symptoms can clearly be attributed to bradycardia	IB
	Symptomatic chronotropic incompetence	IC	SND who have the documentation of symptomatic bradycardia due to sinus arrest or sinus atrial block	IB
	Symptomatic sinus bradycardia that results from required drug therapy for medical conditions	IC		
	SND with heart rate less than 40 bpm when a clear association between significant symptoms consistent with bradycardia and the actual presence of bradycardia has not been documented	IIaC	When symptoms are likely to be due to bradycardia, even if the evidence is not conclusive	IIbC
	Syncope of unexplained origin when clinically significant abnormalities of sinus node function are discovered or provoked in electrophysiological studies	IIaC	With history of syncope and documentation of asymptomatic pauses >6 s due to sinus arrest, sinus-atrial block or AV block	IIaC
	In minimally symptomatic patients with chronic heart rate less than 40 bpm while awake	IIbC		
AV block	Third-degree and advanced second-degree AV block at any anatomic level associated with bradycardia with symptoms (including heart failure) or ventricular arrhythmias presumed to be due to AV block	IC	Third- or second-degree type 2 AV block irrespective of symptoms	IC
	Third-degree and advanced second-degree AV block at any anatomic level associated with arrhythmias and other medical conditions that require drug therapy that results in symptomatic bradycardia	IC		
	Third-degree and advanced second-degree AV block at any anatomic level in awake, symptom-free patients in sinus rhythm, with documented periods of asystole greater than or equal to 3.0 s (86) or any escape rate less than 40 bpm, or with an escape rhythm that is below the AV node	IC	Asymptomatic pauses > 6 s due to sinus arrest, sinus-atrial block or AV block	IIaC
	Third-degree and advanced second-degree AV block at any anatomic level in awake, symptom-free patients with AF and bradycardia with 1 or more pauses of at least 5 seconds or longer	IC		
	Third-degree and advanced second-degree AV block at any anatomic level associated with postoperative AV block that is not expected to resolve after cardiac surgery	IC	High degree or complete AV block after cardiac surgery and TAVI. A period of clinical observation up to 7 days is indicated in order to assess whether the rhythm disturbance is transient and resolves. However, in case of complete AV block with low rate of escape rhythm this observation period can be shortened since resolution is unlikely	
	Third-degree and advanced second-degree AV block at any anatomic level associated with neuromuscular diseases with AV block, such as myotonic muscular dystrophy, Kearns-Sayre syndrome, Erb dystrophy (limb-girdle muscular dystrophy), and peroneal muscular atrophy, with or without symptoms	IB		
	Second-degree AV block with associated symptomatic bradycardia regardless of type or site of block	IB		
	Asymptomatic persistent third-degree AV block at any anatomic site with average awake ventricular rates of 40 bpm or faster if cardiomegaly or LV dysfunction is present or if the site of block is below the AV node	IB		
	Second- or third-degree AV block during exercise in the absence of myocardial ischemia	IC		
	Asymptomatic type II second-degree AV block occursing with a wide QRS, including isolated right bundle-branch block	IC		

(*Continued*)

Table 1 International guidelines ESC vs. AHA/ACC/HRS (Continued)

	AHA/ACC/HRS	*Level*	*ESC*	*Level*
	Advanced second-degree AV block or intermittent third-degree AV block	IB	Intermittent/paroxysmal intrinsic third- or second-degree AV block.	IC
	For type II second-degree AV block	IB		
	Persistent third-degree AV block with an escape rate greater than 40 bpm in asymptomatic adult patients without cardiomegaly	IIaC		
	Asymptomatic second-degree AV block at intra- or infra-His levels found at electrophysiological study	IIaB	Second-degree type I AV block which causes symptoms or is found to be located at intra- or infra-His levels at EPS	IIaC
	First- or second-degree AV block with symptoms similar to those of pacemaker syndrome or hemodynamic compromise	IIaB		
	Asymptomatic type II second-degree AV block with a narrow QRS	IIaB		
	For neuromuscular diseases such as myotonic muscular dystrophy , Erb dystrophy (limb-girdle muscular dystrophy) , and peroneal muscular atrophy with any degree of AV block (including first-degree AV block), with or without symptoms, because there may be unpredictable progression of AV conduction disease	IIbB		
	For AV block in the setting of drug use and/or drug toxicity when the block is expected to recur even after the drug is withdrawn	IIb		
Conduction disturbances	For alternating bundle-branch block	IC	In patients with alternating BBB with or without symptoms	IC
	Bifascicular block with for syncope not demonstrated to be due to AV block when other likely causes have been excluded, specifically ventricular tachycardia (VT)	IIaB	In selected patients with unexplained syncope, BBB and non diagnostic investigations	IIbB
	Bifascicular block and an incidental finding at electrophysiological study of a markedly prolonged HV interval ($\geq$ 100 ms) or non-physiological pacing induced infra-His block in asymptomatic patients	IIaB	in patients with syncope, BBB and positive EPS defined as HV interval of $\geq$70 ms, or second- or third-degree His-Purkinje block demonstrated during incremental atrial pacing or with pharmacological challenge	IB
	In the setting of neuromuscular diseases such as myotonic muscular dystrophy, Erb dystrophy (limb-girdle muscular dystrophy), and peroneal muscular atrophy with bifascicular block or any fascicular block, with or without symptom	IIbC		
Contra-indications for pacing				
Sinus node disease	SND in asymptomatic patients	IIIC	SND which is asymptomatic or due to reversible causes	IIIC
	SND in patients for whom the symptoms suggestive of bradycardia have been clearly documented to occur in the absence of bradycardia	IIIC	In patients with unexplained syncope without evidence of bradycardia or conduction disturbance	IIIC
	SND with symptomatic bradycardia due to nonessential drug therapy	IIICC	In reversible causes of bradycardia	IIIC
AV block	Asymptomatic first-degree AV block	IIIB	AV block which is due to reversible causes	IIIC
	Asymptomatic type I second-degree AV block at the supra-His (AV node) level or that which is not known to be intra- or infra-Hisian	IIIC		
	AV block that is expected to resolve and is unlikely to recur (100) (e.g., drug toxicity, Lyme disease, or transient increases in vagal tone or during hypoxia in sleep apnea syndrome in the absence of symptoms)	IIIB		
Conduction disturbances	Fascicular block without AV block or symptoms	IIIB	Asymptomatic BBB	IIIB
	Fascicular block with first-degree AV block without symptoms	IIIB		

Recommendation: I, is indicated; IIa, is reasonable; IIb, may be considered; III, not indicated.

Evidence: B, data derived from a single randomized clinical trial or large non-randomized studies; C, Consensus of opinion of experts and/ or small studies, retrospective studies, registries.

Sinus Node Disease

In sinus node disease, a clear relation between symptoms and bradycardia should be demonstrated in most cases to warrant PM implantation. A PM however is reasonable if a patient presented with syncope and if sinus node dysfunction can be demonstrated on an electrophysiological study or by severe bradycardia on ECG monitoring. The ESC guidelines put forward an asymptomatic pause >6 s as a cut-off in this case, while the ACC/AHA/HRS guidelines leave more room for interpretation and mention the presence of clinically significant abnormalities of sinus node function without specific rate of pause cut-off. Another demonstration of a somewhat more liberal position on the indication of PM for sinus node disease in the North-American guidelines is the fact that implantation may be considered in minimally symptomatic patients with chronic heart rate less than 40 bpm while awake.

AV Block

Symptomatic AV block of second degree or higher at any location is generally accepted as an indication for PM implantation. Asymptomatic AV block located to an infranodal location, either by electrocardiographic characteristics (second-degree AV block type II or third-degree AV block) or confirmed on an invasive electrophysiological study are also uniformly accepted as indication for PM implantation. In case of an asymptomatic second-degree AV block or higher the ESC guidelines ask for the documentation of a pause >6 s, while the ACC/AHA/HRS guidelines state a pause >3 s in sinus rhythm or 5 s in atrial fibrillation is sufficient to warrant the implantation of a PM.

Conduction Disturbances

Since conduction disturbances are an electrocardiographic manifestation of infranodal disease, a combination of any second-degree AV block with conduction disturbances is considered an indication for the implantation of a PM. Asymptomatic alternating bundle branch block is also a clear PM indication. Finally, in case of syncope in patients with severe conduction disturbances on the surface ECG and either the exclusion of other causes or the demonstration of prolonged infranodal conduction on an invasive electrophysiological study, the implantation of a PM is indicated.

Choice of Device

It has been clearly shown that addition of leads adds significantly to the procedural risk of device implantations (Poole et al., 2010). It is therefore of utmost importance to choose the right hardware at the initial implantation: single-chamber pacemaker, dual-chamber pacemaker, and cardiac resynchronization pacemaker (**Fig. 1**).

This hardware can be programmed in different pacing modes. The pacing modes are described by a four-letter abbreviation. The first letter describes where the PM stimulates the heart (V = ventricle; A = atrium; D = dual). The second letter describes where the PM senses (V = ventricle; A = atrium; D = dual). The third letter describes what the PM does when it senses an intrinsic heartbeat (I = inhibit; T = trigger; D = dual). The fourth letter describes if the PM is programmed in a rate responsive mode (R = rate response). In this case the PM estimates the metabolic need of the patient using build in sensors and automatically increases the heart rate in case of a higher metabolic need. The most commonly used PM programming for single-chamber PMs is AAI (atrial based) or VVI (ventricular based) and DDD in dual-chamber or cardiac resynchronization PMs.

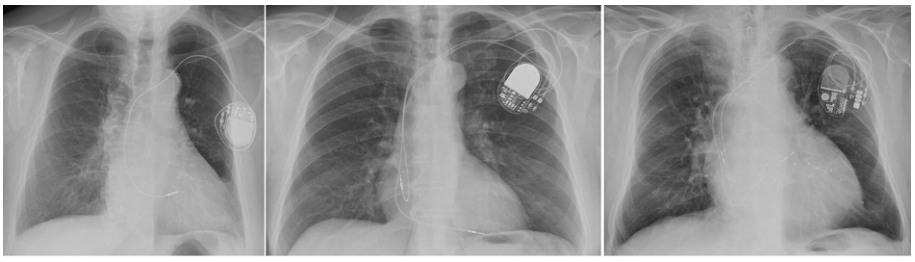

Fig. 1 Chest radiography of the different types of devices.

Single- vs. Dual-Chamber Pacing

It was presumed that physiologic AV sequential dual-chamber pacing (AAI or DDD) would have clear clinical advantages over single-chamber ventricular pacing (VVI). Initial results of Andersen et al. comparing single-chamber atrial (AAI) with single-chamber ventricular (VVI) pacing in 225 patients with sinus node disease showed a significant decrease in atrial fibrillation, stroke, and mortality (Andersen et al., 1994). The PASE trial moreover showed that quality of life was significantly better with physiologic pacing (Lamas et al., 1998). These results set the path for three large randomized clinical trials: CTOPP, MOST, and UKpace (Connolly et al., 2000; Lamas et al., 2002; Toff et al., 1997). The outcome was disappointing. The main results of these seminal trials are summarized in **Table 2**. None of the trials could show a mortality benefit of dual-chamber pacing, not even with an extended follow-up up to 6.4 years (Kerr et al., 2004). The individual patient data of these trials were bundled in one meta-analysis (Healey et al., 2006). There was statistical heterogeneity among the results of the individual trials. This meta-analysis confirmed that there was no effect on mortality (HR 0.95 (0.87–1.03); $P=0.19$) or heart failure (HR 0.89; (0.77–1.03); $P=0.15$) with physiologic pacing. There was however a significant reduction in atrial fibrillation (HR 0.80 (0.72–0.89); $P<0.001$) leading to a borderline significant reduction in stroke (HR 0.81 (0.67–0.99); $P=0.035$). There was no clear evidence that patients with sinus node disease would benefit more than patients with AV block. So compared with single-chamber ventricular pacing (VVI), dual-chamber physiologic pacing (AAI or DDD) does not improve survival or reduce heart failure, but it decreases the risk for atrial fibrillation and possibly stroke.

To further elucidate the perceived difference between the early positive experience with single-chamber atrial pacing (AAI) versus the disappointing results with mainly dual-chamber pacing (DDD) in these trials the DANPACE trial randomized 1415 patients with sinus node disease between AAIR and DDDR pacing (Nielsen et al., 2011). The primary endpoint was all-cause mortality. Secondary endpoints were similar as in the previous discussed trials and included atrial fibrillation, stroke, heart failure, but also the need for re-intervention. There was no difference in mortality (HR 1.06 (0.88–1.29); $P=0.53$). Surprisingly paroxysmal atrial fibrillation was increased with AAIR pacing (HR 1.27 (1.03–1.56); $P=0.024$). AAIR pacing was also associated with a significant increased necessity for re-intervention (HR 1.99 (1.53–2.59); $P=0.001$). There was no difference in the incidence of permanent atrial fibrillation, stroke, and heart failure between AAIR and DDDR. So DANPACE did not show a difference in all-cause mortality between AAIR and DDDR, but single-chamber atrial pacing was associated with an increased risk for paroxysmal atrial fibrillation and reoperation. The authors conclude that their findings support the routine use of DDDR pacing in patients with sinus node disease.

Table 2 Randomized clinical trials comparing VVI vs. DDD pacing

	Andersen et al.	*PASE*	*CTOPP*	*MOST*	*UKPACE*	*DANPACE*	*BLOCKHF*	*BIOPACE*
Pacing mode	AAI-VVI	VVI-DDD	VVI-DDD/AAI	VVI-DDD	VVI-DDD	AAI-DDD	DDD-CRT	DDD-CRT
Year of publication	1994	1998	2000	2002	2005	2011	2013	2014[a]
Pathology	SSS	SSS/AVB	SSS/AVB	SSS	AVB	SSS	AVB	AVB
Randomization	Hardware	Software	Hardware	Software	Hardware	Hardware	Software	Hardware
N	225	407	2568	2010	2021	1415	691	1810
Age (years)	76	76	73	74	80	73	73	73.5
Follow-up (years)	5.5	1.5	3.5 (6.4)	2.8	3	5.4	3.1	5.6
Mortality	Less with AAI	ns	ns	ns	ns	ns	ns	ns
Stroke	Less with AAI	Not reported	ns	ns	ns	ns	Not reported	Not reported
Atrial fibrillation	Less with AAI	ns	Less with DDD/AAI	Less with DDD	ns	More with AAI	Not reported	Not reported
Heart failure	Not reported	Not reported	ns	Less with DDD	ns	ns	Less with CRT	ns
Pacemaker syndrome with VVI	1.5%	26%	7%	30%	Not reported			Not reported
QoL	Not reported	Not reported						
		Overall ns, but better with DDD in SSS	ns	Better with DDD	Not reported			Not reported
	Better with CRT	Not reported						

[a]Presented at hotline session at the European Society of Cardiology, no peer reviewed publication.

Dual-Chamber Pacing vs. Cardiac Resynchronization Therapy

Cardiac resynchronization therapy (CRT) has undoubtedly proven in patients with heart failure and severe conduction disturbances (LBBB and QRS >130 ms or all BBB and QRS >150 ms) that remain symptomatic despite adequate drug treatment, that is, increases quality of life, exercise capacity, and left ventricular ejection fraction and decreases hospitalizations for heart failure and mortality (Ponikowski et al., 2016).

This positive impact of CRT has fuelled the idea that CRT might replace dual-chamber pacing for a broader range of indications, which led to several randomized trials to broaden the indication of CRT to regular bradycardia-related indications for pacing. These focus on AV block-related indications, leading to a high necessity of possible detrimental right ventricular pacing.

In the small PREVENT-HF trial, 108 patients with AV block and expected high ($\geq$80%) pacing percentage were randomized between DDD and CRT pacing (Stockburger et al., 2011). The primary endpoint was echocardiographic remodeling as assessed by change in LV end-diastolic volume after 1 year. Secondary endpoints were other echocardiographic parameters but also a combination of heart failure-related events and cardiovascular hospitalizations. This trial however showed no significant difference in any of the predefined primary or secondary endpoints.

Despite these negative results the authors found their results encouraging and informative for the design of subsequent larger trials with a higher statistical power.

The larger BLOCK-HF trial randomized 691 patients with AV block and a left ventricular ejection fraction of 50% or less (Curtis et al., 2013). All patients received a CRT-P, but were programmed either to DDD or CRT pacing. The primary endpoint was all-cause mortality, heart failure requiring intravenous therapy, or increase $\geq$15% in the left ventricular end-systolic volume index. The average follow-up was just over 3 years. Patients programmed to CRT had a significantly lower incidence of the primary endpoint (HR 0.74 (0.60–0.90)). In a prespecified analysis, BLOCK-HF also examined functional outcomes, including quality of life (Curtis et al., 2016). CRT patients showed greater improvement in NYHA functional class, quality of life, and clinical composite score. Implantation was unsuccessful in 5.5% of patients (51/918) and CRT-related complications occurred in 6.4% of randomized patients. For patients with AV block and a left ventricular function <50%, in BLOCK-HF CRT not only reduces the risk of heart failure and mortality, but also leads to functional improvement. This however comes at the cost of an increased complication rate. Finally it is important to point out that 30% of patients in BLOCK-HF had an ejection fraction <35% and as such had a guideline-based indication for CRT (**Table 3**).

Another large trial BIOPACE randomized 1810 patients with AV block to DDD or CRT to determine if CRT could prevent the deleterious effects of RV pacing. The first results were presented in 2014, but the study has not been published (Blanc, 2014; Funck et al., 2006, 2014). During a follow-up of 5.6 years there was no difference in the composite primary endpoint of mortality and heart failure hospitalization. Up to 14% of CRT implants were unsuccessful in this study. The positive results of BLOCK-HF could not be confirmed by this first analysis of BIOPACE, but because the investigators saw a trend in favor of CRT (HR 0.87; $P=0.08$) to our knowledge the follow-up was extended. Although it seems attractive and there are some encouraging results, a preventive role of CRT to avoid the development of heart failure in patients with a conventional bradycardia-related PM indication remains controversial. The guidelines for now do not recommend routine use of CRT in AV block patients with an expected high percentage of right ventricular pacing, but certainly in patients with a decreased left ventricular function the advantages of a one-stage approach should be weighed against the possibility of upgrading in a second intervention.

Choice of Programming

AV Delay Management

The disappointing results of the trials comparing single chamber ventricular pacing with more physiologic atrial-based pacing raised the question if right ventricular pacing might be detrimental. This hypothesis gained evidence by a post hoc analysis of the CTOPP

Table 3 Complication rate of the different types of implantations in clinical trials

Trial	*VVI*	*DDD*	*CRT*
CTOPP	3.8%	9.0%	
MOST		4.8%[a]	
UKPACE	3.5%	7.8%	
Meta-analysis	3.2%	6.2%	
DANPACE		11.9%	
CARE-HF			12.4%
COMPANION			10.0%
BLOCKHF			10.3%[a]

[a]Limited to 30 days postprocedure.

trial that evaluated if pacemaker-dependent patients, defined as having an unpaced heart rhythm ≤60 bpm, could have an increased benefit from physiological pacing compared with nonpacemaker-dependent patients, defined as having an unpaced heart rhythm >60 bpm (Tang et al., 2001). This analysis confirmed that the unpaced heart rhythm had an important influence on how pacing mode selection affected mortality. Also in MOST the effect of right ventricular pacing induced desynchronization was studied (Sweeney et al., 2003). This analysis revealed that patients were more frequently paced in the right ventricle in the DDD group compared with the VVI group (90% vs. 58%, $P=0.001$). The percentage of right ventricular pacing was a strongly associated with the risk for heart failure hospitalization and atrial fibrillation in both groups. Finally a defibrillator trial (The dual-chamber and VVI implantable defibrillator (DAVID) trial) in 506 patients (without a bradycardia-related PM indication) prospectively demonstrated a worse outcome of the composite endpoint of death or hospitalization for heart failure (HR 1.61 (1.06–2.44)) with DDDR at 70 bpm compared with VVI backup pacing at 40 bpm (Wilkoff et al., 2002). Pacing was more frequent in the DDDR group and the percentage RV pacing was correlated with the primary endpoint. In a subanalysis, the cut-off of right ventricular pacing resulting in the best prediction of endpoints was 40%, with patients with ≤40% pacing in DDDR having an equal outcome as patients in the VVI group (Sharma et al., 2005).

This notion that right ventricular pacing has harmful effects on ventricular function led to the development of different AV delay management (AVM) algorithms in modern pacemakers, developed to minimize right ventricular pacing. These algorithms are either based on prolongation of the AV delay or on AAI pacing with monitoring of the ventricular response and the possibility to switch from AAI to DDD pacing at the occurrence of second-degree AV block.

Several studies evaluated the effect of AVM. The SAVE PACE trial randomized 1065 patients with sinus node disease (Sweeney et al., 2007). The primary endpoint time to persistent atrial fibrillation developed in 12.7% in the conventional DDD pacing and 7.9% with AVM (HR 0.60 (0.41–0.88); $P=0.009$). The median percentage of ventricular pacing was significantly lower (9.1% vs. 99.0%) with AVM. These positive results could however not be confirmed in several more recent trials. The MINERVA trial randomized 1166 patients with tachy-brady syndrome, to conventional DDD pacing, AVM with or without atrial preventive pacing algorithms (Boriani et al., 2014). A combined endpoint of death, cardiovascular hospitalizations, or permanent AF, after 2 years of follow-up occurred in 26.5% with conventional pacing, 19.9% with AVM with atrial preventive pacing algorithms, and 21.4% with AVM only. Compared with conventional DDDR pacing, this was significant for the combination of AVM with atrial preventive pacing algorithms (HR=0.74 (0.55–0.9); $P=0.04$), but not for AVM only (HR 0.89 (0.77–1.03); $P=0.125$). The PREFER MVP trial compared DDD with and without AVM (Botto et al., 2014). A total of 605 patients were randomized, but no significant differences in the primary endpoint or cardiovascular hospitalization nor the secondary endpoint of atrial fibrillation were found, despite a clear reduction in right ventricular pacing percentage (5% vs. 86%; $P<0.001$). The ANSWER trial enrolled 650 patients between conventional DDD and AVM. AVM led to a significant decrease ventricular pacing (11.5% vs. 93.6%; $P<0.001$). No significant difference was found in the co-primary composite of hospitalization for HF, AF, or cardioversion (HR 0.78 (0.48–1.25); $P=0.30$), nor in the individual components. There was however a slightly significant reduction in the secondary combined endpoint of cardiac death or heart failure hospitalization (HR 0.49 (0.27–0.90); $P=0.02$).

The mixed results of these trials have to be interpreted carefully because they included different patient populations and the programming of the AV delay in conventional DDD was not uniform. It is clear that AVM decrease the percentage of right ventricular pacing and that this might reduce the risk for atrial fibrillation and heart failure, but the mixed results together with the previously discussed DANPACE trial make a strategy of avoiding right ventricular stimulation and allowing very long AV intervals highly controversial. Currently the optimal AVM strategy to optimize clinical outcomes remains unknown. Use of these algorithms may be not appropriate in patients in whom atrial pacing leads to a long first-degree AV block. How long is too long however is a matter of debate. The optimal AV delay varies individually, but varies up to 250 ms, a cut-off proposed also in HRS/ACCF Expert Consensus Statement on Pacemaker Device and Mode Selection (Strohmer et al., 2004; Gillis et al., 2012).

Rate Responsive Pacing

Patients with sinus node disease commonly have problems to adapt their heart rate to exercise. Antiarrhythmic drugs often exacerbate this chronotropic incompetence. All current PMs are equipped with sensor to estimate the metabolic need of the patients. These sensors are mainly based on three principles: motion detection, estimation of the respiratory rate, or estimation of the contractile force of the ventricle. The PMs have auto-adaptive software that can increase the paced rate in case of a higher metabolic demand. This rate adaptive pacing was used in most of the patients included in the trials comparing VVI/R with DDD/R pacing.

Over the short term, a benefit of rate adaptive pacing on exercise tolerance is reported, but the long-term benefit is the subject of debate (Abi-Samra et al., 2013; Coman et al., 2008; Duff et al., 2003; Lamas et al., 2007).

The large ADEPT trail was a single-blind randomized controlled trial comparing dual-chamber with rate-modulated dual-chamber pacing with the purpose to determine whether dual-chamber rate-modulated pacing improves quality of life (Lamas et al., 2007). The randomized patient cohort included 872 patients of which 64% had sinus node disease. Patients with DDDR reached a higher peak heart rate during exercise test at 6 months (DDD 113.3 ± 19.6 vs. DDD 101.1 ± 21.1; $P<.0001$), however total exercise time was not different. At 1 year, there were no significant differences in any quality-of-life assessment.

It is also not clear if any sensor technology has advantage over the other. This was tested in the MOST trial. In a selection of 1245 patients with three sensors (accelerometer, piezoelectric, and blended (ventilation rate and accelerometer)) the clinical characteristics and quality of life were followed during the trial (Shukla et al., 2005). A limitation is that since there was no randomization the clinical characteristics of the patients were not equally distributed. After a follow-up of almost 3 years mortality, heart failure hospitalization, atrial fibrillation, and the combined endpoint of mortality and stroke were not different between the sensor types, even after adjustment for baseline differences. Patients with blended sensors had worse physical function and quality of life. However, a selection bias cannot be ruled out. The most sophisticated sensors could have been used in the sickest patients.

Therefore rate adaptive pacing is recommended only for patients with symptomatic chronotropic incompetence and should be re-assessed every visit (Gillis et al., 2012). There seems no evidence one type of sensor is absolutely better than another, however there is some logic in using devices with two types of sensor in the more difficult patients to allow more means of fine-tuning.

Clinical Implications

When you consider implanting a device first make sure there is no indication for an implantable defibrillator (ICD) or CRT by taking into account the left ventricular ejection fraction, QRS-width, and the co-morbidity of the patient. In case of a left ventricular ejection fraction <35% or between 35% and 40% with nonsustained VT on monitoring there could be an indication for an ICD. In case of a left ventricular ejection fraction <35% and the intrinsic QRS width is broader 130 ms or the patient will be continuously paced, there could be an indication for CRT. As discussed previously if the EF is between 35% and 50% and you expect continuous right ventricular pacing in a patient with a good life expectancy the BLOCK-HF trial suggests that CRT might be an option as well.

If you decide to implant a PM you can use the algorithm of Fig. 2 as guidance. If the underlying rhythm is either permanent atrial fibrillation or there is a history of recurrent atrial arrhythmia treated with a rate control strategy, a single-chamber pacemaker is indicated. In case of symptomatic chronotropic incompetence, this should be programmed in VVIR, in the other case programming in VVI is sufficient. In case of sinus rhythm or recurrent atrial arrhythmia treated with a rhythm control

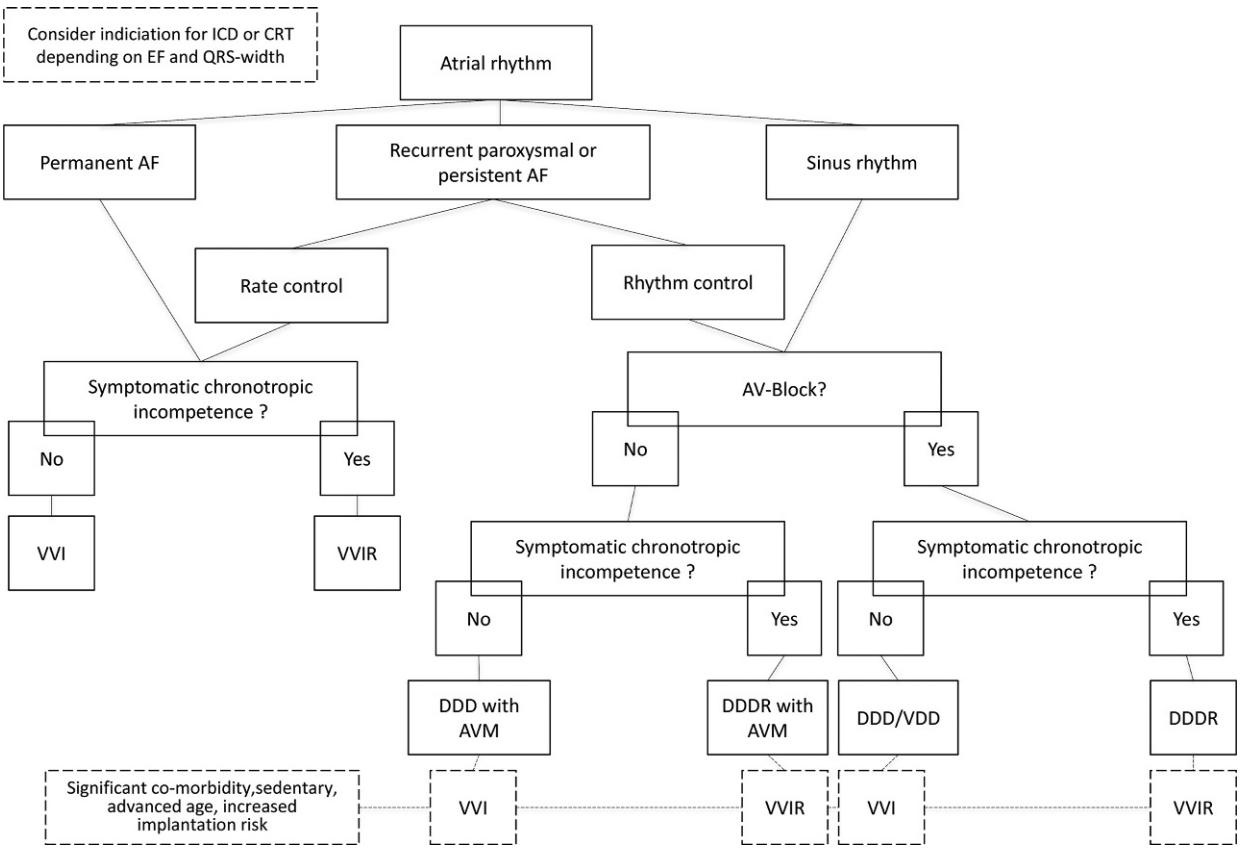

Fig. 2 Clinical decision algorithm.

strategy, a dual-chamber pacemaker is indicated. If there is underlying AV block depending on the presence of concomitant sinus node disease, the best programming is DDD (normal sinus node function) or DDDR (symptomatic chronotropic incompetence). If there is no problem with AV nodal conduction DDD or DDDR with AVM to minimize right ventricular pacing is indicated. In case of frail elderly sedentary patients or increased implantation risk (e.g., patients on hemodialysis, re-implantation post infection, and venous obstruction) cross over to single-chamber pacing always seems warranted in view of the equal mortality between both options.

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Induced Pluripotent Stem Cell–Derived Cardiomyocytes in Advancing Cardiovascular Medicine

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Introduction to iPSCs

Pluripotency is the ability to give rise to all cell types that comprise the organism (except placental cells, which necessitates totipotency) with unlimited potential for self-renewal. Embryonic stem cells have this innate characteristic, but over the past 10 years, scientists have unraveled a mechanism to coax fully differentiated somatic cells back to their precursor states.

The origins of induced pluripotent stem cells (iPSCs) began with Yamanaka and his team in 2006, who identified four crucial transcription factors, *Sox2*, *Oct4*, *Klf4*, *and c-Myc*, that were necessary and sufficient to induce pluripotency from murine fibroblasts, in turn generating the first set of iPSCs (Takahashi et al., 2006). Within a year of their discovery, Yamanaka had successfully generated iPSCs from primary human somatic cells (Takahashi et al., 2007).

These versatile cells have the added benefit of being manipulated and propagated without the ethical concerns surrounding the use of embryonic stem cells. The notion of isolating human cells from anywhere in the body, then reconditioning them to a more primitive, labile state, and, finally, diverting them to a new lineage provide ample opportunity to study diseases with tropisms for specific cell types as well as detecting underlying molecular markers of disease.

The potential of hiPSCs is immense, but the technology is still far from perfect, with several prominent barriers before they can be routinely integrated into human clinical trials. In 2014, the first iPSC clinical trial led by Dr. Takahashi involved transplantation of patient-derived retinal pigment epithelial cells (RPC) to treat age-related macular degeneration (Kimbrel and Lanza, 2015). However, the trial was put on hiatus in 2015 after the discovery of six genetic mutations in the first patient's iPSCs with concern for tumorigenesis prior to transplantation into a second patient (Trounson and DeWitt, 2016). In June 2016, the trials were given the green light to resume after further investigations and regulatory changes (Scudellari, 2016).

The utility of iPSCs stems from their in vitro versatility and in vivo applicability. They serve as excellent models of heritable and inducible disease states, functional platforms for gene-editing innovation (especially in the wake of CRISPR-Cas9 technology), and are safe surrogates for drug testing that help bridge the translational divide from bench to bedside.

The Role of iPSCs in Cardiovascular Research

Heart disease is the leading cause of death in the United States (Roth et al., 2015). Because cardiac myocytes have severely limited capacity for regeneration and self-renewal, dying cardiomyocytes (CMs) due to ischemic injury or aging are replaced by scar tissue, leading to worsening heart function and ultimately death (Chin and Murry, 2012). A smaller subset of heart diseases involves inherited conditions in which typically young patient populations present with potentially fatal arrhythmias or heart failure (Watkins et al., 2011). For many with advanced heart failure, there is no definitive treatment besides heart transplantation. While early enthusiasm for human iPSCs involved their potential in cell therapies for heart diseases, these efforts are still years away from human trials. By contrast, human iPSCs have already had a major impact in the study of human heart diseases (Karakikes et al., 2014).

Traditionally, the study of human heart diseases has been hampered by the extremely limited access to live human heart tissues. Moreover, while animal models have been extremely helpful, they have important species differences, and CMs isolated from adult animals are highly catabolic (Louch et al., 2011), making them less than ideal as an in vitro model. Therefore, there has been tremendous interest and effort in developing patient-specific iPSC-derived cardiomyocytes (hiPSC-CMs) as an in vitro platform to study human heart diseases.

Generating Cardiomyocytes From Human iPSCs

There are a number of differentiation protocols for generating CMs from human iPSCs. For instance, Kattman et al. found that robust differentiation of hiPSCs to CMs depended on conditions that recapitulated key commitment points in embryonic heart development (Kattman et al., 2011). This initially involved modulation of Activin A/TGF-beta superfamily signaling, which in turn modulated the Wnt signaling pathway (Wang et al., 2014b). Today, the most widely utilized cardiac induction protocols involve sequential activation and inhibition of the canonical Wnt signaling pathway (Lian et al., 2013; Burridge et al., 2014). With the use of small molecule Wnt pathway modulators, chemical defined media, and metabolic selection (Burridge et al., 2014), highly purified populations of hiPSC-derived cardiomyocytes (hiPSC-CMs) can now be routinely generated in large quantities from virtually all iPSC lines.

Despite rapid advances in the field, a major hurdle remains the immaturity of hiPSC- CMs. For instance, hiPSC-CMs generated by most methods are small and circular, and express the fetal isoform of troponin (Yang et al., 2014). Moreover, hiPSC-CMs often display weak contractile properties and are unable to respond appropriately to adrenergic stimulation. This contrasts with adult CMs, which are larger and rod-shaped, and exhibit robust contraction and cell shortening (Lundy et al., 2013). Therefore, a number of approaches involving tissue engineering, biomaterials, mechanical loading, and electric stimulation have been utilized to promote hiPSC-CM maturation (Yang et al., 2014; Chun et al., 2015). Recently, Feaster et al found that hiPSC-CMs plated on an undiluted Matrigel of at least 0.4 mm in thickness, termed a "Matrigel Mattress," elongated into a rod-like shape and exhibited robust cell shortening, a phenomenon that was not observed previously (**Fig. 1**) (Feaster et al., 2015). In addition, hiPSC-CMs plated on the Matrigel Mattress adopted more mature characteristics and exhibited contractile properties akin to acutely isolated rabbit and mouse CMs. Moreover, these Mattress hiPSC-CMs exhibited the intracellular calcium handling and electrophysiological properties similar to that of adult CMs, and expressed mature CM molecular markers with reduced fetal markers. Innovations such as these toward the development of hiPSC-CM models that faithfully recapitulate characteristics of native human adult CMs represent a significant step toward utilizing these cells as a valuable platform for human heart disease modeling, drug testing, and regenerative therapies (**Fig. 2**).

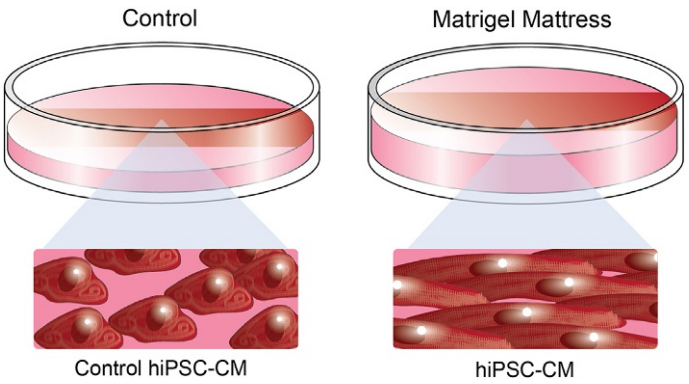

Fig. 1 hiPSC-CMs plated on the Matrigel Mattress recapitulates adult cardiomyocyte properties. Seeding of hiPSC-CMs on undiluted Matrigel promotes elongation and contractile shortening when compared to hiPSC-CMs plated using standard substrate (control).

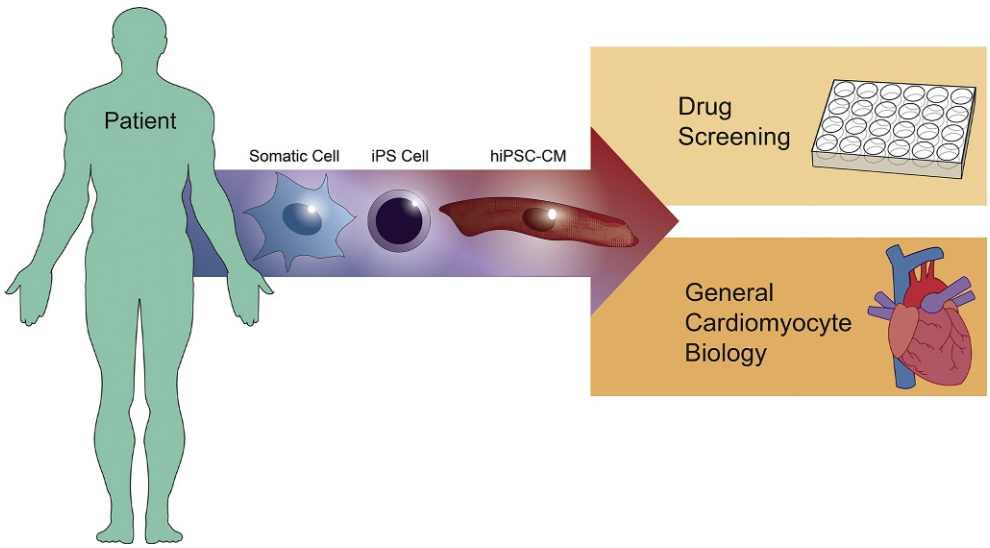

Fig. 2 Patient-derived iPSCs enable in vitro and in vivo applications of hiPSC-CMs in drug discovery and cardiomyocyte biology. HiPSCs can be generated from human somatic cells. HiPSCs can be differentiated into hiPSC-CMs for drug discovery for toxicity and drug efficacy. HiPSC-CMs can also be used for disease modeling and general cardiomyocyte physiology.

Modeling Human Diseases With iPSC-Derived Cardiomyocytes

Among the earliest efforts to use patient-specific iPSC-derived CMs involved the study of inherited arrhythmias caused by specific gene mutations. In one such condition named congenital long QT syndrome, patients present with markedly prolonged QT intervals on electrocardiograms and delayed repolarization, which predisposes them to sudden cardiac death from ventricular tachyarrhythmias such as *torsades de pointes* (Roden, 2008). For instance, CMs derived from a patient with type-1 long QT syndrome (LQTS1), associated with a mutation in the *KCNQ1*, which encodes a subunit of the voltage-gated potassium channel involved in action potential termination, recapitulated the electrophysiological features of the disorder, including the diminution in I(Ks) potassium current and susceptibility to catecholamine-induced tachyarrhythmia (Moretti et al., 2010). Similarly, hiPSC-CMs from patients with type-2 long QT syndrome (LQTS2), associated with a mutation in KCNH2, which encodes another voltage-gated potassium channel, recapitulated the electrical derangements, including propensity for early–after depolarization triggered arrhythmias that is aggravated by potassium-channel blockers (Itzhaki et al., 2011).

In addition, hiPSC-CMs from patients with familial hypertrophic cardiomyopathy (HCM), an autosomal-dominant disorder of the cardiac sarcomere associated with left ventricular wall thickening and predisposition to arrhythmias and sudden cardiac death, recapitulated many of the hallmarks of HCM in vitro (Lan et al., 2013). Patient-specific hiPSC-CMs carrying the missense mutation in the *MYH7* gene, encoding the beta-myosin heavy chain, exhibited cellular hypertrophy, sarcomere disarray, contractile arrhythmias, and abnormal calcium handling. Furthermore, the team investigated the role of calcium dysregulation in the progression of disease and found that treating these CMs with calcium inhibition significantly mitigated the disease phenotype and reduced electrophysiological irregularities.

Patient-derived iPSC-CMs have been used to model familial dilated cardiomyopathy (DCM), in which the heart chamber becomes enlarged along with the loss of contractile function. For instance, Sun et al. demonstrated that hiPSC-CMs from patients with a point mutation in the gene encoding sarcomeric protein troponin T exhibited altered calcium regulation, decreased contractility, and abnormal sarcomere structure (Sun et al., 2012). Interestingly, just as seen in DCM patients, beta-adrenergic stimulation of mutant hiPSC-CMs exacerbated these abnormalities, while beta-adrenergic blockade improved cardiac function. Recently, Hinson et al. generated hiPSCs from familial DCM patients with a titin-truncating variant, which as a group is thought to be the most common genetic cause of DCM, and demonstrated poor sarcomere contractile performance in their engineered microtissue model (Hinson et al., 2015). Besides these studies, patient-derived hiPSCs have provided important pathophysiologic insights into many other inherited heart diseases, including the mitochondrial cardiomyopathy of Barth syndrome (Wang et al., 2014a), the metabolic derangements of arrhythmogenic right ventricular cardiomyopathy (Kim et al., 2013), abnormal TGF-β signaling in left ventricular noncompaction cardiomyopathy (Kodo et al., 2016), and aberrant AMP-activated kinase signaling in PRKAG2 cardiomyopathy (Hinson et al., 2016), just to name a few.

Drug Discovery, Efficacy, and Toxicity in Cardiovascular Medicine

Drug discovery is an integral aspect of translational medicine that applies basic science understanding of disease physiology to pharmaceutical outcomes. In the broadest sense, there are two major routes for drug discovery—phenotypic and target-based screening (Swinney and Anthony, 2011). Phenotypic screening involves evaluating the effects of a candidate molecule on phenotypes of a biological system. For millennia, this was the primary route of drug discovery that relied on serendipitous observations. With advances in cellular and molecular technologies, target-based screening, which entails high-throughput screens for hits that modulate a target, came to the forefront. The compounds that perturbed the target of interest would then be further evaluated for desired effects in in vitro and in vivo studies. While target-based screening has been the dominant paradigm for decades, in recent years, there has been a revival in phenotypic screening (Wagner, 2016). The iPSC technology dovetails nicely with this trend.

The versatility of iPSCs has already proven fruitful in evaluating potential cardiovascular therapeutics. Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited rhythm disorder characterized by potentially fatal ventricular arrhythmias that manifest during high adrenergic, or stressful, states in otherwise healthy adults with structurally normal hearts. The disease is caused by autosomal-dominant mutations in *RYR2*, which encodes the cardiac ryanodine receptor (CPVT-1), or by recessive mutations in calsequestrin, a calcium-binding protein in the sarcoplasmic reticulum (CPVT-2) (Napolitano et al., 1993). Both are sarcoplasmic reticulum proteins that control CM contractility by regulating calcium cycling within the CM, and mutations that disrupt them result in the loss of calcium regulation, reduced myocyte electrical stability, and a predisposition to ventricular tachyarrhythmias under catecholaminergic states. Jung et al. discovered that Dantrolene, a muscle relaxant that binds to ryanodine receptors and inhibits calcium release from the sarcoplasmic reticulum, reduced the arrhythmogenic potential of CPVT-1 iPSC CMs harboring a mutation in the *RYR2* gene (Jung et al., 2012). In the hiPSC-CM model of LQTS2 mentioned above, the calcium channel blocker Nifedipine and the potassium channel opener Pinacidil markedly reduced early–after depolarizations, correcting the underlying pathology that predisposes these patients to life-threatening arrhythmias (Itzhaki et al., 2011).

While it is important to recognize the potential of hiPSC-CMs on guiding cardiovascular drug discovery and contributing to proof-of-principle models for certain monogenic disorders, it is also important to acknowledge current limitations. Many in vitro experiments are conducted in controlled, single, immature CM settings and do not take into consideration the multicellular and organ system interactions that would be present on an organismal level. The single cell cardiac models may not adequately reproduce the contractile dynamics observed in mice or human hearts experiencing daily alterations in the level of adrenergic innervation and suppression (Garbern et al., 2013). Technical variability that arises during differentiation may also mask true pathologic differences among CMs and blur the lines between medication efficacy and individual variation, especially in sporadic diseases that have poorly characterized baselines.

Drug development is a highly risky and costly endeavor. Unforeseen adverse outcomes, especially during late-stage clinical trials, can derail a multibillion dollar effort. Certain medications, especially cytotoxic chemotherapeutic agents with a proclivity for accumulating in metabolically active tissues, are notorious for contributing to heart disease and heart failure. For example, many cancer survivors previously treated with anthracyclines such as Doxorubicin go on to develop heart failure. Burridge et al. used hiPSC-CMs from breast cancer patients who developed cardiomyopathy following Doxorubicin therapy to demonstrate that patient-derived hiPSC-CMs reproducibly elicited Doxorubicin-induced injury responses, including decreased cellular viability and increased production of reactive oxygen species, in contrast to healthy control cells (Burridge et al., 2016). Tyrosine kinase inhibitors (TKIs) are another class of anticancer agents that have also been implicated in cardiovascular toxicities, including arrhythmias and heart failure. Using hiPSC-CMs, hiPSC-derived endothelial cells, and hiPSC-derived cardiac fibroblasts, Sharma et al. carried out a high-throughput screen of FDA-approved TKIs and found that VEGFR2/PDGFR-inhibiting TKIs were associated with cardiotoxicity in this model (Sharma et al., 2017). Interestingly, they also identified insulin and insulin-like growth factor (IGF) signaling as cardioprotective signaling mechanisms that could alleviate TKI-mediated cardiotoxicity. Thus, in addition to modeling disease phenotypes, hiPSC-CMs also serve as effective platforms for investigating collateral effects of novel therapeutic compounds.

The most feared, though rare, cardiotoxicity of a drug is QT prolongation, which may progress to potentially fatal *torsades de pointes* (Fermini and Fossa, 2003). The drug-induced QT prolongation is typically brought about by inhibition of delayed rectifier potassium channels, the alpha subunit of which is encoded by the hERG/KCNH2 gene, which causes LQT2 (Yu et al., 2015). Several drugs, which cause QT prolongation/*torsades de pointes*, have been withdrawn from the market, while others have garnered black box warnings, which preclude use by many patients who would otherwise benefit from these medications.

Presently, assays involving whole heart specimens or tissue slices as well as animal testing are the gold standard in industry testing prior to human clinical trials. Early screening of cardiotoxicity is still heavily reliant upon single channel hERG models expressed within cell lines such as Chinese hamster oocytes (CHO) or human epithelial kidney cells (HEK). The screening of proarrhythmic drug effects can be simulated through patch-clamp trials, which involve electrophysiological measurements of current passing through hERG channel before and after the additional of the compound being tested (Yao et al., 2008).

Implementation of hiPSC-CMs to assess cardiotoxicity of drugs offers several potential benefits. In principle, cells from patients who developed drug-induced arrhythmias may provide a greater sensitivity for detecting potential proarrhythmic drugs as well as greater negative predictive value. Several groups have now developed an iPSC-based approach using a panel of cells from subjects with a range of susceptibility to drug challenge, including those with extremely low or high changes in cardiac repolarization in response to Sotalol challenge (Stillitano et al., 2017) as well subjects with specific inherited cardiomyopathies, such as LQTS and familial HCM (Liang et al., 2013). These studies showed that hiPSC-CMs from healthy and diseased patients exhibited different susceptibilities to cardiotoxic drugs and their use accurately modeled drug-induced proarrhythmic responses compared to the traditional hERG inhibition assay.

Precision Medicine and Personalized Therapies

While much of the focus has been on the deployment of hiPSCs for modeling heart diseases and for screening and testing new drugs, the application of hiPSCs in cell replacement therapy remains an exciting, albeit formidable, next frontier. Generation of virtually any cell or tissue type from hiPSCs on a large scale seems well within the horizon, and the use of healthy hiPSC-derived tissues to replace those lost or injured has tremendous medical implications. Currently, the only definitive therapy for end-stage heart failure is heart transplantation (Katz et al., 2015). However, given the dearth of available donor hearts and the extraordinary skills and resources required, transplantation cannot hope to meet the ever-growing demand. In this light, autologous, or patient-specific, hiPSC-CM may 1 day serve as an alternative to orthotopic heart transplantation. Recent studies involving the transplantation of hiPSC-CMs encapsulated in hydrogel or in cardiac "patches" or sheets have shown promise in rodent models (Wang et al., 2015; Wendel et al., 2015; Kawamura et al., 2012). In these studies, hiPSC-CM transplantation tissue engraftment significantly improved cardiac function and reduced left ventricular remodeling. However, it is worth noting that the long-term survival of these transplanted cells was very small, despite still being able to be detected after 8 weeks (Kawamura et al., 2012). Recently, Shiba et al. carried out an allogeneic transplant of MHC-matched primate iPSC-CMs into the heart of monkeys that were subjected to myocardial infarction (Shiba et al., 2016). The iPSC-treated monkeys had improved cardiac function at 4- and 12-week of transplantation, and grafted cells survived for 12 weeks, showing electric coupling to the host CMs. Similarly, Murry and colleagues

introduced human embryonic stem cell–derived cardiomyocytes (hESC-CMs) via intramyocardial injection to monkeys that were subjected to myocardial ischemia–reperfusion injury. The donor cells had complete electrical coupling to the recipient primate hearts and significantly remuscularized the infarcted monkey hearts (Chong et al., 2014). In contrast to small animal studies, nonfatal ventricular arrhythmias are noted in the primate studies, indicating that additional efforts are needed to minimize posttransplant arrhythmic complications.

These myocardial cell transplantation experiments, particularly those in primates, provide proofs-of-principle that much of the logistic challenges of cell manufacturing and delivery can be overcome. Nevertheless, the long-term durability of engraftment and beneficial effects remain unknown and continue to be an ongoing challenge. Allogeneic cell transplants or tissue grafts will carry the attendant risk of rejection despite optimal immunosuppression. Given this concern, autologous hiPSCs may become a mainstay of truly personalized therapy for regenerating myocardium. However, even autologous hiPSCs, from epigenetic and genetic changes incurred during reprogramming and culturing, have been found to incite an immune response that could lead to rejection (Scheiner et al., 2014).

Until the effectiveness of generating and maintaining hiPSCs improves to the point where the associated costs are greatly reduced, the use of allogeneic donor hiPSCs on multiple patients with immunosuppression may be more practical. With recent revolution in the CRISPR/Cas9 gene-editing technology, it may soon be possible to design a universal donor hiPSC line with modified human leukocyte antigen (HLA) genes that can overcome the immune (Riolobos et al., 2013).

In January of 2016, President Obama announced the Precision Medicine Initiative (PMI), the fundamental goal of which is to develop preventative and treatment regimens that account for phenotypic variations within the general population. Individual genetic composition and environmental differences can account for variations in responsiveness among individuals to disease therapies. The PMI strives to identify these interindividual discrepancies in order to develop patient-specific treatments. In principle, patient-derived hiPSCs offer an unlimited source of cells and tissues with patient-specific disease susceptibilities and treatment responses, which may better predict a patient's individual response in the clinical setting (Karakikes et al., 2015).

Future Directions

Although iPSCs have tremendous potential in the field of personalized and regenerative medicine, there are currently a number of obstacles to their widespread use in the clinic. Concerns regarding mutagenesis and tumorigenic potential of hiPSCs must be thoroughly addressed (Ghosh et al., 2011). An obvious solution is to fully differentiate iPSCs into tissue cells prior to transplantation, but even then, there still exists more than a trivial risk of transformation of trace undifferentiated iPSCs into malignant cells or teratomas. In addition, since each of the reprogramming factors Oct4, Sox2, Klf4, and c-Myc is associated with oncogenesis (Kumar et al., 2012; Lundberg et al., 2016; Yu et al., 2011; Miller et al., 2012), research is ongoing to identify induction factors that are less carcinogenic, to streamline the number of factors required to achieve pluripotency, and to develop ways to completely eliminate exogenous DNA once the somatic cell has been reprogrammed (Medvedev et al., 2010). Low efficiency and slowness of the reprogramming process are other hurdles to a widespread implementation of the iPSC technology (Medvedev et al., 2010). In this light, several recent studies have shown that the use of small molecules that alter epigenetics through the modulation of histone methyltransferases, or histone deacetylases, can significantly increase reprogramming efficiency (Shi et al., 2008; Huangfu et al., 2008). Given the breathtakingly rapid advances in cellular reprogramming, directed differentiation, and genomic editing (Hajjar and Hulot, 2014), there is little doubt that these hurdles can be overcome in the near future. In summary, human iPSCs and hiPSC-CMs offer an unprecedented window to human heart disease pathogenesis and individualized response to therapy as well as the opportunity for transformative new therapies.

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Infective Endocarditis in 21st Century

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Introduction

Infective endocarditis (IE) is the infection of the endocardial surface of the heart, native valves, prosthetic valves, and indwelling cardiac devices. IE can result in injury to multiple organ systems of the body and is associated with high mortality and morbidity unless treated early and properly. IE was described as early as 16th century. In 1885, Sir William Osler described this disease as malignant endocarditis in Gulstonian lectures. (Osler, 1885) Since that time the pattern of this disease has continuously changed.

Epidemiology

Incidence

IE is a rare disease with annual incidence of about 3–11 patients per 100,000 population (Murdoch et al., 2009; Duval et al., 2012; Hoen et al., 2002; Pant et al., 2015). The incidence of IE has changed over the years. The incidence of IE from 1988 to 1990 in Philadelphia metropolitan area was approximately 11.6 cases per 100,000 person-years (Berlin et al., 1995). Between 2000 and 2011, the incidence of IE in the United States increased from 11 per 100,000 population to 15 per 100,000 population (Pant et al., 2015).

Risk Factors

The risk factors for the disease vary worldwide. Access to healthcare, living conditions, migration of population, development of antibiotics, and advances in therapy has created ever changing epidemiology of IE. The epidemiology in low and high income countries was similar during the early antibiotic age and has changed since then (Yew and Murdoch, 2012). In developing countries, rheumatic heart disease (RHD) still remains as the major risk factor and is responsible for about two-thirds of the cases (Yew and Murdoch, 2012). RHD is predominantly caused by group A streptococcus from a preceding respiratory infection and patients are typically younger. In the developed countries, RHD contributes to <5% cases, and the most common heart disease associated with IE is degenerative valve disease (Murdoch et al., 2009). Prosthetic valve was a predisposing condition in 22.2% of the patients with IE, while implanted devices such as permanent pacemakers and implantable cardioverter-defibrillators (ICD) were implicated in 11% of the cases (Murdoch et al., 2009). Other predisposing causes of IE are congenital heart disease, intravenous drug use, diabetes, and cancer.

Age

The mean age of patients with IE has also changed significantly overtime. Historic data from John Hopkins Hospital showed the median age of patients with IE was <30 years in 1926 (Thayer, 1926). Patients with IE appear to be older in recent times, with the average age of patients was >70 years in 2001–2006, compared to mid-40s during the early 1980s (Correa de Sa et al., 2010).

This change in age at which IE presents is due to falling incidence of RHD in the economically developed world. RHD seen mostly in developing or undeveloped countries occurs primarily in younger age group, but even in those countries the incidence of degenerative valve disease is rising with increases in life expectancy.

Hospital acquired IE

Healthcare acquired IE accounts for 25%–30% of contemporary cohorts (Murdoch et al., 2009). Increase in the use of chronic indwelling intravenous lines and invasive procedures has led to increase in rates of staphylococcal bacteremia, which is a risk factor for IE (Lyytikäinen et al., 2005). Prosthetic valves and indwelling cardiac devices such as permanent pacemakers, ICD can also be a nidus for infection within the heart. As the rate of implantation of these devices increases, the burden of IE increases (Voigt et al., 2006; Greenspon et al., 2011). In a recent study of 2781 patients with definite IE, 20% of patients had a prosthetic valve and 7% of the patients had a cardiac device (Murdoch et al., 2009).

Congenital heart disease

Increased survival rate of patients with congenital heart disease has resulted in increases in IE in this population (Day et al., 2009). The overall incidence of IE in adults with congenital heart disease is 11 per 10,000 person-years (Verheugt et al., 2011). The most common conditions associated with IE among children with cyanotic congenital heart disease are endocardial cushion defect and left-sided lesions (Rushani et al., 2013). Cardiac surgery within 6 months of birth is associated with 5-fold increased risk for IE (Rushani et al., 2013). IE risk appears to be reduced after repair that leaves no residual shunt or prosthetic material, and the highest risk during follow up is found in patients with left-sided outflow obstruction (Morris et al., 1998). In the cohort mentioned earlier (Murdoch et al., 2009), congenital heart disease accounted for 12% of all patients with definite IE.

Other risk factors

Other risk factors include intravenous drug use, which results in blood stream seeding of skin flora and other contaminated organisms from the materials used for injection. Also, injection of particulate matter (e.g., talc, which may be present in drug solutions or introduced by intravenous injection of talc containing drugs intended for oral use) may cause valvular endothelial damage predisposing to infection. Poor dentition and dental infection are also thought of as risk factors for IE (Lockhart et al., 2009).

Microbiology

As the spectrum of risk factors has changed overtime, the microbiology has also changed. Staphylococcus and Streptococcus bacteria still account for majority of cases, although the proportion of these two organisms varies by region.

The increase in healthcare-associated IE has resulted in an increase in the incidence of *Staphylococcus aureus* (Federspiel et al., 2012) and coagulase-negative staphylococci (Duval et al., 2012) associated with IE. *Staphylococcus aureus* is the most common cause of IE in developed countries accounting for 30% of the cases (Murdoch et al., 2009; Selton-Suty et al., 2012).

Coagulase-negative Staphylococci are ubiquitous skin commensals and can colonize indwelling lines and devices. They are the most common cause of early prosthetic valve IE (López et al., 2007) and frequently noted in hospital-acquired native valve IE (Chu et al., 2008). These organisms are associated with biofilm production, high rates of abscess formation, and multiantibiotic resistance (Chu et al., 2009).

Enterococcus is now the third leading cause of IE (Pericas et al., 2015). It accounts for about 10% of cases of IE (Murdoch et al., 2009). Some recent data suggest that the frequency of Enterococcal IE is actually increasing, attributed to rise in hospital-associated infections caused by these organisms. Enterococcus spp. cause as much as 30% of hospital-acquired cases of IE (Giannitsioti et al., 2007). Two main species of Enteroccocus species *Enterococcus faecalis* and *Enterococcus faecium* account for most cases of IE in humans, *Enterococcus faecalis*, can cause both native valve and prosthetic valve IE in the elderly or chronically ill patients, and the vast majority of this species are still responsive to penicillin. On the other hand, *Enterococcus faecium* poses significant clinical challenge, and most clinical isolates in the United States are resistant to ampicillin and vancomycin. (Nigo et al., 2014)

With the decline in RHD, the proportion of *Streptococcus viridans* as a cause of IE has declined in developed countries (Duval et al., 2012), although it still remains the most common cause in developing and underdeveloped countries (Yew and Murdoch, 2012).

Other rare causes of IE are fastidious bacteria, zoonotic bacteria and fungi (Cahill and Prendergast, 2016), which are usually associated with healthcare (Morpeth et al., 2007; Baddley et al., 2008). These rare organisms vary significantly with geographical, epidemiological, and risk factors. Fastidious organisms include the HACEK bacteria (*Haemophilus*, *Aggregatibacter*, *Cardiobacterium*, *Eikenella corrodens*, *Kingella*), which account for about 3% of cases (Das et al., 1997; Fournier et al., 2010). Zoonotic IE is caused by *Coxiella burnetii* (*livestock contact*, *tick borne*), *Brucella* spp. (livestock, unpasteurized dailry products), *Bartonella henselae* (*cats*), and *Chlamydia psittaci* (*parrots*, *pigeons*). Other rare causes include Gram-negative bacteria (such as *Acinetobacter* spp., *Pseudomonas aeruginosa*), *Legionella* spp., *Mycoplasma* spp., and *Tropheryma whippelii* (Cahill and Prendergast, 2016; Holland et al., 2016; Brouqui and Raoult, 2001). Fungal endocarditis, usually caused by *Candida* or *Aspergillus*, is rare but is seen in patients with prosthetic valves who are immunosuppressed (Baddley et al., 2008).

About 10% of the cases have "blood culture negative" IE (Holland et al., 2016), most of which are due to patient receiving antibiotics before the culture. Fastidious organisms are difficult to isolate with traditional techniques. Specialized methods such as serological testing, histological examination, and polymerase chain reaction (PCR) using blood or valve biopsies can suggest a causative pathogen in up to 75% of these cases (Fournier et al., 2010).

Clinical Features

Clinical presentation of IE is often nonspecific, and this disease should be suspected in anyone presenting with fever with risk factors for IE. It can also present with complications arising from IE, especially stroke and systemic embolism. About 80%–90% of cases of IE have fever (Murdoch et al., 2009; Duval et al., 2012; Cahill and Prendergast, 2016). New murmur and worsening of a known murmur are reported in 48% and 20% of cases, respectively (Hoen and Duval, 2013). Less common presentations include hematuria (25% of cases), splenomegaly (11%), and splinter hemorrhages (8%). Janeway lesions, Roth's spots, and conjunctival hemorrhage are rare, presenting in 5% of cases. (Richet et al., 2008). Septic emboli can affect any organ system in the body and can present in several ways.

Cardiac manifestations include perforation of a valve leaflet or rupture of the papillary muscle, chordae tendineae, or the interventricular septum. Perivalvular abscesses and conduction abnormalities may result, especially with IE due to *Staphylococcus aureus*. Also, myocardial infarctions may occur as an embolic complication, especially in aortic-valve IE (Holland et al., 2016).

Neurologic manifestations arise most frequently due to cerebral emboli, clinically apparent in about 20%–30% of the people (Holland et al., 2016). The incidence of stroke in IE is 4.82 cases per 1,000 patient-days during the first week and declines quickly after initiation of antibiotics (Dickerman et al., 2007). Other complications include mycotic aneurysms formation, which tend to arise at bifurcation points, most commonly in the cerebral vessels, although almost any vascular bed can be affected.

Renal manifestations include infarction from a septic emboli, abscess, and immune complex glomerulonephritis. Renal biopsies performed during active IE are consistently abnormal, even if clinical renal disease is absent (Morel-Maroger et al., 1972).

Pulmonary manifestations include formation of septic pulmonary emboli from showering of emboli from the vegetation, which is more common in tricuspid valve IE. Pneumonia, pleural effusions, or empyema often accompany septic pulmonary emboli.

Other manifestations include petechiae, cutaneous infarcts, Osler's nodes (arteriolar intimal proliferation with extension to venules and capillaries), Janeway lesions (caused by septic emboli), and Roth spots in their eyes (immunological phenomena presenting as retinal hemorrhages with a pale centre). Also, direct bacteremic seeding of the eye may occur, causing endophthalmitis. Splenic infarcts may also be clinically occult, although splenic abscesses may develop which presents with pain, fever, and leukocytosis. Splenomegaly is found in about 10% of patients with IE (Murdoch et al., 2009) in the developed world, possibly due to prolonged immunological response.

Any patient with unexplained sepsis should be evaluated for IE, regardless of presentation. *Staphylococcus aureus* bacteremia is associated with IE in 25%–30% of cases, and all cases with *Staphylococcus aureus* bacteremia should undergo echocardiography (Fowler et al., 1997; Joseph et al., 2013).

Diagnosis

The diagnosis of IE is usually based on a combination of clinical, microbiologic, and echocardiographic findings. Any patient presenting with IE should undergo evaluation of risk factors such as previous IE, presence of prosthetic valve, valvular or congenital heart disease, intravenous drug use, indwelling intravenous lines and cardiac devices, immunosuppression, or a recent dental or surgical procedure. The Duke criteria have sensitivity and specificity of more than 80% and can aid in diagnosis along with clinical judgment (**Table 1**) (Li et al., 2000). According to the modified Duke criteria, definite IE is defined by pathologically proven IE or fulfillment of the clinical criteria (either two major criteria or one major and three minor criteria or five minor criteria) (Li et al., 2000). Possible IE is defined by either one major and one minor criteria or three minor criteria. Rejected IE is defined by firm alternative diagnosis, resolution of IE symptoms with antibiotic therapy for $\leq$4 days, no pathological evidence of IE at surgery or autopsy with antibiotic therapy for $\leq$4 days, or patient does not meet clinical criteria for possible IE (Li et al., 2000).

Identification of the microorganism with blood culture remains the mainstay in microbiological diagnosis. Three sets of blood cultures can identify organisms in 96%–98% of the cases and should be obtained before starting antibiotics (Lee et al., 2007). As mentioned earlier, 10% of the patients may have negative blood cultures. If blood cultures are negative for 5 days, risk factor-driven testing should be performed. Serological testing for coxiella, bartonella, brucella, mycoplasma, legionella, and chlamydia should be undertaken (Gould et al., 2012).

In blood culture-negative cases, molecular techniques may be helpful to identify the organism if the excised valve tissue is available. Broad range PCR combined with sequencing can identify specific organism (Goldenberger et al., 1997; Vondracek et al., 2011), and this can be helpful in patients with prior antibiotic exposure and with organisms difficult to culture. PCR combined with electrospray ionization mass spectrometry has been used recently to detect and characterize the amplified DNA and offers the possibility of direct detection of the organism from the tissue (Brinkman et al., 2013).

Routine laboratory investigations are usually nonspecific. Elevated inflammatory markers are detected in two-thirds of cases, and leukocytosis and anemia are noted in about half the cases (Murdoch et al., 2009; Crawford and Durack, 2003). Urinalysis may show

Table 1 Modified Duke clinical criteria for diagnosis of IE.

Major criteria

1. Positive blood cultures for one of the following:
   A. Typical microorganisms consistent with IE from two separate blood cultures:
   Staphylococcus aureus, Viridans streptococci, *Streptococcus gallolyticus*, HACEK organisms, Community-acquired enterococci, in the absence of a primary focus.
   B. Persistently positive blood culture for microorganisms consistent with IE:
      - At least two positive blood cultures from blood samples drawn >12 h apart
      - Three or a majority of ≥4 separate blood cultures positive >1 h apart
   C. Single positive blood culture for *Coxiella burnetii* or antiphase I IgG antibody titer >1:800
2. Evidence of endocardial involvement (one of the following):
   A. Echocardiogram positive for IE:
   Findings of oscillating intracardiac mass attached on a valve or on supporting structures, in the path of regurgitant jets, or on implanted material, in the absence of an alternative anatomic explanation) OR Abscess OR New partial dehiscence of prosthetic valve
   B. New valvular regurgitation

Minor criteria

1. Predisposing conditions such as intravenous drug use or presence of a predisposing heart condition
2. Fever: Temperature ≥38.0°C (100.4° F)
3. Vascular phenomena: Major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial hemorrhage, conjunctival hemorrhages, or Janeway lesions
4. Immunologic phenomena: Glomerulonephritis, Osler nodes, Roth spots, or rheumatoid factor
5. Microbiologic evidence: Positive blood cultures that do not meet major criteria or serologic evidence of active infection with organism consistent with IE

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microscopic hematuria and occasionally red cell casts or pyuria (Cahill and Prendergast, 2016). New conduction abnormalities noted in EKG are suggestive of paravalvular or myocardial extension of IE (Meine et al., 2001).

Echocardiography plays a crucial role in the diagnosis of IE, detecting complications, assessing severity of lesions, and assessing underlying heart function. Transthoracic echocardiography (TTE) has 75% sensitivity and more than 90% specificity for detection of a vegetation (Habib et al., 2010). TTE is better than transesophageal echocardiography (TEE) for identifying prosthetic aortic valve abscess and to determine the hemodynamic consequences of valvular dysfunction (Hoen and Duval, 2013) (**Fig. 1**). TEE is recommended if the results of TTE are equivocal or negative and if there is high clinical suspicion for IE. TEE has higher sensitivity (more than 90%) compared to TTE and is superior in detection of complications such as abscess, leaflet perforation, and pseudoaneurysm, and should be done for most cases, even when the diagnosis of IE is already established by TTE (De Castro et al., 2000). A normal TEE favors ruling out IE; however it should be repeated in 7–10 days if clinical suspicion remains high. TEE should also be repeated if a complication is suspected and at the completion of therapy. In suspected cardiac device infection, TEE is the investigation of choice. Three-dimensional echocardiography is especially useful in assessment of paravalvular regurgitation, paravalvular abscess, valve perforation, and dehiscence and is helpful if providing a surgical view to aid in the surgical planning (Bruun et al., 2014) (**Fig. 2** and **3**).

There are few studies, which assess the utility of Cardiac CT, cardiac MRI, and ^{18}F-fluorodesoxyglucose (^{18}F-FDG) PET-CT in diagnosis of IE. Cardiac CT is useful in patients with extensive calcifications, perivalvular abscess, pseudoanuerysms, and also in prosthetic valve IE, which can produce acoustic shadowing in echocardiography; however, TEE is superior to CT in detecting smaller vegetations and valvular perforations. Cardiac CT is also useful in assessment of coronary anatomy before valve surgery, when cardiac catheterization carries a risk of producing septic embolism. Cardiac MRI has sensitivity of about 85% compared to

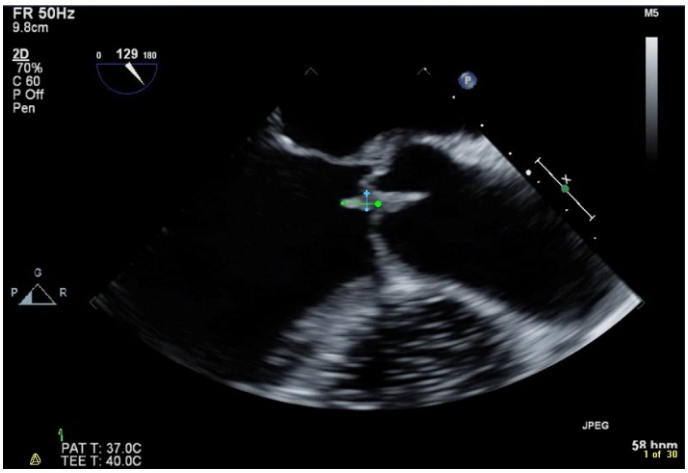

Fig. 1 TEE image of aortic valve vegetation. Differential diagnosis involves Lambl's excrescences/fibrous strands, tumor, or thrombi. Together clinical and echocardiographic characteristic often helps clinch accurate diagnosis and guide therapy.

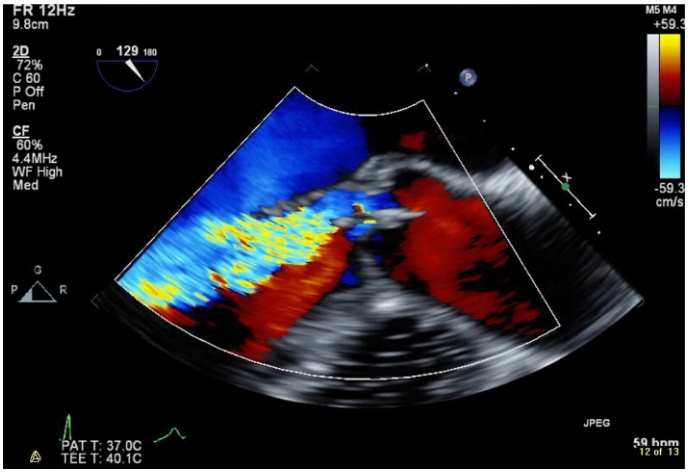

Fig. 2 TEE image showing valve perforation from aortic valve infective endocarditis. Valve perforation appears as an interruption in the tissue continuity (2-D echo) and often leads to eccentric regurgitation jet (color doppler).

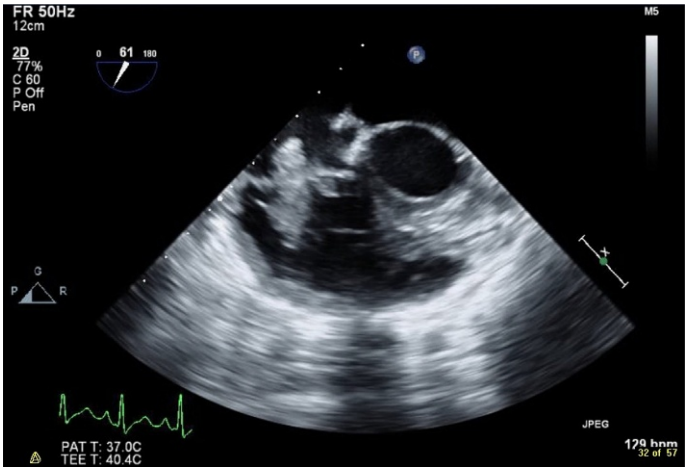

Fig. 3 TEE image shoeing tricuspid valve infective endocarditis. Usually occurs in intravenous drug users, device, or catheter. Valve destruction leading to significant tricuspid regurgitation and septic emboli to lungs are common complications and may lead to right heart failure.

echocardiography in detecting IE. Late gadolinium enhancement was noted in the perivalvular tissue in 43% of patients suggesting perivalvular extension even before abscess or fistula developed (Wong et al., 2016). Few reports have shown utility of ^{18}F-FDG PET-CT in diagnosis, mostly in prosthetic valve IE, even in some cases with negative TEE (Dickerman et al., 2007; Duval et al., 2012). An abnormal FDG uptake in PET-CT around a prosthetic valve, when added to the modified Duke criteria as a new major criterion, increases the sensitivity of diagnosis at admission from 70% to 97% (Wong et al., 2016). Its utility in native valve IE may be limited.

Management

Management of patient with IE should involve a multidisciplinary team comprising of cardiologists, cardiac surgeons, and infectious disease experts. Recent international working group recommendations emphasized the importance of the team approach (Chambers et al., 2014), and it has been shown that the team approach decreases 1-year mortality from 18.5% to 8.2% in IE (Botelho-Nevers et al., 2009). The cornerstone in treatment of IE includes prolonged antibiotic therapy to eradicate the causative organism and early surgery when indicated.

Antibiotic Therapy

Empiric antibiotics should be initiated as soon as the cultures are obtained. Empirical antibiotic regimens for native valve IE and prosthetic valve IE are based on established guidelines (Baddour et al., 2015) which are updated regularly. Choice of empiric therapy should depend on the most likely suspected pathogens. Ideal empiric therapy should cover Staphylococci (including

methicillin resistant, when suspected), Streptococci, and Enterococci. Vancomycin is an appropriate choice for initial therapy for most patients, and antibiotic regimen can be modified base on culture results, resistance patterns, infection severity, and the presence of prosthetic valves. It is reasonable to obtain repeat blood cultures 24–48 h apart until bacteremia resolves (Baddour et al., 2015). Duration of therapy depends on the pathogen, site of valvular infection, complications, and native or prosthetic valves. Duration of therapy should be counted from the first day of negative blood cultures. When valve replacement is performed, duration of antibiotics should remain the same as the duration of native valve IE, not prosthetic valve IE (Hoen and Duval, 2013).

Most viridans group Streptococci are highly penicillin susceptible. AHA guidelines recommend aqueous crystalline penicillin G or ceftriaxone for 4 weeks for viridans and bovis strains of Streptococci IE. The duration can be reduced to 2 weeks if combination therapy is used with gentamycin plus either aqueous crystalline penicillin G or ceftriaxone (Baddour et al., 2015). For viridans and bovis strains of Streptococci relatively resistant to penicillin G (defined by AHA as MIC >0.12 mcg/mL and <0.5 mcg/mL), the recommendation is aqueous penicillin G for 4 weeks with gentamycin added to the first 2 weeks of therapy. Ceftriaxone can be used as monotherapy if susceptible. Vancomycin is a reasonable alternative for patients with beta lactam allergy (Baddour et al., 2015).

Enterococcus exhibits narrow spectrum of susceptibility. Whenever the strain exhibit high level of resistance to gentamycin, it should be used in combination with other antibiotics. The AHA guidelines recommend that *Enterococcus faecalis* with typical low-level penicillin resistance should be treated with combination therapy with penicillin/ampicillin plus gentamicin for 4–6 weeks, or ceftriaxone plus ampicillin for 6 weeks, and for beta lactam allergic patients, vancomycin plus gentamicin for 6 weeks. Vancomycin-resistant enterococcal IE is rare and is treated with linezolid (Falagas et al., 2006) and daptomycin (Kullar et al., 2013) for more than 6 weeks (Baddour et al., 2015).

Staphylococcal endocarditis treatment is dependent on multiple factors including susceptibility, and type and location of the valve. Treatment for native valve IE due to methicillin-susceptible *Staphylococcus aureus* consists of a semisynthetic penicillin, such as nafcillin or oxacillin or flucloxacillin for 6 weeks. Gentamycin is not indicated for Staphylococcal native valve IE as there is no evidence of clinical benefit and there is a risk of nephrotoxicity (Cosgrove et al., 2009). Native valve IE due to methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci should be treated with vancomycin for 6 weeks.

Other organisms like HACEK organisms are treated with ceftriaxone, ampicillin (if organism is susceptible), or ciprofloxacin for 4 weeks (Baddour et al., 2015). Fungal IE is rare and outcomes are poor. Treatment of fungal IE consists of antifungal therapy and valve replacement. After initial therapy with amphotericin or an echinocandin, indefinite azole therapy is recommended, especially when valve surgery is not performed (Cornely et al., 2012).

Culture-negative IE is difficult to manage. Apart from patients receiving antibiotics before culture and inadequate microbiological technique, the most common cause of culture negativity is fastidious organisms. Empiric therapy should cover both Gram-positive and Gram-negative organisms. According to AHA guidelines (Baddour et al., 2015), in patient with acute clinical presentation (days), initial empiric therapy should include vancomycin and cefepime. For patient with subacute presentation (weeks), initial therapy should include vancomycin and ampicillin–sulbactam. This regimen can be modified for individual patient circumstances.

Prosthetic valve infective endocarditis (PVIE) is a serious infection which can occur early or late after surgery (**Fig. 4**). Most common pathogens in early PVIE are *Staphylococcus aureus* and coagulase-negative staphylococci, while streptococci and *Staphylococcus aureus* are frequently encountered in late PVIE (Wang et al., 2007). No randomized-controlled trials have assessed the optimal duration of antibiotics for PVIE. AHA guidelines recommend PVIE should be treated with bactericidal agents against the isolated microorganism for at least 6 weeks. Implantable device (pacemakers and defibrillators) infection can occur with or without associated valvular IE and the complete device and lead explanation is recommended (Baddour et al., 2010).

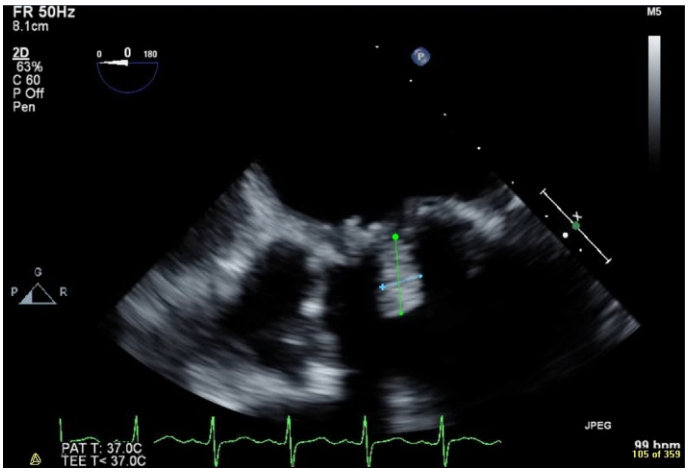

Fig. 4 TEE image showing Infective endocarditis of prosthetic mitral valve. Prosthetic valve endocarditis is a life-threatening condition and is associated with poor prognosis. It is difficult to detect on transthoracic echo, and TEE is indicated if there is clinical suspicion. Suture material or patch may mimic prosthetic valve endocarditis.

Surgery

The rate of early valve repair or replacement has increased over the last 30 years to about 50% (Murdoch et al., 2009). Main indications for early surgery include valve dysfunction leading to heart failure, paravalvular extension of the infection, uncontrolled or persistent infection, and prevention of embolism (Baddour et al., 2015). The criteria for prevention of emboli include patients presenting with recurrent emboli and persistent or enlarging vegetations despite appropriate antibiotic therapy or mobile vegetations >10 mm associated with severe valve regurgitation or involvement of anterior mitral leaflet (Joseph et al., 2013). Surgery is not contraindicated after ischemic stroke as long as cerebral hemorrhage has been ruled out by cerebral imaging (Habib et al., 2009).

Despite advances in diagnosis and management in recent year, the mortality rate for IE patients still remain high at around 15%–20% (Murdoch et al., 2009).

Prevention

Use of antibiotic prophylaxis for prevention of IE is based on expert opinion rather than data from clinical trials. The 2007 AHA guideline for the prevention of IE made major revisions to the previous 1997 AHA guidelines. The new guidelines recommend antibiotic prophylaxis only for patients who have a prosthetic valve, a history of IE, or unrepaired cyanotic congenital heart disease and who are planning to undergo an invasive dental procedure. Prophylaxis is no longer recommended for gastrointestinal or genitourinary procedures (Wilson et al., 2007).

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Inflammatory and Infectious Disorders of the Aorta

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Background

Inflammatory aortitis is a term which encompasses a number of disease processes of both infectious and noninfectious etiology. Quite literally, aortitis means inflammation of the aorta. While aortic inflammation may occur in response to any injury, including trauma, the most well-described causes are infectious in nature or secondary to underlying vasculitis, such as giant cell arteritis (GCA) and Takayasu arteritis (TA). Aortitis can cause aortic dilatation, resulting in aortic valve insufficiency and it has become an uncommon but increasingly recognized cause of abdominal aortic aneurysms and dissections. In addition, it can also cause fibrous thickening and ostial stenosis of major aortic branches. This can lead to end-organ ischemia with important clinical complications.

Inflammatory aortitis is a rare, but potentially life-threatening disorder with a highly variable clinical presentation (Gornik and Creager, 2008).

Epidemiology

The epidemiology of aortitis is poorly classified (Gornik and Creager, 2008). In the United States and Europe, the incidence of inflammatory aortitis is said to be 1–3 new cases per year per one million populations. This number is likely higher as many cases are incidentally identified at the time of histopathologic examination following surgery for aortic aneurysms, suggesting that many cases go unreported.

The most common causes of inflammatory aortitis have been well described (**Table 1**). Giant cell arteritis (GCA) is the most common form of vasculitis among elderly with a reported yearly incidence of 18.8 cases per 100,000 adult males aged 50 or older and this is said to be two-fold higher among females (Espitia et al., 2016; Hall et al., 1985). Worldwide, the greatest prevalence of vasculitis is among Asians. One survey of Japanese hospitals determined an incidence of 1 case per million children per year. Another study conducted in Japan in 1984 identified 20 cases per million population and by 2000 Takayasu arteritis (TA) there was a 2.5-fold increase in prevalence (Vanoli et al., 2005).

To this day, the prognosis for inflammatory aortitis is difficult to predict; however survival rates for vasculitis have been well described with the major predictors of death being vasculitis-related end-organ sequelae, aortic aneurysm, dissection, and a progressive course.

Pathophysiology

Inflammatory aortitis is a pathologic term for inflammation of the aortic wall of various etiologies with similar, nonspecific pathologic, radiologic, and clinical features. A standardized classification system has yet to be adopted. Several classification systems have been published, including one by the Society for Cardiovascular Pathology and the Association for European Cardiovascular Pathology. This classification breaks inflammatory aortitis into three categories including atherosclerosis, atherosclerosis with excessive inflammation, and aortitis/periaortitis (Ladich et al., 2016). This classification is not helpful clinically as many cases are thought to be idiopathic and only become apparent after pathologic evaluation of an excised specimen (Rojo-Leyva et al., 2000). Practically, the entity may be simply classified as infectious or noninfectious (Bossone et al., 2015).

Table 1 Common causes of inflammatory aortitis

(1) Inflammatory, likely auto-immune
  (A) Large vessel vasculitides
    a. Giant cell or temporal arteritis
    b. Rheumatoid Associated
    c. Systemic lupus Associated
    d. Takayasu's Arteritis
    e. Spondyloarthropathy associated
  (B) Other vasculitides
    a. Anca Positive vasculitides
      i. Granulomatosis with polyangitis, formerly known as Wegener's granulomatosis
      ii. Polyarteritis nodosa or panarteritis nodosa
    b. Behcet's disease
    c. Relapsing polychondritis
    d. Cogan syndrome
  (C) Isolated aortitis
    a. Ormond disease, or idiopathic retroperitoneal fibrosis
    b. Inflammatory abdominal aortic aneurysms
    c. Idiopathic isolated aortitis
(2) Infectious aortitis
  (A) Bacterial
    a. Salmonella
    b. Streptococcal
    c. Staphylococcal
    d. Neisseria gonorrhea
  (B) Mycobacterial (Tuberculosis)
  (C) Syphilitic
  (D) Fungi
    a. Aspergillosis
    b. Mucormycosis
  (E) Viruses
    a. Hepatitis B and C
    b. Herpes
    c. Varicella zoster
(3) Iatrogenic aortitis
  a. Post implantation syndrome

Infectious Aortitis

While less common than noninfectious inflammatory aortitis, the possibility of infectious aortitis must always be considered. A number of organisms have been described to be associated with infectious aortitis, most commonly *Staphylococcus*, *Streptococcus*, and *Salmonella*. Tuberculous aortitis, although uncommon in the Western world, is a common organism seen in aortitis in underdeveloped countries (Foote et al., 2005a). Other infectious agents that have been described include *Neisseria* (gonorrhea), *Rickettsia* (Rocky Mountain Spotted Fever), spirochetes (syphilis), fungi (*aspergillosis*, *mucormycosis*), and viruses (hepatitis B and C, herpes, varicella-zoster) (Gornik and Creager, 2008).

Regardless of infectious etiology, the pathophysiology is similar. Typically, involved aorta has underlying baseline pathologic changes, such as atherosclerosis or aneurysmal degeneration. The infectious agent seeds via the vasa vasorum, either through direct seeding from infected adjacent tissue, hematogenous spread during sepsis, infectious endocarditis producing septic emboli, or an underlying immunocompromised state (Foote et al., 2005a).

Noninfectious Aortitis

As described previously, the most common cause of inflammatory aortitis is vasculitis, the two most common being giant cell arteritis (GCA) and Takayasu's arteritis (TA) (Loricera et al., 2014). GCA primarily involves arteries of the head such as temporal and ophthalmic arteries, and TA primarily involves aortic arch and great vessels. Although considered two separate clinical entities with differing arterial involvement, there is significant overlap at the pathologic level. Both cause cellular infiltrate of the intima, media, and adventitia causing arterial wall thickening and both can have granuloma formation and multinucleated giant cells, making their histologic appearances indistinguishable (Kumar et al., 2010). Both can cause an inflammatory aortitis, which confers increased risk for both thoracic and abdominal aneurysms and aortic dissection (AD). Of the two vasculitides, GCA more commonly results in dissection and/or rupture (Tavora and Burke, 2006). Although many theories have been postulated including a cell-mediated autoimmune process and genetic predisposition, the pathogenesis of GCA and TA remains to be elucidated (Gravanis, 2000).

Another less common cause of noninfectious aortitis is idiopathic isolated aortitis. Idiopathic isolated aortitis is a disease process characterized by giant cell or lymphoplasmacytic inflammation of the aorta. While this phenomenon may involve the entire aorta, it most commonly involves the ascending aorta and accounts for 2% to 4% of ascending aortic aneurysms (Warrington and Cooper, 2010). Although rare, these disorders are unique as they are limited to the aorta and peri-aortic tissue. The primary histopathologic findings include patchy necrosis of the aortic media as well as inflammatory cellular infiltrate, which may include multinucleated giant cells, again making it difficult to distinguish from other vasculitides. While it has been postulated to be associated with IgG4-related systemic disease, the underlying pathophysiology is still poorly understood (Venkata et al., 2012).

In addition to vasculitis and idiopathic isolated aortitis, rheumatologic disorders (rheumatoid arthritis, systemic lupus erythematosus) have also been implicated in this phenomenon. While stenosing lesions are more common among patients with TA, dilatations or aneurysms of the aorta are a more common pattern among patients with rheumatic disease and aortic involvement. Both the thoracic and/or abdominal aorta may be involved and it can present in a variety of specific and nonspecific forms of aortitis. Because of this, there is a diverse clinical presentation representing both aneurysmal as well as ischemic symptoms in addition to the constitutional symptoms seen in rheumatic diseases. With thoracic aortic involvement, once may see aortic valve incompetence and heart failure, while involvement of the abdominal aorta may lead to leak, rupture, or thrombus formation and thromboemboli. While much less common and not well described, there should be a high index of suspicion of inflammatory aortitis in patients with rheumatic disease with new unexplained aortic regurgitation, arterial embolic events, or pain distribution concerning for aortic involvement (Slobodin et al., 2006).

With the advent of endovascular repair of abdominal aortic aneurysms (AAA), a newer phenomenon of noninfectious inflammatory aortitis has surfaced known as postimplantation syndrome (PIS). This poorly understood syndrome is a clinical and biochemical expression of an inflammatory response following EVAR. The incidence varies depending on literature quoted and is said to be as high as 60%. Nearly one-third of patients undergoing EVAR will suffer from PIS. It presents as a systemic inflammatory response induced by implantation of endoprosthesis. It was first described in 1999 with fever and leukocytosis following EVAR. Although many postulated mechanisms exist, the leading theory implicates manipulation of aneurysm leading to activating of the leukocyte response and cytokine release. PIS is frequently well tolerated and thought to be a benign process; however its impact on long-term outcomes is still unknown (Arnaoutoglou et al., 2015).

Presentation

The clinical presentation of inflammatory aortitis varies significantly and can range from asymptomatic to aortic insufficiency with acute aortic syndromes (Guard et al., 1995; Nuenninghoff et al., 2003). The underlying cause of aortitis and concomitant manifestations determine presentation. This can make diagnosis difficult as both infectious and noninfectious causes have nonspecific signs and symptoms.

Inflammatory aortitis causes inflammation of the aorta which can lead to aortic dilatation. This can occur at any level of the aorta. When it involves the proximal aorta, symptoms can be related to aortic valvular insufficiency and regurgitation or thoracic aortic dissection. More distally in the abdominal aorta this more commonly manifests as aneurysm formation. Inflammation may also cause fibrous thickening and ostial narrowing of major aortic branches. This is common in Takayasu arteritis, giving it the classic name "pulseless disease" with absence of peripheral pulses and lower arm blood pressures and arm fatigue (**Fig. 1**).

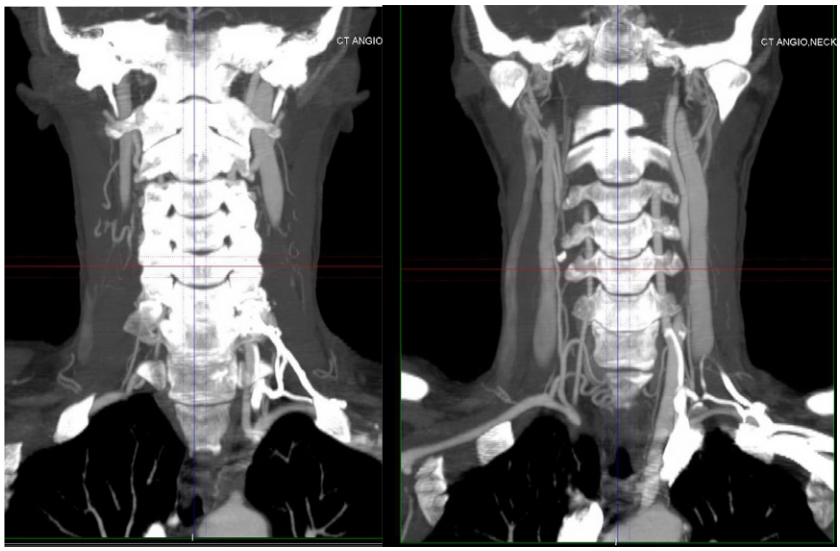

Fig. 1 CT angiogram of patient with Takayasu's Arteritis, demonstrating occluded left subclavian artery, occluded brachiocephalic artery, and occluded right common carotid.

Many other manifestations may occur depending on the vascular beds involved. They can range from claudication if involving lower extremity, ocular disturbances if involving retinal vessels, neurologic deficits if involving intra- or extracranial cerebrovascular beds, and others (Bossone et al., 2015). Systemic clinical manifestations include constitutional symptoms such as fevers, chills, weight loss, and general malaise. Aortitis may also be completely asymptomatic and discovered incidentally on imaging for other purposes or seen on autopsy.

Differential Diagnosis

Clinicians must maintain a high index of suspicion for inflammatory aortitis, especially in patients with nonspecific inflammatory manifestations.

Infectious Aortitis

Before the era of antibiotics, infective endocarditis from organisms such as *group A Streptococci*, *Streptococcus pneumoniae*, or *Haemophilus influenza* was the most common cause of infectious aortitis. Currently, a diverse number of bacteria and fungi have been associated with infectious aortitis, most commonly *Salmonella* species and *Staphylococcus aureus* (Foote et al., 2005b). Although less common, other infectious diseases, including syphilis, tuberculosis and mycosis, should be considered as well if infectious etiology is suspected. It is also important to keep in mind that even after treatment of infectious source, patients may still have sequelae of the inflammatory process (Fabio et al., 2006).

Infectious aortitis generally presents in older patients often with preexisting aortic pathology (e.g., abdominal aortic aneurysm). It often presents with back or abdominal pain as well as constitutional symptoms. Bacteremia may or may not be present. Imaging should be performed early to exclude possible infectious aortitis as it may lead to mycotic aneurysms and subsequent rupture with high rate of mortality.

Noninfectious Aortitis

Various noninfectious causes have been identified, including systemic vasculitides (e.g., polyarteritis nodosa), granulomatous diseases [e.g., giant cell (temporal) arteritis and Takayasu arteritis], rheumatologic diseases (e.g., systemic lupus erythematosus and ankylosing spondylitis), as well as idiopathic and they can all manifest with aortic lesions.

Giant cell arteritis (GCA) or temporal arteritis often has a nonspecific presentation. Common symptoms include headache, polymyalgia rheumatic, scalp tenderness, jaw claudication, and visual changes in addition to abnormal temporal artery biopsy and elevated inflammatory markers. There have been associations made between GCA and aortic aneurysms, particularly thoracic aortic aneurysms (Laco et al., 2011).

Takayasu arteritis is a less common disorder associated with inflammatory aortitis. It has a predilection for younger women. Versus GCA which presents with more nonspecific symptoms, Takayasu arteritis more often presents with arterial occlusive disease of the aorta, aortic arch and large vessel branches (most commonly subclavian, innominate, renal, and carotid arteries). It has often been called "pulseless disease" due to occlusive disease of subclavian arteritis demonstrating pulseless upper extremities. Many present with associated large vessel manifestations, such as renovascular hypertension and lower extremity claudication. Like giant cell arteritis there have been associations made between Takayasu arteritis and aortic aneurysm, however versus GCA which more often involves the thoracic aorta, Takayasu more often involves the abdominal aorta. Takayasu arteritis may also present with nonspecific symptoms and elevated inflammatory markers.

Although less common, rheumatologic disorders can be associated with inflammatory aortitis. This should be suspected when there is evidence of vasculitis in setting of underlying rheumatologic disorders such as in younger patients with longstanding rheumatologic diseases (e.g., ankylosing spondylitis).

Idiopathic isolated aortitis can present with symptoms associated with aortic inflammation but is more often discovered incidentally on pathology for aortic procedures. Idiopathic isolated aortitis can present with nonspecific symptoms as well as elevated inflammatory markers, similar to GCA and Takayasu arteritis (Miller et al., 2006).

Evaluation

Whenever there is suspicion for inflammatory aortitis on clinical findings, imaging is warranted as missing the diagnosis can be associated with high morbidity and mortality.

Initial evaluation should include basic labs. These include a measurement of inflammatory markers, erythrocyte sedimentation rate (ESR), and c-reactive protein (CRP), which are often times elevated above normal limits complete blood count, which may demonstrate leukocytosis, kidney, and liver function tests, which may be elevated if there is end organ malperfusion, and blood cultures, which may be positive in setting of an infectious etiology. If rheumatologic cause is suspected, a rheumatologic panel should also be considered (e.g., anti-nuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA) and rheumatoid

factor (RF)). If infectious etiologies are suspected, plasma protein derivative (PPD) and RPR-VDRL should also be done to rule out tuberculosis and syphilis.

Several imaging modalities are available and there continue to be emerging studies that show promise for diagnosis in the future. Although angiography has traditionally been the gold standard for diagnosis of inflammatory aortitis, advances in less-invasive imaging techniques have led to its replacement. Noninvasive angiographic imaging, including Computed tomography with iodinated contrast (CTA), and magnetic resonance angiography (MRA) are now widely available, allow for rapid identification or exclusion of aortic pathology, and adequately visualize surrounding structures.

CTA allows for assessment of stenotic lesions in aorta and large arterial branches as well as presence and extent of aneurysms or dissections. CTA is useful for long-term follow-up of patients with aortitis or treated for aortitis and for surveillance of known aneurysms. It is also useful for assessing extent of calcification. Its primary limitation is the requirement of iodinated contrast in patients with renal insufficiency or allergy to iodinate contrast as well as need for radiation exposure.

MRA with gadolinium contrast has emerged as a noninvasive imaging modality for inflammatory aortitis, particularly those associated with GCA and Takayasu arteritis. It allows for detailed assessment and characterization of aortic involvement, areas of active aortic inflammation as well as wall edema or wall thickening. One benefit of MRA over CTA is lack of radiation exposure and iodinated contrast. One limitation of MRA with gadolinium is the risk of nephrogenic systemic sclerosis in patients with renal dysfunction.

Positron emission tomography (PET) scanning is yet another imaging modality that can discern vascular inflammation; however it has limited availability and its utility in diagnosis is still under investigation.

Treatment

Once the diagnosis has been established, approach to treatment depends on the underlying etiology and presence of associated ischemic or aneurysmal complications. The spectrum of therapy varies from immediate treatment to chronic management and long-term surveillance.

When infectious aortitis has been diagnosed, treating the underlying infectious source is essential. Initial treatment includes intravenous antibiotics with broad spectrum antimicrobial coverage for the most common organisms, including staphylococcal species and gram negative rods. Once the organism has been identified, the antibiotic coverage should be narrowed appropriately. If aortic involvement is diagnosed (e.g., mycotic aneurysm), antimicrobial therapy alone is insufficient and surgical debridement is required; however despite surgical management, the mortality rate remains high largely due to aortic rupture at time of presentation. Muller et al. reported an operative mortality rate of 36%, which related to a rupture rate of 85 (Muller et al., 2001). While several surgical approaches have been described including aggressive debridement and extra-anatomic bypass, aortoiliac reconstruction with cryopreserved arterial homograft or rifampin-soaked dacron and a neoaortoliliac system (NAIS) with aortoiliac reconstruction using the deep venous system of the lower extremities for mycotic abdominal aortic aneurysms, there is no established optimal surgical management as an infected field puts any reconstruction at high risk of breakdown. Dubois et al. compared extra-anatomic reconstruction with in situ repair. In-hospital mortality rate was 22.7%, 50% in the extra-anatomic reconstruction group and 18.9% in the in situ repair group (Dubois et al., 2010).

For inflammatory aortitis associated with systemic vasculitis or rheumatologic disorders, immunosuppressive therapy with glucocorticoids is the primary mode of treatment in the active phase (Chan et al., 2001; Hunder et al., 1975). The recommended initial dose of glucocorticoid therapy is 60 mg/day for GCA and 1 mg/kg/day for Takayasu arteritis (Salvarani et al., 2002; Kerr et al., 1994; Koening and Langford, 2008). Intravenous glucocorticoids are recommended when visual symptoms are present in GCA and in any patients with symptomatic aortitis. Duration of treatment must be tailored to each individual patient's condition and disease activity and tapering is required to reduce severe side effects.

Despite glucocorticoid therapy, relapse rates remain high, especially in GCA and Takayasu arteritis, and steroid-related adverse effects are not insignificant. While there have been several studies investigating potential steroid-sparing regimens for immunosuppression, including monoclonal antibodies and antitumor necrosis factor agents, steroid therapy remains the superior treatment.

For idiopathic isolated aortitis, treatment is not well established and surgical versus immunosuppressive therapy should be considered on a case-to-case basis.

When deciding the appropriate surgical management, several factors need to be taken into account. It is preferable for inflammation of the aorta to be treated with immunosuppressive therapy and patients to be in quiescent state as evidenced by lack of constitutional symptoms and normal inflammatory markers prior to surgery. There is a large body of evidence that demonstrates improved patency rates of surgery when patients are not in an active inflammatory state. Open vascular reconstruction has been the standard treatment for inflammatory aortitis, although endovascular techniques have been employed with some success (Zehr et al., 2005; Engelke et al., 2002; Baril et al., 2006). Endovascular surgery has the advantage of avoiding a large surgical procedure as well as reducing extensive manipulation of inflamed aortic tissue; however there is yet to be a randomized controlled trial comparing surgical versus endovascular management of inflammatory aortitis and this is an area that deserves further investigation especially with the advent of improved endovascular techniques and lower profile systems.

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Inotropes in Heart Failure

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Introduction

Heart failure (HF) is a complex condition characterized by periods of exacerbations and stability, and often with an overall progressive decline, resulting in deterioration of quality of life, worsening of symptoms, and functional limitation. Patients with HF struggle with physical symptoms including dyspnea, fatigue, and edema, as well as emotional symptoms such as depression and anxiety.

As a result, patients with HF often have frequent hospitalizations especially with progression of their disease severity. Currently there are almost 6 million people with HF in the United States, with 3 million HF-related hospitalizations per year and an associated cost of 40 billion dollars (Norton et al., 2011).

As the disease progresses, patients with advanced HF have limited options including cardiac transplantation, permanent mechanical circulatory support, home inotropes, and hospice care. These patients with end-stage HF are refractory to conventional HF drug and device therapy and are classified under the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) Guideline for the management of HF as stage D HF. Many patients may not be candidates for a heart transplant or left ventricular assist device (LVAD) or may not want to avail of these therapies. These patients with poor quality of life scores often choose to improve their quality of life over longevity. Intermittent or continuous use of inotropic therapy is an option that may be advantageous in this setting (Kraai et al., 2013; Fruhwald et al., 2016).

For the patient with advanced HF, inotropic therapy may be beneficially used in several different scenarios (**Table 1**). Inotropes may be used in acute settings such as acute cardiogenic shock (CS) with or without multiorgan failure, or as a "bridge" to transplantation or LVAD implantation. Inotropes may also be used as a palliative "destination therapy" measure in patients with end-stage HF to improve their functional capacity and quality of life.

Inotropes and Their Pharmacology

Inotropic agents improve myocardial contractility through a variety of mechanisms. Each agent impacts the systemic and pulmonary vasculature differently leading to characteristic hemodynamic effects. Furthermore, the metabolism and side effect profile of each agent differ. A thorough knowledge of both biologic effects and available outcomes data are needed to guide optimum clinical use. **Table 2** compares different inotrope characteristics and **Table 3** offers a practical approach to the use of one inotrope over the others.

Milrinone

Milrinone is a bipyridine derivative that indirectly increases intracellular cyclic adenosine monophosphate (cAMP) via inhibition of phosphodiesterase-III, the enzyme responsible for hydrolytic cAMP breakdown. Increased intracellular cAMP, the secondary messenger of the beta agonist system, leads to increased intracellular calcium via phosphorylation of substances responsible for calcium handling. Milrinone is also thought to have additional direct effects on calcium channel activity, vascular cAMP, and

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Table 1 Indications for inotrope use

1. Acute decompensated heart failure
2. Cardiogenic shock with or without multiorgan failure
3. Cardiogenic shock complicating acute myocardial infarction
4. Acute heart failure as bridge to transplant or mechanical support
5. Right heart failure
6. Perioperative use for cardiac and other surgeries
7. Palliative inotropes in end-stage heart failure

Table 2 Inotrope pharmacology

	Mechanism	*Dose*	*T 1/2*	*Major route of elimination*	*CO*	*HR*	*SVR*	*PVR*	*Side effects*
Dopamine	Synthetic catecholamine	1–5 mcg/kg/min	2 min	COMT	0	+	0	0	Tachyarrhythmias
		5–10 mcg/kg/min			+	++	+	0	Tissue necrosis
		10–15 mcg/kg/min			++	+++	++	+	
Dobutamine	Synthetic catecholamine	2.5–5 mcg/kg/min	2 min	COMT	+	+	−	−	Tachyarrhythmias
		5–20 mcg/kg/min			++	++	0	0	Hypersensitivity
Milrinone	PDE-III inhibitor	0.125–0.75 mcg/kg/min	2–3 h[a]	Renal	++	+	−−	−	Hypotension
Levosimendan	Calcium sensitizer	6–12 mcg/kg over 10 min[b]	1 h[c]	Renal	++	+	−	−	Hypotension
		0.05–0.2 mcg/kg/min							

COMT, Catechol-*O*-methyl transferase.
+, Increase; 0, no change; −, decrease.
[a]Longer if GFR decreased.
[b]If SBP >100 mmHg.
[c]Active metabolite (OR-1896) 80 h.

Table 3 Practical tips for inotrope selection

Patient characteristic	*Milrinone*	*Dobutamine*	*Dopamine*	*Levosimendan*
Vasoplegia	−	+	++	−
Hypotension	+	−	++	−
SVR reduction[a]	++	+	Dose dependent	+
PVR reduction[b]	++	+	+/−	+
Arrhythmias	+	−	−	−
Beta-blocker use	++	−	−	+
Severe renal dysfunction	−	+	++	+
Cost/availability	+	+	+	−

SVR, systemic vascular resistance; *PVR*, pulmonary vascular resistance.
+, Ideal choice; −, poor choice.
[a]SVR = systemic vascular resistance
[b]PVR = pulmonary vascular resistance

perhaps cGMP, which may explain its slightly different impact on chronotropy and vascular relaxation compared with dobutamine (Hess, 1996).

Milrinone decreases systemic and pulmonary vascular resistances (PVR), decreases left and right ventricular (RV) filling pressures, and increases cardiac output without increasing myocardial oxygen consumption (Grose et al., 1986). Electrophysiologic changes associated with milrinone use include increased conduction through the atrioventricular (AV) node and decreased atrial refractoriness (Tisdale et al., 1995a).

Beta receptor downregulation, common in chronic HF (Bristow, 1993), reduces the inotropic efficacy of beta-agonists, but does not diminish the efficacy of milrinone. Likewise, milrinone remains efficacious in patients previously or concomitantly receiving beta-blockers (Travill et al., 1994). Furthermore, milrinone enhances the effects of both endogenous and exogenous catecholamines, which also increase cAMP through first messenger beta-adrenergic stimulation (Honerjäger, 1991).

Milrinone is approved for administration via a continuous intravenous drip, however inhaled delivery forms are being studied (Haglund et al., 2015). Current ACC/AHA guidelines recommend against initiation bolus dosing (Yancy et al., 2013), which has

been associated with hypotension (Baruch et al., 2001). The recommended maintenance dose is between 0.125 and 0.75 mcg/kg/min. The onset of action is approximately 5–15 min, although full hemodynamic impact may take hours (Baruch et al., 2001).

Unlikely dobutamine, tachyphylaxis is not exhibited with milrinone. Milrinone is renally excreted, largely via active tubular secretion in an unchanged drug form. Only minor hepatic metabolism takes place and dose adjustments for hepatic impairment are not necessary. The elimination half-life in HF patients is about between 2 and 3 h, although this may be prolonged in patients with renal insufficiency (Rocci and Wilson, 1987). The elimination half-life in patients undergoing continuous venovenous hemofiltration is approximately 20 h (Taniguchi et al., 2000). Conservative dosing strategies should be considered in patients with kidney disease.

The most common serious side effects are arrhythmias and hypotension. Ventricular premature contractions and nonsustained ventricular tachycardia are not uncommon during short-term milrinone use, however sustained ventricular tachycardia or ventricular fibrillation typically occur in about 1%–3% with short-term use. Supraventricular tachycardia occurs in about 4% of patients, worsening control of atrial fibrillation is not unusual. Headache has been reported in up to 3% of patients. Due to the drugs prolonged half-life, hypotension or arrhythmias may persist even after discontinuation, particularly in patients with renal insuffiency. Uncommon side effects include thrombocytopenia, tremor, elevated liver function tests, rash, and allergic reactions. Concomitant use of riociguat may enhance hypotensive effects and should be used with caution and monitored closely. Concomitant use of furosemide is common but the two drugs have a notable Y-site administration incompatibility and administration through the same IV line should be avoided (Lexicomp, 2016). Milrinone may be associated with increased costs in the current healthcare system compared with other inotropic agents (Hauptman et al., 2006).

Dobutamine

Dobutamine is a synthetic catecholamine that exists as a racemic mixture. FDA approved for clinical use in 1978, dobutamine has served as the most commonly employed inotrope for several decades. At typical doses, the predominant hemodynamic effects of increased contractility and mild peripheral vasodilation are due to a strong affinity for the beta-1 and beta-2 receptors, which are bound in a 3:1 ratio (Overgaard and Dzavík, 2008). The negative enantiomer of the racemic mixture leads to vasoconstriction via alpha-1 antagonism, however at usual doses this appears to be more than counterbalanced by beta-2 stimulation leading to a mild net reduction in systemic vascular resistance. At doses greater than 15 mcg/kg/min, vasoconstriction becomes more prominent leading to increased systemic vascular resistance (Ruffolo, 1987). PVR response is variable, but overall a mild decrease can be expected within the lower dose range, while moderate to high doses result in little appreciable PVR change (Kerbaul et al., 2004; Bradford et al., 2000; Romson et al., 1999).

Unlike milrinone, dobutamine is known to increase myocardial oxygen consumption (Grose et al., 1986). Also tachyphylaxis occurs with infusions greater than 48 h. Electrophysiologic changes associated with dobutamine include increased sinoatrial node automaticity and decreased atrial and AV node refractoriness as well as decreased AV node conduction time (Tisdale et al., 1995a).

Patients taking chronic beta-blocker therapy may have an attenuated response to dobutamine until the beta-blocker has been metabolized. Limited data, in compensated individuals, suggests that concomitant dobutamine and metoprolol administration can lead to a favorable but blunted hemodynamic response. Concomitant dobutamine and carvedilol does not result in favorable hemodynamics and is associated with elevated filling pressures, mean pulmonary artery pressure, systemic vascular resistance, and PVR (Metra et al., 2002).

The manufacturer recommended starting dose is 0.5–1 mcg/kg/min, however initiation at 2.5 mcg/kg/min is commonly employed. Current ACC/AHA guidelines recommend a maintenance dose between 2.5–20 mcg/kg/min. Onset of action is between 1 and 10 min, with peak effect in 10–20 min. Unlike milrinone, dobutamine has an extremely short elimination half-life of 2 min and no dose adjustments are necessary in patients with kidney disease. The predominant route of metabolism is tissue methylation via catechol-*O*-methyltransferase (COMT) and monoamine oxidase (MAO), as well as hepatic conjugation (Yan et al., 2002). Caution should be exercised in patients concurrently receiving MAO inhibitors or tricyclic antidepressants, as paradoxical, prolonged hypertensive episodes may result. Notable drug interactions leading to exaggerated hypertensive reactions also include linezolid and atomoxetine.

Arrhythmias are the major life-threatening side effects of dobutamine. Ventricular ectopy is observed in at least 5% of patients during short-term use. Dose-dependent sinus tachycardia is common and approximately 10% of adult patients in clinical studies have a rate increases of 30 beats/minute or more. Increased frequency of atrial fibrillation and worsening control over ventricular response are not uncommon. Headache is reported in 1%–3% of patients. Less common side effects include hypotension, dyspnea, paresthesias, thrombocytopenia, nausea, and leg cramps. Fever can occur in 1%–3% of patients, while infrequent peripheral eosinophilia and rash have also been reported. Rare allergic reactions ranging from mild bronchospasm to anaphylaxis can ensue due to the sulfite component of drug preparation (Lexicomp Online Dobutamine, 2016). Although thought rare, about 2%–8% of patients receiving prolonged dobutamine infusions while waitlisted for heart transplant have evidence of eosinophilic myocarditis on explantation (Takkenberg et al., 2004; Yoshizawa et al., 2013).

Dopamine

Dopamine, the immediate precursor to norepinephrine, is an endogenous catecholamine that exhibits dose-dependent physiologic effects. Dopamine activate dopaminergic receptors leading to naturiesis and vasodilation of the renal arteries, as well as the

mesenteric, coronary, and cerebral beds (Cheung and Barrington, 199). Doses of <2.5 mcg/kg/min are thought to activate dopaminergic receptors and doses in this range are commonly referred to as "renal-dosed dopamine." Controversy exists as to the legitimacy of renal-dosed dosing strategies (Denton et al., 1996), particularly given the neutral results of ROSE AHF (Renal Optimization Strategies Evaluation in Acute Heart Failure), a randomized trial in which 2mcg/kg/min of dopamine failed to increase urine output, preserve renal function, or improve clinical outcomes against placebo or neseritide (Chen et al., 2013).

Conversely, a recent meta-analysis of trials using variable dopamine doses <5 mcg/kg/min suggests efficacy in preventing renal deterioration (Xing et al., 2016). With increasing doses of dopamine from 1 to 10 mcg/kg/min, renal artery cross-sectional area continues to increase and renal vascular resistance progressively decreases (Elkayam et al., 2008). HF patients may require dopamine doses greater than 2.5 mcg/kg/min to achieve favorable renal changes (Ungar et al., 2004), however the impact of improved cardiac index with these doses confounds attributable benefit.

At intermediate doses, beta-adrenergic inotropic effects are thought to predominate, while at higher doses (>10 mcg/kg/min) alpha-adrenergic vasoconstrictor effects increase. Dopamine improves cardiac index and mean arterial blood pressure, but at the expense of a greater increase in heart rate compared with dobutamine (Stoner et al., 1977). Also dopamine does not effectively decrease filling pressures and at higher doses may actually increase left ventricular end diastolic pressure (Francis et al., 1982). Dopamine has a variable impact on PVR and at low to intermediate doses PVR is largely unaffected in patients with cardiomyopathy (Stoner et al., 1977) as well as pulmonary hypertension (Holloway et al., 1975). Dopamine does however increase the pulmonary vascular resistance/systemic vascular resistance (PVR/SVR) ratio to a greater extent than other inoconstrictors (Barrington et al., 1995). Electrophysiologic changes associated with dopamine include dose-related sinus tachycardia, increases automaticity in Purkinje fibers, and at high doses reduced atrial repolarization and action potential durations. Arrhythmias associated with dopamine occur at lower inotropic doses than dobutamine-associated arrhythmias (Tisdale et al., 1995b).

Dopamine is typically begun at 2 mcg/kg/min (base on ideal body weight) and uptitrated to desired hemodynamic effect. ACC/AHA guidelines recommend a maximum dose of 15 mcg/kg/min although the manufacturer label maximum dose is 50 mcg/kg/min. Ideally the drug should be administered into a large vein via central line to prevent the possibility of extravasation. The onset of action is between 1 and 5 min and the elimination half-life is 2 min. Dopamine is metabolized in the liver, kidney, and plasma primarily by MAO as well as COMT. No dose adjustments are necessary in renal or hepatic disease. Like dobutamine, caution should be exercised in patients concurrently receiving MAO inhibitors or tricyclic antidepressants, as prolonged hypertension may result. Notable drug interactions leading to exaggerated hypertensive reactions also include linezolid, atomoxetine, hyaluronidase, serotonin norepinephrine reuptake inhibitors, or ergot derivatives.

Arrhythmias are the major life-threatening side effect of dopamine. Dose-dependent sinus tachycardia is common, although paradoxical bradycardia can also occur. Arrhythmias including atrial fibrillation as well as ventricular arrhythmias are not uncommon. QRS widening may be observed with low dose dopamine. Increased intraocular pressure and mydriasis have been reported. Contraindications to use include known hypersensitivity to sulfites or known pheochromocytoma. Extravasation of dopamine leads to tissue necrosis with the potential for gangrene. If extravasation occurs, immediately stop infusion and disconnect line while leaving the vascular catheter in place. Then gently aspirate through the catheter before removing it. Elevate the arm and apply a dry, warm compress. Quickly infiltrate the area with 5–10 mg of phentolamine diluted in 10–15 cc of normal saline using a fine hypodermic needle, alternatively topical nitroglycerin ointment or diluted terbutaline can be used (Lexicomp Online Dopamine, 2016).

Levosimendan

Levosimendan is a calcium-sensitizing drug that is currently approved for use in Europe and South America, but remains investigational in the United States. All conventional inotropes increase intracellular calcium leading to increased inotropy as well as potential unwanted toxicity related to calcium overload (Allen et al., 1985; Hove-Madsen et al., 2004). Levosimendan does not cause increased intracellular calcium and instead cause increased myofibrillar calcium responsiveness. The drug selective binds to calcium-saturated cardiac troponin, which enhances the sensitivity of the myofilament and facilitates actin–myosin cross-bridge formation (Sorsa et al., 2004). Additional mechanisms of action include opening of adenosine triphosphate dependent-potassium (K^{ATP}) channels on the sarcolemma of the vasculature smooth muscle cells and cardiac myocyte mitochondria. K^{ATP} channel activation in the vascular smooth muscles leads to arterial (Yokoshiki et al., 1997) and venous (Pataricza et al., 2000) vasodilation, thus decreased preload and afterload, as well as coronary vasodilation (Kaheinen et al., 2001). Cardiac myocyte K^{ATP} channel activation is thought to protect against ischemia–reperfusion injury (Scheiermann et al., 2011).

Characteristic hemodynamic response includes dose-dependent increased stroke volume and heart rate, while decreasing systemic vascular resistance, mean pulmonary artery pressure, and left- and right-sided filling pressures. Compared with dobutamine, levosimendan produces a marginally greater increase in cardiac output but a significantly greater decrease in pulmonary capillary wedge pressure (Follath et al., 2002). These hemodynamic effects are achieved without any significant increase in myocardial energy consumption (Ukkonen et al., 2000). Levosimendan remains active in patients receiving beta-blocker therapy and may be considered to reverse the effect of beta-blockade induced hypotension with hypoperfusion (Ponikowski et al., 2016).

Levosimendan can be initiated with a loading dose of 6–12 mcg/kg over 10 min if the systolic blood pressure is >100 mmHg, followed by a maintenance infusion rate of 0.05–0.2 mcg/kg/min. If a loading dose is utilized effects are seen in minutes. No tachyphylaxis is observed with levosimendan use. The major route of elimination is via conjugation forming an inactive metabolite excreted in the urine and feces. However, a small but significant proportion of the drug undergoes reduction in the small

bowel and is further acetylated to an active metabolite (OR-1896). The active metabolite has an elimination half-life of up to 80 h, thus a degree of drug effect may be maintained for days after stopping the infusion. Significant heterogeneity in acetylation status exists in the population and could theoretically contribute to a differential duration of drug effect, although limited clinical studies to date have failed to demonstrate this phenomenon (Kivikko et al., 2011).

Hypotension is the major side effect of levosimendan use and may persist following infusion discontinuation. This is considered to be related to an initial bolus. Currently, it is recommended that a bolus be administered initially only if immediate effects are required and its systolic blood pressure is over 100 mmHg.

Although levosimendan does not lead to increased intracellular calcium overload, arrhythmias continue to be a significant side effect. In the SURVIVE (Survival of Patients With Acute Heart Failure in Need of Intravenous Inotropic Support) trial, levosimendan was actually associated with a significantly greater incidence of atrial fibrillation and a similar incidence of ventricular tachycardia compared with dobutamine (Mebazaa et al., 2007). Other common side effects include headache, nausea, and diarrhea.

An oral formulation of levosimendan has been evaluated in chronic advanced HF as a palliative measure. Early studies showed a reduction in NT-proBNP was recorded along with improvement in quality of life assessed by the Minnesota Living with Heart Failure quality of life score (Nieminen et al., 2008).

Digoxin

Digoxin can be considered a weak oral inotrope. Digoxin inhibits the sodium–potassium ATPase pump. This inhibition promotes sodium–calcium exchange, which increases the intracellular calcium concentration available for myofilament cross bridging (Gheorghiade et al., 2004). Digoxin may also have beneficial effects on baroreceptor sensitivity (Wang et al., 1990). Digoxin has been shown to improve symptoms and reduce hospitalizations, but it does not convey a mortality benefit in patients with chronic systolic HF (Group TDI, 1997). A level between 0.5 and 0.8 ng/mL is recommended, and levels greater than 1.2 ng/mL are associated with increased mortality rate. The use of digoxin therapy in HF is outside the scope of this article and will not be covered further.

Investigational Agents

Istaroxime is a novel intravenous agent with inotropic and lusitropic properties related to inhibition of Na/K adenosine triphosphatase (ATPase) and stimulation of sarcoplasmic reticulum calcium ATPase. The HORIZON-HF investigators demonstrated improvements in cardiac index, pulmonary capillary wedge pressure, blood pressure, heart rate, and end-diastolic volume in a phase 2 trial of patients hospitalized with decompensated heart failure (Gheorghiade et al., 2008). Istaroxime is currently being evaluated in another clinical trial (https://clinicaltrials.gov/ct2/show/NCT02617446). Gene therapy manipulating calcium cycling changes in the failing heart have been attempted. The sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) regulates cardiomyocyte contraction and relaxation via intracellular calcium transportation, and SERCA2a deficiency is associated with progression of myocardial dysfunction (Hasenfuss et al., 1994; Hasenfuss and Pieske, 2002). Although a pilot study of SERCA2a gene transfer showed promising findings (Zsebo et al., 2014), a subsequent randomized, phase 2b trial failed to show a reduction in heart failure events at 12 months. Further studies of gene transfer are currently ongoing.

Clinical Uses

Acute Decompensated HF or Chronic HF as Bridge to Other Therapies

Inotropic therapy should be considered in hospitalized patients with severely depressed cardiac output complicated by systemic hypoperfusion and end-organ dysfunction. The goal of inotropic therapy is to preserve end-organ function, while the acute precipitating insult is being corrected and/or until definitive therapy (coronary revascularization, mechanical circulatory support, heart transplantation) is undertaken (Yancy et al., 2013). Outside of this setting, inotrope use is associated with increased mortality (Abraham et al., 2005; Cuffe et al., 2002; Packer et al., 1991).

The survival impact of temporary inotrope use as a bridge to definitive therapy in patients in extremis with signs of hypoperfusion has never been adequately studied (Guglin and Kaufman, 2014), thus its impact in this setting is unknown but likely lifesaving. As such evidence is lacking, choice of inotropic agent in the setting of low-output HF is largely based upon desired hemodynamic effects. Milrinone causes relatively larger decreases in both systemic vascular resistance (Grose et al., 1986) and PVR (Eichhorn et al., 1987) than dobutamine. In cases of severe hypotension or vasoplegia, dobutamine may be preferable. Conversely, in cases of high systemic vascular resistance or PVR, milrinone may be more appropriate. Milrinone may also be preferable in patients recently taking beta-blockers or those suspected of beta-blocker induced low-output syndrome (Travill et al., 1994). Prolonged elimination half-life, particularly in the setting of renal dysfunction, may make milrinone a less attractive choice in patients with significant renal dysfunction and hemodynamic instability. Use of inotropic therapy in acute decompensated HF improves cardiac output and enhances diuresis by improving renal blood flow (Al-Hesayen and Parker, 2008). Literature documenting improved outcomes with inotropic therapy in acute cardiorenal syndrome is lacking (Shchekochikhin et al., 2013), nonetheless inotropic agents are commonly used in this setting. Although logical inotropic agent choices should be guided by patient and drug characteristics,

data from the Get With The Guidelines-Heart Failure Registry demonstrated that inotropic agent choice in the United States is largely attributable to institutional preference alone (Allen et al., 2014).

The OPTIME (Outcomes of a Prospective Trial of Intravenous Milrinone for Exacerbations of Chronic Heart Failure) trial of short-term milrinone use in acute decompensated HF patient without CS failed to show a benefit in days spent hospitalized for cardiovascular cause, short- to mid-term mortality, nor a composite of death or readmission. Milrinone use in this setting was however associated with increased rates of atrial arrhythmias and sustained hypotension requiring intervention (Cuffe et al., 2002). Subgroup analysis of the trial suggested those with ischemic cardiomyopathy had worse outcomes compared to placebo, while those with nonischemic cardiomyopathy may have a more favorable response to such therapy (Felker et al., 2003). Regardless of HF etiology, use of inotropes in patients without significantly depressed cardiac output and hypotension or impaired perfusion remains contraindicated by AHA/ACC HF guidelines (Yancy et al., 2013).

Compared to placebo, short-term use of levosimendan for acute decompensated HF with systolic blood pressure >90 mmHg was associated with improvements in symptoms but no mortality benefit in REVIVE II (Randomized Evaluation of Intravenous Levosimendan Efficacy). Also levosimendan use in this setting was associated with an increased incidence of hypotension and cardiac arrhythmias (Packer et al., 2013). The largest levosimendan trial, SURVIVE (Survival of Patients With Acute Heart Failure in Need of Intravenous Inotropic Support), randomized 1327 patients with acute decompensated HF and SBP>85 mmHg to short-term levosimendan versus dobutamine. Levosimendan failed to improve the primary outcome of all-cause mortality at 180 days and was associated with increased incidence of atrial fibrillation, headache, and hypokalemia (Mebazaa et al., 2007).

Levosimendan is currently being evaluated for use in decompensated and high-risk patients with severely reduced left ventricular ejection fraction undergoing cardiac surgery. Early data suggested benefit with preoperative use of levosimendan in patients with severely reduced LVEF undergoing cardiac surgery (Levin et al., 2012); however, a recent, larger randomized trial failed to demonstrate any benefit of preoperative use with regard to the incidence of short-term death, MI, dialysis, or need for mechanical assist device (Mehta et al., 2017). Likewise, postoperative use of levosimendan in patients with LV dysfunction following cardiac surgery also failed to improve short-term mortality, duration of mechanical ventilation, or length of stay compared to standard postoperative hemodynamic support (Kolte et al., 2014).

CS Complicating Acute Myocardial Infarction

Current revascularization strategies and adjunct medical therapy have led to a dramatic reduction in mortality from acute myocardial infarction (AMI) to as low as 3%–4% of cases. That said CS remains the leading cause of death from AMI, occurring in up to 10% of all myocardial infarctions (Kolte et al., 2014) and in-hospital mortality with current approaches may be as high 40%–50% (Goldberg et al., 2009). In the SHOCK (Should We Emergently Revascularize Occluded Coronaries for Cardiogenic Shock) trial, emergency revascularization, as opposed to immediate medical stabilization, lead to reduced mortality rates at 6 and 12 months (Sleeper et al., 1999; Hochman et al., 2001), thus management should focus on immediate revascularization followed by adjunct therapy. Adjunct therapy for pump failure largely consists of temporary mechanical circulatory assist devices and intravenous vasoactive medications, including inotropes. Several studies have demonstrated improvements in end-organ function with the use of temporary mechanical support device, however none have demonstrated a survival advantage (Thiele et al., 2005, 2013; Seyfarth et al., 2008), thus inotropes remain a viable alternative or adjuvant to such devices. Strategies to reduce extension of the infarct area should be undertaken, yet both catecholaminergic and PDE inhibitors have been shown to induce cardiac myocyte cell death (Burniston et al., 2005; Garcia-Cazarin et al., 2008). Furthermore, vasoactive medications utilized in the peri-AMI period are likely to increase the incidence of ventricular arrhythmias.

Current ACC/AHA STEMI guidelines recommend individualized support with inotropes guided by invasive hemodynamic monitoring, but do not specify a preferred agent (O'Gara et al., 2013). These guidelines do however go on to comment that the use of dopamine may be associated with excess hazard (De Backer et al., 2010), and dopamine is contraindicated for use in the German-Austrian S3 Cardiogenic Shock guidelines (Werdan et al., 2012). Both recommendations were largely based on the results of the SOAP (Sepsis Occurrence in Acutely Ill Patients) II study, in which a subgroup analysis of CS revealed a higher rate of death, ascribed to a higher incidence of arrhythmic events, in patients treated with dopamine compared to norepinephrine (De Backer et al., 2010). Notably this was a subgroup analysis of a neutral trial involving predominantly septic shock patients. Furthermore, neither the etiology, nor the remaining therapy of CS was reported in this study. In contradistinction, the ESC 2012 STEMI guidelines make a IIa recommendation for the use of dopamine in STEMI patients with hypotension and hypoperfusion (Steg et al., 2012).

Dobutamine carries a IIa recommendation in the ESC 2012 STEMI guidelines (Steg et al., 2012) and is deemed the drug of choice in the German-Austrian C3 guidelines (Werdan et al., 2012) for intropic support in AMI complicated by CS. Although dobutamine has served as the inotrope of choice in this situation for several years, little prospective evidence exists, and no compelling evidence demonstrates the superiority of dobutamine over another agent. Furthermore, several authors (Overgaard and Dzavík, 2008; Khalid and Dhakam, 2008) have suggested a hemodynamic benefit of combined dobutamine and dopamine to improve hemodynamics and limit side effects, yet the source commonly cited for this practice is from a study of eight patients (Richard et al., 1983), thus an evidence base for this practice is severely limited. Nonetheless, dobutamine seems a well-suited agent for CS complicating AMI from a hemodynamic perspective and is the most commonly used inotrope worldwide in the management of CS (Allen et al., 2014).

In contrast to dobutamine, milrinone is associated with less tachycardia and does not increase myocardial oxygen consumption (Grose et al., 1986), which could theoretically provide a benefit in peri-MI care. However, cell death is not solely due to effects on myocardial oxygen consumption, and cAMP mediated increased intracellular calcium common to dobutamine and milrinone likely

plays a role in the activation of apoptosis (Opie, 1993). Systemic inflammatory response syndrome (SIRS) leading to lower than expected systemic vascular resistance has been described in a subset of patients suffering from CS following AMI (Kohsaka et al., 2005). Introduction of milrinone, known to have significant arterial vasodilatory effects, could be problematic in patients with a SIRS response. No large scale randomized, controlled trials have been performed to assess the utility of milrinone for use in CS complicating AMI. A meta-analysis of four small studies suggests that treatment with milrinone may be safe and effective for patients with CS following AMI (Tang et al., 2015). Subgroup analysis from the OPTIME CHF (Outcomes of a Prospective Trial of Intravenous Milrinone for Exacerbations of Chronic Heart Failure) study demonstrated increased need for short-term cardiovascular rehospitalization and an increased composite of death or rehospitalization in ischemic cardiomyopathy patients treated with milrinone (Felker et al., 2003). Importantly, this was not a trial of patients with CS, nor recent AMI. Furthermore this was a subgroup analysis of trial yielding neutral results, thus these findings are hypothesis generating at best. As it is unlikely that prospective trials with milrinone will be performed in the AMI setting, future registry data will likely help elucidate this drugs role in AMI complicated by CS.

Levosimendan improves myocardial contractility without increasing cAMP or intracellular calcium, thus provides potential biochemical advantages from a cellular perspective. RUSSLAN, a randomized, placebo-controlled trial of levosimendan in patients with left ventricular failure following AMI, use of the calcium-sensitizing drug lead to a significant decrease in both short- and mid-term survival (Moiseyev et al., 2002). Notable exclusion criteria for this trial included SBP<90 mmHg, thus unlikely a true CS population. Levosimendan was associated with improved 30-day survival compared with enoximone, a phosphodiesterase type III inhibitor, in a small randomized trial of refractory CS complicating AMI (Fuhrmann et al., 2008). Another very small trial failed to show a long-term survival benefit of levosimendan over dobutamine in patients with CS following PCI for AMI (Samimi-Fard et al., 2008). Registry data also failed to show a beneficial effect of levosimendan on mortality in patients with CS due to AMI (Omerovic et al., 2010). None the less, the ESC 2012 STEMI guidelines give levosimendan a IIb recommendation, citing particular usefulness in patients on chronic beta-blocker therapy (Steg et al., 2012).

A recent Cochrane Review of randomized controlled trials in patients with AMI complicated by CS concluded that at present there are no robust and convincing data to support a distinct superior inotropic or vasodilator drug to reduce mortality (Unverzagt et al., 2014). Therefore, pharmacologic support with inotropic agents for CS complicating AMI should be individualized and used as an adjunct to prompt revascularization with or without concomitant temporary mechanical support.

Inotropic Support of the Failing Right Heart

RV dysfunction may occur in isolation or in combination with left heart dysfunction. Isolated RV failure may occur due to primary pump failure as is seen in acute RV infarction or may fail secondary to elevated afterload from pulmonary vascular disease. Few clinical studies evaluating the performance of specific inotropes in the setting of primary RV failure have been performed, thus choice of inotropic agent is largely guided by pharmacologic properties and patient characteristics. In addition to the agent impact on contractility and ventriculo-arterial coupling, the PVR/SVR ratio is an important factor in determining RV performance and systemic perfusion.

At low doses, dobutamine leads to improved RV–pulmonary artery coupling via reduced PVR and increased RV contractility (Kerbaul et al., 2004). Dobutamine has been successfully used for RV failure in the setting of acute MI (Ferrario et al., 1994), pulmonary arterial hypertension (Sztrymf et al., 2010), and pulmonary hypertension in the setting of liver transplantation (Romson et al., 1999). RV failure-induced congestive hepatopathy or renal disease may be improved with dopamine therapy via splanchnic vasodilation (Sztrymf et al., 2010). At high doses, dopamine increases the PVR/SVR ratio, thus may be less effective as a primary inoconstrictor in the setting of RV failure with pulmonary hypertension and systemic hypoperfusion (Barrington et al., 1995). Similar to dobutamine, milrinone augments RV performance via increased contractility and decreased PVR. Milrinone decreases RV afterload to a greater extent than dobutamine (Eichhorn et al., 1987), thus many consider milrinone the inotrope of choice in patients with RV failure secondary to high pulmonary afterload (Price et al., 2010; Green and Givertz, 2012). The limiting factor for milrinone use is the drugs impact on systemic vascular resistance and subsequent hypotension, which often requires coadministration of a vasopressor. Vasopressin may be the ideal vasopressor in this setting as concomitant use with milrinone preserves a favorable PVR/SVR ratio compared with other agents (Jeon et al., 2006). Limited data with inhaled milrinone suggests favorable effects on RV afterload and intrapulmonary shunt fraction while avoiding systemic hypotension (Wang et al., 2009). Levosemindan is known to increase CO and decrease PVR in patients with left HF (Slawsky et al., 2000). Experimental data suggest that levosemindan improves RV–pulmonary artery coupling more effectively than dobutamine (Kerbaul et al., 2006). In patients with left ventricular dysfunction and pulmonary hypertension, levosimendan causes similar reductions in PVR and mean pulmonary artery pressure as milrinone. However, levosimendan causes more tachycardia and hypotension requiring norepinephrine than milrinone (Mishra et al., 2016).

Palliative Inotrope Use

Long-term, continuous intravenous inotropic support may be considered as palliative therapy for symptom control in select patients with stage D HF on optimal therapy who are not candidates for durable mechanical circulatory support or transplant (Yancy et al., 2013). A right heart catheterization documenting a favorable hemodynamic response is typically required for reimbursement. Medicare coverage requires the study be performed within 6 months prior to the initiation of home inotropic

therapy and a documented cardiac index $\leq$2.2 L/m/m^2 and/or pulmonary capillary wedge pressure $\geq$20 mmHg before inotrope infusion on maximum medical management and a $\geq$20% increase in CI and/or a $\geq$20% decrease in PCWP during inotrope infusion at the dose initially prescribed for home infusion. The median survival of patients on chronic continuous inotropic therapy is improved in the recent era to 9–18 months as compared to 3–6 months previously. This may be related to the higher use of guideline-based HF therapy including beta-blockers and presence of defibrillators (Hershberger et al., 2003; Hashim et al., 2015; Ginwalla, 2016).

Suitable candidates must understand the goals of palliation and have adequate caregiver support. Given the well-known arrhythmogenic potential of inotropic therapy, it is important to discuss patient preference for ongoing ICD therapy. Almost one-fifth of patients on chronic inotropic therapy will receive an appropriate ICD shock if the device remains activated (Acharya et al., 2016). Other issues include infection risk involved with a chronic indwelling intravenous catheter and availability of caregiver support. Assistance with dressing care and inotrope infusion bags changes is required. Also availability of community and hospice resources is important. Unfortunately, financial coverage for continuous inotropes is often limited if the patient transitions to hospice care.

Conclusion

Varying hemodynamic profiles and clinical scenarios require different inotropic strategies. Each agent causes a unique hemodynamic response and carries a slightly different side effect profile. Knowledge of both biologic effects and clinical outcomes data is required for effective inotropic use.

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Further Reading

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Interventions: Endomyocardial Biopsy

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Case Report

A 34-year-old male patient was admitted to an external hospital with signs of acute heart failure. The patient's medical history was empty. Transthoracic echocardiogram (TTE) showed a highly reduced ejection fraction (EF <20%) with global hypokinesia (data not shown) and the patient underwent coronary angiography revealing no signs of coronary artery disease. Following angiography the patient deteriorated on the same day, went into cardiogenic shock, and was transferred to our hospital for further diagnostic work up and treatment. Upon arrival TTE showed a hypertrophic septal wall and a reduced left ventricular ejection fraction of 22%–25%. (Online **Video 1A**). The patient's respiratory and haemodynamic condition worsened and we stabilized his condition by means of extracorporeal life support (Extracorporeal membrane oxygenation (VA-ECMO)) the following day. On the basis of his clinical presentation and the TTE findings, the patient was felt to require further diagnostics and an endomyocardial biopsy (EMB) was performed the same day and we decided to initiate an immunosuppressive therapy with azathioprine (50 mg/12 h for 6 months) and methylprednisolone (1 mg/kg for 4 weeks) (Frustaci et al., 2009). EMB revealed eosinophilic myocarditis with markedly increased cytotoxic cells (**Fig. 1**). TTE after 5 days of immunosuppressive treatment showed an improved left ventricular systolic function (LVEF-35%) and we could take the patient off the extracorporeal life support (Online **Videos 1B**). TTE performed after 9 days and after one month showed a normalized left ventricular ejection fraction of 60% (Online **Video 1C**).

Introduction

Endomyocardial biopsy (EMB) is the technique of choice in the diagnosis of adults presenting with cardiomyopathy of unknown origin. The toxic, infectious, inflammatory, infiltrative, or autoimmune processes that cause cardiac dysfunction occur at a cellular level and no other diagnostic techniques can establish the nature of the etiological agent. In addition to detection of inflammation or viral genomes in the acute phase of myocarditis, EMB adds important prognostic information during the follow-up of patients that can influence therapeutic decisions (Caforio et al., 2013; Dominguez et al., 2016; Escher et al., 2016; Savvatis et al., 2015).

Indication and Recommendation

The 2007 American Heart Association/American College of Cardiology Foundation/European Society of Cardiology scientific statement on EMB limited its class I recommendations to unexplained new-onset heart failure of less than two weeks duration associated with hemodynamic compromise or unexplained new onset heart failure of two weeks to three months duration associated with a dilated left ventricle (LV) and new ventricular arrhythmias or conduction disturbances (Cooper et al., 2007). In a position statement from the ESC (Caforio et al., 2013), the recommendation for EMB was extended, including patients with a pseudo-infarct presentation after excluding coronary artery disease, myocarditis, and inflammatory cardiomyopathies, as well as for patients with rapidly advancing cardiomyopathy refractory to conventional therapy. Hence, an increasing number of patients can benefit from specific treatment. Moreover, recently the 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure recommends EMB in patients with rapidly progressive heart failure despite standard therapy when there is a probability of a specific diagnosis which can be confirmed only in myocardial samples and specific therapy is available and effective (Ponikowski et al., 2016).

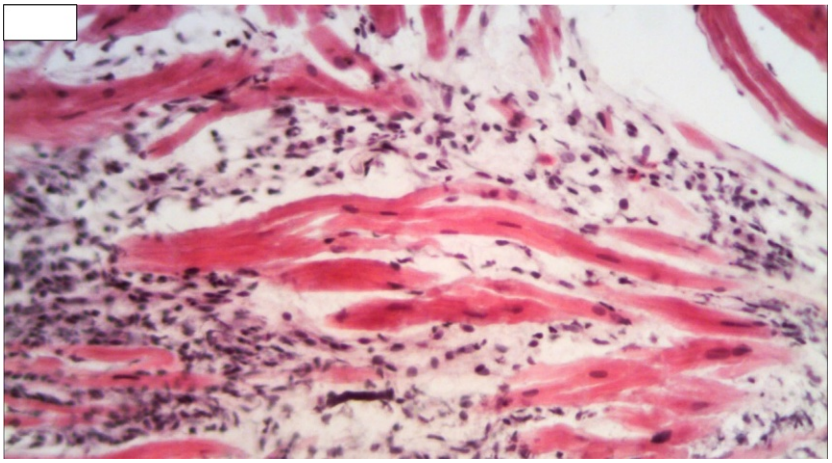

Fig. 1 Histological results of endomyocardial biopsy. H & E Stain. Histology revealed eosinophilic myocarditis with markedly increased eosinophilic invaded cytotoxic inflammatory cells (*red*: myocytes).

These changes respond to the more widespread availability of immunohistochemical and viral genome detection techniques, which improve the ability to detect the underlying cause of myocarditis.

Patient Preparation

EMB is performed in a supine position under local anesthesia. The patient must be monitored with 3-lead ECG, noninvasive blood pressure monitoring, and oxygen saturation. International normalized ratio (INR) of <1.5 is required before the EMB, and anticoagulation therapy should be discontinued 16 h before and 12 h after the procedure. Echocardiogram is recommended before and immediately after the procedure to exclude pericardial effusion. If possible, we recommend a 12–24 h telemetric observation after the procedure. Furthermore, we recommend an in-house transthoracic echocardiography prior to the biopsy to confirm the diagnosis of cardiomyopathy, estimation of the LV ejection fraction, LV hypertrophy, detection of altered myocardial texture and to rule out any obstacles for left ventricular EMB such as a diameter of the lateral LV wall <8 mm or noncompaction cardiomyopathy, LV thrombus, or relevant aortic valve stenosis.

Left Ventricular Versus Right Ventricular Endomyocardial Biopsy

The choice of the ventricular site for EMB is still widely discussed. Whereas some observe that diagnostic yield of LV EMB is superior to right ventricular (RV) EMB when routine immunohistochemistry and viral genome amplification are used in suspected LV myocarditis, more recent data state that both procedures are similar when assessing inflammation or viral genome in the myocardium (Chimenti and Frustaci, 2013). However, we recently showed that morphological changes such as interstitial fibrosis and cardiac collagen type I expression were more reliably found in LV EMB (Escher et al., 2014).

RV-EMB approached by right internal jugular or femoral vein and LV-EMB approached by the left or right femoral artery are commonly used and when performed by experienced operators, both right and left EMBs have very low complication rates. In a single center study that analyzed 3048 EMB in a nontransplant setting, risk of major complications including cardiac tamponade and AV block requiring permanent pacemaker implantation was 0.12% (Holzmann et al., 2008; Tschope et al., 2015). Chimenti et al. documented that over a 28-year period and over 4000 EMB procedures performed complications appeared in only 0.33% of patients who underwent LV-EMB (Chimenti and Frustaci, 2013).

We strongly believe that for this analysis not the localization but the number of taken EMBs is important to get a definite diagnosis and to reduce the sampling error.

Since we could demonstrate that at least morphological changes such as interstitial fibrosis and cardiac collagen type I expression were more reliably found in LV EMB, we perform routinely LV EMB especially in cases where a coronary angiogram is performed prior to LV biopsy to rule out relevant coronary artery disease as the underlying pathology for heart failure.

Access Site

When LV-EMB is performed, the femoral artery is usually the access site (Cooper et al., 2007; Yilmaz et al., 2010). The transfemoral site, however, is associated with bleeding complications form the access site and limited due to postprocedural immobilization.

The radial artery is increasingly used for diagnostic coronary angiography and percutaneous coronary interventions and has been recently recommended according to ESC guidelines. Lower direct costs, fewer vascular complications, better patient acceptance, and earlier ambulation are some of the direct benefits from using radial access (Schueler et al., 2013). When compared to the femoral approach, less vascular access site complications and reduced major bleeding have been reported (Jolly et al., 2011; Rao et al., 2008; Bertrand et al., 2010).

Access-site vascular complications in patients undergoing transradial coronary procedures are rare but may have relevant clinical consequences (Burzotta et al., 2013). Complications include spasm, occlusion or perforation of radial artery (RA), hematoma, pseudoaneurysm, arteriovenous fistula, and nerve injury (Kanei et al., 2011).

We previously showed that transradial LV-EMB is feasible (Schulz et al., 2015). We recently showed in 18 patients undergoing transradial LV-EMB that the success rate was 100%. There were no periprocedural cardiac complications. Immediate postprocedural ambulation could be achieved in all patients. Although radial artery pulse was confirmed by ultrasonic vascular Doppler after removal of the guide in 100% of the patients, 50% (9/18) of the patients showed occlusion of the radial artery (RAO) by duplex sonography proximal to the access site. 33% (3/9) of the patients in the RAO group and 11.1% (1/9) of the patients in the patent radial artery (RAP) group, respectively, experienced mild pain after the procedure in the right lower arm. Color Doppler ultrasonography of the right radial artery performed 24 h after the procedure revealed radial occlusion in 50% (9/18) of the patients. The diameter of the radial artery was significantly smaller in the RAO group ($P=0034$), and peak systolic velocity (PSV) of the right ulnar artery was significantly higher in the RAO group ($P=0.012$). Peak systolic velocity of the opposite radial artery was significantly lower in the RAO group ($P=0045$) (Kherad et al., 2016). Gender, diabetes, and radial artery inner diameter $\leq$2.5 mm are known predictors of RAO (Uhlemann et al., 2012) and we have shown that lower PSV of $<$50 cm/s is also associated with RAO and used these parameters to create the *B*erlin *RAD*ial Access *S*core, $BRAD_2$Score. The $BRAD_2$Score is the sum of the clinical parameters (age, gender, and diabetes) and the ultrasound-based parameters (Radial artery diameter and PSV), whereas the ultrasound parameters count twice to the overall sum. We postulate that with a clinical $BRAD_2$Score of 1 radial access can be performed without the risk of RAO and without an ultrasound evaluation of the radial artery. With a clinical $BRAD_2$Score of 2 we recommend ultrasound evaluation of the radial artery. With a remaining $BRAD_2$Score of 2 after ultrasound evaluation radial access can be performed, whereas with a $BRAD_2$Score of $>$2 after ultrasound evaluation we recommend alternative access sites. All patients with RAO had a $BRAD_2$ Score of $>$3. The $BRAD_2$Score might be a valuable novel tool to identify patients at risk for postprocedural occlusion of the radial artery and will be analyzed in a prospective study (**Table 1**).

Transradial Left Ventricular EMB

Following local anesthesia, a 6 F sheath (Radifocus Introducer II, 10 cm, Terumo, Japan) is introduced into the right radial artery. Following sheath introduction every patient receives 3000 IU unfractionated heparin and 5 mg verapamil i.a. to prevent radial artery occlusion or spasm. A 5 F pigtail catheter (Boston Scientific, USA) is then placed into the LV. A long J-wire (260 cm, 0.03500) is now advanced over the pigtail catheter into the LV to hold the ventricular position, the pigtail and the 6F radial sheath are removed, and a 7.5F sheathless multipurpose guiding catheter (MP1.0, Asahi Intecc, Japan) is introduced and its dilatator is removed as soon as the sheathless guiding catheter reaches the ascending aorta and carefully advanced over the wire into the LV cavity. The J-wire is now removed and a Y-connector (Copilot, Abbott Vascular, USA) is connected. The correct position of the guiding catheter tip is checked in 20 degree left anterior oblique (LAO) projection with the tip of the catheter pointing to the lateral LV wall. Once the positioning of the catheter is confirmed 6 mL of contrast agent are injected to visualize the distance of the tip to the lateral LV wall (**Fig. 2B**). The guiding catheter should not touch the wall. Prior to the biopsy, activated clotting time is checked (ACT: 200–250 s) to prevent thromboembolism during the procedure. After the bioptome forceps (B-18110; 1100 mm, 1.8 mm, Medizintechnik Meiners, Germany; **Fig. 3**) have been rinsed in water to prevent air embolism, they are inserted into the MP1.0 guiding catheter via the Y connector.

Table 1 Berlin RADial access score $BRAD_2$Score

Risk factors	*Points*
R Radial peak systolic velocity (PSV) $\leq$50 cm/s	2
A Age $\leq$ 50 years	1
D Diameter radial artery $\leq$2.6 mm	2
D Diabetes mellitus	1
S Sex (female)	1
$BRAD_2$Score 1 point → Radial access	
$BRAD_2$Score 2 points → Perform color-coded duplex ultrasonography (CCD)	
$BRAD_2$Score 2 points after CCD → Radial access	
$BRAD_2$Score $\geq$3 points → Nonradial access	

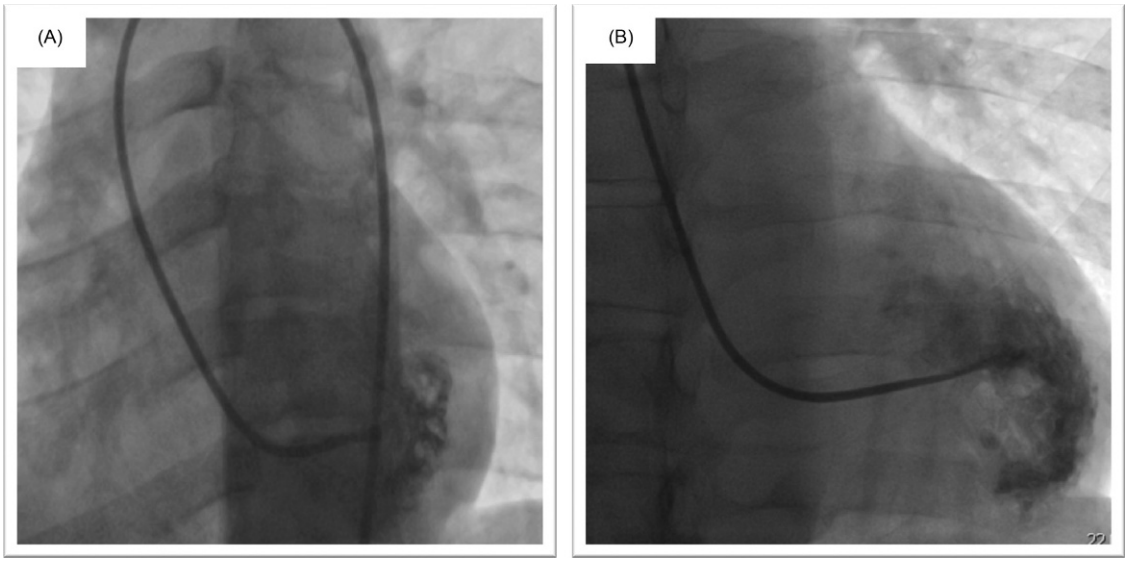

Fig. 2 LV angiography Fluoroscopy in LAO 20 degree view with injection of 6 mL contrast agent to visualize the position of the 7.5F MP-1 guiding catheter inside the left ventricle with the tip pointing to the lateral LV wall. 1A: Transfemoral approach. 1B: Transradial approach.

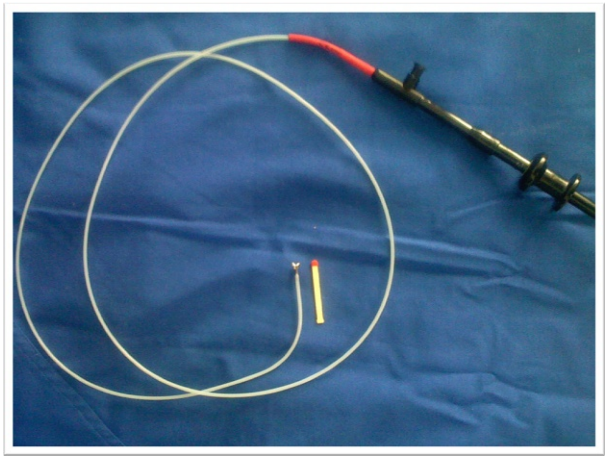

Fig. 3 Biotome (B-18110; 1100 mm, 1.8 mm, Medizintechnik Meiners, Germany).

There are a number of bioptomes available from different companies and they all differ in prize, the possibility of resterilization, and in stiffness and flexibility, respectively. We use a very flexible bioptome (B-18110, Medizintechnik Meiners, Germany). It has a 6F diameter and a length of 1100 mm. The 2 closed jaws have a total diameter of 1.8 mm, a length of 2.8 mm, and a volume of 4.5 mm^3. Compared with other bioptomes, it has a more flexible polytetrafluoroethylene (Teflon) tube instead of an inflexible steel spiral and smaller jaws.

The forceps of the bioptome are advanced under fluoroscopy close to the tip of the guiding catheter. The forceps are opened inside the guiding catheter and carefully advanced toward the lateral left ventricular wall. As soon as resistance is recorded or fluoroscopically seen, the jaws are closed and the forceps are immediately retracted into the guiding catheter (Online **video 2**). Upon completion of the procedure, the sheathless guiding catheter is removed and a vascular closure device (TR Band, Terumo, Japan) is applied for haemostasis. Each patient receives low-dose aspirin for 4 weeks to prevent embolisms from the area of biopsies.

Transfemoral Left Ventricular EMB

Following local anesthesia, an 8 F sheath (Radifocus Introducer II, 10 cm, Terumo, Japan) is introduced into the right or left femoral artery. Upon sheath introduction every patient receives 3000–4000 IU unfractionated heparin (ACT: 200–250 s). A 5 F pigtail catheter (Boston Scientific, USA) is now advanced into the LV. A long J-wire (260 cm, 0.03500) is advanced over the pigtail catheter to hold the ventricular position, the pigtail is removed, and an 8 F multipurpose guiding catheter with side holes

(MP1.0 SH, Medtronic, USA) is introduced, and the guiding catheter is carefully advanced over the wire into the LV cavity. The J-wire is now removed and a regular Y-connector (Copilot, Abbott Vascular, USA) is again connected. The following steps are identical to the transradial approach (Fig. 2A).

Upon completion of the procedure, the guiding catheter and the 8F sheath are removed and an 8F vascular closure device is applied for haemostasis. Each patient receives a low-dose aspirin prophylaxis for 4 weeks.

Transfemoral Right Ventricular EMB

For this access no heparinization or aspirin prophylaxis is recommended. Following local anesthesia, an 8F sheath (Arrow Flex, 30 cm, Tereflex, USA) is introduced into the right or left femoral vein. The left vein access might have an advantage in younger patients where the position of the heart is often steep. We use the described bioptome of Meiners (B-18110), which allows due to its flexibility, an easy modulation of the Teflon tube according to individual anatomy of the patient. In contrast to others, we do not use a guiding catheter under these conditions, which would destroy the flexibility of the tube and increase the perforation risk. Principally, we do not recommend a use of any stiff bioptomes for RV biopsies. Under fluoroscopic control at 0° right anterior oblique (RAO) projection the bioptome is advanced to the right atrium. After carful turning into the direction of the tricuspid valve it can be advanced into the RV. The ideal position of the bioptome in the RV has to be checked at 90 degree LAO (Fig. 4). It is not recommended to take RV biopsies at RAO views, since these views cannot exclude that the bioptome is still in the (large) right atrium or was introduced into the sinus coronaries. Before opening the forceps of the bioptome one should make sure that the bioptome is not touching the wall to prevent uncontrolled tissue damage. Usually a short backward move of the bioptome is enough to open the forceps in the cavum of the RV, followed by a new forward move with an opened forceps to the lower part of septal wall to get the biopsy. Taking biopsies at higher parts of the septum would increase the risk of AV blocks, especially in patients with established left bundle block. Upon completion of the procedure, the bioptome and the 8 F sheath are removed and haemostasis is established by manual compression for several minutes.

Tissue Analysis

On average 6–8 EMBs are necessary to be able to perform histology, immunohistology, and molecular analysis and to overcome the sampling error.

Light microscopic examination is routinely performed on formalin-fixed, Paraffin-embedded, and Tissue-Tek® (Sakura Finetek, USA)-embedded frozen tissues. Histology includes H&E-, EvG-, PAS-, and Azan-stained sections to evaluate tissue alterations such as fibrosis, scars, granulomas, pathological vascular conditions, infiltrative and storage diseases (e.g., amyloidosis, iron deposits, enhanced glycogen storage, M. Fabry), or distinct inflammatory cell infiltrates (lymphocytes, leukocytes, eosinophiles, monocytes, macrophages, giant cells), which may cause acute or chronic myocyte damage, myocytolysis or apoptosis and replacement fibrosis, in inflammatory cardiomyopathy and myocarditis (Dallas-Classification). Immunohistochemistry and digital morphometric analyses improve diagnostic sensitivity and allow a more quantitative and specific differentiation of, for example, amyloid subtypes

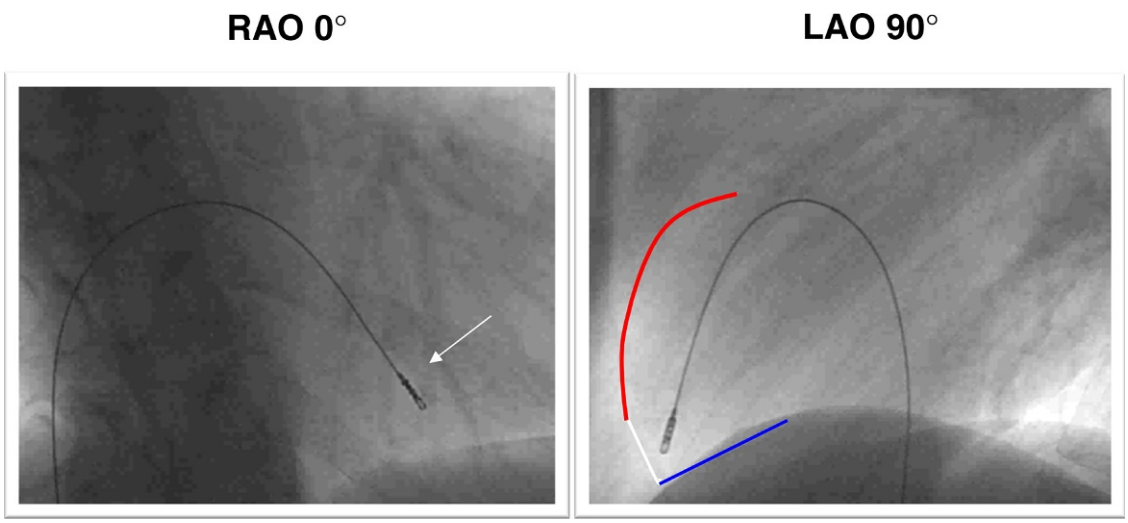

Fig. 4 RV angiography fluoroscopy in RAO 0 degree and LAO 90 degree view. Visualization of the position of the biotome within the right ventricle. *Red*: septum with increased risk of transient or permanent third degree or second degree type 2 AV Block; *White*: optimal position, *Blue*: free wall with increased risk of perforation.

(IgG λ or κ light chains) and of inflammatory infiltrates using monoclonal and polyclonal antibodies (including CD3, CD4, CD8, CD45R0, CD20 CD68/PGM1, HLA-DR. HLA-ABC, or cellular adhesion molecules) in frozen cryostat sections. Whereas the number of cellular infiltrates (lymphocytes, macrophages) is varying in different biopsies (sampling error), the expression of adhesion molecules (e.g., ICAM-1/2, VCAM-1) is more homogeneously distributed and indicates the global tissue activation. The introduction of these markers in routine biopsy diagnostics improves identification of inflammatory processes in myocardium even without on-site identification of infiltrative cellular foci. The timely presence of activated or cytotoxic lymphocytes and proinflammatory or antiinflammatory macrophages furthermore differentiates chronic active from healing inflammation (myocarditis) and thus may be used as a marker of autoimmune myocarditis where immunosuppression may be considered (Leone et al., 2012; Maisch et al., 2005).

Microbial genomes are determined, quantified, and sequenced using PCR-based methods. Depending on the two types of viral nucleic acids the isolation of DNA and RNA is performed in separate extraction procedures needing multiple biopsy specimen. The viral load is determined by quantitative PCR. The transcriptional activity of virus in myocardial tissue or peripheral blood cells is performed by nested RT-PCR and QPCR. These sensitive techniques detect the most common cardiotropic viruses such as enteroviruses, adenoviruses, erythrovirus, human herpesvirus 6, Epstein-Barr virus, and in the Far East also hepatitis C, in about 50%–60% of patients clinically presenting as myocarditis or DCMI. At present the clinical significance is, however, demonstrated for few viruses only (incl. entero/adenoviruses) (Bock et al., 2010; Caforio et al., 2007; Kuhl et al., 2012).

In order to exclude systemic infection, peripheral blood (one sample (5–10 mL) collected in EDTA or sodium citrate tubes) should be investigated in parallel with EMB (Leone et al., 2012; Caforio et al., 2007). The assessment of the viral replication status and parallel studies of the blood compartments (peripheral cells, plasma, serum) allows us to differentiate between acute and latent infections. This additional virus diagnostics allows us to identify subgroups of patients with viral persistence that might benefit from specific antiviral therapy strategies (Caforio et al., 2007; Kuhl et al., 2003, 2005, 2012, 2015; Magnani et al., 2006).

In general, genetic testing is unable to detect viral infections or inflammation in myocardium. In the future next-generation sequencing technology that reads out millions of sequence fragments within in a very short time will significantly improve the EMB-based diagnostics by parallel identification of pathological mutations in genomic DNA and the involved microbial genomes in one single biopsy sample. Treatment strategies for inflammatory cardiomyopathies have been recently summarized by Dominguez et al. (2016).

Conclusion

EMB is central in diagnosing suspected myocarditis, inflammatory cardiomyopathies, and rapidly advancing cardiomyopathy thereby allowing in many cases specific treatment of the underlying cause. Both right and LV EMBs, when performed by experienced operators, are safe and have very low complication rates.

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Intra-aortic Balloon Pumps (IABP) and Percutaneous Ventricular Assist Devices (VADs)

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Introduction

Approximately 6 million adults in the United States suffer from heart failure. Predictions have shown that from 2012 to 2030, the prevalence of heart failure will increase to more than 8 million people in the United States (1 in every 33) (Heidenreich et al., 2013). The clinical spectrum of heart failure encompasses a broad range of pathology and begins with an initial event such as acute myocardial infarction (AMI), progressive valvular heart disease, myocarditis, or onset of a primary dilated cardiomyopathy. Due to improvements in medical therapy, these patients can survive and may eventually develop chronic heart failure, which is characterized by neurohormonal activation, increased sympathetic tone, and maladaptive cardiac remodeling that unfortunately precipitate readmissions for heart failure and eventually lead to mortality. Low cardiac output and multi-organ hypoperfusion refractory to medical therapy are primary indications for the use of advanced therapies including durable mechanical circulatory support (MCS) devices, including surgically implanted left ventricular, right ventricular, or biventricular assist devices or cardiac transplantation.

In the United States, the number of patients receiving cardiac transplant has remained stable at approximately 2000–2500 per year. In contrast, based on data from the HeartMate-II and HeartWare bridge to transplant trials and the HeartMate-II destination therapy trial, the number of LVAD implants has increased to over 2500 implants in 2013 in the United States alone (Kirklin et al., 2015). According to registry data from the Interagency for Mechanically Assisted Circulatory Support (INTERMACS), there has been a reported increase in the use of LVADs for "destination therapy" and a decrease in their utilization as part of a "bridge to recovery" or "rescue" approach. This redirection of LVAD implantation away from unstable, high-risk INTERMACS categories (profiles 1 and 2) toward more stable candidates (profiles 3 and 4) is partially guided by data that demonstrated increased mortality after LVAD implantation for INTERMACS profiles 1 and 2 above the age of 65 and the increasing accessibility of nondurable MCS devices, which include temporary, percutaneously inserted devices without the need for surgery.

Supporting this trend of an increase in the use of acute device support, a recent analysis of the Nationwide Inpatient Sample from the Healthcare Cost and Utilization Project showed a 1511% increase in the implantation of nondurable MCS, including the TandemHeart (Cardiac Assist Inc.) and Impella (Abiomed Inc.), while there was no significant change in intra-aortic balloon pump (IABP) use between 2007 and 2011 compared with 2004–07 (Stretch et al., 2014). Use of the TandemHeart and Impella devices was associated with a reduction in the length of stay and cost for patients admitted with a diagnosis of congestive heart failure, but no change in their in-hospital mortality. The objective of this review is to describe the various options for acute, percutaneous MCS device therapy and the recent clinical trial data supporting their use for patients presenting with an ST-segment elevation myocardial infarction (STEMI), cardiogenic shock, and advanced heart failure.

Intra-aortic Balloon Pumps

The IABP is the most commonly used acute MCS device with over 50,000 implants per year in the United States alone (Cohen et al., 2003; Stone et al., 2003; Williams et al., 1982; Kern et al., 1993a,b). The IABP is a catheter-mounted balloon that inflates during diastole, thereby displacing blood volume in the descending aorta and increasing mean aortic pressure (Fig. 1). The IABP may assist in augmenting coronary perfusion during diastole. The IABP then deflates during systole, which creates a pressure sink that is then filled by blood from the left ventricle. The ideal hemodynamic effects from an IABP are increased diastolic aortic pressure, reduced aortic and LV systolic pressure, increased systemic mean arterial pressure, reduced LV diastolic volume and pressure, and increased coronary perfusion pressure. The magnitude of diastolic augmentation and systolic unloading can be determined from measurements from the IABP console tracing.

Previous studies have shown that the hemodynamic impact of balloon counterpulsation is primarily determined by four factors: (1) the magnitude of diastolic pressure augmentation, (2) the magnitude of reduced systolic pressure, (3) the magnitude of volume

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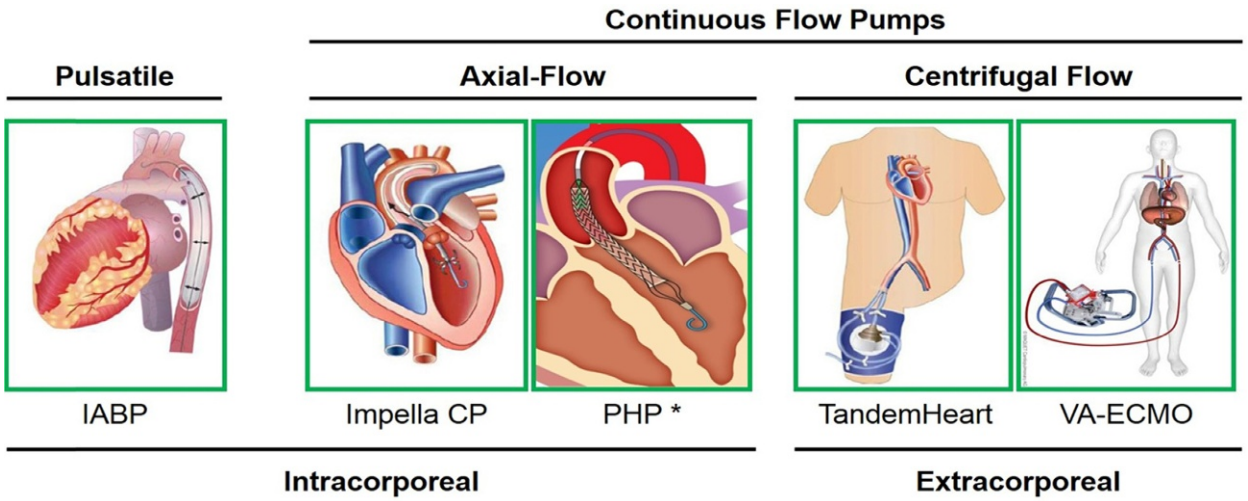

Fig. 1 Acute mechanical circulatory support device options for left-sided ventricular support.

displacement, and (4) the timing of balloon inflation and deflation (Kern et al., 1993b; Braunwald et al., 1958; Sarnoff et al., 1958a, b; Welch et al., 1958; Schreuder et al., 2005a,b). IABP balloon capacity ranges from 34 to 50 cm^3. Larger capacity IABPs potentially offer better hemodynamic support than standard 40 cm^3 IABPs (Kapur et al., 2015a). We recently reported that larger capacity, 50 cm^3 IABPs, provide greater diastolic augmentation and systolic unloading compared with the 40 cm^3 IABP in a retrospective analysis of IABP tracings from 52 consecutive patients. We further showed that the magnitude of systolic unloading correlates directly with the magnitude of diastolic augmentation and inversely with pulmonary artery occlusion pressure. These observations suggest that future clinical trials employing the 50 cm^3 IABPs may demonstrate better clinical outcomes than studies predominantly using the 40 cm^3 IABP.

Benefits of the IABP are its relative cost compared with other acute MCS devices, ease of insertion, and widespread familiarity among the interventional community. Major complications, including acute limb ischemia, severe bleeding, IABP failure or leak, or death directly related to IABP insertion, occurred at a frequency of 2.6% in 16,909 patient case records in the Benchmark Registry (Ferguson et al., 2001). Clinician expertise, sheathless insertion, and smaller IABPs are associated with decreased incidence of vascular complications (Eltchaninoff et al., 1993; Erdogan et al., 2006).

The data from multiple registries have traditionally been in favor of IABP usage (Stone et al., 2003; Abdel-Wahab et al., 2010; Curtis et al., 2012); however, recent studies attempting to identify optimal candidates for IABP support in high-risk percutaneous coronary intervention (PCI), acute MI, or cardiogenic shock have shown no significant benefit associated with elective IABP insertion. The Counterpulsation Reduces Infarct Size Acute Myocardial Infarction (CRISP-AMI) trial showed that IABP implantation immediately prior to revascularization for an anterior ST-elevation myocardial infarction did not reduce infarct size or improve short-term survival (Patel et al., 2011). The IABP-SHOCK II study suggested that not all patients presenting with an ACS with marginal blood pressures and clinical evidence of hypoperfusion benefit from IABP activation (Thiele et al., 2012). In HR-PCI patients, the PROTECT II study showed no difference in major adverse cardiovascular events between IABP and Impella 2.5 axial-flow catheter (O'Neill et al., 2012). The balloon pump-assisted coronary intervention study (BCIS)-1 also showed no reduction in short-term mortality with IABP insertion prior to HR-PCI; however, follow-up data suggested a possible long-term benefit up to 5 years after PCI (Perera et al., 2010, 2013). Recent analysis of trends for acute MCS use has reported an increase in the cost of hospital stays associated with IABP use (Stretch et al., 2014). No clinical studies have rigorously examined the clinical utility of IABP therapy in advanced heart failure.

The Impella Microaxial-Flow Catheter

The Impella devices are catheter-mounted microaxial-flow pumps that are deployed into the LV across the aortic valve via retrograde insertion (Fig. 1). Blood enters the pump though the left ventricle and is moved via a circulating impeller across the aortic valve into the ascending aorta. The Impella 2.5 LP and CP devices can be deployed percutaneously, while the Impella 5.0 device requires surgical vascular access through a conduit graft. In contrast to LV apical cannulation with durable MCS, transvalvular delivery may initially increase LV stroke work (LVSW) until the Impella pump is activated by inducing transient aortic insufficiency (Valgimigli et al., 2006). Remmelink and colleagues reported decreased LV end-diastolic pressure, but no change in LVSW, LV volumes, or cardiac output with maximal activation of the Impella 2.5 LP device in 11 patients presenting for high-risk coronary intervention (Remmelink et al., 2010). In 2007, Sauren et al. reported a 50% reduction in LVSW with a larger Impella prototype device that generated 3.8 LPM of flow in a preclinical model of acute LV injury (Sauren et al., 2007). Consistent with this observation, we recently reported that activation of the Impella CP device generated 3.1 LPM of flow and reduced LVSW by 32% in a bovine model of AMI (Kapur et al., 2015b).

The utility of the Impella 5.0 device has been examined in few studies. RECOVER-I was a prospective, single-arm feasibility study using the Impella 5.0 for postcardiotomy cardiogenic shock (Griffith et al., 2013). The device significantly improved cardiac index from 1.65 to 2.7 L/min/m^2 ($P < 0.01$) and mean arterial pressure (71–83 mmHg), while reducing pulmonary artery diastolic pressure (28–20 mmHg, $P < 0.01$). Ninety-three percent of patients survived to hospital discharge. A larger retrospective analysis of Impella use in cardiogenic shock included 37 of 47 total patients receiving an Impella 5.0 pump. In this study, 1-year survival was 71.8% for postcardiotomy cardiogenic shock and 42.9% for patients with shock complicating AMI or dilated cardiomyopathy (Lemaire et al., 2014).

The TandemHeart Left Atrial-to-Femoral Artery Centrifugal Pump

The TandemHeart device is an extracorporeal centrifugal flow pump that decreases left ventricular preload via bypassing oxygenated blood from the left atrium (LA) into the descending aorta via two cannulas: a 21Fr transseptal inflow cannula in the LA and an outflow cannula into the femoral artery (FA) (Fig. 1). The device can provide up to 3.0–5.0 L/min of flow via percutaneous application depending on the size of the outflow (arterial) cannula, which range between 15Fr and 19Fr (Kar et al., 2011). Prior reports have shown that the positioning of the outflow cannula affects the degree of LV unloading. Specifically, LA-to-ascending aortic bypass will increase LVSW, while LA-to-descending aortic bypass greatly reduces LVSW. By bypassing blood from the LA to the arterial system, the TH device increases pressure in the aorta (Burkhoff and Naidu, 2012). In the ascending aortic position, this increase in afterload limits the magnitude of LV unloading (Kono et al., 2003). In the descending aorta, the increase in afterload is diminished by retrograde perfusion of smaller, branching vessels including the mesenteric, renal, and great vessels of the aortic arch, thereby allowing for a reported 66% reduction in LVSW (Goldstein et al., 1994). Consistent with this study, we recently reported that activation of the TandemHeart pump at 5500 rpm with a 17Fr arterial cannula generates 3.1 LPM of flow and reduces LVSW by 38%, while maximal activation at 7500 rpm generates 4.4 LPM of flow and reduces LVSW by 67% in a bovine model of AMI (Kapur et al., 2015b). Unlike the Impella CP, the primary effect of the TandemHeart was a reduction in native LV stroke volume leading to reduced LVSW.

The safety and efficacy of the TandemHeart device compared with the IABP was clinically assessed in small multicenter randomized trials in which the TandemHeart significantly improved hemodynamic variables without any mortality benefit compared with IABP (Burkhoff et al., 2006; Thiele et al., 2005). The largest data series with TandemHeart was reported by Kar et al. who studied the effects of the TandemHeart in 117 patients with cardiogenic shock refractory to IABP and medical therapy (Kar et al., 2011). The TandemHeart increased mean arterial pressure, pulmonary capillary wedge pressure, mixed venous oxygen saturation, and end-organ perfusion. In this study of critically ill patients with advanced heart failure and shock, only 40.2% of patients died within 30 days after device implantation. An important meta-analysis of smaller studies evaluating these continuous flow devices for cardiogenic shock showed improved hemodynamic profiles with the use of the Impella and TandemHeart devices compared with IABP, yet there was no difference in short-term mortality (Cheng et al., 2009).

Venoarterial Extracorporeal Membrane Oxygenation

Venoarterial extracorporeal membrane oxygenation (VA-ECMO) is more commonly used to improve systemic oxygenation during cardiorespiratory collapse or biventricular failure. When deployed percutaneously, an extracorporeal centrifugal pump (i.e., TandemHeart, CentriMag, Rotaflow, or Biomedicus pumps) removes venous blood from the right atrium and circulates it through an oxygenator into an artery (most commonly the FA) (Fig. 1). As a result, VA-ECMO reduces right-sided ventricular volumes with a simultaneous increase in mean arterial pressure and both LV systolic and diastolic pressures. The potential risk of increased LV afterload may lead to progressive acute lung injury and thus lead to worse outcomes (Boulate et al., 2013). This increase in LV afterload or wall stress occurs in contrast to the Impella or TandemHeart devices since there is no direct unloading ("venting") of the LV with VA-ECMO. Several approaches to "vent" the LV include atrial septostomy, insertion of an Impella or IABP device, or the insertion of an additional venous cannula into the LA.

The majority of the clinical studies involving VA-ECMO in cardiogenic shock are for extracorporeal life support (ECLS) in cardiac arrest. These data suggest a possible benefit with VA-ECMO if "door-to-ECLS" times are shorter than 30 min, which has led to increasing interest in the application of VA-ECMO by specialized teams of first responders (Leick et al., 2013). A systematic review of the literature for the use of VA-ECMO identified 67 manuscripts published between 1966 and 2008 with a reported median survival of 41% (range, 13%–78%) for cardiogenic shock (Allen et al., 2011). The best median survival was associated with VA-ECMO for the indication of myocarditis (73%; range, 40%–83%).

Clinical Trial Data: Acute MCS in STEMI, Cardiogenic Shock, and Heart Failure

Strategies for enhancing cardioprotection in STEMI are limited (Schreuder et al., 2005b). Previous studies have shown that implantation of a cardiopulmonary bypass circuit reduces myocardial oxygen demand and has been associated with improved clinical outcomes and a reduction in infarct size in AMI complicated by cardiogenic shock (Kanter et al., 1982; Allen et al., 1993).

Based on these observations, studies involving the use of an IABP or catheter-mounted axial-flow pump prior to coronary occlusion, known as primary unloading, were found to be associated with reduced infarct size in preclinical models of AMI (Smalling et al., 1992; Achour et al., 2005; Meyns et al., 2003). We and others have previously reported that insertion of the TandemHeart left atrial-to-femoral artery bypass pump and thereby delaying coronary reperfusion by 30 min significantly decreased infarct size in a preclinical model of AMI (Kapur et al., 2013; Catinella et al., 1983). This preclinical study led to the TandemHeart to Reduce Infarct Size (TRIS) trial in the United States, which was later withdrawn prior to enrollment due to insufficient patient recruitment (Cardiac Assist, Inc., 2015).

Myocardial perfusion is influenced by multiple factors, including coronary perfusion pressure versus ventricular filling pressure and myocardial oxygen supply versus demand. The net effect of acute circulatory support may affect these factors in favor of optimal myocardial perfusion. We can also learn from our surgical colleagues, who often approach STEMI and shock management by first initiating cardiopulmonary bypass to unload both the right and left ventricles, followed by a period of time to harvest bypass conduits (during which time the culprit artery remains occluded), and ultimately reperfusion. Third, we know from a recent analysis of the USPELLA registry that implantation of an Impella device before PCI in STEMI and shock may improve survival (O'Neill et al., 2014). Based on these preclinical and clinical data, the Door-to-Unloading trial was initiated in the United States in early 2017 to study the concept of first unloading the LV before reperfusion in STEMI (Abiomed Inc., n.d.).

An unfortunate complication of AMI is the development of cardiogenic shock. In addition to the persistent problem of high mortality associated with AMI complicated by CS, the profile of patients with CS has changed over the past three decades to now include both AMI and a growing population of individuals with advanced heart failure.

While the IABP has been the cornerstone of management for AMI complicated by CS for several decades, the data indicate that IABP is not indicated for routine use in CS, but may be considered in select cases. The randomized, multicenter IABP-SHOCK-II trial is the largest study of IABP therapy in AMI complicated by CS to date. Compared with medical therapy alone, this study did not identify any improvement in 30-day mortality or after 12 months with IABP therapy (Thiele et al., 2012). Data from the SHOCK trial and registry indicate a nearly 60% rate of incomplete hemodynamic recovery after IABP insertion in CS complicating AMI with an increased associated mortality compared with responders (Hochman et al., 1999). The absence of a survival benefit and heterogeneous hemodynamic effects has dampened enthusiasm for the use of IABPs for CS.

A randomized clinical trial comparing the Impella 2.5 with IABP (ISAR-SHOCK) demonstrated a greater increase in cardiac index and MAP, a greater reduction in lactate, and a neutral effect on mortality with the Impella 2.5 (Seyfarth et al., 2008). The multicenter EUROSHOCK registry studied 120 patients receiving the Impella 2.5 for severe CS after failure of conventional therapy (Lauten et al., 2013). A reduction in lactate levels after device activation was observed after Impella 2.5 activation: however, cardiopulmonary resuscitation prior to Impella placement occurred in 41% of patients and 30-day mortality remained high. A more recent analysis of the USPella registry reported improved survival to hospital discharge with implantation of an Impella device immediately before compared with after coronary revascularization in AMI and cardiogenic shock. In this registry analysis, mean door-to-balloon time was delayed by 60 min in patients receiving the Impella device before versus after primary reperfusion (O'Neill et al., 2014). The recently published IMPella versus IABP reduces mortality in STEMI patients treated with primary PCI IN SEVERE and deep cardiogenic SHOCK (IMPRESS) trial (Ouweneel et al., 2017) and highlights the lack of benefit with the use of IABP or Impella device therapy for patients presenting with end-stage CS.

For patients with heart failure undergoing elective PCI, the PROTECT II study randomized patients to the Impella 2.5 LP or IABP before PCI (O'Neill et al., 2012). The trial was terminated early due to expected futility of the 30-day major adverse event primary endpoint. Subsequent analyses of the trial have shown potentially important benefits favoring Impella support associated with more complete revascularization and 90-day major adverse events.

Given the success of medical therapy in the advanced heart failure population with New York Heart Association class III and IV symptoms and the subsequent improvements in longevity for this sick cohort of patients, the role of device therapy in the road to recovery is more crucial than ever. Acute MCS options not only offer hemodynamic support but act as bridges to LVAD, transplant, recovery, and even as a bridge to decision, so patients presenting anywhere along the cardiogenic shock/heart failure spectrum may derive benefit. Future studies are needed to examine the effects of acute MCS device implantation on long-term patient survival and to potentially halt and reverse the progress of end-stage heart failure.

Conflict of Interest Disclosures

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Ion Channelopathy Genetics

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Introduction

The channelopathies are a group of rare inherited cardiac disorders characterized by a propensity for the development of life-threatening ventricular arrhythmias due to cardiac ionic channel dysfunction. They include long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), Brugada syndrome (BrS), short QT syndrome (SQTS), and early repolarization syndrome (ERS). Idiopathic ventricular fibrillation (VF), as its name implies, is characterized by unexplained cardiac arrest (CA) and therefore is not truly a channelopathy but most probably a heterogeneous group of disorders sharing a final common pathway (i.e., VF). Nevertheless, in a minority of these cases an underlying genetic abnormality effecting ionic currents has been implicated and therefore it is included in this article.

As patients with channelopathies, with rare exceptions, have a structurally normal heart and do not have extra-cardiac manifestations, symptoms are almost exclusively arrhythmia related. When the arrhythmia is nonsustained and extremely short or occurs during sleep it may go unnoticed, however, if it is more prolonged it may result in loss of consciousness. In the case of sustained arrhythmia, resuscitated CA or sudden cardiac death (SCD) is the inevitable consequence. In some cases syncope may serve as a warning sign facilitating diagnosis and implementation of sudden death preventive measures. In others, however, SCD is the first symptom. It is this risk of a devastating event striking without warning in an otherwise healthy and frequently young individual that makes diagnosis, risk stratification, and treatment of patients with channelopathies most challenging. Genetic testing, which is useful for diagnoses of presymptomatic individuals, is therefore of special importance in these inherited disorders.

In this article we briefly review the clinical aspects of the various channelopathies and focus on their genetic background and the way genetic testing may be utilized in the clinical setting

Terminology note: For simplicity the term "mutation" in this article is used to denote pathogenic or likely pathogenic genetic variants.

General Approach to Genetic Testing for Channelopathies

Genetic testing may be used for four main indications: support of diagnosis, estimation of prognosis, personalized therapy, and family screening. The latter is the most robust indication for genetic testing in channelopathies (Ackerman et al., 2011).

Family screening—Whenever a pathogenic or likely pathogenic variant is found in a patient diagnosed with one of these syndromes, it is recommended that all first-degree family members be genetically screened in order to facilitate presymptomatic diagnosis and appropriate management. A word of caution is appropriate, though, in this context—in recent years we have become increasingly aware of the limitations of current knowledge in regard to classification of rare variants uncovered by genetic testing. The realization that some of the variants previously classified as pathogenic or likely pathogenic are not rare in large populations (and therefore unlikely to be truly pathogenic) and that many benign variants are also rare has raised the bar for robustness of evidence required for such classification (Richards et al., 2015). Re-evaluation of previously classified variants should therefore be considered depending on the clinical likelihood and details of the genetic variants found.

Evaluation of prognosis and personalized therapy—the use of genetic testing for these indications in channelopathies is still limited mainly to the classification of LQTS types. As LQTS type 1 (LQT1), 2, and 3 differ in their natural history and response to therapy, this genetic classification has an impact on management decisions (see below).

Support of diagnosis—in most cases, the diagnosis of a channelopathy should be made based on phenotype alone and genetic testing is reserved for the other indications (mainly family screening). Nevertheless, in some situations the clinical data does not

allow for a definite diagnosis to be made and the finding of a pathogenic variant may support the diagnosis and lead to change of management.

In recent years there has been a growing interest in the use of molecular autopsy for determination of the cause of SCD. As clinical data in these cases is frequently scarce genetic testing may offer important information leading to diagnosis. Indeed, a recent study demonstrated that pathogenic and likely pathogenic variants in genes associated with channelopathies (*KCNQ1*, *KCNH2*, *SCN5A*, or *RYR2*) were found in 3% and 6%, respectively, of unexplained SCD cases (Bagnall et al., 2016). A detailed discussion of the role of molecular autopsy in the era of next generation sequencing may be found elsewhere (Lahrouchi et al., 2016).

Long QT Syndrome

LQTS is characterized by prolongation of the QT interval and an increased risk for the development of a specific type of ventricular arrhythmia—torsades de pointes (TdP—Fig. 1). The extremely rare and highly lethal autosomal-recessive form of LQTS was first

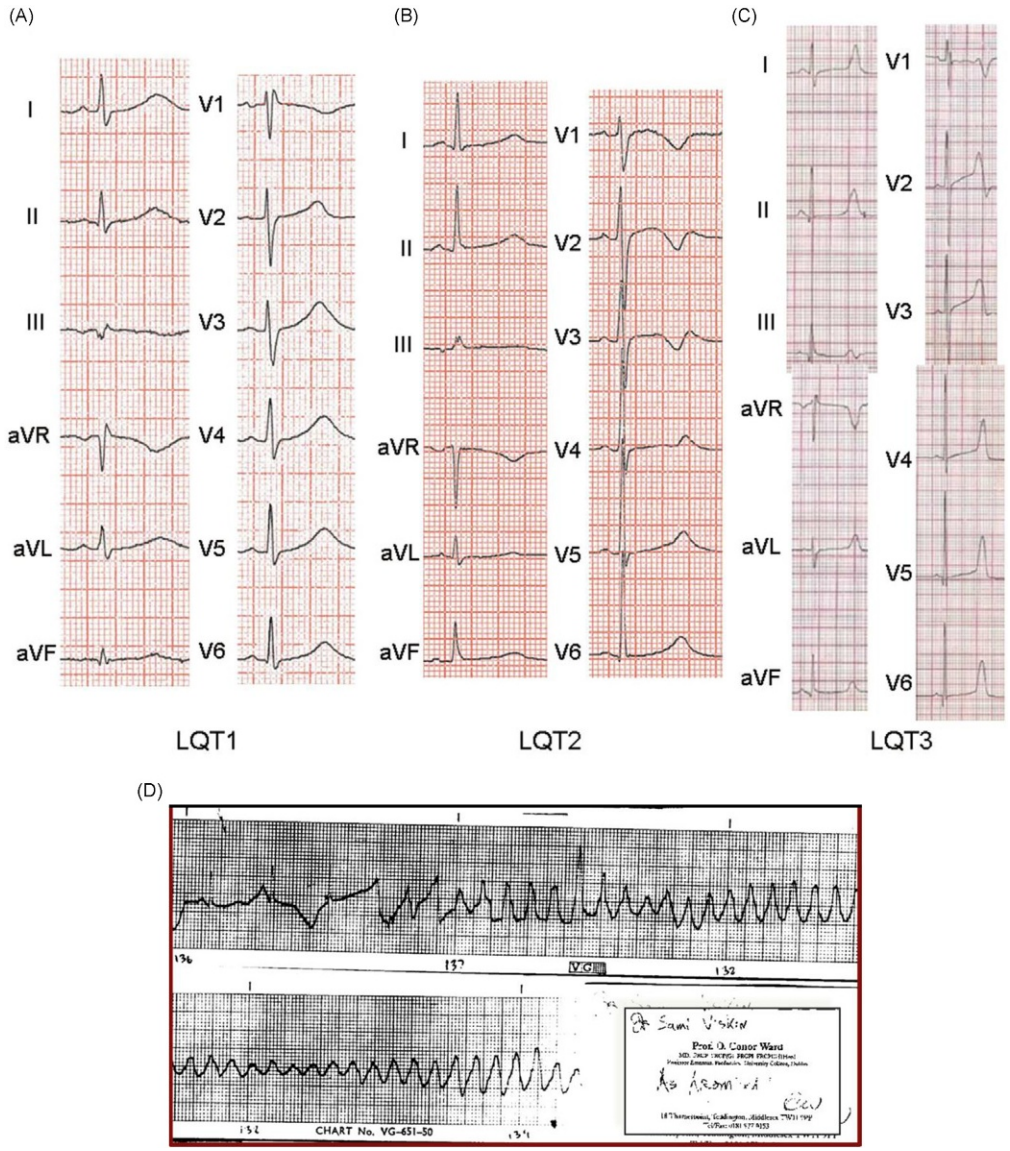

Fig. 1 Typical electrocardiographic patterns in long QT syndrome. (A) QT prolongation with typical broad-based T wave in LQT1. (B) QT prolongation with typical bifid T wave in LQT2. (C) QT prolongation with typical long ST-segment in LQT3. (D) Onset of torsades de pointes in the patient described by Connor Ward in 1964 (C) Adapted with permission from Mizusawa, Y., Horie, M. and Wilde, A. A. (2014). Genetic and clinical advances in congenital long QT syndrome. *Circulation Journal* **78**, 2827–2833. (D) Adapted with permission from Havakuk, O. and Viskin, S. (2016) A Tale of 2 diseases: The history of long-QT syndrome and Brugada syndrome. *Journal of the American College of Cardiology* **67**, 100–108.

described in 1957 by Jervell and Lange-Nielsen (1957). This type also includes sensorineural deafness which is absent from the much more common autosomal dominant form described only a few years later by Romano et al. (1963) and then by Ward (1964).

Typically, symptoms (syncope or CA) develop during episodes of adrenergic stimulation. In LQTS type 1 (LQT1) common scenarios include exercise (especially swimming). In LQT2 sudden startling, particularly due to auditory stimulus (e.g., alarm clock), is a typical trigger for arrhythmias with higher risk during the postpartum period in female patients. In contrast, patients with the less common LQT3 are atypical in the sense that they tend to develop arrhythmias during rest.

Management includes lifestyle changes for avoidance of arrhythmogenic triggers (e.g., exercise, QT-prolonging drugs, hypokalemia) and drug therapy (mainly beta blockers). Left cardiac sympathetic denervation (LCSD) and implantable cardioverter defibrillators (ICDs) are used in higher risk patients.

Genetics of Congenital LQTS

To date, 15 genes have been associated with LQTS, the majority of which encode for subunits of cardiac ionic channels (**Table 1**). Mutations in these genes lead to perturbation of ionic currents which causes prolongation of the repolarization phase of the action potential and its surface ECG manifestation, the QT interval (**Fig. 1**). Differences in the degree of this prolongation between layers of the myocardium (i.e., transmural dispersion of repolarization) create a substrate predisposing for the development of TdP (Antzelevitch and Shimizu, 2002). Mutations in the *KCNQ1*, *KCNH2*, and *SCN5A* genes, responsible for LQTS type 1, 2, and 3, respectively, are found in 75%–80% of patients. Mutations in other genes are found in a small minority of patients while ≈15% remain genotype-negative.

KCNQ1 and *KCNH2* encode for the α-subunit of the slowly and rapidly activating delayed rectifier channels which are responsible for voltage-gated outward potassium currents (I_{Ks} and I_{Kr}, respectively). These are the major repolarization forces in the cardiomyocyte's action potential and therefore it is not surprising that loss-of-function mutations in these genes lead to QT prolongation. *KCNE1* and *KCNE2* encode for the β-subunits of these channels and mutations in these genes result in the less common LQT5 and LQT6, respectively (**Table 1**). Patients with Jervell-Lange-Nielsen syndrome are homozygous for mutations in *KCNQ1* or *KCNE1*.

SCN5A encodes for the α-subunit of the sodium channel, which is responsible for the inward sodium current (I_{Na}). This current is the main depolarization force; however, it has a late component which persists into the repolarization phases of the action potential. This late current ($I_{Na\text{-}L}$) opposes the repolarizing outward potassium currents but is normally very small. In LQT3, however, gain-of-function mutations augment the $I_{Na\text{-}L}$, thereby prolonging repolarization. Interestingly, loss-of-function mutations in this gene are the main genetic substrates in BrS (see below). Even more intriguing is the fact that some specific variants have been demonstrated in both LQT3 and BrS, suggesting that these variants' effect is variable and depends on yet poorly understood factors (Bezzina et al., 1999).

Table 1 Genes associated with long QT syndrome

Gene		*Protein*	*Effect on ionic current*	*% of index cases*
LQT1	*KCNQ1*	Potassium voltage-gated channel subfamily KQT member 1	$I_{Ks}\downarrow$	30–35
LQT2	*KCNH2*	Potassium voltage-gated channel subfamily H member 2 (hERG)	$I_{Kr}\downarrow$	25–30
LQT3	*SCN5A*	Sodium channel protein type 5 subunit alpha	$I_{Na}\uparrow$	5–10
LQT4[a]	*ANK2*	Ankyrin-2	Multiple (disrupts binding and assembly of membrane proteins)	Rare
LQT5	*KCNE1*	Potassium voltage-gated channel subfamily E member 1 (Mink)	$I_{Ks}\downarrow$	Rare
LQT6	*KCNE2*	Potassium voltage-gated channel subfamily E member 2 (MiRP1)	$I_{Kr}\downarrow$	Rare
LQT7[b]	*KCNJ2*	Inward rectifier potassium channel 2	$I_{K1}\downarrow$	Rare
LQT8[c]	*CACNA1C*	Voltage-dependent L-type calcium channel subunit alpha-1C	$I_{Ca\text{-}L}\uparrow$	Rare
LQT9	*CAV3*	Caveolin-3	$I_{Na}\uparrow$	Rare
LQT10	*SCN4B*	Sodium channel subunit beta-4	$I_{Na\text{-}L}\uparrow$	Rare
LQT11	*AKAP9*	A-kinase anchor protein 9 (Yotiao)	$I_{Ks}\downarrow$	Rare
LQT12	*SNTA1*	Alpha-1-syntrophin	$I_{Na}\uparrow$	Rare
LQT13	*KCNJ5*	G protein-activated inward rectifier potassium channel 4	$I_{K\text{-}ACh}\downarrow$	Rare
LQT14	*CALM1*	Calmodulin	Impaired Ca^{2+} binding	Rare
LQT15	*CALM2*	Calmodulin	Impaired Ca^{2+} binding	Rare

[a]Ankyrin B syndrome.
[b]Andersen-Tawil syndrome.
[c]Timothy syndrome.

Notable among the rest of the genes implicated in LQTS (**Table 1**) is *KCNJ2*. Mutations in this gene have been demonstrated to underlie Andersen-Tawil syndrome (LQT7). This syndrome differs from other types of LQTS by extra-cardiac symptoms (periodic paralysis and dysmorphic features), ventricular arrhythmias other than TdP and QT-U rather than QT prolongation (Zhang et al., 2005). These variances have led some experts to exclude Andersen-Tawil syndrome from the LQTS group.

Genotype-tailored management

As mentioned above, arrhythmogenic triggers differ between patients with mutations in *KCNQ1*, *KCNH2*, and *SCN5A* (leading to LQT1, LQT2, and LQT3, respectively). Accordingly, genetic results are incorporated into the clinical decision algorithm regarding lifestyle changes recommended for these patients: LQT1 patients are likely to receive stricter exercise restrictions while women with LQT2 may be followed more closely in the postpartum period. Furthermore, the impact of gender and age seems to be genotype dependent. In LQT1, boys have a higher risk of CA or SCD when compared to girls but after onset of adolescence this difference disappears (Costa et al., 2012). In LQT2, however, women were found to have a higher risk of CA/SCD up to the age of 40 (Migdalovich et al., 2011) and a higher risk of syncope in the peri- and postmenopausal period (Buber et al., 2011). These genotype-specific age and gender-dependent factors may be included in risk stratification schemes that guide therapy in LQTS.

Genotype may also have an impact on effectiveness of drug therapy in LQTS. The protective effect of BB is thought to be most robust in patients with LQT1. One study analyzed data of over 300 patients with LQT1 and LQT2 treated with BB (Priori et al., 2004). It demonstrated that 10% of LQT1 patients had cardiac events (mainly unexplained syncope), including 1% with CA, during 5 years of follow-up. In LQT2 patients, BB offered less protection with 23% having cardiac events, including 7% with CA. Of note, LQT1 patients in this study had shorter QTc intervals, an alternative explanation for their lower risk; however, genotype remained a predictor of cardiac events in treated patients on multivariable analysis. Furthermore, these results are in line with a previous smaller study demonstrating 19% and 36% cardiac event rate over a similar period of follow-up in LQT1 and LQT2, respectively (Moss et al., 2000).

Among patients with LQT1, patients with mutations demonstrated to have a negative-dominant effect had longer QT intervals and were at higher risk of cardiac events when compared to those with haploinsufficient mutations (Moss et al., 2007). Mutations effecting amino acids incorporated into the cytoplasmatic loops (C-loops) may also be at increased risk of CA or SCD. One study, including 860 LQT1 patients, demonstrated a 33% risk of these events up to the age of 40 in patients with C-loop mutations compared with <16% in patients with other mutations (Barsheshet et al., 2012). On BB, however, there was an 88% reduction in events in patients with C-loop mutations but a nonsignificant reduction of 18% in LQT1 patients with other mutations. These findings are possibly limited to female patients (Costa et al., 2012). In LQT2, missense mutations in the pore region of the hERG channel were associated with a higher risk of cardiac events (Shimizu et al., 2009), an observation that may be limited to male patients only (Migdalovich et al., 2011).

Based on studies which included a limited amount of LQT3 patients (28 in each study) (Moss et al., 2000; Priori et al., 2004) the protection derived from BB therapy in LQT3 was thought to be limited. However, a recent study, including 391 LQT3 patients, demonstrated otherwise with an 83% reduction in cardiac events in women with LQT3 (Wilde et al., 2016). Although no statistically significant benefit was demonstrated in men, this may have resulted from a lower event rate in the male subpopulation in this study.

Because of the unique pathophysiology in LQT3 (mutations leading to increase in $I_{Na\text{-}L}$) sodium channel blockers have been investigated for therapy of patients with this LQTS subtype. Indeed small studies have demonstrated benefit using mexiletine (Mazzanti et al., 2016) and ranolazine [ChorinCircEP16-pending] (Chorin et al., 2016). Studies using specific $I_{Na\text{-}L}$ blockers are currently under way [NCT02300558].

Genetics of Acquired LQTS

Acquired LQTS results from prolongation of repolarization due to factors other than pathogenic genetic variants. Common causes of acquired LQTS include drugs blocking the *KCNH2*-encoded hERG channel, hypokalemia, and bradyarrhythmias. In most cases, however, a single factor is insufficient for the development of significant QT prolongation and TdP (Zeltser et al., 2003). For symptoms to occur a combination of several factors is usually necessary, one of which may be genetic predisposition. Indeed, single nucleotide polymorphisms (SNP) in *SCN5A* (Ser1103Tyr) (Splawski et al., 2002), *KCNE1* (Asp85Asn) (Nishio et al., 2009; Kaab et al., 2012), and *KCNE2* (Thr8Ala & Gln9Glu) (Sesti et al., 2000; Abbott et al., 1999) have been associated with increased risk of acquired LQTS and TdP. The *SCN5A* and *KCNE1* variants were found to be polymorphisms in African and European populations, respectively. Both were demonstrated to be overrepresented in arrhythmia cases. The *KCNE2* variants are polymorphisms in European (Thr8Ala) and African (Gln9Glu) populations and were shown to induce sensitivity to the QT-prolonging effect of sulfamethoxazole (Thr8Ala) and clarithromycin (Gln9Glu). Thus, these SNPs may induce an effect which is insufficient for the development of congenital LQTS; however, they probably increase the risk for acquired LQTS and TdP if patients are exposed to other QT-prolonging factors.

Genetic Modifiers

As in many genetic syndromes patients with LQTS demonstrate a wide range of phenotypic severity. This is true not only for patients with different mutations but also within families harboring a single pathogenic variant. Unfortunately, the mechanisms influencing

severity of phenotype are poorly understood. Research in the past decade and a half uncovered multiple genetic variants that have been associated with longer QTc intervals and poorer prognosis in patients with LQTS. Most of these variants have been found in one of the LQTS-associated genes (Amin et al., 2012; Crotti et al., 2005; Makielski et al., 2003) (**Table 1**) but, similar to the abovementioned polymorphisms specifically associated with acquired LQTS, they are regarded as modifiers rather than truly pathogenic. The validity of these associations and their impact on clinical management of patients awaits further studies, however. For instance, a recent publication (Crotti et al., 2016) could not corroborate previous findings implicating SNPs in the 3′ untranslated region of *KCNQ1* as disease modifiers (Amin et al., 2012).

Perhaps surprisingly, the strongest evidence supporting genetic variants as modifiers of disease was found for polymorphisms in the *NOS1AP* gene, which encodes for nitric oxide synthase 1 adaptor protein. Multiple studies have demonstrated association of these polymorphisms with QT interval (Arking et al., 2006; Newton-Cheh et al., 2007; Aarnoudse et al., 2007) and prognosis in patient with LQTS (Crotti et al., 2009; Tomas et al., 2010). Other modifiers in genes not otherwise associated with LQTS have been suggested in *ADRA2C* and *ADRB1* (Schwartz et al., 2008).

Catecholaminergic Polymorphic Ventricular Tachycardia

Patients with CPVT usually present with recurrent syncope, CA, or SCD triggered by physical or emotional stress. Typically, patients develop symptoms during childhood although milder phenotypes presenting only later in life have been described (van der Werf et al., 2012; Priori et al., 2002; Hayashi et al., 2009) and may become increasingly recognized with expansion of genetic family screening. Unlike in other channelopathies patients with CPVT cannot be diagnosed based on ECG findings. Although sinus bradycardia (Postma et al., 2005) and prominent U waves (Aizawa et al., 2006) have been described in this disorder these findings are not diagnostic. The most helpful diagnostic tool is an exercise test, which typically demonstrates incremental ventricular arrhythmias with increased exercise. The typical arrhythmia characteristic of CPVT is bidirectional VT (**Fig. 2**) although it appears only in a minority of patients.

Management of CPVT includes avoidance of exercise and, if possible, emotional stress. Medical therapy includes BB and, in case this proves insufficient or intolerable, flecainide (van der Werf et al., 2011). LCSD (De Ferrari et al., 2015) and ICD implantation are utilized in higher risk patients.



Fig. 2 Typical bidirectional ventricular tachycardia in CPVT. Note the 180 degrees alternating axis change during tachycardia. Copied with permission from Leenhardt, A., Denjoy, I. and Guicheney, P. (2012). Catecholaminergic polymorphic ventricular tachycardia. *Circulation. Arrhythmia and Electrophysiology* **5**, 1044–1052.

Table 2 Genes associated with catecholaminergic polymorphic ventricular tachycardia

Gene	*Protein*	*% of index cases*
RYR2	Ryanodine receptor 2	65
CASQ2	Calsequestrin-2	2–5
TRDN	Triadin	Rare
CALM1	Calmodulin	Rare
CALM2	Calmodulin	Rare
CALM3	Calmodulin	Rare
TECRL	Trans-2,3-enoyl-CoA reductase-like	Rare

RYR2 is the only gene associated with CPVT that encodes for a protein directly incorporated into an ionic channel (the cardiac ryanodine receptor). All other genes encode for proteins that influence the function of the ryanodine receptor. Data on the association of the *TECRL* gene with CPVT is very preliminary.

In 2001 mutations in *RYR2* were identified as the culprits of CPVT in seven families demonstrating cosegregation with the disease (Priori et al., 2001; Laitinen et al., 2001). This gene, mutations in which are found in 65% of CPVT patients (**Table 2**), encodes for the cardiac ryanodine receptor, an ionic channel responsible for calcium flow from the sarcoplasmic reticulum into the cytosol. Gain-of-function mutations in *RYR2* result in a calcium leak during diastole which raises the cytosolic calcium levels. This increased calcium concentration activates the calcium–sodium exchanger leading to an inward sodium current and delayed-after-depolarizations triggering VT (Jiang et al., 2004). As the calcium leak is enhanced by β-sympathetic stimulation, it is not surprising that CPVT patients usually present with stress-induced arrhythmias. The majority of mutations in *RYR2* are found in three regions within the gene (Medeiros-Domingo et al., 2009) located in the N-terminus (amino acids 77–466), central domain (amino acids 2246–2534), and channel region (amino acids 3778–4959). This has led in the past to genetic testing focusing on these domains with optional testing for the rest of the gene in negative cases. However, with introduction of next generation sequencing and declining cost of genetic testing this approach has been largely replaced by a single tier approach of testing the whole gene.

The second gene that was discovered in CPVT is *CASQ2*, mutations in which are found in 2%–5% of CPVT patients. This gene encodes for calsequestrin which is responsible for calcium binding and storage within the sarcoplasmic reticulum and interacts with the ryanodine receptor. Its causality in CPVT was first described in seven families found to harbor a missense mutation cosegregating with the disease (Lahat et al., 2001). Although families with *CASQ2* mutations usually demonstrate an autosomal-recessive pattern of inheritance, an autosomal-dominant form was recently described (Gray et al., 2016).

Other genes associated with rare cases of CPVT are listed in **Table 2**. Patients with Andersen-Tawil syndrome (LQT7) and Ankyrin-B disease (LQT4) caused by mutations in *KCNJ2* and *ANK2*, respectively, may present with an atypical phenotype resembling CPVT (Kimura et al., 2012; Tester et al., 2006). Therefore, these genes should be considered in the evaluation of otherwise gene-negative CPVT cases (and are indeed included in many CPVT commercial genetic testing panels).

Like in other channelopathies, genotype–phenotype correlations in CVPT have been described. Patients with *CASQ2* (CPVT2) mutations are generally thought to express more severe disease than those with *RYR2* mutations (CPVT1). Within the CPVT1 group, patients with *RYR2* mutations located in the C-terminus channel-forming domain were demonstrated to have more nonsustained VT than those harboring mutations in the N-terminus (van der Werf et al., 2012). Nevertheless, these correlations have little impact on clinical management of patients, which is currently mainly phenotype-driven.

Brugada Syndrome

BrS is characterized by typical coved ST-segment elevation in the right precordial leads (type 1 Brugada pattern—**Fig. 3**). This pattern may be either spontaneous or induced by fever or drugs. Indeed, sodium channel blockers are used for diagnostic purposes in order to uncover a type 1 Brugada pattern in patients suspected of BrS who do spontaneously demonstrate this pattern. BrS is more common in males who typically develop symptoms during rest or sleep in their third to fifth decade of life.

Despite more than two decades of research the pathophysiology of BrS is still a matter of debate (Wilde et al., 2010). Some argue that BrS is primarily the result of transmural dispersion of repolarization in the right ventricle due to abnormal ionic repolarizing currents. Others argue that BrS is mainly a depolarization disorder with conduction delay and, possibly, subtle structural abnormalities of the right ventricular outflow tract being responsible for arrhythmogenesis.

Management of BrS is mainly dependent on ICD implantation for secondary prevention. ICDs may also be implanted for primary prevention in patients deemed to be at high risk, although methods for risk stratification remain a topic of heated debate (Adler et al., 2016). As fever and certain drugs may be arrhythmogenic, using antipyretics during febrile illness and avoiding drugs that may be deleterious is also important. Other management options include the use of pharmacological agents [e.g., quinidine (Belhassen et al., 2004; Hermida et al., 2004), isoproterenol (Maury et al., 2004)], and epicardial ablation of the right ventricular outflow tract (Nademanee et al., 2011).

The gene in which mutations are most commonly found and which has the most robust evidence associating it with the disease is *SCN5A*. Mutations in this gene, which encodes for the α-subunit of the cardiac sodium channel, cause loss of function with

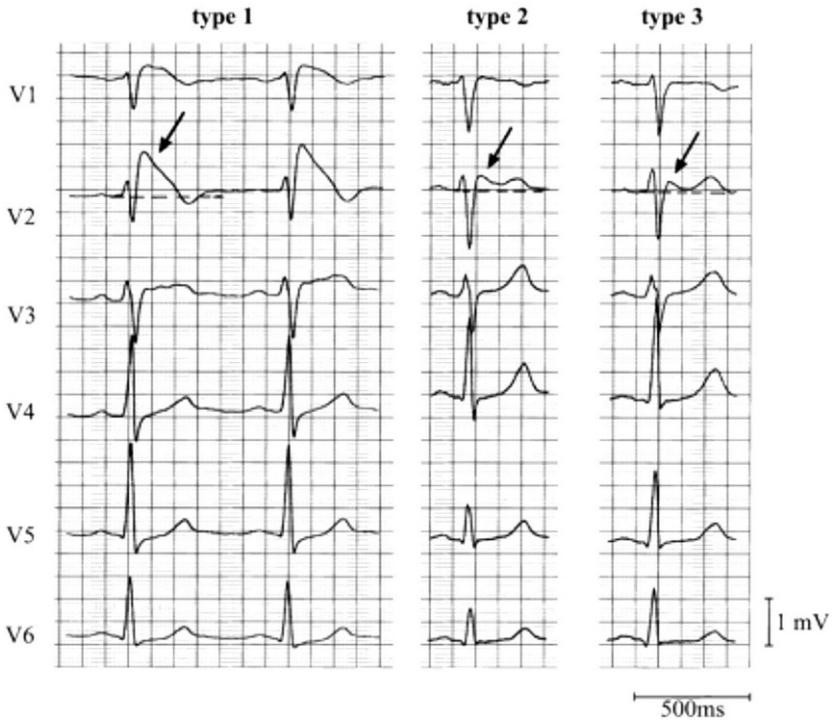

Fig. 3 Brugada ECG patterns. Type I pattern is defined as a coved ST-segment elevation in $\geq$2 mm in lead V1 or V2. Type II is defined as a saddle-back ST-segment elevation of $\geq$1 mm. Type III is defined as an ST-segment elevation $<$1 mm. Type II and III are suggestive of the diagnosis but only documentation of a type I pattern is regarded as diagnostic. Adapted from with permission from Brugada, P., Brugada, R. and Brugada, J. (2005). Should patients with an asymptomatic Brugada electrocardiogram undergo pharmacological and electrophysiological testing? *Circulation* **112**, 279–292; discussion 279–292.

reduced inward current and are found in up to 28% of patients. As mentioned above, gain-of-function mutations in this same gene are responsible for LQT3.

A specific mutation (Ala280Val) in *GPD1L* has been described in a large family of Italian descent and demonstrated cosegregation with phenotype (London et al., 2007). In vitro analysis demonstrated it reduces the inward sodium current (I_{Na}), possibly by impeding trafficking of the channel protein to the membrane. There is little information regarding the pathogenicity of other genetic variants in this gene.

Mutations in *CACNA1C* and *CACNB2b*, encoding for the α_1- and β_{2b}-subunits of the cardiac L-type calcium channel, have been associated with a phenotype consisting of Brugada pattern, a relatively short QT interval and SCD (Antzelevitch et al., 2007). Although supported by in vitro studies, cosegregation of genotype with phenotype was limited due to the small size of families studied.

Rare variants in *SCN10A* have been identified in 17% of patients in one study (Hu et al., 2014). However, subsequent studies comparing burden of rare variants in cases vs. controls casted doubt on whether variants in this gene contribute to disease in a significant percentage of BrS patients (Le Scouarnec et al., 2015; Behr et al., 2015).

The many other genes associated with BrS are listed in **Table 3**. Due to the limited available data supporting the pathogenicity of rare variants in these genes, interpretation of genetic testing utilizing large gene panels for BrS should be performed with extra care.

The use of genetic testing for risk stratification in BrS is currently limited. The presence of a mutation in *SCN5A* has not been associated with a different prognosis when compared with genotype-negative patients in large BrS cohorts (Probst et al., 2010; Conte et al., 2015). Truncating mutations in *SCN5A* and those found to cause a more significant reduction in the peak sodium current (I_{Na}) were associated with a higher risk of unexplained syncope and more pronounced conduction disease in one study (Meregalli et al., 2009). Recently, a SNP in *HEY2* has been associated with a protective effect against VF in BrS patients (Nakano et al., 2016); however, further studies are required for corroboration of this finding. Thus, our knowledge is currently insufficient for integration of genetic data into risk stratification algorithms in BrS.

Short QT Syndrome

SQTS is an extremely rare disorder described for the first time in 2000 (Gussak et al., 2000; Giustetto et al., 2011). It is characterized by a short QT interval (Fig. 4) and an increased risk for the development of atrial and ventricular arrhythmias. Patients are most

Table 3 Genes associated with Brugada syndrome

Gene	*Protein*	*Effect on ionic current*	*% of index cases*
SCN5A	Sodium channel protein type 5 subunit alpha	$I_{Na}\downarrow$	11–28
GPD1L	Glycerol-3-phosphate dehydrogenase 1-like protein	$I_{Na}\downarrow$	Rare
CACNA1C	Voltage-dependent L-type calcium channel subunit alpha-1C	$I_{Ca}\downarrow$	6.6
CACNB2b	Voltage-dependent L-type calcium channel subunit beta-2	$I_{Ca}\downarrow$	4.8
SCN1B	Sodium channel subunit beta-1	$I_{Na}\downarrow$	1.1
KCNE3	Potassium voltage-gated channel subfamily E member 3 (MiRP2)	$I_{to}\uparrow$	Rare
SCN3B	Sodium channel subunit beta-3	$I_{Na}\downarrow$	Rare
KCNJ8	ATP-sensitive inward rectifier potassium channel 8	$I_{K\text{-}ATP}\uparrow$	2
CACNA2D1	Voltage-dependent calcium channel subunit alpha-2/delta-1	$I_{Ca}\downarrow$	1.8
KCND3	Potassium voltage-gated channel subfamily D member 3	$I_{to}\uparrow$	Rare
RANGRF	Ran guanine nucleotide release factor	$I_{Na}\downarrow$	Rare
SLMAP	Sarcolemmal membrane-associated protein	$I_{Na}\downarrow$	Rare
ABCC9	ATP-binding cassette subfamily C member 9	$I_{K\text{-}ATP}\uparrow$	Rare
SCN2B	Sodium channel subunit beta-2	$I_{Na}\downarrow$	Rare
PKP2	Plakophilin-2	$I_{Na}\downarrow$	Rare
FGF12	Fibroblast growth factor 12	$I_{Na}\downarrow$	Rare
SCN10A	Sodium channel protein type 10 subunit alpha	$I_{Na}\downarrow$	5–16.7
HEY2	Hairy/enhancer-of-split related with YRPW motif protein 2	$I_{Na}\uparrow$	Rare
SEMA3A	Semaphorin-3A	$I_{to}\uparrow$	Rare

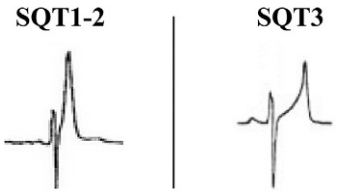

Fig. 4 Typical ECG patterns in short QT syndrome. Adapted with permission from Brugada, R., Hong, K., Dumaine, R., Cordeiro, J., Gaita, F., Borggrefe, M., Menendez, T. M., Brugada, J., Pollevick, G. D., Wolpert, C., Burashnikov, E., Matsuo, K., Wu, Y. S., Guerchicoff, A., Bianchi, F., Giustetto, C., Schimpf, R., Brugada, P. and Antzelevitch, C. (2004). Sudden death associated with short-QT syndrome linked to mutations in HERG. *Circulation* **109**, 30–35 and Priori, S. G., Pandit, S. V., Rivolta, I., Berenfeld, O., Ronchetti, E., Dhamoon, A., Napolitano, C., Anumonwo, J., DI Barletta, M. R., Gudapakkam, S., Bosi, G., Stramba-Badiale, M. and Jalife, J. (2005). A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene. *Circulation Research* **96**, 800–807.

Table 4 Genes associated with short QT syndrome

Gene	*Protein*	*Effect on ionic current*
KCNH2	Potassium voltage-gated channel subfamily H member 2 (hERG)	$I_{Kr}\uparrow$
KCNQ1	Potassium voltage-gated channel subfamily KQT member 1	$I_{Ks}\uparrow$
KCNJ2	Inward rectifier potassium channel 2	$I_{K1}\uparrow$
CACNA1A[a]	Voltage-dependent L-type calcium channel subunit alpha-1C	$I_{Ca}\downarrow$
CACNB2b[a]	Voltage-dependent L-type calcium channel subunit beta-2	$I_{Ca}\downarrow$

Due to the limited number of patients data regarding yield of genetic testing is scarce. See text for further discussion.

[a]This gene has been associated with a phenotype consisting of a Brugada pattern and a relatively short QT interval. A putative mutation has also been described in index cases from short QT cohorts.

commonly males and typically develop symptoms during sleep or rest in the second to fourth decade of life (Giustetto et al., 2011; Mazzanti et al., 2014). The diagnosis of SQTS can be a challenge and requires a high level of suspicion. A diagnostic score has been proposed as an aid for this matter (Gollob et al., 2011).

ICD implantation for secondary prevention is the mainstay of therapy. An ICD may also be considered for asymptomatic patients with family history of SCD (Priori et al., 2013). Pharmacological therapy with quinidine is an option in cases with recurrent ICD interventions.

The first mutations to be associated with SQTS were gain-of-function mutations in *KCNH2* (Brugada et al., 2004) and *KCNQ1* (Bellocq et al., 2004), the same genes in which loss-of-function mutations cause LQT2 and LQT1, respectively (**Table 4**). As a mirror image of LQTS, these mutations are thought to lead to abbreviated repolarization. Once more, it is differences between action potential duration in different layers of the myocardium that are thought to create the vulnerable substrate predisposing to arrhythmogenesis (Patel et al., 2010).

Other genes subsequently associated with SQTS are *KCNJ2*, encoding for the α-subunit of the inward rectifying potassium channel (I_{K1}) (Priori et al., 2005), *CACNA1C*, and *CACNB2b*. As mentioned in the previous section, the last two genes have been associated with an overlap syndrome which includes phenotype of both BrS and SQTS.

Data regarding prevalence of genetic findings in SQTS patients and phenotype–genotype correlations is limited by the small number of patients (the largest series include 53 (Giustetto et al., 2011) and 73 (Mazzanti et al., 2014) patients with only 17 and 45 genotyped index cases). In the larger of these series genetic testing was positive in 13% of patients with none of the genes implicated in more than 5% of patients. In three out of five gene-positive cases familial disease was found (the other two were de novo mutations) and demonstrated an autosomal dominant inheritance. According to the limited data available and in stark contrast to the findings in other channelopathies, families with a positive mutation demonstrated complete penetrance. Mutation carriers were found to have shorter QTc intervals when compared with gene-negative cases but this did not translate into more severe outcomes (Mazzanti et al., 2014).

Early Repolarization Syndrome

ERS is the most recent channelopathy described, first defined in a consensus paper only in 2013 (Priori et al., 2013). It is characterized by the finding of J-point elevation in two consecutive leads other than right precordial leads in a patient resuscitated from CA or the previous documentation of this finding in an SCD victim. Importantly, exclusion of all other reasons for J-point elevation or CA is imperative for diagnosis. Although this prerequisite is true for all channelopathies it is of special importance in ERS due to the relatively high prevalence of J-point elevation (3%–13%) in the general population (Adler and Gollob, 2015). For the same reason diagnosis of asymptomatic patients with ERS is especially difficult but may be suspected in family members of ERS patients or specific and rare clinical scenarios. Similar to the presentation in BrS and SQTS, patients are typically male presenting with symptoms during rest or sleep during their third to fifth decade of life (Haissaguerre et al., 2008; Rosso et al., 2008).

Because most ERS patients are diagnosed after developing symptoms, ICD implantation for secondary prevention is recommended in almost all patients. In the minority of patients with suspected ERS despite being asymptomatic, ICD implantation may be discussed on a case-by-case basis (Antzelevitch et al., 2016). Pharmacological therapy in ERS is similar to that in BrS, which is not surprising taking into account the similarities in patients' characteristics and suspected pathophysiology (Antzelevitch and Yan, 2015).

The molecular basis for ERS is thought to rely on augmentation of the physiological transmural gradient during early repolarization phases of the action potential. The main current responsible for this gradient is I_{to} (transient outward potassium current), which is more prominent in the epicardium than endocardium (Antzelevitch, 2013). It was therefore hypothesized that mutations causing augmentation of outward currents (e.g., I_{to}, I_{KATP}) or diminution of opposing inward currents (e.g., I_{Ca}, I_{Na}) may underlie the pathophysiology of ERS. Studies utilizing a candidate gene approach have indeed uncovered putative mutations in genes encoding for subunits of potassium, calcium, and sodium channels or proteins regulating their activity (**Table 5**). Importantly, however, robust data supporting the pathogenicity of these genetic variants and demonstrating cosegregation of genotype with phenotype is lacking. Furthermore, in a study describing four large families with ERS and multiple SCD cases, analysis of most of the genes associated thus far with ERS (*KCNJ8*, *SCN5A*, *CACNA1C*, *CACNB2*, and *CACNA2D1*) failed to uncover mutations in the probands of each family.

Idiopathic VF

Idiopathic VF is diagnosed in cases resuscitated from CA after exhaustive diagnostic tests have been unable to uncover the cause. As such it is not truly a syndrome but most probably a heterogeneous group consisting of multiple conditions. Nevertheless, many patients with idiopathic VF share common characteristics suggesting a possible common etiology. These include male gender, first presentation during sleep or rest in the third to fifth decade of life, and VF triggered by close-coupled premature ventricular complexes (PVC) (Viskin et al., 1997).

Table 5 Genes associated with early repolarization syndrome

Gene	*Protein*	*Effect on ionic current*	*% of index cases*
KCNJ8	ATP-sensitive inward rectifier potassium channel 8	$I_{K\text{-}ATP}\uparrow$	Rare
CACNA1C	Voltage-dependent L-type calcium channel subunit alpha-1C	$I_{Ca}\downarrow$	4
CACNB2b	Voltage-dependent L-type calcium channel subunit beta-2	$I_{Ca}\downarrow$	8
CACNA2D1	Voltage-dependent calcium channel subunit alpha-2/delta-1	$I_{Ca}\downarrow$	4
ABCC9	ATP-binding cassette subfamily C member 9	$I_{K\text{-}ATP}\uparrow$	Rare
SCN5A	Sodium channel protein type 5 subunit alpha	$I_{Na}\downarrow$	Rare
SCN10A	Sodium channel protein type 10 subunit alpha	$I_{Na}\downarrow$	Rare
KCND2[a]	Potassium voltage-gated channel subfamily D member 2	$I_{to}\uparrow$	Rare

[a]A putative mutation in this gene was found in a patient resuscitated from a cardiac arrest who was found to have large J waves in right precordial leads but not a typical Brugada pattern (i.e., does not answer the diagnostic criteria for Brugada syndrome or early repolarization syndrome).

Table 6 Genes associated with idiopathic ventricular fibrillation

Gene	*Protein*	*% of index cases*
DPP6[a]	Dipeptidyl aminopeptidase-like protein 6	Rare
CALM1	Calmodulin	Rare
ACTN2	Alpha-actinin-2	Rare
RYR2	Ryanodine receptor 2	Rare
IRX3	Iroquois-class homeodomain protein IRX-3	Rare

[a]Dutch founder mutation.

As all patients with idiopathic VF are symptomatic, ICD for secondary prevention is recommended in all. Therapy with quinidine may be considered in patients with frequent ICD shocks or in those who have a contraindication for an ICD (Priori et al., 2013). Ablation of PVCs may be considered in cases with frequent arrhythmias.

As most cases of idiopathic VF are sporadic genetic studies of this population are difficult. One large kindred from Holland, however, does demonstrate multiple cases of SCD and CA. In this kindred a risk haplotype including a variant upstream of the coding region of *DPP6* (c.1-340 C>T) has been identified (Alders et al., 2009). It is thought that this variant results in over-expression of the dipeptidyl peptidase-like protein-6 (DPP6) and subsequent increase in I_{to} which is modulated by it (Xiao et al., 2013).

Others genes that have been implicated with idiopathic VF are listed in **Table 6**. In all of these cases the genes have been found in single cases or small families. Furthermore, in most cases the phenotype has not been typical of idiopathic VF as described above and may be more concordant with an overlap syndrome or an atypical presentation of another syndrome. For instance, a rare variant in *ACTN2* was found in two families with short-coupled PVCs triggering VF; however, in some mutation carriers a variable phenotype including hypertrophic cardiomyopathy and left ventricular noncompaction was demonstrated (Bagnall et al., 2014). Thus, an arrhythmogenic cardiomyopathy rather than idiopathic VF may be the diagnosis in these families. In other studies mutations in *RYR2* were found in patients with CA but without exercise-induced arrhythmias (Cheung et al., 2014). Whether these patients should be best approached as atypical CPVT patients, idiopathic VF patients, or as a unique subset is not clear. Accordingly, the role of clinical genetic testing for rare variants in these genes in the setting of idiopathic VF remains to be elucidated.

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Ischemic Cardiomyopathy

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Introduction

Cardiovascular disease is the leading cause of death in the United States accounting for 801,000 deaths annually, 2200 deaths daily, and a death every 40 s. It causes more deaths than all forms of cancer and chronic lower respiratory tract disease combined. Coronary artery disease (CAD) is the leading cause of and attributed for 45% of all cardiovascular deaths. Further, CAD accounts for 790,000 myocardial infarctions annually, approximately one every 40 s (**Table 1**). Typically, patients with ischemic cardiomyopathy have CAD with or without a past history of myocardial infarction. Ischemic cardiomyopathy is the leading cause for sudden cardiac death that affects 350,000 Americans annually. In this article, we will characterize the transition from CAD to ischemic cardiomyopathy and address its prevention, diagnosis, and management.

Ischemic cardiomyopathy is the most common etiology of heart failure (HF), a disease which affects 6 million patients today and is projected to affect 8 million patients by 2030 (Mozaffarian et al., 2016). In 13 multicenter HF treatment trials involving >20,000 patients, it was the underlying etiology of HF in nearly 70% of patients (Ghoerghiade and Bonow, 1998). Further, the presence of CAD is not only common in patients with HF with reduced ejection fraction (HFrEF), but also in patients with HF with preserved ejection fraction (HFpEF) (Lee et al., 2009; Owan et al., 2006). This is particularly relevant because ischemic cardiomyopathy represents a potentially treatable cause of HF. The importance of assessing CAD in HF is underscored by the observation that prognosis of patients with HF from ischemic cardiomyopathy is considerably worse than that of patients without ischemic cardiomyopathy, and is related to the angiographic severity of the CAD (Bart et al., 1997).

Progression of CAD to Ischemic Cardiomyopathy

There are multiple pathways to ischemic cardiomyopathy that include acute myocardial infarctions, silent ischemia, and recurrent small infarctions (**Fig. 1**). Changes in the myocardium in acute ischemia are dynamic and complex. The acute loss of myocardium results in an abrupt increase in loading conditions in the infarct border zone and the noninfarcted myocardium that triggers neurohormonal activation and a cascade of intracellular signaling processes which induce ventricular remodeling. Ischemia produces a rapid and massive surge in endogenous catecholamines with a deleterious effect on myocytes resulting in myocardial apoptosis, fibrosis, and arrhythmias. This neurohormonal activation and ventricular remodeling can lead to progressive deterioration of further viable myocardium. Ventricular dilation and hypertrophy may continue for weeks or months until the distending forces are counterbalanced by the strength of the collagen scar. The size, location, transmurality of the infarction, patency of the infarct-related artery, and degree of neurohormonal response determine this balance (Rouleau et al., 1993; Sutton and Sharpe, 2000; Warren et al., 1988). Accordingly, in the setting of acute ischemia, therapies are aimed at prompt revascularization to restore infarct-related artery patency and salvage of viable myocardium, as well as ameliorating the neurohormonal activation with the use of ACE inhibitors or angiotensin receptor blockers (ARB), beta-blockers, statins, and aldosterone antagonists. Patients who survive an acute myocardial infarction are at high risk of re-infarction and further loss of myocardial function. This further underscores the importance of risk factor modification to prevent recurrent ischemic events.

While revascularization significantly reduces mortality following acute myocardial infarction, left ventricular remodeling often occurs despite sustained patency of the infarct-related artery and preservation of regional and global left ventricular function (Bolognese et al., 2002) (**Fig. 2**). Sometimes the ischemic event can be asymptomatic or silent and contribute to the progression of left ventricular systolic and/or diastolic dysfunction leading to the development of clinical HF or arrhythmias. Myocardial

Table 1 Fact sheet on ischemic cardiomyopathy

- Cardiovascular disease affects 90 million Americans, accounting for 801,000 deaths annually
- Coronary artery disease is the leading cause of cardiovascular death
- Coronary artery disease leads to ischemic cardiomyopathy with or without myocardial infarction
- Ischemic cardiomyopathy is the leading cause of heart failure
- Ischemic cardiomyopathy is the leading cause of sudden cardiac death

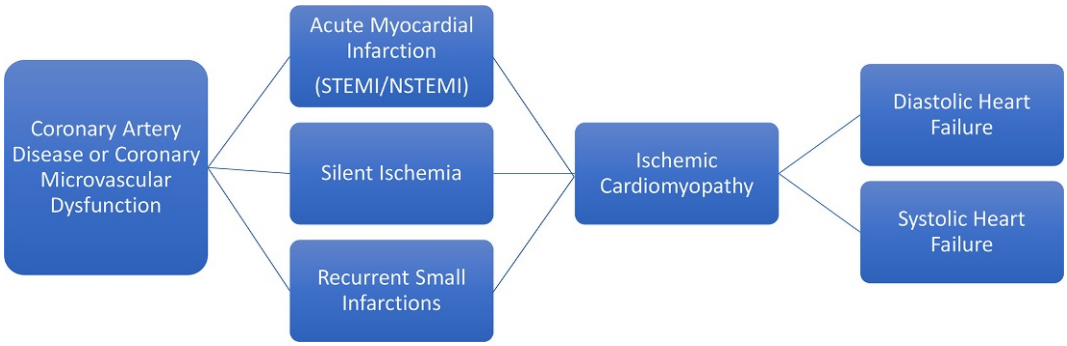

Fig. 1 Progression from coronary artery disease to ischemic cardiomyopathy and heart failure.

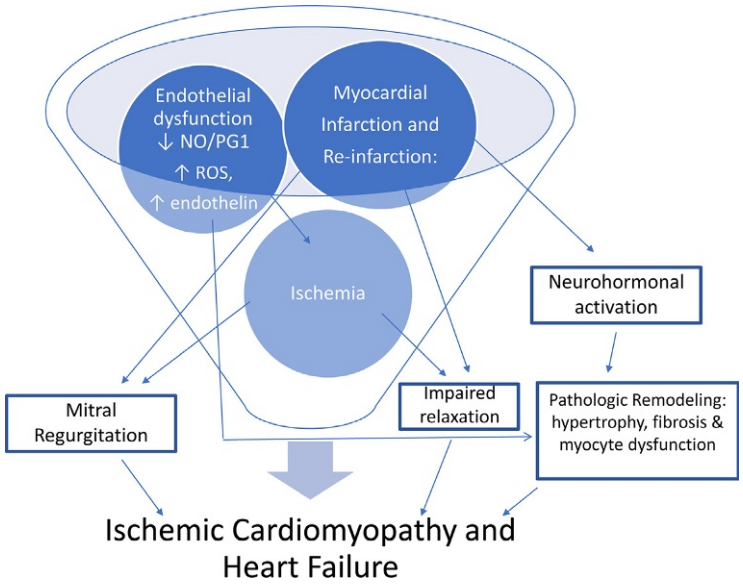

Fig. 2 Patho-biology of Ischemic cardiomyopathy and heart failure. (NO = nitric oxide; PG = prostaglandin; ROS = reactive oxygen species).

hibernation from chronic ischemia may also contribute to left ventricular dysfunction. Coronary microvascular dysfunction, in the absence of obstructive coronary disease, is frequently characterized by heightened sensitivity to vaso-constrictor stimuli and decreased vasodilator reserve, and can cause myocardial ischemia.

The presence and extent of CAD often determines the pace of development and progression of ischemic cardiomyopathy and HF. In a prospective study that longitudinally followed patients for 10 years, the cohort hospitalized for the first time due to HF had documented coronary disease in 25% of patients who developed HFpEF and in 39% of patients with HFrEF (Rusinaru et al., 2014). The vast majority of patients died of cardiovascular causes, and the proportion was comparable between HFpEF and HFrEF. In the presence of coronary disease, sudden cardiac death was the leading cause of death in HFpEF, while refractory HF was the most common cause among HFrEF patients. Coronary disease though was independently predictive of sudden death in both HFpEF and HFrEF and also showed an independent association with HF-related death and with acute myocardial infarction death (Rusinaru et al., 2014).

Mitral regurgitation is a common complication after myocardial infarction, particularly inferior wall infarction, due to changes in ventricular structure and function and often accompanies ischemic cardiomyopathy. Even mild mitral regurgitation after myocardial infarction carries an adverse prognosis and is an independent predictor of HF and death (Lamas et al., 1997; Salukhe et al., 2005). Further, in hemodynamically significant ischemic mitral regurgitation, the left ventricle dilates to preserve forward cardiac output, which in turn worsens mitral regurgitation due to tethering of the papillary muscles and sparks a viscous cycle that often leads to worsening HF.

Prevention of Ischemic Cardiomyopathy

Since patients with and at risk for coronary disease are likely to develop ischemic cardiomyopathy HF, clinicians should seek to control vascular and metabolic risk factors in such patients (Smith et al., 2011). Treatment of hypertension and dyslipidemia can significantly reduce the development and progression of coronary disease and should be controlled in accordance with contemporary guidelines. Long-term treatment of hypertension reduces the risk of HF development by approximately 50% (Kostis et al., 1997). Obesity, diabetes, tobacco use, unhealthy diet, physical inactivity, stress, known cardiotoxic agents, and systemic inflammation that may lead to or contribute to both HF and the progression of underlying coronary disease should be controlled or avoided or addressed.

Diagnostic Strategy in Ischemic Cardiomyopathy

Etiology of HF

Any patient who presents with HF should be evaluated for the presence of CAD. The 2013 ACC/AHA Heart Failure Guideline (**Tables 2–6**) supports seeking the etiology of HF by evaluating for ischemic disease either noninvasively or invasively (Yancy et al., 2013b). Surprisingly, a recent study analyzing the patterns of testing for CAD in patients with new-onset HF showed that only 17% of patients underwent testing for CAD during the index hospitalization and this increased to only 27% at 90 days (Doshi et al., 2016). This underutilization of coronary artery assessment in new-onset HF patients has significant treatment implications.

Given its widespread availability and lack of ionizing radiation, echocardiography is often preferred as the initial imaging diagnostic test in patients with or at risk for HF. Echocardiography should address whether the left ventricular ejection fraction is reduced or whether there are structural or focal wall motion abnormalities that could account for the patient's clinical presentation. Echocardiographic evaluation can also give the clinician insight into the degree and duration of myocardial damage if it is present.

Table 2 ACC/AHA guidelines for imaging in HF

Recommendations	*COR*	*LOE*
A 2-D echocardiogram with Doppler should be performed for initial evaluation of HF	I	C
Repeat measurement of EF is useful in patients with HF who have had a significant change in clinical status or received treatment that might affect cardiac function or for consideration of device therapy	I	C
Noninvasive imaging to detect myocardial ischemia and viability is reasonable in HF with CAD	IIa	C
Viability assessment is reasonable before revascularization in HF with CAD	IIa	B
MRI is reasonable when assessing myocardial scar	IIa	B
When ischemia may be contributing to HF, coronary angiography is reasonable	IIa	C

Table 3 ACC/AHA HFrEF medical therapy guidelines

Recommendations	*COR*	*LOE*
Diuretics for fluid retention	I	C
ACEI	I	A
ARB, if ACEI intolerant	I	A
ARB as alternative to ACEI	IIa	A
ARNI as an alternative to ACEI or ARB for NYHA II-III	I	B
Replacement of ACEI or ARB with ARI for symptomatic patients with NYHA II–III symptoms	I	B
Approved beta-blockers	I	A
Aldosterone antagonist for NYHA class II–IV	I	A
Combination of hydralazine-isosorbide dinitrate for patients who cannot receive ACEI or ARB	IIa	B
Combination of hydralazine-isosorbide dinitrate for African American patients with NYHA II–IV symptoms on GDMT	I	A
Digoxin	IIa	B
Ivabradine to decrease HF hospitalization in NYHA class II–III patients on GDMT (including beta-blocker) with NSR and resting HR $\geq$70 bpm	IIa	B

Table 4 ACC/AHA HFpEF therapy guidelines

Recommendations	*COR*	*LOE*
Blood pressure control according to published guidelines	I	B
Diuretics for volume retention	I	C
Coronary revascularization in patients with angina or demonstrable ischemia despite GDMT	IIa	C
Beta-blockers, ACEI, and ARBs for treatment of hypertension	IIa	C
ARBs to decrease hospitalizations	IIb	B

Table 5 ACC/AHA stable ischemic heart disease guidelines: indications for revascularization

Recommendations	*COR*	*LOE*
Unprotected left main disease		
CABG	I	B
PCI	IIa	B
Three-vessel CAD with proximal LAD disease		
CABG	I	B
PCI	IIb	B
Two-vessel CAD with proximal LAD disease		
CABG	I	B
PCI	IIb	B

Table 6 ACC/AHA HFrEF device therapy guidelines

Recommendations	*COR*	*LOE*
ICD therapy for primary prevention of SCD for LVEF $\leq$ 35%, NYHA II–III symptoms, at least 40 days post-MI, at least 3 months of GDMT	I	A
ICD therapy for primary prevention of SCD for LVEF $\leq$30%, NYHA I, at least 40 days post-MI, at least 3 months of GDMT	I	B
CRT therapy for LVEF $\leq$35%, sinus rhythm, LBBB with QRS $\geq$150 ms, NYHA II–IV symptoms on GDMT	I	A/B
CRT therapy for LVEF $\leq$35%, sinus rhythm, non-LBBB with QRS $\geq$150 ms, NYHA III–IV symptoms on GDMT	IIa	A
CRT therapy for LVEF $\leq$35%, sinus rhythm, non-LBBB with QRS $\geq$150 ms, NYHA II symptoms on GDMT	IIb	B
CRT therapy for LVEF $\leq$30%, sinus rhythm, non-LBBB with QRS $\geq$150 ms, NYHA I on GDMT	IIb	C
CRT therapy for LVEF $\leq$35%, sinus rhythm, LBBB with QRS 120–149 ms, NYHA II–IV symptoms on GDMT	IIa	B
CRT therapy for LVEF $\leq$35%, sinus rhythm, non-LBBB with QRS 120–149 ms, NYHA III–IV symptoms on GDMT	IIb	B

In the setting of an infarction of unclear duration, a thin and akinetic anterior ventricular wall would be managed differently than diffuse global dysfunction or a focal regional wall motion abnormality with normal ventricular thickness.

Assessment of Myocardial Ischemia and Viability

Once the diagnosis of CAD as an etiology of HF is suspected or established, the question then becomes whether further testing to evaluate the ischemic burden or degree of viability is indicated. Noninvasive imaging, such as stress nuclear imaging or stress echocardiography, to detect myocardial ischemia is recommended in patients who have symptoms, and are potentially eligible for revascularization. Exercise–redistribution–reinjection thallium scintigraphy and dobutamine and exercise stress echocardiography are safe, nonvinvasive, and accurate means of identifying viable myocardium and have been shown to predict contractile recovery after redistribution (Arnese et al., 1995; Dilsizian et al., 1990; Vanoverschelde et al., 1995). Additionally, positron emission tomography (PET) enhanced fluorine-18-labeled fluorodeoxyglucose (FDG) uptake in myocardial segments with impaired function and reduced blood flow is evidence of myocardial viability.

Cardiac magnetic resonance (CMR) is now established as a key noninvasive modality for the evaluation of patients with coronary disease. Late gadolinium enhancement (LGE) on CMR identifies infarcted or fibrotic tissue, whereas its absence indicates "viable" myocardium that exhibits a high potential for recovery after restoration of blood flow. LGE on CMR is the gold standard technique for myocardial infarct visualization in both acute and chronic MI and an accurate predictor of recovery of ventricular function after revascularization (Kim et al., 2000). Further, the presence of LGE has been associated with a higher incidence of major adverse cardiac events (MACE) and is an important prognostic indicator (Kwong et al., 2006).

While the results of the STICH (Surgical Treatment for Ischemic Heart Failure) trial cast doubt on the role of myocardial viability assessment to determine the mode of therapy, the presence of viable myocardium was associated with greater likelihood of survival in patients with CAD and left ventricular dysfunction (Bonow et al., 2011).

Coronary angiogram should be completed in all patients with a positive noninvasive study, or patients with obstructive coronary disease who are candidates for coronary revascularization. The utility of intervention can be determined either by effect on viable ischemic myocardium and contractile recovery or by treatment of angina.

Assessment of Coronary Microvascular Dysfunction

Coronary microvascular dysfunction is suspected when patients have angina and no obstructive coronary disease. Noninvasive studies, such as stress echocardiography or stress nuclear imaging, may be normal in these patients. However, CMR with LGE, PET, and myocardial contrast echocardiography may be useful in quantifying myocardial perfusion and coronary blood flow. There is no specific diagnostic test to directly visualize the coronary microvasculature. Coronary angiography with coronary vasoreactivity testing and measurement of coronary flow reserve should be considered in select patients with a high index of suspicion.

Treatment

In patients with ischemic cardiomyopathy and HF, a comprehensive management strategy that draws on all three components of evidence-based therapy is required. Care of the patient should include pharmacologic therapy, revascularization strategies, and assessment for electrophysiological device-based treatment.

Pharmacologic Therapy (Fig. 3)

In the past two decades, numerous clinical trials have examined the effects of medical therapy on clinical outcomes, including survival in patients with chronic HF. Though the majority of these clinical trials that have shaped guideline-directed medical therapy were performed considering HF as a homogenous disease process, the diagnosis of ischemic cardiomyopathy was generally well represented in these trials and subgroup analyses did demonstrate consistent benefit in patients with ischemic cardiomyopathy. Guideline-based medical therapy in HF is based upon whether the patient has HFrEF (LVEF $\leq$40%) or HFpEF (LVEF $\geq$50%), has symptoms, and the degree of functional limitation assessed subjectively by New York Heart Association (NYHA) Class.

Inhibition of the renin–angiotensin system with angiotensin-converting enzyme inhibitors (ACEI), ARB, or angiotensin receptor-neprilysin inhibition (ARNI) has emerged as the clinical strategy of choice for all patients with HFrEF to improve symptoms and functional class, reduce morbidity, risk of hospitalization, and mortality. ACEI or ARB are also recommended for postmyocardial infarction patients with HFrEF. In the SOLVD and SAVE trials, the ACEI enalapril and captopril not only reduced overall mortality in patients with coronary disease but also reduced the rate of nonfatal myocardial infarction and unstable angina (UA) (Pfeffer et al., 1992; Yusuf et al., 1992). In CHARM-Alternative trial, therapy with the ARB candesartan demonstrated a 23% reduction in the relative risk of cardiovascular mortality or hospital admission for HFrEF patients intolerant of ACEI, a reduction similar to that seen in SOLVD with enalapril (Granger et al., 2003). Recent results of the PARADIGM-HF trial showed that the ARNI valsartan–sacubitril was superior to ACEI enalapril in reducing the risk of death and hospitalizations for HF (McMurray et al., 2014).

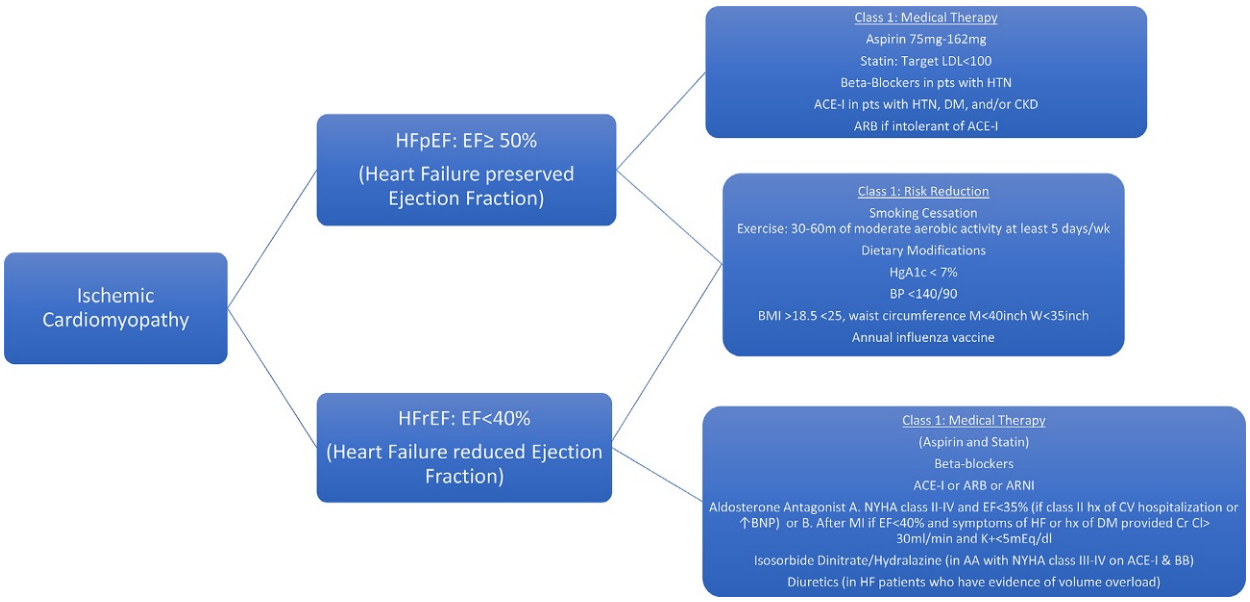

Fig. 3 Pharmacologic therapy.

Based on this data, in symptomatic HFrEF patients with NYHA class II or III symptoms who tolerate an ACEI or ARB, replacement by an ARNI is recommended to further reduce morbidity and mortality (Yancy et al., 2016). According to guidelines, treatment with ACEI and ARB is recommended to treat hypertension in patients with HFpEF. Further, the risk of hospitalization in HFpEF may be reduced by ARBs.

Beta-blockers are indicated in all patients recovering from UA and non-ST segment elevation myocardial infarction (NSTEMI) unless contraindicated and should be started within a few days of the event and continued indefinitely. In patients with ST segment elevation myocardial infarction (STEMI), oral beta-blocker therapy should be initiated in the first 24 h for patients who do not have signs of HF, evidence of a low-output state, increased risk for cardiogenic shock or other relative contraindications for beta blockade such as significant bradycardia. In all patients with a recent or remote history of myocardial infarction or acute coronary syndrome (ACS) and moderate or severely reduced EF (LVEF $\leq$35%), evidence-based beta-blockers (bisoprolol, or carvedilol, or sustained release metoprolol succinate) should be initiated and the daily dose gradually uptitrated. In HFrEF beta-blocker therapy is associated with a 34% relative risk reduction in mortality and a 41% relative risk reduction in HF hospitalizations (Dargie and Lechat, 1999; MERIT-HF Study Group, 1999; Packer et al., 1996, 2001). Beta-blockers have also been shown to improve survival by decreasing risk of re-infarction and ventricular tachyarrhythmia's in patients with preserved EF post-MI who are not at risk for cardiogenic shock (Beta Blocker Heart Attack Trial Research Group, 1983; ISIS Study Group, 1986). Beta-blockers are recommended for treatment of hypertension in HFpEF patients. However, they do not appear to increase exercise capacity or survival in HFpEF patients.

Aldosterone antagonists are recommended to reduce morbidity and mortality following an acute myocardial infarction in patients who have an LVEF of 40% or less who develop symptoms of HF based on the EPHESUS trial which showed a relative 15% reduction in mortality with this therapy (Bertram Pitt et al., 2003). Interestingly, the mortality benefit was primarily driven by a reduction in sudden cardiac death. Aldosterone antagonists are more widely recommended in HFrEF patients with an LVEF <35% with NYHA class II–IV HF to reduce morbidity and mortality based on the RALES and EMPHASIS-HF trials (B Pitt et al., 1999; Zannad et al., 2011). Aldosterone antagonism also decreases hospitalization risk and improves exercise capacity in HFpEF.

While initiating guideline-directed medical therapy is essential, it is as important to be fastidious and uptitrate to the recommended target dose or the highest tolerated dose in order to achieve maximal clinical benefit (Yancy et al., 2013a). Though ACEI, ARB, ARNI, aldosterone antagonists, and beta-blockers improve survival in HFrEF patients, they have not shown similar clinical benefit in HFpEF, even though HFpEF patients experience patterns of functional decline and morbidity that are similar to HFrEF. The differential response to these same therapies suggests that HFrEF and HFpEF are distinctly separate entities with fundamentally different pathophysiologies influenced by different factors in their natural history. In fact, ventricular remodeling in HFrEF is driven by loss of cardiomyocytes, while in HFpEF it is driven by comorbidities, proinflammatory markers, and vascular hyperemic responses. Improved understanding of pathogenesis and identification of new therapeutic targets are probably needed to achieve progress in the clinical outcomes of HFpEF patients.

The combination of hydralazine and isosorbide dinitrate is recommended in patients with HFrEF, who cannot tolerate ACEI or ARB or ARNI to reduce morbidity and mortality. In two trials that compared this vasodilator combination with an ACEI, the ACEI produced more favorable effects on survival and thus should be first line (Cohn et al., 1991; Fonarow et al., 1992). In African Americans with NYHA class III–IV HFrEF already receiving optimal therapy with ACEI or ARB, beta-blockers, and aldosterone antagonist, the addition of hydralazine and isosorbide dinitrate showed significant clinical benefit (Taylor et al., 2004). The particular benefit of this combination in African Americans was also demonstrated in a post-hoc retrospective analysis (Carson et al., 1999). There is no known benefit from the combination of hydralazine and isosorbide dinitrate in HFpEF.

Diuretics are recommended for fluid retention in both HFrEF and HFpEF. Digoxin reduces the need for hospitalization and improves symptoms but not survival in HFrEF (Cody, 1993; Group, 1997). Digoxin has no role in the treatment of HFpEF.

Statins should be used in all patients with a recent or remote history of myocardial infarction or ACS to prevent symptomatic HF and cardiovascular events. High intensity statin should be started or continued as first line therapy in women and men $\leq$75 years of age. In individuals >75 years of age, moderate or high intensity statin is appropriate.

In all patients with coronary disease, a daily aspirin dose of 81 mg (range, 75–100 mg) is recommended. For patients treated with a bare metal stent (BMS), the minimum duration of dual antiplatelet therapy (DAPT) with aspirin and $P2Y_{12}$ receptor inhibitor of 1 month is recommended. BMS is generally reserved for patients who cannot receive DAPT for more than 1 month for reasons of active bleeding, nonadherence to medical therapy, or planned surgery. The recommended minimum duration of DAPT in patients treated with a drug eluting stent is 6 months (Yancy et al., 2016).

Coronary Revascularization Strategies

Myocardial revascularization has been the topic of more randomized clinical trials than almost any other intervention over the past 5 decades and revascularization techniques have undergone continued advances. The timing of angiography and revascularization in ischemic cardiomyopathy should be based on patient risk profile. In both acute and stable CAD, patients should receive guideline-recommended medical treatment, due to its established benefits in terms of symptom relief and prognosis.

Based on extensive trial data, revascularization is recommended to improve survival in patients with (a) >50% left main disease (and ischemia or FFR $\leq$0.80 for stenosis <90%), (b) significant three-vessel disease, (c) significant two-vessel disease with proximal LAD disease, (d) two-vessel or three-vessel disease with stenosis >50% (and ischemia or FFR $\leq$0.80 for stenosis <90%) with impaired LV function (EF <40%), and (e) a large area of ischemia (>10% LV) (Kolh et al., 2014; Levine et al., 2011). Revascularization is recommended to improve symptoms in those patients with significant coronary stenosis in the presence of angina

unresponsive to medical therapy. Revascularization and medical therapy should be seen as complimentary, as the indications for revascularization include persistence of symptoms despite medical treatment.

In patients with stable ischemic heart disease, percutaneous coronary intervention (PCI) decreases angina and the extent of myocardial ischemia but has not shown a survival benefit. In the Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) trial, an initial management strategy of optimal medical therapy alone compared with optimal medical therapy plus PCI among patients with stable ischemic heart disease found no significant difference between the treatment groups with respect to the composite primary end point of death from any cause or nonfatal MI or the secondary endpoint of hospitalization for ACS over a median follow-up period of 4.6 years (Boden and O'Rourke, 2007). During an extended follow-up of up to 15 years, with a median duration of 6.2 years, there was still no difference in survival between an initial strategy of PCI plus medical therapy and medical therapy alone in stable ischemic heart disease (Sedlis et al., 2015). However, freedom from angina was significantly greater in the PCI group at 1 year and 3 years. At 5 years there was a decreased need for additional revascularization in the PCI group, namely, 21% of the PCI group and 33% of the medical therapy received additional revascularization ($P < 0.001$). It is important to note that the severity of coronary disease in COURAGE was moderate and 70% of patients had no or mild ischemia, further, most patients had normal LV function. To date, The Medical, Angioplasty, or Surgery Study II (MASS II) trial is the only RCT comparing medical therapy with PCI (72% with BMS, 28% with balloon angioplasty alone) and with coronary artery bypass graft (CABG) (Hueb et al., 2010). This study compared the 10-year follow-up of PCI, CABG, and medical treatment in patients with multivessel CAD, stable angina, and preserved ventricular function and found that compared with CABG, medical therapy was associated with a significantly higher incidence of subsequent MI, a higher rate of additional revascularization, and a higher incidence of cardiac death. Compared with CABG, PCI was associated with an increased need for further revascularization, a higher incidence of myocardial infarction, and a 1.46-fold increased risk of combined events.

The 10-year outcomes of the STICH trial demonstrated a significant mortality benefit of revascularization with CABG surgery over optimal medical therapy for patients with ischemic cardiomyopathy with an EF of 35% or less and coronary disease amenable to CABG (Velazquez et al., 2016).

In the elective setting, myocardial revascularization is appropriate when the expected benefits exceed the risks of the procedure and should take into consideration coronary anatomy, patient preferences, age and comorbidities, and hospital/operator experience. Whether medical therapy, PCI, or CABG is preferred depends on the risk–benefit ratio of these treatment strategies and should be made as a multidisciplinary team aided by risk models that assess short-term and long-term outcomes.

By contrast, myocardial infarction and myocardial ischemia in the acute setting of ACSs are potentially life threatening and often leads to progressive ischemic cardiomyopathy and HF. In this setting, patients at highest risk (those with refractory angina, severe HF, cardiogenic shock, or life-threatening arrhythmias) should be considered for urgent (<2 h) coronary angiogram and timely intervention. In the management of ACS, there are no specific randomized controlled trials comparing PCI with CABG and the decision whether to perform CABG or PCI is left to the clinician's discretion. With the widespread use of fibrinolytic therapy or primary PCI in patients with STEMI, emergency CABG is generally reserved for patients with (1) left main and/or 3-vessel CAD with proximal LAD disease, (2) ongoing ischemia after successful or failed primary PCI, (3) coronary anatomy not amenable for PCI, (4) a mechanical complication of STEMI, and (5) cardiogenic shock (Hillis et al., 2011). In STEMI, PCI has been shown to increase survival (Patel et al., 2009). Similarly, in NSTEMI PCI has also shown to improve long-term survival by reducing early and late cardiac events (Bavry et al., 2006; Mehta et al., 2005).

Device Therapy

Patients who survive myocardial infarction are at increased risk for sudden death from cardiac causes, largely due to ventricular tachyarrhythmias. In this population and those with chronic ischemic heart disease, a marked reduction of the left ventricular ejection fraction is the most powerful of the known predictors of sudden cardiac death (Myerburg, 2008). The ACC/AHA guidelines recommend ICD therapy for primary prevention of sudden cardiac death in patients with ischemic heart disease at least 40 days post-MI with LVEF of 35% or less and NYHA class II or III symptoms on appropriate medical therapy who are expected to survive more than 1 year. For asymptomatic patients (NYHA class 1) with ischemic cardiomyopathy, the LVEF cut off for implantation of a primary prevention ICD decreases to 30%. MADIT-II (Multicenter Automatic Defibrillator Implantation II) showed a 31% relative risk reduction in all-cause mortality in patients post-MI with LVEF <30% receiving a prophylactic ICD compared with standard of care (Moss et al., 1996). It is not recommended that patients with NYHA class IV HF have an ICD implanted as they are more likely to die from pump failure than ventricular tachyarrhythmias. ICD is not indicated in patients with HFpEF.

HF progression is accompanied by substantial prolongation of the QRS interval, a marker used to define cardiac dyssynchrony, in approximately one-third of patients and is associated with worse outcomes (Young et al., 2003). Cardiac resynchronization therapy (CRT) is an important treatment for patients with HF and aims to restore contractile synchrony at the atrioventricular, interventricular, and intraventricular level. It is a class 1 indication that CRT be placed in patients who have an LVEF of 35% or less, who are in sinus rhythm, and have a left bundle branch block (LBBB) with a QRS duration of 150 ms or greater and NYHA class II, III, or ambulatory class IV symptoms despite optimal guideline-directed medical therapy (Yancy et al., 2013a). Improvement in survival with CRT is evident as early as the first 3 months of therapy and overall CRT has shown a reduction in all-cause mortality of 24%–36% (Bristow et al., 2004). Additionally, a number of studies have demonstrated that CRT results in an improvement in symptoms, exercise capacity, and peak oxygen consumption (Abraham et al., 2002; Saxon and Ellenbogen, 2003). CRT is not indicated in patients with HFpEF.

Advanced HF Treatment in Ischemic Cardiomyopathy

Despite the drugs, devices, and strategies described in the previous sections, a subset of patients with ischemic HF will continue to progress and develop persistently severe symptoms at rest or with minimal exertion. This group of patients with truly refractory HF has Stage D HF. In addition to severe symptoms, the European Society of Cardiology (ESC) has developed a definition of advanced HF with objective criteria that can be useful and include: (1) patients with episodes of fluid retention and/or reduced cardiac output at rest (peripheral hypoperfusion), severe cardiac dysfunction (LVEF <30% or restrictive mitral inflow, PCWP >16 mmHg and/or RAP >12 mmHg, elevated BNP or NT-proBNP), severe impairment of functional ability (inability to exercise or 6MWT <300 m or peak V02 <12–14 mL/kg/min), and HF hospitalization in the past 6 months despite attempts to optimize therapy (Metra et al., 2007). It is important to identify this group of individuals and refer them to a HF specialist to be evaluated for advanced treatment strategies, including mechanical circulatory support with a left ventricular assist device (LVAD), continuous inotropic infusions, cardiac transplantation, or end-of-life care, such as hospice.

While in many ways the treatment of ischemic and nonischemic advanced HF patients is similar, there are some considerations worth noting. Patients with ischemic cardiomyopathy often have comorbidities that are relative contraindications to mechanical circulatory support such as significant chronic kidney disease, diabetes with macro- or microvascular complications, or peripheral arterial disease. These comorbidities can increase procedural risk and may decrease quality of life after mechanical circulatory support. However, no data in the literature to date shows that there is a mortality difference between advanced HF patients with ischemic cardiomyopathy who receive LVADs versus those who have a nonischemic etiology. Continuous intravenous inotropic support is occasionally used in those patients with refractory stage D HF as a bridge to LVAD or cardiac transplantation, and is also considered as palliative therapy in those who are not eligible for either mechanical circulatory support or cardiac transplantation. In patients with ischemic HF, inotropic therapy is associated with significantly higher mortality from ventricular tachyarrhythmias when compared to nonischemic HF patients on inotropic therapy.

In a recent analysis of over 30,000 patients looking at whether or not survival on the heart transplant list depends on the underlying etiology of heart disease, patients with ischemic cardiomyopathy were not shown to be at higher risk of death while waiting transplantation when compared to other patients with dilated, hypertrophic, or valvular cardiomyopathy (Hsich et al., 2016).

Prognosis

The prognosis of patients with HF from ischemic cardiomyopathy is considerably worse than that of patients without coronary disease (Bart et al., 1997). Ischemic etiology from CAD often goes hand in hand with other comorbidities such as peripheral vascular disease and ischemic stroke as a risk factor for poor prognosis. In addition to being a risk factor for the development of HF, progression of ischemic heart disease adversely affects the outcomes of patients with established HF (Shindler et al., 1996). Targeting both HF treatment and prevention of progression of underlying coronary disease through healthy lifestyle habits and appropriate medical therapy remains critical.

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Kidney in Heart Failure

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Introduction

The heart and the kidneys are intimately related, and dysfunction of one organ often leads to dysfunction of the opposite organ. Concomitant dysfunction of the kidney and the heart was first described in 1868 by Moxon and is now termed cardiorenal syndrome (CRS) (Moxon, 1868). Since then, the definition, understanding, and treatment have grown significantly. However, despite nearly 150 years of research, many questions remain unanswered regarding the impact, underlying pathophysiology, and best approaches to the prevention and treatment for CRS.

The generally accepted definition for CRS stems from the National Heart, Lung, and Blood Institute's 2004 report that defined CRS as a condition in which therapy to relieve congestive symptoms of HF is limited by a decline in renal function as manifested by a reduction in glomerular filtration rate (GFR) (https://www.nhlbi.nih.gov/research/reports/2004-cardiorenal-hf-hd, accessed February 22, 2017). The Acute Dialysis Quality Initiative (ADQI) further defines CRS as a disorder of the heart and kidneys whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction of the other (Davenport et al., 2010; Pinheiro da Silva and Vaz da Silva, 2016). CRS is subdivided into five distinct categories, which are described later in this article (Ronco et al., 2008). However, these categories are dynamic, and many patients will transition among the various categories of CRS. These transitions throughout one's disease course reflect the close interplay between the two organ systems (Damman et al., 2011).

Definitions

Acute Heart Failure

The nomenclature for acute heart failure is inconsistent throughout the literature and has been referred to as acute heart failure syndromes, acute decompensated heart failure (ADHF), acute decompensation of chronic heart failure, and others. Broadly speaking, acute heart failure refers to a syndrome of new onset or recurrence of symptoms and signs of heart failure requiring urgent or emergent therapy and resulting in seeking unscheduled care or hospitalization (Pinheiro da Silva and Vaz da Silva, 2016). However, acute heart failure can often times present in a more indolent course, with slow progression of symptoms leading to acute decompensation.

Chronic Heart Failure

Chronic heart failure is a clinical syndrome characterized by a combination of typical signs and symptoms in conjunction with objective evidence of structural or functional abnormalities of the heart (Pinheiro da Silva and Vaz da Silva, 2016). Heart failure ultimately results in the inability of the heart to deliver oxygen at a rate required to meet metabolic demands despite normal filling pressures (Pinheiro da Silva and Vaz da Silva, 2016). The classic signs and symptoms of heart failure are shown in **Tables 1–7**. Heart failure affects approximately 2%–3% of the worldwide population. However, age is an independent risk factor for heart failure, and its prevalence in patients older than 70 years is above 10% (Pinheiro da Silva and Vaz da Silva, 2016).

Table 1 Common signs and symptoms of heart failure

Symptoms	*Signs*
Orthopnea	S3 gallop
Dyspnea	Laterally displaced point of maximal impulse
Paroxysmal nocturnal dyspnea	Elevated jugular venous distention
Poor exercise tolerance	Hepatojugular reflex
Lower extremity edema	Hepatomegaly
Fatigue	Cardiac cachexia

Harrison's Internal Medicine (2015).

Table 2 Diagnostic criteria of heart failure

Heart failure reduced ejection fraction

1. Symptoms of heart failure
2. Signs of heart failure
3. Reduced LVEF $< 45\%$

Heart failure preserved ejection fraction

1. Symptoms of heart failure
2. Signs of heart failure
3. LVEF $> 45\%$
4. Relevant structural heart disease and/or diastolic dysfunction

Table 3 ACC/AHA heart failure classification

ACC/AHA stage		*NYHA functional classification*	
A	At risk for heart failure but without structural heart disease or HF symptoms		
B	Structural heart disease without signs or symptoms of HF	I	No limitations of physical activity
C	Structural heart disease with previous or current symptoms of HF	I	No limitations of physical activity
		II	Patients with cardiac disease resulting in slight limitation of physical activity, causing fatigue, palpitation, dyspnea, or angina. They are comfortable at rest
		III	Patients with cardiac disease resulting in marked limitation in of physical activity. Less than ordinary activities result in fatigue, palpitation, dyspnea, or angina
D	Refractory HF requiring specialized interventions	IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms may be present at rest

Harrison's Internal Medicine (2015).
Reproduced from Pinheiro da Silva, A. L. and Vaz da Silva, M. J. (2016). Type 4 cardiorenal syndrome. *Revista Portuguesa de Cardiologia* **35**, 601–616.

Table 4 Definition of CKD

Criteria for CKD (either of the following for > 3 months)	
Markers of kidney damage (1 or more)	Albuminuria
	Electrolyte disturbances from tubular disorders
	Urine sediment abnormalities
	Radiographic structural abnormalities
	History of renal transplantation
Decreased GFR	GFR $<$ 60 mL/min/1.73 m^2

Reproduced from Webster, A. C., Nagler, E. V., Morton, R. L. and Masson, P. (2017). Chronic kidney Disease. *Lancet* **389**, 1238–1252.

Table 5 Classification of CKD

Stage	*GFR, mL/min/1.73 m^2*
0	$\geq$90 with risk factors for CKD
1	$\geq$90 with evidence of kidney damage
2	60–89
3	30–59
4	15–29
5	$<$15

Webster, A. C., Nagler, E. V., Morton, R. L. and Masson, P. (2017). Chronic kidney Disease. *Lancet* **389**, 1238–1252.

Table 6 Definitions and subtypes of cardiorenal syndrome

Cardiorenal syndrome	A disorder of the heart and the kidneys where acute or chronic dysfunction in one organ may induce acute or chronic dysfunction in the other organ
Cardiorenal syndrome type 1 (acute cardiorenal syndrome)	Acute worsening of cardiac function leading to acute kidney injury
Cardiorenal syndrome type 2 (chronic cardiorenal syndrome)	Chronic abnormalities in cardiac function causing progressive kidney damage
Cardiorenal syndrome type 3 (acute renocardiac syndrome)	Abrupt worsening in kidney function causing acute cardiac dysfunction
Cardiorenal syndrome type 4 (chronic renocardiac syndrome)	CKD contributing to decreased cardiac function and/or increased risk of cardiovascular events
Cardiorenal syndrome type 5 (secondary cardiorenal syndrome)	Systemic conditions contributing to both cardiac and renal dysfunction

Adapted from Ronco, C., Haapio, M., House, A. A., Anavekar, N. and Bellomo, R. (2008). Cardiorenal syndrome. *Journal of the American College of Cardiology 52*, 1527–1539.

Table 7 Categories of biomarkers and examples

Category	*Examples*
Functional markers	SCr, plasma/serum cystatin C
Upregulated proteins	Neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), Liver fatty acid-binding protein (L-FABP), interleukin-18 (IL-18)
Low-molecular-weight proteins	Urine cystatin C
Enzymes	Alpha-glutathione *s*-transferase and pi-glutathione *s*-transferase (α-GST), gammaglutanyl transpeptidase (GGT), alkaline phosphatase (AP), *N*-acetyl-b-D-glucosaminidase (NAG)

Adapted from de Geus, H. R., Betjes, M. G. and Bakker, J. (2012). Biomarkers for the prediction of acute kidney injury: a narrative review on current status and future challenges. *Clinical Kidney Journal* **5**, 102–108

There are two major classification systems of heart failure. The first is the American College of Cardiology/American Heart Association (ACC/AHA) Heart Failure staging approach, which focuses on the severity and progression of disease. The second major classification is the New York Heart Association Functional Classification, which emphasizes the functional burden of heart failure (Kasper, 2015).

Acute Kidney Injury

The definition of acute kidney injury (AKI) has evolved over time. The currently accepted definition is based on the KDIGO Acute Kidney Injury Work Group recommendations; a harmonization of prior RIFLE and AKIN criteria (Palevsky et al., 2013). KDIGO defines AKI as any of the following: increase in serum creatinine (SCr) by $\geq$0.3 mg/dl ($\geq$26.5 μmol/L) within 48 h; or increase in SCr to $\geq$1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or urine volume $<$0.5 ml/kg/h for 6 h (Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group, 2012). AKI represents a broad category of multiple different underlying pathologies. The presentation of AKI can range from asymptomatic to fatal derangements in one's electrolyte and fluid balance. AKI is a common diagnosis complicating an estimated 15% of hospital admissions and up to 60% in critically ill patients (Makris and Spanou, 2016). AKI is also an independent predictor of hospital admission mortality, particularly in the intensive care setting (Makris and Spanou, 2016).

Chronic Kidney Disease

Chronic kidney disease (CKD) is characterized by abnormalities in kidney structure or function or reduced GFR for greater than 3 months duration (Webster et al., 2017). CKD is a public health epidemic, affecting >10% of people aged 20 years or older in the United States, which equates to nearly than 20 million people (National Chronic Kidney Disease Fact Sheet, 2014). Worldwide, CKD affects approximately 8%–16% of the population, and the prevalence is increasing.

CKD is divided into stages, as shown later. The Kidney Disease Outcomes Quality Initiative (K/DOQI) classification system is uniformly utilized in defining not only the concept of CKD but also its subsequent classification the different stages of kidney dysfunction (National, 2002).

Cardiorenal Syndrome

The generally accepted definition for CRS stems from the National Heart, Lung, and Blood Institute's 2004 report that defined CRS as a condition in which therapy to relieve congestive symptoms of HF is limited by a decline in renal function as manifested by a reduction in GFR (https://www.nhlbi.nih.gov/research/reports/2004-cardiorenal-hf-hd, accessed February 22, 2017). The heart and the kidney have a unique ability to initiate and perpetuate injury to the opposite organ, as both organ systems rely upon each other and utilize common pathways including hemodynamics, neurohormonal and immunologic/biochemical pathways (Ronco et al., 2008).

CRS plays a significant role in the morbidity and mortality of patients with kidney and cardiac disease. CKD is disproportionally associated with heart failure. In the Acute Decompensated Heart Failure National Registry (ADHERE), CKD was reported in 30% of patients admitted to hospital for heart failure as compared with just over 10% in the general adult US population (Adams et al., 2005). This is clinically significant, as declining renal function and CKD stage, as defined by the K/DOQI classification system, is associated with increasing mortality in the heart failure population. Furthermore, the rate of decline in kidney function is a strong predictor of increased mortality in this population, independent of worsening heart failure and baseline kidney function. (Khan et al., 2006) Therefore, it is crucial to recognize, prevent, and treat both renal and cardiac dysfunction.

The CRS is divided into five distinct categories (Ronco et al., 2008). These categories are based upon the chronology of the affected organs and the acuity by which they have developed dysfunction. CRS type 1 is known as acute CRS, whereby acute cardiac dysfunction leads to AKI. CRS type 2 is also known as chronic CRS, where chronic cardiac dysfunction progressively causes renal dysfunction. CRS type 3, or acute renocardiac syndrome, is the syndrome by which acute renal dysfunction causes acute cardiac

dysfunction. CRS type 4, also known as chronic renocardiac syndrome, is the process by which CKD contributes to progressive cardiac dysfunction. Lastly, CRS type 5, or secondary CRS, is the process in which a systemic disease causes both cardiac and renal dysfunction. These distinct, though overlapping, syndromes are described below, along with their underlying pathophysiology and the basic approaches to treatment.

Types of CRS

Cardiorenal Syndrome Type 1 (Acute Cardiorenal Syndrome)

Cardiorenal syndrome type 1, or acute cardiorenal syndrome, is defined as acute heart failure, whether new onset or acute decompensation of chronic disease, causing AKI (Ronco et al., 2008). As above, heart failure and AKI are common hospital admission diagnoses. It is estimated that AKI occurs in over 70% of patients presenting to the hospital in cardiogenic shock (Jose et al., 2006). Moreover, AKI has shown to be a poor prognostic factor in patients with acute heart failure, particularly those related to acute coronary syndrome (Goldberg et al., 2005).

The underlying pathophysiology for acute cardiorenal syndrome is complex and equivocal. There are multiple components at play, which are described later in this article. The combination of alterations in hemodynamics, venous congestion, upregulation of renin–angiotensin–aldosterone system (RAAS), activation of the sympathetic nervous system, and the immune-mediated, pro-inflammatory state of acute heart failure and creation of reactive oxygen species from oxidative stress results in a cyclic deterioration of renal and cardiac function (Ronco et al., 2008). The management, as described later in this article, is targeted toward minimizing the deleterious effects of each of the proposed pathophysiological processes.

Cardiorenal Syndrome Type 2 (Chronic Cardiorenal Syndrome)

Cardiorenal syndrome type 2, or chronic cardiorenal syndrome, is defined as chronic abnormalities in cardiac function causing progressive kidney injury (Ronco et al., 2008). An estimated 25% of patients with the diagnosis of chronic heart failure also have a component of CKD (Hillege et al., 2006). Mortality significantly increases with even slight decreases in the GFR in patients with heart failure (McAlister et al., 2004). The pathophysiology of chronic cardiorenal syndrome is multifactorial. Chronic venous congestion plays a crucial role, decreasing effective renal perfusion. Chronic activation of renin–angiotensin–aldosterone pathway results in progressive cardiac dysfunction. The principles of treatment for chronic cardiorenal syndrome include optimization of hemodynamics and inhibition of the RAAS pathway, as described later.

Cardiorenal Syndrome Type 3 (Acute Renocardiac Syndrome)

Cardiorenal syndrome type 3 is defined as abrupt and primary worsening of renal function causing acute cardiac dysfunction (Ronco et al., 2008). Acute changes in renal function have multiple deleterious effects, which contribute to cardiac dysfunction. The Frank–Starling mechanism and the force–tension curve can best explain the hemodynamic effects from acute renal dysfunction. The Frank–Starling mechanism states that as venous return increase, ventricular filling, or end-diastolic volume, increases. The increase in end-diastolic volume results in myocyte and sarcomere stretching and resultant increase in stroke volume. However, the Frank–Starling curve is not linear, and the sarcomere can reach a point where further stretching does not result in an increase in stroke volume, and in patients with heart failure, stroke volume can actually decrease with further myocyte stretching. In patients with acute renal dysfunction, this becomes problematic as RAAS activation leads to sodium and water retention and increases in preload beyond what the cardiac myocytes can accommodate. The other component is explained by the force–tension curve, which describes the relationship between afterload and stroke volume. Afterload increases from acute renal dysfunction through the activation of the RAAS and the sympathetic nervous system. The increase in afterload results in decreases in stroke volume.

Other consequences of acute renal dysfunction can affect cardiac function. For example, electrolyte abnormalities can cause cardiac arrhythmias; nitrogenous waste accumulation can cause pericarditis; and acidemia from severe renal dysfunction is a cardiac depressant. Each of these mechanisms falls under the broad category of acute renocardiac syndrome (Ronco et al., 2008).

Cardiorenal Syndrome Type 4 (Chronic Renocardiac Syndrome)

Cardiorenal syndrome type 4, or chronic renocardiac syndrome, is defined as primary CKD results in cardiac dysfunction (Ronco et al., 2008). CKD is a complex disorder with numerous consequences, many of which affect cardiac function. Electrolyte disturbances, including hyperkalemia, calcium and phosphorus dysregulation, and academia, all affect cardiac function. CKD also activates RAAS pathway, which leads to hypertension, accelerated coronary artery disease, and cardiac remodeling (Ronco et al., 2008). Anemia is also a consequence of CKD, impairing oxygen delivery to cardiac muscle and impairs function (Jie et al., 2006). CKD is also known to cause hyperlipidemia, insulin resistance, endothelin dysfunction, vascular calcification, and accelerated atherosclerosis, all of which are risk factors for cardiac disease.

Slowing the progression of CKD and treating the cardiac risk factors from CKD is the hallmark of treatment for chronic renocardiac syndrome. Specific treatment strategies are discussed later in the article. In patients with ESRD and HF with reduced LVEF (<40%), correction of renal dysfunction has been shown to reduce cardiac comorbidities. Kidney transplantation can result in a considerable increase or even normalization of mean LVEF in a significant number of patients (Wali et al., 2005).

Cardiorenal Syndrome Type 5 (Secondary Cardiorenal Syndrome)

Cardiorenal syndrome type 5 is defined as combined cardiac and renal dysfunction secondary to an underlying acute or chronic systemic disease process. Multiple disease processes are known to cause both cardiac and renal dysfunction, including diabetes, systemic lupus erythematosus, sarcoidosis, and sepsis. The pathophysiology is largely dependent on the underlying systemic disease process.

The treatment for secondary cardiorenal syndrome depends on the underlying systemic disease. There is no common treatment strategy beside the (Ronco et al., 2008) avoidance of further cardiotoxic and nephrotoxic therapies.

Pathophysiology

Hemodynamics and Intra-Abdominal Pressure

Hemodynamics plays a crucial role in the pathogenesis of cardiorenal syndrome. Renal perfusion depends on the transrenal perfusion pressure, which is mean arterial pressure minus central venous pressure. Therefore, venous congestion as reflected in increases in CVP would result in decreased renal perfusion even in the setting of unchanged cardiac output (Gnanaraj et al., 2013). The initial theory was that decreased mean arterial pressure was the primary factor causing cardiorenal syndrome. However, studies have shown central venous pressure is the predominant factor causing renal dysfunction in CRS. The Evaluation Study of Congestive Heart Failure and Pulmonary Artery Catheterization Effectiveness (ESCAPE) trial was the landmark study demonstrating that poor forward flow (mean arterial pressure) did not correlate with baseline creatinine but instead the only predictor was right atrial pressure (Nohria et al., 2008). Subsequent studies have further demonstrated this concept, including a 2009 study of patients with ADHF that concluded that in fact venous congestion, not cardiac output, was the most important hemodynamic factor causing worsening of renal function (Mullens et al., 2009).

Renal perfusion is also dependent on the intra-abdominal pressure (IAP). Renal blood flow is inversely correlated to IAP, and in patients with ADHD, IAP is elevated in up to 60% of patients. A normal IAP is usually 5–7 mmHg but can be affected by venous congestion, visceral edema, ascites, abdominal wall edema, gaseous distention of the bowel, urinary retention, and obesity. Intra-abdominal hypertension is defined as persistent IAP above 12 mmHg. In patients with ADHD, consideration of IAP is crucial to the understanding and management of CRS. In a study of 40 patients admitted for ADHF, elevated IAP (IAP (greater than or equal to) 8 mmHg) was seen in 24 (60%) of the patients and was associated with impaired kidney function (Mullens et al., 2008). One study showed that the greater the IAP was lowered, the more renal function improved, independent of hemodynamic changes.

Neurohormonal Activation

Hormonal activation contributes to further renal and cardiac dysfunction in cardiorenal syndrome. Venous congestion and renal vein distention activates mechanoreceptors and enhances local sympathetic renal nerve activity, leading to intrarenal vasoconstriction and a reduction in GFR (Kostreva et al., 1981; Haddy, 1956; Dilley et al., 1983). Activation of the RAAS also plays a critical role in cardiorenal syndrome. RAAS is activated in response to decreased renal perfusion from venous congestion (Schrier, 2006). RAAS activation has multiple consequences, including peripheral vasoconstriction, sodium and water reabsorption, cardiac remodeling, nonosmotic release of arginine–vasopressin (AVP), and other neuroendocrine hormones, such as endothelin, all of which further promote congestion and renal function (Gnanaraj et al., 2013). RAAS activation ultimately leads to further cardiac dysfunction via increasing afterload by peripheral vasoconstriction and further hypervolemia by sodium and water reabsorption.

Vasopressin and Hyponatremia

In the setting of heart failure, serum AVP increases. This increase has several effects that are deleterious to cardiac and renal function. AVP activated V1 receptors causing subsequent vasoconstriction, with increases afterload and further reduced renal perfusion pressure. However, activation of V2 receptors leads to free water retention leading to hyponatremia. The Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST) trial sought to determine if selective inhibition of V2 receptors using tolvaptan would allow for improved volume overload correction without the potential electrolyte abnormalities of other classic diuretics like loops diuretics. Unfortunately, the EVEREST trial showed no effect of oral tolvaptan on long-term mortality or heart-failure-related morbidity (Konstam et al., 2007).

Endothelin Activation and Inflammatory Effect

Venous congestion results in activation of vascular endothelium to promote inflammation, pro-oxidation, and vasoconstriction (Gimbrone et al., 2000). Pro-inflammatory cytokines like tumor necrosis factor and interleukin-6 are released during venous

congestion, which impair myocardial function and accelerate heart failure (Braunwald, 2008). Renin is also secreted in response to the pro-inflammatory state that, as above, promotes fluid retention and worsening venous congestion, impaired renal function, and progressive worsening of cardiac function (Sánchez-Lozada et al., 2003).

Biomarkers

Cardiac and renal biomarkers are an emerging landscape in the early detection of renal and cardiac dysfunction. The classic cardiac biomarker is brain natriuretic peptide (BNP). BNP correlates with pulmonary capillary wedge pressure and is frequently used in the diagnosis of heart failure. Some data suggest that BNP is an indirect marker for worsening renal function during the treatment of ADHF. However, in the setting of concomitant renal dysfunction, markers such as BNP and NT-proBNP are less accurate (Pfister et al., 2011; Hatamizadeh et al., 2013).

SCr is currently the gold standard with regard to identifying renal dysfunction. However, because a rise in SCr is a late manifestation of renal dysfunction, it has limited utility in the detection of and prevention of kidney disease early in the disease course. Significant research is ongoing investigating alternative markers to detect early renal dysfunction (de Geus et al., 2012).

Functional Markers

Serum creatinine

As mentioned earlier, SCr is the current gold standard for the detection of kidney injury. Creatinine is a degradation product of muscle cells and is filtered in the renal glomerulus. It is therefore a surrogate for glomerular filtration. Unfortunately, following an injurious event like AKI, the rise in SCr is slow and is hours to days after the initial renal insult. It therefore has limited use in the detection of early kidney dysfunction (de Geus et al., 2012; Waikar et al., 2009).

Plasma/serum cystatin C

Cystatin C is a nonglycosylated cysteine protease inhibitor, which is produced by all nucleated cells at a constant rate. It is excreted exclusively by glomerular filtration and metabolized by the proximal tubules. Serum levels of cystatin C are currently being investigated for early detection of glomerular filtration changes, with some evidence suggesting serum cystatin C is superior to SCr in the detection of minor changes in glomerular function (Dharnidharka et al., 2002).

Upregulated Proteins

Neutrophil gelatinase-associated lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein linked to neutrophil gelatins in specific leukocyte granules (Borregaard et al., 1995). It is expressed in a variety of epithelial cells and is associated with antimicrobial defense (Goetz et al., 2002). In normal kidneys, NGAL expression is limited to the distal tubule and the collecting duct. However, in AKI, NGAL staining shows expression in the proximal tubules as well (Mori et al., 2005; Hvidberg et al., 2005). Studies show urine and serum NGAL are early predictors of AKI both in adult and children in either cardiac surgery or patients in the intensive care unit (Haase et al., 2009).

Kidney injury molecule-1 (KIM-1)

Kidney injury molecule-1 (KIM-1) is a type 1 transmembrane glycoprotein expressed by the dilated tubules in acute and chronic renal injury (Ichimura et al., 2008). The proposed function of KIM-1 is this protein is involved in the regenerative processes after epithelial injury and in the removal of dead cells in the tubular lumen through phagocytosis (Bailly et al., 2002; Bonventre, 2009). Several studies have investigated its clinical utility, with some evidence that detection has predictive value in the detection of acute kidney dysfunction (de Geus et al., 2012).

Liver fatty acid-binding protein

Fatty-acid-binding proteins are small, cytoplasmic proteins expressed in tissues with robust fatty acid metabolism. These proteins function to facilitate long-chain fatty acid transportation, regulation o gene expression and reduction of oxidative stress (de Geus et al., 2012). The liver fatty acid-binding protein (L-FABP) is a specific subtype with undetectable urinary levels in healthy individuals. The healthy proximal tubule internalized L-FABP via megalin-mediated endocytosis (Ferguson et al., 2010; Oyama et al., 2005). However, during an ischemic event, upregulation of L-FABP gene expression occurs, and with concomitant renal dysfunction, absorption, and internalization by the proximal tubule is impaired (Oyama et al., 2005; Yamamoto et al., 2007). Limited research has investigated urinary L-FABP levels in the detection of renal dysfunction, and further investigations are needed.

Interleukin-18

Interleukin-18 (IL-18) is an inflammatory cytokine that has proved to be an important mediator in the process of AKI in animal models. Several studies have investigated IL-18's predictive utility for detection of AKI, but further research is needed (de Geus et al., 2012).

Low-Molecular-Weight Proteins

Urine cystatin C

Cystatin C, as mentioned earlier, is a nonglycosylated cysteine protease inhibitor that is produced at a constant rate by all nucleated cells (de Geus et al., 2012). It is excreted via glomerular filtration and is completely metabolized by the proximal tubules (Herget-Rosenthal et al., 2007). Ongoing research is determining if measurement of urine cystatin C can be used to detect early proximal tubular injury. Unfortunately, several factors affect urine cystatin C, including albuminuria, which has limited its diagnostic utility or predictive value of AKI (Nejat et al., 2012).

Enzymes

Alpha-glutathione s-transferase and pi-glutathione s-transferase

Alpha-glutathione *s*-transferase (α-GST) and pi-glutathione *s*-transferase (π-GST) are two enzymes part of a large multigene family responsible for detoxification. These enzymes are not normally expressed or excreted in the urine (de Geus et al., 2012). During injury, α-GST is exclusively expressed in the proximal tubule, and π-GST is expressed in the distal tubule, both of which can be identified in urine during AKI (Harrison et al., 1989).

N-*acetyl*-b-*D*-*glucosaminidase*

N-acetyl-*b*-D-glucosaminidase (NAG) is a large liposomal enzyme produced in the renal tubules. Due to its size, it is impermeable to glomerular filtration, and urinary detection has been associated with tubular injury (de Geus et al., 2012). Measurement of urine NAG suggests tubular injury or increased liposomal activity. However, limited studies to date have examined the predictive value in early tubular injury.

Gammaglutanyl transpeptidase and alkaline phosphatase

Gammaglutanyl transpeptidase and alkaline phosphatase are enzymes expressed in the tubular brush border and are released into the urine during severe renal injury (de Geus et al., 2012). There are few studies investigating their clinical utility and predictive value for AKI.

Management of CRS

The cornerstone of the management of cardiorenal syndrome is based on the minimization of venous congestion. The most appropriate strategy to achieve venous decongestion has been extensively studied. Also, judicious inhibition of RAAS and avoidance of further cardiac or nephrotoxicity are crucial to the treatment of CRS. Emerging strategies continue to be investigated.

Anticipation and Prevention

Cardiorenal syndrome is a cyclic pathophysiological process, by which damage to one organ perpetuates damage to the opposite organ. Breaking this cycle is crucial in the prevention and treatment. Similarly, recognition and minimization of further renal or cardiac dysfunction is imperative. Early recognition of the signs and symptoms of CRS helps aid in preventing further dysfunction and can expedite appropriate treatment. Avoidance of further nephrotoxicity, including avoidance of contrast media and NSAIDs, helps prevent further renal dysfunction. Recognition of early satiety and anorexia as symptoms of splanchnic congestion and resistance to oral diuretics can allow early treatment implementation. Orthopnea, paroxysmal nocturnal dyspnea, and elevated jugular venous distention are all signs and symptoms of volume overload and useful in the diagnosis of heart failure and risk for CRS. Optimization of hemodynamics, including avoiding hypotension, can prevent further renal injury. Also, prevention of intra-abdominal hypertension, which, as above, can further perpetuate renal dysfunction, should be implemented. Strategies include preventing urinary retention, treating constipation, and keeping the head of the bed <30 degrees are all practical strategies to decreased IAP (von Haehling et al., 2013).

Minimization of Venous Congestion

Diuretics are the hallmark of venous decompression in patients with CRS. Administration of intravenous loop diuretics is the initial treatment approach (von Haehling et al., 2013). The Determining Optimal Dose and Duration of Diuretic Treatment in People with Acute HF (DOSE-AHF) trial compared patients receiving high doses of furosemide (2.5 times their usual home oral dose) with patients receiving lower doses (equal to home oral dose) and found to difference in the patient's SCr or prognosis (Felker et al., 2011). Provider preference and experience should be taken into account in the initial diuretic dosing in patients with volume overload and CRS. Intravenous diuretics should be administered due to poor oral absorption due to splanchnic congestion. A 2011 prospective, double-blind randomized study compared continuous intravenous loop diuretics with intermittent, bolus doses of loop diuretics and found no difference in renal function between the two groups (Felker et al., 2011). Long-acting loop diuretics, like torsemide, can be helpful in the management of CRS. Short-acting loop diuretics can cause neurohormonal activation from rebound sodium reabsorption. Long-acting loop diuretics may prevent this occurrence (Salvador et al., 2005).

A common problem in the management of venous congestion, particularly in patients who are not diuretic naive, is diuretic resistance. Several strategies have been proposed to facilitate overcoming diuretic resistance. The addition of thiazide-like diuretics or potassium sparing diuretics in patients who fail to respond to large doses of loop diuretics is a reasonable approach to prevent enhanced sodium reabsorption in the distal tubule (Dormans and Gerlag, 1996). The addition of a mineralocorticoid antagonist like spironolactone or eplerenone in patients with a left ventricular ejection fraction <35% is also a helpful approach to overcome diuretic resistance (van Vliet et al., 1993). Some small studies suggest these medications in high doses overcome diuretic resistance in patients with ADHF without a significant effect on SCr. However, larger, randomized studies are needed.

The addition of natriuretic peptides, for example, nesiritide, is also an effective approach to overcoming diuretic resistance. Nesiritide is a recombinant form of endogenous BNP (von Haehling et al., 2013). It has been shown to rapidly reduce cardiac filling pressure, increase cardiac output, promote diuresis and suppress RAAS, and release of norepinephrine (Cheng, 2002). Some data suggest improved dyspnea symptoms with no increase in mortality or renal dysfunction in patients treated with nesiritide with acute HF.

Neurohormonal Inhibition

Diuresis causes a reflex sympathetic overactivity and activation of the renin–angiotensin–aldosterone pathway. As mentioned previously, RAAS activation leads to sodium and fluid retention, vasoconstriction, and cardiac remodeling, all of which worsen cardiac and renal function. Targeting blockade with beta-blockers, angiotensin-converting-enzyme (ACE) inhibitors, and aldosterone receptor blockers (ARBs) are routinely used in the treatment of heart failure. ACE inhibitors and ARBs are also routinely used in the treatment of CKD and should be used judiciously in patients with CRS. Electrolyte abnormalities, particularly hyperkalemia in patients with CKD, should be closely monitored with ACE inhibitors and ARBs, which may limit their use (von Haehling et al., 2013).

The timing of introduction of beta-blockers, ACE inhibitors, and ARBs in patients with ADHF or worsening renal function is a debated topic. ACE-I and ARBs can make renal function worse in patients undergoing active diuresis, and careful attention to renal function and consideration of withholding in high-risk patients is a reasonable approach (Knight et al., 1999). Similarly, beta-blockade in patients with ADHF can lead to clinical decompensation, and cautious introduction is crucial (von Haehling et al., 2013).

Role of Inotropes

Inotropic medications increase cardiac contractility and were once thought to be useful in patients with cardiorenal syndrome. However, the use of these agents is now known to be associated with higher morbidity and mortality. Their use is now reserved for patients in shock or as a bridge to cardiac transplantation (Goldberg, 1989).

Low-dose continuous dopamine (2–5 mg/kg/min) is known to increase renal blood flow via activation of dopaminergic receptors and also inhibits aldosterone, promoting sodium excretion and diuresis (Goldberg, 1989). Unfortunately, studies comparing the use of dopamine with standard treatment have shown no significant benefit, and dopamine is not currently approved for treatment of heart failure. Further assessment will be available from the ROSE trial, which is an ongoing study investigating the safety and efficacy of adjuvant low-dose dopamine compared with low-dose nesiritide versus standard diuretic therapy in patients with CKD and ADHF (HF Network: ROSE Trial, n.d.).

Emerging Therapies

New, emerging therapies to treat CRS is an area of ongoing research. One area is combining hypertonic saline with furosemide to prevent rebound sodium reabsorption and promote effective diuresis. One study has shown shorter hospital stay, decreased BNP and lower readmission rates in patients with NYHA class IV HF when compared with IV diuretics alone (Paterna et al., 2005).

Relaxin is a peptide hormone that acts on nitric oxide pathways to promote systemic and renal vasodilation. The Efficacy and Safety of Relaxin for the Treatment of Acute HF (RELAX-AHF) trial is ongoing and should provide further information on the impact on congestion, renal dysfunction and overall outcomes in patients with acute decompensated HF (Ponikowski et al., 2012). Early data from the pre-RELAX trial show improvement in dyspnea and greater weight loss compared with smaller doses of diuretics and nitrates (Teerlink et al., 2009).

Hyponatremia is a common electrolyte complication in ADHF and volume overload. This is, in part, from the actions of AVP, which causes vasoconstriction and free water reabsorption via activation of V1 and V2 receptors, respectively. The EVEREST trial sought to compare the use of vasopressin antagonists, specifically V2 receptor antagonist tolvaptan. There was no effect on morbidity and mortality between the two groups, but patients receiving oral tolvaptan did report short-term symptom benefit and weight reduction with worsening renal function compared with their standard heart failure treatment regimen (Konstam et al., 2007).

Iron supplementation is another area of ongoing research in the treatment of ADHF and CRS. The triad of anemia, renal failure, and HF multiplies the mortality rate (Attanasio et al., 2012). The FAIR-HF study has recently shown that among 459 patients with stable chronic HF, those receiving intravenous ferric carboxymaltose were more likely to report an improvement in their quality of life after 24 weeks of follow-up than those receiving placebo (Anker et al., 2009).

Summary

Cardiorenal syndrome is a complex, intertwined, and cyclic interaction between the heart and the kidney that often necessitates the multidisciplinary approach of a cardiology, nephrologist, and critical care physicians. The pathophysiological processes that cause cardiorenal syndrome highlight the intimate nature of these two organ systems. Since the initial description in 1868, significant strides have been made in the understanding and ultimately treatment of cardiorenal syndrome. However, much is still unknown, and further investigations are needed to improve the morbidity and mortality associated with this complex interaction.

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Lead-Related Complications

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Cardiovascular implantable electronic devices (CIEDs) have undergone revolutionary changes in the last decade, resulting in widespread use globally for treatment of both bradyarrhythmias as well as tachyarrhythmias, in both primary and secondary prevention (Connolly et al., 2000; Moss et al., 1996; Moss et al., 2002; Bardy et al., 2005; The Antiarrhythmics versus Implantable Defibrillators (AVID) Investigators, 1997). Cardiac resynchronization therapy is also increasing in use as one of the most effective treatments for advanced heart failure, to prevent progressive heart failure and prevent mortality (Tang et al., 2010; Moss et al., 2009). Pacemakers were the first generation of CIEDs available for implantation in humans, as early as 1958. Implantable defibrillators in the current form (transvenous) have been available since 1997. As the absolute and relative numbers of the elderly population increase, so do the use of CIEDs.

CIEDs in most instances provide life-saving therapy. If a CIED malfunctions or there is a complication, this can result in significant morbidity and/or mortality. They are implanted in the vascular system, so that infection can become a deadly problem leading to infective bacterial endocarditis. If this occurs, complete system removal is required, this may be associated with a mortality risk. The weakest link in any CIED system is the transvenous lead, that is, subject to repeated stress that can result in fracture, insulation breakdown, or other malfunction. Removal of leads can also be fraught with significant morbidity, as laser lead extraction techniques, are often required. The following review deals with lead-related as well as lead extraction-related complications.

Complications With CIEDs

Device Performance

It is well known that there is an inherent failure rate with all devices, described in reliability engineering as the "bathtub curve" (Sohail et al., 2007). The majority of these causes of failure are addressed before devices are released to the market and are identified through bench testing, which occurs prior to in vivo use. Bench testing refers to rigorous experiments replicating how a device may behave in vivo and whether it can withstand such stressors as induced by cardiac motion, shoulder movement, interaction with the bloodstream and interaction with other intravascular devices. It is well known that the number of cardiac cycles averages 100,000 times per day, up to 3.5 billion times in a lifetime. Other interactions, such as metal ion oxidation were discovered after in vivo use, resulting in improvements in bench testing. Metal ion oxidation was found to occur with leads made with polyurethane as the outer conductor. These fluoropolymers can incur micro-damage during the manufacturing process, thereby increasing susceptibility to metal ion oxidation, which is an oxidative degradation resulting from interaction of the micro-damaged polyurethane with the bloodstream. A random failure rate of a device is an accepted failure rate, but occurs at a very low rate. Such random malfunctions are reported in the form of product performance reports that are required to be released by manufacturers at regular intervals, where any abnormalities in performance are reported to the manufacturer. These are published as rates, the denominator being the number of products sold worldwide. Once a device reaches its design lifetime, the rate of "wear out" failures is seen to accelerate. Device advisories are declared if and when the known rate of random failures is exceeded, or if a systematic device flaw has been detected that requires closer monitoring.

A CIED device consists of a battery (pulse generator) that is attached to one, two, or three leads. These leads are typically placed into the right atrium, right ventricle or into a branch in the coronary sinus, in cardiac resynchronization therapy (CRT) devices. Vascular access for these leads is obtained from the subclavian or axillary veins, or through a cut-down of the cephalic vein located in the deltopectoral groove. Once the lead is appropriately placed, it is attached to the battery, which is placed in a pocket that may be submuscular or within the prepectoral fascia. Complications may arise at any stage during the implant: access to the subclavian vein

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may lead to a hemo/pneumothorax, cardiac perforation could occur from the leads once placed, coronary sinus dissection, bleeding with subsequent hematoma formation in the pocket, infection, atrial or ventricular arrhythmias. The intravascular ICD lead has always been the weakest link in the ICD system, and is associated with an increased risk of both early and late complications. Early postimplant, the majority of complications arise from lead dislodgement. Venous thrombosis, persistent pain at the site of the device, set screw dislodgement, development of a seroma, and superficial infection are other complications that may occur in the early (<30 days) peri-implant period.

Complications from initial ICD implants are not insignificant (2.5%–13%)(Tang et al., 2010; Krahn et al., 2011), increasing with complexity of device implant (single chamber < dual chamber < CRT). Initial pacemaker implants run a risk of complication of approximately 4%, primarily due to lead dislodgement (Sweeney et al., 2008). Most patients will require a pulse generator change in their lifetime due to battery depletion (typically 5–7 years for an ICD, 8–12 years for a pacemaker). In this circumstance, the major and minor complication rates are dramatically increased. These rates vary with the type of device (ICD, pacemaker, single versus dual chamber, cardiac resynchronization therapy (CRT)) and whether or not a lead revision is being performed concurrently (Poole et al., 2010). Major complications for a CRT upgrade or revision is as high as 18%, while the rates for ICDs and pacemakers were significantly lower (OR for ICD compared with pacemaker 2.38 $p = 0.004$; CRT versus pacemaker 3.68 $p < 0.001$). At the time of lead insertion, perforation of the lung or heart may occur. In a large, recent multinational study, pneumothorax occurred in 1.5% of ICD implants and cardiac perforation in a further 0.9% (**Table 1**) (Healey et al., 2010). In addition, early lead dislodgement, or loss of adequate lead sensing or pacing are among the most common device-related complications and can occur in 2%–5% of individuals; while late lead fracture or loss of function may occur in another 2%–3% of patients.

In addition to these common complications, there are other vascular complications such as deep venous thrombosis and compromise of dialysis access (**Table 1**). Transvenous ICD leads are also associated with a significant risk of developing tricuspid insufficiency (Baquero et al., 2015). Although this is often an echocardiographic finding, without evidence of right heart failure, existing studies have been too short in duration to adequately capture the potential consequences of this complication. Furthermore, there have not been any randomized trials to evaluate the effect of transvenous ICD leads on this outcome. All of these complications could be avoided (>75% reduction), if one eliminates the need to place a lead through the venous system and into the heart. There are additional common, early complications which are less directly related to the presence of a transvenous lead, including device-related infection, wound hematoma, death stroke, and myocardial infarction (**Table 1**) (Krahn et al., 2011; Healey et al., 2010).

Infectious complications and lead issues are the most concerning and the ones that are fraught with the greatest risk. Device infection, occurs in about 2% of all cases, and is a serious and potentially catastrophic consequence of these procedures (Gould and Krahn, 2006; Sohail et al., 2007; Klug et al., 2007). Device infection rates are increasing (Greenspon et al., 2011) and infection is more common in patients undergoing generator replacement or having more complex procedures where the infection risk is 2%–3%. Device Infection is costly as it is almost always managed with hospitalization with long courses of intravenous antibiotics (Gula et al., 2008; Paulin et al., 2008). System extraction is usually required and is associated with major complications (**Table 2**) (Byrd, 2007; Wilkoff et al., 2009; Wazni and Wilkoff, 2016). A 2008 study of 4.2 million device patients in the USA reported a cost of $146,000 per device system infection, and the risk of death associated with device infection in that study was 4.69% (Greenspon et al., 2011). The major risk factor for device infection is number of prior procedures on the pocket, so that minimizing repeated procedures, due to lead dislodgement, for example, is a major goal to prevent device infection. Given the significant issues surround device infection, there is a randomized clinical trial being conducted in Canada to try and improve outcomes (Prevention of Device Infection (PADIT) NCT01613092).

Lead issues provide an even greater level of complexity. Pacemaker lead failures can lead to loss of pacing which may be life-sustaining. ICD lead failures can lead to not only loss of pacing, but also inappropriate ICD shocks and/or ineffective treatment of

Table 1 Estimated incidence of complications due to pacemaker and ICD transvenous leads

Complication	*Estimated Rate*
Early avoidable, lead-related complications	
Pneumothorax/hemothorax (Healey et al., 2010; Armaganijan et al., 2012; Bernard et al., 2012)	1.5% (30 days)
Cardiac perforation, effusion, tamponade, pericarditis (Healey et al., 2010; Lee et al., 2010; Olde Nordkamp et al., 2016; Kleemann et al., 2007; Russo et al., 2015)	1.0%
Lead dislodgement, loss of sensing/pacing (Armaganijan et al., 2012; Lee et al., 2010; Olde Nordkamp et al., 2016; Kleemann et al., 2007; Russo et al., 2015)	3.0%
New, severe tricuspid insufficiency (Sadreddini et al., 2014)	3%
Ipsilateral upper extremity DVT	0.3%
Need to revise dialysis access	0.2%
Total	*9%*
Other early complications	
Death (Healey et al., 2010)	0.6%
Myocardial Infarction (Healey et al., 2010)	0.1%
Stroke (Healey et al., 2010)	0.2%
Significant wound hematoma (Healey et al., 2010)	2.3%
Device-related infection (Healey et al., 2010; Armaganijan et al., 2012)	1.3%

Table 2 Complications from laser lead extraction

Publication	*Number of leads*	*Major complications (%)*	*Procedural mortality (%)*
Byrd (2007)	2561	1.9	0.8
LExICon study (Wazni et al., 2010)	2405	1.4	0.28
Brunner et al. (2014)	5521	1.8	0.4
Epstein et al. (2013)	2274	0.82	0.31
Bongiorni et al. (2008)	2062	0.7	0.3

Table 3 Major complications: Lead extraction versus lead abandonment in two ICD lead advisories (Parkash et al., 2010, 2016)

	Lead abandonment		*Lead extraction*	
Lead advisory	*Major complications (%)*	*Death (%)*	*Major complications (%)*	*Death (%)*
Sprint Fidelis ($n=468$)	5	0.5	7.3	0.4
Riata ($n=496$)	5.2	0	18.6	1.2



Fig. 1 This demonstrates cable externalization due to inside-out abrasion in a Riata lead under advisory. This lead was removed by laser lead extraction. Courtesy of A. Krahn and R. Yee.

dangerous ventricular arrhythmias. Inappropriate diagnosis of lead failure can also lead to unnecessary lead replacement resulting in unnecessary morbidity or death. Transvenous lead design has had significant improvements over time, including the evolution of materials from silicone rubber to polyurethane, combinations of materials and the creation of copolymers such as Optim, which provides the benefits of silicone rubber (inert, biocompatible) and polyurethane (high tear strength) (Epstein et al., 2009). Lead-to-can insulation defects were previously primarily observed with abdominal implants. Silicone leads are highly durable but are known to have a lower tear strength and ultimate tensile strength than polyurethane. This property led to the development of protective sheaths using thinner coatings of materials with higher tensile and tear strength, to prevent degradation and abrasion.

Advisories affecting pacing or high voltage leads pose a very different clinical management scenario than those affecting the pulse generator alone. Revision of leads with or without a pulse generator change is known to confer an increased risk of complications, as reported in the REPLACE registry where it was associated with a risk as high as 18.7% (Poole et al., 2010). Lead *removal* is not easy to perform, as compared to generator *removal*, with significant morbidity and mortality associated with it; lead *addition* may also be wrought with difficulties, may also have long-term consequences of venous thrombosis, or obstruction, necessitating greater complexity if the need for extraction arises. The Canadian Heart Rhythm Society Device Committee has conducted two large cohort studies examining complications from lead revision due to a lead advisory (**Table 3**), the risks attributable to lead revision and lead extraction are shown in **Table 2** from each of these advisory cohorts (Parkash et al., 2010, 2016). If the lead was abandoned rather than removed, the risk of complications was significantly reduced in both advisory cohorts.

Use of lead extraction versus lead abandonment must take into account several factors including age and clinical status of the patient, comorbidities, number of leads in place, vascular issues and lead model and duration of lead implantation. The Riata lead advisory has proven to be an especially challenging lead to extract given the propensity of this lead to undergo inside-out insulation degradation, resulting in cable externalization, and "snow-plowing" of the lead during extraction attempts (**Fig. 1**) (Parkash and Bennett, 2016). In the case of a lead advisory, careful weighing of risks and benefits is required to prevent harm to patients. In certain

circumstances, such as device infection, total system removal may be unavoidable. Even in the case of infections, the use of laser lead extraction needs to be assessed carefully due to the possibility of life-threatening complications, including superior vena cava and/or right ventricular perforation. Most reports of complications due to laser lead extraction are from high-volume, experienced centers, hence their results may not extrapolate to all centers with this technology available (**Table 2**). Attempts to improve laser lead extraction are underway, with novel technology that may assist in prevention of these life-threatening complications.

Conclusion

Technology is advancing rapidly to eliminate some of the issues we are faced with respect to transvenous leads, however, there are significant limitations with leadless technology as well. Overall, the future is bright for leadless technology, but advances are required to ensure safety and efficacy for patients who require life-saving pacing or defibrillation therapy.

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Left and Right Ventricular Remodeling

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Left Ventricular Remodeling

Molecular Basis

Ventricular remodeling encompasses alterations in ventricular mass, shape, and geometry as a result of an increased hemodynamic load, neurohormonal activation, and genetic factors (Mann, 1999; Cohn et al., 2000). As a result of stressful stimuli, the failing myocardium undergoes cellular changes such as myocyte hypertrophy, decreased myocyte viability, fibroblast proliferation, increased collagen, and fibrosis. These effects manifest as changes in the geometry of the heart, leading to progressive ventricular dysfunction. Pressure and volume overload are the two major inciting stressors that trigger the left ventricular (LV) remodeling process. In the case of pressure overload conditions such as that seen in aortic stenosis or arterial hypertension, the left ventricle has a higher level of resistance to overcome during systole. In conditions where the LV is subjected to handling higher volumes during diastole as in aortic or mitral valve insufficiency, the LV diastolic wall stress increases. Regardless of the type of LV stressor, the consequence is an increase in size of the cardiac myocyte, which is termed LV hypertrophy (LVH). The underlying mechanism of these changes is thought to involve matrix–integrin interactions that are able to activate and/or modify a complex intracellular signaling pathway involving multiple kinases and transcription factors (Heineke and Molkentin, 2006). Intricately connected to this pathogenic process is the role of the renin-angiotensin-aldosterone system (RAAS), namely angiotensin II, which promotes fibroblast activation and potentiates maladaptive LV remodeling (Ahokas et al., 2003; Patel and Mehta, 2012). This chain of events is triggered by the mechanical deformation of cardiac myocytes that occurs with increased ventricular wall stress.

The pattern of LVH differs between that seen in pressure overload and volume overload states. In pressure overload states, there is a parallel addition of sarcomeres (the contractile elements of the myocyte) resulting in overall thickening of the LV, or concentric hypertrophy. Conversely, sarcomeres are added in series when volume overload is the primary LV stressor. This results in lengthening of the cardiac myocyte along with dilatation of the LV and is termed eccentric hypertrophy.

Neurohormonal activation plays a large role in disease progression and the remodeling process. Increasing ventricular wall stress activates the RAAS system, leading to myocardial hypertrophy. The increased level of angiotensin II and aldosterone further promote interstitial remodeling. Angiotensin II was found to increase DNA synthesis in myocardial fibroblasts and increase protein synthesis in both fibroblasts and myocytes. It mediates cell proliferation and growth in response to cellular stretch. In addition, Angiotensin II increases diffusion of growth factors into the myocardial interstitium. Angiotensin II has also been shown to elicit cytotoxic effects on cardiomyocytes leading to necrosis and fibrosis. Increased aldosterone production as a result of RAAS activation stimulates myocardial fibroblasts to synthesis collagen (Brilla et al., 1994). Additionally increased aldosterone has an effect on energy balance that leads to myocyte death (Sheehan and Seelig, 1984). Catecholamine activation also contributes to the process of remodeling.

Myocardial hypertrophy is stimulated by alpha-adrenergic stimulation. Chronic beta-adrenergic stimulation occurs in heart failure (HF) that overtime suppresses beta-adrenergic receptors and augments $G_i\alpha$ protein.

Additional vasoactive peptides such as endothelin, cytokines, and nitric oxide also influence remodeling. Endothelin exerts strong vasoconstrictive properties and is increased in HF. Blockade of endothelin production in animal models of HF has shown to slow the progression of HF. Tumor necrosis factor (TNF) and interleukins are cytokines produced by cells in response to stressful stimuli. Levels of TNF and interleukins are elevated in patients with HF. The elevation of these cytokines has been found to be associated with activation of RAAS. The SOLVD study found an inverse relationship between the increase in TNF and interleukins and clinical decline.

The cardiac myocyte is directly involved in the remodeling process. Cytoskeletal changes of the cardiac myocyte are associated with decreased contractile function in cardiac hypertrophy. In the early reversible stage of hypertrophy, cytoskeletal proteins accumulate to counteract increased myocyte stress. Mitochondria increase in number and decrease in size. As the stress continues, further changes take place that become irreversible. The loss of contractile material and proteins of the sarcomeric skeleton characterize this second stage. Concurrently, there is an increase in desmin and tubulin to compensate for the loss of cellular integrity. The result is a disorganized arrangement of the sarcomeres and disruption of Z bands leading to less effective contraction.

In the failing cardiomyocyte, excitation–contraction coupling is impaired. Normally, calcium influx into the myocyte triggers additional calcium release from the sarcoplasmic reticulum (SR). The released calcium then reenters the SR through the calcium ATPase, the SERCA2a. In diseased myocytes, SERCA2a number is reduced. This leads to high cytosolic calcium and low SR calcium, leading to decrease in calcium transit during contraction. Additionally, increased activity of calcium/calmodulin-dependent protein kinase II in HF leads to leaky ryanodine receptors, further worsening the decrease in calcium transient and contraction.

Another important change is in the phenotype of the failing myocyte. In normal conditions, the expression of myosin heavy chain (MHC) gene is predominantly α-MHC. α-MHC has high ATPase activity and promotes faster shortening velocity of cardiac myofibers. In HF, there is a return to fetal gene expression where α-MHC expression is downregulated and β-MHC is upregulated (Lowes et al., 1997). β-MHC has less ATPase activity and has been shown to correlate with the degree of cardiac hypertrophy.

Fibroblasts in the interstitial compartment modify the volume and composition of the extracellular matrix (ECM). Myocytes are tethered and supported by the connective tissue network formed from fibrillary collagen. Fibroblasts are responsible for the production and resorption of collagen. Fibroblasts are activated in response to ischemic stimuli. Stimulation leads to collagen synthesis and increased fibrosis with effects on ventricular size, structure, and stiffness (Hudlicka et al., 1992). Matrix metalloproteinases degrade ECM proteins and further contribute to ventricular remodeling.

LV Remodeling Effect on Heart Failure Clinical Outcomes

A key early prospective study assessed risk factors for mortality in 36 patients with dilated cardiomyopathy utilizing echocardiography data and demonstrated the significant clinical consequences of pathologic LV remodeling. The investigators found a statistically significant association between an increased degree of spherical LV geometry, as reflected by a higher ratio of short- to long-axis end-diastolic dimensions, and mortality (Douglas et al., 1989). Their other major finding was that smaller baseline LV end-diastolic short-axis dimensions conferred a survival advantage. This was substantiated in a later study assessing patients with decreased LV ejection fraction (LVEF) and symptomatic HF, where it was demonstrated that LV end-diastolic dimension (LVEDD) index was an independent predictor of survival (Lee et al., 1993). Echocardiographic parameters such as LV volume, mass, and ejection fraction have been found to be strong predictors of clinical outcomes (St John Sutton et al., 1997). Even in patients without prior myocardial infarction or HF, a large study consisting of 4744 patients, followed longitudinally over 11 years, found that increased LV end-diastolic and LV end-systolic dimensions were independent risk factors for developing HF (Vasan et al., 1997) with risk factor-adjusted hazard ratios (HR) of 1.47 (95% confidence interval [CI]: 1.25–1.73) and 1.43 (95% CI: 1.24–1.65), respectively (**Fig. 1**).

More recently, the predictive value of LV mass and size was further demonstrated in the Multi-Ethnic Study of Atherosclerosis (MESA) which included 5098 participants who underwent baseline cardiac MRI and followed for a median of 4 years (Bluemke et al., 2008). In this study, a strong association between increased LV mass and incident HF was demonstrated (**Fig. 2**). A later analysis also based on the MESA cohort found that participants with LV dilation (defined as LVEDD > 52 mm) had an increased risk for incident HF compared with those with normal LV size (HR: 2.4; 95% CI: 2.0–5.3) (Yeboah et al., 2014) (**Fig. 3**). Furthermore, a recent study retrospective cohort study (Phan et al., 2016) assessing the risk of sudden cardiac arrest (SCA) with distorted LV geometry in patients with LVEF ≤ 40% and without an implanted cardioverter-defibrillator found that eccentric LVH (elevated LV mass index and normal relative wall thickness (RWT)) was independently predictive of SCA (odds ratio [OR]: 2.15; 95% CI: 1.08–4.29).

Adverse LV remodeling has traditionally been classified as either concentric or eccentric LVH on the basis of increased RWT and LV mass as defined by the American Society of Echocardiography guidelines (Lang et al., 2015) (**Fig. 4** and **Table 1**). While this remains to be the most commonly used schema, a recent prospective analysis of 3,181 elderly participants with predominantly preserved LVEF and without previously diagnosed HF from the Cardiovascular Health Study assessed the prognostic implications of a novel LV geometry grading scheme (**Fig. 4**) that combines LV enlargement (defined as LVEDD > 56 mm), hypertrophy, and concentric remodeling in a sequential fashion (Zile et al., 2014). The incorporation of LV enlargement into this framework proved to be significant as an additive risk of incident HF and death was demonstrated when patients met criteria for both LV enlargement and hypertrophy. This study reaffirmed the prognostic importance of LV enlargement and hypertrophy for incident HF and mortality. Despite preserved LVEF in most of the participants, these alterations in LV structure were statistically significant predictors for both incident HF and death.

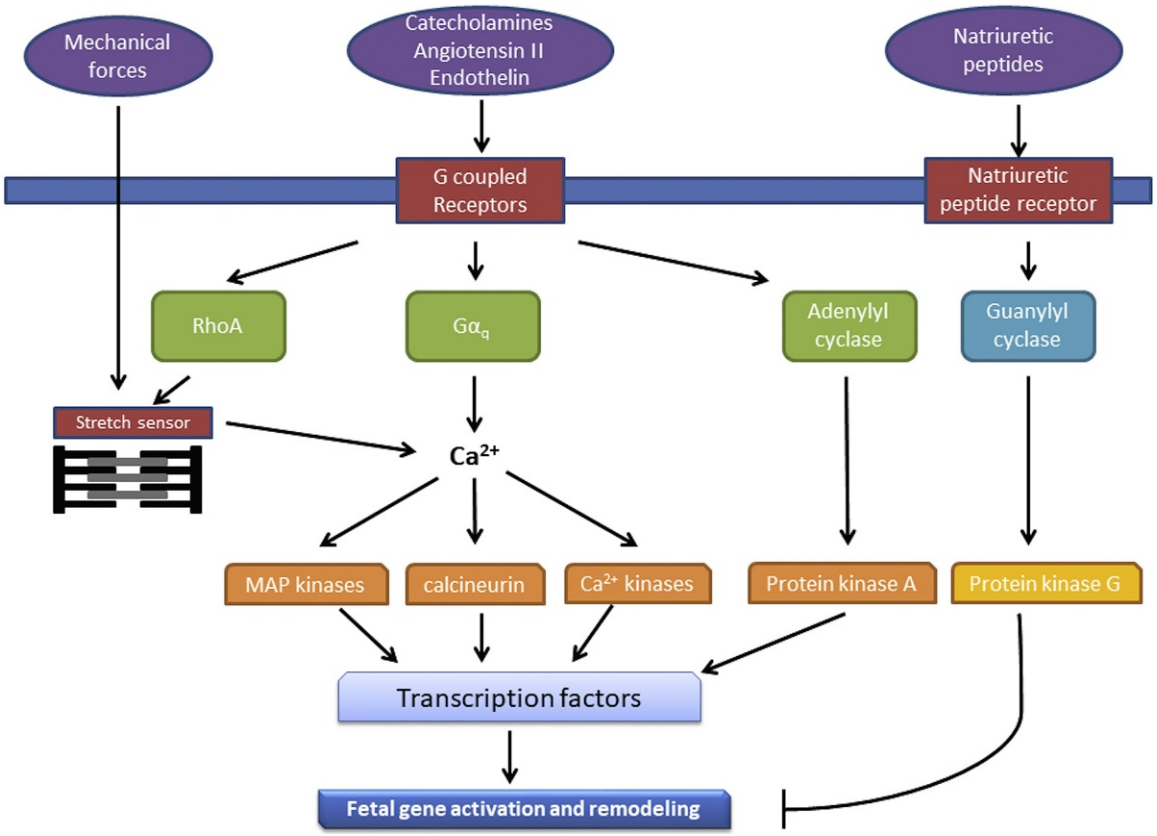


Fig. 1 Simplified diagram of pathways involved in LV remodeling. Mechanical stresses are sensed by stretch receptors and sarcomeric stretch-activated mechanisms. Neurohumoral stress mediators, such as catecholamines, angiotensin II, and endothelins, are sensed by membrane-bound receptors. These receptors signal through G proteins such as $G\alpha_q$, $G\alpha_x$, Rho proteins, and adenylyl cyclase. This affects calcium release and activates downstream phosphatases and kinases. Ca^{2+} kinases and MAP kinases play a role in cellular growth. Calcineurin acts as a key transducing phosphatase. Protein kinase A regulates myocyte contractility. Protein kinase G plays a role in anti-hypertrophy. The activation of these and other signals together affects downstream fetal gene activation and LV remodeling. Adapted with permission from Kehat, I., Molkentin, J. (2010). Molecular pathways underlying cardiac remodeling during pathophysiological stimulation. *Circulation* **122**, 2727–2735; Wolters Kluwer Health, Inc. https://doi.org/10.1161/circulationaha.110.942268.

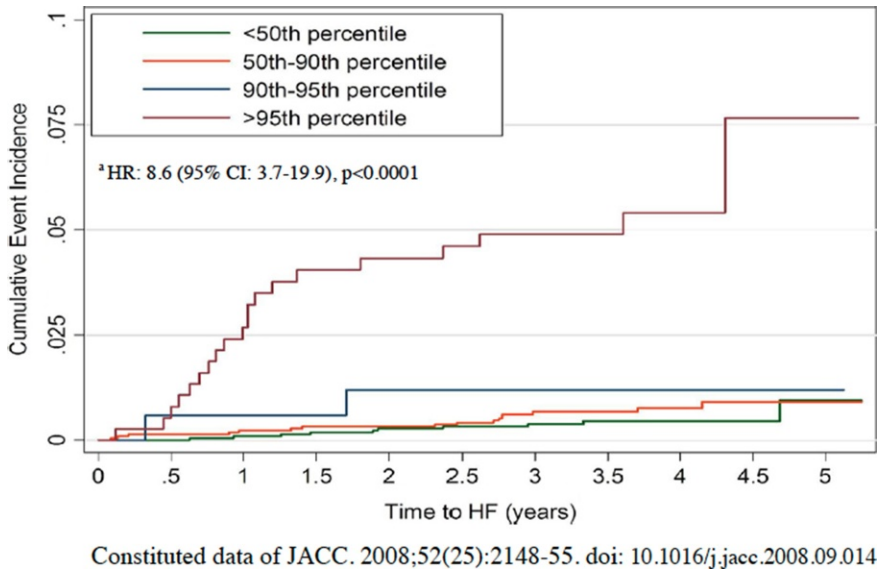
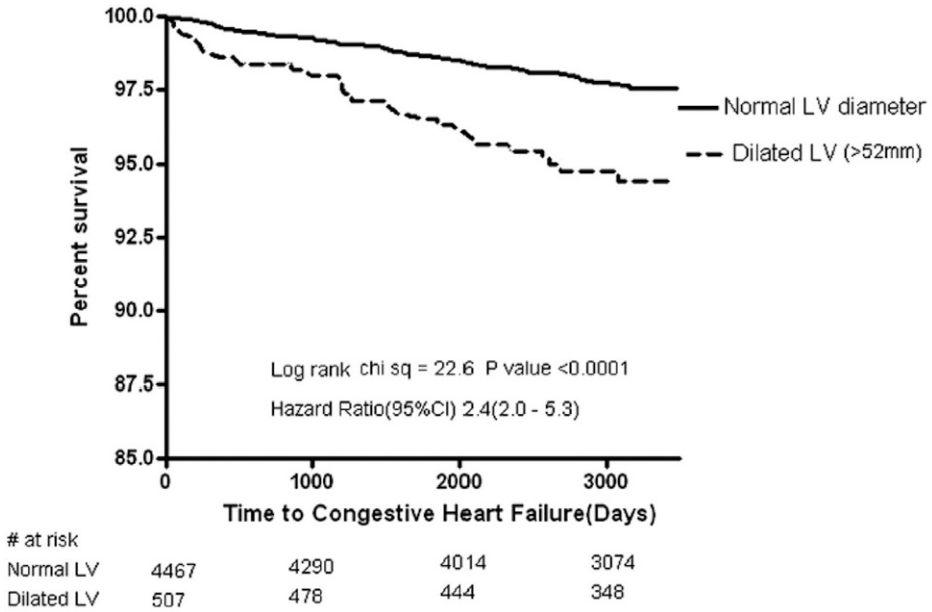


Fig. 2 Cumulative event rates for HF events by intervals of LV mass (body size-adjusted). To examine the relative risk, four categories of LV mass index were compared with the reference category represented by below the 50th percentile of LV mass index. [a]Refers to the hazard ratio (HR) for incident HF in participants with LVH (>95th percentile) versus those below the 50th percentile for LV mass. Constituted data of Bluemke, D. A., Kronmal, R. A., Lima, J. A., et al. (2008). The relationship of left ventricular mass and geometry to incident cardiovascular events: The MESA (Multi-Ethnic Study of Atherosclerosis) study. *Journal of the American College of Cardiology*, **52**(25), 2148–2155. https://doi.org/10.1016/j.jacc.2008.09.014.



Constituted data of J Card Fail. 20(12), 905-11. doi: 10.1016/j.cardfail.2014.09.002

Fig. 3 Kaplan-Meier curves showing the event-free survival for participants with and without dilated left ventricle (LV) for incident HF in the MESA. Constituted data of Yeboah, J., Bluemke, D A., Hundley, et al. (2014). LV dilation and incident congestive heart failure in asymptomatic adults without cardiovascular disease: Multi-Ethnic Study of Atherosclerosis (MESA). *Journal of Cardiac Failure* **20**(12), 905–911. https://doi.org/10.1016.j.cardfail.2014.09.002. Reprinted with permission.

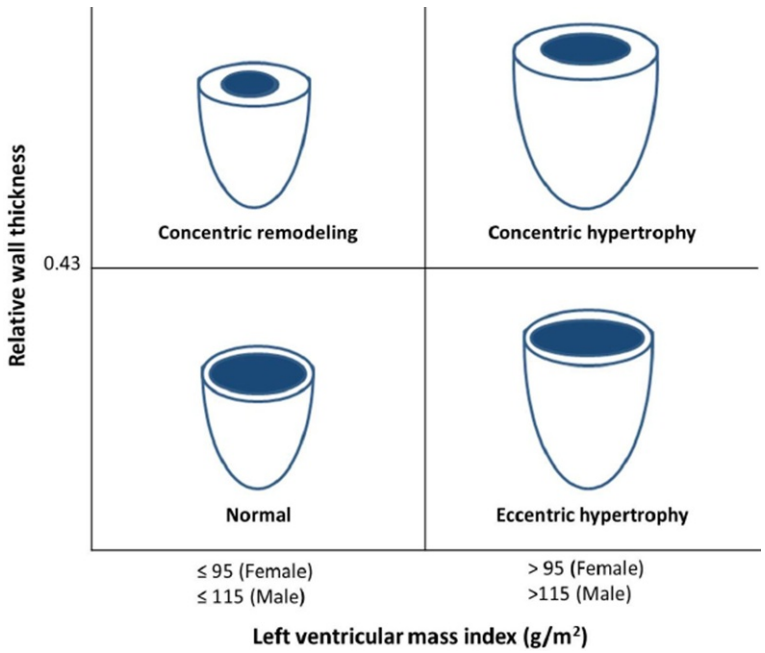


Fig. 4 Abnormal LV geometry in remodeling. The VALLIANT trial used measurements of left ventricular mass index (LVMi) and relative wall thickness (RWT) to define these patterns of LV geometry in patients with HF and ejection fraction $\leq$35%. Concentric remodeling with normal LVMi and increased RWT; eccentric hypertrophy with increased LVMi and normal RWT; and concentric hypertrophy with increased LVMi and increased RWT. Adapted with permission from Verma, A., et al. (2008). Prognostic implications of left ventricular mass and geometry following myocardial infarction: The VALLIANT (VALsartan In Acute myocardial iNfarcTion) Echocardiographic Study. *JACC: Cardiovascular Imaging* **1**(5): 582–591. https://doi.org/10.1016/j.jcmg.2008.05.012.

Table 1 Selected equations in echocardiography for LV geometry assessment

Parameter	*Formula*	*Normal Values*[a]
LVEF (%)	$\text{LVEF} = \frac{\text{LVEDV} - \text{LVESV}}{\text{LVEDV}} \times 100\%$	Male: $62 \pm 5\%$ Female: $64 \pm 5\%$
LV Volume, Simpson's method of discs	$\text{LV Volume} = \sum^{n} A_i T$ A = area; T = thickness of each of the n slices	
LVEDVI (mL/m^2)		Male: 54 ± 10 mL/m^2 Female: 45 ± 8 mL/m^2
LVESVI (mL/m^2)		Male: 21 ± 5 mL/m^2 Female: 16 ± 4 mL/m^2
LVID_d (mm)		Male: 50.2 ± 4.1 mm Female: 45.0 ± 3.6 mm
LVID_s (mm)		Male: 32.4 ± 3.7 mm Female: 28.2 ± 3.3 mm
LV mass index, linear method (g/m^2)	$\text{LV mass index} = 0.80 \times \left[1.04(\text{IVS}_d + \text{PWT}_d + \text{LVID}_d)^3 - \text{LVID}_d^3\right] + 0.6 \,/\text{BSA}$	Male: 49–115 g/m^2 Female: 43–95 g/m^2
LV mass index, 2D method based (g/m^2)	LV mass index $= (5/6 \times A_m \times 0.5 \times L \times 1.055)/\text{BSA}$; where A_m = short-axis myocardial area at the midlevel; L = LV length	Male: 50—102 g/m^2 Female: 44—88 g/m^2
Relative wall thickness (cm)	$\text{RWT} = 2\text{PWT}_d/\text{LVID}_d$ cm	Male: 0.24—0.42 cm Female: 0.22–0.42 cm
Sphericity Index (%)	$\text{SI} = \text{LVEDV}/(4/3) \times \pi \times r\hat{3} \times 100\%$; where $r = 0.5 \times$ LV length at end diastole	Gender—neutral: $26 \pm 3\%$
Strain (%)	Strain $= (L_t - L_0)/L_0$; where L_t = myocardial length at time t, L_0 = initial myocardial length at time 0	
Global longitudinal strain (%)	GLS $= (\text{MLs} - \text{MLd})/\text{MLd}$; where MLs = myocardial length at end-systole; MLd = myocardial length at end diastole	Gender—neutral: -19.7%

[a]Normal values represented as mean ± standard deviation (SD) or range.

BSA, body surface area; IVS_d, intraventricular septal thickness at end diastole; *LVEDV*, left ventricular end-diastolic volume; *LVEDVI*, left ventricular end-diastolic volume index; *LVEF*, left ventricular ejection fraction; *LVESVI*, left ventricular end-systolic volume index; *LV*, left ventricle; $LVID_d$, LV internal dimension at end diastole; $LVID_s$: LV internal dimension at end-systole; PWT_d: posterior wall thickness at end diastole; *RWT*, relative wall thickness; *SI*, sphericity index.

Constituted data of Lang, R. M., Badano, L. P., Mor-Avi, V., et al. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *European Heart Journal of Cardiovascular Imaging* **16**(3), 233–270.

Imaging Modalities for Assessing Ventricular Remodeling in HF

Transthoracic Echocardiography

Two-dimensional echocardiography

Echocardiography utilizes a transthoracic probe that emits ultrasound waves directed at cardiac structures that in turn reflect these ultrasound signals back to the probe. The computer within the ultrasound machine uses an algorithm to reconstruct images of the heart based on characteristics of the returning signal. In the clinical setting, two-dimensional (2D) transthoracic echocardiography (2DE) remains to be the predominant imaging modality for assessing ventricular structure and function in HF patients. It has the capability to provide valuable information on LV geometry and function while offering a high degree of safety, accessibility, and ease of use. Quantitative assessment of LV volumes, mass, geometry, and ejection fraction with 2DE allows clinicians to recognize the progression of adverse LV remodeling in HF patients (**Fig. 5** and **Table 1**). **Table 1** outlines the mathematical basis for these and other relevant parameters as recommended by the most recent American Society of Echocardiography guidelines (Lang et al., 2015). Limitations regarding the reproducibility and accuracy of serial measurements with conventional 2DE have been described on the basis of findings in a recent meta-analysis involving studies on echocardiographic surveillance in patients receiving cardiotoxic chemotherapy (Thavendiranathan et al., 2013).

Speckle-tracking echocardiography

More sensitive measurements can be obtained using newer techniques such as speckle-tracking echocardiography, which involves averaging of myocardial velocities in adjacent segments compared to a single measurement based on a single tracing using conventional 2DE (Sawaya et al., 2011). This allows for the calculation of myocardial strain which is defined as the change in length of a segment of myocardium divided by the segment's resting length and is based on quantitative data on global and regional myocardial deformation obtained on 2DE and the use of a computer algorithm (Amundsen et al., 2006). Strain imaging has been shown to detect early signs of LV remodeling in asymptomatic patients at risk for HF such as patients with diabetes and

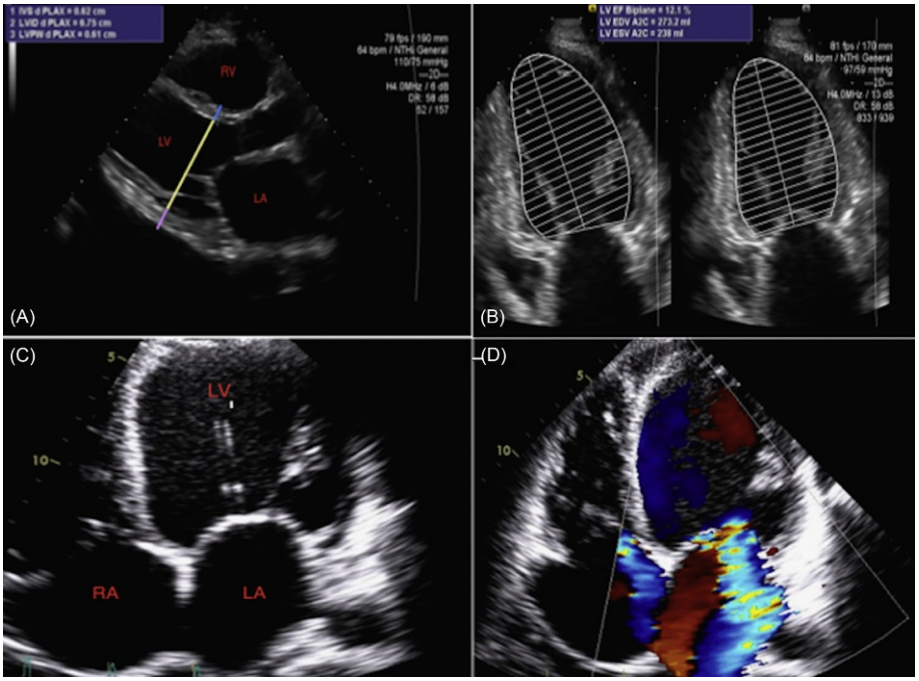


Fig. 5 Echocardiographic assessment of adverse LV remodeling. (A) Transthoracic echocardiogram of the parasternal long-axis view at end diastole showing severe LV dilation of a 58-year-old male with a history of HFrEF secondary to ischemic cardiomyopathy (*yellow line* = LV end-diastolic dimension, *blue line* = interventricular septal thickness, *purple line* = posterior wall thickness). This patient developed end-stage HF and successfully bridged to heart transplantation with home inotrope support 3 months after this study. (B) Apical two-chamber views at end diastole (left) and end systole (right) showing the traced endocardial borders for calculation of biplane LV systolic (238 mL) and diastolic (273.2 mL) volumes and LV ejection fraction (12.1%) in a 43-year-old male with advanced HFrEF secondary to idiopathic nonischemic cardiomyopathy (C) Apical 4-chamber view at end diastole of the same patient showing a severely dilated LV and severe biatrial enlargement. (D) Severe eccentric mitral regurgitation (MR) during systole detected with color flow Doppler in the same patient as in B and C. Secondary MR as seen in this patient is commonly observed in those with severely reduced LVEF and significant LV dilation because the distorted LV geometry with papillary muscle separation results in abnormal coaptation of the mitral valve leaflets and MR.

hypertension. In a prospective study involving 154 type 2 diabetic patients who were followed for 3 years, those with decreased longitudinal systolic strain at baseline were at higher risk of developing adverse LV remodeling (defined as the absence of a decrease in LV end-systolic volume over time and higher RWT) compared to patients with normal longitudinal systolic strain on speckle-tracking echocardiography (Ernande et al., 2014). A preclinical animal study assessing the changes in LV architecture and function associated with systemic hypertension found a striking link between decreased longitudinal myocardial strain and histologic patterns such as myocyte hypertrophy transmurally along with subendocardial fibrosis consistent with adverse LV remodeling (Ishizu et al., 2014). On the basis of their findings, the investigators concluded that longitudinal strain may serve as a surrogate of subendocardial fibrotic changes and may be useful for risk stratification of hypertensive HF.

Three-dimensional echocardiography

Real-time 3D echocardiographic (3DE) image acquisition is a newer imaging modality that is advantageous compared to conventional 2DE because no geometric assumptions are used and it has been shown to be more accurate (using cardiac magnetic resonance (CMR) as the reference standard) and reproducible in the assessment of ventricular chamber size and morphology (Jenkins et al., 2004). A later study calculated LV remodeling index (LVRI), defined as the 3DE-derived ratio between LV mass and LV end-diastolic volume, to describe various patterns of LV remodeling and found significantly lower values of LVRI in patients with dilated cardiomyopathy and significantly higher values in patients with hypertrophic cardiomyopathy (De Castro et al., 2007). Limitations of 3DE include image-quality dependence, lower volume of published data on reference values, and lower temporal resolution. However, 3DE appears to be a promising tool as part of multimodality cardiovascular (CV) imaging for the evaluation of LV remodeling.

Cardiac Magnetic Resonance

High-quality images of the heart can be obtained with CMR by utilizing a strong magnet, which is usually 1.5 or 3.0 Tesla (1 Tesla is equivalent to 20,000 times the strength of the earth's magnetic field). In addition to this main static magnetic field, the other main components of the CMR system are the magnet gradient coils along with radiofrequency transmitter and receiver coils. Gradient coils exist in three different axes and this accounts for the three-dimensionality of CMR. The gradient coils cause slight distortions in

the magnetic field by generating magnetic fields of varying strengths at a given position depending on which gradient coil is used. Positively charged protons mainly from water are aligned in the direction of the magnetic field when placed in the bore of the magnetic field. The transmitting radiofrequency coils act on these protons causing them to transition to a higher energy state. This is followed by a relaxation phase where the protons return to their baseline energy state. A signal is emitted during this relaxation phase that consists of two components, T1 and T2 relaxation. The magnetic gradients produced by the aforementioned gradient coils are applied across the cardiac structure of interest to localize the signals emitted during the relaxation phase. Differences in relaxation properties between extracellular fluid, blood, and different tissues account for the contrast in the final image. Of particular importance when using CMR in HF patients, regions of myocardial tissue that may have undergone adverse LV remodeling also have different relaxation properties compared to normal myocardial tissue. The radiofrequency receiver coil collects these signals that are then transformed into data ultimately used to create these images (**Fig. 6**). A more detailed discussion of the underlying physics and protocols involving CMR is outside the scope of this article but additional resources can be found in the Further Reading section at the end.

The high degree of spatial and temporal resolution, visualization of the endocardial border, and reproducibility provided by CMR have made it widely acknowledged as the reference imaging modality when quantifying LV dimensions, mass, and ejection fraction (Konstam et al., 2011). CMR has also emerged as the ideal noninvasive imaging choice in clinical trials investigating LV remodeling because fewer study participants are required as a result of its outstanding reproducibility and accuracy (Bellenger et al., 2000). The duration of a standard CMR study is roughly 45 minutes and scans are performed in a series of 5 to 15 second breath holds to optimize image quality. An important component of the standard CMR protocol worth noting is the use of intravenously administered gadolinium-based contrast agents to identify regions of myocardial fibrosis. The presence of late gadolinium enhancement (LGE) as seen in **Fig. 6F** accurately identifies the location and extent of myocardial scarring/fibrosis as evidenced by validation against histologic analysis (Kim et al., 1999). The significant clinical impact of CMR in HF patients was demonstrated in a prospective study involving 150 HF patients where 52% of patients had a change in management and 30% of patients had a new diagnosis on the basis of CMR findings (Abbasi et al., 2013). In total, the investigators found that CMR made a significant impact on diagnosis, management, and medical decision-making in 65% of HF patients. Furthermore, the presence of LGE on CMR was found to be a significant independent predictor of producing a significant clinical impact in a multivariable regression model (OR 6.72, 95% CI: 2.56–17.60, P = 0.0001).

In the HF patient population, signs of adverse LV remodeling represented mainly geometric distortions and presence of LGE on CMR as illustrated in **Fig. 6** provide powerful prognostic data on clinical outcomes. A prospective longitudinal study of 472 patients previously diagnosed with nonischemic dilated cardiomyopathy followed for a median of 5.3 years in the UK found that after

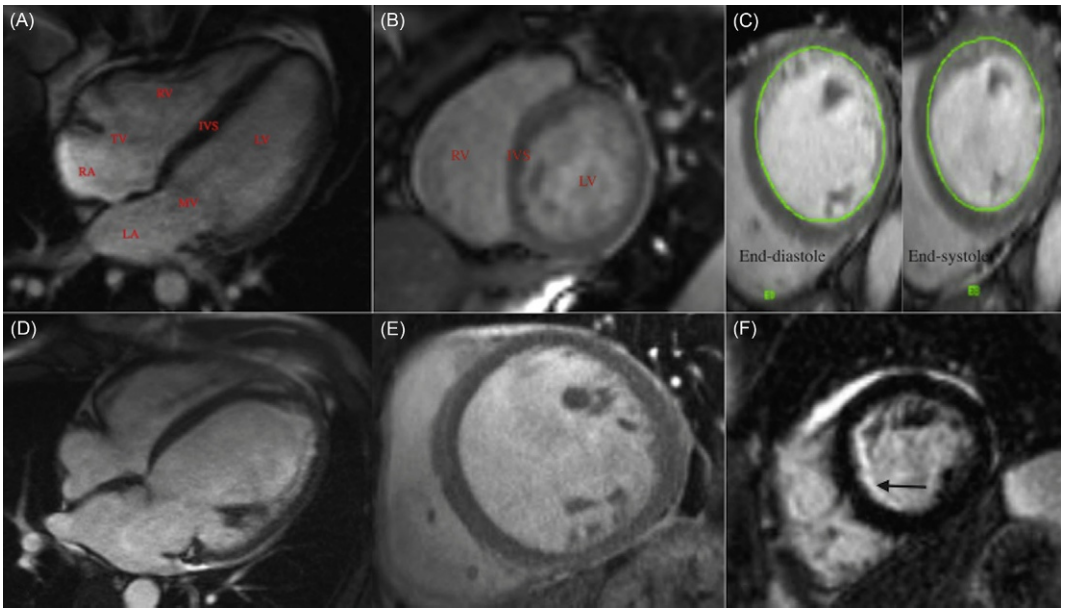


Fig. 6 Selected cardiac MR (CMR) images demonstrating LV remodeling in HF patients. (A) Single-slice four chamber CMR of a normal subject without HF. Note the high degree of soft tissue contrast that allows for clear visualization of intracardiac versus extracardiac structures. (B) End-diastolic short-axis view of a normal heart. (C) Accurate LV dimensions and LVEF can be obtained using contour drawing on short-axis CMR. (D) Significant LV remodeling demonstrated on this four chamber CMR view of a patient with chronic HFrEF. Here a more spherical LV architecture is seen compared to the ellipsoid shaped LV in image A. (E) CMR end-diastolic short-axis view demonstrating a severely dilated (LVEDD = 80 mm) failing LV (LVEF = 8.6%) in a 46 year old patient with significant history of alcohol abuse newly diagnosed with nonischemic cardiomyopathy. (F) Short-axis view using late gadolinium enhancement (LGE) during CMR study of a 64-year-old female cancer survivor with prior chemoradiation exposure presenting with progressive shortness of breath. Diffuse LGE at the mid-septal wall (*black arrow*) was demonstrated and correlates to myocardial fibrosis at this site secondary to a nonischemic etiology. *IVS*: interventricular septum; *LA*: left atrium; *LV*: left ventricle; *MV*: mitral valve; *RA*: right atrium; *RV*: right ventricle; *TV*: tricuspid valve.

adjustment for LVEF and other conventional risk factors, both the presence of fibrosis (HR: 2.43; 95% CI: 1.50–3.92; $P<.001$) and the extent of fibrosis (HR: 1.11; 95% CI: 1.06–1.16; $P<.001$) were independently and incrementally associated with all-cause mortality (Gulati et al., 2013). The presence of myocardial fibrosis was also independently associated with CV mortality or cardiac transplantation (HR: 3.22; 95% CI: 1.95–5.31; $P<.001$) as well as sudden cardiac death (SCD) or aborted SCD (HR: 4.61; 95% CI: 2.75–7.74; $P<.001$).

The high prognostic value of LGE on CMR in patients with nonischemic cardiomyopathy was further demonstrated in a meta-analysis where pooled data from nine studies (eight prospective, one retrospective design) comprising a total of 1488 patients were examined (Kuruvilla et al., 2014). The primary end points included all-cause mortality, HF hospitalization, and a composite end point of SCD or aborted SCD. Mean duration of follow-up was 30 months. Mean LVEF as determined with CMR was 37%. LGE was identified in 38% of patients and patients with LGE had increased overall mortality (OR: 3.27; 95% CI: 1.94–5.51; $P<0.00001$), HF hospitalization (OR: 2.91; 95% CI: 1.16–7.27; $P=0.02$), and SCD/aborted SCD (OR: 5.32; 95% CI: 3.45–8.20 $P<0.00001$) compared with those without LGE. Furthermore, the annualized event rates for mortality was higher in those with LGE at 4.7% versus 1.7% for LGE-negative patients ($P=0.01$), 5.03% versus 1.8% for HF hospitalization ($P=0.002$), and 6.0% versus 1.2% for SCD/aborted SCD ($P<0.001$).

Reversal of LV Remodeling

Role of Pharmacologic Therapy

Inhibitors of the RAAS

It has been well established that treatment with angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) confers improved clinical outcomes and survival rates in HF patients with diminished LV systolic function (Group, 1987; Pfeffer et al., 1992). One of the earliest investigations of this was a substudy analysis as part of the SAVE trial (St John Sutton et al., 1994) that assessed whether captopril mitigates progression of LV enlargement in patients with LV dysfunction (defined as LVEF < 40%) after acute myocardial infarction (MI). In those patients who survived 1 year post MI, LV end-diastolic and end-systolic areas were smaller in the captopril than placebo group ($P=0.038$, $P=0.015$, respectively). Similar improvements in LV dimensions 1 year after MI were also seen in a substudy of the SOLVD trial (Konstam et al., 1992). Of note, the investigators found significant improvement of LV filling pressures as measured on cardiac catheterization and within roughly 2 weeks of enalapril discontinuation, blood pressure, and LV volumes increased to approximately baseline levels but remained below those of the placebo group. The authors concluded that ACEi therapy prevents progression of LV systolic dysfunction and LV dilation probably as a result from a combination of altered remodeling and sustained reduction in preload and afterload. An echocardiographic substudy of the Val-HeFT trial further demonstrated a statistically significant decline in LVEDD index and increase in LVEF after 18 months therapy with the ARB valsartan when added to a beta-blockers (BB) and/or ACEi (Wong et al., 2002). In this study, the valsartan-treated group had a demonstrable decrease in systolic blood pressure (i.e., decreased afterload) compared to placebo groups, which makes the degree of true reverse LV remodeling less clear. Another study further showed that the addition of the aldosterone receptor antagonist spironolactone to the ARB candesartan produced significant improvements in LVEF (35% vs. 26%, $P<0.01$) and reduction of LV end-diastolic volume index (LVEDVI) (121 mL/m^2 vs. 155 mL/m^2, $P=0.001$), LVESVI (88 mL/m^2 vs. 120 mL/m^2, $P<0.0005$), and LV mass index (81 g/m^2 vs. 93 g/m^2, $P=0.002$) in the combination group at 1 year (Chan et al., 2007). Reduction of collagen deposition in the myocardium in experimental HF models with the use of RAAS inhibitors is a possible mechanistic link to the evidence of reverse LV remodeling demonstrated in clinical trials (Liu et al., 1997; Milanez et al., 1997) (**Fig. 7**).

Beta-blockers

Three significant indicators of reversal of LV remodeling (RLVR) with metoprolol succinate were demonstrated in a substudy of 41 patients examined with CMR in the Metoprolol CR/XL Randomized Intervention Trial in HF (MERIT-HF) trial (Groenning et al., 2000): (1) a significant decrease in LVEDVI (150 mL/m^2 at baseline to 126 mL/m^2 after 6 months, P = 0.007), (2) a significant decline in LVESVI (107 to 81 mL/m^2, P = 0.001), and (3) LVEF increased in the metoprolol succinate group (29% to 37%, P = 0.005). A similar significant improvement in LV volumes and function assessed with CMR was demonstrated in a subgroup analysis of 34 patients with stable HF enrolled in the double-blind carvedilol versus placebo CHRISTMAS trial (Bellenger et al., 2004). Compared to placebo, treatment with carvedilol for 6 months significantly decreased LVESVI by nine units, LVEDVI by eight units, and increased LVEF by 3%. Carvedilol's efficacy in terms of contributing to LV reverse remodeling was further supported by Doughty et al. where a 5.8% increase in LVEF and 14 mL/m^2 decline in LVEDD index were observed after 12 months of carvedilol treatment (Doughty et al., 2004). Given carvedilol's inhibition of alpha-1 and beta-2 receptors in addition to beta-1 receptors, it is unclear whether the improvement in LV volumes with carvedilol therapy was a result of true intrinsic LV myocardial remodeling or due to improved LV loading conditions. Evidence of true RLVR occurring with carvedilol therapy was demonstrated by Loews et al. where LV mass decreased (276–247 g) as early as 4 months into carvedilol treatment and continued to decrease over a period of 12 months (Lowes et al., 1999). Improvement in the severity of mitral regurgitation and LV geometry, as manifested by a less spherical configuration, was also demonstrated with 4 months of carvedilol therapy. These changes were associated with an improvement in LVEF (21%–31%) and remained evident at 1-year follow-up. The value of CMR in predicting HF patients more likely to achieve some degree of RLVR with 6 months of BB therapy was demonstrated in a prospective study, where an inverse

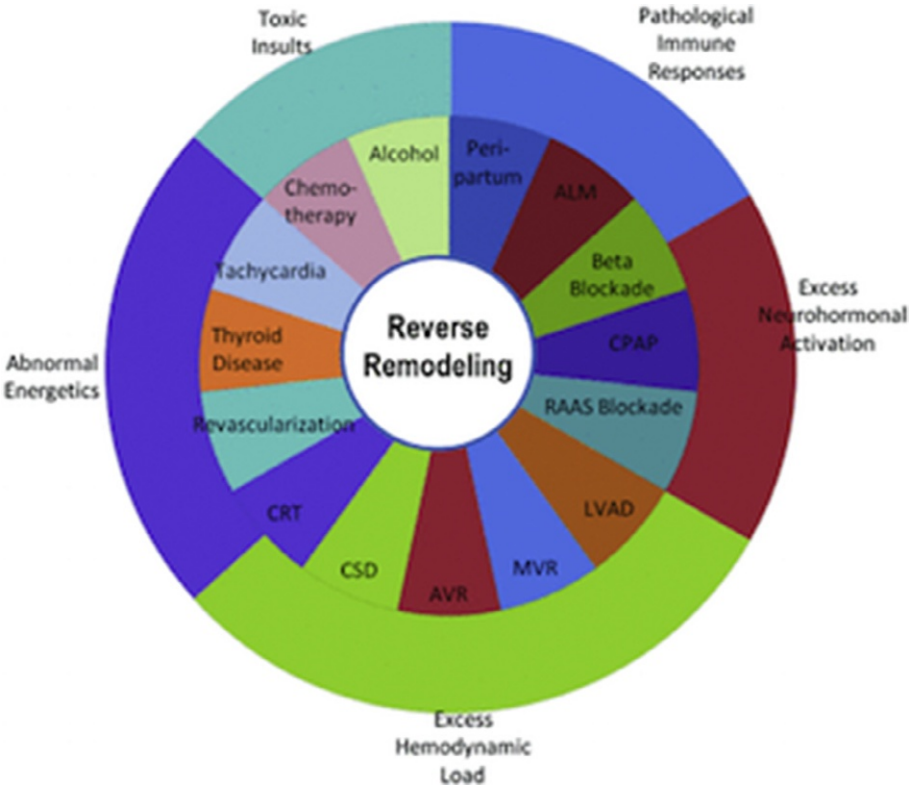


Fig. 7 Clinical scenarios of reverse LV remodeling. The segments of the outermost ring highlight the primary pathophysiologic processes implicated by reverse remodeling in particular clinical settings that comprise the middle ring. *Abbreviations*: *ALM*, acute lymphocytic myocarditis; *CPAP*, continuous positive airway pressure; *RAAS*, renin–angiotensin–aldosterone system; *LVAD*, left ventricular assist device; *MVR*, mitral valve repair/replacement; *AVR*, aortic valve replacement; *CSD*, cardiac support device; *CRT*, cardiac resynchronization therapy. Reprinted with permission from Hellawell, J. L., Margulies, K. D. (2012). Myocardial reverse remodeling. *Cardiovascular Therapeutics 20*, 172–181. doi: 10.1111/j.1755-5922.2010.00247.x.

relationship between the extent of transmural scarring (determined by LGE on CMR) and RLVR was identified (Bello et al., 2003). The investigators found that the amount of dysfunctional but viable myocardium by CMR was an independent predictor of the change in LVEF ($P=0.01$), mean wall motion score ($P=0.0007$), LVEDVI ($P=0.007$), and LVESVI ($P<0.0001$).

Novel pharmacologic agents for HF

Neprilysin is an endopeptidase that breaks down vasoactive peptides within the CV system such as natriuretic peptides, adrenomedullin, and bradykinin (Bayes-Genis et al., 2016). Cleavage of the molecules diminishes their net vasodilatory effect. This understanding of neprilysin's role in neurohormonal regulation in CV biology led to the pharmacologic development of neprilysin inhibitors as a potential treatment for chronic HF. Treatment with an angiotensin receptor-neprilysin inhibitor (ARNI) was shown to significantly reduce CV mortality and HF hospitalizations when compared to ACEi monotherapy among HFrEF (defined as LVEF $\leq$40% until 2010 at which point this was defined as LVEF $\leq$35%) patients in the 2014 PARADIGM-HF (McMurray et al., 2014). The effects of ARNI therapy on LV remodeling in the clinical setting remain largely unknown, but preclinical data has shown that 4-week treatment with the same ARNI used in PARADIGM-HF (LCZ696) attenuates cardiac remodeling and dysfunction after experimentally induced MI in rats (von Lueder et al., 2015). Statistically significant differences in echocardiographic parameters were noted in rats that underwent treatment with ARNI compared to the control group with higher LVEF (60 ± 2 vs. 47 ± 5%, lower LV end-diastolic diameter (9.7 ± 0.2 vs. 10.5 ± 0.3 mm), higher diastolic wall strain (0.23 ± 0.02 vs. 0.13 ± 0.02%), and circular strain (-9.8 ± 0.5 vs. -7.3 ± 0.5%). Compared to the control group, the treatment group was also found to have lower cardiac weights and significantly reduced levels of myocardial fibrosis, despite similar infarct size. Interestingly, the investigators found that the neprilysin inhibitor component of LCZ696, LBQ657, inhibited hypertrophy but not fibrosis. The ARB component of LCZ696 valsartan inhibited both hypertrophy and fibrosis.

Ivabradine is a newer agent that has been shown to reduce CV death and HF-related hospitalizations in patients with HFrEF and basal heart rates greater than 70 beats per minute while on BB therapy (Swedberg et al., 2010). It specifically inhibits the I_f current in the sinoatrial node by entering and binding to a site in the channel pore from the intracellular side (DiFrancesco and Camm, 2004). The SHIFT echocardiographic substudy (Tardif et al., 2011) of 411 patients found that ivabradine therapy led to a reduction in LVESVI compared to placebo (-7.0 ± 16.3 vs. -0.9 ± 17.1 mL/m^2; 95% CI: -8.8 to -2.7, $P<0.001$). LVESVI reduction with ivabradine use was also found to be independent of concomitant BB use, baseline LVEF, and etiology of HF.

The degree of LVESVI reduction correlated with the magnitude of CV risk reduction defined as the composite of CV mortality or hospitalization for progressive HF. Statistically significant improvements in LVEF and LVEDVI were also observed. The BEAUTIFUL echocardiographic substudy (Ceconi et al., 2011) also found a reduction in LVESVI along with a concomitant LVEF increase in ivabradine-treated HFrEF patients with stable CAD (defined as evidence of prior myocardial infarction, prior surgical or percutaneous coronary revascularization, or the presence of one or more major coronary arteries being narrowed by 50% or more on angiography) but failed to detect any significant differences in other echocardiographic parameters. They did demonstrate a clear link between the degree of ivabradine-induced heart rate reduction and reduction in LVESVI. On the basis of these clinical studies, there is evidence suggestive of ivabradine contributing to reversing adverse LV remodeling particularly in patients with ischemic cardiomyopathy.

Role of Device-Based Therapy

Cardiac resynchronization therapy

Patients with HF are commonly found to have concomitant abnormalities in their atrioventricular (AV) and intraventricular conduction pathways that correlate with the presence of left bundle branch block (LBBB) pattern on the electrocardiogram (ECG). These perturbations have been shown to be associated with a reduction in LV filling time compared to the right ventricular (RV) (Grines et al., 1989). The consequence of the delayed LV depolarization and ventricular diastolic imbalance is a dyssynchronous and inefficient pattern of ventricular contraction that contributes to progression of adverse LV remodeling. Cardiac resynchronization therapy (CRT) is defined as simultaneous pacing of both ventricles and its main therapeutic benefit in HF patients is derived from the reestablishment of coordinated interventricular contraction in addition to optimization of conduction within the LV and AV conduction.

On the basis of multiple randomized clinical trials, it is widely accepted that CRT decreases HF hospitalizations and mortality in HF patients who have a wide QRS complex, reduced LVEF, and New York Heart Association (NYHA) functional class I–IV (Moss et al., 2009; Linde et al., 2010; Tang et al., 2010). Its positive effect on halting and even reversing adverse LV remodeling has also been reported in multiple studies. For example, CRT duration of 6 months yielded significant decreases in LV mass, mitral regurgitation, LVEDVI, and LVESVI along with a 3.6% increased in LVEF compared with the control group in the MIRACLE trial (Abraham et al., 2002). A substudy analysis of the REVERSE trial further demonstrated a 68% reduction in mortality in patients who experienced a 15% or greater decrease in LVESVI after 6 months of CRT (Gold et al., 2015). The investigators found that improvement in other LV remodeling parameters such as LVEDVI and LVEF had a similar association with reduced mortality. When LV remodeling was assessed over 5 years in 419 patients in the REVERSE trial, sphericity index was used as a measure of distorted LV architecture (quantitatively derived based on LV shape at end diastole) and was found to be a predictor of death and HF hospitalization (St John Sutton et al., 2017). They also found the mean LV sphericity index to decline to its nadir within 1 year of CRT initiation. A decrease in LVESVI $>15\%$ with CRT occurred in 71% of patients over the 5-year follow-up period with a mean decrease of 22.8 mL/m^2 from baseline. Of note, the greatest decline in LVESVI (mean 18.3 mL/m^2) was observed over the first year of CRT initiation. Similar to prior studies, the decline in LVESVI with CRT was associated with an increase in LVEF compared to baseline (mean $33.2\pm12.7\%$ compared to $27.2\pm6.6\%$, $P<0.0001$) over the 5-year study period.

Predicting which patients respond to CRT (commonly defined as improvement in clinical HF symptoms and/or in LVEF and LV architecture on echocardiogram) has been an area of interest for researchers given the fact that approximately 30% of HF patients are clinical nonresponders to CRT. In addition to the diagnostic data obtained from CMR in HF patients, multiple studies have shown higher CRT failure rates when leads are placed in contact with scarred myocardium identified on CMR by the presence of LGE (White et al., 2006; Bilchick et al., 2014). A higher degree of baseline LV adverse remodeling determined on the basis of derangements in echocardiographic parameters described above and dyssynchrony as measured with CMR techniques have been shown to be reliable predictors of poor response to CRT (Bilchick et al., 2008; Carluccio et al., 2011).

One of the proposed mechanisms for RLVR with CRT is improved efficiency in the cardiomyocyte's cellular metabolism as was demonstrated by an increase in succinate-glutamate ratio, a marker of Krebs cycle activity, in patients undergoing CRT (Nemutlu et al., 2015). Studies have also suggested CRT partially reverses the derangements involving calcium handling in HF patients by inducing structural changes to the T-tubule apparatus, SR, ryanodine receptor, and voltage-gated calcium channels (Sachse et al., 2012). Another potential mechanism of CRT efficacy in RLVR is its ability to improve myofibril calcium sensitivity possible through phosphorylation and reactivation of glycogen synthase kinase-3β (Kirk et al., 2014).

Left ventricular assist device

A significant number of patients progress to end stage HF despite aggressive medical therapies. Implantation of a left ventricular assist device (LVAD) has been demonstrated to be effective in bridging these patients to cardiac transplantation (Miller et al., 2007). Furthermore, the REMATCH study expanded the use of LVADs as destination therapy for patients with end stage HF who are not candidates for cardiac transplantation (Rose et al., 2001). LVAD therapy affects two crucial aspects of pathologic LV remodeling. One, it is placed in parallel to the left ventricle, leading to unloading of pressure and volume, thus reducing both preload and afterload (Bellavia et al., 2017). And second, this unloading restores perfusion to critical organs leading to a decrease in pathologic neurohormonal activation (James et al., 1995). Patients who receive LVADs were found to have improved quality of life, functional status, and hemodynamics. Unfortunately, the long-term survival of patients with destination LVAD implantation remains low, and the use of LVADs as destination therapy remains limited.

Table 2 Mechanistic basis of reverse left ventricular remodeling with guideline-directed pharmacologic and device therapy for heart failure

Impairments in heart failure	*BB*	*ACEi/ARB*	*CRT*	*LVAD*
β-Adrenergic desensitization	↓	↓	↓	↓
Excitation-contraction coupling	↑	↑	↑	↑
Fetal gene expression	↓	↓	↓	↓
Myocyte hypertrophy	↓	↓	↓	↓
Myocyte apoptosis	↓	↓	↓	↓
MMP activation	↓	↓	↓	↓
Angiogenesis	↑	↑	↑	↓
Mitochondrial energetics	↑	↔	↑	↑
LV dilation	↓	↔[a]	↓	↓

Abbreviations. ACEi: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker, BB: beta-blocker, CRT: cardiac resynchronization therapy, LV: left ventricle, LVAD: left ventricular assist device, MMP: matrix metalloproteinase.
[a]Reflects ACEi/ARB effect of stabilizing LV size and preventing further dilation.
Adapted from Mann, D. L., Barger, P. M. and Burkhoff, D. (2012). Myocardial recovery and the failing heart: myth, magic, or molecular target?, *J Am Coll Cardiol*, 60(24), 2465–2472. https://doi.org/10.1016/j.jacc.2012.06.062.

LVADs reduce LV dilation by successfully unloading the LV. Immediate reduction in LV size after implantation has been demonstrated through transesophageal echocardiography (McCarthy et al., 1995). LV pressure–volume recordings have also confirmed lower LV volumes for equivalent pressure loads with LVAD support (Dandel et al., 2005). Moreover, patients with LVAD therapy also demonstrate improvement in ventricular contractile function. In a study by Maybaum et al., a third of patients with partial LVAD support were found to have an EF of greater than 40% at 30 days (Maybaum et al., 2007). Interestingly, the authors found that LVAD support up to 120 days resulted in a lower LVEF. Animal studies have found differential effects in mechanical unloading of normal versus failing hearts (James et al., 1995; Takaseya et al., 2004). Mechanical unloading of normal hearts led to a decrease in contractile function whereas improvement in myocardial contractility results from unloading of failing hearts (Kent et al., 1985; James et al., 1995; Takaseya et al., 2004). Further studies are needed to fully understand the effect of LVAD mechanical unloading on cardiac contractility.

LVADs have been show to help normalize the chronic maladaptive neurohormonal activation through improvement in hemodynamics. Decreased levels of angiotensin II, natriuretic peptide, and TNF-α have been observed after LVAD placement (James et al., 1995; Torre-Amione et al., 1999; Bruggink et al., 2006). Improvement in hypertrophy of the failing myocyte has been attributed to the mechanical unloading of the LV rather than changes in circulating systemic factors. Regression of hypertrophy is seen most prominently in the LV as compared to the RV (Barbone et al., 2001).

LVAD support also improves contractility through increased myocyte force. In vitro studies of myocytes obtained from patients with HF supported by LVAD as compared with patients without, the myocytes from LVAD patients had greater magnitude of shortening, faster time to peak contraction, and faster time to shortening (Dipla et al., 1998). Additional studies have demonstrated improved cardiac myocyte calcium handling and beta-adrenergic responsiveness contribute to in vivo improvements in cardiac function. Together, these studies suggest that the abnormalities seen in failing hearts are largely due to increased myocardial wall stress and abnormal neurohormonal activation. **Table 2** summarizes how each of these pharmacologic or device therapies impacts RLVR.

Right Ventricular Remodeling

Molecular Basis

Similar to LV remodeling, RV remodeling occurs at the structural, histological, and molecular level. RV remodeling and right-sided HF (RHF) have historically been understudied compared to left-sided HF (LHF) but RV function has been shown to be a significant predictor of outcomes in patients with LHF (Haddad et al., 2008). Similar to high afterload states (i.e., systemic arterial hypertension and aortic valve stenosis) triggering LV remodeling, pulmonary hypertension (PH) creates RV pressure overload. Left-sided cardiac abnormalities (i.e., LV systolic and/or diastolic dysfunction, mitral regurgitation) have been shown to be the most common cause of PH, being responsible for as many as 68% of PH cases (Strange et al., 2012). An important difference between RV and LV hypertrophy due to increased afterload is that RV enlargement occurs much earlier because the RV wall thickness is lower than the LV. It has also been shown that the transition to anaerobic metabolism appears to occur earlier in the pressure-loaded RV versus the pressure-loaded LV, resulting in an earlier decrease in net ATP production in the RV (Do et al., 1997). The increased RV wall stress results in reactionary RV hypertrophy and contractility to overcome the high afterload state. Maladaptive remodeling and ultimately RHF, however, ensues after prolonged RV wall stress.

In a swine PH model, investigators analyzed molecular and histologic differences between an RV that had undergone adaptive remodeling versus a failed RV. Decreased expression of RV SR Ca^{2+}-ATPase2a along with increased aldosterone levels was found in

the RV failure group (Aguero et al., 2014). Another important feature detected in the RV failure group was right ventriculoarterial uncoupling (reduced RV pump function despite increased RV contractility of the pressure-overloaded RV) using pressure–volume loop analysis. The progression from compensated RV remodeling to RV failure also appears to be closely involved with the transition from aerobic to anaerobic cellular metabolism due to mitochondrial dysfunction (Watts et al., 2010). When considering the relationship between the molecular basis of RV and LV remodeling, there is a high degree of overlap among them. Apoptosis, dysregulated angiogenesis with decreased capillary density, diminished oxidative capacity and direct cytotoxicity due to reactive oxidative stress, and neurohormonal activation involving RAAS and adrenergic signaling pathways have all been shown to occur with RV remodeling in a similar manner as LV remodeling (Voelkel et al., 2012). Key differences that have been described include myocardial RV-specific expression of phosphodiesterase type 5 (PDE5) in RV pressure-overload states thus making PDE5 inhibitors ideal pharmacologic agents for RV dysfunction (Nagendran et al., 2007). Preclinical investigation has also shown that a specific molecular pathway that is more prominent in RV remodeling is the Wnt pathway that is involved with kinase cascades which regulate cell migration, proliferation, and apoptosis (Urashima et al., 2008). In this experimental model, RV afterload stress was created with pulmonary artery constriction and compared to LV afterload-stressed myocardium. Wnt signaling was more active in the pressure-overloaded RV. Subtle differences in micro-RNA expression between RV and LV remodeling have also been demonstrated, with upregulation of four micro-RNAs (28, 34a, 93, and 148a) in RV hypertrophy/RHF that are downregulated or unchanged in LHF (Reddy et al., 2012).

Imaging Modalities to Assess the RV

Echocardiography is also the predominant imaging tool in assessing RV function and structure. Important prognostic parameters such as tricuspid annular plane systolic excursion, presence of pericardial effusion, RVEF, RV volumes, and RV mass can be obtained using echocardiography (Ghio et al., 2010). CMR has also become recognized as the gold standard in assessing RV function and is the most accurate and reproducible method for determining RV mass, volume with an interobserver variability <7% for the end-diastolic volume, <14% for the end-systolic volume, <7% for EF, and <20% for RV mass (Grothues et al., 2004). Another study identified CMR-derived RVEF as the most predictive parameter of clinical outcomes in patients undergoing PH-specific treatment (van de Veerdonk et al., 2011). This has been supported by a recent meta-analysis using a large sample ($n = 539$) from 21 different studies (Baggen et al., 2016). The significant prognostic value of LGE presence with CMR as seen in the LV has yet to be established in PH patients (McCann et al., 2005).

RV Reverse Remodeling

Currently approved therapy for PH includes PDE5 inhibitors (sildenafil, tadalafil), prostanoids (epoprostenol, iloprost, treprostinil), and endothelin-1 receptor antagonists (ambrisentan, bosentan). Use of these agents yields reverse RV remodeling that is predominantly a result of their afterload reducing and vasodilatory effects (Kerbaul et al., 2007; Holmboe et al., 2013). Recent experimental studies have also demonstrated reverse RV remodeling effects with the BBs carvedilol (Okumura et al. 2015) and bisoprolol (de Man et al. 2012). The proposed mechanism in the Okumura et al. study was that carvedilol reduces biventricular transforming growth factor-β1, connective tissue growth factor signaling, fibrosis, apoptosis and improved fibrosis and hemodynamics occurred despite persistent RV afterload. In the de Man et al. study histologic analysis revealed significantly less RV fibrosis and myocardial inflammation in bisoprolol-treated PH rats.

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Left Ventricular Assist Device (LVAD) and Circulatory Devices in Heart Failure

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Introduction

Incidence and Prevalence

Heart failure continues to be a major public health concern in the United States, contributing to significant morbidity, mortality, and health care costs. An estimated 5.7 million American adults have heart failure, with this number expected to increase to over 8 million by 2030 (Heidenreich, et al., 2013). There is a 1 in 5 lifetime risk of developing heart failure for an adult 40 years of age (Lloyd-Jones et al., 2002). This places a tremendous burden on the health care system with heart failure costs in 2012 estimated to be $30.7 billion, of which 68% was attributable to direct medical costs (Heidenreich et al., 2013). By 2030 this is expected to increase 127% to $67.0 billion (Heidenreich et al., 2013).

For heart failure patients who are refractory to medical therapies, the only remaining therapeutic options are surgical. The ideal treatment is heart transplantation. However, this option is limited by the availability of organs and the strict criteria needed to be met to be a transplant candidate. Despite an increasing number of patients requiring heart transplantations, the number of transplants in North America has remained relatively stable over the past 20 years at approximately 2500 transplants per year (Lund et al., 2015). Therefore, with the aging population and increasing prevalence of heart failure, an alternative to heart transplantation is necessary. For end-stage heart failure patients who are either not a candidate for heart transplantation or cannot wait for a donor heart, mechanical circulatory support (MCS) with a left ventricular assist device (LVAD) can be lifesaving.

History of Mechanical Circulatory Support

The first use of MCS in open heart surgery was in 1953 when John Gibbon used a cardiopulmonary bypass machine to repair an atrial septal defect in an 18-year-old woman (Gibbon, 1954). This was followed by the development of temporary MCS systems for use in patients with low cardiac output after cardiac surgery (Liotta et al., 1963). In 1966, Michael DeBakey and colleagues implanted the first pneumatic paracorporeal pulsatile LVAD (DeBakey, 1971). Early generations of LVADs provided pulsatile flow and were pneumatically or electrically driven. Multiple variations on the pulsatile LVAD concept were developed during the 1980s and 1990s, including the first successful bridge-to-transplantation (BTT) LVAD implant of the Novacor Left Ventricular Assist

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System (LVAS) by Phil Oyer in 1984 (Starnes et al., 1988). This device employed an electromechanically driven dual pusher-plate blood pump. However, probably the most successful of the first-generation LVADs was the Thoratec HeartMate XVE device. Variations of this device were initially approved for BTT, but the XVE ultimately became the first intracorporeal LVAD device for destination therapy (DT) after the landmark REMATCH (Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure) trial which compared LVAD therapy to optimal medical management (Rose, 2001). Despite the clinical advantages of LVAD therapy over medical management, these devices were limited by their large size, infectious complications, and limited durability.

The search for improved LVAD designs led to the development of the second-generation continuous flow pumps. Compared to the pulsatile pumps, these pumps are smaller in size, do not require valves, require less power, do not need a compliance chamber, and are less expensive. The most frequently used second-generation LVAD is the Heartmate II and was FDA approved for BTT in 2008 and for DT in 2010. The impeller of this axial-flow pump, which has a typical rotation rate of approximately 10,000 rpm, is both mechanically and magnetically positioned. This design improves its durability with an expected lifetime of at least five years.

The third-generation LVAD was designed as a radial centrifugal pump that propels blood using a spinning top with magnetic and hydraulic positioning inside a small chamber. Because there is no mechanical contact between the impeller and the chamber, minimal to no wear is expected, further improving the durability of the LVAD which is expected to last at least 10 years. With a further decrease in size, these LVADs are contained within the pericardium and can even be implanted without a sternotomy via bilateral thoracotomies (Schmitto et al., 2012). Two leading examples of this generation of LVADs are the HeartWare HVAD (HeartWare Inc., Framingham, MA, USA) and the HeartMate 3. Because of their reduced size, these pumps have also been used for right ventricular support, either as a standalone right ventricular assist device (RVAD) or in conjunction with a left-sided pump for biventricular failure. However, such application of these pumps is considered off-label use (Shehab et al., 2016).

In instances of biventricular failure, an LVAD alone may be inadequate. However, because of the large size of the earlier generation of LVADs, it was not possible to intracorporeally implant two pumps for biventricular support. In 1969, Denton Cooley and colleagues implanted the first total artificial heart, which was intended as a BTT (Cooley et al., 1969). In 1982, the JARVIK-7 TAH was implanted as DT by William DeVries (DeVries et al., 1984). This device was acquired by CardioWest, Inc, in 1992 and first implanted as a BTT in 1993. The CardioWest TAH was a pneumatically driven biventricular pulsatile pump implanted in the orthotopic position (Copeland et al., 2003). The modern version of this device is the Syncardia TAH, the only FDA approved TAH device currently in use.

Clinical Considerations

Heart Failure Management

The clinical syndrome of heart failure describes a constellation of signs and symptoms resulting from inadequate cardiac function. The traditional discussion of heart failure refers to heart failure associated with severe systolic dysfunction, or heart failure with reduced ejection fraction (HFrEF). However, heart failure can also be associated with normal systolic function, or heart failure with preserved ejection fraction (HFpEF). HFpEF is still not completely understood and ideal therapy is still undefined. However, in the context of MCS for heart failure, the discussion will focus on HFrEF.

Appropriate management of patients with heart failure requires close monitoring and an understanding of the severity of their disease and associated symptoms. To this end, classification systems have been developed to describe the severity of the heart failure. The two most commonly used classifications are the ACCF/AHA stages of heart failure, which emphasizes the development and progression of disease (Hunt, 2009) and the New York Heart Association (NYHA) functional classification, which focuses on exercise capacity and the symptomatic status of the patient. The ACCF/AHA stages represent a progression of disease wherein a patient advances through the stages but does not regress to earlier stages. However, a patient's NYHA functional class can change with time as the disease progresses or as the patient responds to therapy (**Table 1**).

The treatment of patients with heart failure is primarily dictated by the patient's disease etiology, symptoms, and heart failure state. As they progress through the stages of heart failure, patient therapies are escalated, beginning with risk factor management and then progressing to the use of a variety of heart failure medications (**Fig. 1**). Depending on the etiology and nature of their heart failure, they may also benefit from specific surgical intervention (coronary revascularization and valve surgery) and/or cardiac resynchronization therapy. This is followed by inotropic therapy, which may be temporary or chronic. However, as they advance to stage D heart failure with persistent NYHA class III or IV symptoms despite optimal therapy, or they become inotrope dependent, they are left with heart transplantation or MCS as their only remaining therapeutic options.

Stage D heart failure describes an advanced heart failure state that is associated with significant structural abnormalities of the heart and severe symptoms despite optimal therapy. Patients in this stage are faced with limited treatment options, elevated morbidity, and short survival. The selection of appropriate advanced therapeutic options is dictated by a number of factors. To aid in this discussion, a decision tree is provided in **Fig. 2**.

Terminology and Classifications

Among the wide range of MCS devices available, there are several categories of devices. The most fundamental distinction among these devices is whether they are temporary (nondurable) or permanent (durable). Nondurable MCS devices are meant for relatively

Table 1 Comparison of ACCF/AHA stages of HF and NYHA functional classifications (Yancy et al., 2013)

ACCF/AHA stages of HF	*NYHA functional classification*	
A. At high risk for HF but without structural heart disease or symptoms of HF	None	
B. Structural heart disease but without signs or symptoms of HF	I	No limitation of physical activity. Ordinary physical activity does not cause symptoms of HF
C. Structural heart disease with prior or current symptoms of HF	I	No limitation of physical activity. Ordinary physical activity does not cause symptoms of HF
	II	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in symptoms of HF
	III	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes symptoms of HF
	IV	Unable to carry on any physical activity without symptoms of HF, or symptoms of HF at rest
D. Refractory HF requiring specialized interventions	IV	Unable to carry on any physical activity without symptoms of HF, or symptoms of HF at rest

ACCF, American College of Cardiology Foundation; *AHA*, American Heart Association; *HF*, heart failure; *NYHA*, New York Heart Association.

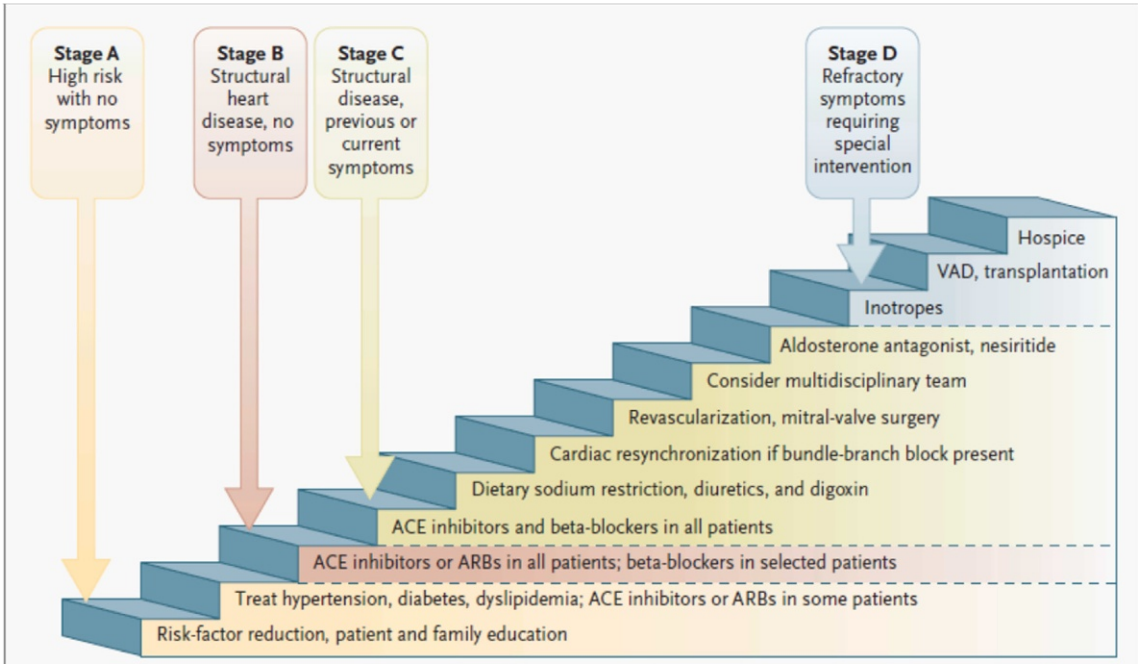

Fig. 1 Heart failure decision tree (Jessup and Brozena, 2003).

short term (days to weeks) use that is restricted to use in the hospital. These devices are typically employed for situations where the patient requires urgent or emergent hemodynamic support but are not yet candidates for heart transplantation or durable devices. A durable device is one that is implanted intracorporeally and meant for permanent or long-term use. Patients with these devices can be discharged from the hospital after recovery.

Another distinction that can be made between devices is whether they are centrally or peripherally inserted. For the most part, peripherally inserted devices are all nondurable devices. These devices can generally be inserted percutaneously via the femoral or axillary arteries, although some cases require surgical cut down for direct insertion into the artery. Centrally inserted devices are placed directly into the heart or associated vessels via a sternotomy or thoracotomy. All durable devices are centrally implanted; however, certain nondurable devices can or require central insertion as well. The advantage of peripherally inserted devices is the decreased invasiveness of the procedure, some of which can be carried out at the bedside. However, one of the primary limitations of any device inserted via the femoral artery is that the patient is bed bound and generally has to remain supine.

Devices can also be classified based on the type of support they provide. LVAD support the left heart, generally bypass the left heart. Typically, blood is diverted from the heart via the left atrium or left ventricle, go through the device, and return back into the aorta. Some devices, such as the Impella family of pumps, are placed across the aortic valve and pump the blood from the left ventricle to the ascending aorta. Similarly, RVAD bypass the right heart and take blood from the right atrium or some part of the

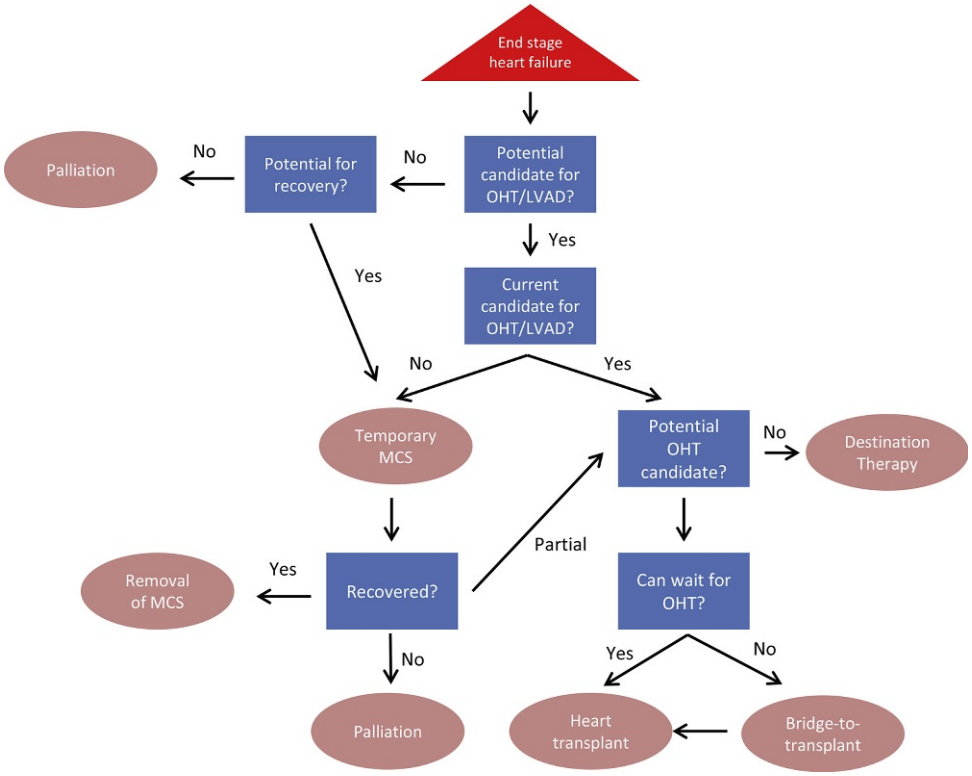

Fig. 2 Decision tree.

venous system, and return it to the pulmonary artery. Patients who have biventricular failure will either require both an LVAD and RVAD, or a total artificial heart (TAH). A TAH involves replacing both ventricles and provides pulsatile flow from the right and left atria to the pulmonary artery and aorta, respectively. An alternative to a TAH for biventricular support that is temporary and potentially percutaneous is Extracorporeal Membrane Oxygenation (ECMO). Here, the entire cardiopulmonary system is bypassed as blood is diverted from the right atrium or some part of the venous system to the arterial system. One other aspect that makes ECMO unique among MCS devices is that ECMO also includes an oxygenator, and can both oxygenate the blood and remove carbon dioxide.

Another important distinction that is made with MCS implantation is the intended purpose of the device. Devices that are meant for a patient who is a candidate for heart transplantation but is not able to wait long enough for the transplant are deemed to be for bridge-to-transplant (BTT). Those who are not candidates for heart transplant or do not want one, can have durable devices implanted for destination therapy (DT). BTT and DT are formal designations that are made prior to implanting any durable device. Sometimes, a patient may not be a transplant candidate at the time of implantation but has the potential to be one in the future. Such a patient can be implanted with a device with the intention of being a bridge-to-candidacy (BTC). Finally, a device can be implanted with an expectation of recovery or bridge to recovery (BTR). However, recovery is uncommon in patients implanted with durable devices. More commonly, recovery is seen in patients who need temporary support with a nondurable device for an acute problem.

Indications for MCS

Although the precise indications for nondurable versus durable MCS may differ, the main principles underlying the indications for MCS in general are the same. MCS is indicated in patients with advanced heart failure and/or cardiogenic shock who continue to have inadequate tissue perfusion despite optimal medical therapy. The International Society for Heart and Lung Transplantation (ISHLT) published guidelines for MCS in 2013 (**Table 2**) that outline recommendations for who should be considered for MCS (Feldman et al., 2013).

Decision Tree

Once a determination has been made that a patient requires MCS, a series of questions have to be answered to determine the ideal course of action (**Fig. 2**). And although the precise criteria and protocols involved in the decision making process may differ from institution to institution, the basic principles are universal. It is also important to note that having an indication for MCS does not necessarily make a patient a candidate for MCS.

Table 2 Recommendations for risk stratification for consideration of MCS (Feldman et al., 2013)

Class IIa

1. Long-term MCS for patients who are in acute cardiogenic shock should be reserved for the following:
   a. Patients whose ventricular function is deemed unrecoverable or unlikely to recover without long-term device support.
   b. Patients who are deemed too ill to maintain normal hemodynamics and vital organ function with temporary MCSDs, or who cannot be weaned from temporary MCSDs or inotropic support.
   c. Patients with the capacity for meaningful recovery of end-organ function and qualty of life.
   d. Patients without irreversible end-organ damage.

Level of evidence: C

2. Patients who are inotrope-dependent should be considered for MCS because they represent a group with high mortality with ongoing medical management.
   Level of evidence: B
3. Patients with end-stage systolic heart failure who do not fall into recommendations 1 and 2 above should undergo routine risk stratification at regular intervals to determine the need for and optimal timing of MCS. This determination may be aided by risk assessment calculators and cardiopulmonary stress testing.
   Level of evidence: C
4. Heart failure patients who are at high-risk for 1-year mortality using prognostic models should be referred for advanced therapy including heart transplant, or MCS (bridge to transplantation [BTT] or destination therapy [DT]) as appropriate.

1. One of first questions to answer is whether a potential candidate for MCS has a viable "exit strategy." The potential clinical outcomes for these patients (from best to worst) are recovery, heart transplantation, durable device implantation, or palliation. If the patient is not a potential candidate for either heart transplantation or durable device, then the next question is whether the patient has a reasonable potential for recovery. If he/she does not, then palliation may be the appropriate option. However, if there is a chance of meaningful recovery or transplant/durable MCS candidacy, then the patient may be offered a trial of temporary MCS.
2. If a patient is a potential heart transplant or durable device candidate, then they should be evaluated to see if they are currently a candidate for either therapy. If not, then they should be considered for temporary MCS with the hopes of either complete recovery (less likely), palliation if they do not have any meaningful recovery, or recovery to the point where they become candidates for heart transplantation or durable device implantation.
3. Patients who are current candidates for heart transplantation or durable device implantation should then be evaluated specifically for heart transplantation. If they are a candidate and are stable enough to wait for a suitable donor organ, then they can be listed for heart transplantation. If they are a candidate for transplantation but are not stable enough to wait for a donor heart, they can have a durable device implanted as BTT. Under certain circumstances, centrally cannulated extracorporeal pumps may also be appropriate for BTT. If they are not a candidate for transplantation, they can then have a durable device implanted as DT.

Nondurable Mechanical Circulatory Support

Clinical Considerations

Patients who require nondurable MCS generally fall under two categories. They may be patients with acute cardiogenic shock due to any one of a wide range of possible causes who require hemodynamic support despite maximal medical therapies. The other group of patients requiring nondurable MCS is those with stage D heart failure who are being considered for heart transplantation or durable MCS but need urgent support before they can be a candidate for these therapies. Various medical societies, including the ISHLT, the American Heart Association (AHA), and the Heart Failure Society of America (HFSA), have devised guidelines for when temporary MCS should be instituted (Kapur and Esposito, 2015).

ISHLT Mechanical Circulatory Support Guidelines 2012

- Class 1: Nondurable MCS indicated for acute decompensated heart failure failing maximal medical therapy, multi-organ failure, sepsis, or ventilator-dependent patients to optimize hemodynamics and evaluate status.
- Class 1: Right ventricular assist device is indicated for postoperative right ventricular failure refractory to maximal medical therapy after LV assist device implantation.

AHA Heart Failure Guidelines 2013

- Class 2A (level of evidence, B): Nondurable MCS is reasonable as a bridge to recovery or decision for carefully selected patients with heart failure and acute profound disease.

HFSA Heart Failure Guidelines 2010

- Urgent MCS is indicated for bridge to decision in unstable heart failure refractory to maximal medical therapy complicated by multi-organ dysfunction and with a relative contraindication to transplant or durable MCS.

Cardiogenic shock is typically characterized by severe refractory myocardial dysfunction that leads to inadequate tissue perfusion and progressive end-organ dysfunction in the setting of adequate preload. There is a wide range of causes of cardiogenic shock, many of which have specific medical or surgical treatments. However, sometimes the severity of their acute condition is such that they are either not candidates or cannot wait for these treatments (**Table 3**). These patients may be candidates for urgent or emergent nondurable MCS, allowing them to recover to the point where they can receive the definitive treatment they need, or even completely recover without needing further intervention.

Nondurable MCS Devices

Although the selection of nondurable MCS devices depends on a number of factors, they all have the common goals of (1) increasing vital organ perfusion, (2) augmenting coronary perfusion, and/or (3) reducing ventricular volume and filling pressures in order to reduce wall stress, stroke work, and myocardial oxygen consumption (Kapur and Esposito, 2015). The four most common percutaneous MCS devices that support the systemic circulation (as opposed to pulmonary circulation and right heart) are (1) the intra-aortic balloon pump (IABP), (2) extracorporeal membrane oxygenation (ECMO), (3) the Impella family of devices, and (4) the TandemHeart (**Fig. 3**). In addition to these peripherally placed devices, there are also centrally placed nondurable MCS devices.

Intraaortic balloon pump

IABP is a counterpulsation pump and is the most widely used nondurable MCS device. It can be considered the least invasive of the nondurable devices in that they can be placed percutaneously, at the bedside, and does not involve blood circulating through the device itself. It also has the advantage of being less expensive than other devices and relatively easy to insert. IABP comprises a double-lumen catheter mounted cylindrical polyethylene balloon that is inserted into the aorta and placed in the descending aorta just distal to the takeoff of the left subclavian artery. It is usually inserted percutaneously through a sheath placed via the femoral artery, although it has also been placed through the axillary artery (H'Doubler et al., 2000). The balloon is rapidly inflated and deflated using helium gas and is timed to inflate at the onset of cardiac diastole and deflate at the onset of systole. The timing is accomplished by synching the device with either the patient's electrocardiogram or direct arterial blood pressure. Inflation during diastole theoretically augments coronary perfusion by displacing blood volume in the descending aorta and increasing the mean aortic pressure. When the balloon deflates during systole, a vacuum effect is produced thereby decreasing afterload and augmenting cardiac output.

Table 3 Causes of cardiogenic shock (Hollenberg et al., 1999)

Acute myocardial infarction
Pump failure
Large infarction
Smaller infarction with preexisting left ventricular dysfunction
Infarction extension
Reinfarction
Infarction expansion
Mechanical complications
Acute mitral regurgitation caused by papillary muscle rupture
Ventricular septal defect
Free-wall rupture
Pericardial tamponade
Right ventricular infarction
Other conditions
End-stage cardiomyopathy
Myocarditis
Myocardial contusion
Prolonged cardiopulmonary bypass
Septic shock with severe myocardial depression
Left ventricular outflow tract obstruction
Aortic stenosis
Hypertrophic obstructive cardiomyopathy
Obstruction to left ventricular filling
Mitral stenosis
Left atrial myxoma
Acute mitral regurgitation (chordal rupture)
Acute aortic insufficiency

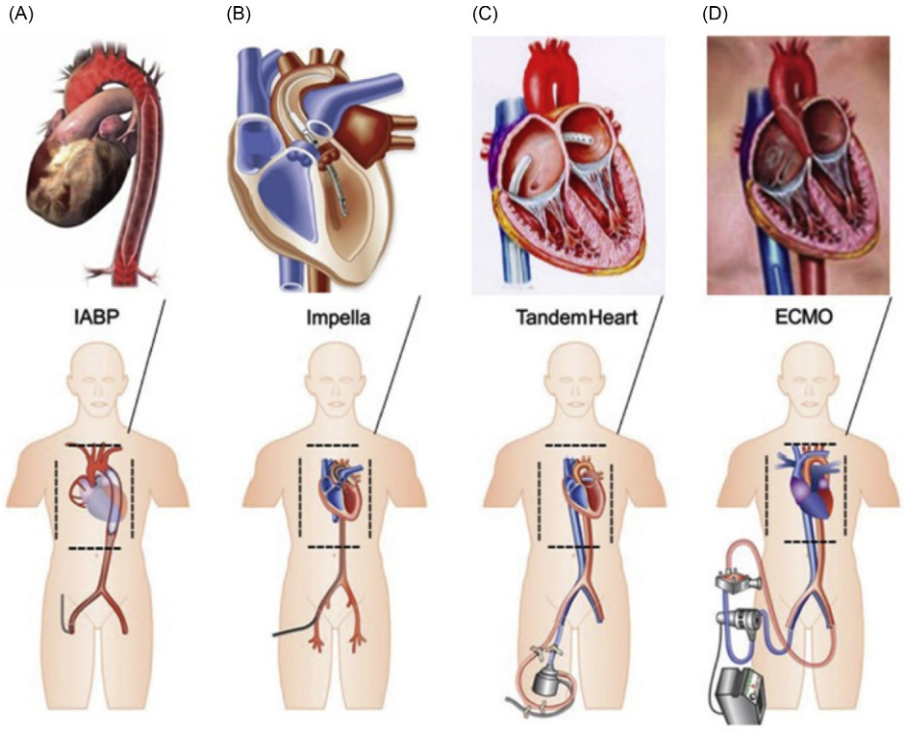

Fig. 3 Four most common percutaneous MCS devices that support the systemic circulation (Brown and Estep, 2016).

The standard balloon capacity is 40 cc, although the larger capacity 50 cc balloons potentially offer greater hemodynamic support (Kapur et al., 2015). The frequency of inflation can be adjusted with the 1:1 setting (inflation with every heart beat) being most common. As with all MCS devices, patients with IABPs should be anticoagulated, although this can be held on the 1:1 setting for brief periods of time should the clinical situation require such a measure. Caution should be taken in patients with severe peripheral arterial disease due to the risk of vascular complications and limb ischemia. Additionally, because afterload is increased during diastole, IABP is contraindicated in patients with more than mild aortic regurgitation. The risk of major complications including major limb ischemia, severe bleeding, balloon leak, and death directly due to insertion or leak has been reported to be 2.6% (Ferguson et al., 2001).

Impella circulatory support system

The Impella family of axial flow catheters (Abiomed, Danvers, MA) includes three devices of varying flow capacities to support the left heart and one for the right heart. These catheter-mounted devices use an axial flow pump containing an Archimedes screw impeller. The left-sided devices are placed in the left ventricle and are inserted in a retrograde fashion across the aortic valve, generating continuous blood flow from the left ventricle to the ascending aorta. Depending on the degree of hemodynamic support needed, there are three sizes to choose from: the Impella 2.5 provides up to 2.5 L/min of flow whereas the Impella CP provides up to 4 L/min of flow. The largest is the Impella 5.0 and can provide up to 5 L/min of flow. The 2.5 and CP devices can generally be inserted percutaneously via the femoral artery with an introducer sheath. The larger Impella 5.0 requires a surgical cut down and is frequently placed through the right axillary artery via a vascular graft, thus allowing the patient to sit up and even ambulate. The Impella is typically guided retrograde into the left ventricle with the use of fluoroscopy in the catheterization laboratory, although transesophageal echocardiography can also be used for positioning. Complications include vascular complications from the peripheral artery insertion, bleeding, ventricular arrhythmias, perforation from the catheter being placed too far into the left ventricle, and increased hemolysis, especially if the device is malpositioned.

The Impella provides hemodynamic support by unloading the left ventricle, with a resultant increase in mean arterial pressure, reduced myocardial oxygen demand, and reduced pulmonary capillary wedge pressure. ISAR-Shock (Efficacy Study of LV Assist Device to Treat Patients With Cardiogenic Shock) was a small randomized clinical trial comparing the Impella 2.5 to IABP in patients presenting with acute myocardial infarction and cardiogenic shock. In this study, the Impella 2.5 led to a greater improvement in cardiac index compared to the IABP (0.49 ± 0.46 L/min/m^2 for Impella 2.5 versus 0.11 ± 0.31 L/min/m^2 for the IABP; $P < .01$) (Seyfarth et al., 2008). In a multicenter, prospective, single-arm feasibility study (RECOVER I) of the Impella 5.0 for postcardiotomy cardiogenic shock, the device improved cardiac index from 1.65 to 2.7 L/min/m^2 $P = .0001$), mean arterial pressure from 71.4 to 83.1 mm Hg ($P = .01$), and decreased pulmonary artery diastolic pressure from 28.0 to 19.8 mmHg ($P < .0001$) (Griffith et al., 2013).

For right-sided support in the context of right ventricular failure, the Impella RP can be used. This pump can be inserted percutaneously via the femoral vein and can provide up to 4 L/min of flow. It is positioned with the inlet in the inferior vena cava and the outlet in the main pulmonary artery. This device can be used alone for right ventricular failure or in conjunction with left-sided support for biventricular failure.

TandemHeart

The TandemHeart device (Cardiac Assist, Inc; Pittsburgh, PA) is an extracorporeal centrifugal flow pump that can be placed percutaneously via the femoral artery and vein. A 21-Fr inflow cannula is inserted via the femoral vein, advanced into the right atrium and then the left atrium via transseptal puncture. Oxygenated blood is pumped by the continuous flow centrifugal pump to the descending aorta, which is inserted via the femoral artery using a 15-Fr to 19-Fr outflow cannula. The device can provide 3.0–5.0 L/min of flow, depending on the size of the arterial cannula, and serves to essentially bypass the left heart. The TandemHeart has been shown to increase cardiac output, unload the left ventricle, and decrease PCWP, central venous pressure, and pulmonary artery pressure, and reduce cardiac workload and oxygen demand (Kar et al., 2011; Burkhoff et al., 2006) In a meta-analysis of controlled trials comparing percutaneous Impella and TandemHeart devices to IABP, the percutaneous LVADs provided superior hemodynamic support, but was not associated with improved early survival (Cheng et al., 2009). Anticoagulation is required to prevent thrombotic complications. Complications include bleeding, pump thrombosis, limb ischemia, stroke, atrial perforation, and creation of atrial septal defects with an associated shunt.

Furthermore, by placing the inflow cannula in the right atrium, instead of the left atrium, and adding a membrane oxygenator to the circuit, the TandemHeart effectively becomes an ECMO circuit providing biventricular support. The TandemHeart can also be useful for right ventricular or biventricular failure. By positioning the inflow cannula in the right atrium and the outflow cannula in the pulmonary artery, inserted via a femoral vein and the right internal jugular vein, the pump bypasses the right heart and can serve as a right ventricular assist device, either as a stand-alone device or in conjunction with an LVAD (Kapur et al., 2013; Prutkin et al., 2008).

Extracorporeal membrane oxygenation

Extracorporeal support comes in two varieties. For patients who primarily require oxygenation support, the system can be set up as a veno-veno system (VV ECMO). VV ECMO requires a relatively normally functioning heart as it does not provide any systemic circulatory support. In this set up, the inflow cannula is placed in the inferior vena cava providing drainage of blood which is carried through a continuous flow pump and a membrane oxygenator for gas exchange. The oxygenated blood is then returned to the right atrium. The two cannulas are inserted via a combination of the two femoral veins and the right internal jugular vein. Alternatively, the Avalon bi-caval dual lumen catheter can be inserted via the right internal jugular vein. The dual-lumen cannula includes an inflow cannula with the tip inserted into the inferior vena cava and the outflow cannula that pumps oxygenated blood via side ports into the right atrium.

Unlike the VV ECMO system, the veno-arterial (VA ECMO) system provides total cardiopulmonary and biventricular support. In VA ECMO, the venous inflow cannula is placed via the femoral vein and/or the right internal jugular vein and advanced into the right atrium. More than one inflow cannula can be placed and Y-connected together if there is inadequate drainage. Cannula sizes range from 15 to 29 Fr. The deoxygenated blood is circulated through the centrifugal pump and a membrane oxygenator and then returned to the abdominal aorta via an arterial outflow cannula (ranging in size from 15 to 23 Fr), which is typically inserted via the femoral artery. Depending on the size of the cannulas, they can be inserted percutaneously or via surgical cut down. Like all other MCS devices, ECMO requires anticoagulation, although short periods without anticoagulation can be tolerated when necessary.

VA ECMO can provide flows of up to 6 L/min depending on the sizes of the inflow and outflow cannulas. VA ECMO is particularly useful in cases of cardiopulmonary failure or biventricular failure. The system effectively decompresses the cardiopulmonary system, reducing both the right and left ventricular volumes. This leads to not only an increase in mean arterial pressure but also an increase in both left ventricular systolic and diastolic pressures. This increase in LVEDP and PCWP has the potential risk of progressive acute lung injury. To alleviate this, LV decompression strategies can be employed, which include percutaneous options such as IABP, Impella, TandemHeart, and atrial septostomy. Surgical options include direct left ventricular apical cannulation and direct left atrial cannulation. Noninvasive options include inotropic support and reducing the VA ECMO flow. Based on a meta-analysis of VA ECMO treatment of cardiogenic shock or cardiac arrest, complications include major bleeding, lower extremity ischemia, compartment syndrome, and stroke (Cheng et al., 2014). Distal limb ischemia can occur, particularly with the use of large arterial cannulas. To minimize this risk, an antegrade sheath can be placed in the distal SFA connected to the arterial cannula to provide improved distal limb perfusion.

Centrally cannulated extracorporeal pumps

Central cannulation of extracorporeal pumps allows for a patient to be mobilized after implantation. These devices can generally also be used for longer periods of time compared to percutaneously placed devices. Additionally, these pumps are more versatile as they can be used for left, right, or bi-ventricular support. In patients who are candidates for heart transplantation, these devices can also be used as a bridge-to-transplantation. When used as an LVAD, the inflow cannula is placed in the left atrium, typically via the right superior pulmonary vein and the outflow cannula in the ascending aorta. The apex of the left ventricle can also be a cannulation site for the inflow cannula. For right ventricular support, the inflow cannula is placed in the right atrium with the

outflow cannula in the main pulmonary artery. The cannulas can be tunneled subcutaneously to exit the upper abdomen and connected to an external pump. With this configuration, the chest can be closed, allowing the patient to be mobilized postoperatively. Some currently available external pumps for this purpose include the CentriMag by Thoratec (formerly Levitronix) and the Thoratec paracorporeal ventricular assist device (PVAD).

Durable Mechanical Circulatory Support

Clinical Considerations

Even after a patient has been determined to have indications for a durable MCS device, careful consideration is required to determine if they are indeed good surgical candidates, what clinical treatment strategy should be employed, which devices should be implanted, and the ideal timing of the surgery.

Generally speaking, durable MCS is indicated in patients with stage D heart failure who have NYHA class III or IV symptoms. These are patients whose heart failure has led to severe circulatory compromise refractory to optimal medical therapy and thus requiring advanced therapy such as continuous inotropic therapy, heart transplantation, or MCS. Criteria for LVAD implantation include LVEF of less than 25%, systolic blood pressure $<$80 mm Hg, cardiac index $<$2.0 L/min/m^2, pulmonary capillary wedge pressure $>$20 mm Hg, and dependency on intra-aortic counterpulsation (Williams and Oz, 2001; Fitzpatrick et al., 2008). The Centers for Medicare and Medicaid Services have also developed an approved list of indications for LVAD support (**Box 1**).

Device strategy

The two primary payer-approved indications for MCS device implantation are as a bridge-to-transplant (BTT) and destination therapy (DT). A patient that is a candidate for heart transplantation but is clinically not stable enough to wait for a suitable donor heart would be implanted with a durable MCS as a BTT. These patients are usually listed as a heart transplant candidate and made inactive at the time of device implantation. They then wait to recover from surgery before being reactivated on the transplant list, typically at least three to six months after surgery. If a patient is not a heart transplant candidate, then they are implanted as DT, whereby the durable device is their definitive treatment. Sometimes patients may not be a candidate for heart transplantation at the time of evaluation but has the potential to become one after a durable device is implanted. These patients can be implanted with the subcategory designation of bridge-to-candidacy (BTC). Examples of BTC include patients who are too obese to be a heart transplant candidate but may become one once they have lost enough weight. Durable device implantation may allow them to exercise or get bariatric surgery to help them lose the weight they need to get off. Another typical example is a patient with elevated pulmonary artery pressures (PA), which may decrease after LVAD implantation, thus making the patient a heart transplant candidate. In rare instances, patients may be implanted with a durable MCS with the expectation or at least hope of recovery and eventual explantation of the device. Unfortunately, true bridge-to-recovery (BTR) implantations are not common due to the nature of end-stage heart failure.

Unfortunately, even when a patient has clear indications for durable MCS support, they may also have contraindications that make them ineligible for surgery. It is important to recognize these contraindications so that patients are not being placed at undue risk. The list of absolute and relative contraindications differs from program to program. However, general principles are universal. Examples of contraindications include major irreversible neurologic deficits, active or recent malignancy with poor prognosis, contraindications to anticoagulation, irreversible end-organ damage, and advanced age. The patient's overall fitness for surgery should be evaluated to ensure that they can tolerate the procedure and the postoperative recovery (**Box 2**).

INTERMACS

The Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) database collects outcomes on patients implanted with Food and Drug Administration (FDA)-approved durable MCS devices in the United States (Kirklin et al., 2015). Although the National Heart, Lung and Blood Institute (NHLBI) provides the primary funding and oversight of INTERMACS, this registry is a collaboration that includes not only the NHLBI but also the professional heart failure community, industry, and

Box 1 Centers for medicare and medicaid services—Approved indications for LVAD support

DT: FDA approved only for patients with NYHA class IV HF who are not candidates for heart transplant and:

1. Patients who have failed optimal medical therapy for at least 45 of the last 60 days or have been balloon pump dependent for 7 days or intravenous inotrope dependent for 14 days
2. LV ejection fraction less than 25%
3. Peak oxygen consumption of $\leq$14 mL/kg/min or unable to perform test

BTT

1. Use of an FDA-approved device, implanted according to FDA approval criteria, including NYHA class IV HF
2. Listed for transplant at the time of VAD implant

Data from Centers for Medicare & Medicaid Services. Decision memo for ventricular assist devices as destination therapy (CAG-00119 N).

Box 2 Selected contraindications for left ventricular assist device support

- Major irreversible neurologic deficits
- Active malignancy without reasonable intent for cure
- Inability to tolerate anticoagulation
- Active drug use, psychological instability, or noncompliance
- Nonreversible end-organ dysfunction, including end-stage renal disease and hepatic cirrhosis
- Pregnancy
- Advance age (center-dependent thresholds)
- Severe dementia
- Mechanical aortic valve or severe aortic insufficiency without surgical plan to address
- Severe RV dysfunction without options for RVAD support

Data from Feldman et al. (2013).

Table 4 INTERMACS level of limitation at time of implant (Stevenson et al., 2009)

Level	*Description*	*Time to MCS*
1	"Crashing and burning"—critical cardiogenic shock	Within hours
2	"Progressive decline"—inotrope dependence with continuing deterioration	Within a few days
3	"Stable but inotrope dependent"—describes clinical stability on mild-moderate doses of intravenous inotropes (patients stable on temporary circulatory support without inotropes are within this profile)	With a few weeks
4	"Recurrent advanced heart failure"—"recurrent" rather than "refractory" decompensation	Within a few months
5	"Exertion intolerant"—describes patients who are comfortable at rest but are intolerant of exercise	Variable
6	"Exertion limited"—a patient who is able to do some mild activity but fatigue results within a few minutes or any meaningful physical exercise	Variable
7	"Advanced NYHA 3"—describes patients who are clinically stable with a reasonable level of comfortable activity, despite history of previous decompensation that is not recent	Not a candidate for MCS

hospitals (Kirklin et al., 2015). One of their main goals is to use the extensive device and patient database to identify predictors of outcomes and risk factors for complications by stratifying device candidates. Because the timing of LVAD implantation with respect to the severity of the heart failure correlates with postoperative survival, proper patient selection is a critical factor in determining postoperative outcomes. INTERMACS stratifies patients into one of seven profiles or levels that describes the patient's severity and acuity (**Table 4**). Compared to higher levels (i.e., more stable), patients who are at lower levels (i.e., less stable) at the time of device implant have worse outcomes (Kirklin et al., 2015).

Outcomes

The landmark REMATCH (Randomized Evaluation of Congestive Heart Failure) trial was the first to compare survival outcomes of patients implanted with a durable MCS device and medical therapy (Rose et al., 2001; **Table 5**). This study randomized 129 patients with end-stage heart failure who were ineligible for cardiac transplantation to receive a HeartMate XVE (St. Jude Medical-Thoratec, Little Canada, Minnesota) LVAD or optimal medical management. The one year survival was 52% in the device group compared to only 25% in the medical therapy group ($P = .002$). However, the frequency of serious adverse events was 2.35 times higher in the device group compared to the medical-therapy group, with infection, bleeding, and device malfunction being the predominant complications. But despite these adverse events, the device group had a significantly improved quality of life at one year.

With the introduction of second- and third-generation LVADs, there have been great strides in LVAD outcomes since the first-generation XVE LVAD. One year survival has improved from 52% for the first-generation XVE to as high as 86% with third-generation LVADs (Aaronson et al., 2012).

MCS outcomes extend well beyond survival gains (Waqas and Cowger, 2016). LVAD support has demonstrated improvements in 6-minute walk distances, NYHA functional class, quality-of-life measures, and cardiac output (Aaronson et al., 2012; Starling et al., 2011; Rogers et al., 2017; Pagani et al., 2009; Slaughter et al., 2013).

Durable MCS Devices

HeartMate II

Although the first-generation LVADs, such as the XVE, paved the way for future generations of LVADs, these older models are now primarily of historical interest. The second-generation HeartMate II LVAD was FDA approved for BTT in 2008 and for DT in 2010. The device is implanted just below the heart and has a flexible inflow conduit that is attached to the apex of the left ventricle. Its size requires that it be implanted in the upper abdomen anterior to the peritoneum, which necessitates dividing the left diaphragm. The

Table 5 Survival after left ventricular assist device in major clinical trials and INTERMACS

		Survival		
Trial	*Device(s) studied,* c	*Operative (%)*	*6 months (%)*	*1 year (%)*
REMATCH[a]	68 XVE	88	61	52
	61 OMM	–	52	25
HMII-DT[b]	134 HMII	93	78	68
	66 XVE	89	64	55
HMII-BTT[c]	133	89	75	68
HMII-BTT Pivotal[d]	281	92	82	73
INTERMACS-HMII BTT[e]	169	96	91	85
ADVANCE-Heartware BTT[f]	140 HVAD	99	94	86
	499 HMII	97	92	85
INTERMACS[g]	12,030 CF-LVADs	95	85	80

[a]Rose et al. (2001).
[b]Slaughter et al. (2013).
[c]Miller et al. (2007).
[d]Pagani et al. (2009).
[e]Starling et al. (2011).
[f]Aaronson et al. (2012).
[g]Kirklin et al. (2015).

outflow graft extends from the pump and is anastomosed to the ascending aorta. A percutaneously placed driveline connects the pump to an external controller. The pump itself consists of an axial-flow rotary pump containing a magnet and is driven by the electromotive force generated by the motor. The rotational speed of the pump is determined by the clinician but the actual pump output is pre- and after-load dependent. For a fixed pump speed, the pump flow is inversely related to the pressure gradient across the valve. The HeartMate II is continuous-flow LVAD and therefore patients typically have little to no pulsatility in their arterial waveform. Outcomes with the HeartMate II are significantly improved over the XVE with a 30-day operative mortality of 4% and a one-year Kaplan-Meier survival of 85% (Starling et al., 2011). The HeartMate II also provides significant functional improvements. Compared to a baseline where most patients had NYHA class IV symptoms, over 80% of patients had class I or II symptoms by 6 months, a finding that was sustained at 24 months (Rogers et al., 2010).

Third-generation LVADs

The HeartWare ventricular assist device (HVAD) is a third-generation LVAD and is a continuous flow pump. It was FDA approved for BTT in 2012. The device is small enough to be implanted directly into the left ventricular apex within the pericardial cavity. An outflow graft connects the pump to the ascending aorta. The titanium housing contains an elevated impeller that rotates utilizing magnetic and hydrodynamic forces. The driveline is tunneled subcutaneously and exits the upper abdomen on one side and connects the pump to the external controller. The small size of this pump has also led some surgeons to implant them via bilateral mini-thoracotomies, thus avoiding a sternotomy and making surgery during a subsequent heart transplant potentially easier in BTT patients (Schmitto et al., 2012). The ADVANCE clinical trial implanted 140 HVADs as BTT as an investigational device (Aaronson et al., 2012). Six-month success (survival, transplantation, recovery with LVAD explantation) was comparable between the HVAD and commercially available pumps at 90.7% versus 90.1% ($P < .001$ for noninferiority), respectively. At 6 months, median 6-minute walk distance and quality-of-life scores improved significantly as well. Because of its small size, the HVAD is particularly useful for smaller patients and in patients for whom a minimally invasive approach would be helpful.

The HeartMate III is also a third-generation continuous flow LVAD. It is currently under investigational use for BTT and DT. Similar to the HeartWare HVAD, it is implanted into the apex of the left ventricle within the pericardial cavity. The outflow graft is anastomosed to the ascending aorta and the driveline is tunneled subcutaneously and exits the upper abdomen to an external controller. The device has a magnetically levitated centrifugal pump. An additional feature of this pump is that it can generate pulsatile flow patterns which would theoretically reduce thrombosis formation. The MOMENTUM 3 trial (Multicenter Study of MagLev Technology in Patients Undergoing Mechanical Circulatory Support Therapy with HeartMate 3) compares the HeartMate III to the HeartMate II for advanced heart failure patients as BTT or DT (Mehra et al., 2017). Initial 6-month results are promising with the HeartMate III demonstrating a decrease in the primary end point (composite of survival free of disabling stroke or reoperation to replace or remove the device) of 86.2% compared to 76.8% for the HeartMate II (two-tailed $P = .04$ for superiority). There was no difference in rates of death or disabling stroke but reoperation for pump malfunction was less frequent and there were no pump thrombosis events with the HeartMate III.

Total artificial heart

Patients requiring biventricular MCS require two separate ventricular assist devices or a total artificial heart. Currently, there are no FDA approved durable RVAD devices, although the smaller third-generation LVADs have been used for this purpose (Shehab et al.,

2016). The SynCardia (Syncardia Systems, Inc. Tucson, AZ) total artificial heart (TAH) is the modern version of the CardioWest artificial heart from the 1990s. Unlike LVADs, the TAH provides biventricular support and is orthotopically implanted by replacing the native ventricles. It contains a pneumatically driven diaphragm pump containing valves. Two ventricular cannulas are tunneled subcutaneously and connect to an external drive system. It is available in two sizes, the original 70 cc version and the newer and smaller 50 cc version. The 70 cc temporary TAH (TAH-t) was FDA approved in 2004 for BTT for in-hospital use and is undergoing investigational studies for use as DT. The smaller 50 cc TAH is undergoing investigational studies for BTT use. In addition to the larger hospital driver, the Freedom Portable Driver is available for patients who are discharged home. In a prospective nonrandomized study of the TAH, 79% of the implanted patients survived despite 94% of the patients being INTERMACS level 1 (Copeland, 2014). Similarly matched historical control patients had a 46% survival ($P < .001$). The overall one-year survival was 80% for TAH patients compared to 31% for controls ($P < .001$).

Complications

While there have been significant improvements in patient survival with newer generation LVADs, these devices are unfortunately associated with a significant rate of complications. Freedom from major adverse events at 12 and 36 months is only 30% and 14%, respectively (Kirklin et al., 2013). The most common complication after LVAD implantation is bleeding. In the early postoperative period the reoperation rate for bleeding and the rate of blood transfusions of at least 2 units was 31% and 53%, respectively (Miller et al., 2007). During this early phase, postoperative bleeding may be due to number of factors, including diffuse coagulopathy, hemodilution, or consumption of coagulation factors, and the residual effects of antiplatelet therapies (Despotis et al., 2001). In the later period, bleeding is most commonly associated with the gastrointestinal (GI) tract. In addition to the chronic antiplatelet and anticoagulant therapy, this GI bleeding may also be due to an acquired von Willebrand syndrome secondary to mechanical damage within the pump.

Infection is another significant complication of LVAD therapy. This likely has much to do with the fact that the driveline allows for potential communication of external infectious hazards to the implanted device. The rate of infection is 7.3 events per patient year (EPPY) (Kirklin et al., 2015) and is associated with obesity, diabetes, malnutrition, younger age, and driveline trauma (John et al., 2014; Koval et al., 2014). Driveline infections may initially be treated with antibiotics but may also require surgical debridement. Infection involving the pump itself may require a device exchange, which is associated with increased morbidity and mortality (John et al., 2014).

Strokes are a potentially devastating complication of LVAD therapy, with a freedom from stroke rates of 89% at one year (Kirklin et al., 2013). They can be hemorrhagic or ischemic. Hemorrhagic strokes are likely associated with anticoagulation therapy and if severe enough or recurrent in nature, the anticoagulation goals may need to be reduced or event discontinued. However, this has to be balanced with the risk of thrombosis. Ischemic strokes, on the other hand, are likely related to thromboembolic events associated with pump thrombosis. Strokes are suspected based on clinical findings and confirmed with imagining by head CT and/or MRI, and are treated accordingly with the guidance of neurology. In hemorrhagic strokes, the degree of anticoagulation and antiplatelet therapy has to be weighed against the risk of device thrombosis.

LVADs require anticoagulation to prevent device thrombosis, a complication that had increased in HM II devices at 3 months post-implantation to 8.4% after 2011 compared to 2.2% before this date (Starling et al., 2011). The reason for this is not clear but may be multifactorial and include better recognition and diagnosis of the problem, modifications in anticoagulation intensity, and variable monitoring. The diagnosis of pump thrombosis involves a combination of clinical findings, laboratory assessments, device values, and imaging. Patients may present with symptoms of heart failure and hemoglobinuria. They may also have TIAs or strokes due to emboli from the pump. Pump thrombosis is associated with elevated power consumption with elevations in the power readings. Laboratory findings consistent with hemolysis can be expected and include an elevated lactate dehydrogenase and serum-free hemoglobin, and reduced haptoglobin and hemoglobin (Cowger et al., 2014). Echocardiography may show reduced unloading of the left ventricle by the LVAD that can be quantitatively demonstrated with a ramp protocol whereby the LV dimensions can be plotted against sequential increases in pump speed. A dampened decrease in LV dimensions is suggestive of pump thrombosis (Uriel et al., 2012). Initial management of pump thrombosis includes adequate anticoagulation and renal protection with IV fluids and sodium bicarbonate infusion. More aggressive measures include the use direct thrombin inhibitors or thrombolytics. If all this fails, the patient may require a surgical pump exchange.

Right ventricular (RV) failure is the Achilles heel of LVADs and is associated with poorer outcomes (Baumwol et al., 2011). Proper functioning of the LVAD requires adequate functioning of the right ventricle. RV failure can occur acutely in the OR at the time of implant, in the perioperative period, or even months after implantation. However, in a patient with known RV dysfunction, RV failure in the early perioperative period is of greatest concern. The published rates of acute RV failure ranges from 15% to 35% (Slaughter et al., 2009; Aaronson et al., 2012; Slaughter et al., 2013; Matthews et al., 2008). Careful preoperative assessment of RV function is critical and is primarily based on echocardiography and right heart catheterization. Aggressive volume management with diuretics and the use of pulmonary vasodilators and inotropes may be necessary to support the RV both preoperatively and postoperatively. If a patient has acute RV failure post-LVAD implantation, he/she may require temporary right-sided support with MCS. Off-label use of durable third generation LVADs has been reported for special circumstances (Shehab et al., 2016). Several risk scores have been published to assess the risk of RV failure after LVAD implantation. In a patient with significant biventricular failure at high risk of RV failure requiring MCS, a total artificial heart should be considered (Kasirajan et al., 2012).

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Left-Sided Obstructive Congenital Heart Lesions: Including Hypoplastic Left Heart

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Overview

Lesions causing left ventricular outflow obstruction typically result in acyanosis and adequate pulmonary blood flow. Characteristic lesions include aortic valve stenosis, coarctation of the aorta, and interrupted aortic arch. In the most extreme form of left-sided obstruction, hypoplastic left heart syndrome, cyanosis will occur resultant from intracardiac mixing at the atrial level.

Left-sided obstructive lesions result in a clinical presentation that correlates with the severity of obstruction. Those with significant obstruction may have an audible cardiac murmur, symptoms of congestive heart failure, and electrocardiographic changes consistent with myocardial hypertrophy or ischemia. This chapter focuses on the prevalence, physiology, clinical findings, and management of left-sided obstructive lesions including hypoplastic left heart syndrome.

Aortic Stenosis

There are three general classifications of aortic stenosis: below the level of the aortic valve (subvalvar or subaortic stenosis), at the level of the aortic valve (valvar aortic stenosis), and above the aortic valve (supravalvar aortic stenosis) (**Fig. 1**) (Park, 2014).

Valvar Aortic Stenosis

Definition and Prevalence

Obstruction to left ventricular outflow due to an abnormal aortic valve is termed valvar aortic stenosis (**Fig. 1B**) (Park, 2014). The reported incidence ranges from 0.16 to 0.38 per 1000 live births (Hoffman and Kaplan, 2002). A normal aortic valve has three

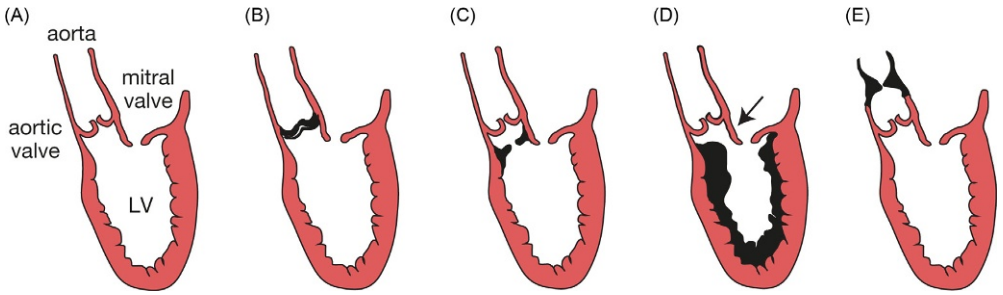

Fig. 1 Aortic stenosis subtypes. (A) Normal structure of left ventricle (LV) leading to the aorta, with the mitral valve and aortic valve indicated. (B) Valvar stenosis. (C) Discrete membranous subaortic stenosis. (D) Hypertrophic subaortic stenosis (*arrow indicates the anterior leaflet of the mitral valve*). (E) Supravalvar stenosis. Adapted from Park, M. K. (2014) Park's pediatric cardiology for practitioners (6th edn.). St. Louis: Mosby.

cusps or leaflets (termed tricuspid or trileaflet) and three commissures (termed tricommissural). As opposed to elderly patients, where stenosis is often due to a calcified and immobile trileaflet valve, young patients usually have a congenitally malformed, but noncalcified valve. Due to the abnormal valve development, the aortic valve can be unicuspid, bicuspid, tricuspid, or quadricuspid.

Physiology

Any obstruction to outflow results in an increased afterload on the left ventricle. This means the ventricle must generate an increase in pressure during systole proportional to the degree of obstruction to provide the required cardiac output. The effect the increased afterload has on the heart not only depends on the severity of the obstruction but also the timing of the stenosis. During fetal life, severe aortic valve stenosis will result in dilation and decreased contractility of the left ventricle followed by development of endocardial fibroelastosis. As the fetus matures to term, the growth of the left heart is below normal, and the fetus may develop hypoplastic left heart syndrome.

After birth upon closure of the ductus arteriosus, the left ventricle needs to support the entire systemic arterial circulation. If the degree of aortic valve stenosis is severe enough to result in the left heart being unable to support the systemic circulation, due to ventricular dysfunction and/or inadequate growth of the left heart, then the infant will develop cardiovascular collapse. In these circumstances when the systemic circulation is dependent on a patent ductus arteriosus, it is termed "critical" aortic stenosis. In keeping the ductus arteriosus patent, the right ventricle can support the systemic circulation.

Clinical Manifestations

If the degree of obstruction is not severe enough to interfere with the ability of the left ventricle to provide adequate systemic perfusion, a child may remain asymptomatic with normal growth and development. A heart murmur may be appreciated incidentally during a routine physical examination. Less commonly, a child with valvar aortic stenosis reports chest pain and exercise intolerance. A history of syncope in a child with aortic stenosis is a sign of clinically significant disease and is considered a high risk of sudden death.

The physical exam of a child may reveal weaker than normal peripheral pulses, a decreased pulse pressure, and a systolic thrill in the suprasternal notch. On auscultation, a systolic ejection click may precede the systolic ejection murmur. The heart murmur is normally loudest at the upper right sternal border with potential radiation to the neck. There is a delay in the timing of the aortic component of the second heart sound, since the duration of ventricular systole is in the direct proportion to the degree of stenosis, resulting in a narrowly split second heart sound. If the degree of stenosis is severe enough, the closure of the aortic valve may occur after the pulmonary valve, which is termed "reverse splitting."

Evaluation

Chest X-ray

A chest X-ray may reveal a prominent ascending aorta due to poststenotic dilation.

Electrocardiogram

Oftentimes, an electrocardiogram is normal in mild aortic valve stenosis. However, if the degree of obstruction is severe, changes such as left ventricular hypertrophy, T-wave changes, and/or ventricular ectopy may be present. In patients who remain asymptomatic with concerning findings or in those who want to participate in athletics, a stress test can be helpful. Significant obstruction can produce ST-segment and T-wave abnormalities during times of exercise.

Echocardiogram

The primary tool used to diagnose aortic valve stenosis is echocardiography. This imagining modality provides a detailed noninvasive assessment of the cardiac anatomy. The primary goals of the echocardiographic assessment include determining the severity of valve stenosis using Doppler interrogation; determining the morphology of the aortic valve; evaluating all left heart structures (determine if there are associated anomalies); and obtaining systemic ventricular dimensions, wall thickness, and function (Lai et al., 2015). The severity of valve stenosis is estimated by measuring the Doppler-derived maximum instantaneous and mean pressure decrease across the aortic valve. It is important to remember that these measurements are only valid if systemic ventricular function and cardiac output are normal.

Current guidelines define mild aortic valve stenosis as a peak velocity of 2.0–2.9 m/s or a mean pressure gradient of $<$20 mmHg. Moderate stenosis is defined as a peak velocity of 3.0–3.9 m/s or a mean pressure gradient of 20–39 mmHg. Severe stenosis is defined as a peak velocity of $>$4 m/s or a mean pressure gradient of $\geq$40 mmHg. In patients with decreased cardiac output, significant aortic stenosis can be present with lower peak flow velocities and pressure gradients. In these instances, an aortic valve area should be calculated to determine severity of stenosis (Feltes et al., 2011; Nishimura et al., 2014). An effective valve orifice of >1.4 cm^2 is considered mild aortic valve stenosis, 1.0–1.4 cm^2 is considered moderate aortic valve stenosis, and <1.0 cm^2 is considered severe aortic valve stenosis.

Cardiac catheterization

As the ability to accurately evaluate the severity of aortic valve stenosis using noninvasive diagnostic methods has improved greatly, the role of cardiac catheterization has become primarily one of therapeutic intervention.

Management

Valvar aortic stenosis is a difficult disease entity to treat, with repeated interventions often required, and necessitates lifelong follow-up as it is often progressive. Not counting infants in heart failure and/or those with "critical aortic valve stenosis," current recommendations for intervention are based on the published natural history studies (Keane et al., 1993). In many pediatric institutions, if the degree of aortic insufficiency is not significant, balloon valvotomy has become the initial intervention of choice. Current class I indications for aortic valvuloplasty include in a newborn with critical aortic stenosis or a child with depressed left ventricular systolic function regardless of valve gradient; children with a resting peak to peak systolic valve gradient of $\geq$50 mmHg; children with a resting peak systolic valve gradient of $\geq$40 mmHg if there are symptoms of angina, syncope, or ischemic ST-T wave changes at rest or with exercise. Although balloon valvuloplasty is considered an effective intervention, it is palliative and one must always be cautious about creating significant aortic valve regurgitation (Baim and Grossman, 2006; Warnes et al., 2008).

For infants with "critical" aortic stenosis, the main aspect of management is to ensure ductal patency as to allow for systemic and cerebral perfusion. Balloon aortic valvuloplasty is the primary method of treatment. Care is taken to avoid creating significant aortic valve insufficiency and/or injury to the mitral valve (Baim and Grossman, 2006).

Although not commonly performed for valvar aortic stenosis in children, surgical options include valvotomy and valve replacement. Valve replacement may consist of either a mechanical or bioprosthetic valve or with a pulmonary autograft (known as the Ross procedure) (Jonas, 2014).

Subvalvar Aortic Stenosis

Definition and Prevalence

Obstruction to left ventricular outflow from below the aortic valve is termed subaortic stenosis. The incidence of subvalvar aortic stenosis is 0.09 per 1000 live births and can occur either in isolation or in association with a multitude of other congenital heart defects (Kitchiner et al., 1994). These include but are not limited to ventricular septal defects (VSD), atrioventricular septal defect (AVSD), aortic valve stenosis, coarctation of the aorta, interrupted aortic arch (IAA), and double-chambered right ventricle.

There is variation in the morphologic types of subaortic obstruction. The most common type is due to a discrete, thin ridge, or membrane located beneath the aortic valve, known as discrete membranous subaortic stenosis (**Fig. 1C**) (Park, 2014). Due to the abnormal flow of blood through the obstruction, the aortic valve may be deformed, resulting in aortic valve insufficiency. It is important to remember that the abnormal obstructive tissue may not only involve the ventricular septum but can extend onto the anterior leaflet of the mitral valve. Although this type of obstruction is rare in neonates, significant progression is often noted in childhood.

Hypertrophic subaortic stenosis results from hypertrophy of the interventricular septum causing muscular obstruction of the ventricular outflow tract. This form of subaortic stenosis can often result in systolic anterior motion of the mitral valve (**Fig. 1D**) (Park, 2014).

Tunnel-type subaortic obstruction is characterized by a long-segment narrowing of the left ventricular outflow tract due to muscle. This type of stenosis often occurs early in life and is frequently associated with other left-sided obstructive lesions.

Subaortic obstruction is also seen in other congenital cardiac lesions such as interrupted aortic arch, which is often associated with posterior deviation of the infundibular septum, or complete atrioventricular septal defect, which may have accessory endocardial cushion tissue obstruct the left ventricular outflow tract.

Physiology

Although the development of subaortic stenosis is believed to be secondary to abnormal flow across the left ventricular outflow tract resulting in abnormal growth of tissue (either fibrous or muscular), the physiologic consequences may be like that of aortic valve stenosis as there is increased afterload on the left ventricle. Due to the obstruction, there is a compensatory ventricular hypertrophy to reduce the wall stress. Ventricular function is usually maintained; however, it can worsen overtime if the obstruction is not alleviated. Not only can the abnormal jet of flow across the subaortic region interfere with proper motion of the aortic valve, the fibrous tissue may extend onto the aortic valve leaflets and result in impaired functionality.

Clinical Manifestations

The physical exam of a child with subaortic stenosis will produce a crescendo–decrescendo systolic ejection murmur usually appreciated best at the left mid sternal border. There may be an additional blowing early diastolic murmur if significant aortic regurgitation is present. In severe cases, the peripheral pulses may be weak and delayed, and the left ventricular impulse is displaced and sustained.

Evaluation

Chest X-ray

Chest X-ray is often not diagnostic. If there is significant aortic regurgitation, cardiomegaly may be appreciated due to left ventricular dilation.

Electrocardiogram

Like aortic valve stenosis, an electrocardiogram may be normal in mild subvalvar stenosis. However, if the degree of obstruction is severe, changes such as left ventricular hypertrophy, T-wave changes, and/or ventricular ectopy may be present.

Echocardiogram

The primary tool used for diagnosis and assessment of disease severity is echocardiography. The primary goals of the echocardiographic examination include assessment of the severity of left ventricular outflow tract obstruction, determine the morphology of the outflow tract and the type of obstruction, and identify any associated anomalies. It is important to note that the severity of subaortic obstruction may be underestimated in cases of depressed left ventricular function or if there is a large, nonrestrictive ventricular septal defect (Lai et al., 2015).

Cardiac catheterization

Although cardiac catheterization will provide an accurate measurement of the systolic peak-to-peak gradient across the left ventricular outflow tract, this diagnostic tool is not used as much as echocardiography, which has become a reliable imaging modality and is noninvasive.

Management

As opposed to valvar aortic stenosis, subvalvar obstruction does not respond to balloon angioplasty. Therefore, surgical resection of the subvalvar membrane or fibrous ridge, or myectomy in patients with muscular or tunnel-like obstruction, is performed. However, due to the high rate of recurrence, the timing of surgical intervention can be controversial, particularly in the first decade of life. Subvalvar aortic obstruction tends to be progressive in most, and there is a risk of developing of aortic regurgitation (particularly if the mean echocardiographic Doppler gradient is >30 mmHg). Most recommend deferring surgery if the degree of obstruction is moderate or less (mean Doppler gradient <30 mmHg or peak instantaneous systolic gradient <50 mmHg) and there is no more than trivial aortic valve insufficiency. Consideration for surgery may be considered in those with lesser degree of obstruction if there is concomitant left ventricular dysfunction and/or significant aortic valve regurgitation. If a patient desires to become pregnant or participate in athletic activity, surgical intervention is considered in the setting of less significant obstruction. Although prevention of aortic valve insufficiency alone is not a criterion for surgical intervention, the development and progression of significant aortic valve insufficiency is commonly used as criteria for surgery (Warnes et al., 2008).

Supravalvar Aortic Stenosis

Definition and Prevalence

Obstruction to left ventricular outflow from above the aortic valve is termed supravalvar aortic stenosis (**Fig. 1E**) (Park). The incidence of supravalvar aortic stenosis is 0.05 per 1000 live births and can be associated with an abnormal aortic valve, coronary artery stenosis, coarctation of the aorta, and pulmonary artery stenosis (Kitchiner et al., 1994). It is commonly seen as a feature of Williams syndrome. Additionally, mutations in the ELN gene, which leads to a decrease in production of tropoelastin, are often seen in patients with supravalvar aortic stenosis (Health and Medicine).

Supravalvar aortic stenosis usually occurs at the sinotubular junction and is visualized as a discrete narrowing of the vessel lumen due to thickening of the aortic media. There is frequently prestenotic dilation of the sinuses of Valsalva, tethering of the aortic valve leaflets, and stenosis of the coronary ostia. There may be complete occlusion of the coronary ostia if a leaflet of the aortic valve adheres to the obstructing ridge of tissue. Additionally, there may be hypoplasia of downstream vascular structures such as the ascending aorta, aortic arch, and head and neck vessels.

Physiology

In general, the physiology is like that of valvar aortic stenosis as there is a hypertrophied left ventricle in the setting of significant stenosis. However, it is important to remember that due to the location of the narrowing above the aortic root, there is elevated systolic pressure proximal to the site of obstruction, which is believed to play a role in the development of coronary ostial stenosis. Therefore, as the demand from a hypertrophied ventricle increases, an inadequate degree of myocardial perfusion may result, particularly during periods of increased myocardial oxygen demand.

Clinical Manifestations

The physical exam will often reveal a basilar crescendo–decrescendo systolic ejection murmur with radiation to the suprasternal notch and may be associated with a thrill. There may be preferential flow into the right brachiocephalic artery, which results in a blood pressure and strength of pulse differential between the right and left upper extremities (known as the Coanda effect) (Lai et al., 2015; Eidem et al., 2015). A dynamic apical impulse may be present and there can be paradoxical splitting of the second heart sound. Additionally, these patients tend to display poor growth, exercise intolerance, and potentially angina with effort and/or syncope.

Evaluation

Chest X-ray

The chest X-ray is often normal, however left ventricular hypertrophy or a prominent aortic knob may be present.

Electrocardiogram

If the stenosis is significant, left ventricular hypertrophy and/or ischemic changes may be present.

Echocardiogram

The diagnosis of supravalvar aortic stenosis is usually confirmed by echocardiography. The aims of the echocardiogram include assessment of the aortic root, sinotubular junction, and aortic valve morphology, Doppler evaluation of the severity of stenosis, anatomic assessment of the aortic arch (including head and neck vessels), and functional assessment of the left ventricle. Magnetic resonance imaging with angiography can also be used and provides detail of the supravalvar obstruction and aortic arch anatomy (Lai et al., 2015; Eidem et al., 2015).

Cardiac catheterization

Cardiac catheterization may be performed, particularly if additional disease is suspected such as branch pulmonary artery stenosis. If there is severe bilateral outflow obstruction, catheterization is extremely high risk. As there is often coronary ostial stenosis, selective coronary angiography is high risk.

Management

Therapy for supravalvar aortic stenosis consists of surgical repair of the obstruction, regardless if it is discrete versus long-segment narrowing. The lesion does not reliably respond to balloon angioplasty, and stent placement has mixed results and is considered an inferior therapy. Current recommendations support operative repair for patients with a mean Doppler gradient or systolic peak-to-peak gradient ≥ 50 mmHg. Surgical repair is also recommended in those who have a lower gradient and are symptomatic, have left ventricular hypertrophy or systolic dysfunction, or who desire to become pregnant or engage in athletic activity (Warnes et al., 2008).

Current Topics in Aortic Stenosis

Understanding the development heart valve disease is an interesting area of research. Recently, efforts have been directed at the use of novel mass spectrometry techniques to evaluate lipid biology in heart valve tissues (Angel et al., 2016). Interventional research has focused on understanding the long-term outcomes of aortic valvuloplasty in pediatric patients with aortic stenosis (Awasthy et al., 2016). Others have focused on novel methods of monitoring and risk stratification of patients with aortic stenosis (Domanski et al., 2017).

Coarctation of the Aorta

Definition and Prevalence

Coarctation of the aorta is defined as a narrowing of the aorta, most typically in the descending aorta at the insertion point of the ductus arteriosus (referred to as juxtaductal, **Fig. 2**) (Townsend et al., 2016). It has been estimated to account for 4%–6% of all congenital heart disease (4 per 10,000 live births) (Reller et al., 2008; Hoffman and Kaplan, 2002). Coarctation of the aorta is associated with Turner syndrome and will occur in 5%–15% of girls with this syndrome (Eckhauser et al., 2015; Wong et al., 2014).

Physiology

In utero, coarctation has limited hemodynamic consequence as 60% of the cardiac output is via the ductus arteriosus. During transition, obstruction becomes increasingly problematic as the ductus closes from the pulmonary artery toward the aorta after birth (Rudolph, 2009; Johnson and Moller, 2014; Moller and Neal, 1981). Progression of obstruction to left ventricular outflow leads to increased pressure (afterload) in the left ventricle and ascending aorta. This stress on the left ventricle results in increased myocardial

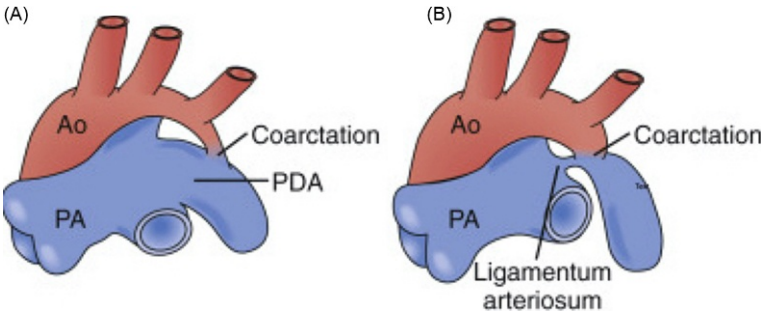

Fig. 2 Coarctation of the aorta (Ao). (A) Preductal aoarctation. (B) Juxtaductal coarctation. *PA*, Pulmonary artery; *PDA*, patent ductus arteriosus. Reprinted with permission from Townsend, C. M., Beauchamp, R. D., Evers, B. M. and Mattox, K. L. (2016). Sabiston textbook of surgery: The biological basis of modern surgical practice (20th edn.). Philadelphia, PA: Elsevier.

oxygen consumption as well as compensatory hypertrophy. In cases of severe obstruction in a neonate, compensatory hypertrophy may be inadequate. This, coupled with the inability of the neonate to augment myocardial contractility, can result in left ventricular failure (Johnson and Moller, 2014; Moller and Neal, 1981).

Coarctation of the aorta may also result in the formation of collateral vessels. In the setting of a pressure gradient across the aorta, blood vessels may enlarge and result in communications with the descending aorta as a means of bypassing the obstruction. Such collateral blood vessels aid in compensation and may mask the development of symptoms even in the setting of a significant coarctation (Moller and Neal, 1981).

Clinical Manifestations

Symptoms are directly related to the degree of obstruction and typically develop as the ductus arteriosus closes in the first several days of life. A majority will remain asymptomatic in childhood. However, 10% of patients will present in infancy with signs of failure (Moller and Neal, 1981; Johnson and Moller, 2014). Those with severe obstruction will have poor perfusion. Others may gradually develop symptoms of heart failure.

Physical exam findings also vary with the degree of obstruction. In the absence of cardiogenic shock, a blood pressure gradient may be noted with lower systolic pressure measured in the lower extremities when compared with the upper extremities. A systolic murmur may be audible; however, in infants with cardiogenic shock this may be absent.

A patient may elude diagnosis until presentation for other findings such as hypertension. If the hypertension is severe, there may be report of headaches and/or epistaxis, and the individual may be at risk for heart failure and dissection of the aorta.

Evaluation

Chest X-Ray

The chest X-ray findings correlate with the severity of obstruction. In asymptomatic children and young adults, the only findings may be rib notching and indentation of the aorta resulting in a "3" sign. In infants with severe obstruction and congestive heart failure, the chest X-ray will reveal cardiomegaly and pulmonary venous congestion (Park, 1996).

Electrocardiogram

The electrocardiogram will also vary with the severity of obstruction. The EKG may be normal. A rightward QRS axis or right bundle branch block may be present in symptomatic infants. Older children with coarctation may have evidence of left ventricular hypertrophy (Park, 1996).

Echocardiography

Echocardiography is the diagnostic test of choice for evaluation of coarctation given its noninvasive nature, ability to quantify obstruction, detect additional lesions, and provide follow-up after repair. In addition to identifying coarctation, echocardiography can be used to describe additional intracardiac findings that suggest arch obstruction including left ventricular obstruction, right or left ventricular hypertrophy, or ventricular dysfunction. Doppler evaluation will demonstrate flow acceleration proximal to the coarctation. With the addition of color Doppler, a turbulent flow pattern around the area of narrowing will be evident. Doppler evaluation can also be used to demonstrate an abnormal flow pattern in the descending aorta. Following arch repair, the echocardiogram can be used to monitor and quantify the presence and degree of residual obstruction (Eidem et al., 2015).

Cardiac catheterization

The use of cardiac catheterization is largely interventional as noninterventional methods of diagnosis, such as echocardiography, are quite accurate. Cardiac catheterization may be used for stent placement, balloon dilation of residual coarctation following repair, or evaluation of additional cardiac defects such as coronary abnormalities. The use of catheter-based interventions varies widely among institutions.

Management

Medical management

Infants with coarctation of the aorta and severe obstruction require medical management prior to surgical intervention. The goals of medical management are to maintain patency of the ductus arteriosus to ensure systemic cardiac output, support ventricular function, and address additional consequences of cardiac failure such as metabolic acidosis. Ductal patency is achieved with infusion of prostaglandin E1. Patients on prostaglandin infusion need to be monitored for the development of apnea and fever as the most common side effects. Support of ventricular function can be achieved with inotropes when necessary (Moller and Neal, 1981).

If an older child presents with hypertension and is discovered to have a coarctation, the hypertension should be controlled by medical therapy. Current recommendations advocate for intervention when the peak-to-peak gradient is $\geq$20 mmHg or in the presence of a significant coarctation with evidence of substantial collateral flow (Feltes et al., 2011).

Surgical intervention

Surgery remains the mainstay of intervention for coarctation of the aorta in infants and children. Societal guidelines recommend that surgical repair be performed as early as possible to avoid long-term complications (Warnes et al., 2008; Feltes et al., 2011; Silversides et al., 2010). Surgical approach varies widely from institution to institution. Options for surgical repair include resection with end-to-end anastomosis, subclavian flap aortoplasty, bypass graft placement across an area of coarctation, and prosthetic patch aortoplasty. Overall, the mortality for surgical repair is low (Warnes et al., 2008; Burch et al., 2009; Mery et al., 2015).

Percutaneous alternatives to surgery in older children and adolescents include balloon angioplasty or stent placement. Balloon angioplasty is the treatment of choice in patients, regardless of age, with recoarctation.

Current topics in coarctation of the aorta

At present, coarctation of the aorta remains the most common ductal-dependent lesion missed on neonatal screening. As such, ongoing research is being done to identify methods and tools to improving fetal diagnosis of the disease (Beattie et al., 2016). Additionally, methods of improving aortic arch dimensions in utero are ongoing (Zeng et al., 2016).

Interrupted Aortic Arch

Definition and Prevalence

Interrupted aortic arch (IAA) represents a rare, extreme form of coarctation of the aorta in which the aortic arch is atretic or absent (**Fig. 3**) (Eidem et al., 2015; Townsend et al., 2016). There are three types of IAA categorized by the location of the defect (Celoria and Patton, 1959):

1. Type A: interruption of the aorta occurs distal to the left subclavian artery (40%).
2. Type B: interruption of the aorta occurs between the left common carotid and the left subclavian artery (most common, >50%).
3. Type C: interruption of the aorta occurs proximal to the origin of the left common carotid artery (rare).

Interrupted aortic arch often occurs in association with other congenital heart defects such as patent ductus arteriosus (PDA), bicuspid aortic valve, atrial septal defect (ASD), ventricular septal defect (VSD), transposition of the great vessels, and truncus arteriosus. In almost every patient, a VSD and PDA are present (Moller and Neal, 1981; Johnson and Moller, 2014). DiGeorge syndrome is a commonly associated syndrome (Health and Medicine).

Physiology

The physiology of interrupted aortic arch is like that of coarctation of the aorta with a VSD. Left ventricular output is directed across the aortic valve, into the ascending aorta, and through the head and neck vessels. Additionally, left to right shunting occurs at the level of the VSD. Right ventricular output is directed across the pulmonary valve with a portion crossing the PDA and into the descending aorta.

Left to right shunting through the VSD is influenced by the pulmonary vascular resistance (PVR) and the degree of subaortic stenosis. As the PVR falls after birth, an increasing amount of blood flows through the VSD and into the pulmonary circulation. Subaortic stenosis results in increased resistance to left ventricular output and augments left to right shunting through the VSD. Finally, as the patent ductus arteriosus narrows, blood flow to the pulmonary bed is increased. It should be noted that, in the setting of an unrestrictive VSD, the pulmonary artery pressure and ascending aorta pressure will be equal.

Clinical Manifestations

The presentation of IAA is like that of other left-sided obstructive lesions. Many patients will develop symptoms of congestive heart failure in the first several weeks of life due to pulmonary overcirculation. If the ductus arteriosus constricts, there will be inadequate systemic perfusion and shock will ensue.

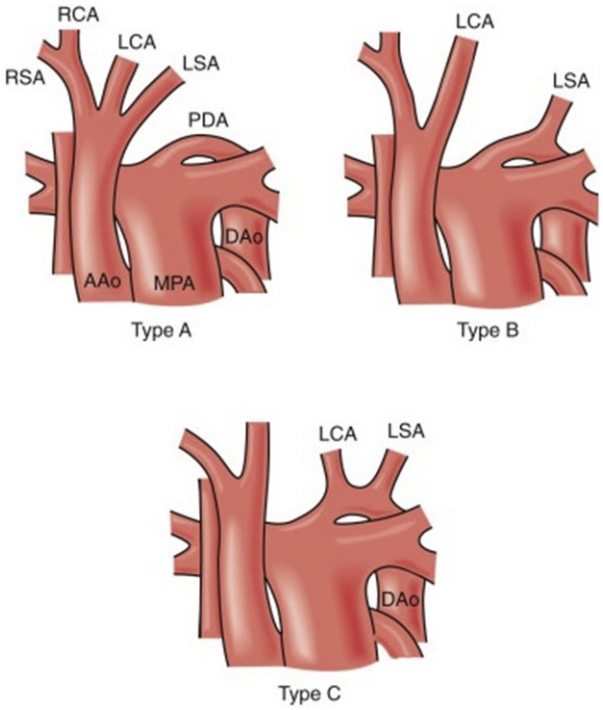

Fig. 3 Classification of interrupted aortic arch. *AAo*, Ascending aorta; *Dao*, descending aorta; *LCA*, left common carotid artery; *LSA*, left subclavian artery; *MPA*, main pulmonary artery; *RCA*, right common carotid artery; *RSA*, right subclavian artery. Reprinted with permission from Townsend, C. M., Beauchamp, R. D., Evers, B. M. and Mattox, K. L. (2016). Sabiston textbook of surgery: The biological basis of modern surgical practice (20th edn.). Philadelphia, PA: Elsevier.

The physical exam may reveal a blood pressure gradient from upper to lower extremities suggestive of progressive ductal closure. Differential cyanosis may be absent as a large VSD results in a large left to right shunt. In the case of Type B interrupted aortic arch with an anomalous right subclavian artery, all limb pulses may be absent. A strong carotid pulse may help differential IAA from aortic stenosis (Johnson and Moller, 2014; Moller and Neal, 1981).

Evaluation

Chest X-ray

The chest X-ray will reveal cardiomegaly, pulmonary vascular congestion, and pulmonary edema. In the case of DiGeorge Syndrome, an absent thymus results in a narrow mediastinum.

Electrocardiogram

The electrocardiogram demonstrates a normal to rightward axis. Right atrial and ventricular enlargement may be evident. Left ventricular hypertrophy may be evident in the setting of subaortic stenosis.

Echocardiogram

As with coarctation of the aorta, the goals of echocardiography include delineation of anatomy, quantification of cardiovascular function, operative planning, and postoperative follow-up. In the presence of a patent ductus arteriosus, the color Doppler pattern in the descending aorta may be normal with normal pulsatility by spectral Doppler. Following surgical repair, the echocardiogram can be used to identify and quantify the degree of residual obstruction (Eidem et al., 2015).

Cardiac catheterization

There currently are no percutaneous-based interventions that are indicated in an infant with IAA prior to surgical repair. If there is a postoperative narrowing of the repaired aortic arch, balloon angioplasty is often beneficial.

Management

Medical management

As with coarctation of the aorta, initial management focuses on maintaining/establishing ductal patency, ensuring adequate fluid resuscitation, and correcting metabolic derangements such as acidosis. This lesion is not compatible with life unless the ductus arteriosus is patent. Ductal patency is achieved with infusion of prostaglandin E1. Inotropes may be necessary for support of

ventricular function. To ensure adequate perfusion to the lower half of the body, strategies to increase the pulmonary vascular resistance are often employed (see HLHS section).

Surgical intervention

A variety of surgical interventions have been proposed for repair. As with coarctation of the aorta, the surgical approach will vary by institution. In general, the goals of surgical repair center on relieving left ventricular outflow obstruction, providing aortic continuity, and removing pulmonary over circulation (Jonas, 2014).

Current Topics in Interrupted Aortic Arch

Novel techniques for surgical intervention of IAA are continually evaluated (Wu et al., 2017; Kato et al., 2016). As with coarctation of the aorta, fetal detection can be challenging and ongoing efforts are being made to improve the tools and strategies for detection on IAA in the fetus (Axt-Fliedner et al., 2011).

Hypoplastic Left Heart Syndrome

Definition and Prevalence

Hypoplastic left heart syndrome (HLHS) is applied to underdeveloped mitral and/or aortic valves resulting in an inadequate left ventricle that is unable to support systemic circulation. The three major anatomic types of HLHS include mitral and aortic stenosis, mitral stenosis and aortic atresia, and mitral and aortic atresia (**Fig. 4**) (Davis et al., 2011). The aortic and mitral valves may be hypoplastic, atretic, or severely stenotic. Although the ascending aorta is also hypoplastic, it remains sufficient to supply coronary arterial blood flow. Oftentimes, the coronary arterial and brachiocephalic vessel flow is supplied in retrograde fashion via a patent ductus arteriosus (which is essential to survival). The incidence of HLHS is 266 per 1 million live births (Hoffman and Kaplan, 2002).

Physiology

Survival after birth is predicated on the ductus arteriosus remaining patent, meaning the right ventricle supplies both the pulmonary and systemic circulations. Even in cases with prograde aortic flow, it is insufficient to meet the demands of the systemic circulation. Therefore, the ratio of pulmonary to systemic blood flow is determined by the relative resistances of each vascular bed. There is a direct relationship between the volume of pulmonary blood flow and the systemic saturation. Meaning the more the pulmonary blood flow, the higher the systemic saturation, as the volume of oxygenated blood returning from the lungs is greater compared to the volume of deoxygenated systemic venous return from the body.

Immediately after birth, the ductus arteriosus is widely patent and does not restrict systemic blood flow. Although the aortic arch may be hypoplastic, if there is no constriction of the ductus, the aortic arch should be adequate to ensure proper coronary and

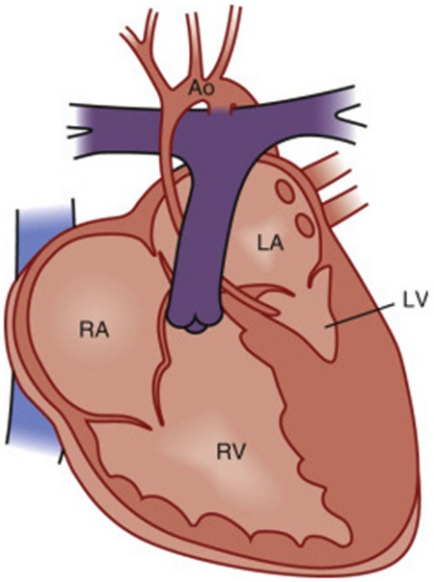

Fig. 4 Hypoplastic Left Heart Syndrome Reprinted with permission from Davis, P. J., Cladis, F. P. and Motoyama, E. K. (2011). Smith's anesthesia for infants and children (8th edn.). Philadelphia: Mosby

cerebral perfusion. Normally the descending aorta is of appropriate caliber, allowing for adequate systemic perfusion. Importantly, during the newborn period there is a normal elevation in pulmonary vascular resistance which naturally limits the quantity of pulmonary blood flow, often limiting pulmonary overcirculation.

Infants with HLHS become critically ill because of normal physiologic changes: closure of the ductus arteriosus and a decrease in the pulmonary vascular resistance. As the ductus becomes restrictive, the balance of blood flow favors the pulmonary vascular bed as the systemic vascular resistance increases. This occurs alongside the physiologic decrease in pulmonary resistance. Both changes decrease systemic output significantly leading to weak peripheral pulses, tachypnea, lethargy, metabolic acidosis, and cardiogenic shock. These profound changes occur in the setting of increased systemic oxygen saturation since there is an increase in pulmonary blood flow.

The physiologic changes on the right (systemic) ventricle are significant. As the ductus closes there is an increase in afterload, which results in a pressure load on the ventricle. The resultant increase in pulmonary blood flow results in a volume load on the ventricle as well. As the systemic output decreases, the coronary perfusion falls, resulting in decreased oxygen delivery to the organ at a time when metabolic demand increases. These changes lead to right ventricular dysfunction and dilation, which leads to worsening atrioventricular valve insufficiency, which further increases the volume load. Clinically these patients may present in respiratory distress (due to pulmonary overcirculation and elevated pulmonary venous/left atrial pressure), cardiogenic shock, metabolic acidosis, and end-organ failure (due to inadequate systemic output).

Inadequate systemic perfusion can develop in the setting of a widely patent and unobstructed ductus arteriosus. The right ventricle is volume loaded as it is pumping a more than normal volume of blood to adequately perfuse the systemic vascular bed. As mentioned previously, the relative flows to the pulmonary and systemic circuits are determined by the relative resistances. As the pulmonary vascular resistance decreases, the decrease in systemic blood flow is due to the right ventricle's inability to meet the metabolic demand of the systemic circuit as the pulmonary circuit becomes overcirculated. The increase in pulmonary blood flow results in a volume load to the left atrium and if there is obstruction to atrial egress (such as a restrictive atrial septal communication) then pulmonary venous hypertension develops. In response to the volume load, the right ventricle becomes dilated and the ventricular end-diastolic pressure increases. As the ventricular dilation worsens, tricuspid valve insufficiency develops, which further lessens cardiac output.

Clinical Manifestations

Clinically, an infant with HLHS appears to be healthy at birth. Oftentimes a heart murmur is not present. Shortly after birth, hour to days, the infant becomes pale, gray, tachypneic, develops feeding intolerance, and the peripheral pulses are weak or nonpalpable. Essentially, the infant is in cardiogenic shock with circulatory collapse.

Evaluation

Chest X-ray

Similarly, a chest X-ray is often nonspecific as well, potentially demonstrating cardiomegaly and/or increased pulmonary vascularity.

Electrocardiogram

An electrocardiogram is often not diagnostic. It may reveal low left-sided voltages and amplified right ventricular forces for a newborn.

Echocardiogram

The diagnosis of HLHS is made by echocardiography, either postnatally or more commonly prenatally by a fetal echocardiogram. The abnormal mitral and aortic valves with resultant left ventricle and ascending aorta hypoplasia should be definitively seen. Additionally, the patency of the atrial septum and ductus arteriosus, with retrograde flow visualized in the aortic arch, and ventricular function need to be adequately interrogated. Color Doppler interrogation is used to determine if a valve is atretic and to assess the competency of the tricuspid and pulmonary valves.

Cardiac catheterization

If there is either an intact or significantly restricted atrial septum resulting in hemodynamically significant left atrial hypertension, a cardiac catheterization is often necessary to create an atrial septal defect via balloon angioplasty or atrial septal stent placement. Otherwise, a catheterization is usually not required prior to initial surgical palliation.

Management

Medical management

The initial management of an infant with HLHS is aimed at maintaining a balance between pulmonary and systemic blood flow. After the airway is secured, if applicable, the infant with HLHS is started on prostaglandin E_1 to maintain ductal patency and ensure systemic perfusion. As the pulmonary vascular resistance decreases and pulmonary overcirculation (with systemic hypoperfusion)

ensues, medical management is required to increase pulmonary vascular resistance. Oftentimes, the infant will be intubated while minimizing the Fi02 to achieve controlled ventilation. The PC02 will be allowed to rise in order to increase pulmonary vascular resistance. The positive end-expiratory pressure may be increased as well to raise the total pulmonary resistance. Usually sedation is required as well to achieve this goal, as the intrinsic respiratory drive will increase the respiratory rate in response to an elevated PC02. Additionally, the use of vasoactive agents, such as dopamine and milrinone, will have beneficial effects on the contractility of the right ventricle and decrease systemic vascular resistance, both of which are physiologically beneficial to a patient with this anatomy.

As mentioned previously, there are patients with HLHS with a restrictive interatrial communication which result in clinically significant left atrial hypertension. Meaning that the severity of left atrial hypertension leads to a substantial reduction in pulmonary blood flow that results in inadequate oxygenation. In these cases, decreasing the degree of obstruction across the atrial level communication, usually by balloon atrial septostomy or stent placement, is necessary.

Surgical intervention

In general, there are three main aims in the surgical palliation of HLHS. It is important to note that the overall goal is surgical palliation and not surgical repair, as a patient with HLHS will not have a "normal" heart after the surgical interventions.

The first aim is to establish unobstructed systemic ventricular outflow to the systemic vascular bed. Meaning there is no obstruction between the right ventricle and the aorta. The second aim is to provide a stable and adequate source of pulmonary blood flow. The degree of pulmonary blood flow should be adequate but limited, as to protect the pulmonary vascular bed from overcirculation. The third aim is to ensure unobstructed pulmonary venous return.

The initial surgical procedure is referred to as a Norwood palliation (**Fig. 5**) (Davis et al., 2011). This procedure consists of creating an anastomosis between the proximal main pulmonary artery, the hypoplastic ascending aorta, and the aortic arch. The main pulmonary artery is transected at the distal end and oversewn. Pulmonary blood flow is most commonly ensured through a modified Blalock–Taussig shunt or a right ventricle to distal pulmonary artery shunt. In addition, the ductus arteriosus is ligated and if applicable, the atrial septum is opened. It is important to note that some centers advocate for primary cardiac transplantation rather than proceeding with staged surgical palliation. This initial surgical palliation not only allows for the infant to grow, but also adjust to the decrease in pulmonary vascular resistance without resulting in pulmonary overcirculation. The patency of the interatrial septum, reconstructed aortic arch, and branch pulmonary arteries along with the function of the right ventricle and competency of the tricuspid valve are all followed closely.

The second surgical palliation, a bidirectional Glenn shunt or a hemi-Fontan, is usually performed at 4–6 months of age (**Fig. 5**). With this surgical palliation, superior cava blood is directed to the pulmonary arteries. Prior to this surgery, a cardiac catheterization is performed for hemodynamic assessment and assess candidacy for proceeding with single-ventricle palliation. In addition, percutaneous intervention can be performed to address inefficiencies in the single ventricle physiology, such as clinically significant aorto-pulmonary and/or veno-venous collaterals.

The third and final surgical procedure is referred to as the Fontan operation (**Fig. 5**). There have been many modifications to the original procedure described in 1971 (Fontan and Baudet, 1971). Currently, the two most common techniques to achieve total cavopulmonary anastomosis are the lateral tunnel or extracardiac conduit (Driscoll, 2007; Feinstein et al., 2012). Oftentimes a

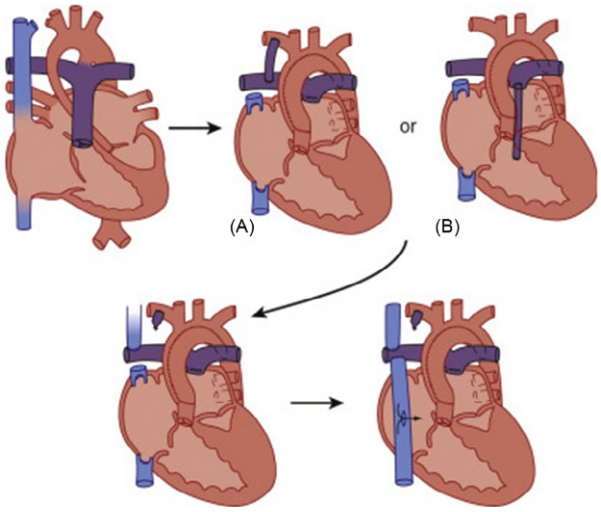

Fig. 5 Staged Repair of Hypoplastic Left Heart Syndrome. *Upper right*: (A) Damus–Kaye–Stansel (DKS) anastomosis of main pulmonary artery to ascending aorta, homograft augmentation of aortic arch, and a right modified Blalock–Taussig shunt (BTS). (B) Alternate initial repair involving a right ventricle-to-pulmonary artery conduit. *Lower left*: Superior cavopulmonary shunt or bidirectional Glenn (BDG). *Lower right*: Completed total cavopulmonary connection or Fontan procedure. Reprinted with permission from Davis, P. J., Cladis, F. P. and Motoyama, E. K. (2011). Smith's anesthesia for infants and children (8th edn.). Philadelphia: Mosby

small communication (or fenestration) is created between venous pathway and the common atrium. Although there is a resultant mild systemic desaturation, this allows for decompression of the venous pathway while maintaining cardiac output.

Outcomes

Mortality is most notable following the first stage with overall 1-year mortality approaching 30% (Ohye et al., 2012). Mortality rates with stage II and III are low (McGuirk et al., 2006; d'Udekem et al., 2012). As more and more patients survive into adulthood, more attention is paid to long-term morbidity.

An important area of morbidity is neurodevelopmental outcomes (Fogel et al., 2016). Multiple studies have demonstrated that patients with HLHS are at risk for impaired neurodevelopmental outcomes, regardless of type of surgical intervention (Mahle et al., 2006; Tabbutt et al., 2008; Newburger et al., 2012; Goldberg et al., 2014; Marino et al., 2012). As such, these children require evaluation, monitoring, and early intervention for the suspected neurodevelopmental delays (Marino et al., 2012).

Current topics in hypoplastic left heart syndrome

Research in the field of hypoplastic left heart syndrome is varied. The development of the Pediatric Heart Network has resulted in completed studies regarding optimal surgical approach medical management, and the outcomes following the Fontan Procedure (Bradley, 2016). Others are focusing on improving our understanding of neurodevelopmental outcomes in these patients (Cheatham et al., 2015; Lloyd et al., 2016). Additional topics of interest include the use of ventricular assist devices in Fontan physiology, and stem cell therapy in single ventricle physiology (Imielski et al., 2017; Ishigami et al., 2017).

Conclusion

Obstruction to left ventricular outflow compromises a group of lesions that result in inadequate systemic perfusion. In certain instances, patency of the ductus arteriosus is crucial to survival until either surgical or percutaneous intervention is performed. Specific management will be determined based on the location and severity of the obstruction.

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Lipid-Mediated Mechanisms in Atherosclerosis

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Introduction

Cardiovascular disease (CVD) is the number one cause of death worldwide. The increased prevalence and cost of treatment for CVD and associated risk factors have placed a huge economic burden on society. The primary cause of CVD is atherosclerosis (Bhatnagar et al., 2015), which is a lipoprotein-driven disease that involves vascular resident cells and immune cells. The complex interactions between those cells and various lipoproteins lead to plaque formation in large and medium-sized arteries (de Jager and Pasterkamp, 2013).

Atherosclerosis is initiated by the retention and accumulation of apolipoprotein B (apoB)-containing lipoproteins, mainly low density lipoprotein (LDL), in the intimal layer of arteries (Khalil et al., 2004). The dysmetabolism of lipids fuels atherogenesis (Gustafsson et al., 2007). As cholesterol influx exceeds its efflux, foam cells form, which serve as the building blocks of an atheroma. Atherosclerosis is also recognized as a chronic inflammatory disease in which a broad spectrum of inflammatory cytokines and chemokines participate. In the past decade, it has been revealed that immune cells participate in the interactions with the vascular resident tissue cells. Thereby, the immune system plays a crucial role in the development and progression of atherosclerotic plaques (Hansson and Hermansson, 2011). In this review, we summarized the current knowledge of the lipid-mediated mechanisms of atherosclerosis including metabolic perturbations, inflammation, and immune responses, and the interactions of these factors (Schaftenaar et al., 2016; Hansson, 2001).

Overview of Pathology of Atherosclerosis

When the risk factors for atherosclerosis were analyzed, both genetic and environmental components were revealed in epidemiological studies (Musunuru and Kathiresan, 2016). Among them, the abundance of atherogenic lipoproteins was found to be a prerequisite, and thus hypercholesterolemia plays a key role in the development of atherosclerosis. The increase of apoB-containing LDL and its accumulation in the arterial intima is sufficient for atherosclerosis to develop (Khalil et al., 2004). The interactions between hypercholesterolemia and other risk factors (e.g., smoking, hypertension, and diabetes) can further accelerate atherogenesis (McIntyre and Hazen, 2010).

Among the cells responding to LDL, monocytes and macrophages are essential in atherogenesis. During this process a series of changes occur in the vessel wall. After the endothelial cells are injured by turbulent blood flow or oxidized LDL (oxLDL), blood-derived inflammatory monocytes and other innate immune cells are recruited to the injured sites, adhere to the endothelial cells, and penetrate into the intima where monocyte-derived macrophages as well as the resident vascular smooth muscle cells (VSMCs) phagocytose lipids and become foam cells, which are the main components of the atherosclerotic plaque (Davidson and Rotondo, 2003). Morphologically speaking, there are continuous stages in the development of an atherosclerotic plaque, from the fatty streak formation resulting from intracellular lipid accumulation, to the visible intermediate lesion and atheroma formation, which is composed of an extracellular lipid core filled with an abundance of foam cells; these stages progress mainly by lipid addition (Subbotin, 2016). Then a fibroatheroma can be formed from single or multiple lipid cores with a fibrotic cap and increased VSMCs and collagen. The last stage in the development of a thrombosis usually comes from the disruption of complicated lesions, with the potential to result in hematoma and hemorrhage (Usman et al., 2015). Theoretically, atherosclerotic plaques can be classified into two types depending on their composition: the stable plaques which are less prone to thrombotic complications and the vulnerable plaques which are susceptible to rupture and complications. From an examination of more than 800 cases of sudden coronary death at autopsy, 55%–60% of them resulted from plaque rupture (Finn et al., 2010).

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Lipoprotein Metabolism in Atherosclerosis

Based on the density, which is determined by the lipid to protein ratio in the lipoproteins, plasma lipoproteins are classified into five major categories: LDL, intermediate-density lipoproteins (IDL), high-density lipoproteins (HDL), very low density lipoproteins (VLDL), and chylomicrons. The larger the size is, the lower the density. Key components of these lipoprotein particles are apolipoproteins. Apolipoproteins are structural components of lipoprotein particles, cofactors for enzymes, and ligands for cell-surface receptors in lipid transport.

There are six major classes of apolipoproteins with several subclasses, that is, apoA (apo A-I/II/IV/V), apoB (apo B48/B100), apoC (apo C-I/II/III/IV), apoD, apoE, and apoH. These apolipoproteins can be classified into two major types. ApoB forms LDL particles and associates with lipid droplets irreversibly; thus it is called a nonexchangeable apolipoprotein (Plakkal Ayyappan et al., 2016). All other apolipoproteins form lipoprotein particles and associate with lipids reversibly, and thus are called exchangeable apolipoproteins. ApoB is the main apolipoprotein of LDL and is required for LDL formation and metabolism. ApoB exists in two isoforms: apolipoprotein B100 (apoB100), which is located on liver-derived VLDL, IDL, and LDL, and apoB48, which mainly resides on intestine-derived chylomicrons (Khalil et al., 2004). With the help of apoB, lipoproteins retained in the intima adhere to vessel walls by binding to proteoglycans in the extracellular matrix (ECM) during the pathogenesis of atherosclerosis. ApoA-I is the major protein component of HDL, and apoE is also found on some HDL particles (Feig et al., 2014). Since apoE has high affinity for LDL receptors (LDLR); defects in apoE result in experimental hyperlipoproteinemia, with accumulation of plasma cholesterol and triglycerides (TGs), the result of impaired clearance of VLDL and chylomicron remnants (Xu et al., 2016).

Metabolism of lipoproteins and apolipoproteins involves the process of synthesis, secretion, uptake, cholesterol transport, and reverse cholesterol transport (RCT), accomplished by multiple organs (Tall, 1998). The liver and intestine are major organs for synthesizing lipoproteins and apolipoproteins; the immune cells residing in these organs can assist in their synthesis and secretion. Lipoproteins can be taken up by many cell types through certain receptors; for example, LDL can be internalized by cells via LDLR. LDLR is a trans-membrane protein that diffuses freely within the cell membrane until binding to the membrane dynamic protein clathrin-coated pits. After the ligation of LDLR with LDL, the complex of LDL-LDLR and clathrin-coated pits can be brought into the cell through endocytosis. Similar to LDL, oxLDL can be internalized via the scavenger receptor B-3 (SR-B3, also named CD36) (Chistiakov et al., 2016). These LDL uptake receptors are essential in cellular cholesterol homeostasis; and a variety of mutations in the receptor genes have been suggested to contribute to dyslipidemia and increased plasma cholesterol. About 10 years ago an enzyme named proprotein convertase subtilisin kexin type 9 (PCSK9), which is synthesized along with LDLR, was demonstrated to act on LDLR and prevent it from being recycled back to the plasma membrane (Giunzioni and Tavori, 2015); instead, PCSK9 marks the LDLR for degradation in lysosomes. Antibodies that neutralize PCSK9 have become potent drugs that lower plasma cholesterol (Li and Li, 2015).

RCT is a process through which cholesterol is transported from nonhepatic peripheral tissues back to the liver (Jukema et al., 2004). It is mainly mediated by apoA-1-containing HDL and the ATP-binding cassette transporters (ABC), such as ABCA1 and ABCG1 (Feig et al., 2014). The cholesterol can be converted to cholesteryl ester (CE) by the enzyme lecithin-cholesterol acyltransferase in HDL particles and selectively taken up by the liver through SR-B1 or be transferred by CE transfer protein in exchange for TGs to other lipoproteins (such as LDL and VLDL), and these lipoproteins can then be taken up by the liver (Shao and Heinecke, 2009).

When investigating the metabolic functions of lipoproteins and apolipoproteins in mediating the pathogenesis of atherosclerosis, four kinds of lipids, LDL cholesterol, HDL cholesterol, lipoprotein(a) (Lp(a)), and TG are mainly considered (Aluganti Narasimhulu et al., 2016).

LDL Cholesterol

LDL cholesterol is generally referred to as bad cholesterol because it can be transported in LDL particles into the artery wall, initiating foam cell formation and atherogenesis (Segrest et al., 2001). This process seems to be self-accelerated, as the LDL-stimulated inflammatory cells can release cytokines to promote VSMC to secrete proteoglycans, thus increasing the affinity for LDL. In addition, engulfing of LDL enables macrophages to produce lipoprotein lipase (LPL) and thus further enhances LDL retention (Hasham and Pillarisetti, 2006; Rutledge et al., 1997).

After invading the endothelium, LDL becomes oxidized and/or aggregated. The formation of oxLDL is regulated by a complex set of biochemical reactions. Both necrotic cell debris and free radicals participate in these reactions in the endothelium (Khalil et al., 2004). Compared to the native LDL, the oxidized forms of LDL are more easily retained in the intima to be later recognized, engulfed, and accumulated by macrophages, forming foam cells and initiating inflammatory responses (Libby, 2002). In this process, oxLDL triggers VSMC to produce more proteoglycans and helps to retain more LDL in the arterial wall; while the retention of LDL leads to oxidative modification of LDL, forming a vicious cycle (Kiyan et al., 2014). OxLDL exerts a wide range of effects to activate various cell types and drive the development of atherosclerosis. As a ligand for the scavenger receptors on macrophages, oxLDL can be engulfed for foam cell formation and initiate the proinflammatory properties of macrophages (Boullier et al., 2001). Moreover, the degree of oxidation of LDL determines the differentiation of naïve macrophage to either an M1 or M2 phenotype (Seo et al., 2015). Minimally oxidized LDL induces the production of TNF-α and IL-12p40 in macrophages, indicating an M1 macrophage phenotype; while heavily oxidized LDL induces mannose receptor CD206 expression that indicates an M2 phenotype (Seo et al., 2015). OxLDL also affects the apoptosis of macrophages and plaque rupture (Hundal et al., 2001, 2003). OxLDL can also

induce VSMC proliferation, migration, and transition to a proinflammatory phenotype from a contractile smooth muscle phenotype (Chien et al., 2003; Zettler et al., 2003). For endothelial cell (EC), oxLDL enhances the expression of adhesive molecules such as VCAM-1 and the production of several chemokines (Takei et al., 2001).

Clinically, the increased plasma LDL cholesterol level is associated with increased incidence and severity of atherosclerosis (Plakkal Ayyappan et al., 2016). Significant elevation of oxLDL was observed in patients with acute coronary syndromes, while treatment with a statin drug markedly reduced its levels (Ramadan et al., 2009). Thus, the oxLDL levels are widely used to predict the development of atherosclerosis and other CVDs, based on the correlation between oxLDL levels and CVD outcome (Shimada et al., 2004). However, it is critical to note that oxLDL is highly heterogeneous, which suggests more complex functions of oxLDL in mediating the progression of atherosclerosis (Levitan et al., 2010).

HDL Cholesterol

HDL is the driving force of RCT, a process that removes excess cholesterol from cells in peripheral tissues (e.g., foam cells in arterial wall) to the liver (Ohashi et al., 2005). Therefore, HDL exerts strong protection against atherosclerosis and has a strong negative correlation with the risk of atherosclerotic CVD. Plasma level of cholesterol carried by HDL (HDL-C) reflects the availability of functional HDL (Rosenson et al., 2016; Ansell et al., 2005).

Functionally, HDL and lipid-poor apoAI promote the regression of atherosclerosis due to the antioxidant properties of their enzymatic and nonenzymatic components and the ability to remove normal and toxic lipid species from cells (Feig et al., 2014). HDL interferes with the oxidative modification of LDL and inhibits the biological activity of oxLDL. This is attributed to many antioxidative and antiinflammatory enzymes, such as paraoxonase 1 (PON1), an esterase carried on HDL that can degrade certain biologically active oxidized phospholipids (oxPL) (Shao and Heinecke, 2009). Another enzyme, myeloperoxidase (MPO), also contributes to the anti-atherogenic function of HDL. The functionality of HDL, conferred by these antioxidative and antiinflammatory components, is considered more important than the quantity of HDL in peripheral blood (Calabresi et al., 2015). HDL can also protect the arterial endothelium via nitric oxide synthase activation (Mineo and Shaul, 2003), and exert antiinflammatory effects through dampening toll-like receptor (TLR) signaling by modulating the content of plasma membrane cholesterol (De Nardo et al., 2014). Regarding the phenotypes of macrophages, HDL can decrease proinflammatory cytokine production and promote the differentiation of M2 macrophages. This is associated with the promotion of the phosphorylation of STAT6, an essential cell signaling pathway for M2 polarization (Sanson et al., 2013). Nevertheless, the anti-atherogenic functions of HDL may be compromised once HDL is trapped in the arterial wall or when modified in the context of kidney disease or diabetes.

Lipoprotein(a)

After being assembled at the hepatocyte cell membrane surface and released into the plasma, Lp(a) consists of two components: an LDL-like particle and apo(a). The latter is covalently bound to apoB of the LDL-like particle (Fless et al., 1985). However, unlike LDL, Lp(a) contains lower amounts of antioxidants and exhibits a high affinity to ECM and fibrin. Lp(a) contributes to the pathological processes of both atherogenesis and thrombogenesis, depending on Lp(a) plasma concentrations and the variable antifibrinolytic capacity of different apo(a) isoforms. Thus an increased plasma level of Lp(a) has been recognized as an independent risk factor for an atherothrombotic event (Kronenberg et al., 1996; Nordestgaard et al., 2010).

Lp(a) promotes the pathogenesis of both the early and advanced stages of atherosclerosis. Lp(a) is the major carrier in plasma of oxPL, which attracts inflammatory cells to infiltrate into arterial walls and promotes the proliferation of VSMCs (Bergmark et al., 2008). Lp(a) can also inhibit anti-atherogenic transforming growth factor-β (TGF-β) activation by impairing the synthesis of plasmin (Bogavac-Stanojevic et al., 2003). Since it usually is retained in the subendothelial space of the arterial wall, Lp(a) can stimulate endothelial cells to increase the expression of intercellular adhesion molecule-1, which promotes circulating monocyte adhesion to the arterial endothelium (Takami et al., 1998). Moreover, Lp(a) may also act as a potent chemoattractant for monocytes in a dose-dependent manner in the development of atherosclerosis (Syrovets et al., 1997). Based on these functions, plasma levels of Lp(a) can be used to predict the risk of early atherosclerosis in subjects with high LDL cholesterol levels, and it was found that this effect is independent of the size polymorphism of apo(a) because the distribution of apo(a) is similar in subjects with or without early atherosclerotic plaques (Kronenberg et al., 1999).

Lp(a) also promotes thrombogenesis (Lip et al., 1997). Because the structure of Lp(a) is similar to plasminogen and tissue plasminogen activator, it can competitively reduce fibrinolysis. In addition, Lp(a) can enhance the secretion of plasminogen activator inhibitor(PAI) (Rouy et al., 1991). Moreover, Lp(a) can enhance coagulation by inhibiting the effect of tissue factor pathway inhibitor (van der Stoep et al., 2014). Lp(a) is also observed to be involved in wound healing and tissue repair by interacting with the ECM in vascular wall.

Triglyceride

TG is an ester derived from glycerol and fatty acids. There are two different types of TG: saturated and unsaturated (Pundir and Narang, 2013). There is a renewed interest in investigating increased levels of TGs in atherogenesis in recent years. The intestine is the main location for TG metabolism (Mattson and Volpenhein, 1964). Bile salts are used to emulsify dietary TG and cholesterol in the intestinal lumen. This results in the enhanced uptake of TG. After uptake, TGs can be digested by lipases into free fatty acids

(FFA) and monoacylglycerides (MAG) in the intestinal lumen and absorbed into the enterocyte for the synthesis of new TG, phospholipid, and CE. Subsequently, the synthesized materials can be packaged into chylomicrons, secreted, and enter the lymphatic system.

Similar to both atherogenic apoB-containing lipoproteins and Lp(a) (Kimak and Solski, 2002), TG-rich remnant lipoproteins also promote atherothrombosis (Krauss, 1998a). It is known that high levels of TGs in the bloodstream have been linked to atherosclerosis, which is attributed to multiple mechanisms. TG can directly influence the development of atherosclerotic plaque, as TG-rich lipoproteins carry abundant cholesterol and thus promote foam cell formation (Krauss, 1998b). Because TGs can be degraded by most cells, while cholesterol cannot, cholesterol content of TG-rich lipoproteins is more likely to be the cause of atherosclerosis and CVD (Nordestgaard and Varbo, 2014). Besides cholesterol delivery, TG-rich lipoproteins could also promote atherosclerosis by inducing lipid toxicity (Goldberg et al., 2012). After lipolysis cholesterol in the TG-rich lipoproteins can be concentrated, and FFA and MAG are produced. This product is cytotoxic as demonstrated in vitro, as these lipids can promote cell death and local inflammatory injury, especially in the arterial wall where the lipoprotein-lipase activity at the surface of remnants is active (Liu and Czaja, 2013). TG-rich lipoproteins could also accelerate hypertriglyceridemia, because these lipoproteins contain apoCIII, which inhibits LPL and remnant uptake by the liver (Larsson et al., 2013). Moreover, an elevated TG level can result in increased plasma levels of thrombotic factors including the fibrinogen, PAI, and inflammatory mediators (McLaughlin et al., 2005).

Inflammation Caused by Dysfunctional Lipoprotein Metabolism in Atherosclerosis

During the pathogenesis of atherosclerosis, native LDL can be modified into oxLDL at early stages, and oxLDL is rapidly taken up by macrophages to generate foam cells. By exposure to the oxidative waste of vascular cells, LDL is mildly oxidized with the help of an enzyme called 12/15-lipoxygenase (LO), which is an important source of the reactive oxygen species in LDL oxidation (Huo et al., 2004). It is found that 12/15-LO is also an inducer for proinflammatory molecule production and thus promotes atherosclerosis. 12/15-LO induces the production of TNF-α, IL-6, and CCL2, and 12/15-LO deficiency exhibits diminished fatty streak formation and atherosclerotic lesions in mice (Wen et al., 2007). Therefore, there are multiple connections between the metabolic functions of lipids and cytokine-mediated inflammatory responses in atherosclerosis (Buckley and Ramji, 2015).

Abundant oxLDL generated in the context of hyperlipidemia accelerates cholesterol accumulation in macrophages and thereby provokes inflammation. The inflammatory responses to oxLDL include the activation of TLRs (Goldklang et al., 2012), inflammatory cell infiltration, inflammatory cytokine and chemokine production, and inflammasome activation. Subsequently, TLR-mediated inflammatory responses can decrease cholesterol efflux (Tall and Yvan-Charvet, 2015) and lead to increased cholesterol accumulation, resulting in a vicious cycle.

TLR signaling is a bridge from innate immune response and inflammation to atherogenesis, while the cholesterol-enriched atherogenic microenvironment augments the TLR signaling activation. For example, LPS-activated TLR4 signaling can be enhanced by plasma membrane cholesterol enrichment via promoting the formation of TLR4-MD2 and TLR4-CD14 complexes (Dauphinee and Karsan, 2006) and TLR-activated macrophage inflammatory responses can be suppressed by ABCA1 and ABCG1-mediated macrophage cholesterol efflux (Zhu et al., 2010).

The activation of TLRs and the uptake of oxLDL and phospholipids by macrophages result in the release of proinflammatory cytokines such as TNF-α, IL-1, IL-18, IL-6, IL-12, IL-15, and MCP-1, as well as some antiinflammatory cytokines including IL-10, IL-4, and TGF-β (Han and Boisvert, 2015). The proinflammatory cytokines could enhance inflammatory responses in vessels and further induce immune cell infiltration and exacerbate atherosclerosis (Aluganti Narasimhulu et al., 2016). Three of the most important proinflammatory cytokines are TNF-α, IL-1, and IL-6. TNF-α promotes the development of atherosclerosis at the early fatty streak stage. IL-1β is associated with inflammasome activation (Duewell et al., 2010), and IL-1 can stimulate VSMCs to produce IL-6 in atherosclerotic lesions (Ait-Oufella et al., 2011). However, the role of IL-6 in atherogenesis is still controversial, as the administration of exogenous IL-6 can increase the formation of fatty streaks in apoE$^{-/-}$ mice (Huber et al., 1999); however, IL-6$^{-/-}$apoE$^{-/-}$ mice showed elevated atherosclerosis and decreased leukocyte homing (Elhage et al., 2001). To some extent, these effects of cytokines are achieved by controlling lipid metabolism associated genes. For example, IL-6 downregulates scavenger receptor-A, IL-4 upregulates CD36, while TNF-α and TGF-β affect the expression of the lectin-like oxidized LDL receptor (LOX-1) (Minami et al., 2000).

In hyperlipidemia, the accumulated cholesterol crystals in macrophages can promote inflammasome activation during atherogenesis, while inflammasome activation can subsequently exert pro-atherogenic effects. On the one hand, the ligation of oxLDL with TLR4 or CD36 could provide a priming signal, while the phagocytosis of extracellular cholesterol crystals via CD36 could provide a second signal for inflammasome activation (Abderrazak et al., 2015). On the other hand, the inflammasome activation could further lead to the maturation and secretion of the proinflammatory cytokines IL-1β and IL-18 (Buckley and Ramji, 2015). Both of these cytokines are pro-atherogenic molecules. IL-1 initiates leukocyte adhesion to ECs and transmigration, and it can also provoke vascular tissue cells to produce other cytokines (Tsouknos et al., 2003), while IL-18 can increase lymphocyte infiltration.

Furthermore, the exposure of macrophages to the inflammation and oxidative stress within atherosclerotic lesions can also activate endoplasmic reticulum stress pathways and result in macrophage apoptosis and necrosis. With TNF-α and IL-1 stimulation, pentraxins such as C-reactive protein can be produced, and exert direct effects on leukocyte recruitment and cell apoptosis induction in the vessel wall (Buckley and Ramji, 2015).

Immune Responses to Lipids in Atherosclerosis

Over the past few decades, the roles of the immune system including both the innate immunocytes, such as monocytes, macrophage, mast cells, nature killer cells, and dendritic cells (CDs), and adaptive immunocytes including T cells and B cells, in the pathological process of atherosclerosis have been gradually revealed.

Native LDL does not trigger an immune response, unless oxPL or aldehyde-modified breakdown fragments of apoB expose neo-antigens, which break self-tolerance and induce inflammation (Matsuura et al., 2014). Cells of the innate immune system recognize and respond to pathogens in a generic and immediate way, but this defense is relatively transient. Their major functions are to recruit immune cells by producing cytokines and chemokines and activate the adaptive immune system through antigen presentation (Hansson and Hermansson, 2011). Atherosclerosis is now recognized as a disease associated with immune responses since antigen presentation occurs in atherosclerotic lesions.

It is believed that monocyte-derived macrophages are the main cellular component of an atheroma, and these cells are the major precursors of foam cells. OxLDL affects the migration of monocytes to the vascular wall by switching from CCR2 to CX3CR1 expression in a peroxisome proliferator-activated receptor-γ-dependent manner (Yu et al., 2015). After infiltrating the arterial wall, monocytes can differentiate into macrophages, ingest modified lipids, predominantly oxLDL, and then store them in lipid droplets (Plakkal Ayyappan et al., 2016). As short-lived phagocytic cells, neutrophils possess a variety of biological enzymes such as MPO and proteinases. The infiltration of these cells is recognized as a driving force for atherosclerotic lesion growth (Tiyerili et al., 2016). The infiltrated neutrophils are produced in bone marrow under the control of granulocyte colony stimulating factor and IL-23. Neutrophils express the G protein-coupled receptor CXCR4, whose ligand is CXCL12, and their interaction plays an essential role in neutrophil egress from bone marrow and migration to the atherosclerotic lesions (Bot et al., 2014). Mast cells and natural killer (NK) cells are both major effector cells in host defense responses and are also found to be involved in atherogenesis. The activated mast cells release abundant proinflammatory cytokines, proteolytic enzymes, growth factors, and vasoactive substances (Bot and Biessen, 2011). These molecules participate in altering lipid metabolism via affecting the cholesterol efflux mediated by apoE and apoAI, and they also modify lipoproteins and digest matrix components, thereby taking part in plaque formation and rupture. The NK cells were mainly found in the shoulder regions of the atherosclerotic plaques. An adoptive transfer experiment indicates that the deficiency of NK cells can result in dramatic reduction of atherosclerotic lesion formation (Selathurai et al., 2014).

As the professional antigen-presenting cells, DCs are essential in the initiation of immune responses to lipid antigens (e.g., oxLDL) in atherogenesis. DCs share phenotypic and functional properties with macrophages, which enables them to have the ability to phagocytize and present antigens (Manthey and Zernecke, 2011). DCs take up lipids via scavenger receptors and form a dendritic cell-like phenotype of foam cells, contributing to the development of atherosclerotic lesions. In the meantime, more importantly, the uptake of lipid antigens such as oxLDL and apoB100 results in DC maturation, migration, and antigen presentation to T cells in the draining lymph nodes, subsequently initiating the activation of subpopulations of T cells and B cells (Paulson et al., 2010; Hermansson et al., 2011). For example, after the intravenous injection of oxLDL or apoB100 coupled splenocytes, the Th1 polarization was dramatically enhanced in both WT and apoE$^{-/-}$ mice (Steinmetz et al., 2015). By contrast, if these lipid antigens including apoB100-derived peptides are recognized and presented to regulatory T cells, they will reduce the development and progression of atherosclerosis in mice (Herbin et al., 2012). These reports indicate that different subsets of T cells exert different functions in modulating atherogenesis even after having been exposed to similar lipid antigens.

Summary

Increased plasma LDL levels cause LDL entry into and accumulation in the arterial intima. After the modification by oxidation and aggregation, these lipoproteins can activate both the vascular resident tissue cells and immunocytes, driving pathogenic evolution of the plaque by forming lipid-loaded foam cells as well as releasing proinflammatory cytokines and chemokines. As the atherosclerotic lesion evolves, other immune inflammatory cells such as DCs, neutrophils, NKs, mast cells, T cells, and B cells are recruited by the chemokine and cytokine stimulation and also accumulate in the lesion area. The adaptive immunocytes like T cells and B cells recognize specific epitopes on lipid antigens presented by the innate immunocytes, leading to lymphocyte activation and secretion of cytokines and lipid antigen-specific antibodies. As a result, a positive feedback loop of inflammatory cell infiltration and foam cell formation further exacerbate the pathological process of atherogenesis.

Although the mechanisms of metabolism, inflammation, and immunity in lipid-mediated atherogenesis are discussed individually, these factors in the network cannot be separated. Many of the details in this network need to be investigated further, and questions must be raised such as: can we use our current knowledge of these mechanisms in atherosclerosis to prevent or treat this disease? Or will immunomodulation during atherogenesis be helpful to improve the treatment of atherosclerosis? Immunotherapies are attractive as a possible therapeutic approach for CVDs (Hermansson et al., 2011; Shah et al., 2004), although no such immunotherapy is clinically available today. The success in protective immunization with oxLDL as an immunogen in experimental models suggests that this might be a fruitful approach in humans. However, broadly acting immunosuppressants are unsuitable for this purpose; instead the development of more specific immunomodulators that act on the key mechanisms in the pathogenesis of atherosclerosis should be encouraged. We assert that, with the further understanding of the lipid-mediated mechanisms of atherogenesis, more promising therapeutic methods will be developed.

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Lipids and Cardiovascular Diseases: Epidemiologic Perspectives

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Introduction

Average life expectancy in ancient Greek and Roman times was 27 years in 200–300BC. By the year AD1900, it had increased to 47 years. Thus, life expectancy had increased by only 20 years during more than 2000 years. However, looking back to the end of the 20th century, life expectancy had increased by a further 30 years in a mere century as a consequence of the control of epidemic diseases, treatment of infections with antibiotics, vaccination, and improvements in food and environmental safety. Prolongation of life expectancy more recently has been due mostly to prevention of cardiovascular disease (CVD), and for many years plasma cholesterol has been recognized as one of its most important risk factors.

This article reviews aspects of lipids and CVD from the viewpoint of epidemiology, from the first epidemiologic studies to recent clinical trials.

The Dawn of Epidemiologic Studies on Lipids and CVD

The Framingham Heart Study: The Advent of CVD Risk Factors

The onset of prospective studies of cholesterol and CVD was heralded by "The Framingham Heart Study" (Dawber et al., 1951). It was planned in 1948, just after the end of World War II, when CVD accounted for 80% of total mortality in the United States. The National Heart Institute of the United States Public Health Service organized a large-scale prospective study in the town of Framingham, Massachusetts, with a population of 28,000, to consider measures for preventing a further increase in CVD complications. The study followed the causes of coronary heart disease (CHD) for the first time by epidemiology, which up to then had been a research method mostly for infectious diseases. The study contributed to a halving of the incidence of CVD in the United States during the following 30 years. The Framingham Offspring Study targeting the first participants and their children and spouses was initiated in 1971, the Omni study targeting minority races was started in 1994, and the Third-Generation Cohort targeting grandchildren of first participants was initiated in 2002. Moreover, in 2003 the New Offspring Spouse Cohort and the Second-Generation Omni Cohort were started. These studies are still in progress today.

In the Framingham Heart Study, 5070 subjects aged 35–64 years were followed for 30 years to examine the relation between candidate risk factors and the development of CHD. The results showed clearly that CHD incidence was strongly affected by systolic blood pressure (SBP), serum cholesterol concentration, and smoking habit (Stokes et al., 1987). In doing so, the study created the concept of "risk factors" and had a major impact on clinical practice for CVD prevention worldwide. The message that CVD might be preventable by risk factor control had a great and far-reaching impact. The outcome of the study has had a remarkable impact on life expectancy through prevention of CVD during the 20th century (Futterman and Lemberg, 2000).

"The Seven Countries Study," the First International Collaborative Study of CVD Epidemiology

The Seven Countries Study is a prospective study that began in 1957 (Verschuren et al., 1995). It targeted men aged 40–59 years in seven countries (United States, Finland, Yugoslavia, Greece, Italy, Netherlands, Japan), using a unified survey procedure. It was the pioneer of international collaborative studies that have identified associations between CHD incidence and lifestyle, especially dietary habits. One of the pioneering features of the study was the application of standardized food surveys, mostly using duplicate meals. The study confirmed that total cholesterol (TC) is related to CHD in a manner similar to that identified in the Framingham Heart Study and also showed that the association partly explained regional differences in CHD mortality, that is, CHD mortality was lowest in Japan where serum cholesterol levels were very low and was very high in the United States and Finland (Verschuren et al., 1995). Many associations with CHD and stroke were observed, including those of saturated fatty acid intake, smoking, and blood pressure. The follow-up was completed at the 25th year. Two similar studies are in progress in different parts of the world: the FINE Study in Finland, Italy, and the Netherlands, and the Tanushimaru Study in Japan.

"The Whitehall Study," Targeting Socioeconomic Status

The association between social class and health status has been one of the classic topics of social epidemiology. A number of studies have been undertaken to date. One of the most notable is the Whitehall Study in the United Kingdom. "The Whitehall I Study" commenced in 1967 with the aim of studying the association between socioeconomic status (SES) and all-cause mortality in 18,403 male civil servants working in public offices in London (Reid et al., 1974). An association between the occupation class of civil servants and their mortality risk was observed, with the risk being higher in lower class occupations than in higher class occupations. Men in the lowest class had a threefold greater all-cause mortality risk than men in higher grades during more than 10 years of follow-up (Marmot et al., 1984). "The Whitehall II Study," which began in 1985 and targeted 10,308 male and female civil servants aged 35–55 years (Marmot et al., 1991), was undertaken to explore the relations between SES, stress, and CVD. It found that mortality due to CHD had increased along with the rise in serum TC levels, and differences in CHD risk by SES were partly explained by differences in risk factor levels such as TC (Rose and Shipley, 1986; Whitty et al., 1999).

The Cohort Study of CVD in Asia (Hisayama Study)

The Hisayama Study is the oldest cohort study targeting CVD in Japan and might be the oldest in all Asian populations. Since 1961, an epidemiologic survey of CVD, of which the main target was stroke, has been performed in Hisayama town (approximate population 8400) in Fukuoka prefecture located in the western part of Japan (Kiyohara et al., 1989). The residents of Hisayama have almost the same age and occupational distribution as the national average and accordingly are almost a representative sample of the Japanese population. The justification for this study was uncertainty about the credibility of Japanese death certificates expressed by US researchers. According to the World Congress on Neurology in Rome in 1960 (Goldberg and Kurland, 1962), the annual mortality from stroke in Japan was the highest among 33 countries with reliable vital statistics. Moreover, when looking into stroke subtypes, intraparenchymal hemorrhage was conspicuously high in Japan. Goldberg and Kurland had questioned whether there were inaccuracies in the completion of death certificates by Japanese physicians. There was no evidence to refute this at the time. The Hisayama Study had started with the aim of clarifying the natural history of stroke of Japanese. In the initial data from the first follow-up population (autopsy rate 80%) in 1961, mortality due to intraparenchymal hemorrhage was high as expected, but 1.1-fold that of cerebral infarction. The study recruited residents aged 40 years or more from the town in the years 1961, 1974, 1988, and 2002, after which there was continuous follow-up. The initial endpoints were stroke and they have since been expanded to include cancer, diabetes, dementia, and others. As an example, one recent publication reported that increased risks of atherothrombotic cerebral infarction and CHD were observed with increasing low-density lipoprotein cholesterol (LDL-C) or non-high-density lipoprotein cholesterol (non-HDL-C) concentrations, each in a linear manner (Imamura et al., 2009; Imamura et al., 2014).

Subsequent to the Pioneer Studies

It has been shown that the incidence/mortality of CVD is associated with dyslipidemia, such as a raised serum TC level in cohort studies in many countries. Evidence provided by these studies has become the foundation for the National Cholesterol Education Program (NCEP)-Adult Treatment Panel III (ATP III) of the US National Institutes of Health, which provides guidelines for atherosclerotic disease prevention (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). This program developed the Framingham Score, which predicts the future development of CHD. In Japan, the Hisayama Risk Score was developed (Arima et al., 2009).

Advances in Cohort Studies and CVD Risk Assessment Worldwide

TC Cut-Off Points by Cohort Studies

In 1986, Stamler et al. reported the relationship between serum TC and CHD death rate in the Multiple Risk Factor Intervention Trial (MRFIT) (Stamler et al., 1986). In this study, 356,222 men aged 35–57 years who had no history of hospitalization for myocardial

infarction (MI) were recruited at 22 clinical centers in 18 US cities and then followed for 6 years. The relative risk (RR) of 6-year CHD mortality in the second to fifth quintiles of TC (182–202, 203–220, 221–244, and ≥245 mg/dL) were compared with that in the lowest quintile. The RR results were 1.29, 1.73, 2.21, and 3.42, respectively. These findings contributed to the determination of the criteria for TC levels in the US National Cholesterol Education Program. In the National Cholesterol Education Program Adult Treatment Panel II and III (NCEP-ATP II and III), a categorical high TC was defined as ≥240 mg/dL (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 1994, 2001), as subjects with such TC levels have almost twice the RR for CHD death as those with TC ≥200 mg/dL.

Risk Assessment for Future CVD Based on Cohort Studies

Since the dawn of CVD epidemiology, several cohort studies have identified multiple risk factors for CVD. These findings have given rise to the strategy of CVD prevention in populations at risk by the treatment of those with established risk factors. To this end, it is important to detect individuals who are at high risk owing to the magnitude of each risk factor and the combination of risk factors. In order to estimate individual CVD risk, risk assessment tools have been introduced. In 1998, the Framingham Risk Score for CHD in the United States was published (Wilson et al., 1998). Subsequently, the SCORE risk charts in Europe and the Pooled Cohort Equations validated by the 2013 ACC/AHA guidelines on the assessment of CVD in the United States were promulgated using a pooled dataset from several cohort studies (Conroy et al., 2003; Goff et al., 2014). In addition, several CVD risk assessment tools were developed in Asian countries (NIPPON DATA80 Research Group, 2006; Nishimura et al., 2014).

The American College of Cardiology (ACC) and American Heart Association (AHA) jointly published the 2013 ACC/AHA guidelines on the assessment of cardiovascular risk in collaboration with the National Heart, Lung, and Blood Institute (NHLBI) (Goff et al., 2014). The 2013 ACC/AHA guidelines were based on evidence from a systematic review and metaanalysis of relevant randomized controlled trials with the aim of reducing cardiovascular risk. As a consequence, statin therapy is now widely recommended for TC reduction. There are four major statin benefit groups: (1) atherosclerotic CVD (ASCVD; defined as history of acute coronary syndrome (ACS), MI, stroke, transient ischemic attack, or peripheral arterial disease), (2) LDL-C ≥190 mg/dL and age >21 years, (3) ages 40–75 years with diabetes and LDL-C 70–189 mg/dL without ASCVD, and (4) ages 40–75 years without ASCVD or diabetes and with LDL-C 70–189 mg/dL and estimated 10-year ASCVD risk ≥7.5%.

The 10-year ASCVD risk was defined as the risk of developing a first ASCVD event, nonfatal MI, CHD death, or fatal or nonfatal stroke, over a 10-year period among people with no history of ASCVD at the beginning of the period. The estimated 10-year risk of ASCVD was calculated using the new Pooled Cohort ASCVD Risk Equations. The pooled cohorts included the ARIC (Atherosclerosis Risk in Communities) study, the Cardiovascular Health Study, the CARDIA (Coronary Artery Risk Development in Young Adults) study, the Framingham Heart Study, and the Offspring Study cohorts, which are NHLBI-funded community-based cohorts. Participants were 24,626 non-Hispanic, Caucasian, and African-American women and men aged 40–79 years who were without a history of CVD, nonfatal MI, heart failure, stroke, percutaneous coronary intervention, coronary artery bypass surgery, and atrial fibrillation. The ASCVD risks were estimated using sex- and race-specific proportional hazards models that included the covariates of age, treated or untreated SBP, TC, HDL-C, current smoking status, and diabetes.

The ACC/AHA guidelines focus on statin therapy (high or moderate intensity) without the need for lipid monitoring ("fire and forget" approach) to assess therapeutic efficacy (Stone et al., 2014) and identifying the target population by means of the Pooled Cohort ASCVD Risk Equations.

Ten-year risk assessment of CVD using The SCORE risk chart was introduced in the 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias (Catapano et al., 2016). This assessment was based on pooled data from 12 European cohort studies, which included 205,178 participants free of a history of CVD. The RRs of each risk factor for CVD were calculated using a cohort- and sex-stratified Weibull proportional model, with the covariates of age, SBP, TC, HDL-C, and smoking status. Risk estimates have been produced as charts for high- and low-risk regions of Europe.

The targeted approach for lipid management is mainly to reduce LDL-C. For patients with a *very* high total CVD risk, the targeted value is an LDL-C of 70 mg/dL. For subjects with a high total CVD risk, the goal is an LDL-C of 100 mg/dL. Among populations with moderate total CVD risk, the LDL-C goal is 115 mg/dL. Regardless of risk levels, at least a 50% reduction from baseline is recommended in the 2016 ESC/EAS Guidelines.

It can be seen that there is a clear difference in lipid management between the United States and Europe. The latter recommends treatment of the targeted population in order to reduce the value of cholesterol to a targeted value, that is, a "target-to-target" approach. They are both controversial approaches for the prevention CVD in clinical practice.

Investigating the Impact of Lipid Parameters on CVD in Asian Countries

Recently, many cohort studies have investigated the association between various lipid parameters and CVD in Asian countries. The Asia Pacific Cohort Studies Collaboration (APCSC) is a pooled analysis from prospective cohort studies in several Asian countries, Australia, and New Zealand (Asia Pacific Cohort Studies Collaboration, 2004). The inclusion criteria were as follows: (1) all participants were from Asia or the Pacific region (including Australia and New Zealand); (2) age, sex, and blood pressure of each individual had been measured at baseline; and (3) ≥5000 person-years of follow-up had been completed.

There are three representative studies that have shown the association between lipid parameters and CVD in the APCSC. It was reported that serum triglycerides (TG) were an important and independent predictor of CHD and stroke risk in the Asia-Pacific

region (Asia-Pacific Cohort Studies Collaboration, 2004). For this study, the APCSC analyzed data from 96,224 individuals (median follow-up period, 7.9 years) in 26 cohort studies. Participants in the highest 5th of TG (≥168 mg/dL) had a 70% greater risk of CHD death, an 80% greater risk of fatal or nonfatal CHD, and a 50% increased risk of fatal or nonfatal stroke when compared to participants in the lowest 5th of TG (≤62 mg/dL). On the other hand, Huxley et al. reported that an isolated low HDL-C was significantly associated with CHD in 220,060 individual data from the APCSC (Huxley et al., 2011). In that study, during 6.8 years of follow-up, participants with isolated low HDL-C had a 63% greater risk of CHD death relative to those with normal and high HDL-C level in Asians. Hirakawa et al. (2016) recently reported the impact of body mass index (BMI) on the associations of lipid parameters with CHD risk in the Asia-Pacific region by using data from the APCSC. The authors showed that the risk of CHD increased with increasing TC and with decreasing HDL-C independent of BMI. On the other hand, the impact of TG on CHD events significantly increased as BMI increased, and this effect was still evident after adjusting for TC and HDL-C.

In China, a multicenter prospective cohort study of cardiovascular risk factors in Chinese, the Chinese Multiprovincial Cohort Study (CMCS), was performed from 1992. Chinese subjects ($n=30,378$) aged 35–64 years and free of CVD participated. Ren et al. (2010) reported that a very low density density lipoprotein cholesterol (VLDL) level was significantly associated with an elevated 15-year CVD risk. After the CMCS, the China Cardiovascular Health Study and the China Multicenter Collaborative Study of Cardiovascular Epidemiology (ChinaMUCA) were conducted during 1998–2001. A total of 11,480 participants were followed in the ChinaMUCA study. Gu et al. (2015) reported that a higher non-HDL-C level was associated with an increase in CVD incidence in Chinese and had a similar effect on cardiovascular risk as LDL-C.

The Korea Medical Insurance Corporation (KMIC) Study was conducted in 1990. This study aimed to assess risk factors for ASCVD in Korean men and women and included more than 100,000 men and 50,000 women aged 35–59 years. Suh et al. (2001) reported that low-serum TC was not an independent risk factor for either intracerebral or subarachnoid hemorrhagic stroke in Korean men. In addition, Jee et al. (1999) have indicated that a low-cholesterol level did not protect against smoking-related ASCVD in Korean men. The Korea National Health and Nutrition Examination Survey (KNHANES) was established in 1998, and many reports concerning cardiovascular risk factors including lipid parameters have been published. KNHANES is a national surveillance system to assess the health and nutritional status of Koreans, and the surveys have been conducted by the Korea Centers for Disease Control and Prevention (KCDC) (Kweon et al., 2014). In this project, Lee et al. (2014) have reported the descriptive data in Korea, the age-standardized mean levels of serum cholesterol, and the prevalence, awareness, treatment, and control rates of high LDL-C levels according to age. Compared with elderly participants, significant proportions of young and middle-aged adults were unaware of their hypercholesterolemia and were not being treated with effective lipid-lowering medications.

Cohort studies for CVD risk including lipid parameters have been reported in several countries. However, there have not been sufficient studies to elucidate the differences between races, regions, or health behaviors. In light of these differences, future cohort studies are warranted to clarify more specific individual risks.

Evidence From Lipid-Lowering Clinical Trials

Clinical Trials of Primary Prevention

Before the advent of the 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors, also known as the stains, several lipid-lowering therapies had been evaluated in controlled clinical trials. LRC-CART (Lipid Research Clinics-Coronary Prevention Program) was the first trial that succeeded in the prevention of CHD by lipid-lowering therapy, using cholestyramine to reduce LDL-C (Lipid Research Clinics Program, 1984). The study was a double-blind randomized controlled trial (DB-RCT) with a placebo control. It included 3806 patients with no history of CHD and randomly assigned 1906 patients to cholestyramine (daily use: 24 g/day) and 1900 patients to a placebo. Both groups were recommended to take a moderate cholesterol-lowering diet. The primary outcome variable was the first occurrence of CHD death or nonfatal MI. During 7.4 years, 155 events (8.1%) of primary outcomes occurred in the cholestyramine arm and 187 events (9.8%) in the placebo arm. The estimated relative risk reduction (RRR) for the primary endpoint was 19% (90% confidence interval (CI): 3%–32%). However, the RRR for all-cause death was not statistically significant as deaths due to accidents and violence were greater in the cholestyramine arm.

Since their first market appearance in 1987, statins have been the most used lipid-lowering drugs. The first trial that tested a statin in the primary prevention of CHD was WOSCOPS (West of Scotland Coronary Prevention Study) (Shepherd et al., 1995). It was a DB-RCT and included 6595 men with hypercholesterolemia and no history of MI aged 45–64 years. Their mean TC was 7.0 ± 0.6 (standard deviation, SD) mmol/L. The subjects were randomly assigned to pravastatin (daily use: 40 mg each evening) or a placebo and followed for 4.9 years. In the pravastatin arm, serum TC and LDL-C were lowered 20% and 26%, respectively, at the end of the trial. The event numbers for the primary endpoint were 248 of 3293 in the placebo arm and 174 of 3302 in the pravastatin arm, corresponding to a 31% risk reduction (95% CI: 17%–43%, $P<0.001$). In addition, there was no difference in deaths due to non-CVD such as suicide, trauma, and cancer. Overall, a 22% reduction of total deaths was observed in the pravastatin arm (95% CI: 0%–40%, $P=0.051$). The WOSCOPS study was the first trial to show not only a preventive effect for primary CHD events but also no evidence for an increase in adverse events, which until then had been a concern about use of statins.

The AFCAPS/TexCAPS (Air Force/Texas Coronary Atherosclerosis Prevention Study) (Downs et al., 1998) evaluated the efficacy of lovastatin in a population that had no history of CHD and had normal serum TC (4.65–6.82 mmol/L) and LDL-C (3.36–4.91 mmol/L) levels but low HDL-C (≤1.16 mmol/L for men, ≤1.22 mmol/L for women). The study was a DB-RCT with placebo. The total number of subjects was 6605, who were divided into a lovastatin arm (daily use 20–40 mg, $n=3304$) and a

placebo arm ($n=3301$). The primary endpoint was a major CHD event defined by fatal or nonfatal MI, unstable angina, or sudden cardiac death. The mean follow-up period was 5.2 years. Lovastatin reduced LDL-C by 25% and increased HDL-C by 6% during follow-up. 116 major CHD events were observed in the lovastatin arm and 183 events in the placebo arm. The estimated RR for lovastatin vs. placebo was 0.63 (95% CI: 0.50–0.79, $P<0.001$). The results of this study suggested that lipid-lowering treatment with a statin may be beneficial even for subjects with low-serum HDL-C and normal TC/LDL-C levels.

The target of CARDS (Collaborative Atorvastatin Diabetes Study) (Colhoun et al., 2004) was patients with type 2 diabetes mellitus without high-serum LDL-C ($\leq$4.14 mmol/L). The participants also each had at least one of the following risk factors: retinopathy, albuminuria, current smoking, and hypertension. This DC-RCT with a placebo arm enrolled 2838 patients aged 40–75 years who had no previous history of CVD. They were randomly assigned to atorvastatin (daily use 10 mg, $n=1428$) or placebo ($n=1410$), and the primary endpoint was the first event, defined as acute CHD, coronary revascularization, or stroke. The trial was stopped 2 years earlier than expected because the early stopping rule for efficacy was adopted. At the end of the trial, 83 primary events had occurred in the atorvastatin arm (5.8%, 1.54 per 100 person-years at risk) and 127 events in the placebo arm (9.0%, 2.46 per 100 person-years at risk). The estimated RRR between the two arms was 37% (95% CI: −52 to −17), $P=0.001$). In addition, subgroup analyses showed risk reductions for both stroke and acute CHD.

These studies were performed in Western countries, where the incidences of CHD are relatively high. MEGA (Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese) (Nakamura et al., 2006) was the first trial to evaluate the effectiveness of pravastatin for the primary prevention of CHD in Japan, where the incidence of CHD is low. This trial was based on the PROBE design (prospective, randomized, open-labeled, blinded study) and included 7832 patients with hypercholesterolemia (serum TC, 5.69–6.98 mmol/L) and no history of CHD or stroke. The primary endpoint was the first episode of CHD, defined as fatal or nonfatal MI, angina, cardiac sudden death, and coronary revascularization. The mean follow-up period was 5.3 years, and 3966 patients were assigned to the diet therapy arm and 3866 to the diet therapy plus pravastatin arm (daily use 10–20 mg). At the end of the trial, the reductions of serum TC and LDL-C were 2.1% and 3.2%, respectively, in the diet only arm and 11.5% and 18% in the diet plus pravastatin arm. For the primary endpoint, 66 events were observed in the diet plus pravastatin arm and 101 in the diet only arm. The estimated hazard ratio (HR) indicated that the diet plus pravastatin arm had significantly fewer CHD events (HR: 0.67, 95% CI: 0.49–0.91, $P=0.01$).

A metaanalysis of randomized control trials (RCTs), including the trials mentioned earlier, confirmed that statins prevented primary CVD (Taylor et al., 2013) (**Table 1**). Totally 20 to 30% relative risk reductions for CVD, CHD and stroke were observed in this metaanalysis.

The results of epidemiological studies have suggested that a high intake of long-chain n-3 fatty acids may reduce the risk of CVD events (Burr et al., 1989; Lemaitre et al., 2003). To test this hypothesis, the PROBE trial called JELIS (Japan EPA Lipid Intervention Study) was performed in Japan (Yokoyama et al., 2007), where consumption of fish is higher than in Western countries. The primary endpoint was a major coronary event, defined as sudden cardiac death, fatal or nonfatal MI, and other nonfatal events including unstable angina pectoris, angioplasty, stenting, or coronary artery bypass grafting. The participants were 18,645 patients with serum TC$\geq$6.5 mmol/L and were composed of a primary prevention group ($n=14,981$) and a secondary prevention group ($n=3664$). A total of 9326 patients were assigned to eicosapentaenoic acid (EPA) plus a statin (intervention arm), and 9319 patients were assigned to a statin only (control arm). The mean follow-up period was 4.6 years. At the end of the trial, there were 262 events (2.8%) in the intervention arm and 324 events (3.5%) in the control arm. The estimated HR for intervention vs. control was 0.81 (95% CI: 0.69–0.95, $P=0.011$). However, when the analysis was limited to primary prevention, there was no significant difference between the intervention and control groups (1.4% vs. 1.7%, respectively, $P=0.132$).

Clinical Trials of Secondary Prevention

The first DB-RCT trial that evaluated secondary prevention for CVD with a statin was 4S (Scandinavian Simvastatin Survival Study) (Scandinavian Simvastatin Survival Study group, 1994). The primary endpoint was all-cause death, and the secondary endpoint was a major CHD event (coronary death, nonfatal MI, resuscitated cardiac arrest, or definite silent MI confirmed by electrocardiogram). 4444 patients with hypercholesterolemia (serum TC 5.5–8.0 mmol/L) who had experienced angina pectoris or had suffered a MI were randomly assigned to simvastatin (daily use 20–40 mg, $n=2221$) or placebo ($n=2223$). The median follow-up period was 5.4 years. For the primary endpoint, 182 deaths (8%) were observed in the simvastatin arm and 256 deaths (12%) in the placebo arm. The estimated RR for total death in the simvastatin arm was 0.70 (95% CI: 0.58–0.85, $P=0.0003$). For the secondary endpoint,

Table 1 The results of the metaanalysis for primary prevention of CVDs

Endpoint	*No. of studies*	*No. of participants*	*Summary RR (95% CI)*
Total number of CVD events	9	23,805	0.75 (0.70–0.81)
Total number of CHD events	14	48,049	0.73 (0.67–0.80)
Total number of stroke events	10	40.295	0.78 (0.68–0.89)
Total mortality	13	48,060	0.86 (0.79–0.94)[a]

CVD, cardiovascular disease; *CHD*, coronary heart disease; *RR*, risk ratio; *CI*, confidence interval.
[a]Estimated as summary odds raio.

there were 431 events (19%) in the simvastatin arm and 622 (28%) in the placebo arm, demonstrating a significant risk reduction in the simvastatin arm (RR: 0.66, 95% CI: 0.59–0.75, $P<0.00001$).

The object of CARE (Cholesterol and Recurrent Events) (Sacks et al., 1996) was to investigate a secondary prevention effect using a statin in patients who had a history of MI and a normal serum profile. The primary endpoint was a fatal coronary event or nonfatal MI. This DB-RCT included 4159 patients, who were randomly assigned to pravastatin (daily use 40 mg/day, $n=2081$) or a placebo ($n=2078$). During a mean follow-up of 5 years, 212 events (10.2%) were observed in the pravastatin group and 274 events (13.2%) in the placebo group, corresponding to a RRR of 24% (95% CI: 9%–36%, $P=0.003$). However, in those patients with serum TC $\leq$ 3.2 mmol/L, pravastatin had not significantly prevented recurrent CHD events. On the other hand, additional posthoc analysis of CARE (Plehn et al., 1999) showed that pravastatin also reduced stroke and stroke or transient ischemic attack in patients with a history of MI.

A metaanalysis of the RCTs including 4S and CARE confirmed that statins were effective in the secondary prevention of CVD (Gutierrez et al., 2012) (**Table 2**). The estimated relative risk reductions were about 15% to 25% for CVD, CHD and stroke.

In contrast to the result in the primary prevention group of JELIS (Yokoyama et al., 2007), major secondary coronary events in the intervention arm were reduced by 19% relative to the control arm (intervention arm: 8.7%, control arm: 10.7%, $P=0.048$). The discrepancy between the results obtained for primary and secondary prevention suggests that the benefit of EPA treatment may be stronger for higher-risk individuals than it is for lower-risk ones.

Recently, new evidence was provided by IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial) (Cannon et al., 2015). The participants of this DB-RCT were 18,144 patients who had been hospitalized because of ACS within the preceding 10 days. Their serum LDL-C levels were in the range of 1.3–2.6 mmol/L if receiving lipid-lowering therapy, or 1.3–3.2 mmol/L if not receiving such therapy. Both of these ranges were within guideline recommendations (Grundy et al., 2004; Perk et al., 2012). The trial compared the combination of simvastatin (40 mg/day) and ezetimibe (10 mg/day) with simvastatin alone (40 mg/day) for the secondary prevention of CVD events, defined as cardiovascular death, nonfatal MI, unstable angina requiring rehospitalization, coronary revascularization ($\geq$30 days after randomization), or nonfatal stroke. After the standard treatment for ACS, the participants were randomly assigned to a combination arm ($n=9067$) and a monotherapy arm ($n=9077$). The median follow-up period was 6 years. The median time-weighted average serum LDL-C level was 1.4 mmol/L in the combination group and 1.8 mmol/L in the monotherapy group ($P<0.001$). The Kaplan–Meier event rate for the primary endpoint was 32.7% (combination) versus 34.7% (monotherapy). The estimated absolute risk difference was 2.0%, and the HR was 0.936 (95% CI: 0.89–0.99, $P=0.016$). The rates of adverse events such as hepatic disorder, myopathy, and cancer were not different between the two arms. IMPROVE-IT was the first trial that supported the treatment concept "the lower, the better" for reducing CVD events by lowering serum LDL-C levels below guideline recommendations and showed the prevention effect for recurrent CVD events of adding another lipid-lowering drug to a statin.

Conclusions From Clinical Trials

The evidence that lipid-lowering therapies, especially statins, can reduce the risk of CVD events through reduction of serum lipid levels is now well established. However, the estimated risk reduction rates for CVD events were at most only 30%. Therefore, control of the residual risk by new therapies or treatment concepts is needed for greater prevention of CVD.

Traditional but Long-Standing Problems

Is HDL-C a Causal Protective Factor for CHD?

Serum HDL-C concentration has been known to be a protective factor for CHD since the first the epidemiologic report from the Tromsø heart-study (Miller et al., 1977). The principal mechanism postulated for the protective effect of HDL is reverse cholesterol transport, which opposes cholesterol accumulation in artery walls due to LDL-C deposition. This may show its protective effect only in the presence of a high-serum level of LDL-C. Recently, a large pooled analysis from Japan where mean LDL-C levels were lower than those of Westerners showed that isolated low HDL-C was not associated with an increased risk of CHD (Hirata et al., 2016).

The results of a large Mendelian randomization analysis reconfirmed the causal effect of LDL-C on CHD, but a causal association of HDL-C with CHD was less clear (Holmes et al., 2015). Among patients with ASCVD and normal LDL-C levels, there was no clinical benefit from addition of niacin to statin therapy, despite a significant increase in HDL-C (AIM-HIGH Investigators

Table 2 The results of the metaanalysis for secondary prevention of CVDs

Endpoint	*No. of studies*	*No. of participants*	*Summary RR (95% CI)*
Total number of CVD events	11	43,191	0.81 (0.78–0.85)
Total number of MI events	4	13,916	0.73 (0.65–0.81)
Total number of stroke events	7	27,746	0.84 (0.76–0.93)
Total mortality	5	19,059	0.81 (0.75–0.88)

CVD, cardiovascular disease; *MI*, myocardial infarction; *RR*, risk ratio; *CI*, confidence interval.

et al., 2011). Furthermore, a clinical trial using a cholesteryl ester transfer protein inhibitor failed to show a protective effect for CHD (Barter et al., 2007).

These findings indicate two possibilities. One is that a low HDL-C per se is not a risk factor for CHD but is instead a surrogate marker of metabolic syndrome with impaired glucose tolerance. The other is that it is not so much the serum level of HDL-C that matters for CHD but the antiatherosclerotic function of HDL particles. Accordingly, we need clinical trials or Mendelian randomization studies that focus on improvement of HDL function, in other words the quality of HDL. Further studies that provide such information are warranted.

Weak Association Between Hypercholesterolemia and Stroke

In contrast to the case of CAD, relatively little attention has been given to the relations between hypercholesterolemia and stroke, even though stroke has been the most common CVD in East Asia, the most populated region in the world (Ueshima et al., 2008). The majority of cohort studies in Japan have failed to demonstrate a positive relation between hypercholesterolemia and stroke (Tanaka and Okamura, 2012). Indeed, even in the Western populations, the relation between hypercholesterolemia and stroke is controversial and less clear. A positive association between TC and cerebral infarction was observed in the MRFIT trial in the United States (Iso et al., 1989) and in the Copenhagen City Heart Study in Europe. On the other hand, the ARIC Study did not find TC to be associated with the incidence of cerebral infarction. Hypercholesterolemia was associated only with nonlacunar, nonembolic stroke, in other words, atherothrombotic cerebral infarction (Kurth et al., 2007). A large metaanalysis of data from 61 prospective studies, most of which were carried out in the United States and Europe, showed the absence of independent positive associations between TC and ischemic and total stroke mortalities (Prospective Studies Collaboration, 2007). Consequently, the relation between hypercholesterolemia and cerebral infarction may be modified by the proportion of atherothrombotic cerebral infarctions in the populations surveyed.

On the other hand, recent RCTs using statins have shown substantial reductions in cerebral infarction (Mills et al., 2008; Vergouwen et al., 2008). However, caution is needed when interpreting the results of these trials. In the protocols of most clinical trials, the primary endpoint was CHD, and cerebral infarction was usually set as a secondary endpoint. To our knowledge, the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) and the Japan Statin Treatment against Recurrent Stroke Trial (J-STARS) are only two trials that were designed with stroke as the primary endpoint (Amarenco et al., 2006; Hosomi et al., 2015). Although both found a protective effect of statins against stroke, the first study found a relatively small effect (16% risk reduction), and the second found a reduction of only atherothrombotic infarction occupying one-fourth of cerebral infarction. Furthermore, statins have pleiotropic effects, such as antiinflammatory actions, that may reduce atherosclerosis. This may be the reason why we observed a discrepancy between cohort studies and clinical trials, which to date has not been adequately explained, and which was not observed in relation to hypercholesterolemia and CHD, or to hypertension and stroke.

Low Blood Cholesterol and Noncoronary Mortality

In 1980, Japanese investigators reported that community dwellers with low-serum TC levels had a high risk of intraparenchymal hemorrhage (Ueshima et al., 1980). At the end of the 1980s, similar results were observed in the United States (Iso et al., 1989). In 1990, an NHLBI conference was held to discuss associations between low blood cholesterol and noncoronary mortality (Jacobs et al., 1992). Most of the inverse associations between cholesterol and noncoronary mortality, such as malignancy and chronic pulmonary diseases, were thought to be explained by latent preexisting disease, unconsidered confounding factors, and SES. The only concern expressed in the report was the possibility of a causal relationship between low TC and intraparenchymal hemorrhage. The associations between low TC levels and intraparenchymal hemorrhage in previous cohort studies were not rare findings, although they were scarcely observed in populations with a very low incidence of intraparenchymal hemorrhage such as the United States. Furthermore, even in a metaanalysis of clinical trials the results were controversial (Boekholdt et al., 2014; Cholesterol Treatment Trialists' (CTT) Collaboration (2015)). In SPARCL, intraparenchymal hemorrhage was more frequent in those treated with a statin, especially in those with a past history of hemorrhagic stroke or hypertension (Amarenco et al., 2006) although similar results were not observed in the J-SATARS trial (Hosomi et al., 2015).

The pathologic link between hypercholesterolemia and coronary artery disease is atherothrombosis, but that between hypercholesterolemia and intraparenchymal hemorrhage is angionecrosis in a penetrating arteriole, which is also the main focus of lacunar infarction (Konishi et al., 1990). Low blood cholesterol may causally induce angionecrosis although there is little experimental evidence. As another hypothesis, low blood cholesterol might reflect nutritional status, which might influence mortality rate after the event. An inverse association between low cholesterol and intraparenchymal hemorrhage seems to have been well documented when the endpoint was mortality (Tanaka and Okamura, 2012). Nevertheless, further experimental, clinical, and epidemiological studies are warranted to examine these hypotheses.

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Long QT Syndrome and Torsade de Pointes

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Abbreviations

AD	Autosomal dominant
ECG	Electrocardiogram
I_{Ca}	Calcium channel
I_{Na}	Sodium channel
SCD	Sudden cardiac death
TS	Timothy syndrome
ABS	Ankyrin-B syndrome
AP-A	Anthopleurin-A
APD	Action potential duration
AR	Autosomal recessive
ATS	Anderson–Tawil syndrome
GWAS	Genome-wide association studies
HERG	Human ether-a-go-go-related gene
ICD	Implantable cardioverter defibrillator
JLNS	Jervell Lang-Nielsen syndrome
LQTS	Long QT syndrome
SNP	Single-nucleotide polymorphism
TdP	Torsade de Pointes
VT	Ventricular tachyarrhythmias

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Introduction

Since its initial description by Jervell and Lange-Nielsen (1957) the congenital long QT syndrome (LQTS) has been the most investigated cardiac ion channelopathy. A prolonged QT interval in the surface electrocardiogram (ECG) is the *sine qua non* of the LQTS and is a surrogate measure of the ventricular action potential duration (APD). Congenital as well as acquired alterations in certain cardiac ion channels can affect their currents in such a way as to increase the APD and hence the QT interval. The inhomogeneous lengthening of the APD across the ventricular wall results in dispersion of APD, that is, of ventricular repolarization (VR). This together with the tendency of prolonged APD to be associated with oscillations at the plateau level, termed early afterdepolarizations (EADs), provides the substrate of ventricular tachyarrhythmia (VT) associated with LQTS, usually referred to as Torsade de Pointes (TdP) VT (El-Sherif and Turitto, 1999). The term was first coined by Dessertenne who described its ECG pattern of continuously changing morphology of the QRS complexes that seem to twist around an imaginary baseline (Dessertenne, 1966). The quasimusical term (**Fig. 1**) and the intriguing ECG pattern have caught the attention of electrophysiologists for years and have been, to some extent, a driving force behind the recent focused interest into the role of genetics and cardiac ion channel pathophysiology in cardiac arrhythmias (El-Sherif and Turitto, 1999).

Acquired LQTS is by far more prevalent than congenital LQTS. The vast majority of acquired LQTS is the result of adverse effect of drugs (Kannankeril et al., 2010) and/or electrolyte abnormalities (El-Sherif and Turitto, 2011) that interact in the majority of cases with the Human Ether-à-go-go-Related Gene (HERG) encoding the pore-forming subunits (Kv11.1) of the rapidly activating delayed rectifier current, I_{Kr}.

This article review the genetic, molecular, and phenotype characteristics of both congenital and acquired LQTS as well as current management strategies and future directions in the field.

ECG Characteristics of TdP

In an updated analysis of 150 different episodes of non-sustained VT obtained from 62 patients with the acquired LQTS (El-Sherif and Turitto, 1999), the arrhythmia ranged in length from 3 beats (the definition of nonsustained VT) up to 117 beats (**Fig. 2A**) with an average length of 16 ± 8 beats. The cycle length (CL) of these episodes ranged from 193 to 364 ms with an average of 279 ± 47 ms. The VT was frequently preceded by a variable period of bigeminal rhythm due to one or two premature ventricular beats coupled to the prolonged QT segment of the preceding basic beat (**Fig. 2**, panels A and C). This so called short-long cardiac sequence is seen in both the acquired and congenital LQTS, and the arrhythmogenic mechanism is related to increased dispersion of repolarization (DR).

Following termination of an episode of fast VT, it was not uncommon to see one or more ectopic beats of variable configuration occurring at much longer CL compared to that of the VT (see beats marked by stars in **Fig. 2**). The change in QRS configuration during VT can take several forms. During a very fast VT, periodic decrease in the amplitude of the entire QRS-T complex is seen with less distinct shifts in QRS axis (**Fig. 2A**). In VTs with slower rates, the classic twisting of the QRS axis from a predominantly positive to a predominantly negative configuration with a variable number of transitional complexes and vice versa is commonly seen (**Fig. 2B**). Sometimes, a polymorphic QRS configuration is seen without any of the two previously characteristic patterns (as verified in multiple simultaneous leads, **Fig. 2C**, middle recording). Different patterns can be seen in different VT episodes from the same patient (**Fig. 2C**).



Fig. 1 The daily utterance of the term "torsade" in French refers to different bakery items, including bread loaves with characteristic twisting configuration. The above card displays one advertisement for such products.

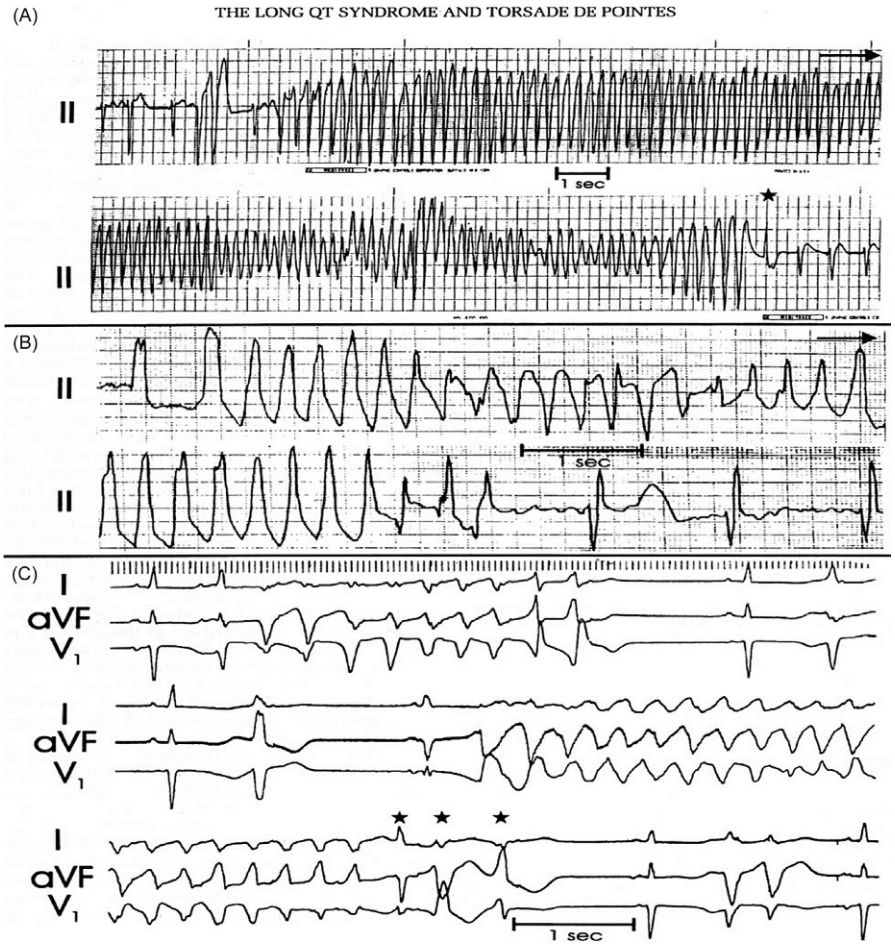

Fig. 2 Three electrocardiographic examples of acquired long QT syndrome (LQTS) and torsade de pointes (TdP). Panel A was obtained from a 23-year-old woman who had human immunodeficiency virus (HIV) positive and was receiving pentamidine. The patient was admitted with severe diarrhea and hypokalemia. Panel B was recorded from a 62-year-old man with hypertension and chronic atrial fibrillation who was receiving digoxin and hydrochlorothiazide and had a potassium level of 3.2 mEql. The TdP tachycardia developed 12 h after the patient received a total of four tablets of quinidine gluconate in an attempt to restore normal sinus rhythm. Panel C was obtained from a 64-year-old man who was receiving procainamide for suppression of very frequent ventricular premature complexes. N.B. pentamidine, quinidine, and procainamide are considered a group of drugs with high risk of drug-induced acquired QT syndrome and their use is currently curtailed. Reproduced with permission from El-Sherif N, Turitto G, Pace 1999;22:91–110.

QT/T Wave Alternans and TdP

It has long been known that tachycardia-dependent T wave alternans (TWA) occurs in patients with the congenital or acquired form of the LQTS and may presage the onset of TdP (Fig. 3).

In analysis of 1103 LQTS patients with QTc interval >0.44 s from the International LQTS Registry, TWA was recorded in 30 patients (Moss, 1995). The frequency of occurrence of TWA was directly proportional to the length of the QTc interval on the enrollment ECG. TWA occurred in one or more occasions during an average 4-year follow-up in 21% of the patients with QTc > 0.60 s, but in <0.2% of the patients with QTc <0.50 s. Patients with advanced forms of TWA (those with bidirectional beat-to-beat changes in T wave polarity; $n = 21$) were younger, had longer QTc values, had a higher incidence of complex VT, and were more likely to experience a cardiac event (syncope or cardiac arrest) than those with less advanced forms of TWA (those without bidirectional beat-to-beat changes in T wave polarity; $n = 9$).

Although overt TWA in the ECG is not common, in recent years, digital signal-processing techniques have made it possible to detect subtle degrees of TWA. This suggests that the phenomenon may be more prevalent than previously recognized and may represent an important marker of vulnerability to VT (Verrier et al., 2011). Interest in TWA is attributed to the hypothesis that it reflects a greater degree of underlying DR (Chinushi et al., 1998).

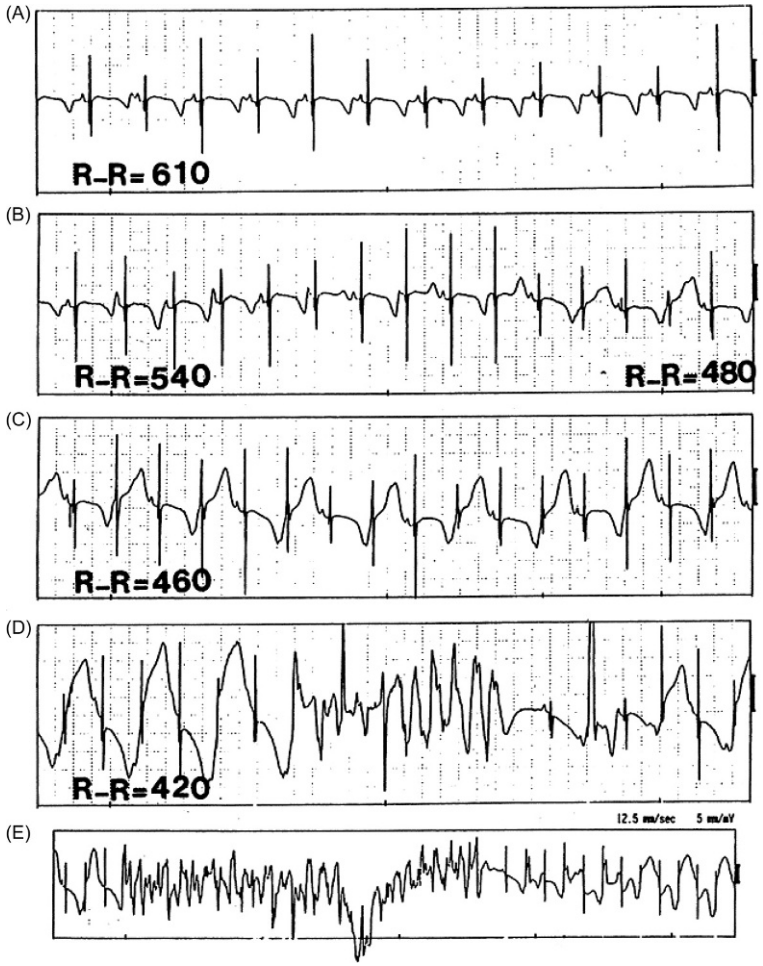

Fig. 3 Tachycardia-dependent QT alternans from a newborn baby with the Romano-Ward long QT syndrome (LQTS). The figure illustrates the development of TWA with gradual acceleration of the heart rate. At a cardiac cycle length (CL) of 610 ms (panel A), the QTU interval was 440–480 ms and TWA alternans was not discernible. Gradual shortening of the CL to 540 ms resulted in TWA alternans that became more prominent at shorter CLs (panels B and C). In panel D, on further shortening of the CL to 420 ms, TWA alternans was markedly exaggerated and was associated with the onset of nonsustained polymorphic ventricular tachycardia (VT) with a twisting QRS morphology characteristic of torsade de pointes (TdP). Panel E was recorded at reduced paper speed to illustrate the onset and termination of a longer run of TdP that was also associated with marked TWA alternans at a sinus CL of 420–440 ms. Reproduced with permission from Habbab MA, El-Sherif N, Pace 1992;15:916–931.

Genotype-Specific T-Wave Morphology

The ventricular myocardial action potential changes are based on the affected ion channels along with the distributive pattern of channels in the layers of the myocardium. Multiple ST-segment and T-wave morphologies associated with a particular genotype have been delineated (Zareba, 2006). Due to the small available sample sizes, this association has been restricted to the most common genotypes (LQT1, LQT2, and LQT3). Presence of broad-based T-waves in LQT1, low-amplitude T-waves in LQT2, and late-peaking T-waves in LQT3 was initially demonstrated by Moss et al. (1995). This association was demonstrated in-vitro, based on models affecting particular ion channels (Antzelevitch, 2003). Further delineation of the T-wave morphology was done by Zhang et al. using 284 LQTS carriers; demonstrating 10 typical patterns (4 LQT1, 4 LQT2, and 2 LQT3) (Zhang et al., 2000). In this study, LQT1 carriers demonstrated normal or broad-based T-wave pattern consistent with the homogenous action potential prolongation caused by affecting I_{Kr}. LQT2 carriers had a bifid T-wave pattern, thought to be a result of increased transmural dispersion due to greater increase in APD in the M cells of the myocardium compared to the endocardium or the epicardium. Due to the delay in opening of the sodium channels and hence phase 2 of the action potential, LQT3 patients had a late-peaked T-wave (Fig. 4).

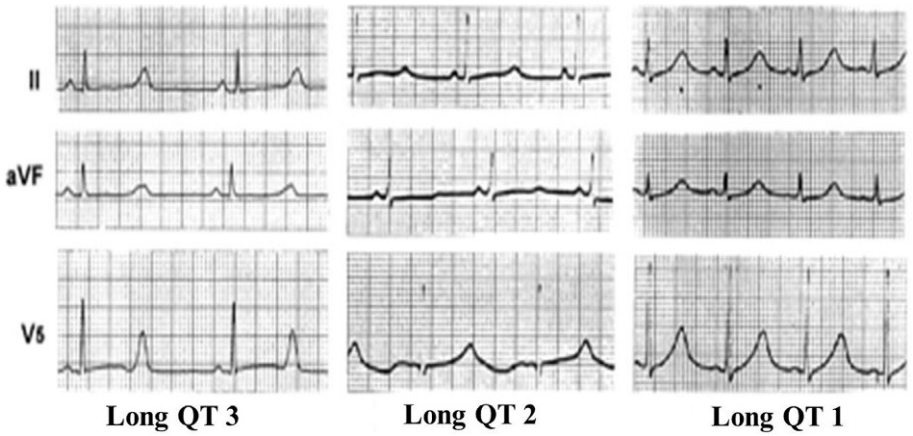

Fig. 4 Genotype-specific T-wave morphology of the common genotypes: LQT1 is characterized by broad-based T-waves; LQT2 by low-amplitude T-waves, and LQT3 by late-peaking T-waves. Reproduced with permission from Moss et al., Circulation 1995;92:2929–2934.

A Paradigm of TdP From Ion Channels to ECG

Figs. 5 and 6 are a composite that is intended to briefly summarize the correlation between the molecular mutation of an ion channel, the electrophysiological consequence, and the final phenotype presentation of LQTS. The gene selected encodes for the α subunit of the Na channel SCN5A whose mutation can result in LQT3, together with the *β* subunit of the channel, SCN4B, whose mutation results in LQT10 (Medeiros-Domingo et al., 2007). Over 200 different mutations of the SCN5A have been identified, the majority giving rise to LQT3. There is wide variation in the phenotypic presentation of different mutations. In general, mutations involving subunits results in a milder form of the disease as compared to mutations involving the pore forming region of the channel. An interesting aspect of the cardiac Na channel is that some mutations can result in different electrophysiological syndromes other than LQTS. This phenomenon is referred to as cardiac Na channel overlap syndrome (Remme et al., 2008). Certain mutations can result in Brugada syndrome, sick sinus syndrome, atrial stand still, atrial fibrillation, and cardiac conduction defects. Thus, although these syndromes were originally considered separate entities, various SCN5A-related arrhythmia syndromes are now known to display mixed phenotypes. This understanding could have an important impact on the management of cardiac ion channelopathies.

Fig. 5, panel A illustrates an ECG obtained from an infant with LQT3 that shows a nonsustained run of TdP. The first four beats of the recording are sinus rhythm and show the marked prolongation of the QT interval, the hall mark of the LQTS. Fig. 5, panel B is a diagrammatic illustration of the α subunit of the Na channel SCN5A and its β subunit, SCNB4.

Fig. 6, panels A–D illustrates the whole spectrum of the electrophysiological consequence of Na channel mutations causing LQT3. The panels represent data obtained from a surrogate model of LQT3 that faithfully reproduced the genetic counterpart. The model is created by the neurotoxin Anthopleurin-A (AP-A) (El-Sherif et al., 1988). Of interest, the experimental surrogate model of LQT3 anticipated the first description of the clinical LQT3 (Wang et al., 1995) by 7 years. Panel A illustrates the effect of ATXII on the behavior of single unitary Na channels. The control Na channel opens briefly, on average only once. In contrast, Na channels exposed to ATXII, a similar neurotoxin to AP-A, show long-lasting burst consisting of repetitive long openings interrupted by brief closures. The effects of ATXII on the Na channel (El-Sherif et al., 1992) are almost identical to the effects of the clinical mutations of the SCN5A a subunit on the function of the channel (Bennett et al., 1995). This abnormality of Na channel relaxation has the consequence of inducing consistent Na current during the plateau phase of the AP resulting in significant prolongation of the APD as shown in panel B. Panel B shows AP recordings from a Purkinje fiber and a mid-myocardial (M) fiber. Both fibers show marked prolongation of APD. The Purkinje fiber, in particular, shows the development of multiple oscillatory responses at the plateau level characteristic of EADs. Panel C shows the differential prolongation of APD of different myocardial fibers across the left ventricular wall. It illustrates AP recordings from an epicardial (EPI), mid-myocardial (M), and endocardial (END) myocardial fiber from a transmural slice from a canine left ventricular wall. Because of the different ionic constituents of different myocardial fibers, the drug results in differentially more marked prolongation of the M cell compared to EPI and END cells (Sicouri and Antzelevitch, 1995).

Panel D represents tridimensional activation maps of a 12-beat run of TdP from the in vivo canine surrogate AP-A model of LQT3 and helps to summarize the final electrophysiological mechanism of TdP in the LQTS. The first beat of a TdP is due to EAD-triggered beat from the subendocardial Purkinje network that acts on the transmural dispersion of myocardial repolarization to induce reentrant excitation in the form of circulating tridimensional wave fronts (the thick continuous line that traces the activation wave front from one beat to the next (El-Sherif et al., 1996). The twisting QRS pattern of the classic TdP is attributed in this example to transient bifurcation of a predominately single rotating wavefront into two separate simultaneous wave fronts rotating around the left and right ventricular cavities (El-Sherif et al., 1997).

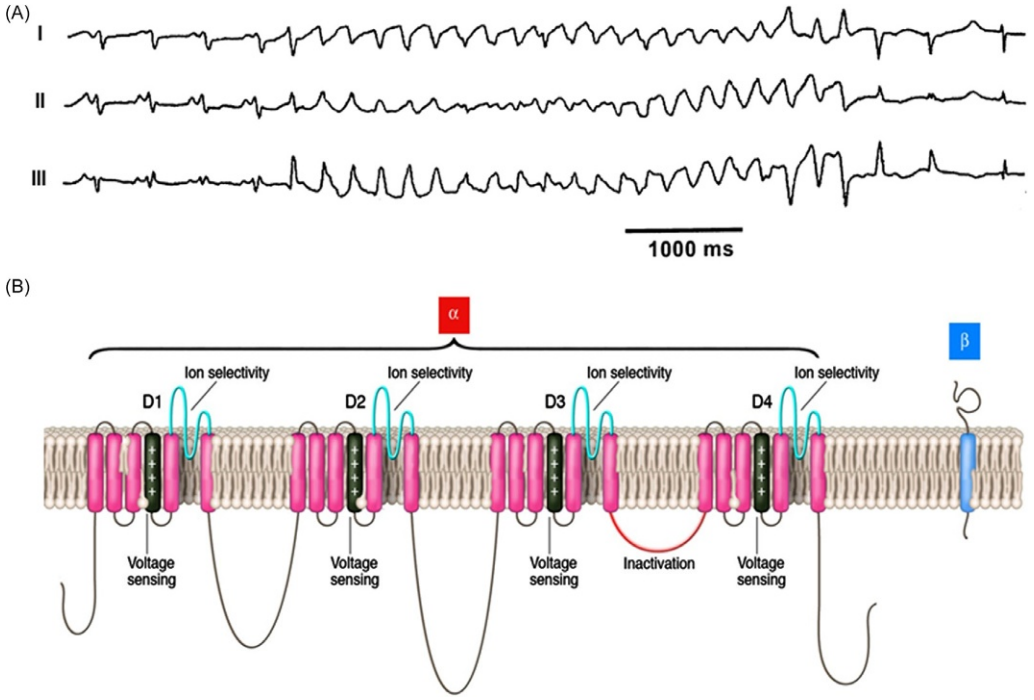

Fig. 5 (A) Electrocardiographic recording from an infant with LQT3. The first four beats are sinus showing prolonged QT interval followed by a nonsustained run of polymorphic ventricular tachycardia with the characteristic Torsade de Pointes pattern. (B) Diagrammatic presentation of the α subunit of the Na channel KCN5A whose mutation can result in LQT3, together with the β-subunit of the channel, SCN4B, whose mutation results in LQT10.

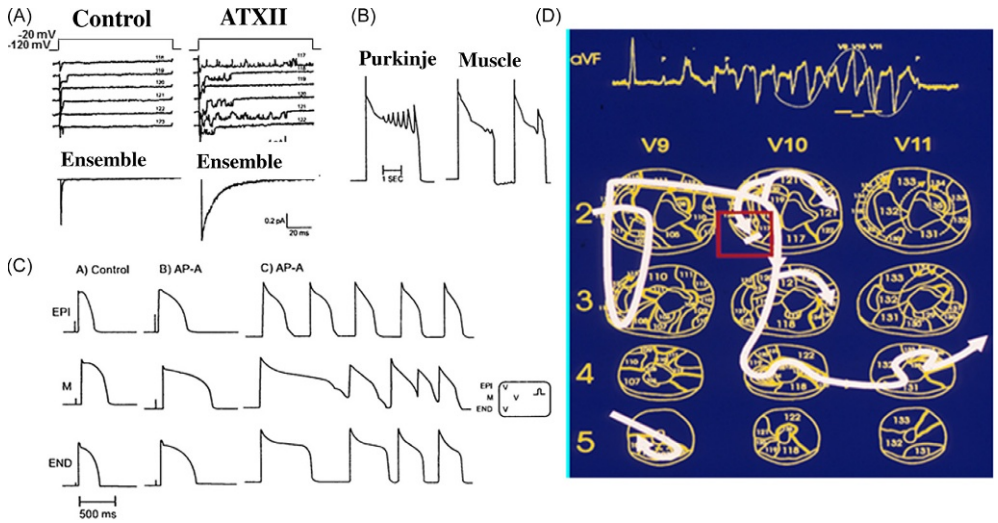

Fig. 6 Composite figure that illustrates the correlation between the specific molecular changes of the Na channel that result in LQT3, the electrophysiological consequence, and the final phenotype presentation of LQTS. (A) Cell-attached patch clamp recordings of the Na channel during control and following superfusion with ATXII. This neurotoxin faithfully reproduces the molecular changes associated with the clinical mutations of the Na channel in patients with LQT3. It illustrates sequential recordings of single Na channel current responses during depolarizing steps from −120 to −20 mV from rabbit cardiomyocytes. The left panel shows control recordings and the right panel shows recordings from a patch exposed to 100 nM of ATXII that resulted in long-lasting burst consisting of repetitive long opening interrupted by brief closures. The ensemble current from this patch shows markedly slowed relaxation. (B) Action potential recordings from a Purkinje fiber (PF) and a mid-myocardial (M) cell; both isolated from a 10-week-old puppy and placed in the same chamber perfused with 50 mg/L AP-A and stimulated at 3000 ms. The PF shows a series of early afterdepolarizations (EADs) that increased gradually in amplitude before final repolarization. (C) Simultaneous recordings from a subepicardial cell (EPI), M cell, and a subendocardial cell (END) from a transmural strip isolated from the left ventricle of a 12-week-old puppy and transfused with 50 mg/L AP-A and stimulated at 4000 ms. AP-A resulted in marked differential prolongation of the action potential of the M cell resulting in asynchronous activation in the preparation which is a substrate of reentrant excitation. (D) Illustration of cardiac tridimensional activation maps of a 12-beat run of TdP VT from the in vivo canine surrogate AP-A model of LQT3 and helps to summarize the final electrophysiological mechanism of the characteristic twisting QRS configuration of the TdP electrocardiogram in the LQTS.

Genotype of LQTS

Table 1 illustrates the heterogeneity of the genetic, molecular, and ionic basis of the congenital LQTS (Giudicessi and Ackerman, 2013). To date mutations of 16 different genes have been identified in patients clinically diagnosed with LQTS. Since approximately 20% of LQTS eludes genetic classification, this list is expected to grow. Loss of function of one of several potassium (K^+) channels, gain of function of the sodium (Na^+) and calcium (Ca^{2+}) channels are the three abnormalities that can result in prolongation of APD and LQTS. The three channels abnormal behavior can be traced to either gene mutations involving the channel protein itself or mutations of membrane structural proteins that can indirectly affect the function of the channel (El-Sherif and Boutjdir, 2015). LQT1, LQT2, and LQT3, account for an estimated 75%–85% of genotype-positive LQTS cases, while minor LQT syndromes account for less than 5%. LQT1 is due to loss of function of the KCNQ1 that encodes for I_{Ks} (Barhanin et al., 1996; Sanguinetti et al., 1996) LQT2 is due to loss of function of KCNH2 channel that encodes for I_{Kr} (Sanguinetti et al., 1995), while LQT3 is due to gain of function of SCN5A α subunit of the Na channel that encodes for I_{Na} (Wang et al., 1995).

Homozygous or compound heterozygous mutations of KCNQ1 or KCNE1 occurring in recessive form underlie the relatively rare Jervel and Lang-Nielsen syndrome (JLNS) (Splawski et al., 1997; Duggal et al., 1998).

Some LQTS types are associated with somatic and neurological changes in addition to prolongation of the LQT interval. Loss of function of the KCNJ2 gene which encodes the inward rectifier K channel Kir2.1 (I_{K1}) results in a syndrome termed Anderson–Tawil syndrome (ATS) or LQT7. In addition to a long QT interval, periodic paralysis and dysmorphic features are present (Andersen et al., 1971; Tawil et al., 1994) Similarly, gain of function of mutations in the CA CNA1C gene which encodes the L-Type Ca channel Ca_v

Table 1 Genetic basis of Long-QT syndrome and multisystem syndromes associated with QT prolongation

Gene *(genotype)*	*Locus*	*Protein*	*Functional effect*	*Mode of inheritance*	*Frequency*	*References*
Long QT syndrome (major)						
KCNQ1 (LQT1)	11p15.5	Kv7.1	Reduced I_{Ks}	AD; AR	30%–35%	Wang et al. (1995), Sanguinetti et al. (1996)
KCNH2 (LQT2)	7q35-46	Kv11.1	Reduced I_{Kr}	AD	25%–30%	El-Sherif et al. (1992)
SCN5A (LQT3)	3p21-p24	Nav1.5	Increased I_{Na}	AD	5%–10%	Bennett et al. (1995)
Long QT syndrome (inor)						
AKAP9 (AKAP9-LQTS)	7q21-q22	Yotiao	Reduced I_{Ks}	AD	<1%	Khositseth et al. (2004)
CACNA1C (CACNA1C-LQTS)	12p13.3	Cav1.2	Increased $I_{Ca,L}$	AD	<1%	Boczek et al. (2012)
CAV3 (CAV3-LQTS)	3p25	Caveolin 3	Increased I_{Na}	AD	<1%	Tomas et al. (2010)
KCNE1 (KCNE1-LQTS)	21q22.1	MinK	Reduced I_{Ks}	AD	<1%	Wilde et al. (1999)
KCNE2 (KCNE2-LQTS)	21q22.1	MiRP1	Reduced I_{Kr}	AD	<1%	Mizusawa et al. (2014)
KCNJ5 (KCNJ5-LQTS)	11q24	Kir3.4	Reduced $I_{K,ACh}$	AD	<1%	Goldenberg et al. (2006)
SCN4B (SCN4B-LQTS)	11q23.3	Nav1.5 β4-subunit	Increased I_{Na}	AD	<1%	Arking et al. (2006)
SNTA1 (SNTA-LQTS)	20q11.2	Syntrophin-α 1	Increased I_{Na}	AD	<1%	Schwartz et al. (2006)
Jervell and Lange-Nielson syndrome						
KCNQ1 (JLNS1)	11p15.5	Kv7.1	Reduced I_{Ks}	AR	Very rare	Barhanin et al. (1996)
KCNE1 (JLNS2)	21q22.1	MinK	Reduced I_{Ks}	AR	Very rare	Stramba-Badiale et al. (2000)
Ankyrin-B syndrome						
ANKB (ABS)	4q25-q27	Ankyrin-B	Aberrant ion channel/transporter localization	AD	<1%	Westenskow et al. (2004)
Andersen–Tawil syndrome						
KCNJ2 (ATS)	17q23	Kir2.1	Reduced I_{K1}	AD	<1%	Barsheshet et al. (2012)
Timothy syndrome						
CACNA1C (TS)	12p13.3	Cav1.2	Increased $I_{Ca,L}$	Sporadic	Very rare	Chinushi et al. (1998)
Recurrent infantile cardiac arrest syndrome						
CALM1	14q24-q31	Calmodulin 1	Dysfunctional Ca^{2+} signaling	Sporadic	<1%	Windle et al. (2001)
CALM2	2p21	Calmodulin 2	Dysfunctional Ca^{2+} signaling	Sporadic	<1%	Windle et al. (2001)

AD, autosomal dominant; *AR*, autosomal recessive; *ABS*, ankyrin-B syndrome; *ATS*, Anderson–Tawil syndrome; *LQTS*, Long QT syndrome; *TS*, Timothy syndrome.
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1.2 leads to immunodeficiency and Autism in addition to prolonged QT interval and is termed Timothy syndrome (TS) or LQT8 (Splawski et al., 2004; Splawski et al., 2005). JLNS results in severe congenital bilateral sensorineural hearing loss in addition to marked QT interval prolongation with very high risk of cardiac events (Splawski et al., 1997).

Examples of mutations of structural proteins that affect the function of ion channels are LQT4 due to mutations of ankyrin gene encoding the anchoring β subunit ANK2 that leads to intracellular Ca overload (Mohler et al., 2003). LQT9 due to mutations of CAV3, which encodes caveolin-3 that alters the biophysical properties of the Na channel (Vatta et al., 2006), and LQT11 due to mutations of an A-kinase-anchoring protein (AKAP9-Yotiao) that binds to KCNQ1, responsible for the most common LQT1 syndrome, and depresses the function of the channel by reducing the c-AMP-induced phosphorylation (Chen et al., 2007).

Genotype-Specific Triggers for Cardiac Events

Sympathetic activation caused by conditions such as physical exercise triggers the activation of IKs which results in shorter VR at fast heart rates, which prevents afterdepolarizations caused by β1-adrenergic stimulation. Patients with LQT1, who have diminished IKs, are prone to develop prolonged VR during exercise, whereas patients with LQT2 and LQT3 who have intact IKs are relatively protected against this phenomenon. This failure of the QT interval to adapt at faster heart rates results in a high risk for afterdepolarization which can culminate in TdP. In a study of 670 asymptomatic LQTS patients, Schwartz et al. demonstrated that the majority of LQT1 patient's lethal and nonlethal cardiac events were triggered by exercise or stress, especially swimming (Schwartz et al., 2001). LQT2 patients are exquisitely sensitive to sudden auditory stimuli which is thought to mimic a fright response resulting in an abrupt release of local catecholamines leading to EADs and resultant TdP (Schwartz et al., 2001; Wilde et al., 1999).

Women with LQT2 are also at higher risk for cardiac events in the postpartum period. This is thought to be multifactorial, which likely includes hormonal changes, sleep disruption, and the sudden auditory stimuli caused by the crying neonate (Khositseth et al., 2004). In contrast, LQT3 patients seem to be more prone for cardiac events during sleep; this is hypothesized to be secondary to amplification of the inward flow of Na+ at low heart rate when time spent at a negative potential is increased (Schwartz et al., 2001; Stramba-Badiale et al., 2000).

Genetic Modifiers of LQTS Risk

In the last several years it became obvious that LQTS shows incomplete penetrance and variable expressivity. In other words, different clinical phenotypes (sudden cardiac death (SCD), syncope, asymptomatic) are observed in family members carrying the same familial mutation, which may suggest the influence of multiple genetic and environmental factors affecting the pathophysiology of LQTS (Mizusawa et al., 2014). The discovery of common genetic variants that explain the heritable component of the human ECG, including the QT interval, generated the hypothesis that genetic modifiers may account for phenotypical variability in LQTS. In LQTS, variants in the coding regions of LQTS-related genes such as KCNE1 D85N (Nishio et al., 2009), KCNH2 K897T (Crotti et al., 2005), and SCNA5A H558R (Makielski et al., 2003) are known to influence the QT interval. Single-nucleotide polymorphisms (SNPs) in noncoding regions (intron, 3′UTR) of KCNQ1 are also reported as an important QT interval modifier (Duchatelet et al., 2013). Genome-wide association studies (GWAS) have revealed SNPs in nitric oxide synthase 1 adaptor protein gene (NOS1AP) as modulator of QT interval in LQTS patients (Tomas et al., 2010), as well as in the general population (Arking et al., 2006). However, despite the fact that multiple SNPs have been linked to QT interval duration, the clinical applications of any findings are limited by the small effect sizes conferred by SNPs and incomplete knowledge of their functional consequences. Nevertheless, there is continuing efforts to understand the complexity of genotype–phenotype relationship in LQTS.

Risk Stratification of Congenital LQTS

Both genotypic (e.g., LQTS genetic, intragenic, and mutation-specific subtype) and phenotypic (e.g., gender, QTc, and history of cardiac events) characteristics are used to guide the risk stratification, and ultimately the clinical management, of patients with congenital LQTS (**Fig. 7**) (Giudicessi and Ackerman, 2013). Those patients who harbor LQT1-causative mutations on >1 KCNQ1 allele (e.g., JLNS and AR-LQT1) (Schwartz et al., 2006; Goldenberg et al., 2006) have suffered ≥ 10 cardiac events before the age of 18 (Sauer et al., 2007) or have TS at highest risk ($\geq 80\%$) of suffering from one or more LQTS-associated cardiac events before the age of 40, including a high rate of sudden cardiac arrest/death. Those patients require aggressive clinical management, which often involves more invasive approaches such as left cardiac sympathetic denervation (LCSD), and/or use of the implantable cardioverter defibrillator (ICD).

Similarly, individuals with a QTc ≥ 550 ms regardless of LQTS genotype (Sauer et al., 2007), a QTc ≥ 500 ms with an LQT1, LQT2, or males with an LQT3 genotype (Priori et al., 2003), non-JLNS patients with LQTS-causative mutations on >1 major LQTS-susceptibility allele (e.g., compound heterozygosity or digenic heterozygosity) (Westenskow et al., 2004; Mullally et al., 2013), or individuals who have suffered ≥ 2 but <10 cardiac events before the age of 18 (Sauer et al., 2007) are at higher risk ($\geq 50\%$) for suffering an LQTS-associated cardiac event(s) before the age of 40 and often require a combination of medical, surgical, or device-related management.

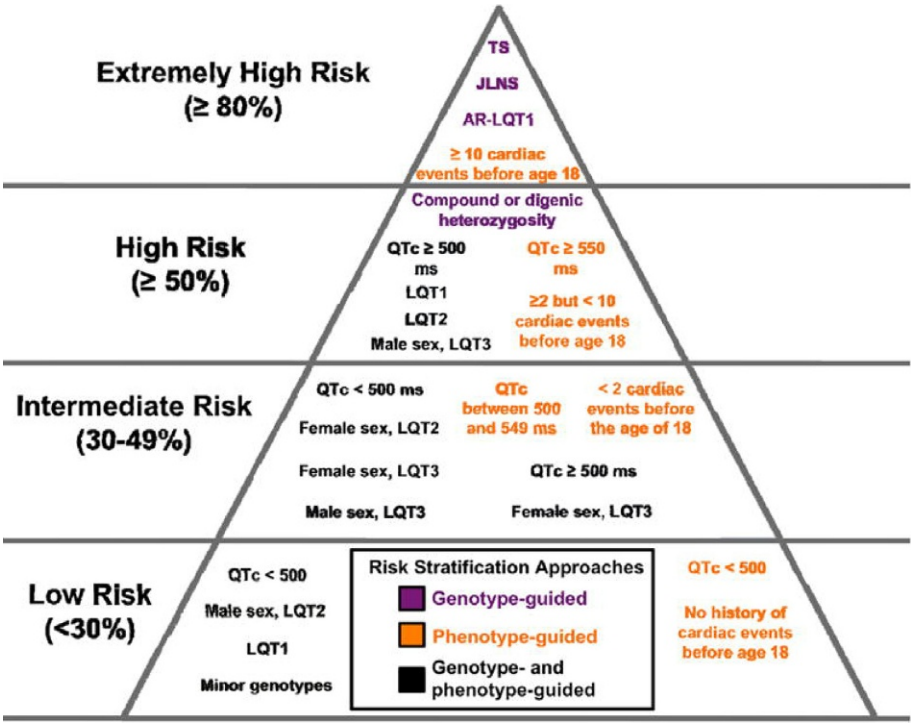

Fig. 7 Genotype- and phenotype-guided risk classification of long-QT syndrome patients: Risk groups have been defined based on the previously published probability of suffering a first or recurrent cardiac event (syncope, seizure, sudden cardiac arrest, or sudden cardiac death) before 40 years of age without appropriate therapeutic interventions. A probability of suffering a first cardiac event >80% defines the extremely high-risk group, >50% the high-risk group, between 30% and 49% as the intermediate-risk group, and below 30% as the lowest-risk group. Genotype-guided recommendations are indicated by purple text, phenotype-guided recommendations are indicated by orange text, and a combination of genotype- and phenotype-guided recommendations is indicated by black text within the figure. Reprinted with permission from Giudicessi JR, Ackerman MJ, Curr Probl cardiol, 2013;38(10):417–455.

Those individuals with a QTc between 500 and 549 ms regardless of genotype, females with major genotype-positive LQTS, male LQT3 patients with a QTc <500 ms, and any individual who has experienced <2 cardiac events before the age 18 are at intermediate risk for experiencing an LQTS-associated cardiac event(s) before the age of 40 and require some form of treatment, typically β-adrenergic blockers (Sauer et al., 2007; Priori et al., 2003).

Management of Congenital LQTS

Beta-blocker therapy is the first choice for LQTS. It dramatically decreases event rates from 0.97 to 0.31 events per patient per year (Moss et al., 2000). In addition to beta-blockers, there is a large number of adjuvant drugs that are tailored for a specific genetic abnormality (El-Sherif and Boutjdir, 2015). Patients who fail pharmacotherapy, patients with history of resuscitated cardiac arrest, and patients with J-LNS or TS are at particularly high risk and require other management modalities that is LCSD or the ICD. Besides medical treatment, life style modification is important, as well as meticulous avoidance of other drugs that can prolong the QT interval.

Detailed Pharmacotherapy in the LQTS

β-Blockers

β-Blockers are the main drugs of choice for LQTS irrespective of the genotype (Sauer et al., 2007; Moss et al., 2000). However, clinical data from the International Registry of LQTS found that β-blockers are more effective in preventing arrhythmias and SCD in LQT1 patients (81%) compared to LQT2 patients (59%) and LQT3 patients (50%) (Schwartz et al., 2001). This is due to the fact that arrhythmias in LQT1 patients are more frequently triggered by enhanced sympathetic stimulation. Despite β-blockers' efficacy in LQTS, a minority of patients remain symptomatic and in those patients additional therapeutic options like LCSD has been advocated (Schwartz et al., 2004). Alternatively, those patients may be candidates for the ICD. β-Blockers may be harmful in some patients with LQT3 if the heart rate is significantly slowed since bradycardia-dependent arrhythmias are more common in LQT3 patients.

Congenital LQTS in adults requires special risk stratification. A report from the International LQTS Registry has shown that the severity of LQTS in adulthood can be risk stratified with information regarding genotype, gender, QT duration, and history of cardiac events. β-Blockers effectively reduce but do not eliminate the risk of life-threatening cardiac events in congenital LQTS in adults (Sauer et al., 2007).

In a study including 382 LQT1 and LQT2 patients it was shown that not all β-blockers are equally effective (Chockalingam et al., 2012). In this regard, propranolol seems to be the most effective drug from this class, due to Na channel blocking action with limited effects on cardiac K channels. However, not only different properties on the various channels may influence the effectiveness of the β-blocker therapy, the precise location of a mutation in the underling gene and the specific type of resultant amino acid exchange has an impact on the effectiveness of the β-blocker therapy. For example, LQT1 patients with a missense mutation in a specific cytoplasmic loop region seem to benefit more from the β-blocker therapy than patients with other mutations (Barsheshet et al., 2012).

Pharmacotherapy in LQT2

Although LQT2 patients experience more life-threatening events under β-blocker therapy when compared with LQT1 patients, it still remains the first choice of therapy (Crotti et al., 2008). Analyzing 204 cardiac events of 634 genetically confirmed LQT2 patients from the US cohort of the International LQTS Registry, β-blocker therapy was associated with a pronounced reduction of exercise-triggered events but had a nonsignificant effect on the risk of arousal- and nonexercise/nonarousal events (Kim et al., 2010). Beside β-blockers, Na channel blockers such as mexiletine may also have a positive effect on the recurrence of cardiac events in LQT2 patients. In a small study of four LQT2 patients recurrent syncope and polymorphic VT occurred, although they received β-blocker therapy at full dose. An additional therapy with mexiletine resulted into a significant reduction in polymorphic VT and no syncope episodes occurred during follow-up (Ildarova et al., 2012).

Na Channel Blockers in LQT3 Patients

Since LQT3 syndrome is caused by gain of function due to delayed Na channel inactivation resulting in increased Na current during the plateau phase of AP, it comes to reason that Na channel blockers may be beneficial in LQT3 patients. Schwartz et al. demonstrated that a single oral dose of the Na channel blocker, mexiletine, administered to seven LQT3 patients with the AKPQ deletion produced significant shortening of the QTc interval within 4 h (Schwartz et al., 1995). Similar QTc shortening in LQT3 patients with the AKPQ deletion has been reported with lidocaine and tocainide (Rosero et al., 1997). Benhorin et al. reported QT shortening with flecainide in LQT3 patients with the D1790G mutation involving the SCN5A gene (Benhorin et al., 2000). Windle et al. showed normalization of the QTc interval with low doses of oral flecainide administered over 48 h in five patients with the AKPQ deletion (Windle et al., 2001). In vitro studies have shown that flecainide has high affinity for the Na channel protein and provides almost complete correction of the impaired inactivation associated with the AKPQ deletion (Nagatomo et al., 2000). Moss et al. have shown that chronic low-dose flecainide significantly shortens the QTc interval in LQT3 subjects with the AKPQ mutation with no major adverse drug effects, but the sample size was not large enough to evaluate the safety of flecainide therapy in patients with this mutation (Moss et al., 2005).

Several case reports describe that also phenytoin has antiarrhythmic effects in LQT3 patients (Vukmir and Stein, 1991). Phenytoin is thought to restore normal membrane responsiveness, to decrease basic sympathetic activity, to increase atrioventricular conduction velocity, and ventricular automaticity.

The Na channel inhibitor ranolazine was also shown to shorten the QT-interval effectively in five LQT3 patients (Moss et al., 2008). The underlying mechanism is the inhibition of late component of Na currents (predisposing indirectly to intracellular Ca overload) with only minimal effects on peak Na current during upstroke of the action potential.

Pharmacotherapy in LQT7 and LQT8

In patients with ATS (LQT7), β-blockers are often prescribed, although the International ATS Registry showed that in most cases the frequency of ventricular ectopy did not change (Tristani-Firouzi and Etheridge, 2010). A single case report indicated the efficacy of verapamil, by blocking voltage-dependent Ca channel, suppressing bidirectional ventricular tachycardia in a patient with ATS (Tristani-Firouzi and Etheridge, 2010). There is more convincing evidence of the efficacy of flecainide in ATS patients (Bokenkamp et al., 2007). The underlying antiarrhythmic action may result from flecainide's activation of I_{K1} as observed in ventricular myocytes (Caballero et al., 2010). However, in another case report flecainide was ineffective in a patient with genetically confirmed ATS, but after administration of amiodarone ventricular tachycardia was suppressed (Junker et al., 2002). In some cases the substitution of K and thereby increasing the serum K level to a high normal value may reduce the occurrence of ventricular arrhythmias and/or neurological features such as the episodic attacks of periodic paralyses (Sansone and Tawil, 2007).

Timothy syndrome (LQT8) is associated with a high mortality rate; therefore, if the diagnosis is confirmed, ICD implantation is the most important therapeutic approach. Although there are no specific data concerning antiarrhythmic therapy, β-blockers are used on the basis that they are generally effective in LQTS patients. However, mexiletine (Krause et al., 2011), verapamil (Jacobs et al., 2006), and ranolazine (Shah et al., 2012) have been proposed to shorten repolarization by directly or indirectly suppressing the L-type Ca channels and reduce the risk and burden of ventricular arrhythmias and ICD shocks.

K Supplement and K Channel Openers in LQTS

Currently 5 out of 15 LQTS are caused by mutations that result in reduction of net K current. Compton et al showed that administration of oral K to raise plasma concentration can reduce the QTc interval in patients with LQT2 (Compton et al., 1996). However, the long-term clinical efficacy of this approach is not clear. On the other hand, several experimental studies have shown the efficacy of K channel openers like nicorandil (Shimizu et al., 1998), and novel HERG current enhancers like RPR260243 (Kang et al., 2005) to shorten the QT interval in LQT1. The future role of this category of drugs in the clinical management of LQTS remains to be determined.

Drugs That Correct Trafficking Defects in LQTS

Transport of ion channel proteins through the secretory pathway involves a selective mechanism in which cargo molecules are concentrated into carrier vesicles. The importance of protein trafficking abnormalities is the recognition that this can be corrected. An early example was the observation that culturing cells expressing the key trafficking-deficient cystic fibrosis mutation AF 508 at reduced temperature could restore the cellular processing of CFTR protein and Cl transport (Lenhart et al., 2007). This has resulted in an intense search for chemical chaperones that can act as ligands to cause pharmacological correction of trafficking-deficient mutations. It is estimated that as little as a 10%–20% improvement in protein trafficking would ameliorate most of the clinical syndrome. Compared with a monogenetic defect in cystic fibrosis (1:2000) that occurs at a frequency similar to that in LQTS (1:2500), LQTS is a multigenetic syndrome, and probably because of the complexity of mechanisms and defects, industry interest in LQTS mechanism-specific drug development has been limited (Lenhart et al., 2007). However, recent studies have indicated that channel trafficking defects are common in LQT2 (Anderson et al., 2006), and therefore, pharmacological agents like thapsigargin (Delisle et al., 2003), and fexofenodine (Rajamani et al., 2002), or other trafficking rescue approaches are promising therapeutic rationales.

Left Cardiac Sympathetic Denervation

LCSD is a surgical procedure to denervate cardiac sympathetic innervation to the heart. It is indicated as an adjuvant therapy in patients who are refractory to b-blocker therapy. LCSD has been shown to significantly reduce cardiac events (Schwartz et al., 2004; Olde Nordkump et al., 2014). In patients with history of syncope, post-LCSD QTc <500 ms predicts efficacy of the procedure. However, those with persistent QTc prolongation (>500 ms) have a higher chance of SCD and are candidate for the ICD.

Implantable Cardioverter Defibrillator

The ICD is indicated in patients who have frequent syncopal episodes despite maximal pharmacotherapy and in those at high risk of recurrent cardiac arrest, for example, symptomatic infants <1 year of age or patients with JLNS.

Acquired LQTS

Acquired LQTS is by far more prevalent than congenital LQTS. The vast majority of acquired LQTS is the result of adverse effects of drugs that interact with the HERG gene encoding the pore-forming subunits (Kv11.1) of the rapidly activating delayed rectifier current, IKr (Kannankeril et al., 2010; Schwartz and Woosley, 2016). A recent report suggests that some drugs that block IKr and are known to cause TdP (dofetilide, E-4031, d-sotalol, thioridazine, and erythromycin) also increase the late sodium current, which may contribute to their proarrhythmic effect (Yang et al., 2014). Other causes of acquired LQTS include electrolyte abnormalities (hypokalemia, hypomagnesemia, and hypocalcemia), hypothyroidism, hypothermia, and marked bradycardia. Any of these factors can cause acquired LQTS or contribute to the risk of drug-induced LQTS.

In addition, there is recent evidence of the high prevalence of QTc interval prolongation in patients with anti-SSA/Ro antibody, autoimmune, and inflammatory diseases (Lazzerini et al., 2015; Boutjdir et al., 2016; Lazzerini et al., 2016). A recent report from this laboratory provided strong evidence for a pathogenic role of anti-SSA/Ro antibody in the development of QTc prolongation. Anti-Ro antibody from patients with autoimmune disease was shown to inhibit I_{Kr} by directly cross-reacting with the HERG channel likely at the pore region where homology between 52Ro antigen and HERG channel was demonstrated (Yue et al., 2015). In addition, an animal model of autoimmune-associated QTc prolongation was established, for the first time, whereby induction of anti-SSA/Ro antibodies by immunization resulted in QTc prolongation on the surface ECG (Yue et al., 2015).

Incidence of Drug-Induced LQTS

The overall incidence of drug-induced LQTS in a given population is difficult to estimate. One study estimated that between 5% and 7% of reports of VT, ventricular fibrillation, or SCD were in fact drug-induced LQTS and TdP (Molokhia et al., 2008). European

pharmacovigilance centers in Sweden, Germany, and Italy have found an annual reporting rate of drug-induced LQTS or TdP of approximately 0.8–1.2 per million person-years (Schwartz and Woosley, 2016; Molokhia et al., 2008). In an epidemiological study of drug-induced LQTS in Germany found the reporting rate for symptomatic acquired LQTS to be 2.5% per million person-years for men and 4.0% per million person-years for women, with 60% attributed to drugs (Sarganas et al., 2014).

QT prolongation is one of the most common reasons for drug withdrawal from the market despite the fact that these drugs may be beneficial for certain patients and not harmful in every patient (Niemeijer et al., 2015). Since 1989, 14 clinically important drugs have been removed from the market due to TdP (Ninan and Wertheimer, 2012), and development of an unknown number has been stopped due to concerns that these might pose a risk of causing QT prolongation and TdP (Schwartz and Woosley, 2016). In the 1990s, the US Food and Drug Administration (FDA) and the European Medicine Agency (EMA) began requiring routine preclinical and clinical testing to determine whether drugs have the potential to cause QT prolongation. Today, according to CredibleMeds website, which has become the standard reference for drug-induced TdP, marketed drugs are now recognized for their potential to cause TdP and another 72 to cause QT prolongation (Woosley and Romero, 2013).

Pharmacogenetics of Acquired LQTS

The susceptibility to acquired QT interval prolongation can be influenced by genetic variation (Mahida et al., 2013). This is supported by the fact that the heritability of QT interval duration in the general population (excluding congenital LQTS patients) is estimated to be around 35% (Hong et al., 2001; Newton-Cheh et al., 2005). Further, first-degree relatives of patients with congenital LQTS have a higher risk of drug-induced QT prolongation than nonrelated individuals (Kannankeril et al., 2005). In GWAS, a large number of genes associated with QT interval duration have been identified (Duchatelet et al., 2013; Pfeufer et al., 2009). The gene with the strongest signal related to QT interval duration is the NOS1AP, located on chromosome 1 (Pfeufer et al., 2009; Jamshidi et al., 2012), and inhibit L-type calcium channel and influence impulse propagation (Kapoor et al., 2014). Other finding from GWAS included polymorphisms within genes known to be mutated in congenital LQTS, genes associated with intracellular calcium handling, as well as genes previously not known to influence cardiac repolarization. In a recent study that compared 188 patients with drug-induced LQTS and more than 1000 patients with congenital LQTS found disease causing mutations in 28% of patients with drug-induced LQTS. Of interest, under basal conditions, the QTc of drug-induced patients (453 ± 39 ms) is significantly longer than that of control subjects (406 ± 26 ms).

It is important to consider pharmacogenetics of drug-induced LQTS as related to both *Pharmacokinetic* and *Pharmacodynamic* properties (Mahida et al., 2013). Pharmacokinetics constitute the effect of the body on the drug, which is usually categorized into effect on absorption, distribution, metabolism, and elimination of the drug. Pharmacokinetic genetic susceptibility is mainly characterized by variation in genes encoding drug-metabolizing cytochrome P450 or drug transporter like the P-glycoprotein. On the other hand, the pharmacodynamics component of genetic susceptibility is mainly characterized by genes known to be associated with QT prolongation in the general population and genes in which the causal mutations of congenital LQTS are located.

Ethnicity and Gender Differences in Drug-Induced LQTS

As ethnic differences ultimately reflect genetic variation, it is useful to study ethnicity with regard to susceptibility to drug-induced QT interval prolongation and TdP. However, the role of ethnic differences has not been well established in published studies on drug-induced QT interval prolongation. In 20 QT/QTc studies, only 10% of the total study population was African American and only 7% was Asian (Florian et al., 2011). Nevertheless, the frequency of polymorphisms in genes known from congenital LQTS had varying distribution among ethnic groups (Ackerman et al., 2004). African Americans had the highest risk of prolonged QT interval after acute overdose of QT-prolonging drugs while Hispanics had the lowest risk compared to all other ethnic groups (Manini et al., 2014).

Adult females have a longer QT interval, greater drug and bradycardia-induced QT prolongation, and approximately twofold greater incidence of cardiac arrhythmias (Drici and Clement, 2001). Several studies have shown female gender as a risk factor for TdP with drugs that suppress I_{Kr} (Makkar et al., 1993; Burke et al., 1997; Coker, 2009; Liu et al., 1998). These differences have been attributed most likely due to regulation of cardiac ion channels expression by sex steroids. For example, sex differences in QTc interval are not present at birth, but postpubertal women have longer baseline QTc interval than men (Goldberg et al., 1991). This change may be due to shortening of the male QT interval (Rautaharju et al., 1992). Further, the QTc interval of women is prolonged during pregnancy (Lechmanova et al., 2002). In fact, the definition of the upper limit of normal QTc interval is sex specific (440 ms in adult males and 460 ms in adult females).

The concept of reduced repolarization reserve in females compared to males has been used to explain sex differences in arrhythmia risk in LQTS (Roden, 2004). The reduced repolarization reserve of the female heart is attributed to lower repolarizing $K+$ currents. This difference was thought to be primarily due to testosterone-mediated increase in I_{Kr} and I_{K1} resulting in shorter APD and QTc interval in male hearts. However, recent experimental data that include, among others, estrogen upregulation of Ica-L by a classical genomic mechanism (Yang et al., 2012), may challenge the concept of repolarization reserve. Instead, Ca^{2+} homeostasis (Slama and Bett, 2014) and voltage-Ca^{2+} uncoupling (El-Sherif et al., 2017) may provide more attractive electrophysiological mechanisms for the increased vulnerability of female to drug-induced LQTS.

Future Directions and Concluding Remarks

There is increased recognition that a sizable minority of patients who are susceptible to VT and SCD, and who are usually young and have structurally normal heart have genetically inherited abnormality of the cardiac ion channels. This recognition has heightened interest in the diagnosis and management of this group of patients. Of all malignant channelopathies, the LQTs is the most widely investigated. Although some of the patients may receive the ICD, pharmacotherapy remains the first line of management for majority of patients. Looking ahead into the future, the role played by modifier genes in determining the phenotype along with studies on induced pluripotent stem cells (Savla et al., 2014) for treatment strategies will continue to enhance our understanding and help optimal management of those patients.

Although congenital LQTS continues to remain the domain of cardiologists, cardiac electrophysiologists, and specialized centers, the by far more frequent acquired drug-induced LQTS is the domain of all physicians and other members of the health care team who are required to make therapeutic decisions. To support better prescribing of medicines, clinical decision support systems to date have issued alerts that warn of potential harm from a prescribing decision (Schwartz and Woosley, 2016; Payne et al., 2015). However, the impact of these systems has been limited. Moving away from the use of alerts to signal prescribing errors, the concept of "medical autopilots" has been suggested as a preferred approach (Woosley et al., 2016). These programs will monitor the electronic medical record and send signals to guide prescribers toward decisions that result in maximum benefit and minimal risk of TdP.

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Management and Care of Older Cardiac Patients

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Abbreviations

ACCF	American College of Cardiology Foundation
ACE	Angiotensin-converting enzyme
ACS	Acute coronary syndrome
AHA	American Heart Association
ARB	Angiotensin II receptor blockers
BP	Blood pressure
CAD	Coronary artery disease
CVD	Cardiovascular disease
CHD	Coronary heart disease
CRT	Chronic resynchonization therapy

DOAC	Direct oral anticoagulation
ECG	Electrocardiogram
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HF	Heart failure
IHD	Ischemic heart disease
ISH	Isolated systolic hypertension
MRA	Mineralocorticoid receptor antagonists
NSTE-ACS	Non-ST elevation acute coronary syndrome
NYHA	New York Heart Association
PAD	Peripheral arterial disease
PCI	Percutaneous coronary intervention
SAVR	Surgical aortic valve replacement
SBP	Systolic Blood Pressure
STEMI	ST-segment elevation myocardial infarction
TAVR	Transcatheter aortic valve replacement
TIA	Transient ischemic attack

Background

Despite major therapeutic advances, cardiovascular disease (CVD) remains the leading cause of morbidity and mortality in the United States and throughout the world. Older adults are particularly susceptible to CVD; prevalence extends to more than two-thirds of adults >60 years of age and to more than 85% of those aged ≥85 years (Writing Group Members et al., 2016). Of the 23.6 million adults that the American Heart Association predicts will die from CVD by 2030 (Writing Group Members et al., 2016), advanced age is leading mortality risk (Sahyoun et al., 2001). Moreover, risks of CVD-related hospitalizations, poor quality of life, functional decline, and other detrimental sequelae are also disproportionate in older adults. While numerous trials have focused on treatment and prevention of CVD in younger adults, care for elderly that best achieves survival as well as improved quality of life and function remains relatively uncertain (Forman et al., 2011). Despite a tradition of evidence-based CVD management, therapeutic choices for older candidates are commonly confounded by the presence of multimorbidity (i.e., concomitant cardiac and noncardiac comorbidities), as well as interrelated issues of polypharmacy, cognitive impairment, frailty, falls, functional decline, and other age-related complexities of care. In this chapter, we highlight the physiologic changes of aging and the intertwined issues of aging that both exacerbate CVD risks and transmute management considerations. Some of the many research opportunities to address these gaps will also be discussed.

Physiology of Cardiovascular Aging

While a comprehensive account of cardiovascular aging is beyond the scope of this chapter, **Table 1** provides an overview of characteristic age-related physiologic changes that fundamentally predispose to CVD. With rising age, distinctive changes within the vascular structure, myocardium, valvular apparatuses, and conduction system are common and have important implications on development of CVD (Lakatta and Levy, 2003a; Lakatta and Levy, 2003b; Lakatta, 2003). Aging itself is the foremost risk factor for CVD pathophysiology, and adults who survive into old age are fundamentally more vulnerable to CVD.

In the vascular system, deposition of calcium and collagen and loss of elastin fibers within the media of larger vessels contribute to increased stiffness, hypertrophy of vessel walls, and decreased arterial compliance in older adults (Fleg and Strait, 2012). In addition, the reduction of nitric oxide production and increases in angiotensin II have been associated with endothelial dysfunction, resulting in decreased coronary blood flow and reduction of flow-mediated vasodilation in exercising muscles. As a result of such structural and functional changes, arterial systolic pressure tends to increase (Lakatta, 1999). Even in older adults without clinical hypertension, systolic blood pressures may increase by as much as 20 mmHg between the ages of 60 and 80 (Lakatta and Levy, 2003a). Smaller arteries are also affected by increased lipid deposition, resulting in a more heterogeneous intimal layer which disrupts laminar flow, thereby increasing perfusion pressure (Pepe and Lakatta, 2005).

In the myocardium, an enhanced rate of cell degeneration due to oxidizing radicals and proliferation of interstitial fibrosis contribute to a significant loss of myocytes and increased myocardial stiffness (Olivetti et al., 1991). Although there is a reduction in the overall number of myocytes, the remaining myocytes are increased in their individual size (myocyte hypertrophy), which commonly results in mild thickening of the myocardium (Fleg and Strait, 2012; Hees et al., 2002). Deposition of amyloid, collagen content, lipofuscin, and fat in the myocardium also increases stiffening of ventricles in older adults (Folkow and Svanborg, 1993). These changes result in higher diastolic pressures in the left ventricle, at rest and particularly during exercise (Lakatta and Levy, 2003b; Folkow and Svanborg, 1993; Lakatta, 1994). Patterns of elevated diastolic pressures exacerbate susceptibility to heart failure (HF) with preserved ejection fraction (HFpEF), left atrial enlargement, as well as the development of atrial fibrillation.

Table 1 Age-associated physiologic changes in the cardiovascular system

System	*Physiologic changes*	*Clinical implications*
Vascular system	*Arterial wall*	● ↑↑ isolated systolic hypertension ● ↑ diastolic pressure until 6th decade ● ↓ diastolic pressure after 6th decade ● ↑ pulse pressure[b] ● ↑ cardiac workload ● ↓ renal function ● ↑ vascular encephalopathy ● ↑ in aorto-femoral pulse wave velocity ● ↑ atherosclerotic disease
	● ↑↑ arterial wall media thickness ● ↑↑↑ intimal medial thickness ● ↑ fraying of elastic fibers ● ↑↑ collagen content ● ↓↓↓ arterial dispensability ● ↑↑↑ arterial stiffness[a] ● *Endothelial function* ● ↓ vasodilation ● ↓ nitric oxide production ● ↑ angiotensin II signalling ● *Oxidative stress and inflammation* ● ↑ NADPH oxidase ● ↑ uncoupled nitric oxide synthase ● ↑ xanthine oxidase ● ↓ in anti-oxidant capacity	
Cardiac structure and function	*LV composition and mass*	Left ventricle
	● ↓↓ numbers of myocytes ● ↑↑ myocyte hypertrophy ● ↑ deposition of collagen, fibrous tissue, amyloid, and lipofuscin within connective tissue	● ↑ LV stiffness and fibrosis ● ↑↑ LV dysfunction and heart failure ● ↑↑ LV systolic and diastolic volumes ● ↑↑↑ in LV mass to volume ratio ● ↑↑ susceptibility to HFpEF [c] ● ↑↑ susceptibility to myocardial ischemia
	LV wall thickness, cavity size, and shape	*Left atrium*
	● ↑↑ myocardial thickness ● ↑↑ concentric LVH ● ↑ interventricular wall thickness ● ↑ spherical LV shape	● ↑ atrial size ● ↑ susceptibility to atrial fibrillation
	Left heart valves	*Left heart valves*
	● ↑ calcium deposition & collagen infiltration ● myxomatous degeneration ● fixation of valvular leaflets	● ↑aortic sclerosis and stenosis ● ↑ mitral annular calcification ● ↑ mitral and aortic regurgitation
	LV function	
	● ↓ early diastolic peak filling ● ↑ LV filling facilitated by atrial contraction ● ↑ late LV filling	
Cardiovascular physiology	*Response to exercise*	● ↑ pulmonary hypertension ● ↓ myocardial contractility ● ↓↓↓ functional independence ● ↓↓↓ quality of life ● ↓↓ muscle function
	● ↓ in VO2 max per kg weight at peak exercise ● inability to increase LVEDP during exercise ● ↑↑ sarcopenia ● ↓↓ body weight ● ↑↑ muscle fiber atrophy (fast twitch) ● ↑ intramuscular fat	

LV, left ventricle; *LVH*, left ventricular hypertrophy; *VO2*, oxygen consumptions; *LVEDP*, left ventricular end diastolic pressure; *HFpEF*, heart failure with preserved ejection fraction.
[a]Mainly large and medium sized arteries.
[b]Pulse pressure is defined as the difference between systolic and diastolic blood pressure, which is a potent predictor of cardiovascular events.
[c]Exacerbated by stress-induced tachycardia.

In the valvular apparatus, calcium deposition, collagen infiltration, and myxomatous degeneration are common, especially in the left heart (Cheitlin, 2003a). Though calcification alone rarely affects the function of the mitral or aortic valves, fibrosis can lead to hemodynamically significant stenosis (Cheitlin, 2003b). Fixation of valvular leaflets can also cause substantial valvular regurgitation (Hu et al., 2016; American College of Cardiology et al., 2006).

The conduction system can also be affected by collagen and elastin deposition, which can cause slower propagation of electric impulses and abnormalities in intraventricular conduction (Fleg and Strait, 2012; Folkow and Svanborg, 1993; Kistler et al., 2004). Degeneration of sinus node and pacemaker cells exacerbates risks of sick sinus syndrome as well as supraventricular and ventricular arrhythmia (Fleg and Strait, 2012).

Aging is also associated with important changes in organs outside the cardiovascular system including kidney function, pulmonary reserve, and hemostasis. These changes may also accelerate the development of CVD because of their many interactions with cardiovascular function (Senni et al., 2014; Shah et al., 2016).

Research Opportunities

Aging entails physiological steps that predispose to CVD. Research that distinguishes mechanisms of aging may also provide opportunities to modify these determinant pathways (North and Sinclair, 2012). The burgeoning field of Geroscience focuses on key cellular and subcellular pathways of aging that are potentially modifiable (Kennedy et al., 2014).

Geriatric Domains That Impact CVD Management

Many of the same physiologic processes that predispose to CVD in old age (e.g., increased inflammation, oxidative stress, and related changes in gene expression and mitochondrial function) also predispose to geriatric syndromes (**Table 2**) (Chaudhry et al., 2013). Whereas CVD pathophysiology is generally considered the primary determinant of cardiovascular instability in younger CVD patients, geriatric syndromes become progressively more likely to play a codominant role among older adults. Geriatric syndromes essentially entwine with CVD physiology and presentation, such that diagnosis, prognosis, and management are typically transformed.

Multimorbidity implies a situation in which two or more chronic conditions are active. Multimorbidity increases with age and is prevalent in more than 70% of adults age $\geq$75 years (Dunlay and Chamberlain, 2016). Whereas "evidence-based" CVD guidelines typically rely on investigations that selected study populations with few if any comorbidities, such evidence becomes less reliable when then applied to typical patients who usually have many concomitant diseases (Boyd et al., 2005). Thus, such "evidence-based" regimens become more likely to provoke interactions and/or management complexities that have not been studied.

Polypharmacy is common among older adults with multimorbidity as clinicians prescribe a group of evidence-based medications oriented to each disease, and which often result in a risky accumulation. Common scenarios include combined regimens for multiple pathologies with combinations that jeopardize hemodynamics and stability (e.g., medications for hypertension, heart failure [HF], atrial fibrillation in a context also including frailty, falls, and dehydration). Adverse drug reactions, poor adherence, and exorbitant costs are all more likely to occur among older CVD patients.

Frailty is also common among older adults, particularly among elderly with CVD (Veronese et al., 2017). Despite methodological and conceptual debates regarding the optimal method to assess frailty, there is general agreement in defining frailty as a state of increased vulnerability to stressors, with limited reserves to stabilize declines across multiple physiologic systems (Clegg et al., 2013). Frail adults with CVD typically suffer with worse disease outcomes, as well as increased susceptibility to harmful effects from standard therapies.

Disability refers to a physical or mental condition that limits a person's movements, senses, or activities. Whereas younger adults with CVD are usually able to fully recover after a successful CVD hospitalization or therapy, full recuperation is less certain among older adults with similar disease. Multimorbidity, frailty, polypharmacy, and other geriatric syndromes compound risks of disability in hospitalized elderly, especially in the context of acute illness, deconditioning, cognitive impairment, poor sleep quality, poor nutrition, dehydration, oversedation, and other common geriatrics patterns.

Older patients with CVD have high susceptibility to cognitive impairment and related susceptibility to delirium (Silbert et al., 2007; Dodson et al., 2013). Delirium is a state of an acute disturbance in awareness and attention that commonly arises amidst hospitalizations and postacute care. Delirium predisposes to higher mortality rates, frailty, and disability (McPherson et al., 2013). Efforts should be made to integrate protocols to detect delirium and moderating risks, including efforts to minimize medications (e.g., benzodiazepines) and circumstances (e.g., pain, poor sleep quality) that may precipitate the condition (Falsini et al., 2017; Salluh et al., 2015).

Research Opportunities

While CVD has been extensively studied in terms of disease mechanisms, the impact of nondisease aspects of care becomes increasingly relevant in old age. Geriatric syndromes arise from the same underlying molecular and biological substrate as CVD and inherently confound many precepts of disease-based care. Treating CVD amidst multimorbidity, frailty, polypharmacy, and other geriatric dimensions is now typically left to physician judgment, but research holds the potential to better clarify and respond to these challenges with more information regarding complex interdynamics and implications regarding qualitative (e.g., quality of life, patient reported outcomes) and quantitative (e.g., mortality, function, cognition, lengths of stay, rehospitalization) endpoints.

Ischemic Heart Disease

Atherosclerosis tends to be more severe and diffuse in older adults and is the most common etiology of ischemic heart disease (IHD). Other predisposing factors include microvascular dysfunction, endothelial dysfunction, vascular spasm, or microembolism

Table 2 Overview of geriatric syndromes

Geriatric syndrome	*Definition and prevalence*	*Prognosis and clinical implication*	*Management*
Multiple chronic conditions	• Presence of two or more concomitant chronic disorders • Prevalence: ~2/3 of older adults • Classification: Cardiovascular and non-cardiovascular conditions[a]	• Influence CVD management • ↓↓ QOL • Loss of independence • ↑↑ mortality • ↑↑ disability • ↑ polypharmacy • ↑ falls • ↑ therapeutic burdens	• Geriatric Assessment • ↑ importance of patient-centered (not disease-centered) approach in disease management • ↑ importance of shared decision making between patient and providers • ↑ importance of inter-specialty collaboration and communication • ↑ importance of quality of life and functional outcomes
Polypharmacy	• Use of ≥5 medications • ∝ to number of comorbidities • ~40% of older adults take ≥5 medications	• ↑ Iatrogenesis and medication errors • ↑ Nursing home admissions • Exacerbation of other medical condition (~20%)	• Review drug-drug interaction and indication of therapy • Review of drug-disease interaction • Simplify frequency of medication to maximize adherence. • Incorporate patient goals and preferences in treatment decisions • Gradual deprescribing of non-essential medications to maximize compliance • Adoption of checklist to identify errors and interactions
Frailty	• A state of reduced physiologic reserve in multiorgan system • ↑↑ susceptibility to stressful event • Tends to increase with CVD Prevalence can reach ≥50% of older adults with CVD	• Worsens CVD prognosis and confounds management • ↓↓ QOL • Loss of independence • ↑↑ mortality • ↑↑ disability • ↑ falls • ↑↑ heart failure • ↑↑↑ hospitalization	• Integration of frailty assessment with CVD management in older adults • Treatment of underlying disorders • Physical activity can help to moderate frailty • Nutritional supplementation can help to moderate frailty • Comprehensive geriatric assessment is useful
Cognitive impairment and delirium	• Mild cognitive impairment→↓ cognitive function without loss of function; 10%–15% per year develop dementia • Dementia→Severe memory loss that interferes with daily life and loss of functional independence • Dementia: 8.8%–11.6% in adults ≥ 65 years • Delirium → disturbance in cognition, attention, and consciousness or perception with fluctuating course • Delirium: as high as 14% in older adults living in community; 17%–61% after major surgery; up to 83% at end of life.	• Loss of independence • Difficulty performing complex executive activities • ↓↓ QOL • ↓↓ medication compliance • ↑↑ frailty • ↑↑ hospitalization • ↑↑ mortality	• Weighing risks of invasive procedure in older adults with neurocognitive impairment • Integration of patient and family wishes in CVD care & routine assessment of life expectancy • Routine integration of delirium assessment in cardiovascular care for older adults • Avoidance of precipitating factors (psychoactive medications; benzodiazepines; urinary catheters. . .etc) • ↑ Utility of palliative care service to address goals of care

CVD, cardiovascular disease; *QOL*, quality of life; *no*, number; *CVD*, cardiovascular disease.

[a]Cardiovascular comorbidities include: hypertension, dyslipidemia, ischemic heart disease, heart failure, stroke, valvular heart disease, heart rhythm disorders; Noncardiovascular comorbidities include: diabetes, osteoarthritis, obstructive lung disease, anemia, chronic kidney disease, dementia, depression, and geriatric syndromes.

(Marzilli et al., 2012). Age-related susceptibility to disease relates to increased media and intimal thickness of coronary arteries, endothelial dysfunction, and heightened inflammation and oxidative stress in the arterial wall (**Table 1**). Although IHD can result in classic angina pectoris, in older adults less specific complaints of exercise tolerance and dyspnea are more likely.

Prevalence of IHD among older men and women is rising (Dai et al., 2016a). Age-related pathophysiological susceptibility to IHD pathophysiology is compounded by increased survival from acute ischemic events, as well as improved diagnostic testing (e.g., imaging and biomarker testing) to diagnose IHD. The rising prevalence of IHD in older adults poses significant challenges in clinical decision-making and frequent application of pharmacologic and invasive therapies that have not been well studied in very old adults with multimorbidity, frailty, and other complexities of care.

Given the complexity of care, the 2012 ACCF/AHA/ACC/ACP/AATS/PCNA/SCAI/STS Guidelines for the Diagnosis and Management of Patients with Stable IHD included a section on older adults (Fihn et al., 2012). Although the management of stable IHD is generally similar to that of younger patients, the guideline recommendations acknowledged the complexity of care for older

adults >75 years of age. The presence of concomitant coexisting medical conditions that can result in chest pain including pulmonary, gastrointestinal, and musculoskeletal systems can render the diagnosis of IHD more difficult. Furthermore, older adults are more likely to present with atypical symptoms such as exertional dyspnea, lack of energy, nausea and vomiting, diaphoresis, syncope, and altered mental status rather than typical chest pain (Alexander et al., 2007a). Finally, given the higher prevalence of left-ventricular diastolic dysfunction in older adults, acute HF is a common consequence of acute myocardial ischemia (Lakatta, 2002).

Given the presence of muscle loss, deconditioning, neuropathy, lung disease, and degenerative joint disease, exercise ECG protocols with sufficient intensity to diagnose IHD are often difficult to perform. Moreover, high prevalence of baseline ECG abnormalities, left ventricular hypertrophy, and arrhythmias often limit the value of standard ECG testing (Fihn et al., 2012). For older patients who are unable to exercise, pharmacologic stress imaging is recommended. Older adults referred for coronary angiography often have more extensive atherosclerosis than younger patients, including multivessel disease or left main artery involvement (Alexander et al., 2007a). In general, guideline-directed medical therapy is recommended as an initial approach. Invasive therapies to improve symptoms are considered useful, but only with consideration of each patient's personal preferences, functional capacity, quality of life, and end-of-life preferences (Fihn et al., 2012).

Geriatric Complexities

Challenges in the management of older adults with IHD are numerous. Prior studies revealed that older adults who exhibited frailty were less likely to receive the same extent of therapy as compared to adults without frailty (Lupon et al., 2008). For example, the use of guideline-directed medical therapy with ACE inhibitors and beta-blockers was lower when frailty was encountered, and these patients were less likely to be admitted to coronary care units and to undergo cardiac catheterization or coronary artery bypass surgery (Afilalo et al., 2014; Graham et al., 2013). Furthermore, adults ≥80 years usually have higher prevalence diabetes mellitus, HF, atrial fibrillation, renal failure, anemia, chronic kidney disease, and dementia (Alexander et al., 2007a; Mogensen et al., 2011). As such, treatment with invasive procedures that requires prolonged dual antiplatelet therapy with or without the need for antithrombotic therapy for cerebrovascular vascular accident prevention may not produce the same results in comparison to younger counterparts with a single disease process. The risk of contrast-induced nephropathy after coronary angiography is higher in older adults, which often reinforces rationale for conservative approaches. Generally, pharmacotherapy is challenging in older adults as reduced renal function results in different bioavailability and drug elimination profiles. Drug–drug and drug–disease interactions are common specially in setting of polypharmacy (Fihn et al., 2012). Intolerance to beta-blockers because of underlying fatigue may exert a significant challenge to achieve a reduced work load of the heart. Many older adults are intolerant to statin therapy because of muscle aches (see section on dyslipidemia). Impaired mobility may mask underlying exertional symptoms. Similarly, impaired cognition may diminish adherence to healthful to medications and diet (Arnett et al., 2014).

Research Opportunities

The impact of IHD and management (pharmacological vs invasive revascularization procedures) on symptoms, quality of life, and functional independence must be better elucidated in respect to age-related contextual factors (Rich et al., 2016). Steps to standardize these approaches are needed especially as iatrogenic risks also increase with age. Furthermore, research is needed to clarify therapies that are better tailored to each older patient's functional capacities (physical and cognitive), comorbidities, personal goals, and medication profiles. Studies would also be useful to better clarify the role of noninvasive cardiac imaging to detect IHD associated with atypical symptoms such that under- and overdiagnosis are minimized. Likewise, the optimal duration of antiplatelet therapy or other adjunctive therapies after percutaneous revascularization in older adults merits further analysis to better balance the tradeoffs between therapeutic benefits versus bleeding and other medication-related risks.

Acute Coronary Syndrome

Advanced age is the strongest risk factor for poor outcome following acute coronary syndromes (ACS) (Dai et al., 2016b; Goldberg et al., 1989). The pathophysiology of ACS entails plaque rupture or cap erosion, triggering a thrombotic cascade and supply/demand mismatch (Damluji et al., 2015a). The spectrum of ACS depends on the degree of obstructive disease. Unstable angina and non-ST elevation ACS (NSTE-ACS) result from subtotal occlusion of coronary flow along with associated vasospasm. ST segment elevation myocardial infarction (STEMI) refers to the total occlusion of the coronary artery (Damluji et al., 2015a). While some older adults with ACS present with typical angina symptoms of chest pain, atypical symptoms become more likely with age, including shortness of breath, nausea, vomiting, presyncope or syncope, weakness, or changes in cognitive function. ACS in older adults is also often induced by coexisting conditions (e.g., urinary tract infections, sepsis, chronic lung disease, pneumonia, or anemia) that provoke type II myocardial infarctions.

Although the standards of ACS care do not fundamentally vary between older and younger adults, revascularization complications and medication iatrogenic effects are more likely and often impact care therapeutic choices (Goldberg et al., 1989). Older patients are also more prone to development of mechanical complications, especially after STEMI when immediate revascularization is not available or withheld resulting in higher mortality rates (e.g., free wall rupture, papillary muscle rupture, ventricular septal defects, left ventricular aneurysm, or pseudoaneurysm).

The American Heart Association Council on Clinical Cardiology produced a scientific statement specifically outlining recommendations for older adults with ACS (Alexander et al., 2007a; Alexander et al., 2007b). For patients with STEMI, prompt treatment with reperfusion therapy (e.g., percutaneous coronary intervention or fibrinolysis) is recommended when possible. Delays in reperfusion therapies are common in older patients because of atypical symptoms, abnormal baseline ECG, associated cardiogenic shock, or the presence of multiple chronic conditions that complicate management. Reduced cognition and constricted medical access are also pertinent. Older patients with STEMI who receive percutaneous coronary intervention (PCI) as compared to fibrinolysis have been demonstrated to benefit with reduced rates of reinfarction and target-vessel revascularization (Alexander et al., 2007b). Early invasive strategy (within the first 48 h of presentation) is also recommended for older adults with NSTE-ACS or unstable angina, particularly if high risk features are present (i.e., angina at rest, ischemia at low activity levels despite treatment with two anginal medications, elevated cardiac biomarkers, dynamic ECG changes, congestive HF or depressed ejection fraction (<40%), history of surgical or percutaneous revascularization) (Alexander et al., 2007a). Utilization of techniques to decrease the risk of bleeding in older adults (e.g., transradial catheterization, use of ultrasound/fluoroscopic guided femoral access, bivalirudin infusion) are important considerations when considering invasive percutaneous therapies for older patients. Given their high risk profiles, older adults who are candidates for surgical revascularization particularly benefit from a "Heart Team" approach wherein care is coordinated by experts with synergistic insights and skills (Holmes et al., 2013).

Post ACS medical therapy is inherently complicated by age. Dual antiplatelet therapies are critical post PCI/stenting but with higher risks of bleeding. Beta blockers reduce risks of reinfarction and are especially useful in patients with systolic dysfunction, but with greater risks of chronotropic incompetence, heart block, and fatigue. In patients with systolic dysfunction or diabetes mellitus, the concomitant use of ACE inhibitors and ARB pharmacotherapy improve survival but with increased risks of renal insufficiency (especially in those with renal artery stenosis). Statins for secondary cardiovascular prevention result in improved cardiovascular outcomes even for very older patients >80 years of age (Dai et al., 2016b; Damluji et al., 2015b), but with increased risks of myalgias.

Geriatric Complexities

Multimorbidity and other geriatric syndromes contribute to atypical ACS symptoms that often result in delayed care. Furthermore, management of ACS in the elderly is often impeded by coexisting cardiovascular (e.g., HF, arrhythmia, PAD) and noncardiac (e.g., chronic obstructive pulmonary disease, chronic kidney disease, diabetes, and frailty) factors that increase complexity and instability. Major bleeding, complications of invasive therapies, contrast-induced nephropathy, delirium, and drug–drug interactions are all relatively more common in older versus younger ACS patients. The presence of frailty in very old adults also contributes to longer hospital length of stay, increased rehospitalization, increased bleeding, and greater cardiovascular mortality (Alonso Salinas et al., 2016a; Ekerstad et al., 2011; Alonso Salinas et al., 2016b).

Discrepancies in the management of ACS in older adults have been observed in several clinical studies. In the GRACE registry (Avezum et al., 2005), patients >65 years were less likely to receive standard medical therapy, and in those >74 years, coronary angiogram and PCI were less likely to be performed. Furthermore, major bleeding doubled in patients >85 years. In the CRUSADE study, four different age groups were compared: <65 years, 65–74 years, 75–84 years, and >85 years (Alexander et al., 2005). Approximately 35% of the patients were aged >75 years and 11% were >85 years. The rate of utilization of antiplatelet and antithrombin therapy within the first 24 h of presentation was significantly lower with advancing age.

Because of the higher risk of bleeding, the use of multiple antithrombotic and antiplatelet agents is particularly challenging (Forman et al., 2016). In older adults with ACS and concomitant atrial fibrillation who require oral antithrombotic therapy, prolonged dual antiplatelet therapy after PCI with oral antithrombotic therapy increases the long-term risk of bleeding events. However, recent studies indicate that the use of clopidogrel in combination with oral anticoagulant (i.e., omitting aspirin) was as effective in preventing cardiovascular events, with reduced risks of bleeding (Lamberts et al., 2013). Similarly, in another trial, among patients with atrial fibrillation undergoing stent placement the administration of low-dose rivaroxaban plus $P2Y_{12}$ for 12 months or very low dose rivaroxaban plus dual antiplatelet therapy for 1, 6, or 12 months are associated with lower rate of clinically significant bleeding with efficacy that was similar to traditional standard triple therapy (Gibson et al., 2016).

In older patients with myocardial infarction, early revascularization results in improved clinical outcomes and better symptom control. In TACTICS-TIMI 18, an early invasive strategy was compared to conservative medical therapy, and a subgroup analysis was performed in older patients (Bach et al., 2004; Cannon et al., 2001). This analysis showed a treatment benefit for an early invasive strategy even with advancing age. In patients ≥75 years of age, there was a 56% relative risk reduction with an invasive strategy compared to only 20.4% relative risk reduction in younger patients. However, there was also a 3-fold risk of major bleeding, particularly among older patients. In the After Eight Study, approximately 450 patients 80 years or older with non-ST elevation ACS or unstable angina were randomized to an early invasive strategy with PCI or coronary bypass grafting versus medical therapy alone, and the early invasive strategy was shown to be superior to conservative medical treatment (Tegn et al., 2016). DeBelder et al. examined stent placement in octogenarians for symptomatic angina in 22 centers in the XIMA trial (de Belder et al., 2014). Approximately 800 patients received either drug-eluting stents or bare metal stents, and drug-eluting stents were associated with a lower incidence of myocardial infarction and target vessel revascularization without increased incidence of major hemorrhage. Despite concerns relating to procedural complications, bleeding risks, and worse long-term prognosis, many older adults benefit from invasive therapies.

Data from the GUSTO-I trial showed that 30-day mortality after a STEMI increased 10-fold in patients ≥85 years of age compared to those <65 years of age (White et al., 1996). Older STEMI patients often do not receive revascularization because of late presentation (>6 h after symptoms onset) (Alexander et al., 2007b), cognitive impairment and dementia, nondiagnostic EKG, atypical symptoms, associated geriatric syndromes, poorly controlled hypertension, prior stroke, or chronic anticoagulation (Antman et al., 2004). Nonetheless, older STEMI patients who do not get revascularized then become more susceptible to complications (e.g., HF, ventricular arrhythmias, and cardiogenic shock). Thus, early revascularization in selected older adults with STEMI is typically associated with improved longevity and reduced risks of recurrent events (de Boer et al., 2002; Goldenberg et al., 2003; Grines, 2005).

Research Opportunities

Improved diagnosis of ACS can help overcome notorious delays in initiating care. The utility of revascularization and other ACS therapies in relation to frailty, multimorbidity, and other geriatric syndromes is also a key research consideration (Afilalo et al., 2014). Likewise, the dose, intensity, and duration of pharmacologic therapy after ACS needs greater clarification, with benefits of antiplatelet therapy, statins, beta-blockers, ACE inhibitors/ARB, and other medications all counterbalanced by increased iatrogenic risks (Rich et al., 2016) amidst geriatric vulnerabilities.

Risk stratification tools for better patient selection to undergoing invasive procedures are needed. The influence of nonpharmacologic therapies, including cardiac rehabilitation, may have a role in improving physical function and quality of life parameters in older adults after ACS as well as with stable IHD.

Enrolling older ACS patients into trials to evaluate the effects of interventions on clinical and quality of life outcomes is inherently challenging yet feasible (Sinclair et al., 2016). This will entail creative strategies to enroll and facilitate participation for candidates who may lack sophisticated health literacy or logistical access. Finally, studies to better study advanced care planning and methods for decision-making are needed because it can influence the goals of care and choice of therapy for IHD and ACS (Rich et al., 2016).

Heart Failure

Heart failure is more common in older adults, rising in prevalence from <1.5% of the population in patients <60 years old to approximately 12% (10.6% in men and 13.5% in women) in patients ≥80 years of age (Writing Group Members et al., 2016). Both HF with reduced ejection fraction (HFrEF) and HFpEF increase with age, but incident HFpEF is more prevalent as it is essentially intertwined with the elements of cardiovascular aging (**Table 1**). HFpEF is also exacerbated by the increasing prevalence of CHD, hypertension, diabetes, atrial fibrillation, and other factors which commonly coexist in older patients (Shah et al., 2016; Paulus and Tschope, 2013).

Many pivotal HF trials have systematically excluded older adults given the degree of complexity associated with their management, and caution is warranted when implementing guideline-directed therapies for older patients. Nonetheless, basic precepts of care for older HFrEF and HFpEF patients are similar to younger adults. Routine serum measurements of electrolytes, renal, kidney, and thyroid functions, and surface ECG may provide important clues to the etiology of the disease. Transthoracic echocardiography is a corner stone in the diagnosis given the availability, noninvasive nature, and lower cost. Echocardiograms are useful for the evaluation of structural feature, mechanical function, and/or flow abnormalities. If underlying coronary disease is suspected, coronary angiography can evaluate coronary anatomy and stratify older adults suitable for coronary revascularization. Right heart catheterization may be useful to clarify intracardiac hemodynamics especially for HFpEF.

The goals of management of acute HF in the elderly are similar to younger adults, which are focused on immediate symptomatic improvement and prevention of disease progression. Improvement in shortness of breath with use of diuretic therapy may result in improvement in functional status and maintenance of independence. However, routine screening for electrolytes disturbances, worsening renal function, and evidence of hypoperfusion are important because of coexisting chronic kidney disease, drug–drug interactions, or presence of other medical conditions in older patients (Azad and Lemay, 2014).

In HFrEF, prevention of disease progression is achieved by adherence to pharmacotherapy that have been proven to improve survival (particularly ACE inhibitors, ARB, beta-blockers, and mineralocorticoid receptor antagonists [MRA]) (Yancy et al., 2013). Although beta-blockers are associated with significant mortality reduction in combination with ACE inhibitors or ARB, their use may be confounded by advanced conduction disease, severe obstructive lung disease, or sick sinus syndrome. In general, medication choices must be individualized, with the assumption that guidelines' precepts may not be fully implementable. Moreover, routine assessment of kidney function is prudent with up titration of ACE inhibitors, ARB, or MRA as a significant rise in serum creatinine is expected (Azad and Lemay, 2014).

The recent PARADIGM-HF trial showed that symptomatic HFrEF patients (ejection fraction ≤40%) treated with a combination of the ARB valsartan and the neprilysin inhibitor sacubitril resulted in significantly improved clinical outcomes compared to treatment with enalapril pharmacotherapy. Substantial benefits extended to adults ≥75 years, including reduced cardiovascular death or rehospitalization, all-cause mortality, cardiovascular mortality, heart failure exacerbation, and symptoms related to heart failure (Forman et al., 2016; McMurray, 2014). Thus, it is now felt that many older adults may benefit from combined valsartan–sacubitril instead of ACE inhibitors or ARB, in combination with beta-blockers and possibly MRA. Still, the risks of polypharmacy from this regimen, especially in combination with medications from concurrent diseases, remain an enduring concern.

In contrast to HFrEF, treatment for HFpEF remains relatively more ambiguous. Treatment of hypertension, atrial fibrillation, and other cardiac instability are all recommended, but there is less certainty regarding medications to treat HFpEF on a more fundamental level. Although the TOPCAT trial found that MRA reduced HF hospitalization in the setting of HFpEF, it resulted in higher rates of hyperkalemia and renal impairment (Pitt et al., 2014). Nonetheless, other analyses reinforce the utility of MRA as posthoc analysis of TOPCAT suggest that even greater benefits may result in specific subsets of HFpEF patients (Pfeffer et al., 2015). Ongoing trials continue to explore new pharmacological agents that target other steps in this complex age-related disease (Shah et al., 2016).

The observed benefit of device-based therapy in older HF patients remains controversial (Rich, 2001). Cardiac transplantation and mechanical support devices, such as left-ventricular assist devices (LVAD), improve overall survival for younger adults with advanced HF, but older adults are not routinely considered for these advanced therapies (Richards et al., 2016). In general, selection for implantable cardioverter defibrillator should include overall assessment of quality of life measures, patients' preferences, and life expectancy (North and Sinclair, 2012) Furthermore, end of life care and palliative domains in management are important considerations for older HF patients (Yancy et al., 2013; Yancy et al., 2016). Prognosis is inherently grim for this progressive disease, and discussion regarding quality of life as well as death is an important dimension of care (Forman et al., 2016).

Geriatric Complexities

Exercise intolerance and dyspnea are common symptoms of HF in older adults, but they are nonspecific and thus often attributed to other etiologies. Similarly, HF symptoms may include confusion, weakness, anorexia, cognitive decline, or somnolence which are frequently misinterpreted and/or undertreated (Forman and Rich, 2003). Standard therapy for HF is often complicated by side effects of medications and preexisting chronic conditions (Forman and Rich, 2003). Prior studies have shown that >90% of older adults with HF have at least two comorbidities and 60% have five or more (Chamberlain et al., 2015; Wong et al., 2011), further complicating the presentation, diagnosis, and management of HF.

Objective data may help ascertain the diagnosis of HF in these patients including the use of clinical exam (particularly elevated internal jugular pressure), chest X-ray, and echocardiography. Biomarkers (natriuretic peptide BNP or N-terminal pro-BNP [NT-pro-BNP]) can be informative, but with the caveat that diagnostic cut-points are relatively higher in older versus younger adults. While biomarkers are less specific for diagnosis of HF in old age, they still have valuable prognostic utility (van Veldhuisen et al., 2013).

Given the substantial role of comorbidity in HF pathophysiology and instability, treatment of concomitant cardiac (e.g., CAD, valvulopathy, and atrial fibrillation) and noncardiac (e.g., anemia, diabetes) diseases are critical parts of management, both in respects to symptoms and quality of life. Standardized approaches to comorbid diseases, including choices of medications and procedures, remain an important challenge to care.

Research Opportunities

Studies are needed to evaluate the utility and safety of newer pharmacologic therapies and mechanical support devices in older adults, particularly HFpEF. Furthermore, it is increasingly important to clarify and facilitate more patient-centered approaches for management of HF as it is a disease of complex multimorbidity, frailty, and disability. A broader range of patients (e.g., broader ranges of age, frailty, multimorbidity) and pertinent endpoints (e.g., mortality, hospitalization, quality of life, or functional capacity for older adults) need to be studied. HFpEF remains a particularly dynamic area of research, especially as new mechanisms underlying disease are still being delineated. Based on these expanding insights, many novel medications are being studied (e.g., valsartan/sacubitril in the Paramount trial) (Rich et al., 2016; Solomon et al., 2012).

The impact of frailty and cognitive impairment merits more study in a disease, wherein these risks are endemic in the vulnerable population, and also predictive of iatrogenesis and poor adherence. The impact of nonpharmacologic interventions like supervised exercise programs and diet modification may improve quality of life, symptoms, and function and lead to options for reduced pharmacotherapy. Early introduction of palliative therapy, including consideration for hospice care, minimizes burden associated with advanced heart failure in older adults and may also result in improvement in quality of life.

Valvular Heart Disease

Just as aging physiology affects the coronary vasculature and myocardium, cardiac valves also undergo changes that predispose to disease (**Table 1**). Myxomatous degeneration, collagen infiltration, and calcification are common, especially in the left heart (Cheitlin, 2003a; Cheitlin, 2003b). Fixation of valvular leaflets can also cause substantial valvular regurgitation (Hu et al., 2016; American College of Cardiology et al., 2006). According to the 2014 AHA/ACC Valvular Heart Disease Guidelines, 12%–13% of patients older than 75 years have moderate to severe valvular heart disease. While surgery is often technically indicated, many elderly patients are suboptimal surgical candidates, and thus their treatment options are more limited (Nishimura et al., 2014). In a U.S. population-based study of 11,911 patients, Nkomo et al. showed that 13.3% of patients ≥75 years had valvular disease, with prevalence increasing with age (Nkomo et al., 2006). There were no differences in prevalence between men and women. Approximately 9% of patients had mitral valve disease, and 4.5% had aortic valve disease (Nkomo et al., 2006).

Given the relatively high prevalence of valve disease in older adults and high surgical risks, the option to use innovative transcatheter aortic and mitral valve technologies in lieu of valve surgery have rapidly proliferated. For severe symptomatic aortic stenosis, transcatheter aortic valve replacement (TAVR) is now an alternative treatment in high-risk patients for conventional surgical aortic valve replacement (SAVR). Many of the patients in the initial TAVR implementation efforts have been of advanced age. The PARTNER A & B trials were multicenter, randomized clinical trial that compared standard medical therapy for severe aortic stenosis to TAVR and showed that transfemoral TAVR was superior (30% mortality vs 50% mortality) (Leon et al., 2010). Risks of stroke, heart block, vascular complications, and paravalvular leak are generally higher with TAVR, but these risks have progressively lowered as TAVR technology has advanced. In OBSERVANT, TAVR was compared to SAVR; a substudy of patients aged >80 years showed similar early and midterm mortality (Fraccaro et al., 2016). In the PARTNER 2 trial, TAVR was also demonstrated to be equivalent to SAVR in terms of death or disabling stroke in intermediate risk patients with severe aortic valve disease (Leon et al., 2016).

Over the past several years, transcatheter mitral therapies for repair and replacement of the mitral valve have also advanced. The MitraClip is the device with the widest clinical experience and has been studied on a larger scale in both the TRAMI and ACCESS-EU trials (Baldus et al., 2012; Maisano et al., 2013). In the German trial for percutaneous repair of mitral regurgitation (TRAMI), 486 patients (median age 75 years), most of whom had severe mitral regurgitation and NYHA Class III/IV heart failure, were enrolled, and 94% of these patients successfully underwent MitraClip placement leading to an acute reduction in the severity of mitral regurgitation (Baldus et al., 2012). Functional capacity also improved in approximately 2/3 of patients, which lends support to this therapy as an important nonsurgical option for elderly patients with HF who are high-risk surgical candidates.

The first randomized trial comparing the MitraClip to conventional mitral valve surgery was the EVEREST II study. One-year results showed that the MitraClip was superior in terms of safety, but less effective in reducing mitral regurgitation. However, there were no differences at the end of a 4-year follow-up period in terms of the prevalence of moderate or severe mitral regurgitation or mortality (De Backer et al., 2014; Mauri et al., 2013). There are also multiple transcatheter mitral valve replacement systems that are being tested as an option for severe mitral regurgitation treatment in nonsurgical candidates (De Backer et al., 2014).

Research Opportunities

Although many of the valve studies have included older adult patients, refinement in patient selection and age-tailored care is still critical. The key goal is to pinpoint patients who will most likely recover from valve interventions. Therefore, comorbid disease, frailty, and other dimensions of care often become determinant of outcomes. Management of low-gradient aortic stenosis, multivalve disease, pulmonary hypertension, and amyloid heart disease are other issues which merit additional research and refinement. The long-term durability of the current transcatheter valves and devices in older adults and the utility of anticoagulation are other key issues which warrant further study.

Heart Rhythm Disorders

As the conduction system ages, degenerative changes predispose older patients to sinus node disease, sick sinus and tachycardia–bradycardia syndromes, supraventricular, and ventricular arrhythmias. These include fibrous, fatty, and calcific infiltration of the conduction system, cardiac fibrous skeleton, and reduction in the number of pacemaker cells (**Table 1**) (Dai et al., 2012; Chow et al., 2012). Atrial fibrillation is the most prevalent arrhythmia in older populations, and the incidence only increases with age. Seventy percent of patients with atrial fibrillation are between the ages of 65 and 85 years (Kistler et al., 2004). Atrial ectopy is common in older adults, and ventricular ectopy becomes more prevalence in patients with structural heart disease (Chow et al., 2012). Management of these heart rhythm disorders is complex because many older adults have limited life expectancy, multimorbidity, frailty, and polypharmacy. Benefits of anticoagulation to reduce thromboembolic risks of atrial fibrillation are counterbalanced by burdensome monitoring, dietary restriction, and bleeding risks. Benefits of implantable cardioverter defibrillators (ICD) to reduce sudden death in heart failure patients are often surpassed by the risks created by the multimorbidity and frailty in these patients.

Treatment options for atrial fibrillation range from rate control, systemic anticoagulation, and antiarrhythmic drugs to more invasive therapies such as radiofrequency ablation and left atrial appendage closure devices (Fang et al., 2007; Fuster et al., 2007; Damluji et al., 2015c). Antiarrhythmic medications have disproportionately unfavorable side effect profiles in older adults, (Dayer and Hardman, 2002; Fleg et al., 2011) and rate control with AV nodal blocking agents is a more common strategy of care (Curtis and Rich, 2007). Direct acting oral anticoagulants (DOACs) are generally considered advantageous for prevention of thromboembolic events as they are relatively simpler to administer than warfarin and lack the need for dietary restriction or routine blood monitoring. In a recent metaanalysis, DOACs were superior to warfarin therapy in preventing events in atrial fibrillation patients with reduced adverse events, even in elderly patients (Sharma et al., 2015).

Percutaneous left atrial appendage closure devices such as the Watchman and Amplatzer devices have also emerged as reasonable alternative therapies to chronic anticoagulation for stroke prevention in patients with atrial fibrillation at high risk of bleeding. PROTECT AF showed that left atrial appendage occlusion closure with the Watchman device was noninferior to anticoagulation with warfarin for stroke prevention (Reddy et al., 2013). In the PREVAIL trial, 407 patients (mean age ~75 years old) were randomly assigned to either left atrial appendage occlusion with the Watchman device without warfarin versus chronic warfarin therapy, and again, the Watchman was shown to be noninferior to chronic anticoagulation for ischemic stroke prevention (Holmes et al., 2014).

Implantable cardioverter defibrillators for prevention of sudden cardiac death in older adults are sometimes controversial. A recent metaanalysis of 4,915 patients ≥75 years of age showed that ICDs can reduce all-cause mortality in selected older adults (Fleg and Forman, 2013), but life expectancy of at least one year is necessary for ICDs to achieve such significant clinical benefit. The utility of ICDs becomes less straightforward in the context of mounting comorbidity and complexity. In fact, many now assert the critical importance of deactivating ICDs if comorbidities advance and overwhelm any likelihood of benefit (Kirkpatrick et al., 2012).

Research Opportunities

Research is warranted to better clarify the influence of multimorbidity, inflammation, and other constitutional changes in relation to arrhythmia (Richards et al., 2016) both to better elucidate pathophysiology and optimize therapies. Whereas catheter-based ablation therapy holds much promise in older populations, utility for older adults is still relatively unclear. One study evaluated 587 patients ≥75 years of age with atrial fibrillation; 261 who underwent ablation. Based on this study, ablation therapy for atrial fibrillation was determined to be safe and effective in older populations (Nademanee et al., 2015). However, larger studies with study populations that include more comorbidities are still warranted.

Older adults are at increased risk of bleeding with antithrombin therapies, and thus new percutaneous left atrial appendage closure devices show promise as an effective alternative to chronic anticoagulation, but more research is needed to better guide patient selection and choice of device. New oral anticoagulants also hold much promise to moderate bleeding risks; however more research is needed to ascertain safety amidst frailty and complexity.

Given the many concerns about ICDs for elderly with complex comorbid disease, alternative therapy with chronic resynchonization therapy (CRT) to reduce sudden death in eligible elderly without the risk of shocks is also an important area of investigation (Kron et al., 2009).

Peripheral Arterial Disease

Peripheral arterial disease (PAD) entails noncardiac atherosclerotic disease, and it is common in older adults. The Rotterdam Study showed that the prevalence of PAD, defined as ankle-brachial systolic blood pressure index <0.9 in either leg, was 19.1% overall, and almost 60% in patients ≥85 years (Meijer et al., 1998; Aronow, 2008). The risk factors for PAD are analogous to those of CAD, and thus patients with PAD are at increased risk for cardiovascular events including myocardial infarction and death (**Table 1**) (Ankle Brachial Index Collaboration et al., 2008). Although many older PAD patients are asymptomatic, CVD risks are still significant. Diehm et al. conducted a prospective cohort study to assess the mortality and morbidity risk in patients aged ≥65 years and demonstrated that both symptomatic and asymptomatic patients had similarly high CVD mortality compared to patients without PAD (Diehm et al., 2009).

The management of PAD in older adults is similar to younger patients. A thorough physical examination with attention to pulses, bruits, and ulcerations is fundamental. Assessment of the ankle-brachial index (ABI) is recommended. The toe-brachial index is an alternate consideration for patients with noncompressible ankle arteries. Duplex ultrasound, computed tomography angiography, or magnetic resonance angiogram may be needed to ascertain the diagnosis and extent of PAD. Treatment is mainly focused on symptom relief and risk factor modification, including exercise programs, smoking cessation, and pharmacologic therapy (Armstrong et al., 2014). However, when symptoms persist or critical limb ischemia occur despite optimal medical management, revascularization with surgical bypass, angioplasty, or major amputation may be required (Hirsch et al., 2006).

Stains have been demonstrated to increase walking distance in PAD patients (Aronow et al., 2003). Cilostazol has also been demonstrated to reduced claudication symptoms and increase walking distance (Lee and Nelson, 2014). Exercise is a particularly effective way to improve function and quality of life in older adults with obstructive PAD. The CLEVER study compared medical therapy alone to medical therapy with supervised exercise as well as to medical therapy with stent revascularization. It showed that supervised exercise and stent revascularization each achieved superior outcomes to medical therapy alone. Improvement in claudication onset time was greater for supervised exercise compared with medical therapy, but not for stent revascularization compared with medical therapy (Murphy et al., 2015).

Endovascular intervention including angioplasty, stents, and atherectomy is recommended for those patients with life-limiting claudication with hemodynamically significant aortoiliac occlusive disease or femoropopliteal disease but is recommended only to prevent progression to critical limb ischemia (Gerhard-Herman et al., 2016). Surgical revascularization has also been demonstrated to improve survival and offset the burden of symptoms. In the BASIL trial, 452 patients with severe infra-inguinal limb ischemia were randomized to bypass surgery versus angioplasty, and results showed that either strategy achieved similar mortality benefits and amputation-free survival (Adam et al., 2005).

Research Opportunities

While many PAD trials include patients >65 years of age, those aged >80 years (when PAD is most prevalent) remain noticeably understudied. Therefore, key perspectives regarding quality of life and functional outcomes are not well studied, despite significant implications pertaining to activities of daily living, mobility, frailty, and functional independence (Rich et al., 2016). Similarly, the

effects of pharmacological versus nonpharmacologic interventions in this population warrants much greater understanding. In addition, the interplay between PAD and age-related muscular decline and sarcopenia are important determinants of functional outcomes that have sparked great interest and research opportunity.

Cerebrovascular Disease

Prevalence of cerebrovascular disease increases dramatically with age, rising from 2% in patients 40–59 years of age to almost 15% in patients aged >80 years (Writing Group Members et al., 2016; Fleg and Forman, 2013). Given that the pathophysiology and risk factors for ischemic stroke and CAD overlap, mechanisms of vascular aging are similarly pertinent (**Table 1**), and adequate control of risk factors (e.g., hypertension, hyperlipidemia, and diabetes) are vital as primary prevention, especially as 76% of strokes are first events (Meschia et al., 2014).

In older adults undergoing bypass surgery or with past medical histories of PAD, history of smoking, or stroke or transient ischemic attack (TIA), screening with carotid ultrasound is often useful. The presence of atrial fibrillation increases the risk of thromboembolic disease, and antithrombotic therapy becomes an added consideration. Carotid endarterectomy has shown to be beneficial in reducing stroke risk in patients with 70%–99% stenosis of the internal carotid artery (Adams, 2009). The CREST trial showed carotid endarterectomy was superior to carotid artery stenting for reducing the stroke risk in patients >70 years of age (Brott et al., 2010).

For acutely presenting ischemic stroke, intravenous plasminogen activator inhibitor is recommended for selected patients who can be treated within 3 h of symptoms (door-to-needle time <60 mins from hospital arrival) (Jauch et al., 2013), but many older adults have absolute or relative contraindications for therapy. For patients with noncardioembolic ischemic stroke or TIA, antiplatelet therapy (aspirin monotherapy at a dose of 50–325 mg/day; the combination of aspirin and extended-release dipyridamole at a dose of 25/200 mg twice a day; or clopidogrel monotherapy at 75 mg per day) (Jauch et al., 2013) and high-intensity statin therapy have been demonstrated to reduce the risk of recurrent stroke, nonfatal myocardial infarction, and vascular death; however, even these options tend to be more complicated in the context of geriatric syndromes (Jauch et al., 2013; Chen et al., 2010).

Research Opportunities

Given the common and devastating nature of cerebrovascular disease, research to better ascertain risk and acute management are both essential. Utility of carotid screening, for example, is less certain for adults aged >80 years. Utility of TPA is often surpassed by risks associated with therapy, but alternative mechanical options (arteriography and thrombus extraction) have not been well studied in older populations. While risk factor modification is widely recommended, optimal strategies regarding blood pressure, diabetes, blood pressure targets, and antiplatelet therapy all remain uncertain in advanced age.

Hypertension

Age-associated changes in the arterial wall of the vascular systems, along with endothelial dysfunction, and chronic inflammatory changes result in decreased arterial dispensability and increased in wall stiffness (**Table 1**) and associated predisposition to isolated systolic hypertension. Historically, these elevations were considered "normal" aging and treatment was not advised by cardiovascular experts. However, many recent studies have shown that elevated blood pressure in older adults is associated with increased cardiovascular mortality and morbidity (Damluji et al., 2015b). Isolated systolic hypertension (ISH) is directly associated with development and progression of CAD, cerebrovascular disease, HF, and obstructive PAD. Although treatment of hypertension has been demonstrated to improve outcomes in adults aged into their 80s, it is often complicated by the presence of coexisting conditions, falls, fatigue, renal failure, and other iatrogenic sequelae.

There is heterogeneity in the guideline recommendations regarding the optimal blood pressure targets in older adults. The American College of Cardiology Foundation (ACCF) and American Heart Association (AHA) recommend systolic blood pressure (SBP) goal <140 mmHg in adults ≤79 years of age and SBP 140–145 mmHg in adults >80 years of age (Aronow et al., 2011). Similarly, the American Society of Hypertension and International Society of Hypertension recommend BP <140/90 mmHg in adults <80 years old and BP <150/90 mmHg for adults ≥80 years. The Joint National Commission 8 recommends BP <150/90 for all adults >60 years of age (Weber et al., 2014; James et al., 2014). Although several clinical trials and subgroup analyses examined different antihypertensive drug regimens in older adults, the exact class of medication which is most efficacious and with least adverse events remains ambiguous. Several studies have highlighted benefits of calcium channel blockers and angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARB) in older adults. There is no evidence that elderly women respond to antihypertensives differently than elderly men, and thus both genders are usually treated similarly (Oparil, 2006).

The lack of consensus among these societies reflect the underlying special characteristics of older adult populations, including the potential for polypharmacy, and the greater risk of pharmacokinetic and pharmacodynamics complexities (Damluji et al., 2015b). For older adults with underlying CKD, history of falls, or preexisting conditions, Individualized treatment plans tailored to

achieve a more liberal blood pressure control may be acceptable. Notably, aggressive antihypertensive therapy associated with reduced diastolic blood pressure may also reduce coronary blood flow and exacerbate ischemic symptoms. However, for healthier older adults with fewer comorbidities in the SPRINT trial, low blood pressure (<120 mmHg) led to reduced cardiovascular events and all-cause mortality (Group et al., 2015). As such, a thorough evaluation for the patient's medical, cognitive, and functional status is important for a tailored individualized treatment plan to achieve optimal cardiovascular outcomes.

Geriatric Complexities

While the conceptual value of treating hypertension is clear, older adults are especially sensitive to adverse drug reactions given the prevalence of polypharmacy and hemodynamic lability (Butt et al., 2010). These risks range from postural hypotension (with increased propensity to falls) to increased medication toxicity due to reduced renal clearance. One of the mainstays of the treatment of hypertension in the elderly is to start with the lowest dose of a medication and gradually increase as tolerated (Damluji et al., 2015b). White coat syndrome can often result in the inappropriate escalation of therapy in older adults, and thus ambulatory blood pressure monitoring or ambulatory blood pressure logs can be an effective way to help avoid this common source of error.

Although multiple clinical trials evaluated different antihypertensive regimens in older adults (**Table 3**), therapy is frequently guided by the patient's cardiovascular comorbidities or risk factors. For example, ACE inhibitors are important first-line options for diabetes or HF. Beta-blockers may be relatively more useful in patients with CAD, HF, and/or atrial fibrillation (Jamerson et al., 2008). Multiple trials have shown that combination therapy of a variety of antihypertensives with a diuretic is efficacious in the elderly (Beckett et al., 2008; Staessen et al., 1997; Amery et al., 1985; MRC Working Party, 1992; Dahlof et al., 1991). Thiazide diuretics warrant added caution given the possibility of metabolic derangements and orthostatic hypotension (Beckett et al., 2008; Aronow, 2010), as well as risks pertaining to incontinence and dehydration.

Research Opportunities

Major gaps in knowledge remain target blood pressure goals in the setting of geriatric syndromes. Falls, frailty, cognition, and other dimensions of health may all worsen in some older patients who are treated too aggressively with antihypertensive medication. Outcomes for future trials should not only include the traditional cardiovascular outcomes but treatment related adverse event in older adults, including orthostatic hypotension, fall risks, cognition, functional capacity, and patient-based metrics. The recently released SPRINT Trial has sparked much controversy, as purported benefits of lower blood pressure targets implicit call for greater efforts to tailor care relative to each patient's idiosyncratic vulnerabilities (Patel et al., 2017).

Options to control hypertension also highlight the role of lifestyle modification. Aerobic exercise, intentional weight loss, alcohol moderation, and reduced salt have all been associated with improved blood pressure control in younger subgroups of elderly (Aronow et al., 2011). Nonetheless, the data substantiating these benefits in very old adults are relatively sparse. Furthermore, strategies to best achieve healthy lifestyles amidst the vulnerabilities and limitations of aging are often unclear.

Hyperlipidemia

Vascular aging (**Table 1**) results in increased arterial wall thickness and stiffening, reduced vasodilation, and increased oxidative stress and inflammation. These events lead to faster development and progression of atherosclerotic plaques and reduced microvascular function. Sustained exposure to low-density lipoprotein, along with other metabolic risk factors (e.g., hyperglycemia, tobacco), are implicated in the chronic inflammatory state, which initiate and drive vascular wall injury. Prior studies have shown that higher levels of low-density lipoproteins result in significantly increased proportion of CVD events and cardiovascular mortality, even among older adults (Pepe and Lakatta, 2005). Multiple studies have demonstrated that statin therapy reduces CVD mortlity and morbidity in older adults.

The 2013 ACC/AHA guideline on the treatment of blood cholesterol outlined the treatment algorithms for primary and secondary prevention of cardiovascular events (Stone et al., 2014). In that document, recommendations for adults $\geq$75 years of age without clinical evidence of CAD remain largely based on expert opinion. For older adults with low-density lipoprotein $\geq$190 mg/dL without clinical evidence of cardiovascular disease or diabetes, the consensus is to treat hyperlipidemia unless contraindications or intolerance exist (Stone et al., 2014). Patient preference to initiate, continue, or intensify statin therapy is considered a key part of decision-making.

For older adults with preexisting atherosclerotic CVD, secondary cardiovascular prevention with moderate- or high-intensity statin therapy is recommended, but with associated considerations regarding life expectancy, goals of care, and comorbid conditions. In the Heart Protection Study, which included over 20,000 patients aged 40–80 years with CVD, statin therapy decreased all-cause mortality, CHD, nonfatal myocardial infarction, need for revascularization, and stroke, including older patients (Heart Protection Study Collaborative Group, 2002). A posthoc analysis from the Scandinavian Simvastatin Survival Study showed that simvastatin use in patients >65 years of age provided similar benefits in terms of reducing the relative risk of major adverse cardiac events similar to younger patients (Miettinen et al., 1997). In PROSPER, one of the first randomized controlled trials exclusively conducted in 5,804 older adults (age 70–82 years), pravastatin reduced the risk of CAD by showing a significant 22% reduction in the combined endpoint of CHD death + MI + stroke in the 43% of the study population with known CVD (Shepherd et al., 2002).

Table 3 Main trials in hypertension involving older patients

Trial	*Year*	*Design*	*Drug therapy*	*Outcome*	*Recommendations*
EWPHE Substudy (Amery et al., 1985)	1985	Total 840 pts 691 in substudy Mean age = 71.5	Active tx with HCTZ + triamterene vs placebo	Relation between mortality and treated blood pressure	In elderly patients with HTN given active treatment, total mortality had a U-shaped relation with treated systolic pressure and an inverse association with treated diastolic pressure.
Coope and Warrender (Coope and Warrender, 1986)	1986	884 pts Mean age = 69	Active tx with either atenolol ± bendrofluazide vs control	Primary endpoint—Incidence of stroke or coronary disease; effect on overall mortality Secondary Endpoints—adverse side effects, unwanted morbidity	42% reduction in incidence of stroke, 22% reduction in cardiovascular death (NS); no effect on overall mortality or incidence of fatal or nonfatal heart attacks
SHEP (SHEP Cooperative Research Group, 1991)	1991	4736 pts Mean age = 72	Active tx with Chlorthalidone 12.5 followed by atenolol 25 vs placebo	Primary endpoint—nonfatal and fatal stroke Secondary endpoint—cardiovascular and coronary morbidity and mortality, all-cause mortality, QOL	Antihypertensive stepped-care drug treatment with low-dose chlorthalidone as step 1 medication reduced the incidence of total stroke by 36%; major cardiovascular events were also reduced
STOP-hypertension (Dahlof et al., 1991)	1991	1627 pts 70–84yo	Active tx with atenolol, HCTZ + amiloride, metoprolol, or pindolol vs placebo	Primary endpoint—total stroke, total MI, other cardiovascular death	Active treatment resulted in significantly fewer primary endpoints, lower morbidity and mortality from stroke, and lower total mortality compared to placebo
Medical Research Council (MRC Working Party, 1992)	1992	4396 pts Mean age = 70yo	Atenolol 50 vs HCTZ 25 or 50 and amiloride 2.5 or 5 vs Placebo	Actively treated subjects had 17% reduction in all cardiovascular events and the diuretic group showed significant reduction in all cardiovascular events where beta-blockers did not	HCTZ and Amiloride decrease risk of stroke, coronary events, and all cardiovascular events in older pts
Syst-Eur (Staessen et al., 1997)	1997	6403 patients Mean age = 70	Treatment with nitrendipine with ACE inhibitor/diuretic if necessary to reduce SBP <150 vs placebo	Primary endpoint—fatal and nonfatal stroke Secondary Endpoints—myocardial infarction, CHF, dissecting aortic aneurysm, renal insufficiency, retinal hemorrhage	42% reduction in total stroke and 31% reduction in all fatal and non-fatal cardiovascular endpoints; treatment of BP starting with nitrendipine can reduce the rate of cardiovascular complications
Syst-China (Wang et al., 2000)	1998	2394 pts Mean age = 66.5yo	Nitrendipine + captopril/ HCTZ vs placebo	Primary endpoint—fatal and nonfatal stroke Secondary endpoints—myocardial infarction, CHF, dissecting aortic aneurysm, renal insufficiency, retinal hemorrhage	Active treatment reduced total mortality by 39%, cardiovascular mortality by 39%, and stroke mortality by 58%; Treatment of hypertension in older Chinese patients can prevent cardiovascular complications, especially stroke
LIFE (Dahlof et al., 2002; Lindholm et al., 2002; Kjeldsen et al., 2002)	2002	9193 pts Mean age = 70yo	Losartan vs atenolol	Primary endpoint—cardiovascular event such as death, MI, or stroke	Relative risk reduction Losartan prevents more cardiovascular morbidity and death than atenolol for a similar reduction in BP and is better tolerated
SCOPE (Lithell et al., 2003)	2003	4964 patients Mean age = 76.4	Candesartan vs placebo with open-label antihypertensive therapy as needed	Primary endpoint—major cardiovascular events, composite of cardiovascular death, nonfatal stroke, and nonfatal MI Secondary endpoint—cardiovascular death, nonfatal and fatal stroke and MI, cognitive function	Candesartan-based tx assocated with modest, but not statistically significant reduction in MACE with a marked reduction in nonfatal stroke; cognitive function well maintained in both groups
HYVET (Beckett et al., 2008)	2008	3845 patients Mean age = 84yo	Indapamide + perindopril vs placebo	Primary endpoint—fatal or non-fatal stroke Secondary endpoints—death from any cause, death from cardiovascular causes, death from stroke	30% reduction in rate of any stroke but NS; 21% reduction in rate of death from any cause and 64% reduction in rate of heart failure in treatment group SBP 140–145 for pts >80

Table 3 (Continued)

Trial	*Year*	*Design*	*Drug therapy*	*Outcome*	*Recommendations*
JATOS (Group, 2008)	2008	4418 pts Pts 65–85yo	Efonidipine for strict (SBP <140) vs mild (SBP 140–160) control of BP	Primary endpoint—combined incidence of cerebrovascular dz, cardiac and vascular dz, and renal failure Secondary endpoint—death from any cause	No significant difference in outcomes between strict and mild treatment of BP, but complex clinical features associated with aging thought to have obscured tx effect
CARDIO-SIS (Verdecchia et al., 2009)	2009	Total 1111 pts Mean age = 67	Usual (<140) vs tight control (<130) of BP in non-DM pts with HTN	Primary endpoint—rate of electrocardiographic LVH 2 years after randomization Secondary endpoint—composite of all-cause mortality, fatal or non-fatal MI, fatal or non-fatal stroke, TIA, decompensated CHF Stage III/IV, angina with objective ischemia, new onset Afib, coronary revasularization, aortic dissection, occlusive PAD, ESRD on HD	Lower BP goal is recommended in nondiabetic patients with HTN; hazard ratio of 0.5 between usual and tight control; side effects did not significantly differ
VALISH (Ogihara et al., 2010)	2010	3260 pts Mean age = 76 yo	Valsartan for strict vs moderate control of BP	Primary endpoint—composite of cardiovascular events: sudden death, fatal or nonfatal stroke, fatal or nonfatal MI, death because of heart failure, other cardiovascular death, unplanned hospitalization for CV disease, and renal dysfunction Secondary endpoints—components of the primary end point, total mortality, new onset/exacerbation of angina pectoris	SBP <140 safely achievable in patients >70yo; but trial underpowered to determine if strict better than less strict BP control
INVEST (Denardo et al., 2010; Pepine et al., 2003)	2010	22,576 pts with HTN and CAD enrolled in full trial 2180 looked at in substudy Mean age = 84yo	Verapamil SR vs atenolol	Primary endpoint—first occurrence of all-cause death, total MI, total stroke, and revascularization with PCI/CABG	No difference in morbidity and mortality between antihypertensive drug treatment strategies, but possibility of increased risk of adverse events with BP <140/70
SPRINT (Group et al., 2015; Williamson et al., 2016)	2015	Total 9361 pts with HTN, no DM 2636 patients >75yo Mean age = 80yo	Intensive therapy with SBP <120 vs standard therapy with SBP <140 with any number of antihypertensive agents	Primary endpoint—composite outcome of MI, ACS not resulting in MI, stroke, acute decompensated CHF, death from cardiovascular cause Secondary endpoint—individual components of primary outcome, death from any cause	SBP <120 better than SBP <140

Geriatric Complexities

Despite signals of improved outcomes for secondary cardiovascular prevention with statin therapy, important treatment considerations for older adults include limited life expectancy, physiologic age, neurocognitive status, frailty, comorbidities, and polypharmacy (Table 2). The expected time to benefit of statins is 2–5 years, and this may factor decisively in decisions to discontinue the medication. Nonetheless, statins have also been associated with reduced claudication and other benefits which may occur relatively more quickly. These nuanced differences need greater clarification for a population inherently vulnerable to drug interactions, myalgias, and other complexities of management (Navar-Boggan et al., 2015; Grundy, 2014).

The intensity of LDL reduction in older adults also remains unclear. SAGE, TNT, and PROVE-IT TIMI 22 trials all showed benefit of higher-intensity therapy, but also with reversible worsening in liver function tests (Damluji et al., 2015b; Ray et al., 2006). Older

adults with sarcopenia and deconditioning may experience pain with statin therapy that may only exacerbate functional impairments (Jacobson, 2006). As a result, low-intensity statins, such as pravastatin, are sometimes preferable because of improved safety profile and lower to drug interaction (Grundy, 2014; Han et al., 2011).

Research Opportunities

Primary and secondary prevention in older adults with lipid lowering agents remains a highly controversial area. Benefits include stroke, PAD, and CAD event reduction, and even the potential to moderate many insidious changing of aging that contribute to subclinical disease and functional decline. Novel PCSK9 therapies have erupted into the field with great promise to reduce atherosclerotic risks even more effectively (Sabatine et al., 2017), but at the trade-off of high expense and many uncertain medications effects. However, even statins raise conceptual controversies amidst age-related susceptibility to disease, juxtaposed to risks of polypharmacy, and iatrogenic effects, especially at higher doses. Research can help to better delineate thresholds of age physiology, aggregate risk, cumulative medication burdens, and other dimensions of risk and implementation to better optimize care. Until then, secondary prevention is rarely straightforward as it is confounded by multimorbidity, frailty, exercise intolerance, and other complexities of care. Rationale and therapeutic standards for primary prevention are even less clear. Existing guidelines are derived primarily by expert consensus, but the fact remains that many experts in this medical field do not agree.

For now, a patient-centered approach seems indicated that incorporates each patient's preferences. However, this assumes there is an effective decision-making process for a medication and that has diffuse effects and much controversy associated with its effectiveness and value. Ongoing research seems essential.

Conclusions

Cardiovascular disease and atherosclerosis are mainly diseases of older adults, and as this population grows, the prevalence of CVD worldwide continues to increase. However, many of the trials that have been conducted do not include older adults with typical geriatric complexities such as multimorbidity, polypharmacy, frailty, and neurocognitive impairment. Thus, data pertaining to therapies best suited to this age group are lacking. Furthermore, health outcomes in older adults need be tailored to improve symptoms, health-related quality of life, and functional status that incorporate aging complexities, and that move beyond an exclusively disease-centered approach. The rising costs of healthcare and the increasing prevalence of cardiovascular disease drive the need for preventative cardiovascular care for older adults. Basic science and translational research focused on cardiovascular aging in as well as clinical trials that include representative populations of complex elderly will help develop better treatment insights with greater potential to improve quality of life, reduce adverse side effects, and reduce cardiovascular mortality and morbidity among CVD patients.

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Management of Cardiac Sarcoidosis

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Introduction

Sarcoidosis is a noninfectious granulomatous disorder of unknown cause that affects many organ systems, most often the lungs but not uncommonly the heart. Cardiac sarcoidosis (CS) is more prevalent than previously thought and is the second leading cause of sarcoidosis-related mortality following pulmonary complications. In older observational studies, sarcoidosis was thought to be relatively uncommon, affecting the heart in approximately 5% of patients; however, in autopsy studies and in studies using more sophisticated imaging techniques, the prevalence of cardiac involvement was closer to 25%–30% (Silverman et al., 1978). This variability suggests that the diagnosis of CS is difficult to establish based upon standard clinical criteria and that the methods for disease detection need to be better standardized.

Epidemiology

The prevalence and actual incidence of sarcoidosis are not known with certainty, and this is partly due to the fact that the incidence varies among geographic regions and different ethnicities. Furthermore, differences in environmental exposures, predisposing genetic factors, and different surveillance methods likely play a large role in the variable worldwide incidence. In northern European countries, the incidence of sarcoidosis is estimated to be 5–40 cases per 100,000 people. In the United States, this number varies between 10 and 40 cases for every 100,000 with African Americans three times more likely to be affected than Caucasians. This number is even higher in smaller studies conducted on a city-by-city basis. Moreover, sarcoidosis is more likely to be chronic and fatal in African Americans. Finally, mortality secondary to sarcoidosis in the United States has increased, and cardiac involvement certainly impacts this increase, especially in African American males (Rybicki et al., 1997; Pietinalho et al., 1995; Baughman et al., 2001; Swigris et al., 2011). Therefore, it is imperative that we understand the true burden of sarcoidosis and specifically the impact of CS.

Furthermore, certain ethnic differences in the epidemiology and presentation of CS have been reported. For example, Japanese pathologists reported cardiac involvement in 50%–78% of patients diagnosed with sarcoidosis; this is in comparison with 13%–50% involvement seen in US patients and <2% involvement in patients in Israel (Yoshida et al., 1997; Kim et al., 2009; Yigla et al., 2002).

Etiology

The precise etiology of CS remains unknown; however, numerous hypotheses including environmental, occupational, and infectious causes have been discussed. It is hypothesized that certain agents, such as infectious exposures, trigger an immunologic response in genetically susceptible individuals (Newman et al., 2004). These agents enter via the respiratory tract and incite an inflammatory response that is then carried over into the circulatory system, eventually reaching the heart.

Pathogenesis and Histopathology

Though the exact etiology of sarcoidosis remains unknown, it is thought that repeated exposures to specific antigens in genetically susceptible individuals lead to granuloma formation. This can either resolve or progress to fibrosis. It is thought that sarcoidosis requires at least three major events, including exposure to an antigen, acquired cellular immunity against the antigen and activation of CD4+ cells, and finally a nonspecific inflammatory response, initially with a Th1 response leading to inflammation and later with a Th2 response leading to tissue scarring (Sekhri et al., 2011). Moreover, a recent study suggests that certain lifestyle factors, namely, occupational exposures, may increase the severity of sarcoidosis (Liu et al., 2016).

CS is associated with noncaseating granulomas, which can be found in any part of the heart; however, sarcoidosis has a predilection for the conduction system. The most common areas of involvement include the basal segment of the inferolateral left ventricular free wall and the basal interventricular septum, followed by the atrium, the papillary muscles, the right ventricle, and finally the pericardium (Roberts et al., 1977).

Typical Presentation

Clinical manifestations of CS depend on the location and extent of granulomatous disease (Fig. 1). Given the predilection for the conduction system (the interventricular basal septum), complete heart block (CHB) is the most common manifestation and has been reported in up to one-third of patients with clinical evidence of sarcoidosis. CHB in patients with sarcoidosis occurs at a younger age than in patients with CHB from other causes (Silverman et al., 1978; Fleming, 1974). Moreover, in young to middle-aged, otherwise healthy individuals, who present with CHB, CS should be considered. Furthermore, patients with sarcoidosis who present with presyncope or syncope should be evaluated for CHB with an electrocardiogram (ECG). Sudden cardiac death may be a direct result of CHB.

Sudden cardiac death accounts for 25%–65% of deaths caused by CS and is often due to ventricular arrhythmias or CHB (Sekiguchi et al., 1980). Ventricular tachycardia is one of the most common arrhythmias noted in patients with CS with an incidence of up to 20% (Furushima et al., 2004). Granulomatous inflammation causes abnormal foci leading to ventricular activation and/or reentrant tachycardias. Patients with CS that have documented ventricular tachycardia, syncope, symptomatic congestive heart failure, or high-grade atrioventricular block are at high risk for sudden cardiac death.

Though less common than ventricular arrhythmias, atrial arrhythmias are also associated with CS. Both atrial fibrillation and atrial tachycardia have been noted and are due to atrial dilatation or pulmonary involvement. Atrial arrhythmias occur in 15%–20% of patients with CS (Roberts et al., 1977).

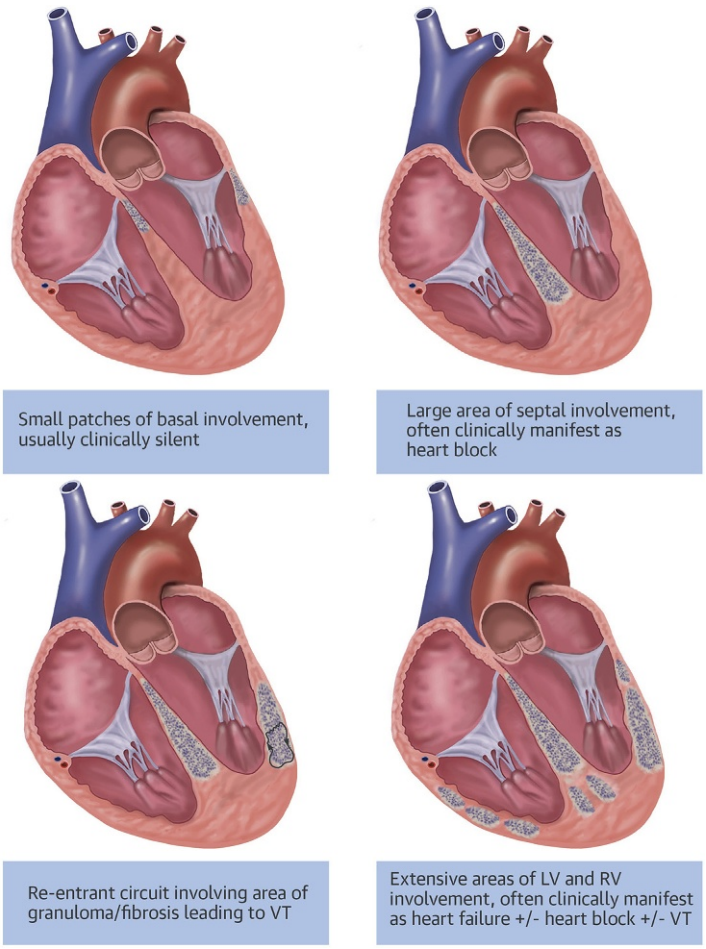

Fig. 1 Clinical manifestations of cardiac sarcoidosis based on myocardial involvement. Reproduced with permission from Birnie, D. H., Nery, P. B., Ha, A. C. and Beanlands, R. S. (2016). Cardiac sarcoidosis. *Journal of the American College of Cardiology* **68**(4), 411–421.

CS can also manifest as congestive heart failure, which accounts for 25%–75% of cardiac deaths (Yazaki et al., 2001). Heart failure can either be diastolic or systolic and is secondary to extensive involvement of the myocardium. During the early inflammatory phase, diastolic dysfunction is more common. In the later fibrotic stages, systolic dysfunction predominates.

Pulmonary hypertension (PH) is another manifestation of CS, and this typically portends a poor clinical outcome. PH may arise as a direct consequence of impaired forward flow and reduced left ventricular function. It may also occur in patients with hypoxic vasoconstriction as alveolar hypoxia can lead to pulmonary vascular remodeling. Finally, PH can be a direct result of infiltration of the vasculature by noncaseating granulomas (Sekhri et al., 2011). The management of PH associated with sarcoidosis is not clear; however, treatment typically involves pulmonary vasodilators, endothelin receptor antagonists, and supplemental oxygen.

Pericardial involvement is relatively uncommon affecting approximately 10% of patients with CS, most of which are asymptomatic (Garrett et al., 1984; Pesola et al., 1987). Though direct valvular involvement is rare, valvular incompetence secondary to papillary muscle dysfunction may occur (Lewin et al., 1985). Ventricular aneurysms, typically resulting from extension of ventricular lesions, can be seen in approximately 10% of patients with sarcoidosis. Furthermore, corticosteroid therapy has been associated with ventricular aneurysms (Lull et al., 1972; Jain et al., 1990). Myocardial aneurysms may lead to complex ventricular arrhythmias; however, these often resolve after resection.

Detection

Sarcoidosis is diagnosed based on clinical symptoms consistent with the disease in addition to noncaseating granulomas seen on biopsy of appropriate tissues. If patients have signs or symptoms suggestive of cardiac involvement, additional testing is required not only to confirm these manifestations are in fact related to sarcoidosis but also to risk stratify patients for specific treatments. However, the diagnosis of CS remains challenging due to nonspecific clinical manifestations and the lack of gold standard diagnostic test secondary to low specificity and/or sensitivity. Furthermore, the current practice of CS diagnosis appears to be insufficient as evidenced by the high prevalence of undetected or undiagnosed CS prior to death.

In keeping with recent recommendations from the Japanese Society of Sarcoidosis and Other Granulomatous Disorders (Table 1), all patients with sarcoidosis should have a detailed history, inquiring about palpitations, presyncope, and syncope, in addition to a thorough physical exam. Moreover, all should be screened with a resting ECG as changes are seen in up to 50% of patients with systemic disease. Any electric abnormality noted on ECG, such as arrhythmia or prolonged QT interval, should be further investigated as it may indicate myocardial involvement. An ECG should be repeated at any time if symptoms develop that suggest cardiac involvement.

Though transthoracic echocardiography lacks the sensitivity for myocardial detection, it helps risk stratify patients with suspected CS based on right and left ventricular function and provides insight into anatomical features (e.g., myocardial thinning) corresponding with CS. Moreover, echocardiography is also helpful in assessing the response of patients with documented ventricular dysfunction to immunosuppressive therapy.

Twenty four hour Holter monitoring should also be considered as it may discover rhythm disturbances not seen on ECG. In patients with sarcoidosis and greater than 100 ectopic ventricular beats per day, Holter monitoring has a reported sensitivity and specificity for detecting CS of 67 and 80%, respectively (Suzuki et al., 1994). Though somewhat controversial, most experts conclude that inducible ventricular arrhythmias should be assessed, and their presence would favor implantation of an implantable

Table 1 Diagnosis of cardiac sarcoidosis

Pathologic diagnosis
Confirmed when noncaseating granuloma seen on endomyocardial biopsy
Clinical diagnosis
If biopsy does not demonstrate noncaseating granulomas, however, the following is true:
1. More than two of four major criteria are satisfied
2. One in four major criteria and more than two in five minor criteria are satisfied
Major criteria
(a) Advanced atrioventricular block
(b) Basal thinning of the interventricular septum
(c) Positive cardiac ^{67}Ga uptake
(d) Depressed ejection fraction of the left ventricle (LVEF $<$ 50%)
Minor criteria
(a) Abnormal ECG findings, including ventricular arrhythmias (ventricular tachycardia, multifocal or frequent premature ventricular contractions), complete right bundle branch block, axis deviation, or abnormal Q-wave
(b) Abnormal echocardiography: regional abnormal wall motion or morphological abnormality (ventricular aneurysm or wall thickening)
(c) Nuclear medicine: perfusion defect detected by ^{201}Tl myocardial scintigraphy or ^{99}Tc myocardial scintigraphy
(d) Gadolinium-enhanced MRI: delayed enhancement of myocardium
(e) Endomyocardial biopsy: interstitial fibrosis or monocyte infiltration over moderate grade

Source: Adapted from Japan Society of Sarcoidosis and Other Granulomatous Disorders.

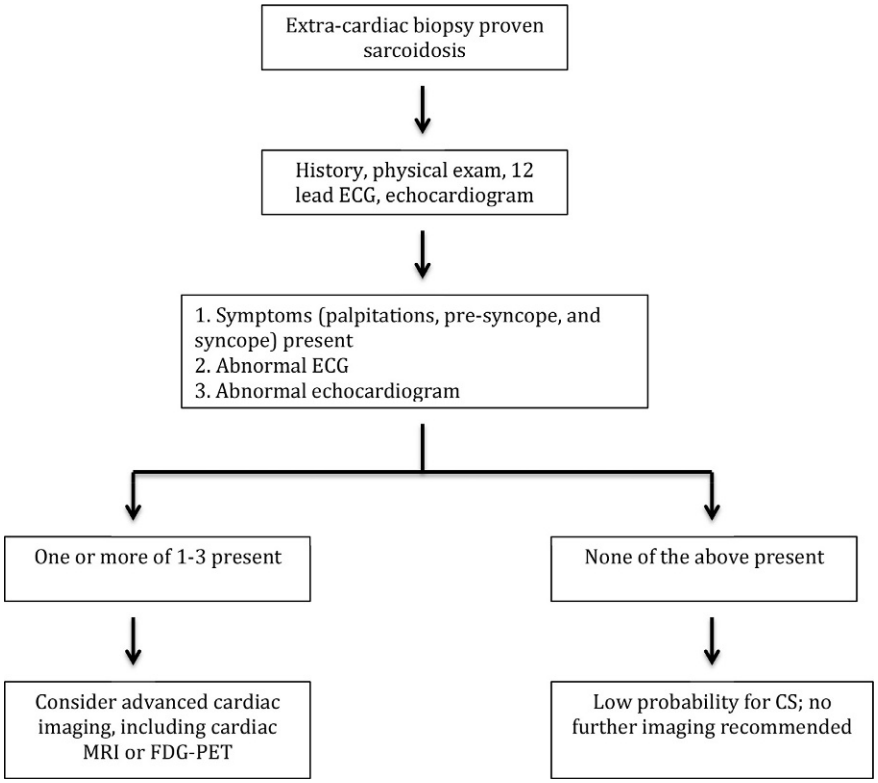

Fig. 2 Algorithm for screening of patients with extracardiac biopsy-proved sarcoidosis. Reproduced with permission from Birnie, D. H., Sauer, W. H., Bogun, F., Cooper, J. M., et al. (2014). HRS expert consensus statement on the diagnosis and management of arrhythmias associated with cardiac sarcoidosis. *Heart Rhythm***11**, 1304–1323.

cardioverter defibrillators (ICD) (Birnie et al., 2014). In combination, ECG, Holter monitoring, and echocardiography are quite sensitive for detecting cardiac involvement; however, they lack specificity for CS.

In summary, per expert consensus on screening for CS with biopsy-proved extracardiac sarcoidosis (Fig. 2), it is a class I recommendation that all sarcoidosis patients be screened for specific symptoms, including presyncope, syncope, and significant palpitations, and should undergo screening with a 12-lead ECG. It is a class IIa recommendation that patients be screened with an echocardiogram and further advanced cardiac imaging, including cardiac MRI (CMR) or FDG-PET (positron emission tomography) scan, if an abnormality is detected on screening ECG, echocardiogram, electrophysiology testing, or based on unexplained cardiac symptoms (Birnie et al., 2014). An abnormal ECG was defined as the presence of at least one of the following: complete left or right bundle branch block, unexplained pathological Q waves in two or more leads, sustained second-degree or third-degree AV block, or sustained or nonsustained ventricular tachycardia. An abnormal echocardiogram was defined as a regional wall motion abnormality, basal septum thinning, left ventricular ejection fraction (LVEF) $<40\%$, or a ventricular wall aneurysm.

Ideally, the diagnosis of CS would rest on confirmatory pathological findings on tissue samples derived from the heart. However, this approach is rarely employed for several reasons. First, due to patchy myocardial involvement, endomyocardial biopsies have low diagnostic sensitivity at $<20\%$ (Uemura et al., 1999). Second, cardiac biopsy is not without risk, and given the low sensitivity of the test and the availability of alternatives serving as surrogates for definitive biopsy, the risk/benefit analysis is not favorable for routine performance of endomyocardial biopsy (EMB) for the diagnosis of CS. However, EMB should still be considered when the diagnosis remains in question to rule out other causes of cardiomyopathy, such as idiopathic dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, giant-cell myocarditis, and viral myocarditis and in situations wherein no other clues to the diagnosis are apparent. In this regard, isolated cases of CS, albeit rare, are well documented (Isobe and Tezuka, 2015). If cardiac biopsy is planned, image guided with CMR or PET scan or using electroanatomical bipolar voltage mapping can improve the diagnostic yield. Though data are limited with regard to yield of EMB using electrogram guidance, it is an approach that allows the physician to differentiate between normal myocardium and areas of active inflammation or chronic fibrosis based on electrogram amplitude (Liang et al., 2014). Further data are needed to determine the role of EMB in the routine diagnosis of CS, particularly isolated CS cases wherein other diagnostic options are limited.

Radionuclide studies can be helpful in patients with clinical or electrocardiographic findings that suggest CS; however, they should not be used to screen patients. Thallium 201 and technetium 99m sestamibi are radionuclides normally absorbed by viable myocardium; however, in areas of inflammation, scarring, or ischemia, they accumulate less. Sestamibi has higher radioactivity than thallium and is therefore more sensitive for CS (Le Guludec et al., 1994; Eguchi et al., 2000). These modalities can be considered as

alternatives to CMR or PET scans in individuals with MRI incompatible implants or with significant renal impairment (those with glomerular filtration rates <30 mL/min are at risk of nephrogenic systemic fibrosis due to gadolinium accumulation) or in whom insurance coverage for a PET scan is not approved.

In CS, granulomatous lesions in the myocardium demonstrate decreased uptake of radionuclide. Improvement or resolution of decreased uptake after dipyridamole or adenosine administration helps differentiate CS from coronary artery disease. Perfusion abnormalities in CS are reversible with pharmacological dilatation due to microvascular vasoconstriction; this phenomenon, though not specific to CS, is called reverse distribution (Fields et al., 1990; Tellier et al., 1988). It is recommended that a baseline resting myocardial scan be performed in addition to scan after exercise or dipyridamole or adenosine administration (Haywood et al., 1982).

With the evolvement of cardiac imaging, CMR has become paramount in establishing the diagnosis of CS. CMR is highly sensitive, low risk, and readily available at most tertiary care centers. Furthermore, CMR is able to differentiate CS from other cardiac processes, and when compared with other imaging modalities, including radionuclide tests, CMR is more than twice as sensitive for detection of CS in patients with biopsy-proved pulmonary sarcoidosis (Smedema et al., 2005). The appearance of CS on CMR depends on the stage of disease. In the early and acute inflammatory stage of CS, myocardial thickening and increased signal intensity on T2-weighted images are often seen, indicating edema (Smedema et al., 2005). In the later chronic stages of CS, characterized by focal areas of scar formation, myocardial thinning and LV dysfunction can be detected with delayed gadolinium enhancement (Smedema et al., 2005). In patients with CS, the presence of delayed gadolinium enhancement was associated with an increased (11-fold) risk of cardiac death (Patel et al., 2009).

PET is another modality used to detect the presence and severity of cardiac involvement. In a meta-analysis, PET-CT was estimated to have a sensitivity of 89% and specificity of 78% for detection of CS (Youssef et al., 2012). PET-CT is a strong alternative to CMR in patients with pacemakers or cardioverter defibrillators that are not MRI compatible. Another advantage of PET-CT is that it is able to detect the active inflammatory stage of the disease and is therefore useful in monitoring and predicting response to immunosuppressive therapy. In contrast, perfusion defects on nuclear imaging and delayed gadolinium enhancement on CMR change minimally with treatment (Treglia et al., 2011). This said, recent data suggest that T2 enhancement on MRI, which presumably represents active inflammation, is reversible to some degree following immune suppression treatment (Crouser et al., 2016).

Finally, cardiac catheterization is indicated to rule out atherosclerotic coronary artery disease in patients with sarcoidosis that present with chest pain, newly diagnosed heart failure, or abnormal imaging. It is important to note that sarcoidosis rarely involves the coronary arteries (Butany et al., 2006).

Treatment

Due to the increased risk of sudden cardiac death, all patients with biopsy-proved or high clinical suspicion of CS should be treated with immunosuppression in order to control inflammation and minimize fibrosis. Glucocorticoids are the initial treatment of choice because the onset of action is rapid and the dose is readily titrated to achieve the desired effect. Studies have shown that long-term glucocorticoid use in patients with normal LVEF may reduce the risk of remodeling. Furthermore, an increase in the LVEF was seen in patients on long-term glucocorticoids and an initial LVEF of <54%; however, in patients with significantly reduced LVEF (<30%), steroid therapy did not improve ventricular function. This suggests that glucocorticoids may have a preventative and therapeutic effect in early and middle stages, at which time active inflammation is present rather, but minimal effect in those with advanced CS, at which point myocardial scarring prevails (Chiu et al., 2005).

The optimal dose of glucocorticoid is not established; however, it is reasonable to start prednisone 60–80 mg daily with a plan to taper to a dose of 10 mg daily over a period of 6 months based upon the clinical response. Given the likelihood that patients will require immunosuppression for months or years, it is reasonable to initiate a steroid-sparing agent during the initial phases of treatment to facilitate the achievement of this goal. If at any time there are signs of recurrence, higher doses of prednisone should be provided; the dose of steroid-sparing agents can be adjusted. Of the steroid-sparing agents, including methotrexate, azathioprine, mycophenolate, and cyclophosphamide (see Table 2), methotrexate is most commonly used. The typical dose of methotrexate is 10–20 mg weekly. Of note, a recent study indicates that the concurrent use of methotrexate with prednisone is superior to prednisone therapy alone and can actually aid in tapering the prednisone dose (Baughman et al., 2000).

Azathioprine is often used if patients fail in methotrexate therapy; the initial dose is 50 mg daily with a target dose of 2 mg/kg daily. Infliximab, a TNF alpha inhibitor, should be considered in patients who have persistent disease despite treatment with glucocorticoids and at least 1 s line immunosuppressive agent. In a randomized, double-blind, placebo-controlled study, infliximab was found to be beneficial in the treatment of extrapulmonary sarcoidosis in patients already receiving corticosteroid therapy (Pacheco et al., 1985; Müller-Quernheim et al., 1999; Baughman and Lower, 2001; Baughman et al., 2006; Rossman et al., 2006; Judson et al., 2008).

Ventricular tachycardia and other arrhythmias are common in patients with CS, and glucocorticoids have not been shown to prevent these. Antiarrhythmic drug therapy for ventricular tachycardia has been associated with a high rate of recurrence or sudden cardiac death. Amiodarone is difficult to use in patients with CS and arrhythmias due to the occurrence of pneumonitis and/or pulmonary fibrosis and the inability to distinguish pulmonary sarcoidosis from medication effect. Beta blockers have been shown to increase the risk of heart block in patients with CS. Given these limitations, ICD and catheter ablation should be considered.

Table 2 Treatment options for cardiac sarcoidosis

All patients with biopsy-proved or high clinical suspicion of cardiac sarcoidosis should be treated
Glucocorticoids: initial treatment of choice
(a) Long-term use may reduce risk of remodeling
(b) Associated with preventative and therapeutic effect in early and middle stages
(c) Typical starting dose of prednisone is 60–80 mg daily with plan to taper to 10 mg daily over a 6-month period
Steroid-sparing agents: used in patients whose disease is refractory to high-dose glucocorticoids, those requiring prolonged glucocorticoid taper, or those unable to tolerate the adverse effects of glucocorticoids
Methotrexate
(a) Most common steroid-sparing agent used
(b) Typical dose starting dose is 10–20 mg weekly
Azathioprine
(a) Often used if methotrexate therapy fails
(b) Initial starting dose is 50 mg daily with target dose of 2 mg/kg daily
Infliximab
(a) TNF alpha inhibitor
(b) Should be considered in patients with persistent disease despite treatment with glucocorticoids and at least 1 s line immunosuppressive agent
Antiarrhythmic drug therapy
(a) Glucocorticoids have not been shown to prevent arrhythmias related to cardiac sarcoidosis
(b) Antiarrhythmic drug therapy is associated with high rate of recurrence or sudden cardiac death
(c) Amiodarone is difficult to use due to medication-related pneumonitis
(d) Beta blockers increase the risk of heart block
ICD implantation
(a) Indicated in patients presenting with sustained ventricular tachycardia or significant LV dysfunction (LVEF $<$ 35%)
(b) Should be strongly considered in patients with mild LV dysfunction (LVEF 35%–50%), RV dysfunction, or high-grade AV block
(c) EP testing may be helpful in patients that are asymptomatic and do not have a high risk of sudden cardiac death
Heart transplantation
(a) Should be considered in patients with refractory heart failure or ventricular tachycardia
(b) Five-year posttransplant survival is $\sim$80%
(c) Sarcoidosis can recur in transplanted patients but is rare

In one study, catheter ablation was successful in terminating clinical or inducible ventricular tachycardia and ventricular storm in the majority of patients. Though VT recurrence after catheter ablation was common, most patients achieved arrhythmia control with repeat ablation and fewer antiarrhythmic medications as compared with patients that did not undergo ablation (Kumar et al., 2015).

ICD implantation is an invasive procedure with potential risks including lead displacement or fracture, inappropriate firing, and infection. In one study, approximately 15% of patients had an adverse event related to ICD implantation for CS (Betensky et al., 2012). Therefore, most experts now agree that not all patients with CS should have an ICD implanted. As per a recent consensus statement (Birnie et al., 2014), an ICD is strongly recommended in patients with spontaneous sustained ventricular arrhythmias and/or reduced LVEF (<35%) despite optimal medical therapy and a period of immunosuppression. An ICD might be helpful in patients with an indication for a permanent pacemaker and/or unexplained syncope or presyncope felt to be arrhythmogenic and/or inducible ventricular arrhythmias. Finally, an ICD should be considered in patients with moderately reduced LVEF (36%–49%) or reduced RVEF (<40%) despite optimal medical therapy and at least 3 months of immunosuppression demonstrating that the arrhythmias are otherwise preventable. A CMR and/or an EP study may be helpful to further risk stratify these patients (Birnie et al., 2016).

Heart transplantation should be considered in patients with refractory heart failure or ventricular tachycardia. One-year survival posttransplant was higher in patients transplanted due to sarcoidosis when compared with all other transplanted patients and 5-year survival is estimated at 80%. Sarcoidosis can recur in transplanted patients; however, the incidence is low, and the recurrence rarely leads to death (Uusimaa et al., 2008; Jefic et al., 2009; Mehta et al., 2011; Zaidi et al., 2007; Valantine et al., 1987; Oni et al., 1992; Diagnostic standard and guidelines for sarcoidosis, 2007).

With the emergence of new imaging modalities, including CMR, CS is being diagnosed more often and earlier than before; however, CS is still often missed or diagnosed in a delayed manner and can present tragically as sudden cardiac death. Furthermore, despite a growing pool of clinical evidence from large retrospective studies, there is a lack of prospective data relating to the course of the disease in response to various treatments, such that the optimal management of CS remains uncertain. Given the intricacies of the disease, a multidisciplinary approach to the patient should be utilized, and standards of care should be established. To this end, it is useful to follow the consensus guidelines provided for the diagnosis (Fig. 2, Table 1) and treatment of CS (Table 2). It is recognized that additional evidence derived from well-designed prospective clinical trials will be required to arrive at more definitive recommendations guiding the diagnosis and optimal management of CS.

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Management of Patients With Implantable Cardiac Devices Referred for Magnetic Resonance Imaging: A Rapidly Changing Landscape

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Introduction

The role of magnetic resonance imaging (MRI) in patient care has greatly expanded over the past several decades, providing unique value across a wide range of illness. Concurrently, the prevalence of implantable cardiac devices has increased and presents a challenge given that such devices have been conventionally been considered a contra-indication to this technique. However, substantial knowledge has been gained, both contextualizing theoretical concerns and migrating technology toward an era of MRI-compatible devices.

In this article we: (i) highlight the potential challenges of performing MR imaging in patients with implantable cardiac devices, (ii) describe contemporary rates of complications among those same patients undergoing MRI, (iii) summarize protocols to safe performance of MRI in these patients, and (iv) summarize the current status of MRI-compatible devices.

Potential Challenges of MRI Scanning in Patients With Implanted Cardiac Devices

Three broad categories of technical challenges encountered when introducing implantable cardiac devices into an external magnetic field are as follow: mechanical effects, device heating, and other electromagnetic effects. (Ainslie et al., 2014; Miller et al., 2016) Contributing to these adverse effects are magnetic fields and radiofrequency energy emitted by MRI systems.

Mechanical Effects

Mechanical effects on devices arise mainly from the static magnetic field of the MRI system. Static fields, as their name implies, do not vary with time. Importantly, these static magnetic fields are always considered to be "on" for all clinical MRI units as scanners must be maintained in their magnetized state. Current clinical MRI scanners range in strength from 1.5 to 3.0 tesla (T). 1 T corresponds to approximately 10,000 G, a unit of measure that can be compared to the earth's magnetic field which is approximately 0.5 G. Thus, standard contemporary clinical MRI machines have a magnetic field that is approximately 30,000–60,000 times stronger than the Earth's magnetic field (Levine et al., 2007). This static magnetic field can result in ferromagnetic objects (i.e., those with intrinsic magnetic field induced when exposed to an external magnetic field) gaining significant kinetic energy while exposed to the magnet. Ultimately this can lead to objects being pulled into the magnet "bore" leading to potential injury to patients and/or MRI staff, a phenomenon known as the "projectile" or "missile" effect (Levine et al., 2007; Yilmaz and Misirlioglu, 2013; Hartwig et al., 2009; Jerrolds JaK, 2009; Gowland, 2005). Alternatively, for implanted ferromagnetic material, this energy may result in device repositioning or dislodgment or may transfer energy to adjacent tissue resulting in trauma (Shellock et al., 1993). The latter concern is primarily focused upon vascular clips, particularly intracranial, where small displacement movement may lead to intracranial hemorrhage (Kanal and Shellock, 1993).

While theoretical concerns for mechanical effects posing risk for the scanning of implanted cardiac devices, these have not proven to be of major concern in clinical practice. While static magnetic fields can produce linear (translational) or rotational (torque) forces on the ferromagnetic components of implanted cardiac devices, these forces are typically insufficient to result in local trauma or device malposition.

Heating

During MRI scanning, a pulsed radiofrequency energy is transferred to the patient at the resonant frequency of protons (typically), this frequency dependent upon the applied field strength. While the primary aim is to alter the net magnetic vector of precessing protons in order to generate signal (also in the radiofrequency range), some of this energy is converted to heat (Ainslie et al., 2014; Levine et al., 2007; Loewy et al., 2004; Achenbach et al., 1997; Gimbel et al., 2005a; Gimbel, 2009; Fontaine et al., 1998). Quantification of this absorbed radiofrequency energy is described as the specific absorption rate (SAR), a value representing the RF power absorbed per unit mass of tissue and is expressed in watts per kilogram (W/kg) and inherently being greater at higher field strengths (Levine et al., 2007). Both the FDA and International Electrotechnical Commission (IEC) provide SAR limit recommendations that range from 2 W/kg in the brain to 20 kg/W in the extremities. However, most scanners are preset to a maximum of 4 W/kg for routine clinical imaging. For patients with implanted cardiac devices SAR levels should, in general, not exceed 2 W/kg for body imaging and 3.2 W/kg for head imaging. As SAR increases approximately fourfold from 1.5 to 3 T, this poses challenges for maintenance of such levels at higher field strengths and therefore 1.5 T is generally preferred.

The transfer of radiofrequency energy to ferromagnetic devices, particularly device leads and wires, is commonly described as the "antenna effect." This energy can theoretically lead to local tissue heating, injury, and fibrosis, which may alter pacing thresholds (Miller et al., 2016; Sommer et al., 2006). Fortunately, device generators innately act as a "sink" to absorb significant amounts of energy. However, this means that abandoned leads (or fractured leads) are especially susceptible to heating and are considered an absolute contraindication to MRI (Langman et al., 2011).

Other Electromagnetic Effects

Electromagnetic effects also occur from nonstatic, gradient magnetic fields created by rapidly turning on and off (often referred to as "switching") gradient coils (Levine et al., 2007). These changing gradients can induce electrical currents within leads that have the potential to invoke arrhythmias (Levine et al., 2007). This is particularly problematic for old unipolar leads which effectively transmit currents, and may result in life-threatening arrhythmias (Verma et al., 2014). Further, the same mechanism can lead to over- and/or undersensing by pacemakers and ICDs through misinterpretation of induced current as native cardiac rhythm. Oversensing can lead to the erroneous "detection" of ventricular arrhythmias leading to inappropriate ICD therapy, while undersensing may lead to an inability to detect true arrhythmia (Verma et al., 2014). Invoked currents within the pulse generator may also lead to power-on-reset events or trigger activation of the reed-switch, leading to asynchronous pacing for permanent pacemakers and the inability to detect tachycardia for ICDs (Miller et al., 2016; Roguin et al., 2004). Finally, electromagnetic interference during imaging can lead to the production of artifacts and their subsequent inappropriate tracking. Ultimately, this may culminate in inappropriate therapy (Ainslie et al., 2014; Miller et al., 2016; Nazarian et al., 2013; Nazarian et al., 2006).

Defining Risk in the Real World—MRI Scanning With "Noncompatible" Pacemakers or ICDs

While the aforementioned concerns led to initial trepidation regarding the scanning of patients with "noncompatible" implantable cardiac devices, many have accepted such theoretical risks in the face of clinical need, resulting in a significant amount of observational data over the past decade. Importantly, all reported data have been from imaging performed at major centers following strict periprocedural protocols, as are outlined later in this article.

Table 1 provides a summary of these studies, the vast majority being reported at 1.5 T. The first study was performed by Martin et al. (2004). They prospectively studied 63 patients with permanent pacemakers (49 dual chamber and 14 single chamber) undergoing MRI examinations at 1.5 T across a variety of indications, including cardiac, vascular, and general body MRI. Overall, 9.4% of the systems studied demonstrated an alteration in pacing threshold following MRI. However, only 1.9% of such cases required reprogramming of output to maintain capture and no other complications were noted (Martin et al., 2004). Within a year, studies emerged describing similar procedures performed in patients with ICDs, also suggesting no significant alteration in pacing, sensing, impedances, charge times, or battery status. However, one patient experienced a "power-on-reset" of his ICD without permanent device malfunction (Gimbel et al., 2005b). Nazarian et al. subsequently evaluated 55 patients (undergoing 68 MRI studies), 31 having a permanent pacemaker, and 24 an ICD. No episodes of inappropriate inhibition or activation of pacing were observed. There were no significant differences between baseline and immediate or long-term sensing amplitudes, lead impedances, or pacing thresholds (Nazarian et al., 2006). Since these sentinel studies, numerous additional reports have confirmed low rates of complications related to scanning devices at 1.5 T MRI. To date, approximately 1100 patients undergoing 1.5 T scanning have been studied with an overall device complication rate of approximately 3.5% (**Table 1**). The vast majority of these cases did not result in patient-related outcomes and were not related to mechanical forces or device heating. Rather, the majority were alterations in system performance due to "other electromagnetic effects" (see section "other electromagnetic effects") (Miller et al., 2016; Verma et al., 2014).

To elaborate on some of these rarely reported complications, several studies warrant highlighting. Sommer et al. examined 82 pacemaker patients in a prospective study and did identify a statistically significant rise in mean pacing capture thresholds from pre- to post-MRI interrogations. Further, in one case a mild, asymptomatic rise in serum troponin was observed (troponin I pre-MRI 0.02 ng/mL to post-MRI 0.16 ng/mL). Both the rise in mean pacing capture threshold and the troponin elevation were not felt to be

Table 1 Summary of studies examining safety of MRI scanning in nonconditional pacemakers and ICDs at 1.5 T or 3 T field strengths as of January 2017

Study	*Number patients*	*Included devices*	*Field strength (Tesla)*	*Adverse events*
(Boilson et al., 2012)	32	Dual chamber PPM ($N=28$) Single chamber PPM ($N=3$) CRT ($N=1$)	1.5	Power-on-reset ($n=5$)
(Buendia et al., 2010)	33	PPM ($N=28$) ICDs ($N=5$)	1.5	Oversensing due to electromagnetic artifact ($n=2$) Safety signal (VOO pacing at maximal frequency and output during the MRI scan) ($n=1$)
(Burke et al., 2010)	38	PPM ($N=24$) CRT-D ($N=4$) ICD ($N=10$)	1.5	None
(Cohen et al., 2012)	109	Single, dual, and biventricular PPMs and ICDs	1.5	Reduced battery voltage ($n=5$) Lead impedance changes ($n=7$) Increase in pacing threshold ($n=4$)
(Del Ojo et al., 2005)	13	Dual chamber PPM	2.0	None
(Gimbel et al., 2005b)	7	ICDs	1.5	Increase in pacing threshold ($n=7$)
(Halshtok et al., 2010)	18	PPM ($N=11$) ICDs ($N=7$)	1.5	Power-on-reset ($n=2$)
(Martin et al., 2004)	63	Dual chamber PPM ($N=49$) Single chamber PPM ($N=14$)	1.5	Change in lead parameters or thresholds ($n=5$)
(Mollerus et al., 2008)	37	PPM ($N=31$) ICD ($N=5$) CRT ($N=1$)	1.5	None
(Mollerus et al., 2009)	52	PPM ($N=42$) CRT ($N=4$) ICD ($N=6$)	1.5	Asymptomatic ventricular ectopy during scanning ($n=4$) [No significant complications]
(Burke et al., 2010; Mollerus et al., 2010)	103	PPM ($N=81$) ICD ($N=22$)	1.5	None
(Naehle et al., 2009)	47	Single chamber PPM ($N=14$) Dual chamber PPM ($N=33$)	1.5	None
(Naehle et al., 2008)	44	PPM (single and dual chamber)	3.0	None
(Nazarian et al., 2006)	55	PPM ($N=31$) ICD ($N=24$) CRT (N-7)	1.5	None
(Nazarian et al., 2011)	438	PPM ($N=237$) ICD ($N=201$)	1.5	Power-on-reset ($n=2$)
(Pulver et al., 2009)	11	Single chamber PPM ($N=5$) Epicardial leads ($N=6$)	1.5	None
(Sommer et al., 2006)	82	PPM (single and dual chamber devices)	1.5	Increase in pacing threshold ($n=1$)
Total	1182			41 (3.5%)

clinically significant by the attending electrophysiologists (Sommer et al., 2006). Boilson et al. examined 32 patients undergoing spine or head imaging in a prospective cohort study. Of these, 28 had dual chamber pacemakers, 3 single chamber pacemakers, and 1 had a biventricular pacemaker implanted. Five patients had episodes of "power-on reset." However, no significant changes in battery voltage, lead impedance, thresholds, or pacing functions were noted following MRI or at 1-month follow-up (Boilson et al., 2012). Buendia et al. also performed a prospective study of electrical and clinical parameters before and after an MRI in 33 patients, 38 with pacemakers, and 5 with ICDs. There were minor electrical changes reported after the MRI. MRI-induced artifact was interpreted as atrial fibrillation in one case and ventricular fibrillation in the other. In both cases, there was no therapy provided by the device as the antitachycardic pacing and defibrillating functions were turned off prior to the scan. Another device, a DDDR pacemaker, began to pace in VOO mode at maximum frequency and output during the MRI scan. After the MRI scan had been completed, the device was reprogrammed to its prior settings. Of note, none of the observed adverse effects resulted in clinically meaningful irreversible changes in PM or ICD function. Further, there were no clinical adverse events that occurred as the result of the MRI scanning (Buendia et al., 2010).

Cohen et al., utilizing a retrospective chart review study design, examined 109 patients with noncompatible devices undergoing 1.5 T MRI. This group was compared to 50 patients with devices who did not undergo MRI. No differences in device-related events

were seen, although statistically significant changes in battery voltage occurred in 4% of patients, pacing thresholds in 3%, and pacing lead impedance in 6%. While statistically significant differences were observed between the intervention and control groups in the earlier-mentioned parameters, these differences were deemed to not be clinically significant by electrophysiologists (Cohen et al., 2012).

A large study examining the safety of devices in MRI scanning was performed by Nazarian et al. in 2011. In this prospective, multicenter study a total of 438 patients (54% pacemakers, 46% ICDs, and 12% being biventricular systems) were prospectively followed for adverse events after undergoing 1.5 T MRI scanning of various regions including in the brain, heart, spine, abdominal, and pelvic. Importantly, in this study no SAR limits were mandated. All patients underwent device interrogations and clinical visits before and following scans, with a median time to follow-up of 214 days (IQR, 64–478 days). Early outcomes registered three patients having "power-on-reset" events with temporary reversion to back-up asynchronous pacing. One study was terminated due to the patient experiencing mechanical forces from the device. Long-term follow up reported reductions in median battery voltage, RV sensing, and RV lead impedance with an increase in median RV capture thresholds. None of these changes were deemed of clinical importance or even sufficient to require device reprogramming (Nazarian et al., 2011).

The largest examination to date of device safety in MRI environments was provided by the MagnaSafe Registry (www.magnasafe.org), recently publishing its results from 1500 patients (1000 pacemaker, 500 ICD) at 21 US sites undergoing nonthoracic imaging at 1.5 T (Russo et al., 2017). This landmark study confirmed successful imaging in these patients with no adverse events occurring during imaging (inclusive of death, lead failure, capture loss, or ventricular arrhythmia) and only six patients having partial power-on reset of their pacemakers. One patient with an ICD experiencing a device malfunction requiring replacement, however, was related to battery depletion due to incorrect programming prior to scanning.

Overall, based on cumulative published evidence to date in over 1200 patients, the risk of device malfunction or requirement for re-programming following a single MRI scan, performed under controlled conditions at 1.5 T, is very low (approximately 3.5%), and is associated with no significant risk of major clinical adverse events.

The New Era of MRI Conditional Devices

Recognizing a necessity to accommodate for expanding MRI demands in patients with a variety of illness, substantial efforts focused on the development of devices compatible with MRI environments. This goal has been achieved by the majority of device vendors through focused efforts to reduce ferromagnetic material, replace components sensitive to rapidly alternative gradient magnetic fields, and integrating dedicated software to protect patient and device.

With respect to lead design, ferromagnetic steel components have been replaced by nickel and copper alloys with the use of platinum lead tip electrodes. Geometric changes, for example, in lead parameters such as area and length, have also resulted in net reductions in heating risk (Ainslie et al., 2014; Miller et al., 2016; Verma et al., 2014; Kalin and Stanton, 2005; Sutton et al., 2008).

A reduction in pulse generator size has inherently reduced the net mass of ferromagnetic material in devices and reduced the potential of both mechanical forces and heating in applied magnetic fields. These changes reduce the amount of heat generated within the generator and also reduce electromagnetic interference (Verma et al., 2014; Roguin et al., 2004). Incremental benefit is realized through a reduction of field-related image artifacts, improving the capacity to maintain diagnostic image quality in regions close to the generator, such as the great vessels, atria, and right ventricle.

Reed switches have been replaced by Hall effect sensors: transducers that modify their voltage output based upon the ambient magnetic field. This prevents the induction of asynchronous pacing that was described with conventional Reed switch-based devices in MRI environments (Verma et al., 2014; Cronin and Wilkoff, 2012).

Finally, significant advancements in pacing software and circuitry have been implemented to improve filtering of MRI-generated signal, provide circuit protection, and enhance heat dissipation. Pacemaker modes (e.g. "MRI-mode") have been introduced to deactivate advanced pacing and defibrillation features during the scan while allowing easy postimaging reactivation. A recognized consequence of this is that patients are considered "unprotected" when in this mode and require monitoring during scanning with external defibrillators or "crash carts" readily available to a supervising physician. It is also therefore recommended that patients not be pacemaker dependent.

While recommendations for imaging noncompatible implantable cardiac devices using MRI have been previously published, (Miller et al., 2016; Verma et al., 2014) we provide in **Fig. 1** a summary outlining a standardized algorithm for minimizing patient and device complications. Given limited experience of imaging noncompatible devices at higher field strengths, these recommendations are specific to hardware operating at a 1.5 T field strength and SAR levels $\leq$2 W/kg.

Currently Available MRI-Conditional Devices

The American Society for Testing and Material certifies a device as MRI conditional if it does not demonstrate any hazards under prespecified imaging conditions. Of note, there may be jurisdictional differences in terms of this approval. While it is our aim to provide a comprehensive list of MRI compatible devices (see **Table 2**), we cannot guarantee its completeness given rapid evolutions in this field. We therefore suggest contacting the manufacturers in order to determine the most up to date recommendations for specific devices.

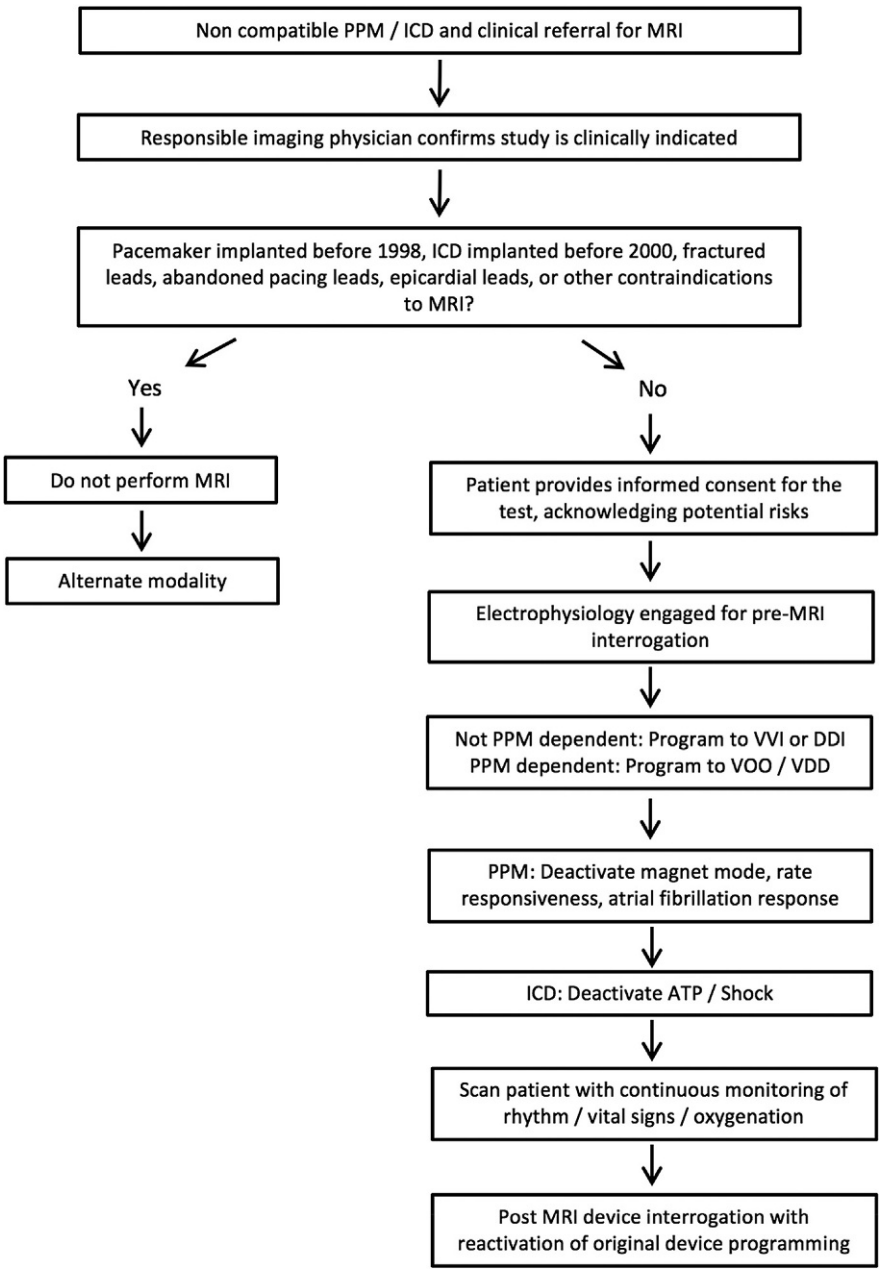

Fig. 1 Recommendations for maximizing safety surrounding MRI imaging of patients with noncompatible cardiac devices. *PPM*, permanent pacemaker; *ICD*, implantable cardioverter defibrillator; *ATP*, anti-tachycardia pacing. Adapted from Miller, J. D.; Nazarian, S.; Halperin, H. R. Implantable Electronic Cardiac Devices and Compatibility With Magnetic Resonance Imaging. *J. Am. Coll. Cardiol.* **2016**, *68*, 1590–15908

Conventional (Lead) Pacemaker Systems

Medtronic delivered the first commercial pacing system with approval in Europe in 2010 and subsequent US Food and Drug Administration (FDA) approval for 1.5 T scanning. This dual-chamber capacity system, called the EnRhythm, MRI™ SureScan™ pacing system was validated in a randomized controlled trial published in 2011 by Wilkoff et al. (2011) Only minor alterations in pacing thresholds were observed with no adverse events following MRI. However, limitations were placed upon this device to prevent imaging within the C1 to T12 vertebral zone (region of pulse generator). Subsequently, Medtronic received FDA approval for the Advisa MRI™ SureScan™ system that lifted such restrictions and was similarly validated in a trial by Gimbel et al. (2013) As with the EnRhythm™ system, no adverse events were identified and only minor alterations in pacing thresholds were observed following MRI imaging.

Table 2 List of MRI conditional devices (pacemakers and ICDs) as of January 2017

Device	*MRI field strength (T)*	*Manufacturer*
Lead pacemakers		
EnRhythm/Advisa MRI Surescan Pacing System	1.5	Medtronic Inc.
Entovis ProMRI Cardiac Pacing System	1.5	Biotronik Inc.
KORA 250 MRI conditional pacemaker	1.5	Sorin Group Inc.
Implantable cardioverter defibrillators leadlesspacemakers		
Evera ICD	1.5	Medtronic Inc.
Iforia 5 & 7 series ICD	1.5	Biotronik Inc.
Lumax 740 Series implantable ICD	1.5	Biotronik Inc.
EMBLEM MRI S-ICD (subcutaneous ICD) system	1.5	Boston Scientific Inc.
Leadless pacemakers		
Micra leadless pacemaker	1.5, 3.0	Medtronic Inc.
Nanostim leadless pacemaker[a]	1.5	St. Jude Medical Inc.

[a]As of October 28, 2016 implants of the Nanostim leadless pacemaker were paused by the manufacturer due to concerns of battery malfunction. No update was available at the time of this review.

A similar pacemaker device, available from Biotronik called the Entovis® ProMRI Cardiac Pacing System, was validated for safety at 1.5 T for imaging of the head and lower lumbar spine. This study reported the freedom from serious device adverse effects to be 99.6% (one adverse event possibly related to MRI; pericardial effusion requiring lead repositioning) with freedom from atrial and ventricular threshold increase being 100% at study completion. Further, freedom from P and R wave amplitude changes were 98.2% and 100%, respectively (Bailey et al., 2016).

Leadless Pacemaker Systems

Rapid growth in leadless pacemaker technology is anticipated over the next decade. All such devices are being developed with MRI compatibility in mind, yet are innately less challenged by external magnetic fields. The latter is owing to their small mass and lack of leads, both of which reduce the potential for heating and current generation.

Two leadless systems, Micra® by Medtronic, and Nanostim® by St Jude Medcial, are currently approved by the FDA, and both are MRI-compatible at 1.5 T. The Micra® system is also approved for imaging at 3 T. Neither device has restrictions placed on the imaging region. Both vendors provide clear recommendations for scanning parameters that should be adhered to comply with FDA labeling.

The safety of MRI imaging in patients with leadless pacemakers was recently reported via modeling data for the Micra leadless pacemaker (Soejima et al., 2016). These models evaluated potential MRI risks including device heating, torque and movement, and device malfunction. In addition, assessments of MRI-induced device heating were conducted using a phantom followed by simulations using various combinations of human body models, device positioning, and coil designs. Overall, this study reported no significant device-related changes or anticipated secondary effects related to device heating or mechanical force. The authors also presented a clinical case study where no adverse effects were identified with MRI scanning of this device (Soejima et al., 2016).

Implantable Cardioverter Defibrillators (ICDs)

Several MRI-compatible ICDs have been approved for clinical use by the FDA. Medtronic's Evera® ICD is compatible at a 1.5 T field strength when combined with MRI compatible leads and was found in a randomized controlled trial to demonstrate noninferior safety and efficacy data (Gold MRS et al., 2015). In this study, 275 patients from 42 centers received either a single- or dual-chamber ICDs and were randomized (2:1) to receive MRI scanning at 1.5 T of chest, cervical, and head regions to a maximal SAR of 2 W/kg. No patients experienced the primary MRI-related adverse outcome (ventricular arrhythmia or device complication within 30 days of scan) and all devices performed appropriately in follow-up for observed tachy-arrhythmias.

Biotronic's ProMRI Iforia® ICD system was tested for safety endpoints in patients undergoing cardiac and thoracic spine imaging at 1.5 T. In this study, 170 patients were enrolled in 39 centers across the United States. 169/170 had freedom from predetermined endpoints (ventricular pacing threshold increases >0.5 V, R wave amplitude decrease $>50\%$ after the MRI scan or significant adverse device effects). One patient had reduced R wave amplitude which was not felt to be of clinical importance (Awad et al., 2015).

Subsequently, another Biotronik ICD system (Lumax) was studied in a single center study consisting of 16 patients. (Kypta et al., 2016) Study endpoints were the absence of (1) serious adverse device events (SADE), or (2) ventricular pacing threshold increase >0.5 V, or of an R-wave amplitude attenuation $<50\%$, or (3) R-wave amplitude <5.0 mV at 1-month follow-up. Freedom from the study endpoints was 100% at 1 and 3 months post MRI scan for all three endpoints.

Finally, Boston Scientific has released the EMBLEM® MRI S-ICD, a subcutaneous ICD system approved for imaging at 1.5 T. This unique design maintains complete independence from leads contacting the cardiovascular system, implanted only within the subcutaneous tissue of the thorax.

Table 3 List of MRI conditional implantable loop recorders (ILRs) as of January 2017

Reveal XT and DX implantable loop recorders	1.5, 3.0	Medtronic Inc.
SJM confirm implantable cardiac monitor (certain models)	1.5	St. Jude Medical Inc.

Safety of MRI Scanning of Implantable Loop Recorders (ILRs)

Similar to leadless pacemaker systems, owing to low mass and lack of lead components, ILRs pose low risk for mechanical and heating related complications. Two commonly used ILRs (Medtronic's Reveal and St. Jude's medical's SJM Confirm) have each been tested and approved for MRI conditional use following clinical testing showing no clinical adverse events (**Table 3**) (Miller et al., 2016). Of note, while scanning is safe, artifact mimicking arrhythmia is common. Therefore, it is important to collect all data prior to imaging and clear device recordings immediately following MRI scanning to avoid misdiagnoses of arrhythmia (Gimbel et al., 2005c).

Conclusions

While migration toward MRI-compatible implantable cardiac devices is underway, a comprehensive knowledge of acceptable versus unacceptable risk surrounding MR imaging, and available approaches to mitigate such risk, is required for both imagers and cardiovascular specialists. With independently expanding clinical indications for MRI and device therapy within our aging population, equally expanded attention to appropriate imaging protocols and device monitoring will inherently be required.

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Management of Pregnancy With Underlying Congenital and Acquired Cardiac Disease

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Introduction

This article will review the general principles in the management of pregnancy in women with both congenital heart defect (CHD) and acquired heart disease (AHD). As survival in women with congenital and AHD improves, more women of childbearing years are considering having a family and as a consequence face many issues related to pregnancy. CHD is now the predominant form of heart disease encountered during pregnancy in developed countries (Pitkin et al., 1990) in contrast to rheumatic heart disease in developing countries. While CHD does not preclude pregnancy in most cases, it does increase the risk to both the mother and fetus, requiring specialized knowledge of the burden of pregnancy and appropriate management of such patients.

Epidemiology

Heart disease complicates less than 1% of all pregnancies, but this number is increasing. In a UK study, a quarter of women who died during pregnancy or in the first 6 weeks after delivery died from a cardiovascular cause (the leading cause of death) (Knight et al., 2016). Interestingly, more than half the deaths were deemed to be due to substandard care—highlighting the need for a greater understanding of the burden of pregnancy on the heart. While cardiac death in pregnancy is rare, the main causes have been similar for years: peripartum cardiomyopathy, myocardial infarction, aortic dissection, and pulmonary hypertension. Morbidity is much more common.

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In developing countries, rheumatic heart disease is the predominant lesion not congenital anomalies. In developed countries, women present with both congenital and acquired cardiac conditions. Congenital heart disease occurs in 0.8% of live births, and 85% of these patients now reach adulthood. Success of cardiac surgery has led to an increase in the number of women presenting for obstetric care. As surgeries have improved, this has reduced the maternal and fetal risks associated with pregnancy and has increased the underlying fertility rate in such women. That said, CHD remains a significant source of mortality and morbidity risk for women. In a registry from the European Society of Cardiology covering 28 countries and 1321 patients, CHD was the most prevalent form of structural heart disease (66%) affecting pregnancy worldwide (Roos-Hesselink et al., 2013). Furthermore, the precise impact of multiple pregnancies on long-term outcome is as yet unclear but probably varies depending on the underlying lesions. Arrhythmias are the most common acquired problem seen. Coronary disease may be seen in older, obese women who smoke and have increased rates of diabetes. Historically, myocardial infarction in pregnancy has been due to coronary dissection, but the rates of atheromatous disease are increasing.

In a review of maternal morbidity from 1985 to 2006, in 2491 pregnancies, cardiac complications were documented in 11% of completed pregnancies, with heart failure in 5% and arrhythmias in 4.5%. Patients with Eisenmenger syndrome and palliated or unrepaired cyanotic heart disease were at highest risk of myocardial infarction, stroke, and cardiovascular mortality(Drenthen et al., 2007). A more extensive report in the United States from 1995 to 2006 revealed that the prevalence of deliveries complicated by chronic heart disease was stable at 1.5%. However, during the same period, the frequency of specific CHD, arrhythmias, cardiomyopathy, heart failure, and severe complications had increased (Kuklina and Callaghan, 2011), suggesting that the rate of maternal morbidity will continue to increase over the coming years.

Hemodynamic Changes During Pregnancy, Labor, and Delivery

A clear understanding of the hemodynamic changes associated with pregnancy will allow for better management of patients with underlying cardiac abnormalities. In general, systemic vascular resistance falls, and there is an increase in cardiac output (Regitz-Zagrosek et al., 2011). Absorption, excretion, and bioavailability of drugs are also altered (Anderson, 2005). Management of specific lesions is beyond the scope of this article although the issue of anticoagulation is addressed in some detail.

Red blood mass increases 20%–30%, and plasma volume increases 30%–50%. This usually begins at 12–14 weeks' gestation and peaks at 32–34 weeks. As a consequence, there is an increase in total blood volume with the development of relative anemia (Elkayam and Gleicher, 1998). Cardiac output increases by 30%–50% during pregnancy again peaking around 32 weeks. In early pregnancy, the increase in cardiac output is related to a rise in stroke volume; in late pregnancy, heart rate is the primary driver. Heart rate starts to rise at 20 weeks and increases until 32 weeks. It remains higher until 2–5 days after delivery. With increasing metabolic needs of the mother and the fetus, oxygen consumption rises by approximately 30%. During late pregnancy, the physical size of the fetus affects cardiac output due to compromised venous return. Positioning in a left lateral decubitus position will alleviate that to some degree. In those patients with myocardial dysfunction or stenotic valves such adaptations are compromised leading to congestive heart failure signs and symptoms. These patients frequently start to present with complaints from 18 to 22 weeks.

Systemic BP usually falls early in gestation, and diastolic BP is usually 10 mmHg below baseline in the second trimester. This is in part due to local mediators such as prostacyclin and nitric oxide. In the third trimester, diastolic BP returns to prepregnancy levels. The heart size can increase by 30%. Data on myocardial systolic and diastolic function are limited, but changes in both have been reported (Mone et al., 1996; Moran et al., 2002).

Pregnancy is associated with an increased incidence of thrombosis. Factors influencing that include—lower extremity stasis due to inferior vena cava compression and a hypercoagulable state due to increases in vitamin K-dependent clotting factors and a reduction in protein S activity. Patients with congenital heart disease appear to have a 40-fold increase in thrombosis risk compared with normal pregnancies (Drenthen et al., 2013; Toglia and Weg, 1996). This is of particular importance in patients with prosthetic valves, atrial arrhythmias, Fontan surgery, and prior thromboembolic events.

Normal labor and delivery also lead to changes in cardiac output. There is up to a further 50% increase in cardiac output during the first stage of labor into second stage (Robson et al., 1987). The documented increase in blood pressure and heart rate during labor reflects this. Systolic and diastolic BP can increase during uterine contractions by 15%–25% and 10%–15%, respectively, elevating ventricular afterload. An increase in venous return with the release of vena caval obstruction and from uterine contraction (300–500 mL) is also noted. If postpartum hemorrhage occurs, volume shifts can be seen. Administration of epidural anesthesia can reduce cardiac output, with general anesthesia reducing cardiac output even further. Blood loss at delivery will also impact on hemodynamics. It is estimated that 500 mL of blood is lost at the time of vaginal delivery and 1000 mL during a normal cesarean section. After delivery, venous return increases, and spontaneous diuresis occurs during the first 24–48 h. It takes 2–8 weeks for hemodynamics to return to normal.

Risk Stratification

Maternal Risk Stratification

To date, there are three scoring systems available for maternal risk stratification—the modified WHO classification, the CARPREG score, and the ZAHARA scoring system. All three provide insights into at-risk women and what issues should be assessed when such

women present to the cardiologist for preconception counseling or when pregnant. A prospective study of patients with congenital heart disease (203 women) reported maternal events in 10% of pregnancies. The study found that the modified WHO system performed best with an area under the cover (AUC) level of 0.77. The CARPREG system performed no better than chance with an AUC score of 0.57 (Balci et al., 2014). More recently, the use of biomarkers has been studied (N-terminal pro-B-type natriuretic peptide, NT-proBNP) and has been shown to help with maternal risk stratification (Kampman et al., 2014). An NT-proBNP greater than 128 pg/mL at 20 weeks' gestation in patients with congenital heart disease was independently associated with an increased incidence of cardiovascular events (10.3%, odds ratio of 10.6). A level less than 128 pg/mL had a negative predictive value of 96.9%.

Modified WHO classification

The modified WHO classification groups conditions into four classes based on mortality and morbidity risk for the mother. This has been adopted by the European Society of Cardiology and is central to their guidelines on the management of such patients (Regitz-Zagrosek et al., 2011; see **Table 1**).

Class I conditions have no increase in mortality with no or mild increase in morbidity. Class II conditions carry a small increase in mortality or moderate increase in morbidity. Class III conditions carry a significant increase in mortality or severe increase in morbidity. Finally, class IV conditions carry such an extremely high maternal mortality risk or severe morbidity risk that pregnancy is essentially contraindicated. Conditions classed as WHO class IV would include severe mitral stenosis, symptomatic severe aortic stenosis, bicuspid aorta with an aortic diameter >50 mm, Marfan syndrome with an aortic diameter >45 mm, severe LV systolic dysfunction (EF <30%, NYHA III–IV), native severe coarctation, and significant pulmonary hypertension (systolic pulmonary artery pressure >25 mmHg at rest or >30 mmHg with exercise).

ZAHARA score

The ZAHARA score is a risk score derived from weighting of factors that are used to predict adverse maternal cardiac events. It is derived from a retrospective observational study of 1302 completed pregnancies in women with congenital heart disease (Drenthen et al., 2010). Arrhythmias (4.7%) and heart failure (1.6%) were the most prevalent complications. Factors associated with maternal cardiac complications included cyanotic heart disease, cardiac medications used prior to pregnancy, left heart obstruction,

Table 1 Modified WHO classification of maternal cardiovascular risk (Regitz-Zagrosek, 2011; Thorne et al., 2006)

Conditions in which pregnancy risk is WHO I
- Uncomplicated, small, or mild
  - Pulmonary stenosis
  - Patent ductus arteriosus
  - Mitral valve prolapse
- Successful repair of simple lesions (ASD, VSD, PDA, PAPVR)
- Atrial or ventricular ectopic beats, isolated

Conditions in which pregnancy risk is WHO II or III

WHO II
- Unoperated atrial or ventricular septal defect
- Repaired tetralogy of Fallot
- Most arrhythmias

WHO II–III (depending on patient)
- Mild left ventricular impairment
- Hypertrophic cardiomyopathy
- Native or tissue valvular heart disease not considered WHO I or II
- Marfan syndrome without aortic dilation
- Aorta <45 mm in aortic disease associated with bicuspid aortic valve

WHO III
- Mechanical valve
- Systemic right ventricle
- Fontan circulation
- Cyanotic heart disease (unrepaired)
- Other complex heart disease
- Aortic dilation 40–45 mm in Marfan syndrome
- Aortic dilation in 45–50 mm in aortic disease associated with bicuspid aortic valve

Conditions in which pregnancy risk is WHO IV (pregnancy contraindicated)
- Pulmonary arterial hypertension of any cause
- Severe systemic ventricular dysfunction (EF <30%, NYHA III or IV)
- Previous peripartum cardiomyopathy with any residual impairment of left ventricular function
- Severe mitral stenosis, severe symptomatic aortic stenosis
- Marfan syndrome with aorta dilated >45 mm
- Aortic dilation >50 mm in aortic disease with bicuspid aortic valve
- Native severe coarctation

Table 2 ZAHARA score and risk factor weighting

Risk factor weighting included
- Mechanic valve—4.25 points
- Severe LV outflow obstruction (peak gradient >50 mmHg or aortic valve area <1.0 cm^2)—2.50 points
- Cardiac medication use prior to pregnancy—1.50 points
- Cyanotic heart disease (repaired or unrepaired)—1.00 points
- Moderate to severe pulmonary or systemic AV valve regurgitation—0.75 points
- Symptomatic heart failure before pregnancy (NYHA ≥ II)—0.75 points

Five categories of risk were created
- 0–0.5 points: 2.9%
- 0.51–1.50 points: 7.5%
- 1.51–2.50 points: 17.5%
- 2.51–3.50 points: 43.1%
- ≥3.51 points: 70.0%

mechanical valve replacement, and systemic or pulmonary AV valve regurgitation related to moderately complex heart disease. This study also identified adverse fetal outcomes. Others have yet to validate this scoring system in a prospective manner (**Table 2**).

CARPREG scoring system

Siu et al. (1997) created a risk score based on a retrospective study of women with cardiac disease. It was then applied prospectively to 562 women with congenital, AHD or arrhythmias. Seventy-four percent of those pregnancies were in women with congenital heart disease (Siu et al., 2001).

Four predictors were identified:

- Poor functional class (NYHA III or IV) or cyanosis
- Prior CV event including heart failure, TIA, stroke, or arrhythmia
- Left heart obstruction (mitral valve area <2 cm^2, aortic valve area <1.5 cm^2, and peak LV outflow gradient of >30 mmHg)
- LV systolic dysfunction (EF <40%)

One point is assigned to each finding. An overall rate of cardiac event (pulmonary edema, arrhythmia requiring treatment, stroke, cardiac arrest, or death) was 13% with 55% occurring antepartum. The risk of events in the prospective study increased with increasing point score: 0 point, 5%; 1 point, 27%; ≥2 points, 75%.

A subsequent study evaluating this scoring system in patients with CHD showed similar findings with adverse events occurring in 25% of pregnancies. The presence of subpulmonary ventricular dysfunction and/or severe pulmonary regurgitation further increased the risk of adverse events (Khairy et al., 2006).

Pulmonary Hypertension

Given the significant hemodynamic loads of pregnancy and labor, the presence of pulmonary hypertension is a major risk factor for poor outcome. Great care should be placed on avoiding a sudden drop in systemic vascular resistance as this can lead to an increase in right to left shunting and a resultant drop in oxygenation. The poor prognosis is particularly true for patients with Eisenmenger syndrome. Maternal mortality in patients with Eisenmenger syndrome was originally reported to be as high as 50% (Kahn, 1993). More recent studies suggest that the mortality rate while remaining high at 25% has improved with the advent of newer therapies (17% mortality rate in the idiopathic pulmonary hypertension group and 28% mortality rate in the CHD–pulmonary hypertension groups) (Bedard et al., 2009). Preterm labor and fetal growth restriction occur in 50% of such patients with only 15%–20% progressing to term. Maternal mortality appears to be highest during delivery and in the first postpartum week. In general, women with Eisenmenger syndrome should be counseled to avoid pregnancy. If they become pregnant, then termination should be discussed. If termination is declined, then heparin therapy and the use of novel pulmonary vasodilators should be considered. Invasive hemodynamic monitoring would be indicated at the time of delivery at a center with appropriate expertise.

Fetal Risk Stratification

Two elements of risk should be considered for the fetus—(i) the likelihood of successful completion of pregnancy and (ii) the risk of a cardiac fetal anomaly. To date, a fetal risk assessment score has not been established. Adverse outcomes include spontaneous abortions, intrauterine growth retardation, and prematurity (Siu et al., 2001; Khairy et al., 2006; Drenthen et al., 2007). Preterm delivery and growth restriction lead to an increase in perinatal morbidity and mortality. The growth of the fetus is influenced by the functional class of the mother; the degree of maternal cyanosis; the maternal medications; and the presence of congenital heart disease, AHD, and arrhythmias. Siu et al. found that adverse fetal events including premature birth, small-for-gestational age, respiratory distress syndrome, intraventricular hemorrhage, and fetal/neonatal death were associated with poor maternal functional class (class III or IV), cyanosis, maternal left heart obstruction, maternal age <20 or >35 years, obstetric risk factors (history of

premature delivery, premature rupture of membrane, incompetent cervix, cesarean section, intrauterine growth retardation, antepartum bleeding beyond 12 weeks' gestation, febrile illness, and uterine/placental abnormality), multiple gestation, tobacco use during pregnancy, anticoagulant therapy, and known lesion-specific risk (Siu et al., 2001).

Risk of adverse events during pregnancy

The maternal functional class appears to correlate with the risk of fetal mortality. Those with serious symptoms have a 30% fetal mortality risk (Child, 2009). Patients with Eisenmenger syndrome have only a 15%–25% chance of reaching term (Kahn, 1993). Spontaneous abortion risk is increased.

While a woman with cyanosis and no pulmonary hypertension can go through pregnancy with low maternal risk, the effect on the fetus is increased. The level of maternal hypoxia (cyanosis) has a direct effect on fetal growth, risk of spontaneous abortion, and risk of prematurity. Presbitero et al. (1994) studied 44 women (96 pregnancies) with cyanosis and found that only 26 pregnancies reached term while 49 spontaneous abortions and 6 still births were recorded. The best possible outcomes were seen when the hemoglobin was less or equal to 16 g/dL and/or arterial oxygen saturation equal or greater than 90%. In addition, the underlying anatomical lesion influenced the likelihood of a live birth (**Table 3**).

Risk of congenital heart disease in offspring

Offspring of women with congenital heart disease are at increased risk of congenital heart disease. This risk will vary depending upon the mother's own cardiac condition (Drenthen et al., 2007; Burn et al., 1998). The incidence of a cardiac anomaly in offspring is as high as 10.7% (Whittemore et al., 1994). In a study by Burn et al. (1998), cardiac defects were seen in 4.1% of offspring with the risk being higher if the mother has the cardiac defect (5.7% if mother vs. 2.2% if father). Whittemore et al. (1994) however found no difference between offspring of a father or mother with congenital heart disease. The incidence of the same defect occurring is approximately a third (Gill et al., 2003). Parental left heart obstructive lesions are associated with higher rates of transmission (13%–18%). Autosomal dominant conditions such as Noonan syndrome, William syndrome, Holt–Oram syndrome, Marfan syndrome, and 22q11 deletion syndrome confer a 50% risk of recurrence in the offspring. Genetic syndromes can be associated with specific lesions. For example, Holt–Oram is associated with atrial septal defects and DiGeorge syndrome with conotruncal abnormalities. Preconception use of multivitamins containing folic acid has been shown to decrease the incidence of congenital defects and should be encouraged (Czeizel, 1996). A fetal echocardiogram is indicated when a parent has congenital heart disease to assess the fetal cardiac anatomy and is typically completed between 18 and 22 weeks' gestation.

Preconception and Prenatal Management

Preconception/Initial Evaluation

Ideally, a comprehensive preconception evaluation should be performed, but if the patient presents during pregnancy, the initial evaluation should be performed at that time by someone knowledgeable about the implications of pregnancy for both the fetus and the mother. Frequently, the woman is unaware of the risks so that education of the mother and spouse is critical (Kovacs et al., 2008).

The initial evaluation should include a detailed history (including prior interventions/surgeries), symptoms, complete physical examination, 12-lead ECG, and transthoracic echocardiogram. Where appropriate, a more comprehensive assessment of functional status should be considered (exercise testing). The transthoracic echocardiogram will help determine underlying repairs, residual lesions, right-sided pressures, and ventricular function. This evaluation allows for a comprehensive risk assessment of the woman and fetus (see prior sections for details) with appropriate counseling to follow.

If significant lesions are present, then correction or palliation prior to pregnancy is ideal using percutaneous and surgical techniques. Such an approach should improve fertility, improve the woman's ability to tolerate the aforementioned hemodynamic changes, and reduce fetal risks. Such discussions are complex and require thoughtful discussion with the woman. This is particularly true when valve replacement is needed. The risk benefit trade-off with tissue valves versus mechanical valves must be discussed and adequate time provided for the patient to make an informed decision.

Surgery and percutaneous interventions during pregnancy while possible should be avoided. The maternal risks are similar during cardiac surgery to the nonpregnant state, but the risks to the fetus are increased (Weiss et al., 1998; Parry and Westaby, 1996; Strickland et al., 1991; Chambers and Clark, 1994; John et al., 2011). Similarly, catheter interventions including balloon

Table 3 The impact of the underlying anatomy in mothers with cyanosis on pregnancy outcome

Group	*Examples*	*% Live birth*
I	Single ventricle	31
II	Tetralogy of Fallot or pulmonary atresia with aortopulmonary collaterals	33
III	Ebstein anomaly with an ASD	86
IV	Corrected transposition (L-TGA) VSD and pulmonary stenosis	60

valvuloplasty have been reported. Specific consideration should be given to reduce radiation exposure to the fetus with a greater reliance on transesophageal echocardiography for imaging (Bhargava et al., 1998; Esteves et al., 2006; Myerson et al., 2005).

Prenatal Care

Prenatal care is determined by the underlying conditions of the woman and fetus. The frequency of follow-up will depend on the severity of the heart condition(s). Using the WHO classification of maternal risk assessment, the following are general rules: class I, one to two visits; class II, every trimester at a minimum; class III, once or twice a month, with consideration for alternatives to pregnancy; class IV, pregnancy is contraindicated and termination considered, but if pregnancy continues, then once or twice a month evaluations. The management of arrhythmias is beyond the scope of this article. The management of anticoagulation is discussed later.

An understanding of normal pregnancy is important to help detect the presence of abnormal hemodynamic changes. Peripheral edema due to an increase in total exchangeable sodium and water is commonly seen and a decrease in air entry bibasally in the lungs. Early development of pitting edema or the development of fine crackles on auscultation should be thoughtfully evaluated. In addition, anemia occurs, but the routine use of iron during pregnancy should be avoided, in particular in cyanotic patients.

Pregnancy leads to an increase in thromboembolic risk and requires careful follow-up, especially in high-risk patients (prosthetic valves, atrial fibrillation, and prior thromboembolic events). Such patients need appropriate anticoagulation. The therapy chosen will balance the risk for the fetus (teratogen effect in early pregnancy and later bleeding) and the woman (bleeding risk vs. risk of thrombus formation).

Careful and comprehensive evaluation of the fetus at 18–22 weeks' gestation should be offered to all at-risk women, especially those with congenital heart disease.

Labor, Delivery, and Postpartum Care

Endocarditis

Both the European Society of Cardiology and the American Heart Association do not recommend endocarditis prophylaxis during uncomplicated cesarean sections or vaginal delivery (Habib et al., 2015; Wilson et al., 2007). Endocarditis is rare during and after a completed pregnancy (Drenthen et al., 2007) but carries with it both high maternal and fetal mortality (Campuzano et al., 2003). Some centers do however consider endocarditis prophylaxis in higher-risk groups as defined by the American Heart Association and American College of Cardiology in their 2008 adults with congenital heart disease guidelines (prosthetic valve, cyanotic heart disease, residual lesions near prosthetic material, and those with surgical repair within the prior 6 months) (Warnes et al., 2008).

Labor

Typically, repaired cardiac conditions or those with minor residua can tolerate vaginal delivery and are allowed to go into spontaneous labor. If there are concerns about the ability of the heart to tolerate the stresses of labor, then induction should be considered. Timing should be dictated by cardiac status, suitability of the cervix and fetal maturity with every effort made to avoid long durations of induction. Typically, oxytocin and artificial rupture of membranes are well tolerated. Ripening of the cervix is generally done using mechanical methods although many use misoprostol. Avoiding a sudden drop in BP or systemic vascular resistance is important especially in patients with cyanotic heart disease. This concern would support not using dinoprostone.

During labor, the woman should be placed in a left lateral decubitus position to avoid compression of the abdominal aorta and IVC, which would exacerbate the hemodynamic changes seen during uterine contraction. Pushing by the mother should also be avoided as such Valsalva maneuvers will affect blood circulation. Delivery is frequently done using a low forceps or vacuum extraction for similar reasons.

Anesthesia

Effective regional anesthesia that abolishes the pain of labor and the associated cardiovascular changes has allowed for controlled delivery during stage two of labor. This has made vaginal delivery the preferred mode of delivery for the majority of women with heart disease. Low concentrations and gradual introduction of anesthesia agents cause minimal hypotension (Suntharalingam et al., 2001). In general, noninvasive monitoring of blood pressure, heart rate, oxygen saturation, and rhythm is performed in all patients. Invasive blood pressure monitoring can be used in high-risk patients. During the third stage of labor (delivery of the placenta), oxytocic agents are administered at lower doses and with slow infusions to minimize systemic vasodilation (Pinder et al., 2002). Regional anesthesia is contraindicated if the patient is receiving anticoagulation therapy.

During cesarean sections, the majority are now performed under regional anesthesia. The choice of regional versus general anesthesia should be considered well before the day of delivery, necessitating an elective consultation with anesthesia for patients with heart disease. If regional anesthesia is chosen, then deliberation between epidural and spinal anesthesia is made. Typically, catheter techniques allow for the titration of effect (Durbridge and Yentis, 2011) making this approach the preferred one.

Oxygen

Oxygen is often used during labor although maternal benefit has not been demonstrated. Typically, transcutaneous oxygen monitoring is performed.

Preterm labor

The incidence of preterm labor is increased in patients with heart disease (especially women with cyanotic heart disease). Several pharmacological therapies are used to manage premature contractions including the use of magnesium, indomethacin, nifedipine, beta-adrenergic agonists, or an oxytocin receptor antagonist (atosiban). An understanding of their pharmacological effects is important to predict adverse effects on the woman. Beta-agonists will increase heart rate, increase volume, and can trigger heart failure (Armson et al., 1992). Magnesium has a limited role and is generally used for the prolongation of pregnancy for brief time periods (2016). Nifedipine and indomethacin are general preferred although nifedipine due to its reduction in systemic vascular resistance may be harmful in aortic stenosis or cyanotic heart disease.

Cesarean section

In general, vaginal delivery is the mode of choice even in the presence of heart disease. Compared with a cesarean section, vaginal delivery has less blood loss, more rapid recovery, the absence of abdominal surgery, lower infection risk, and reduced thrombogenic risk. Cesarean delivery is reserved for obstetric indications (breech, failure to progress, placenta previa, abnormal fetal heart rates, etc.). The European Society of Cardiology states that cesarean delivery should be considered for patients on oral anticoagulants in preterm labor, women in severe heart failure, Marfan syndrome with an aortic root diameter >45 mm, and patients with acute or chronic aortic dissection (Regitz-Zagrosek et al., 2011). Some consideration for cesarean section is also given to severe aortic stenosis and severe forms of pulmonary hypertension.

Despite the recommendations from professional societies, women with cardiac disease have a higher likelihood of delivering by cesarean section compared with those without heart disease (Josefsson et al., 2011; Karamlou et al., 2011). A recent study of 1262 women showed that in the absence of a firm indication for cesarean section, a vaginal delivery can be safely attempted and if circumstances change, the outcome from an emergency cesarean section is no worse than in patients with a planned cesarean section (Ruys et al., 2015). In a single-center study of 116 patients with CHD, severity of CHD was an independent determinant of risk for primary cesarean section. Functional status was a predictor of the risk of a secondary cesarean section. Higher rates of cesarean section were also found to follow the induction of labor (Hrycyk et al., 2016).

Postpartum care

After the placenta is delivered, bleeding is reduced by uterine massage and slow infusion of oxytocin (to avoid hypotension). Early ambulation and preventative measures to reduce the risk of deep venous thrombosis are also important. In patients who are cyanotic, heparin use should be used judiciously as it can paradoxically worsen the intrinsic hemostatic abnormalities present leading to fatal hemorrhage (Child, 2009). Heparin therapy is mandated in patients with prosthetic valves.

Contraception

The approach and options for contraception are beyond the scope of this article.

Anticoagulation During Pregnancy

The management of patients with prosthetic valves is complex as all the available therapeutic options pose risks and benefits to both the mother and fetus (Salazar et al., 1984). Thoughtful preconception counseling is best done to allow the mother to make a fully informed decision on the best therapeutic approach for their circumstance. In general, heparin does not completely prevent valve thrombosis, and warfarin can cause an embryopathy.

Pregnancy results in a hypercoagulable state in part related to an increase in clotting factors and platelet adhesiveness and a decrease in the degree of fibrinolysis and protein S activity. This leads to an increase in the risk of thrombosis and embolism.

Warfarin

Warfarin does cross the placenta and leads to fetal anticoagulation. The warfarin effect is greater in the fetus due to reduced K-dependant factors. Increases in spontaneous abortion, fetal deformity, stillbirth, retroplacental hemorrhage, and intracranial hemorrhage have all been seen.

The risk of warfarin embryopathy was thought to be as high as 30%, although recent reports suggest that the incidence is lower at 4%–10% (Oakley, 1995; Sbarouni and Oakley, 1994). Characteristic features include bone and cartilaginous abnormalities with chondrodysplasia, nasal hypoplasia, optic atrophy, blindness, intellectual impairment, and seizures. The teratogenic risk is highest during warfarin exposure between 6 and 12 weeks' gestation. The risk is also dose-dependent. The risk is significantly reduced if the dose is less than 5 mg/day (3% vs. 8%) during the first trimester (Vitale et al., 1999). The risk of CNS effects (spasticity and optic atrophy) continues throughout pregnancy.

Heparin

Heparin does not cross the placenta. The use of subcutaneous heparin is associated with a 12%–24% incidence of thromboembolic complications including fatal valve thrombosis and so is not recommended in either American or European guidelines. Intravenous

unfractionated heparin is used especially in the late third trimester. Risks associated with intravenous heparin include valve thrombosis, heparin-induced thrombocytopenia, and osteoporosis. Monitoring is usually with APTT levels, but periodic checks of their antifactor Xa level are important. Typically, unfractionated heparin is given as a continuous infusion. Low-molecular-weight heparin is now being used, with an increasing number of reports supporting its use, although it continues to carry a black box warning from the FDA that was issued in 2004 when valve thrombosis occurred at higher rates than seen with Coumadin use. Benefits of low-molecular-weight heparin include the following: It does not cross the placenta, it has a lower incidence of heparin-induced thrombocytopenia compared with unfractionated heparin, and its half life is more predictable than unfractionated heparin (Melissari et al., 1992). If used, meticulous monitoring of the woman's antifactor Xa level aiming for a level of 0.8–1.2 U/mL is recommended. Even with therapeutic levels of antifactor Xa levels 4–6 h post administration, the predosing levels can be subtherapeutic. The need to maintain predose levels over 0.6 U/mL has not been sufficiently studied (Friedrich and Hameed, 2010) but should be considered.

First-trimester anticoagulation

Numerous guidelines have been published including from the American Heart Association, American College of Cardiology (Nishimura et al., 2014), American College of Chest Physicians (Hirsh et al., 2008), and the European Society of Cardiology (Regitz-Zagrosek et al., 2011). In general, warfarin doses are less than or equal to 5 mg/day that provides the best protection for the mother with the lowest risk to the embryo. If the dose is higher than 5 mg/day, then warfarin is typically discontinued and either intravenous unfractionated heparin (with a target APTT two to three times control) or low-molecular-weight heparin given subcutaneously (with a target antifactor Xa level of 0.8–1.2 U/mL 4 h after drug administration) is used. Aspirin therapy is typically continued throughout pregnancy especially during the second and third trimesters.

Second-trimester anticoagulation

Warfarin is typically used during the second trimester with usual INR targets. If unfractionated heparin or low-molecular-weight heparin is used, then the mother needs to be aware of the higher risk of valve thrombosis.

Third-trimester anticoagulation

Warfarin can be continued until the 36th week of pregnancy. At that time, the patient should be transitioned to unfractionated heparin (with the earlier targets). The early transition is required since it takes 3 weeks for warfarin to be cleared from the fetal liver. If low-molecular-weight heparin is chosen, then hospitalization is not required until 36 h prior to delivery when the patient is transitioned to intravenous unfractionated heparin.

During the peripartum period, delivery needs to be planned, and intravenous heparin is discontinued 6 h prior to delivery. Heparin can be resumed 4–6 h after delivery. A cesarean section would be necessary if labor occurs during the use of warfarin to minimize the risk of fetal intracranial hemorrhage. Heparin can be resumed 4–6 h after delivery in the absence of ongoing bleeding.

Conclusion

Improvements in surgical and medical care of patients with cardiac anomalies have led to an increase in women of childbearing age becoming and successfully navigating pregnancy. A clear understanding of the hemodynamic changes (fall in systemic vascular resistance and increase in cardiac output) associated with pregnancy and labor allows for a more targeted approach to patient care. Pregnancy also increases the hypercoagulable state of the woman necessitating an anticoagulation regimen that protects both the mother and fetus.

Appropriate risk assessment of the mother (using the modified WHO risk classification scheme) allows for better counseling of women and helps determine the frequency of follow-up needed. High-risk maternal conditions include pulmonary artery hypertension, mitral stenosis, aortic stenosis, bicuspid aortic valves with aortic aneurysms >50 mm, Marfan syndrome with aortic aneurysms >45 mm, severe left ventricular dysfunction (EF <30%, NYHA III or IV), and native severe coarctation. Impaired maternal functional class, maternal cyanosis, and maternal medications can put the fetus at risk.

Labor and delivery carry with it certain risks, but vaginal delivery with the appropriate use of epidural and assisted delivery is typically preferred over cesarean section. Cesarean section is generally reserved for obstetric reasons and some unique cardiac issues including active labor while on oral anticoagulants, severe heart failure, aortic diameter >45 mm in patients with Marfan syndrome, and patients with acute or chronic aortic dissection.

Where appropriate, at-risk women should be managed in a multidisciplinary way at a center with high level of comfort and expertise in such conditions.

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Management of Ventricular Tachycardia in Ischemic and Nonischemic Cardiomyopathy

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Introduction

Ventricular arrhythmias, ranging from asymptomatic ventricular premature beats (VPBs) to ventricular fibrillation (VF), are common in patients with ischemic and nonischemic cardiomyopathy(NICM) (Holmes et al., 1985). VPBs, nonsustained ventricular tachycardia, and accelerated idioventricular rhythm were shown to be associated with an increased risk of sudden cardiac death (SCD) in patients with ischemic cardiomyopathy but not in NICM (Packer, 1992). In contrast, sustained monomorphic ventricular tachycardia (SMVT)/VF occur in less than 5% of cardiomyopathy patients (Teerlink et al., 2000; Meinertz et al., 1984) and are associated with high risk of sudden death (Poll et al., 1984; Milner et al., 1988). SMVT in this setting is most commonly a result of reentry through myocardial scar, most commonly following prior infarction. This type of VT typically occurs in the absence of acute ischemia (Wit et al., 1982; El-Sherif et al., 1981). Ventricular scars leading to reentrant VT also occur in nonischemic cardiomyopathies, including idiopathic dilated cardiomyopathy, hypertrophic cardiomyopathy, infiltrative heart diseases (e.g., sarcoidosis), arrhythmogenic right ventricular cardiomyopathy, and repaired congenital heart disease or valvular heart disease.

Prognosis of patients with ventricular tachycardia is generally dependent on the nature of the underlying heart disease. Persons with VT and a structurally normal heart have an excellent prognosis. In contrast, VT in ischemic cardiomyopathy has worse survival (Trappe et al., 1988).

Management of VT in Ischemic and NICM

Therapy for SMVT involves acute termination and long-term treatment to prevent recurrences and SCD. The initial therapy should be directed based on hemodynamic status and the presence or absence of symptoms. Additionally, treatment of any underlying precipitating factors (e.g., myocardial ischemia, heart failure, electrolyte disturbances) is an important part of the therapy.

Acute Management

For patients with SMVT who are hemodynamically *unstable*, severely symptomatic, or pulseless, urgent synchronized electrical cardioversion should be performed (Link et al., 2015). Antiarrhythmic therapy (intravenous amiodarone, procainamide, lidocaine, or oral agents such as sotalol or amiodarone) is generally indicated if SMVT recurs. For repetitive episodes of SMVT, consideration should be given to catheter ablation.

For patients who are hemodynamically *stable* during SMVT, intravenous pharmacologic therapy with procainamide, amiodarone, or lidocaine may be attempted prior to electrical cardioversion (Link et al., 2015; Ortiz et al., 2016). Sometimes, additional antiarrhythmic therapy may be administered (e.g., a second bolus of amiodarone, adding lidocaine to amiodarone, etc.).

Treatment of underlying conditions, which may precipitate VT, such as myocardial ischemia, electrolyte disturbances, and heart failure, as well as decreasing the sympathetic facilitation of SMVT, are important components of the acute management of VT. However, it should be recognized that most episodes of SMVT do not have identifiable precipitating factors, and SMVT is rarely precipitated by acute ischemia, although it most commonly occurs in patients with prior myocardial infarction (MI). Thus,

appropriate treatment of heart failure and ischemia is important for improving the overall prognosis of patients with SMVT after MI, but they may not directly affect the occurrence of VT.

Adjunctive treatment

- Adjunctive use of beta blockers, in combination with antiarrhythmic agents, is effective in reducing sympathetic facilitation of SMVT, especially in patients with frequent recurrences of VT within a short time period (VT storm) (Hirsowitz et al., 1986).
- In the postoperative or intensive care unit setting, eliminating or decreasing the use of inotropic or adrenergic agents may facilitate pharmacological control of SMVT (Jazayeri et al., 1989). Similarly, the judicious use of sedative-hypnotic agents to lower anxiety, as the patient's hemodynamic status allows, may be beneficial.
- Electrolyte disturbances, particularly hypokalemia and hypomagnesemia, are not usually responsible for precipitating SMVT, but when present, they should be treated aggressively.
- In appropriate patients, insertion of an intraaortic balloon pump (IABP) may help stabilize patients with refractory recurrent VT until ablation or surgical treatment is performed. The IABP can reduce ischemia and help in maintaining blood pressure until definitive therapy is implemented.

Prevention of Recurrent Arrhythmias and SCD

Long-term therapy of patients with SMVT usually requires utilization of multiple therapeutic modalities, including the implantable cardioverter defibrillator (ICD), antiarrhythmic drugs, and/or radiofrequency (RF) catheter ablation (Pedersen et al., 2014) (**Fig. 1**).

Revascularization is usually not antiarrhythmic in patients with SMVT, although such patients should be investigated for myocardial ischemia, which, if present, should be treated aggressively. Effective treatment of congestive heart failure does reduce both heart failure mortality and sudden death (Domanski et al., 2003; Deedwania and Carbajal, 2012; Pitt et al., 1999; Das and Zipes, 2010) but has not been effective in reducing recurrences of VT. It had been hoped that cardiac resynchronization therapy would significantly reduce VT; unfortunately, this only appears to be the case in those without a history of VT (submitted) (Sapp et al., 2016), particularly if the EF improves (Ruwald et al., 2014). Once the substrate for VT becomes manifest, reduction of VT requires specific therapy, either antiarrhythmic drug therapy or catheter ablation.

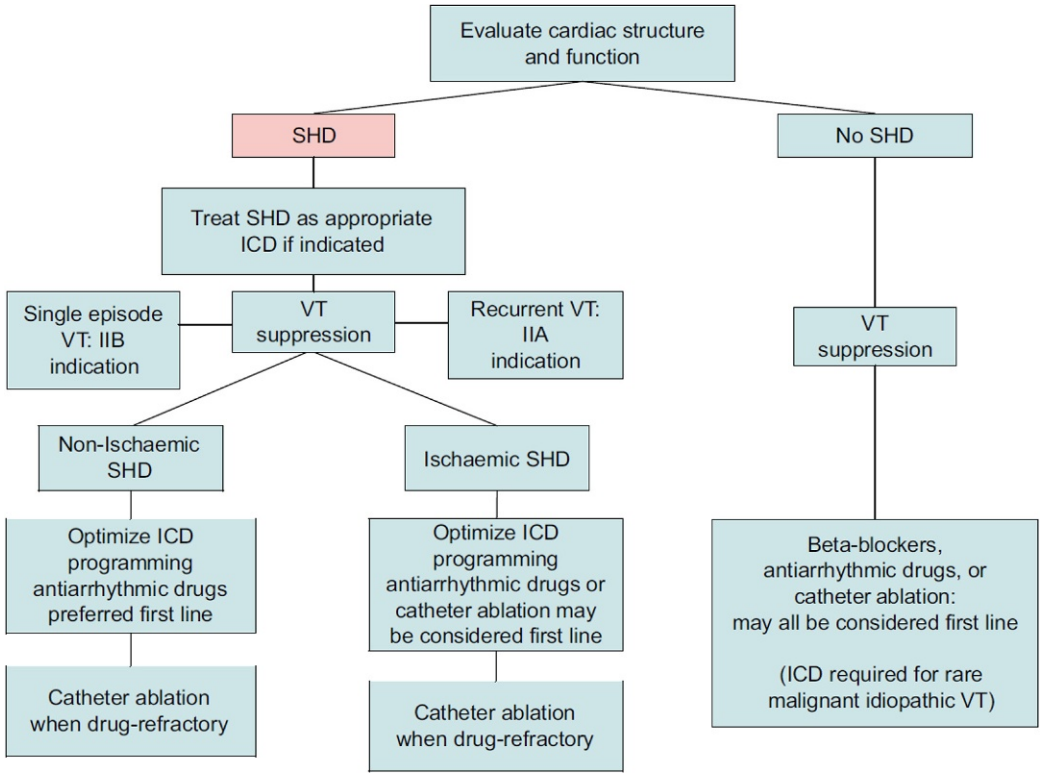

Fig. 1 Sustained monomorphic ventricular tachycardia evaluation and management. Adapted from Pedersen, C. T., Kay, G. N., Kalman, J., Borggrefe, M., Della-Bella, P., Dickfeld, T., Dorian, P., Huikuri, H., Kim, Y. H., Knight, B., Marchlinski, F., Ross, D., Sacher, F., Sapp, J., Shivkumar, K., Soejima, K., Tada, H., Alexander, M. E., Triedman, J. K., Yamada, T., Kirchhof, P., Lip, G. Y., Kuck, K. H., Mont, L., Haines, D., Indik, J., Dimarco, J., Exner, D., Iesaka, Y. and Savelieva, I. (2014). EHRA/HRS/APHRS expert consensus on ventricular arrhythmias. *Heart Rhythm 11*(10), e166–e196. With permission from Elsevier Science.

ICD therapy

Three large prospective trials (AVID, CASH, and CIDS) evaluated ICD therapy compared to amiodarone or other antiarrhythmic drugs in survivors of cardiac arrest or life-threatening ventricular tachyarrhythmias, including hemodynamically unstable SMVT. Although the AVID trial was the only one to demonstrate a statistically significant mortality benefit, similar point estimates of benefit were seen in all three, and a meta-analysis of all three trials showed a significant improvement in overall mortality (Connolly et al., 2000a). These trials did not address patients with hemodynamically stable SMVT who may be more challenging to manage because of frequent VT recurrences.

There is evidence and/or general agreement that ICD therapy may be beneficial in patients with structural heart disease and spontaneous sustained VT, whether hemodynamically stable or unstable (Epstein et al., 2008; Priori et al., 2015; Zipes et al., 2006). The principal benefit in patients with hemodynamically stable VT derives from the ability to reduce the need for antiarrhythmic drugs in patients with infrequent tachycardia episodes, as well as the ability to terminate SMVT with antitachycardia pacing rather than shocks. Earlier trials of programming ICDs to treat VT without shocks were successful and form the basis for modern programming (Wilkoff et al., 2006; Wathen et al., 2004). It was hoped that further shock reduction could be achieved with prolongation of detection—this was successful for patients with primary prophylactic ICDs (Moss et al., 2012), but less so for patients who already have VT (Gasparini et al., 2013; Sterns, 2015; Sterns et al., 2016).

There remains some debate regarding the optimal management of sustained, hemodynamically stable VT in persons with a prior MI but normal or near normal left ventricular systolic function, in whom there is increasing consensus that VT ablation should be considered for primary or initial therapy (Aliot et al., 2009).

Long-term antiarrhythmic drug therapy

Both empiric (Waldo et al., 1996) and tailored antiarrhythmic drug therapy based on electrophysiologic studies (Buxton et al., 1999; The Cardiac Arrhythmia Suppression Trial II Investigators, 1992; The Cardiac Arrhythmia Suppression Trial (CAST) Investigators, 1989) have been disappointing. Only four drugs have been studied in randomized trials in the ICD era to reduce recurrent VT:

1. *Amiodarone* is the most effective available antiarrhythmic drug for the reduction of VT (Ferreira-Gonzalez et al., 2007). It substantially reduces 1 year recurrent VT for patients with ICDs who have experienced VT (HR 0.27, $P<0.001$) (Connolly et al., 2006). It is however, plagued by the long-term risk of complications. When used as preventive therapy for patients with heart failure, it has resulted in reductions in sudden arrhythmic death, but no effect on overall mortality (Burkart et al., 1990; Cairns et al., 1997; Doval et al., 1994; Julian et al., 1997). In the SCD-HeFT trial, patients with reduced EF and heart failure were randomly allocated to placebo, amiodarone, or ICD implant, stratified by functional class. In the functional class III stratum, amiodarone resulted in increased risk of mortality (Bardy et al., 2005) suggesting that some of amiodarone's benefits are offset by harm. Potential amiodarone side effects are numerous and often relate to longer tem exposure. Two of the largest amiodarone trials (AVID and CIDS) reported frequent amiodarone-related adverse events: AVID (The Antiarrhythmics versus Implantable Defibrillators (AVID) Investigators, 1997), during 2 years follow-up, reported a 21% adverse event rate, including pulmonary toxicity in 5%, and thyroid toxicity in 16%, while CIDS (Connolly et al., 2000b), over 3 years follow-up, reported 5.7% pulmonary toxicity, 14.5% ocular symptoms, 6% skin toxicity, 10.3% photosensitivity, 17% ataxia, 15% tremor, 19% insomnia, and 0.5% neuropathy. In an 11-year long-term extension of this study, side effects were seen in 82% of patients and drug was discontinued in 50% over a mean follow-up 5.6 years, and within 8 years, 100% of patients assigned to amiodarone had either died, had recurrent VT, or did not tolerate the drug (Bokhari et al., 2004). In a meta-analysis which included studies with follow-up ≥ 1 year, even relatively low-dose amiodarone was significantly associated with frequent side effects (Vorperian et al., 1997).
2. *Sotalol* demonstrated a trend to reduced recurrences of VT at a 1-year follow-up in comparison to beta-blocker in the OPTIC trial (HR 0.61, $P=0.055$) (Connolly et al., 2006) and a significant reduction in recurrent VT in the trial by Pacifico et al. (49% relative risk reduction, $P<0.001$) (Pacifico et al., 1999). Other smaller ($n\leq 100$) single center trials previously estimated the relative reduction in VT with sotalol at 39% in comparison to placebo (Kuhlkamp et al., 1999) or found either a neutral or increased risk in comparison with beta-blockers (Seidl et al., 1998; Kettering et al., 2002). Sotalol has been similar to other antiarrhythmic drugs, conferring a dose-dependent risk of ventricular proarrhythmia (Waldo et al., 1996) necessitating a careful assessment of risk versus benefit before its use. Sotalol thus has moderate effectiveness compared to amiodarone, but in the presence of an ICD, it is considered to have a relatively good safety profile and is thus a frequent choice for first line therapy for patients without high risk of sotalol-induced proarrhythmia (Pedersen et al., 2014). The risk of mortality related to proarrhythmia from sotalol may be reduced in patients with ICDs. Sotalol should be avoided in patients with severe reactive airways, severe heart failure, severe renal insufficiency, or severe QT prolongation (Fuster et al., 2011; Piccini et al., 2014; Wolbrette, 2003).
3. *Dofetilide* (Mounsey and DiMarco, 2000) was studied in a multicenter randomized trial, which failed to demonstrate a reduction in incident VT, and revealed a risk of torsade de pointes in 10% in the Dofetilide arm (O'Toole et al., 1999; Food and Administration, 2015). When tried in heart failure, it did not demonstrate any benefit (Kober et al., 2000; Torp-Pedersen et al., 1999) and has not been approved for this indication.
4. *Azimilide* successfully reduced VT, but is not clinically available (Dorian et al., 2004; Hohnloser et al., 2006).

Catheter ablation for VT in ischemic cardiomyopathy

Ablation is a nonpharmacologic alternative to antiarrhythmic drug therapy for patients with recurrent VT and ischemic cardiomyopathy, and is recommended, like antiarrhythmic drug therapy, as a possible first line therapy (Pedersen et al., 2014). Its use for post-MI VT has tripled in the last decade (Palaniswamy et al., 2014). During an ablation, intracardiac electrode catheters are placed via the central vasculature. Catheter mapping techniques have been developed, which can identify myocardial scar, and the surviving conduction channels within scar, which form the substrate for reentrant VT. RF energy can then be applied to these sites, interrupting VT circuits (**Fig. 2**). Efficacy and adverse effects of this procedure are summarized in **Table 1**.

Three randomized trials of ablation for scar-related VT have been published. The SMASH-VT trial compared ICD implant with prophylactic ablation to ICD implant alone in patients with recent VT (Reddy et al., 2007). In this trial, ablation of VT reduced ICD shocks from 31% to 9% over a mean follow-up of 22.5 ± 5 months ($P = 0.003$), and reduced VT from 33% to 12% ($P = 0.007$), but no antiarrhythmic therapy was used in the control group. The VTACH trial (Kuck et al., 2010) studied the effect of catheter ablation in patients with ischemic cardiomyopathy, again without specific antiarrhythmic drugs in the control arm (although 1/3 of patients in both arms were treated with amiodarone). Over a mean follow-up of 22.5 months, ablation prolonged time to recurrent VT significantly (HR = 0.61), with a relative risk reduction of 25% and an absolute risk reduction of 18% at 2 years. The VANISH trial was the first trial to randomize VT patients to either catheter ablation or escalation of antiarrhythmic medical therapy (Sapp et al., 2016). During 28 months of follow-up, catheter ablation was associated with 28% relative risk reduction in the composite endpoint of death, VT storm, and appropriate ICD shock ($P = 0.04$).

While catheter ablation for VT is "minimally invasive," it does require instrumentation of the left ventricle in patients who have significant ventricular dysfunction and frequent comorbidities. In a contemporary cohort experience (unselected patients undergoing VT ablation), procedural risks included a mortality risk of 0.4%, as well as risks of cardiac perforation (1.4%), systemic embolism including stroke/MI (0.8%), vascular complications (2%), and other complications, with a major complication rate in the 5%–6% range (Bohnen et al., 2011). This is in the same range as the observed complication rate in multicenter experiences: in the Multicenter Thermocool prospective cohort study of 231 patients, 30-day mortality was 3%, mostly due to VT which was not successfully ablated rather than due to complications, while overall there were significant complications in 7.3%. In phase 2 of this study, when investigators had experience with the catheter, the complication rate fell to 5.1%, with the major contributors including

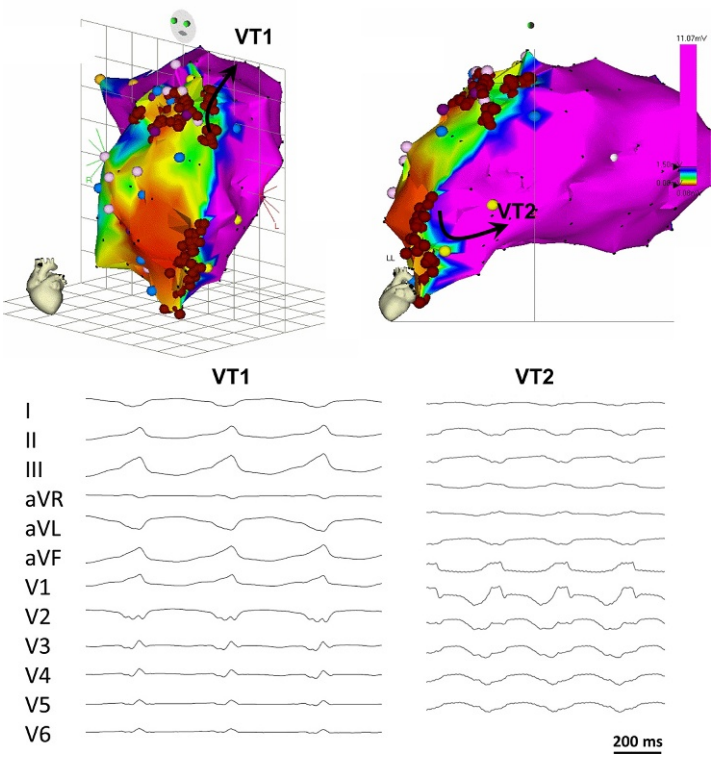

Fig. 2 Bipolar voltage map of the left ventricle during sinus rhythm in left anterior oblique cranial view (Top-left) and left lateral view (Top-right). This patient had a previous anterior STEMI that resulted in a large anteroapical aneurysm and presented with multiple ICD shocks. Reduced sinus rhythm bipolar contact electrogram signal amplitude (<1.5 mV) was recorded throughout the aneurysm. Two poorly tolerated VT morphologies were induced, VT1 (Bottom-left) exiting from the basal margin of the scar and VT2 (Bottom-right) exiting from the lateral margin. VT1 had RBBB morphology with TCL of 380 ms, precordial transition in V2 and QRS axis of 120, while VT2 had RBBB morphology with TCL of 280 ms, precordial transition in V3 and QRS axis of −120. Ablation lesions (*dark red dots*) were delivered along these exits with the endpoint of exit block across the lines. VTs were rendered noninducible at the end of the procedure.

Table 1 Clinical series of catheter ablation for ventricular tachycardia in ischemic heart disease (Modified from Stevenson and Soejima, 2007)

Authors	*Year*	*N*	*EF*	*Complications*	*F/U*	*VT free in f/u*
Sapp et al. (2015)	2016	259	31	Tamponade 1.5%, vasc 2.3%	28	42%
Kumar et al. (2016)	2016	358	28	Tamponade 1.1%, vasc 1.9%, emboli 1.9%, CHF 1.3%, HB 1.3%	72	54%
Di Biase et al. (2015)	2015	118	32	Tamponade 4.2%, vasc 0.8%	12	52%–84%
Peichl et al. (2014)	2014	473	33	Tamponade 0.6%, emboli 0.8%, heart block 1.3%, vasc 4.7%	n/a	n/a
Silberbauer et al. (2014)	2014	160	32	3%	18	70%
Dinov et al. (2014)	2014	300	32	Vasc 2.3%, tamponade 1.7%	24	43%
Proietti et al. (2015)	2014	87	29	Vasc (5), CHF (8)	21	74%
Carbucicchio et al. (2013)	2013	126	33	TIA (1), vasc (3), tamponade (3), CHF(8)	12	88%
Kuck et al. (2010)	2010	107	34	Emboli 3.8%	22	47%
Carbucicchio et al. (2008)	2008	95	36	TIA (2), pericard eff (4), groin (2)	22	66% (92% free recurrent storm)
Reddy et al. (2007)	2007	128	31	1 Effusion, 1 CHF, 1 DVT	22	88%
Abdelwahab et al. (2007)	2007	37	35	1 CHF, 2 HB	16	79
Bogun et al. (2006)	2006	48	27	None reported	16	88
Deneke et al. (2005)	2005	25	37	None	9	78
Segal et al. (2005)	2005	40	36	28% shock/CVA/tamponade/hemothorax/AV block/vascular	36	50
Della Bella et al. (2004)	2004	137	31–36	None reported	36	51%
Arenal et al. (2003)	2004	26	31	None	17	73
Kottkamp et al. (2003)	2003	28	29	3.5% TIA	15	64
O'Donnell et al. (2003)	2003	112	<0.35 in 42%	2% tamponade	72	78%
Kautzner et al. (2003)	2003	28	28	3.5% TIA	11	79
Reddy et al. (2003)	2003	11	31	9% tamponade	13	81
Borger van der Burg et al. (2002)	2002	89	29	9% CVA/incess VT/tamponade/block	34	73
Soejima et al. (2002)	2002	14	29	None	6	71
de Chillou et al. (2002)	2002	21	34	None reported	16	81
Soejima et al. (2001)	2001	40	29	10% minor groin	10	63
Sra et al. (2001)	2001	19	27	5% tamponade	7	66
Calkins et al. (2000)	2000	146	31	Death 3%, 8% CVA/tamponade/AV block/AoV injury	8	54%
Strickberger et al. (2000)	2000	13	33	23% tamponade/CVA	1	71

cardiac perforation 0.4%, CHF 1.3%, and respiratory complications 0.8% (Waldo et al., 2012); concordant with other multicenter experiences (Tanner et al., 2010).

Catheter ablation for VT in nonischemic cardiomyopathy

Nonischemic cardiomyopathy represents a heterogeneous group of patients with multiple underlying etiologies. Hence, the resultant scar has a wider distribution in the endocardium, epicardium, and mid-myocardium (**Fig. 3**). The long-term success rate is modest and ranges between 26% and 86% (**Table 2**). Reentry circuits require epicardial ablation in more than a third of patients (Soejima et al., 2004). Advances in electroanatomic mapping, cardiac imaging, and epicardial access have improved ablation strategies. Occasionally, incessant idiopathic VT or PVCs cause a cardiomyopathy that improves after ablation (Yarlagadda et al., 2005).

Bundle branch reentry represents the second most common mechanism of VT in this subset of patients. In the two largest series reported, acute success rates were 100% (Blanck et al., 1993; Lopera et al., 2004). Intraprocedural high grade AV block has been reported to occur in 10%–30% of patients after successful ablation (Lopera et al., 2004). Recurrence of the BBR VT appears to be uncommon but has not been thoroughly documented with follow-up testing. The long-term prognosis is usually more guarded, however, reflecting the underlying heart disease. Of 20 patients treated, Lopera et al. (2004) reported need for pacing or ICD implantation in 19. Of the 14 patients who required an ICD, 7 (50%) had VT recurrence within 16 months. Five of these patients had VTs of myocardial origin which, were different than the original BBR VTs.

Recent retrospective multicenter analyses examining the outcomes of catheter ablation in the setting of structural heart disease are suggesting a possible survival benefit among those with a successful procedure. Tung et al. (2015) analyzed 2061 SRVT ablation

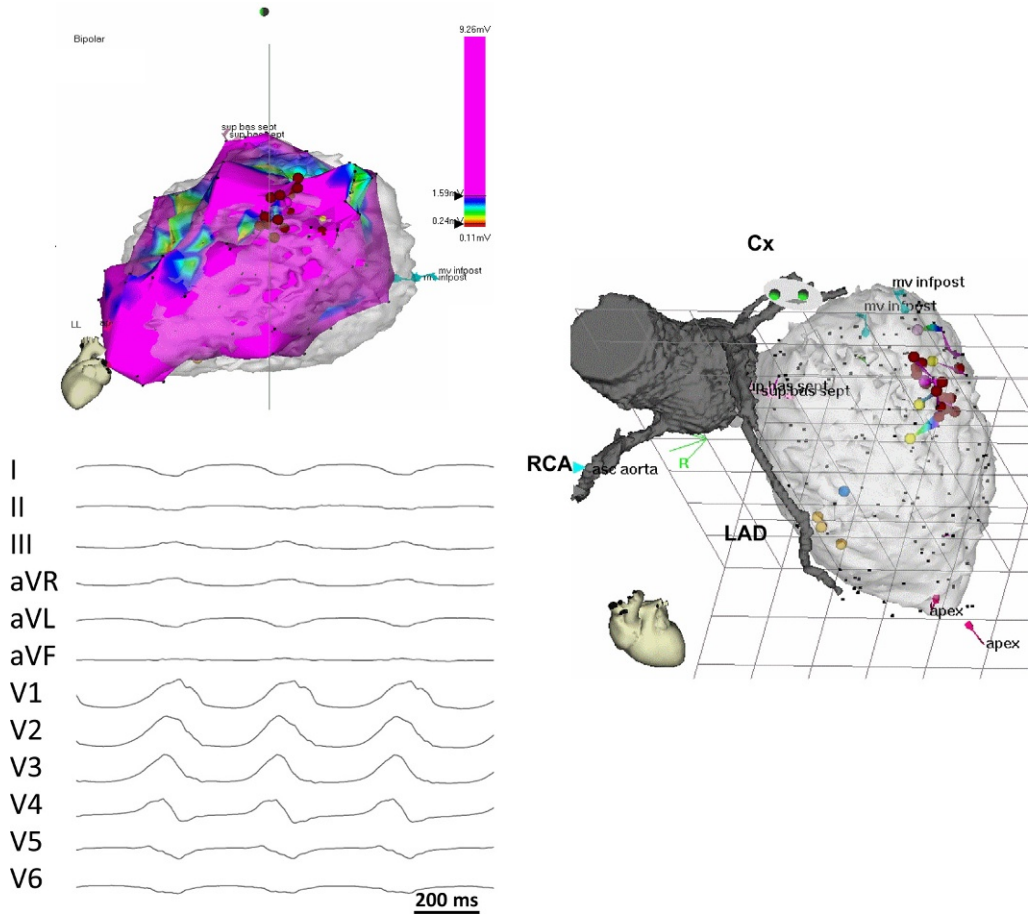

Fig. 3 Bipolar voltage map of the left ventricle during sinus rhythm in left lateral view (Top-left) and the corresponding integrated CT scan reconstruction of the LV and aorta in a left anterior oblique cranial view (Right). This patient had nonischemic dilated cardiomyopathy and presented with multiple ICD shocks. Notice the normal proximal coronary arteries in the reconstructed CT scan image. Small patchy areas of endocardial reduced bipolar voltage (<1.5 mV) were recorded over the anterolateral LV. A single poorly tolerated sustained monomorphic VT (Bottom-left) was inducible with RBBB morphology, TCL of 355 ms, precordial transition in V4 and QRS axis of 170. Successful ablation was performed at the basal lateral LV wall (*dark red dots*) in an area of relatively preserved endocardial bipolar voltage suggesting deeper midmyocardial or epicardial scar with endocardial exit. VT was rendered noninducible at the end of the procedure.

Table 2 Clinical series of catheter ablation for ventricular tachycardia in nonischemic cardiomyopathy

Authors	*Year*	*N*	*EF*	*Epicardial mapping and/or ablation*	*F/U*	*VT free on F/U (%)*
Kumar et al. (2016)	2016	239	40	30% (sarcoid and ARVC included)	72	38
Dinov et al. (2014)	2013	63	34	30%	20	23
Vergara et al. (2012)	2012	14	35	79%	13	86
Haqqani et al. (2011)	2011	31	30	45%	20	68
Kuhne et al. (2010)	2010	24	31	20% (sarcoid and ARVC included)	18	67
Nakahara et al. (2010)	2010	16	27	75%	14	50
Schmidt et al. (2010)	2010	16	32	94%	12	53
Cano et al. (2009)	2009	22	30	100%	18	71
Cesario et al. (2006)	2006	8	34	100%	12	75
Soejima et al. (2004)	2004	28	30	29%, all failed prior endo cardial ablation	12	57
Hsia et al. (2003)	2003	19	34	16%, coronary venous mapping, no ablation	22	26
Marchlinski et al. (2000)	2000	7	44	0	8	57

patients from 12 international centers. At 1 year of follow up, transplant-free survival was significantly higher in patients without VT recurrence than in those with recurrence (90% vs. 71%, $P<0.001$). This observation was independent of ejection fraction or heart failure severity. Similarly, Yokokawa et al. (2015) analyzed data from 1064 patients who underwent VT ablation for postinfarction VT at seven international centers. Noninducibility of VT at the end of the procedure was found to be independently associated with lower mortality (adjusted hazard ratio: 0.65; 95% confidence interval: 0.53–0.79; $P<0.001$) over 2-year follow-up period.

Conclusions

Ventricular arrhythmias are common in patients with ischemic and NICM. Their prognosis is often dependent on the nature of the underlying heart disease. SMVTs are associated with high risk of sudden death. They are usually related to reentry involving an established myocardial scar. While antiarrhythmic drugs can reduce the arrhythmia burden, they are not associated with survival benefit and some of them can be harmful. ICDs are effective in reducing the risk of sudden death but they do not prevent recurrences. Catheter ablation can significantly reduce arrhythmic burden by selectively targeting reentrant circuits within the scar. It has a reasonable long-term efficacy and a possible survival benefit but still has a significant complications rate.

Call for Further Research

Our knowledge of the underlying substrate responsible for VT in patients with ischemic and nonischemic cardiomyopathies has grown remarkably in the past 3 decades. Further research is needed to better understand the underlying pathophysiology that promotes the development of VT many years following myocardial scarring. Noninvasive characterization of the underlying scar as well as localization of potential VT circuits within the scar is another field of research that is showing remarkable progress, particularly with the advances in cardiac scar imaging and ECG imaging. Moreover, treatment options for VT still need further investigation. New antiarrhythmic medications that are both effective and have a favorable safety profile are strongly needed. Catheter ablation for VT is playing an increasing role in treating those patients. Well-designed randomized studies comparing antiarrhythmic drugs and catheter ablation as a first line therapy for VT are ongoing. Further refinement and improvement of ablation tools, procedure technique, and mapping technology will hopefully improve the efficacy of the procedure and lower its risk. Finally the signal toward better survival with catheter ablation needs further investigation in prospective well-designed large studies.

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Managing Cardiovascular Disease in Sport and Athletes

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Introduction

Participation in competitive sport and vigorous recreational exercise has grown rapidly worldwide. USA Triathlon membership has increased by over 350,000 members in the last 15 years (2015 Running USA Annual Marathon Report, 2015) and nontraditional running events (themed races and obstacle events) have had over 3 million finishers from 2009 to 2013 (State of the Sport—Part I: Non-Traditional Running Events, 2014). Contemporary athletes are a heterogeneous group ranging from dedicated lifelong athletes to weekend warriors alike that encompass a broad spectrum of age, athletic prowess, performance goals, and underlying cardiovascular risk profiles. Athletes become patients in one of two principal ways, either following "abnormal" findings during preparticipation screening and general medical care or after the development of symptoms suggestive of underlying cardiovascular disease (Baggish and Wood, 2011).

In 2015, updated guidelines on the eligibility and disqualification for competitive athletes were published by the American College of Cardiology and American Heart Association (Maron et al., 2015a). These guidelines serve as an update to the 36th Bethesda Conference published in 2005 (Maron and Zipes, 2005). The purpose of the guidelines is to ensure the health and safety of young competitive athletes with the end goal of preventing sudden cardiac death (SCD). The intent of this chapter is not to review the guidelines, but to provide clinicians valuable insight into the unique approach and management of athletes with cardiovascular disease that may be encountered in everyday practice. We will highlight sport classification, the incidence, causes and prevention of SCD, the impact of exercise on cardiovascular risk factors, and the management of specific cardiovascular diseases that are common among athletes.

Sport Classification

Each athletic endeavor has its own associated hemodynamic conditions with unique changes in cardiac output and peripheral vascular resistance. In general, exercise activities are separated into two forms with characteristic hemodynamic differences. Isotonic

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exercise or endurance exercise, involves sustained elevations in cardiac output, with normal or reduced peripheral vascular resistance. The principal effect is a volume challenge that affects all four heart chambers and downstream cardiac remodeling results in eccentric remodeling (Fig. 1). Endurance activities include, but are not limited to, long-distance running, cycling, and swimming. In contrast, isometric exercise is defined by an increase in peripheral vascular resistance without significant change in cardiac output leading to concentric hypertrophy. This can cause transient but marked systolic hypertension with increased left ventricular (LV) afterload. Isometric exercises are synonymous with strength training and are the dominant form of exercise in weightlifting, track and field throwing events, and American-style football. Many sports, including team sports such as soccer, lacrosse, basketball, hockey and field hockey, and specific endurance disciplines including rowing, cycling, and triathlon involve significant elements of both isotonic and isometric physiology. Sport classification may provide a useful clinical framework for the provision of eligibility recommendations following a cardiovascular diagnosis in a competitive athlete (Fig. 2) (Levine et al., 2015).

Shared Decision-Making

It is important to recognize that the updated practice guidelines necessarily provide a conservative management approach to athletes as they are based on limited existing research and expert consensus that are applied by physicians with or without expertise in treating athletes with cardiovascular disease. For clinicians who routinely care for competitive athletes it is clear that these guidelines are not always in line with an athlete's goals, ambitions, and livelihoods. Additionally, many habitual athletes use sport as a means to treat depression and anxiety so limitation to IA sport could expose other pathologic conditions. Lastly, there is clear evidence that living a sedentary lifestyle results in weight gain, hypertension, diabetes, depression, and cardiovascular disease. Thus, sports cardiologists are encouraged to rely on guidelines as a reference while simultaneously recognizing the potential long-term consequences of athletic disqualification during shared decision-making with an athlete and his/her family.

Shared decision-making is the process by which an athlete engages in an open dialogue with his/her physician to make a collaborative decision regarding exercise eligibility and prescription. The clinician's role is to review the underlying cardiovascular condition and management strategies with an emphasis on the risks and benefits of all available strategies. The sports cardiologist's final recommendation should ultimately take into account all factors including the athlete's underlying cardiovascular risk, athletic aspirations, and unquantifiable benefits such as stress relief, personal satisfaction, and avoidance of unhealthy habits. Importantly however, safety must be considered the ultimate priority and is the shared responsibility of the provider, athlete, and his/her family.

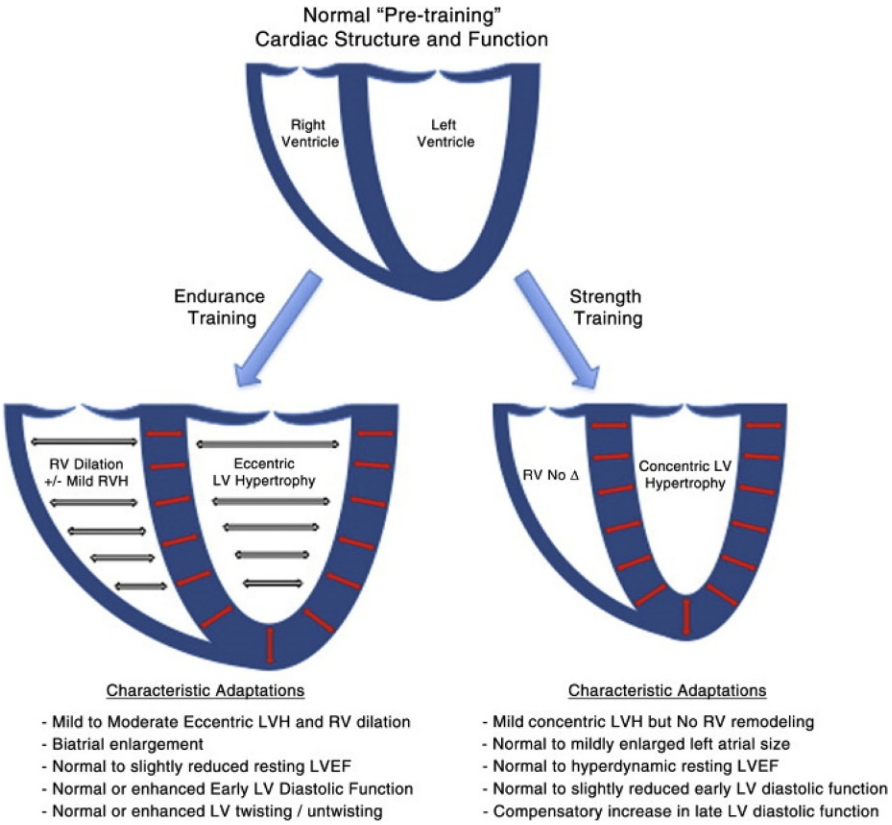

Fig. 1 Summary of ventricular remodeling during sustained exercise training, highlighting the sport-specific nature of exercise-induced cardiac remodeling. Reprinted from Weiner, R. B. and Baggish, A. L. (2012). Exercise-Induced cardiac remodeling. *Progress in Cardiovascular Diseases* **54**, 380–386, 2012 with permission from Elsevier.

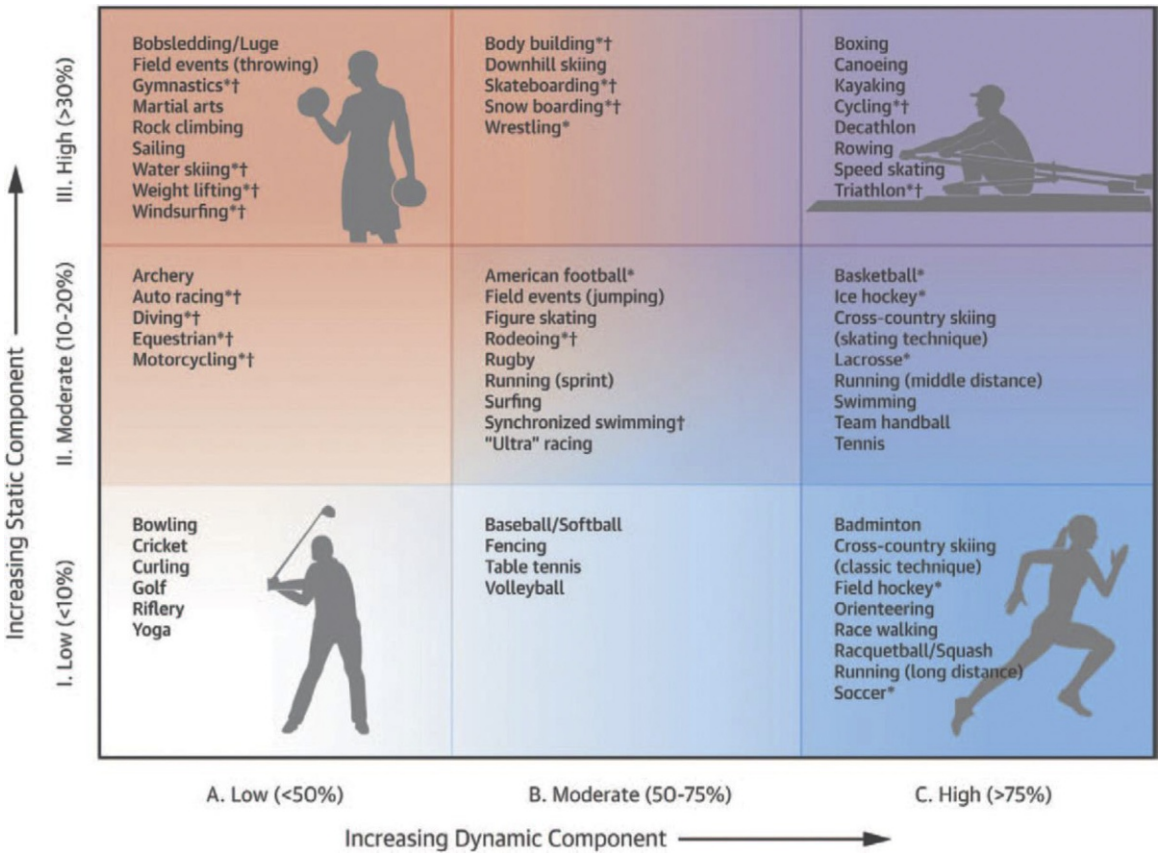

Fig. 2 *Classification of sport.* This classification is based on peak static and dynamic components achieved during competition; however, higher values may be reached during training. The increasing dynamic component is defined in terms of the estimated percentage of maximal oxygen uptake (VO_{2max}) achieved and results in an increasing cardiac output. The increasing static component is related to the estimated percentage of maximal voluntary contraction reached and results in an increasing blood pressure load. The lowest total cardiovascular demands (cardiac output and blood pressure) are shown in the *palest color*, with increasing dynamic load depicted by increasing *blue intensity* and increasing static load by increasing *red intensity*. Note the graded transition between categories, which should be individualized on the basis of player position and style of play. *Danger of bodily collision. †Increased risk if syncope occurs. Journal of the American College of Cardiology by American College of Cardiology Reproduced with permission of Elsevier Inc.

All athletes should identify care providers that understand the breadth of cardiovascular disease pathogenesis and competitive sport and then partner with this provider to identify and address individual risk.

Sudden Cardiac Death: Incidence and Causes

SCD in the athlete is a rare but tragic event that receives significant attention from the media. Such unexpected deaths are often met with a sense of confusion as being an athlete is thought to be synonymous with health and immunity from cardiovascular disease. In reality, acute bouts of vigorous physical exercise transiently increase the risk of sudden death among people with and without underlying cardiovascular disease (Thompson et al., 1982; Siscovick et al., 1984). However, routine vigorous physical activity lowers overall risk of sudden death in a dose dependent fashion, meaning the fittest people are at the lowest risk. Among athletes, absolute risk of sudden death is highest among those with underlying genetic or acquired cardiovascular disease (Mittleman et al., 1993; Albert et al., 2000; Kim et al., 2012). Incidence data defining the risk of sudden death during running come from the RACER study which cataloged cardiac arrests among US-based runners participating in organized marathon or half marathon races over a decade long period of study. The overall incidence of sudden death in the RACER study was 1 per 259,000 runners (Kim et al., 2012), which has been confirmed in smaller studies (Webner et al., 2012). In addition to defining sudden death risk among competitive runners, the RACER study confirmed higher rates among men, increasing risk with longer race distance, and a strong clustering of events in very close proximity to the finish line. Of note, the incidence of cardiac arrest among male marathon runners increased threefold during the study period which almost certainly reflects changes in the underlying health status of men turning to marathon running. It is important to note that sudden death incidence appears to vary across different athletic populations with statistics including 1 death per 43,770 participants/year among NCAA athletes (Harmon et al., 2011), 1 death per 52,630 among US triathlon participants (Harris et al., 2010), and 1 death per 7620 previously healthy middle aged joggers (Thompson et al., 1982).

Consideration of population specific risk has important implications during implementation of screening strategies and emergency action planning.

Causes of SCD among athletes vary as a function of age. Younger athletes, defined as <35 years of age, most commonly succumb to sudden death attributable to congenital or genetic cardiovascular conditions including hypertrophic cardiomyopathy (HCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), congenital coronary artery anomalies (CCAA), and inherited arrhythmia syndromes. Mild forms of these diseases may not impair cardiac function enough to limit exercise capacity rendering them "silent diseases" but may sufficiently increase the risk of malignant, potentially fatal arrhythmias. Contemporary autopsy series derived from athletic victims of sudden death increasingly include cases without clear anatomic abnormalities. Such cases, often termed sudden unexplained death, are likely attributable to occult cardiac channelopathies or other less well understood arrhythmia syndromes.

In contrast, sudden death among athletes above the age of 35 years is more commonly caused by diseases acquired during an athlete's lifetime. In practice, the vast majority of sudden death among older athletes is caused by atherosclerotic coronary disease. The accumulation of coronary artery plaque may lead to sudden death via one of two distinct mechanisms. Coronary plaques, usually those of only mild to moderate severity, may rupture during exercise causing a thrombotic occlusion, myocardial infarction, and subsequently a malignant arrhythmia. In our experience, this pathophysiologic process typically leads to symptoms characteristic of classic myocardial infarction; chest pain, nausea, increases in perspiration, etc., rather than unheralded sudden death. In contrast, moderate to severe coronary plaques rupture relatively infrequently but promote sudden death from demand or ischemia-mediated arrhythmia. Here, fixed blood supply that is limited by the diseased artery segment may sufficiently supply the heart muscle at low to moderate exertion but become insufficient at higher workloads. When this threshold is exceeded, a common occurrence during the often unavoidable "finish line surge," oxygen starved myocardium may become sufficiently unstable to precipitate ventricular arrhythmias. Thus, timely diagnosis and effective management of atherosclerotic coronary artery disease (CAD) is of paramount importance in aging athletes.

Sudden Cardiac Death: Prevention

Sudden death may be a preventable tragedy. As discussed above, underlying diseases of the cardiovascular system cause most cases of sudden death during exercise. Athletes with these conditions may experience warning symptoms including exertional chest pain, inappropriate exertional breathlessness, or a new inability to keep up with peers. It is thus of vital importance that athletes of all ages are educated to be on vigilant watch for such symptoms and to report their occurrence immediately to medical professionals. Unfortunately however, warning symptoms may be absent and the first manifestation of disease may be catastrophic collapse.

Recognition of this fact has been the driving force behind efforts to design, develop, and disseminate preparticipation cardiovascular screening (PPCS) programs. The rationale for PPCS is predicated on the concept that some, if not most, forms of high-risk heart disease fail to produce warning symptoms but are detectable by some form of screening. The purpose of PPCS is thus to identify cardiovascular disorders or abnormalities that can potentiate sudden cardiac arrest during athletic participation, and to take steps to reduce this risk by sport restriction and/or medical intervention. The American Heart Association and American College of Cardiology recommend a mandatory PPCS protocol for young competitive athletes that is limited to a focused medical history and physical examination (Maron et al., 2014a). The impact of this PPCS approach on sudden death incidence is unknown and its ability to detect culprit diseases may be of limited value (Maron et al., 2013, 2014b; Maron, 2012). This has led to some expert consensus panels recommending the inclusion of a 12-lead electrocardiogram (ECG) as part of a more comprehensive PPCS protocol (Corrado et al., 2005, 2006). Indeed, data comparing PPCS confined to medical history and physical examination to one that includes ECG demonstrate that the ECG significantly increases PPCS's ability to detect high-risk conditions. However, it must be emphasized that the inclusion of ECG comes with increased risk of false positive testing. This is a problem of decreasing magnitude in the era of increasingly accurate athlete ECG interpretation algorithms but one that will remain substantial for the foreseeable future. As such, the addition of the ECG to a medical history and physical examination-based PPCS remains controversial and the interested reader is directed to several excellent discussions on this topic (Maron et al., 2009, 2014a, 2015b; Steinvil et al., 2011). All practitioners and organizations considering implementation of a PPCS program, with or without ECG, are urged to consult recent documents that address the strengths and limitations of this process (Maron et al., 2015b).

Screening of older athletes is less comprehensively dictated and receives comparatively little attention. We encourage runners above the age of 35 to work closely with primary care and sports cardiology experts to identify and treat cardiovascular disease risk factors. For older athletes planning to embark on or to intensify a training program, exercise testing should strongly be considered based on its ability to screen for many high-risk, often occult forms of acquired cardiovascular disease.

No PPCS approach will eliminate the problem of sport-associated SCD. Thus, the ability to resuscitate cardiac arrest victims is a critical component of preparedness for those engaged in or those sponsoring races and events. Survivors of cardiac arrest uniformly receive early bystander-administered cardiopulmonary resuscitation (CPR) and have rapid access to automated external defibrillators (AED) at the scene of cardiac arrest (Kim et al., 2012). An effective resuscitation strategy, one key component of an overall emergency action plan, must include ample team members trained in CPR, the availability of AEDs, and rapid access to Emergency Medical Services with advanced cardiac life support capacities. Development and practice of an emergency action plan that integrates these key elements is mandatory for individuals and organizations that host athletic events.

Benefits of Exercise

Participation in regular- and moderate-intensity exercise attenuates cardiovascular risk factors, reduces incident cardiovascular disease, and reduces cardiovascular morbidity and mortality (Sattelmair et al., 2011; Sofi et al., 2008; Wen et al., 2011; Paffenbarger et al., 1993; Wasfy and Baggish, 2016). However, routine exercise does not confer a free pass for dietary indiscretion and does not undo the risks associated with bad habits such as smoking and excessive alcohol intake. It is also noteworthy that exercise used in isolation as a risk factor reduction technique is often inadequate and many athletes therefore benefit from a comprehensive approach that focuses on exercise dose, dietary modification, stress reduction, and conventional medical therapy. The following sections address the current knowledge base defining the impact of exercise on traditional cardiovascular risk factors.

Lipids

Aerobic exercise training has been demonstrated to have a favorable impact on serum cholesterol. The strongest relationship seems to be with increased levels of high-density lipoprotein (Kodama et al., 2007). While data is less consistent, exercise results in decreased low-density lipoprotein and triglycerides (Zilinski et al., 2015; Kelley et al., 2005). It should be noted that lowering total cholesterol by even 10% through diet or medication has been shown to result in a 27% reduction in the incident of cardiovascular disease (Law et al., 1994). Beneficial effects of this magnitude on cholesterol can be seen with adherence to recommended guidelines of 150 min of moderate-intensity or 75 min of vigorous intensity exercise weekly (Eckel et al., 2014). Additionally, incremental benefit may be achieved with exercise routines that exceed the minimum recommended levels (Wasfy and Baggish, 2016). It must be emphasized that many athletes with a genetic predisposition to high cholesterol will be unable to obtain optimal cholesterol profiles through diet and exercise alone. Thus, many athletes will benefit from the addition of medication, most commonly the use of an HMG-Co-A reductase inhibitor, which is well tolerated by most athletes, particularly when flexible dosing schedules are employed.

Blood Pressure

Hypertension is common among otherwise healthy athletes and is associated with the development of numerous forms of heart disease. While the beneficial impact of exercise training on blood pressure is well established, its impact is typically small with reductions in both systolic and diastolic blood pressure in the 2%–4% range (Fagard, 2001; Cornelissen and Fagard, 2005; Whelton et al., 2002; Halbert et al., 1997; Kelley and Tran, 1995). Reductions in blood pressure are more pronounced in those with preexisting hypertension (Fagard, 2001; Cornelissen and Fagard, 2005; Kelley et al., 2001). Endurance athletes routinely exceed the minimum recommended weekly exercise which should confer this beneficial effect on blood pressure. As such, endurance athletes with documented hypertension rarely reverse this problem with increasing training mileage and therefore should be treated with antihypertensive medications that have minimal effect on exercise physiology such as ACE-inhibitors or calcium channel blockers.

Body Mass

The degree of change in body mass related to exercise is dependent on the net energy balance and thus requires increased energy expenditure while concomitantly relying on reduced caloric intake. Effective weight loss or stability is best achieved through a weekly exercise dose that is at least double the baseline recommended weekly activity (Irwin et al., 2003; Slentz et al., 2005; McTiernan et al., 2007; Donnelly et al., 2003). There is a direct dose–response relationship between exercise and body mass with higher exercise workloads resulting in a greater decrease in body mass. Endurance sporting activities (i.e., jogging, cycling, swimming, etc.) are therefore one very effective component of an overall weight management strategy. It must be emphasized that athletes seeking to reduce body mass must couple with deliberate control of caloric intake and should expect sustainable weight loss to occur slowly over months not weeks.

Metabolic Syndrome and Diabetes Mellitus

Exercise training improves metabolic health, including increased insulin sensitivity and noninsulin-mediated skeletal muscle glucose metabolism (Goodyear and Kahn, 1998; Henriksen, 2002). The dose of exercise is directly associated with improvements in insulin sensitivity in a graded fashion with greatest improvements seen in those with high intensity and high duration of exercise (Johnson et al., 2007; Dube et al., 2012). Studies evaluating the impact of exercise on prevention and treatment of metabolic syndrome and diabetes mellitus have shown that weight loss is the most important determinant to reduce the incidence of diabetes mellitus (Tuomilehto et al., 2001; Knowler et al., 2002). Athletes with the greatest exercise exposure have the lowest risk of developing diabetes mellitus even after controlling for weight loss and diet (Tuomilehto et al., 2001). Taken together, comprehensive lifestyle choices including exercise, diet and, weight loss have been shown to prevent metabolic syndrome and diabetes mellitus.

Physical Fitness

There is an inverse relationship between cardiorespiratory fitness and both all-cause and cardiovascular mortality (Blair et al., 1989, 1996; Kokkinos et al., 2010). Put simply, fitter people tend to live longer than less fit people. Fitness can be improved by increasing exercise frequency, intensity, or duration among people of all ages and both sexes (Spina, 1999; Seals et al., 1984). Physical fitness is determined by many factors in addition to exercise including age, sex, body mass, and genetics (Bouchard and Rankinen, 2001). Physical fitness is far more responsive to exercise training than any of the traditional cardiovascular risk factors mentioned above. Additionally, routine physical activity attenuates the risk of cardiovascular disease and mortality more than that which can be explained by its impact on individual risk factors.

Management of Cardiovascular Disease in the Athlete

Clinicians must be thorough with their history taking, as athletes may be less willing to be forthright with symptoms or exertional limitations. When exercise stress testing is utilized in athletes it is imperative that they are maximal effort tests designed to simulate race type workloads and efforts. Additionally, cardiovascular limitations, ischemia, and arrhythmia may only be seen at peak efforts so tests should not be terminated after achieving a submaximal target heart rate. As endurance athletes can achieve much higher workloads than their sedentary counterparts, achieving a normal workload during an exercise test may represent a significant functional impairment in an athlete. Thus, all data should be interpreted in the context of an athlete and clinicians should have an appreciation for basic exercise physiology.

Coronary Arteries

Coronary Artery Disease

Despite the cardiovascular benefits of routine moderate intensity exercise, vigorous exertion actually transiently increased the risk of acute myocardial infarction and SCD in individuals with underlying CAD (Siscovick et al., 1984; Mittleman et al., 1993; Giri et al., 1999). CAD remains the leading cause of exercise-related cardiac events, defined as those occurring during or within one hour of strenuous exercise, in adults (Rai and Thompson, 2011). Additionally, it has been shown that there are higher event rates among those who are occasionally active (i.e., weekend warriors) as compared with those who are habitually active (Mittleman et al., 1993; Willich et al., 1993). Thus, routine and habitual moderate aerobic activity is not only the best for cardiovascular health and longevity but also the safest from a cardiac event perspective. Furthermore, the relative cardiovascular stress of exercise will vary from individual to individual based on underlying fitness, cardiovascular disease, and age. Athletes with obstructive CAD generally present with chest discomfort that reproducibly develops with exertion, resolves with rest and is located in the substernal and/or left chest area. The constellation of symptoms are typical of stable angina with the discomfort characterized as an ache, pressure, or tightness that can radiate to the jaw or arm and may be accompanied with a sense of air hunger.

In addition to routine questions regarding chest pain, pressure, and dyspnea on exertion, more subtle changes and symptoms should be assessed. Due to their competitive nature, athletes may minimize symptoms so careful assessment can provide subtle clues to changes in exercise habits or symptoms. Unexplained decreases in exercise tolerance or an inability to maintain prior training regimens or race paces may indicate underlying CAD. Many victims of exertion-related SCD reported possible cardiac symptoms within a week of their death (Thompson et al., 1979). Some athletes may experience chest discomfort during the initial few minutes of exercise that abates with continued exertion. This is commonly referred to as "warm up angina," a process that reflects focal early exercise coronary artery blood flow insufficiency that is relieved by the subsequent recruitment of collateral arteries, and should prompt comprehensive assessment for underlying atherosclerotic CAD (Parker and Thompson, 2012). Similarly, gastrointestinal symptoms that occur with exertion should be treated as cardiac in nature until proven otherwise.

We prefer functional assessment with maximal effort cardiopulmonary exercise testing (CPET) to evaluate for ischemia, arrhythmia, and cardiopulmonary capacity. In general, we avoid coronary artery calcium scoring (CACS) as in our experience we have seen a high prevalence of left main and proximal left anterior descending calcification in endurance athletes without risk factors, a clinical scenario that is devoid of outcome data and one that we have not anecdotally seen as a marker for future adverse events. Maximal effort exercise testing in these individuals typically reveals supranormal exercise capacity without symptoms, ischemia or arrhythmia. Thus, it is probable that the mechanism and prognostic significance of coronary artery calcification among aging athletes may be different from those with traditional risk factors. Until further literature defining the clinical significance of coronary artery calcification among aging athletes is available, we recommend against the routine use of CACS in this population. In cases when an athlete's degree of CAD is disproportionate to his/her risk factors, an elevated Lipoprotein (a) (Lp(a)) may be a possible explanation (Danesh et al., 2000). Niacin therapy has been shown to reduce Lp(a) levels but there is no data that decreasing Lp(a) improves outcomes at this time (Parker and Thompson, 2012).

Athletes diagnosed with CAD by noninvasive imaging or after a cardiac event benefit from aggressive medical and in some cases interventional management. Prevention of an incident or subsequent event is the goal of therapy. Standard of care requires treatment with platelet inhibition, optimal blood pressure control, aggressive lipid management, and exercise counseling. Recommendations regarding returning to exercise and competitive sport are based on the presence of residual disease, wall motion

abnormalities, LV systolic function, and adherence/tolerability of comprehensive medical therapy. We recommend that all athletes undergo cardiac rehab after an acute coronary syndrome followed by a maximum effort CPET to evaluate for signs of residual ischemia, arrhythmia, or cardiopulmonary limitations. If testing is unremarkable, we recommend a gradual increase in activity with the goal of returning to competitive sport by 1 year after the initial event.

Coronary Anomalies

While the majority of people with CCAA live a full life without symptoms and die of unrelated causes, CCAA are the second most frequent cause of SCD in young athletes, accounting for about 20% of cases (Cheitlin et al., 1974; Liberthson et al., 1979; Taylor et al., 1992; Basso et al., 2000). Malignant anomalies, with either the left main or right coronary artery arising from an inappropriate coronary sinus, are those that traverse in an anterior course between the aorta and pulmonary trunk leading to transient compression during strenuous exercise (Taylor et al., 1997). The prevalence of wrong sinus coronary anomalies is thought to be less than 0.2% but prevalence data are sparse. Additional risk factors dictating sudden death risk include a stenotic or a slit-like ostium, an intramural arterial course, and the presence of distal arterial atresia (Pelliccia, 2001). The mechanism of SCD is thought to be triggered by myocardial ischemia and is supported by pathologic evidence of myocardial damage in the region supplied by the anomalous coronary artery (Liberthson et al., 1979; Taylor et al., 1997). In those with sudden death, ventricular fibrillation is likely the final event (Liberthson et al., 1979; Cheitlin, 1998).

Many people with CCAA go undiagnosed as symptoms of chest discomfort, atypical chest pain, and/or syncope are reported in less than 30% of patients (Basso et al., 2000; Taylor et al., 1997). Symptoms are most commonly reported in those with left main arising from the right coronary sinus and generally occur with exercise (Taylor et al., 1992). CCAA cannot be identified on screening ECG and the absence of ischemia during an exercise stress test is not sensitive enough to rule out the diagnosis in those with symptoms (Basso et al., 2000). In the case of a young athlete with exertional chest pain or syncope, the coronary origins should be identified as part of the work up. Echocardiography has been shown to visualize the ostium and proximal epicardial course of the left main in 97% and right coronary artery in 80% of subjects (Pelliccia et al., 1993; Davis et al., 2001). When the origin of both coronary arteries cannot be appropriately visualized with echocardiography, magnetic resonance imaging (MRI), cardiac computer tomography, or conventional coronary angiography should be employed based on institutional and provider preferences.

Treatment recommendations should be tailored to individual characteristics of the athlete and the type coronary anomaly. In general, young athletes with CCAA presenting with symptoms or documented ischemia should undergo surgery to repair the anomaly. In young asymptomatic athletes without ischemia, a shared decision-making should be undertaken. Despite being asymptomatic, an accurate risk assessment cannot be made and to avoid the risk of sudden death it is reasonable to undergo repair of the anomaly, particularly in the case of left coronary arteries that arise off the right sinus of Valsalva. Patients >35 years old who are asymptomatic may forego surgery as the greatest risk of SCD seems to be at a younger age but again shared decision-making is imperative (Taylor et al., 1997; Kragel and Roberts, 1988).

Arrhythmias

Conduction Abnormalities

It is well established that incomplete right bundle branch block (RBBB) is common, particularly among endurance athletes (Kim et al., 2011). In addition, complete RBBB are not uncommon among endurance athletes and may be a marker of physiologic adaptations in response to the repetitive volume challenge in isotonic exercise (Kim and Baggish, 2015). In a study of collegiate endurance athletes, athletes with incomplete and complete RBBB demonstrated increased biventricular chamber dimensions and relative reductions in right ventricular (RV) systolic function, with the degree of change in RV size and function correlating with increased QRS duration and exercise exposure (Kim et al., 2011). In the absence of clinical symptoms or associated right precordial repolarization abnormalities suggestive of ARVC, the presence of incomplete RBBB should not be considered to represent cardiac pathology (Bussink et al., 2013). In our experience, asymptomatic endurance athletes with isolated RBBB and QRS duration <140 ms without repolarization abnormalities apart from concordant T wave inversions in V1 and V2 are unlikely to harbor significant cardiovascular disease. In contrast, atypical RBBB that have a QRS duration in excess of 140 ms or those that are associated repolarization abnormalities that extend beyond V2 should be pursued further, as they are likely to represent underlying structural cardiac disease. Similarly, there is a low prevalence of left bundle branch block (LBBB) reported in healthy athletic cohorts and although data is sparse, the presence of LBBB in an athlete does not appear to represent a physiologic response to exercise. LBBB is more likely a result of structural heart disease and data suggests that LBBB is a marker of future cardiovascular morbidity and mortality (Rabkin et al., 1980; Schneider et al., 1979).

Bradycardia

Sinus bradycardia is common and normal among trained endurance athletes. Profound bradycardia (<40 bpm), a result of heightened parasympathetic activity, can be seen during sleep or rest and is not associated with a poor prognosis. Bradycardia is not a concern in the asymptomatic well-trained athlete. Documentation of an appropriate increase in heart rate with exercise is typically sufficient to exclude a pathologic process.

Premature Beats

Premature beats (both atrial and ventricular) may be observed in trained athletes. Palpitations caused by premature beats are a common reason for athletes to seek medical assistance. Endurance athletes may be particularly aware of such alterations in rhythm, as they are typically lean and in sync with their bodies. Symptomatic premature beats are often described as a slight pause followed by a heavy, single, central thump. Evaluation with an echocardiogram is often required to exclude structural and valvular heart disease. Treadmill exercise stress testing can be utilized to assess the frequency of premature beats during exercise. Absence of structural heart disease in conjunction with suppression of premature beats during exercise is reassuring, and in this scenario premature atrial and ventricular beats are considered benign and without long-term consequences (Biffi et al., 2002, 2004).

Atrial Fibrillation

Atrial fibrillation (AF) may be problematic in the trained athlete. Studies suggest that AF is more common in endurance athletes, especially in the older athlete, compared to their sedentary counterparts (Sorokin et al., 2011; Mont et al., 2002; Molina et al., 2008). The mechanisms responsible for development of AF in endurance athletes are speculative. Postulated mechanisms include atrial dilatation seen in dynamic sports with increases in preload (Pelliccia et al., 2005), increased vagal tone, and inflammatory changes related to sports participation (Swanson, 2006). AF in endurance athletes presents in two distinct forms. The first is parasympathetically mediated and is characterized by the onset of AF during times of slow resting heart rate that can potentiate atrial escape (Stein et al., 2002). Athletes with this form of AF generally experience bouts of arrhythmia during sleep, quiet rest, or after meals. This is commonly referred to as "vagally-mediated" AF and accounts for about 70% of AF in athletes (Mont et al., 2008). Vagally induced AF almost never occurs with exercise, is four times more common in men (Coumel, 1994), and can be precipitated by alcohol (Yesil et al., 1999). The second form of AF is sympathetically mediated and is commonly triggered by exercise or significant psychological stress. The importance of differentiating these two forms of AF lies in their diametrically opposed treatment algorithms.

Initial evaluation includes a thorough history and physical followed by blood tests with evaluation of electrolytes and thyroid function. Additional testing to evaluate for cardiac structure and function with an echocardiogram is recommended. It is imperative that questioning include the use of supplements, caffeine, alcohol, and drugs as use of any of these may increase the likelihood of developing AF. Evaluation and treatment of underlying hypertension at rest and/or during exercise is required. Treatment is based on risk factors and symptoms with management options that include detraining, which can decrease the number of AF episodes (Hoogsteen et al., 2004), negative chronotropic or antiarrhythmic medications, and catheter-based ablation. For patients with paroxysmal AF and a structurally normal heart, a pill in the pocket approach with flecainide or propafenone can be effective in converting more than 90% of outpatient episodes (Alboni et al., 2004). Verapamil may be effective in athletes with sympathetically mediated AF. If ineffective, we recommend trial of antiarrhythmic agents. We reserve catheter-based ablation for athletes who have failed to remain in sinus rhythm on medications, those who experience side effects on medications, or those who prefer an ablation.

Ventricular Arrhythmias

Healthy athletes undergoing exercise testing may demonstrate short bursts of asymptomatic nonsustained ventricular tachycardia (VT). This finding should prompt testing to exclude structural heart disease which, if absent, portends a benign prognosis and does not require medical therapy or sport restriction. Sustained VT in a structurally normal heart is likely of RV outflow tract or fascicular origin and is generally amenable to catheter-based ablation. If the arrhythmia is successfully ablated and cannot be induced, we recommend that athletes wait a minimum of four weeks before resuming exercise. This may be longer than other recommendations but in our experience there is increased recurrence of arrhythmia if athletes resume play prematurely. VT in a structurally abnormal heart requires many considerations and should be managed according to guidelines (Pedersen et al., 2014). Symptomatic or sustained ventricular arrhythmias carry a high risk of adverse events and represent a medical emergency or at least a reason to prohibit training and competition until a competent sports cardiologist conducts a comprehensive assessment.

Patients deemed to be high risk for SCD independent of sports participation are recommended to have implantable cardioverter defibrillators (ICD)s. Importantly, guidelines specifically state that ICDs should not be placed for the sole purpose to allow sport participation. In those with preexisting ICDs or ICDs placed for high risk individuals there are concerns of device malfunction or injury. The interested reader is directed to a thorough discussion regarding sport participation with ICDs (Lampert, 2012).

Valvular Heart Disease

The evaluation of cardiac murmurs is a frequent source of consultation for the sports cardiologist. Assessing valvular heart disease in athletes is challenging as exercise-induced cardiac remodeling can create physiologic innocent flow murmurs that are difficult to distinguish from pathologic murmurs during physical examination. In young athletes, innocent flow murmurs reflect increased physiologic flow across the pulmonic valve and through the main pulmonary artery, while in older athletes this may be related to aortic sclerosis (Thompson, 2001). Athletes evaluated for valvular disease generally fall into one of three categories. In the first, examination and work up reveal a physiologic flow murmur. In the second, work up reveals severe valve disease or symptoms that

require valve intervention. In the third, there is clear valvular heart disease but treatment and/or activity recommendations are not clearly defined.

Echocardiography is essential in evaluating those with suspected valvular heart disease. However, in cases with equivocal history and echocardiograms, exercise testing can help with timing of intervention as it provides an objective functional assessment of an athletes' cardiopulmonary capacity and an indication as to whether the athlete is truly asymptomatic. The relevant findings include a drop or inadequate increase in blood pressure, arrhythmias, ischemic ECG changes, or impaired exercise tolerance.

Aortic stenosis (AS) is generally a result of degenerative valve disease, but in a younger athletic population bicuspid aortic valve and congenital AS are also commonly seen. The hemodynamic significance of any specified aortic valve area depends on the cardiac output. Since muscle mass is a determinant of resting cardiac output, larger individuals may have more severe hemodynamic impairment despite a larger absolute aortic valve area (Thompson, 2001). This may be a factor in athletes with increased body size and muscle mass. AS typically has a long asymptomatic period, but when symptoms do occur patients present with syncope, exertional intolerance, angina pectoris, and heart failure. Exercise testing can be invaluable in the assessment of athletes with severe AS who suggest that they are asymptomatic. Our practice is to closely observe asymptomatic athletes with severe AS, normal ejection fractions, and appropriate exercise capacity as the risk of surgery and anticoagulation likely outweigh any benefit of proceeding with surgery. In these cases, serial follow-up is paramount with the goal to intervene immediately at the onset of symptoms as a delay in intervention can lead to worse outcomes once there is a reduction in ejection fraction.

Chronic regurgitant lesions, including mitral and aortic regurgitation, can be well tolerated by endurance athletes for extended periods as the LV has undergone exercise-induced cardiac remodeling and thus is well adapted to accept the volume challenge. However, the bradycardia that is also seen in these athletes can worsen aortic regurgitation at rest. Additionally, static exercise, which is accompanied by increases in afterload, can worsen regurgitant valvular disease and thus athletes are advised to avoid isometric exercises. While generally well tolerated, it is important that athletes are queried for exercise-induced symptoms of heart failure.

Managing athletes with valvular heart disease requires a careful history, examination, and review of echocardiogram and exercise testing. Additionally, a sound understanding of each patient's goals and preferences will play a role in final recommendations. Due to high workloads, athletes may become symptomatic at earlier disease stages when compared to less active patients. Such cases are not part of the recently updated valvular heart disease guidelines (Nishimura et al., 2014) as athletes may become symptomatic at high intensity exercise with moderate levels of valve disease. The clinician must weigh the risk of intervening too soon and its downstream consequences of having a mechanical valve or finite life of bioprosthesis. If available, access to prior echocardiograms allows the clinician to follow changes in valve pathology and chamber dimensions over time and can help in determining timing of intervention.

Sports cardiologists should seek out surgeons that have an understanding of exercise physiology and surgeons should be made aware of the athletic ambitions of these patients. For instance, it would be important to discuss with a surgeon prior to mitral valve repair for severe mitral regurgitation that a slightly larger annuloplasty ring with mild residual mitral regurgitation is an acceptable and preferred outcome over facing the challenges of functional mitral stenosis if a smaller annuloplasty ring is placed.

Aortopathies

The hemodynamic load on the aorta is dependent on the type of sport. Endurance activities result in high volume aortic flow with modest systemic hypertension while isometric activities can cause profound systemic hypertension with normal aortic flow (Baggish and Wood, 2011). The impact of sport specific exercise training on aortic dimensions has not been definitively delineated as prior studies have had contradictory findings. Pelliccia et al. (2010) found that endurance trained athletes, specifically swimmers and cyclists, had the largest aortic root dimensions in a heterogeneous group of 2317 Italian athletes. Meanwhile, D'Andrea et al. (2010) measured aortic dimensions in 615 elite athletes (370 endurance-trained athletes and 245 strength-trained athletes) and found that the aortic root diameter was significantly larger among strength trained athletes. While conclusions regarding sport specific effects on aortic dimensions cannot be made, a finding in common among such studies is that the aortic root rarely exceeds the clinically accepted upper limits of normal (40–42 mm) in trained athletes. Thus, pending further studies, it seems that athletic training alone does not cause marked aortic dilation. Readers are referred to a review of this topic (Pelliccia et al., 2012). In athletes with aortic root >40–42 mm in the absence of diagnostic criteria for Marfan syndrome, bicuspid aortic valve, or any identifiable systemic disease, close evaluation and if athletic participation is allowed, close serial assessment is paramount.

Cardiomyopathies

The clinical task of differentiating marked exercise-induced cardiac remodeling from mild forms of HCM, familial or acquired dilated cardiomyopathy and ARVC should be undertaken by clinicians with sound understanding of each clinical entity. Missing a diagnosis of a cardiomyopathy can inappropriately put an athlete at increased risk for SCD. Alternatively, improperly diagnosing an athlete with a cardiomyopathy can have long term social, economic, and cardiovascular and mental health consequences.

There is no single diagnostic test with adequate accuracy to differentiate adaptive from pathological cardiomyopathy. Thus, we encourage clinicians faced with this diagnostic dilemma to begin the assessment with an integrated assessment of personal and family history, 12-lead ECG, and echocardiography. In many cases, information derived from this initial work up is sufficient for

diagnostic decision-making. If the diagnosis remains unclear, further measures including cardiac MRI, CPET, prescribed detraining and disease specific genetic testing may be considered. Cardiac MRI has become an invaluable tool for evaluation of the athlete with indeterminate cardiac enlargement. Cardiac MRI provides highly accurate assessment of myocardial thickness, chamber volumes, tissue composition, extracardiac anatomy and cardiac magnetic resonance-derived reference values in athletes have been published (Prakken et al., 2010). Additionally, use of gadolinium contrast with delayed imaging provides information about the presence and location of myocardial fibrosis. Specific myocardial fibrosis patterns are seen in distinct cardiomyopathies and can lead to the diagnosis in athletes with indeterminate cardiac enlargement (Maron et al., 2008; Prakken et al., 2009; Rubinshtein et al., 2010).

Hypertrophic Cardiomyopathy

HCM, characterized by ventricular hypertrophy in the absence of another cause and myocardial relaxation abnormalities, is a common condition encountered amongst those who care for athletes. With the prevalence of 1 in 500 people and increasing rates of screening and SCD awareness, more and more athletes are being diagnosed and followed. HCM is inherited in an autosomal dominant fashion and is caused by mutations in cardiac sarcomeric proteins. Phenotypic expression of the disease is highly variable within families. Diagnosis can be especially difficult if an athlete is in the "Maron grey zone" (Maron, 2003) and has properties of both athletic remodeling and HCM. A valuable approach to the origin of LV hypertrophy has been outlined (Maron et al., 1995). Tissue Doppler assessment can be valuable as early diastolic relaxation velocity has been shown to be normal or enhanced in athletes with LV hypertrophy resulting from exercise-induced cardiac remodeling (Pela et al., 2004; Zoncu et al., 2002). In contrast, pathologic forms of LV hypertrophy are typically associated with reduced early diastolic relaxation velocity and peak systolic tissue velocity (Matsumura et al., 2002; McMahon et al., 2004; Nagueh et al., 2001).

Management guidelines of symptomatic and obstructive HCM have been published (Gersh et al., 2011). These guidelines also discuss ICD criteria for primary prevention. Sport participation guidelines recommend IA sports for athletes with HCM. Our approach starts with a maximal effort CPET to evaluate for blood pressure response to exercise as well as for arrhythmias. If exercise testing is unremarkable and the athlete does not have any high-risk features as per the guidelines, we use a less conservative approach for our exercise recommendations. Due to the known long-term risks of a sedentary lifestyle and knowledge that arrhythmias tend to be provoked with sympathetic surges, we recommend that athletes with HCM engage in low to moderate sustained aerobic exercise for cardiovascular health. It is vital to stress the importance of an appropriate warm up and a cool down and avoidance of activities that require sprint physiology such as high intensity interval training or cross fit type training. Additionally, we recommend that athletes stay below their ventilatory threshold (determined by CPET) as prior studies in healthy subjects show an initial linear increase in catecholamines with increasing exercise intensity but a sharp increase in catecholamine levels after crossing the ventilatory threshold (Urhausen et al., 1994; Weltman et al., 1994). We review the guidelines with athletes, discuss our approach and rationale, and engage in shared decision-making.

Arrhythmogenic Right Ventricular Cardiomyopathy

Managing athletes with ARVC is complex. While it is clear that patients with ARVC should not participate in competitive or endurance sports, current guidelines also subject those with borderline or even a possible diagnosis of ARVC to these recommendations (Maron et al., 2015c). This is because patients with ARVC have a predisposition to sudden death and RV dysfunction. Exercise has been shown to both increase the incidence of VT and the progression of RV dilation. In heterozygous plakoglobin-deficient mice, vigorous exercise was associated with greater RV enlargement, RV systolic dysfunction, and ventricular arrhythmias when compared to wild-type control (Kirchhof et al., 2006). These findings were corroborated in a study of gene positive endurance athletes. Gene positive athletes had symptoms at a younger age, lower lifetime survival from VT/ventricular fibrillation and heart failure compared to more sedentary gene positive individuals (James et al., 2013).

A recent consensus statement published on the treatment of ARVC gives a IIa recommendation to using beta blockers in all patients with ARVC irrespective of arrhythmias (Corrado et al., 2015). This recommendation stems from the fact that beta blockers may reduce exercise-induced VT, may decrease progression to heart failure, and have a potential to decrease disease progression by lowering RV wall stress.

Dilated Cardiomyopathy

Dilated cardiomyopathy is characterized by dilation and impaired systolic function of one of both ventricles. Individuals may be asymptomatic or have end-stage heart failure, arrhythmias, and/or thromboembolic complications (Saberi and Day, 2016). About 20%–30% are familial and inherited in an autosomal dominant pattern and if present, children and family members should be screened (Grunig et al., 1998; Goerss et al., 1995). Management guidelines have also been published (Yancy et al., 2013).

Left Ventricular Noncompaction

LV noncompaction is characterized by prominent LV trabeculization and deep intertrabecular recesses resulting in an outer compacted and inner noncompacted myocardial layer. Autosomal dominant inheritance is most common but can be sporadic (Zaragoza et al., 2007; Weiford et al., 2004). Clinical expression is variable, and reduced ejection fraction, ventricular arrhythmias,

thromboembolic disease, and sudden death have all been reported (Greutmann et al., 2012; Oechslin et al., 2000). Diagnosis can be made by echocardiography but cardiac MRI can better define the extent of disease and presence of fibrosis. Few competitive athletes with LV noncompaction have been reported clinically and as a result, the consequences of the disease in this population remain unknown. The risk for adverse outcomes seems to be associated with LV systolic dysfunction or VT (Brescia et al., 2013). Similarly, nonathletes with a normal ejection fraction and chamber size have a good prognosis (Murphy et al., 2005). However, without data on long-term follow up, we cannot adequately risk stratify these athletes.

Conclusions

There has been a steady rise in competitive sport participation worldwide. Regular exercise has many cardioprotective effects including mitigating traditional cardiovascular risk factors and reducing cardiovascular death. However, athletes are not immune to cardiovascular disease and the majority of SCD in athletes is in the presence of underlying and previously undetected cardiovascular disease.

Additionally, participants in competitive sport now range from lifelong habitual athletes to weekend warriors of different ages, social habits, and cardiovascular risk factors. Thus, the benefits of increased exercise doses may be offset by underlying risk factor profiles. Updated guidelines on competitive sport participation should be used as a reference but prove to be too conservative for many athletes and thus a shared decision-making model is essential in the care of athletes with cardiovascular disease. As a result, providers caring for athletes need to be aware of the nuances in history taking, appropriate work up and management of cardiovascular disease in this unique patient subset.

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Mechanisms of Cardiac Arrhythmias: Molecular and Cellular Perspective

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Introduction

Cardiovascular diseases account for about one out of three deaths in the industrial world, and about 45% of the cardiac deaths are sudden cardiac deaths attributed to abnormal cardiac electrical activity (arrhythmic events) such as ventricular fibrillation (VFib) (Mozaffarian et al., 2016; Abdelghani et al., 2016). In this article, we will consider underlying mechanisms of the most prominent cardiac arrhythmias such as ventricular tachyarrhythmias (VT and VFib), long QT syndrome (LQTS), short QT syndrome (SQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D), and Brugada syndrome (BrS). To aid understanding of this material, we will start from brief overview of pertinent electrophysiological concepts.

Important Electrophysiological Concepts

Understanding of mechanisms of arrhythmias relies on several important electrophysiological concepts that describe membrane currents, changes in membrane potential (action potential, AP), and how these changes in membrane potential produce macroscopic cardiac electrical activity (electrocardiogram, ECG).

Membrane Currents

Membrane currents are movements of ions across cell membrane that are driven by electrochemical gradients. At its simplest, electrochemical gradient can be viewed as a difference in ion concentration between inside and outside of the cell (**Fig. 1A**). Currents are grouped by carrier ions: Na^+ currents (I_{Na}), Ca^{2+} currents (I_{Ca}), and K^+ currents (I_K) are the major currents underlying electrical activity in cardiac cells (Catterall, 2014; Nerbonne, 2014; Oudit et al., 2004; Rose and Backx, 2014). Depending on the direction of the flow, currents can be inward (current flows from outside to inside the cell) or outward (current flows from inside to outside of the cell). Since currents are driven by ionic gradients, which are generally stable under normal physiological conditions, each type of the major current has a typical direction of the flow. I_{Na} and I_{Ca} are inward, and I_K is outward. Directions of the current flow will determine its action on membrane potential. Inwardly flowing currents will bring more positive ions (Na^+ and Ca^{2+}) inside the cell making inside of the cell less negative or polarized; hence, inwardly flowing currents, such as I_{Na} and I_{Ca}, are depolarizing. Conversely, outwardly

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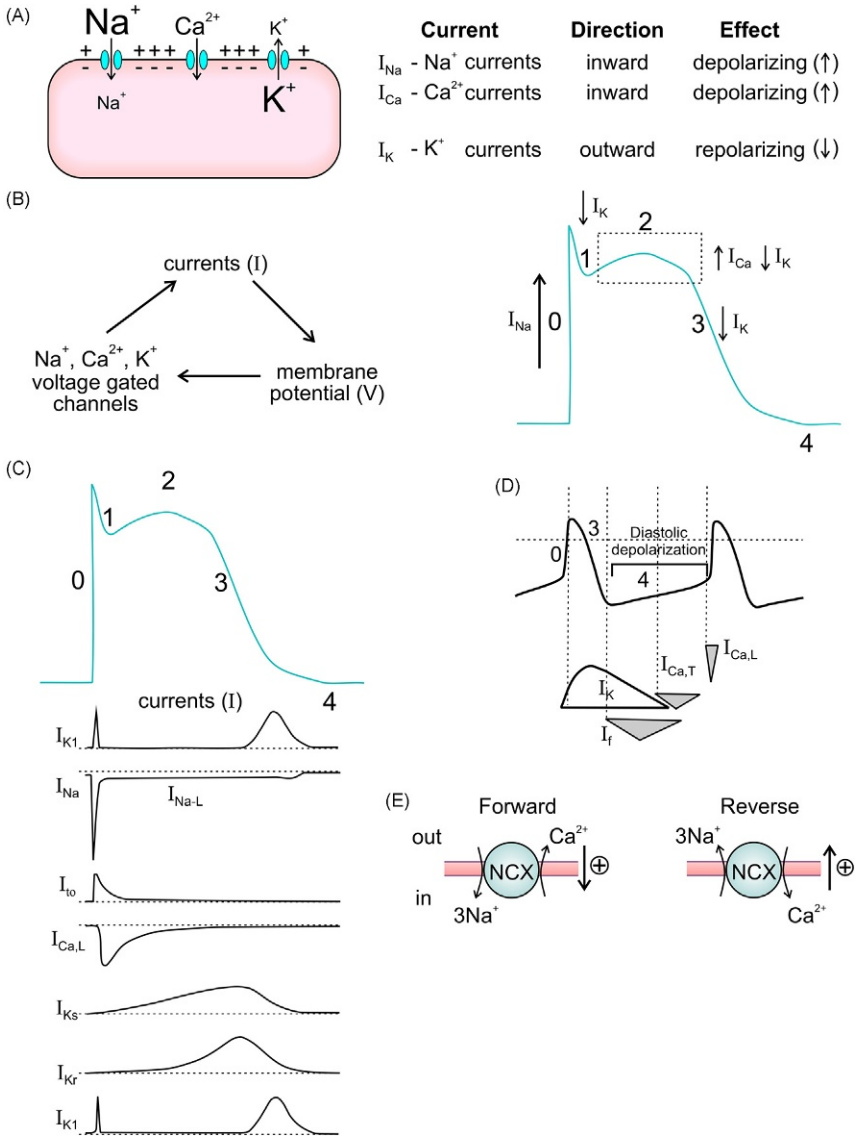

Fig. 1 Electrophysiological concepts of normal electrical activity. (A) Main membrane currents of ventricular myocyte and their effects on the membrane potential. (B) Ventricular action potential and its phases. (C) Currents underlying ventricular action potential. (D) Currents underlying pacemaker action potential. (E) Modes of function of Na^+–Ca^{2+} exchanger NCX and current via NCX.

flowing currents will remove positive ions (K^+) from inside the cell making inside of the cell more negative restoring cell membrane polarization; hence, outwardly flowing currents, such as various I_K, are repolarizing (Fig. 1A).

Membrane Potential: Action Potential

Self-propagating change in membrane potential that goes from depolarization to repolarization is called AP. AP is driven by ionic currents that depolarize and repolarize the membrane and changes in the ionic currents (I) brought by voltage-gated channels reacting to the changes in membrane potential (V) (Fig. 1B). Ventricular AP is divided in phase 0 (fast depolarization), phase 1 (initial repolarization), phase 2 (plateau that is characterized by slow change of membrane potential), phase 3 (repolarization), and phase 4 (resting phase that is characterized by a stable negative potential) (Fig. 1B). During phases 0 through 2 cardiac myocytes are in an absolute refractory period and cannot be re-excited. In phase 3, excitation is possible, but threshold of stimulation is higher. Finally, in phase 4, the myocyte enters a state of normal excitability.

Ionic Currents Underlying Ventricular Action Potential

Normally, ventricular myocytes have stable negative membrane potential due to presence of large number of constituently open K^+ channels (Kir2.1, *KCNJ2*). These channels produce outward repolarizing current (I_{K1}) in response to any depolarization or injection

of inward current counteracting depolarizing influence and stabilizing membrane potential at the resting levels. However, if depolarization is strong enough to produce inward current (I_{Na}) larger, then counteracting outward I_{K1}, AP begins (Fig. 1C). In phase 0, large inward Na^+ current (I_{Na}) via $Na_v1.5$ (*SCN5A*) depolarizes the cell producing upstroke of AP (Fig. 1C). Initial repolarization (phase 1) follows and is driven mainly by repolarizing outward K^+ current I_{to} mainly via $K_v4.1$ (*KCND1*). In the absence of I_{to}, APs do not have "notch" and transition gradually to plateau (phase 2) (Fig. 1C). Plateau phase is characterized by relative balance of inward currents (mainly L-type Ca^{2+} current, $I_{Ca,L}$, via $Ca_v1.2$, *CACNA1C*) and outward K^+ currents I_{Ks} (via $K_v7.1$, *KCNQ1*) and I_{Kr} ($K_v11.1$, *KCNH2*) (Fig. 1C). Once balance is decidedly shifted toward K^+ currents, repolarization (phase 3) begins. This phase is dominated by I_{Ks}, I_{Kr}, and later I_{K1}, which finishes repolarization and stabilizes the cell in the rest phase (phase 4) (Fig. 1C).

Ionic Currents Underlying Pacemaker Action Potential

Pacemaker cells of sinoatrial node (SA) have a distinct AP that is characterized by diastolic depolarization in phase 4 and relatively slow depolarization in phase 0 that is driven by $I_{Ca,L}$ (Fig. 1D) rather than I_{Na} as in ventricular myocytes. Diastolic depolarization is driven by inward "funny" current I_f (via HCN channels, *HCN4*) that unlike many other voltage-gated channels increases with more negative voltages hence "funny." As diastolic depolarization progresses T-type Ca^{2+} current, $I_{Ca,T}$, overtakes as depolarizing current, and once membrane is depolarized enough to activate L-type Ca^{2+} current upstroke or phase 0 of pacemaker AP begins (Fig. 1D). Phase 0 of pacemaker AP transition to repolarization (phase 3) as K^+ currents start to activate. Repolarization phase of pacemaker AP has a large contribution from acetylcholine-activated K^+ current $I_{K,Ach}$ (GIRK4, *KCNJ5*). Pacemaker rate is promoted by β-adrenergic agonists and by reduction of external K^+ (leading to reduction in repolarizing I_{K1} that counteracts the depolarization of membrane potential during diastolic depolarization).

Na^+–Ca^{2+} Exchanger (NCX) as a Modulator of Action Potential

NCX is an exchanger that exchange 3 Na^+ for 1 Ca^{2+}. In forward mode, 3 Na^+ ions move inside the cell extruding 1 Ca^{2+} ion and producing (3+)–(2+)=(1+) net charge transfer that generates inward current (Fig. 1E, left). In reverse mode, 3 Na^+ ions move out of the cell bringing in 1 Ca^{2+} ion resulting in outward current (Fig. 1E, right). Direction of the flow through NCX is determined by gradient of Na^+ and Ca^{2+} across membrane, and normally, during plateau phase, NCX is in reverse mode and in the forward mode during repolarization (Courtemanche et al., 1998). The dependence of NCX on intracellular Ca^{2+} (Ca^{2+} gradient across membrane) creates a feedback link between Ca^{2+} release and AP affecting repolarization of pacemaker and ventricular AP (Antzelevitch and Burashnikov, 2011). Depolarizing current of forward mode of NCX is responsible for the generation of arrhythmogenic responses such as delayed afterdepolarizations (DAD) and late phase 3 early afterdepolarization (EAD).

Variability in Electrophysiological Properties Across Myocardium

Within ventricle, going from outside in, three types of ventricular myocytes can be distinguished functionally and electrophysiologically: epicardial, M-, and endocardial cells (Antzelevitch and Burashnikov, 2011; Antzelevitch et al., 1991). These cells differ in current composition and thus AP shape (Fig. 2A). Epicardial myocytes have very prominent "notch"-shaped early repolarization due to large repolarizing K^+ current (I_{to}) and relatively short duration due to large contribution from repolarizing K^+ current (I_{Ks}) (Fig. 2A, left). M-myocytes have relatively low density of I_{to} and less prominent notch than epicardial myocytes, and their AP is long due to joint effects of lower density of repolarizing K^+ current (I_{Ks}) and higher density of depolarizing Na^+ current I_{Na}-late (I_{Na-L}) (Fig. 2A, middle). Endocardial myocytes have no "notch" phase (the lowest I_{to} density) and AP duration similar to epicardial myocytes (Fig. 2A, right).

From Action Potential to ECG

Spread of excitation through cardiac tissue can be measured at macroscopic level as ECG. ECG reflects important features of underlying AP in different parts of the heart. Spread of excitation through atria produces P-wave of ECG (Fig. 2B). QRS complex of ECG represents spread of excitation through ventricles and followed by normally isoelectric ST segment. At that time, ventricles are fairly uniformly depolarized and membrane potential through ventricular tissue changes little because ventricular myocytes are in plateau phase of AP, and thus heart as a whole becomes isoelectric. Transition of AP from plateau to repolarization produces T-wave of ECG (Fig. 2B). Since Q-wave corresponds to the beginning of ventricular excitation and the T-wave reflects repolarization of ventricles, QT intervals correlate with the duration of ventricular AP.

Mechanisms of Arrhythmias

Arrhythmias can arise from changes in AP, abnormal automaticity, abnormal and triggered electrical activity, and re-entry. In this section, we will consider underlying mechanism of these phenomena and how they manifest themselves at cellular and organ level.

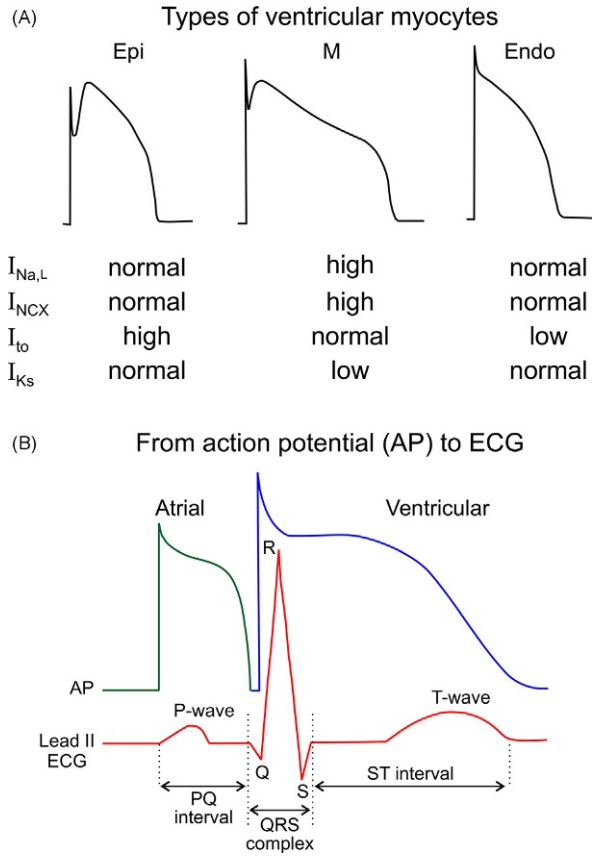

Fig. 2 Action potential in different types of ventricular myocytes (A) and correspondence between cellular excitation of cardiac tissue (action potentials, AP) and electrocardiogram (ECG) (B).

Changes in ECG and Action Potential in QT Syndromes

In long QT (LQT) and short QT (SQT) syndromes, QT interval is prolong or shortened, respectively. These changes arise from changes in AP, which becomes longer (LQT) or shorter (SQT) due to mutations that alter currents underlying AP. In many cases, mutations happen in channel proteins and these mutations are classified as channelopathies. Since AP duration is a delicate balance between opposite influences of depolarizing (I_{Na} and I_{Ca}) and repolarizing (various K^+ currents) currents, the effect of channelopathy depends on the direction of the current and the timing of the current flow within AP. In the case of LQT syndromes, loss-of-function mutation in channels carrying repolarizing K^+ currents will leads to reduction ($-\Delta$) in repolarizing currents, prolongation of AP, and prolongation of QT interval (Fig. 3A). For example, loss-of-function mutations LQT1 and LQT2 affect Ks channels and Kr channels, respectively, producing reductions ($-\Delta$) in corresponding repolarizing K^+ currents I_{Ks} or I_{Kr} and leading to prolongation of AP and in turn QT interval. Conversely, LQT syndrome may arise from the increase ($+\Delta$) in depolarizing currents that will lead to prolongation of AP duration and in turn QT interval (Fig. 3B). For example, gain-of-function LQT3 mutations increase ($+\Delta$) depolarizing Na^+ current (I_{Na}). In the case of SQT syndromes, mutations that cause increases ($+\Delta$) in repolarizing K^+ currents (gain-of-function mutation) will lead to promotion of repolarization, and as a result shortening of AP leading to shorter QT interval (Fig. 3C). Gain-of-function mutations SQTS1, SQTS2, and SQTS3 affect Kr, Ks, and K1 channels, respectively, producing increases in corresponding repolarizing K^+ currents I_{Kr}, I_{Ks}, and I_{K1} shortening AP duration and QT interval as a consequence. Conversely, SQT syndrome can result from decrease ($-\Delta$) in depolarizing currents that will lead to similar effect in shortening of AP duration and in turn QT interval (Fig. 3D). Both SQTS4 and SQTS5 reduce depolarizing $I_{Ca,L}$ by affecting either α (SQTS4) or β (SQTS5) subunit of the L-type Ca^{2+} channel. Abnormal changes in length of AP predispose to arrhythmic events such as EAD in the case of LQT syndromes and re-entrant arrhythmia in the case of SQT syndromes.

Abnormal Automaticity

Abnormal automaticity includes reduced automaticity, enhanced automaticity, and protected pacemaker (parasystole). In the case of *reduced automaticity*, reduction in the rate of primary pacemaker (SA) may lead to shift of the rhythm generation to subsidiary pacemakers (atrioventricular (AV) node or His bundle). The underlying causes might be (i) impaired or blocked impulse

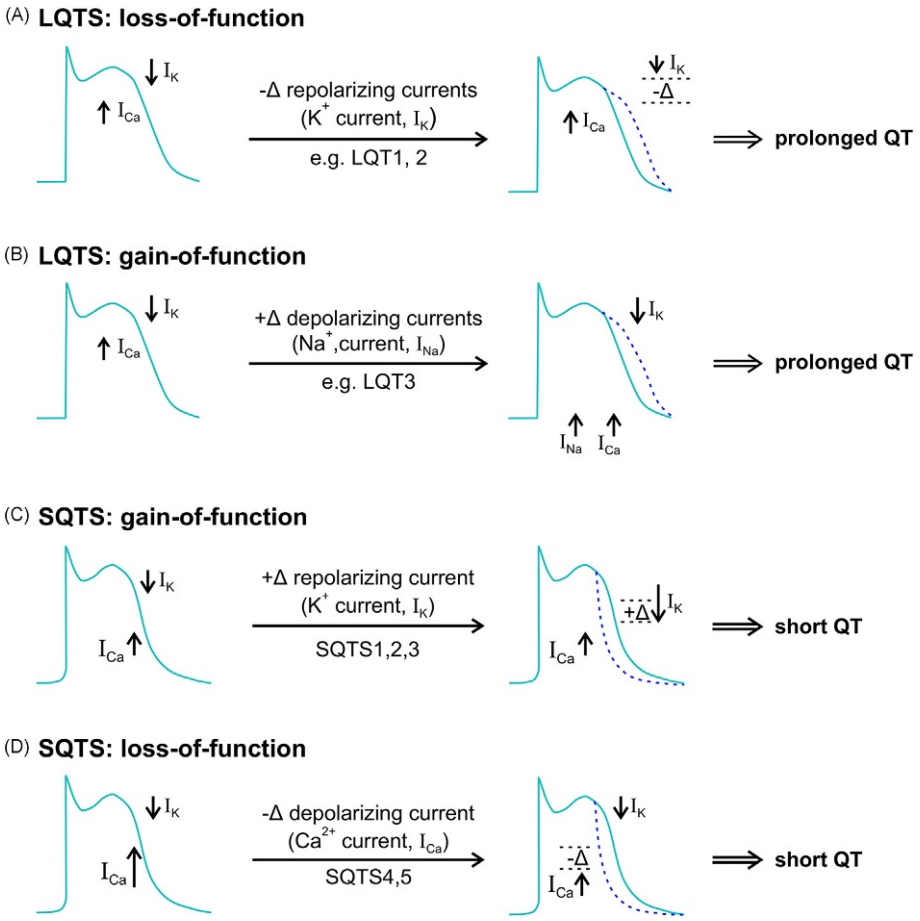

Fig. 3 Changes in action potentials due to long QT syndrome (LQTS) and short QT syndrome (SQT). (A) LQTS: prolongation of action potential due to loss-of-function mutation in repolarizing current. (B) LQTS: prolongation of action potential due to gain-of-function mutation in depolarizing current. (C) SQTS: shortening of action potential due to gain-of-function mutation in repolarizing current. (D) SQTS: shortening of action potential due to loss-of-function mutation in depolarizing current.

conductance out of SA node or (ii) increased vagal activity that suppresses SA node (Antzelevitch and Burashnikov, 2011). In either case, since the intrinsic rate of subsidiary pacemakers is lower, reduced automaticity will manifest itself as bradycardia. Another possible reason for reduced automaticity can be channelopathy of HCN channel responsible for I_f that drives diastolic depolarization. Loss-of-function mutations in *HCN4* gene cause familial bradycardia (Schulze-Bahr et al., 2003; Nof et al., 2007).

Enhanced automaticity arises when atrial and/or ventricular myocytes that normally do not display diastolic depolarization acquire that property. This type of automaticity develops in response to various conditions that lead to more depolarized resting potential (e.g., ischemia, infarction, experimental current injection) and is called depolarization-induced automaticity (Antzelevitch and Burashnikov, 2001). It is characterized by the rates substantially higher than normal automaticity, and similarly to normal automaticity, it arises from time-dependent reduction in repolarizing I_K and time-dependent activation of I_{Ca} that makes it sensitive to external K^+, β-adrenergic agonists, and Ca^{2+} channel blockers (Antzelevitch and Burashnikov, 2001, 2011).

Parasystole is arrhythmia caused by activity of additional secondary pacemaker that is protected (fully or partially conductively isolated) from primary pacemaker (SA node). Normally, secondary pacemakers are reset by excitation front generated by the activity of primary pacemaker. However, damage of the tissue (ischemia, infarction) may leads to creation of parasystolic focus. A region of cardiac tissue that is conductively isolated from entrance of excitation (entrance block) generated by primary pacemaker, but permit excitation generated with isolated tissue to exit. Such parasystolic focus will produce ectopic beats and may mimic re-entry patterns (Antzelevitch and Burashnikov, 2011; Jalife and Moe, 1979; Antzelevitch et al., 1983).

Abnormal and Triggered Electrical Activity at the Cellular Level

Abnormal electrical activity at cellular level can be divided in two major groups by their timing in relation to repolarization phase of AP: EAD and DAD. EAD occurs during phase 2 (late plateau) or phase 3 (repolarization) of AP, whereas DAD happens after repolarization has been completed. In either case, when EAD or DAD reach threshold potential spontaneous AP (triggered activity) is generated that may lead to extrasystole or initiation of re-entrant arrhythmias.

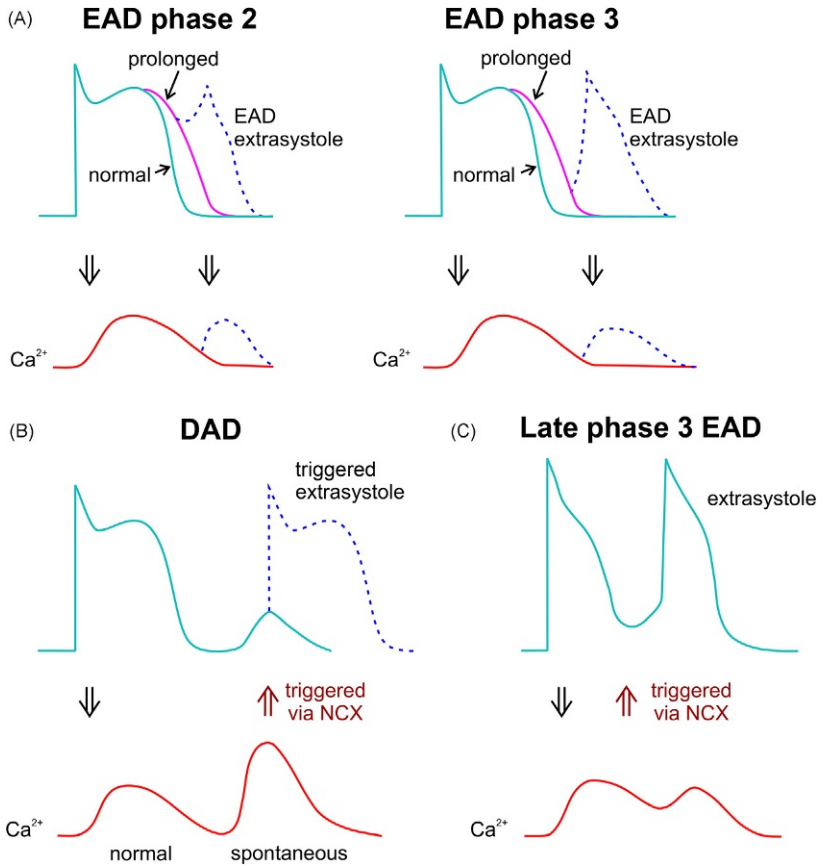

Fig. 4 Abnormal and triggered electrical activity at the cellular level. (A) Depictions of action potential with early afterdepolarization (EAD): phase 2 EAD (left), phase 3 EAD (right) and their corresponding Ca^{2+} releases (bottom). (B) Depiction of action potential followed by delayed afterdepolarization (DAD) and triggered extrasystole (*dashed line*) driven by spontaneous Ca^{2+} release (bottom). (C) Late phase 3 EAD triggered by normal Ca^{2+} release (bottom) from abnormally short action potential.

EAD are mainly arise when AP duration is prolonged due to slowing of the rate of repolarization. Slow repolarization allows inward (depolarizing) currents such as I_{Ca} and/or I_{Na} to recover and re-activate prematurely (**Fig. 4A**). Activation of recovered inward currents create an additional depolarization (abnormal electrical activity). EAD during phase 2 are mainly triggered by recovering of I_{Ca}, whereas EAD during phase 3 are triggered by recovering of I_{Na} (**Fig. 4A**). Pharmacological interventions and pathophysiological conditions promoting EADs act through one or more of four mechanisms: (i) reduction of repolarizing K^+ currents (I_{Kr}, class IA and III antiarrhythmic drugs; I_{Ks}, chromanol 293B; I_{K1}); (ii) increase in the availability of Ca^{2+} current (Bay K 8644, catecholamines); (iii) increase in the Na^+–Ca^{2+} exchanger current (I_{NCX}) caused by increase in intracellular Ca^{2+} or upregulation of the I_{NCX}; and (iv) increase in late Na^+ current (I_{Na-L}) (aconitine, anthopleurin-A, and ATX-II) (Antzelevitch and Burashnikov, 2011). EAD are mainly invoked in M-myocytes due to relatively weak slow delayed rectifier K^+ current (I_{Ks}) and larger contribution of late Na^+ current (I_{Na-L}) and NCX. Block of I_{Ks} (e.g., block with chromanol 293B) allows to generate EAD in other myocardial cells (Aiba and Tomaselli, 2010).

DAD are induced at relatively rapid rates and under conditions that promote increase in intracellular Ca^{2+} (block of Na^+/K^+ pump by digitalis) (Ferrier et al., 1973; Rosen et al., 1973) and build-up of Ca^{2+} in sarcoplasmic reticulum (β-adrenergic stimulation by catecholamines) (Priori and Corr, 1990). DAD is a result of abnormal spontaneous Ca^{2+} release from sarcoplasmic reticulum (**Fig. 4B**), which leads to depolarization of membrane by forward mode of NCX: intracellular Ca^{2+} is exchanged to three extracellular Na^+ producing net positive movement of ions into the cell (depolarizing inward current). This mechanism is responsible for the generation of arrhythmias in CPVT (Priori and Chen, 2011; Kazemian et al., 2011; Zhabyeyev et al., 2013). In CPVT, mutations in cardiac Ca^{2+} release channel (*RYR2*) or cardiac calsequestrin (*CASQ2*) lead to increase in probability of spontaneous Ca^{2+} release (store overload induced Ca^{2+} release, SOICR) that triggers DADs and sometimes EADs via NCX. The hallmark feature of CPVT is that abnormal Ca^{2+} overload happens as a consequence of increased concentration of circulation catecholamines during exercise or stress (Priori and Chen, 2011).

Late phase 3 EAD is abnormal electrical activity that combines properties of classical EAD and DAD (Burashnikov and Antzelevitch, 2003, 2006). Similarly to classical EAD, late phase 3 EAD happens before repolarization is completed. Similarly to DAD, late phase 3 EAD is triggered by Ca^{2+} release from SR, but unlike DAD it triggered by normal and not spontaneous (abnormal) Ca^{2+} release (**Fig. 4C**). The triggering of EAD by normal Ca^{2+} release is possible because AP is shortened considerably

and Ca^{2+} release is enhanced (Burashnikov and Antzelevitch, 2003). Such conditions may arise when both sympathetic and parasympathetic influences are active at the same time. Late phase 3 EADs have been shown to be responsible for atrial and ventricular fibrillations (Patterson et al., 2005; Ogawa et al., 2009).

Re-Entrant Arrhythmias

Besides abnormal electrical activity on cellular level, arrhythmia at tissue/organ level can also be produced through re-entry of excitation. Re-entry can be classified as anatomical or functional. Anatomical re-entry is re-entry around anatomical obstacle like scar or damaged tissue. Functional re-entry requires spatial heterogeneity in repolarization and/or conductivity to arise. Re-entry may or may not require a circular movement of excitation.

Re-entry around anatomical obstacle is conceptually the simplest form of re-entry. In this type of re-entry, excitation travels in a closed circuit within tissue (**Fig. 5A**) generating its own rhythm independent of normal automaticity generated in right atrial node, and this abnormal rhythm can propagate through the heart by conduction system. Re-entry circuit can be very limited and local resulting in local re-entry, or the circuit can encompass the whole heart producing global re-entry. In the simplest instance, ring model (Mayor, 1906), re-entry occurs around an obstacle (e.g., scar) provided criteria of (i) unidirectional block and (ii) size of the circuit are met. In this model, excitation travels by two different pathways: "slow" and "fast" (**Fig. 1F**) (Wit et al., 1972). Difference in conduction may be either geometrical ("slow" pathway is longer than fast) or intrinsic (tissue in "slow" pathway conducts excitation slower). In either instance, initial excitation spreads through both pathways with different durations. In the "fast" pathway excitation spreads and excites tissue in the "slow" pathway from the other end until excitations meet. That creates region of depolarized cells (+), which acts as a barrier for subsequent regular excitations creating a unidirectional block (**Fig. 5B**, initial). When the next regular excitation arrives, it cannot travel through the "slow" pathway because cells in this pathway are still depolarized (+) and cannot be re-excited, so excitation travels via "fast" pathway reaches slow pathway, spreads in the opposite direction, and passes to the "fast" pathway because, by the time the excitation reaches reflection point, the depolarized cells have

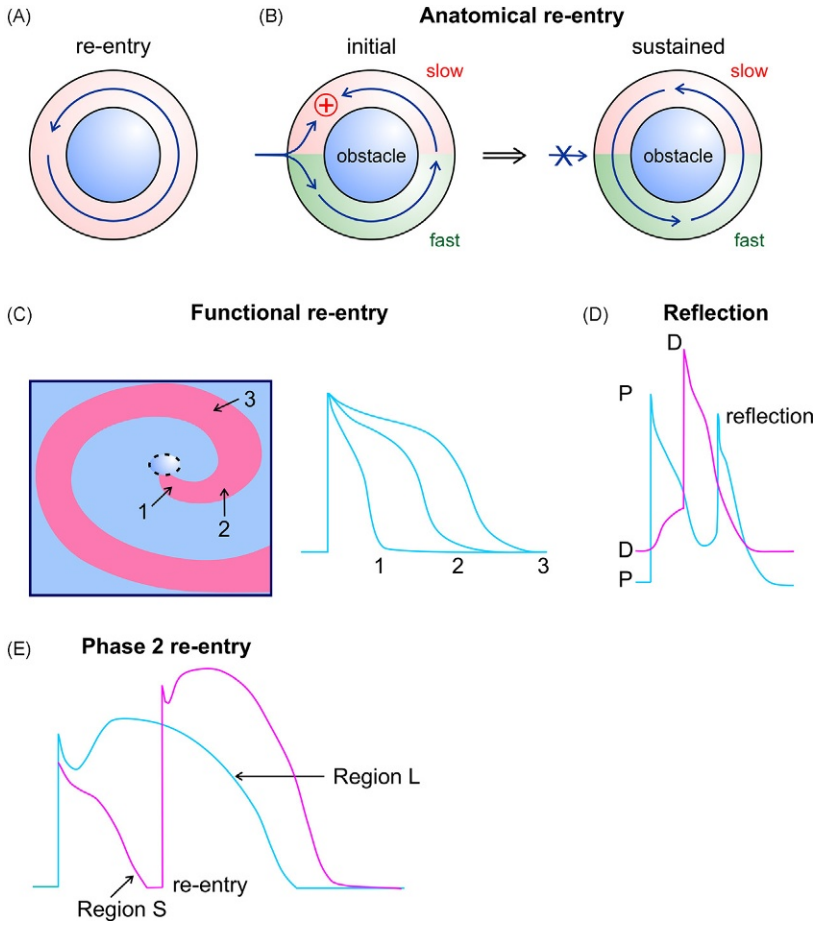

Fig. 5 Mechanisms of re-entry. (A) Re-entry around anatomical obstacle. (B) Formation of re-entry around anatomical obstacle. (C) Functional re-entry: spiral wave of excitation (right: excited regions in *pink* and repolarized regions in *blue*) and corresponding action potentials. (D) Reflection of the excitation across inexcitable segment: electrical activity at the proximal end (P) and electrical activity at distant end (D). (E) Phase 2 re-entry between region with long action potential duration (L) and the region with the short action potential duration (S).

repolarized and became excitable again allowing the excitation to pass to the "fast" pathway producing sustained re-entry (**Fig. 5B**, sustained). Sustained re-entry requires a circuit that is large enough for excitation to travel to allow cells to recover in between excitations. Reduction of conduction velocity (CV) or duration of AP (refractory period) can significantly reduce the required limit of the path length for the development and maintenance of re-entry (Antzelevitch and Burashnikov, 2011).

Re-entry without anatomical obstacle can arise if two conditions are met: (i) considerable heterogeneity in repolarization of AP and (ii) considerable slowing of conduction. This type of re-entry manifest itself as spiral waves (**Fig. 5C**) centered on cores. Slowing of conduction velocity toward the core allows curvature of wave to increase and wave length (WL; spatial extension of the excited state) to decrease toward the core and form a spiral (**Fig. 5C**) (Jalife, 2004). Spiral waves are used to explain the ECG patterns observed during monomorphic and polymorphic ventricular tachycardias (VT) as well as during fibrillation. Monomorphic VT can be explained as the spiral wave with "anchored" core, whereas polymorphic VT produced if the core drift through myocardium. In VFib, there are multiple drifting and breaking up spiral waves (Gray et al., 1995).

Reflection is a re-entry that does not require circular movement of excitation. Reflection arises as a result of back-and-forth impulse transmission across inexcitable segment of the tissue. Inexcitable segment of the tissue (gap) will produce delay in forward (proximal (P) to distal (D)) transmission, and in the distal segment of the tissue AP (D) will arise with considerable delay. That AP in turn will impose back a depolarizing (excitatory) influence across the same gap with a delay, and that delayed depolarization across the gap can produce another AP (reflection) at the proximal end (P) provided that proximal end had repolarized enough to be re-excited again (**Fig. 5D**) (Antzelevitch et al., 1980; Antzelevitch and Moe, 1981).

Phase 2 re-entry is a re-entry of focal origin, and this type of re-entry does not require circular movement of excitation for initiation. Phase 2 re-entry occurs under conditions of severe spatial heterogeneity of repolarization. The re-entry arises when one region with "long" AP (L) that has phase 2 (plateau) is linked and closely located near another region with "short" AP (S) that lack phase 2 (plateau). In this situation, phase 2 of region L will re-excite another AP in region S shortly after termination of normal AP in region S that was initiated in lockstep with original excitation (**Fig. 5E**) (Krishnan and Antzelevitch, 1993; Di Diego and Antzelevitch, 1993; Lukas and Antzelevitch, 1996). Phase 2 re-entry can initiate circular movement re-entry in ischemic conditions due to activation of repolarizing ATP-dependent K^+ current ($I_{K,ATP}$) in ischemic regions of the heart creating necessary steep gradient in repolarization between still perfused (no $I_{K,ATP}$ and long AP with phase 2) and ischemic regions ($I_{K,ATP}$ activated and short AP lacking phase 2) (Lukas and Antzelevitch, 1996). Also, phase 2 re-entry is considered a mechanism responsible for closely coupled extrasystole and initiation of ventricular tachycardia (VT)/ventricular fibrillation (VFib) episodes connected to BrS (Antzelevitch and Burashnikov, 2011; Antzelevitch, 2006; Antzelevitch and Yan, 2010).

Spatial dispersion of repolarization: Ventricular myocardium is electrophysiologically heterogeneous tissue that consists of at least three distinct cell types: epicardial, M, and endocardial myocytes. The cells are differ in phase 1 (I_{to} and I_{Na-L}) and phase 3 (I_{Ks}) repolarization as well as AP duration (Antzelevitch and Burashnikov, 2011; Antzelevitch et al., 1991). Beside different types of myocardial cells, epicardial and M-myocytes differ between left and right ventricle with right ventricle having more prominent I_{to} (Di Diego et al., 1996; Volders et al., 1999). Amplification of normally present transmural differences is responsible for transition of relatively benign changes in APs associated with Brugada, LQT, and sort QT syndromes as well as CPVT to re-entrant arrhythmias such as VT and VFib (Antzelevitch and Burashnikov, 2011; Antzelevitch and Patocskai, 2016). The presence of large dispersion of repolarization within cardiac tissue increase the risk that one still depolarized part of myocardium (long AP duration, EAD, DAD, and/or extrasystole) will re-excite another already repolarized part of myocardium (short AP duration) initiating and sustaining re-entry. In the case of ARVC/D, spatial dispersion of repolarization can arise from mutations of desmosome related genes leading to impaired cell-to-cell coupling, fibrosis, and adiposis (Gerull et al., 2004; Thiene and Basso, 2001). These disruptions give rise to re-entry circuits and local automaticity, which in turn can lead to the development of re-entrant arrhythmias (VT and VFib).

Brugada Syndrome: Action Potential Changes, Dispersion of Repolarization, and Re-Entrant Arrhythmias

BrS is arrhythmia characterized by ST-elevation in the right precordial leads (V1–V3) that produces two distinct ECG patterns "saddle back" and "coved." The syndrome is linked to 4%–12% of sudden cardiac deaths in the general population (Juang and Huang, 2004; Watanabe and Minamino, 2016). Prevalence of the syndrome ranges from 1–5 in 10,000 for western countries (Berne and Brugada, 2012) to 12 in 10,000 for Southeast Asia and Japan with an average age of diagnosis of 41 ± 5 years (Antzelevitch and Patocskai, 2016; Watanabe and Minamino, 2016). Arrhythmic events associated with BrS occur mainly at rest, during sleep, and after meal (Shimizu, 2005). The diagnosis of BrS is based exclusively on the presence of a specific ST segment elevation in one or more right precordial leads spontaneously or due to provocative agents (Richter et al., 2010). BrS is associated with mutation in 19 genes (for review see (Antzelevitch and Patocskai, 2016)).

Mechanism of Brugada syndrome: Electrocardiographic changes associated with BrS has been explained as (i) conduction delay in the epicardium of free wall of right ventricular outflow tract (Tukkie et al., 2004) and/or (ii) premature repolarization in RV epicardium with or without the loss of plateau phase of epicardial action potential (AP) (Antzelevitch, 2001). The cellular mechanisms underlying the latter are summarized in **Fig. 6**. Normally, there is small difference between epicardial (Epi) and endocardial (Endo) APs during plateau (**Fig. 6A**) resulting in little transmural voltage gradient and the isoelectric ST segment (**Fig. 6A**). In addition to that, epicardial repolarization (Epi) precedes endocardial (Endo) producing positive transmural gradient (red arrow) and positive T-wave (**Fig. 6A**). In the case of BrS, two main ECG patterns can appear: "saddle back" or "coved." Saddle back pattern is a result of an increase in early repolarization in epicardium with little or no change in endocardial repolarization (transmural dispersion in early repolarization). That dispersion produces positive transmural voltage gradient during plateau phase

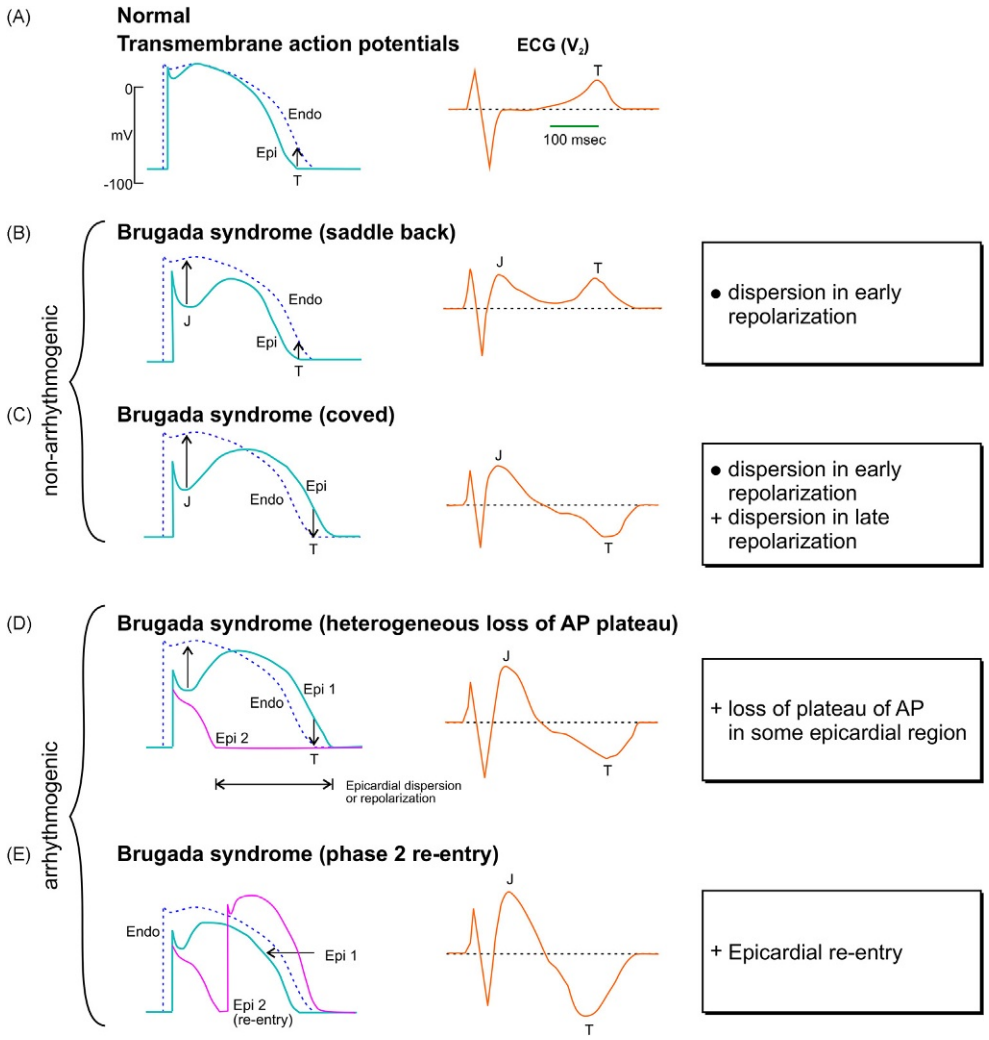

Fig. 6 Brugada syndrome (BrS). (A) Normal transmembrane action potential (Epi, epicardial [*cyan*]; Endo, endocardial [*blue dash*]) and normal ECG. (B) Formation of Brugada syndrome (*saddle back pattern*) ECG due to changes in transmembrane action potentials. (C) Formation of Brugada syndrome (coved pattern) ECG due to changes in transmembrane action potentials. (D) Brugada syndrome with heterogeneous loss of plateau of action potential (AP) in some epicardial regions (epicardial dispersion of repolarization: Epi1 [*cyan*]—epicardial AP with plateau; Epi2 [*magenta*]—epicardial AP without plateau) and corresponding ECG. (E.) Brugada syndrome with epicardial re-entry and corresponding ECG.

of AP resulting in elevated ST-segment (J wave) or "saddle back" pattern of ECG (Fig. 6B). "Coved" patter is generated when, in addition to changes in early repolarization, late repolarization of epicardium is prolonged in comparison to endocardium (transmural dispersion in late repolarization) that leads to inversion of transmural voltage gradient during late repolarization and inversion of T-wave giving a "coved" pattern of ECG (Fig. 6C). However, these changes in itself are not immediately arrhythmogenic. To create arrhythmogenic substrate, further increase in early repolarization is required to achieve a loss of plateau phase of AP in some regions of epicardium (Fig. 6D, Epi2 trace). The regions with abnormally short AP give possibility for re-entry and epicardial extrasystole (Fig. 6E, Epi2 trace with re-entry). The epicardial extrasystole accentuates the inverted T-wave producing an ECG of a more symmetrical appearance. The presence of epicardial regions with abnormally short AP creates a substrate for the development of re-entrant VT and VFib (Lukas and Antzelevitch, 1996; Yan and Antzelevitch, 1999). BrS is mostly right ventricular disorder (Antzelevitch and Burashnikov, 2011; Antzelevitch and Patocskai, 2016) because in this case re-entrant arrhythmia are produced due to transmural dispersion of early repolarization caused by I_{to} syndrome and right ventricle have more prominent I_{to} facilitating early termination of epicardial AP creating possibility for phase 2 re-entry (Di Diego et al., 1996; Volders et al., 1999).

Overview of Ventricular Tachyarrhythmias

Ventricular tachyarrhythmia is one of the most common types of arrhythmia producing cardiac arrest and sudden cardiac death. According to American Heart Association statistics, sudden cardiac arrests happen to over half a million people in North America

(Mozaffarian et al., 2016). Ventricular tachyarrhythmia is an abnormal electrical activity that originates in the ventricular conduction system, ischemic myocardium, or scar tissue and characterized by high rhythm. Spread of electrical impulses through the ventricles can result in either crossing of the atrioventricular (AV) node, capturing the atria retrograde, or bypassing AV node producing ventriculoatrial dissociation. Arrhythmogenic factors responsible for ventricular tachyarrhythmia include alterations in autonomous nervous system activity, myocardial ischemia, and proarrhythmic effects of various drugs; alternatively, ventricular tachyarrhythmia may arise from abnormal electrical automaticity produced by structural heart diseases (Baldzizhar et al., 2016; Parajuli et al., 2015). Ventricular tachyarrhythmias are induced by re-entry mechanism: localized re-entry (local or macroscopic), abnormal automaticity, triggered activity, or combination of the above.

Classification of Ventricular Tachyarrhythmias

Ventricular tachyarrhythmias are subdivided into four types by pattern characteristics (organized vs. disorganized), by pattern duration (non-sustained vs. sustained), and by heart rate. The four types are non-sustained ventricular tachycardia (NSVT), sustained ventricular tachycardia (VT), ventricular fibrillation (VFib), and *torsade de pointes* (TdP) (**Fig. 7**).

- NSVT is characterized by a rapid heart rate ($\geq$120 bpm) with three or more consecutive ventricular beats, but with an overall short duration ($\leq$30 s).
- VT, similarly to NSVT, is characterized by a rapid heart rate ($\geq$120 bpm) with three or more consecutive ventricular beats, but unlike NSVT, sustained VT last more than 30 s.

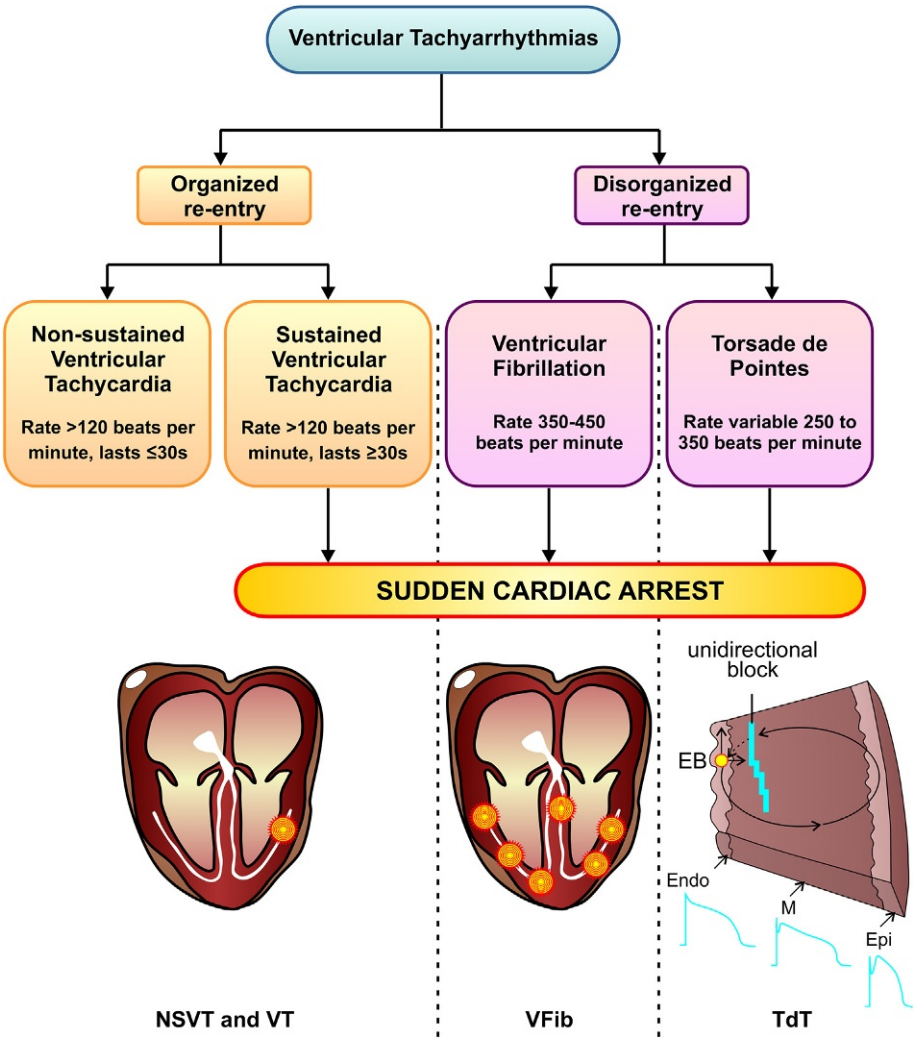

Fig. 7 Classification of ventricular tachyarrhythmias by the type of re-entry with the depictions of their respective mechanisms at the bottom: Left: nonsustained ventricular tachycardia (NSVT) and sustained ventricular tachycardia (VT) are generated by an activity of a single ectopic focus; Center: ventricular fibrillation (VFib) is generated by an activity of a multiple ectopic foci; Right: *torsade de pointes* (TdP) is generated by transmural re-entry with M-myocytes acting as a region of unidirectional block.

- VFib is a chaotic electrical activity with high ventricular heat rate (350–400 bpm) that arises from uncoordinated electrical activity of individual fibers and muscle groups. As a result of such electrical activity, myocyte contractions become disorganized and cardiac output nonexistent leading to hemodynamic collapse followed by cardiac arrest and death.
- Torsade de Pointes (TdP) is an atypical form of ventricular tachyarrhythmia that is closely linked with LQTS and therefore will be considered in the next section.

Mechanisms of Ventricular Tachyarrhythmias

Both NSVT and VT are generated by an activity of a single ectopic focus that sends its impulses through the ventricle(s) bypassing normal conduction pathways (**Fig. 7**). In the case of VFib multiple ectopic foci generate electrical activity that overrides normal rhythm coming from the atria via conductive system (**Fig. 7**).

Torsade de Pointes (TdP)

Initially described by Dessertenne (Dessertenne, 1966), Torsade de Pointes (French "twisting of the spikes") is a distinctive form of polymorphic ventricular tachycardia (VT) that is not related to the spectrum NSVT–VT–VFib and characterized by gradual change in the amplitude or "twisting" of the spikes around isoelectric line (misshaped QRS complexes). TdP is strongly linked with LQTS, medication-induced LQT (e.g., quinidine, procainamide, tricyclic antidepressants) and changes in repolarization due to electrolyte imbalances (hypokalemia, hypomagnesemia, and hypocalcemia) (Baldzizhar et al., 2016; Maisel et al., 1997; Straus et al., 2005). The underlying mechanism of TdP is the increase in transmural dispersion of AP prolongation and EAD associated with excessive prolongation of AP due to slow repolarization rate. At the heart level, transmural differences in repolarization allow M-cells, which have longest AP duration, to act as unidirectional block (**Fig. 7**). Extrasystole (ES) from EAD will propagate in retrograde (toward apex) where cell is already repolarized until it can cross the line of unidirectional block (M-myocytes finished their repolarization) to initiate re-entry by re-exciting M-myocytes and then epicardial myocytes initiating transmural re-entrant arrhythmia *torsade de pointes* (**Fig. 7**) (Antzelevitch and Burashnikov, 2011; Antzelevitch and Shimizu, 2002; Belardinelli et al., 2003).

Conclusions

In general, arrhythmias arise due to involvement of one or more arrhythmogenic mechanisms: (i) abnormal automaticity (decreased or enhanced, as well as isolated pacemaker), (ii) changes in AP duration (LQT and SQTSs, as well as medication-induced LQT), (iii) abnormal and triggered activity (EADs, DADs, late phase 3 EADs that can lead to extrasystole), and (iv) spatial dispersion of repolarization, which allows different parts of the cardiac tissue to re-excite each other and initiate re-entry. In many cases, natural or accentuated heterogeneity in repolarization is required for proarrhythmic changes in AP to become malignant re-entrant arrhythmias.

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Medical Management of Left Ventricular Assist Devices

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Abbreviations

ACE Angiotensin-converting enzyme
ARB Angiotensin receptor blocker
MCS Mechanical circulatory support
VAD Ventricular assist device

Background

The use of ventricular assist devices (VADs) to treat end-stage heart failure is now considered standard of care for appropriately selected patients. The use of mechanical circulatory support (MCS) was first shown to improve survival versus medical therapy in advanced heart failure in the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) trial (Rose et al., 2001). Subsequent trials using newer versions of VADs continued to show benefits as well (Miller et al., 2007; Slaughter et al., 2009; Rogers et al., 2017). In fact, the survival benefit for VADs has continued to improve, even contemporary post-trial use which is different than many other therapies used for heart failure (Jorde et al., 2014). The use of VADs to treat advanced heart failure is recommended as part of both U.S. and European guidelines (McMurray et al., 2012; Yancy et al., 2013).

Current VADs Technologies

Currently, two approved types of MCS are available for clinical use in the United States, with a third option available in certain European regions. The first, the HeartMate II (Abbott/Thoratec, Pleasanton, CA), is a continuous flow device, which currently has approval in the United States for both Bridge to Transplant and Destination Therapy use. The second, the HeartWare HVAD (Medtronic/HeartWare, Framingham, MA), is a centrifugal flow device approved for Bridge to Transplant Use in the United States. The final is the HeartMate 3 (Abbott/Thoratec, Pleasonton, CA) which employs a magnetically levitated rotor to produce flow. The HeartMate 3 is currently an investigational device in the United States but is available in CE mark countries along with the HeartMate II and the HVAD.

Pump Management

Although each of the available pump styles is unique, the basic principles underlying VAD management are generally applicable to all patients with MCS. Each pump operates around a programmed speed; however the operating speed does not singularly define flow through the pump. Rather, flow is determined by several factors, including the afterload on the pump. Pump-operating characteristics, referred to as HQ curves, demonstrate the effect of varying afterload on pump flow (Moazami et al., 2013).

Clinically, pump speeds are selected by balancing the amount of flow desired with secondary effects. Higher pump speeds may decrease pressure and volume within the left ventricle, which can affect the preload to the pump and cause unwanted drops in flow. Likewise, drops in pressure and volume can induce septal shift toward the left ventricular free wall and away from the right ventricle, which may lead to right ventricular failure. Echocardiography and invasive hemodynamics can aid in the selection of the appropriate pump speed for a patient (Slaughter et al., 2010).

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Anticoagulation

All current generation VADs require systemic anticoagulation to prevent pump thrombosis. Oral vitamin K antagonists, such as warfarin, are the recommended agent to achieve adequate anticoagulation. The standard target international normalized ratio (INR) for VADs is 2–3 although specific situations may require adjustments to this goal. Examples include the presence of a mechanical heart valve, which may necessitate an INR goal of 2.5–3.5. Likewise, gastrointestinal bleeding, and in particular, recurrent gastrointestinal bleeding, may prompt a reduction in goal INR if not complete cessation of therapeutic anticoagulation (Feldman, 2013, p. 4455). The risks versus benefits of reduced anticoagulation need to be considered on an individual basis prior to changing targets from device-manufacturer recommended ranges. No evidence exists to support the use of other forms of non-vitamin K oral anticoagulants such as direct thrombin inhibitors or factor Xa inhibitors.

Concurrent with the use of oral vitamin K antagonists is the use of antiplatelet therapies, and in particular the use of aspirin. Recommended practices for centrifugal pumps include the use of 325 mg daily of aspirin, while axial flow and magnetically levitated flow devices currently recommend between 81 and 325 mg of aspirin daily. Additional antiplatelet therapies can be used in conjunction with aspirin if clinically indicated but can increase the bleeding risk in the VAD population similar to other populations. Some evidence exists that antiplatelet therapy may not be required at all for the subset of VAD patients without coronary artery disease; however this hypothesis has not been conclusively shown and therefore routinely withholding aspirin is not yet recommended (Netuka, 2017, p. 4474).

Blood Pressure Control

Systemic blood pressure control remains an important component of VAD patient management. As previously noted, afterload plays a role in determining flow through a VAD. Clinically, afterload is represented by systemic blood pressure; therefore inadequate blood pressure control can have negative effects on pump function and subsequent consequences. Indeed, stroke rates are noted to be higher in VAD patients not on antihypertensive medications versus those who were treated (Lampert et al., 2014).

No data exist to suggest superiority of any given class of antihypertensive agents over others. Generally, antihypertensive agents that concurrently treat heart failure, such as those targeting the renin–angiotensin system (ACE inhibitors, ARBs) or neurohormonal pathways (beta-blockers, mineralocorticoid receptor antagonists) are used before other agents are employed. Often, multiple agents are required to control blood pressure (Lampert et al., 2014). As with the general use of these agents, appropriate monitoring and follow-up is recommended for VAD patients.

Treating blood pressure in MCS patients requires a reliable method of measuring. Current generations of VADs create continuous flow in the arterial system, which creates minimal variations in pressures over time. Whereas the traditional cardiac cycle results in systolic and diastolic pressures, continuous flow VADs may not have discernable differences to label as such. Traditional blood pressure measurement methods that rely on Korotkoff sounds to determine systolic and diastolic pressures may not be accurate in VAD patients. Due to this phenomenon, the recommended practice for measuring blood pressure in a VAD patient is to use Doppler devices to determine a return to flow. In the absence of a detectable pulse, the Doppler return to flow blood pressure is considered a mean arterial pressures (MAP). In the setting of a palpable pulse, the Doppler return to flow pressure is considered to be a systolic blood pressure. Although some doubts to these correlations exist, present clinical practice is to assign the Doppler return to flow based on the presence or absence of a palpable pulse (Lanier et al., 2013; Feldman et al., 2013). The guideline recommended practice is to target a systolic blood pressure less than 130 mmHg or a MAP $<$80 mmHg (Feldman et al., 2013). More recent data correlate a MAP maintained $<$90 mmHg with positive outcomes, particularly for axial flow pumps (Maltais et al., 2017).

Heart Failure Therapies

As noted, medical therapies for heart failure are traditionally utilized when medical therapy is required and tolerated. Some data exist demonstrating the potential benefit of these therapies including improvement in underlying cardiac function (Birks et al., 2015; Dandel and Hetzer, 2015). Despite the use of these medications, and the presence of the VAD itself, episodes of worsening heart failure are still common among MCS patients (Lampert and Emani, 2015). Traditional therapies for acute heart failure, including diuretic therapies, are often required to treat these patients.

Summary

Certain specific considerations are required when considering the management of patients with VADs. These considerations should be incorporated into the overall management of the VAD patients and individualized as appropriate, especially when considering comorbidities that should be concurrently managed. The medical management of a VAD patient should be undertaken in conjunction with a certified VAD center to ensure proper care.

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Relevant Websites

HeartWare Corp.—www.heartware.com.
Thoartec Corp.—www.thoratec.com.

Metabolomics in Cardiovascular Research

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Introduction

Metabolomics is the systematic study of small molecules (metabolites) in biological samples. Metabolomics can be targeted, that is, focused on a few specified metabolites only, or untargeted aiming at detecting and quantifying all metabolites, known or unknown, in a sample. The product of the latter is the metabolome, the collection of all metabolites detectable in the sample. Metabolomics can produce a person's metabolic phenotype ("metabotype"), which reflects the person's metabolic health at the time of sampling. The metabotype is partially a functional output of the person's genotype but, in contrast to genotype, it is highly variable over time due to various environmental influences. Like genomics and other "omics" research, metabolomics has progressed rapidly in recent years and advancements in laboratory technology have now enabled the investigation of thousands of metabolites from thousands of individuals.

In cardiovascular research the utilization of metabolomics is becoming common. In fact, the main risk factors for cardiovascular diseases, total cholesterol, smoking, and blood pressure, have remained amazingly similar for the past 30 years. Since metabolomics technologies can now produce hundreds and even thousands of biomarkers from a single sample, they have the potential to bring new precision to cardiovascular research and clinical treatments. There are diverse applications for metabolomics in cardiovascular medicine: (a) to improve our understanding of molecular mechanisms underlying various cardiovascular disorders; (b) to enable subclassification of cardiovascular disorders and help with patient stratification based on the metabolic pathways impacted; (c) to provide new biomarkers or biomarker profiles which improve cardiovascular risk estimation and improve targeting of preventive efforts; (d) to provide biomarkers which help with efficacious selection and monitoring pharmacological treatment.

Technologies for Metabolomics Profiling

Two principal technologies can be used for high-throughput metabolomic profiling, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). The NMR technology has been described in detail (Jeyarajah et al., 2006; Mora et al., 2009; Soininen et al., 2015; Larive et al., 2015). It can provide quantitative data on lipoprotein subclasses, their lipid concentrations and composition, apolipoproteins A-I and B, multiple cholesterol and triglyceride measures, albumin, various fatty acids as well as on numerous low-molecular-weight metabolites, including amino acids. NMR is not destructive to the sample and can detect metabolites down to the low-micromolar concentration range. The analysis of a single sample takes only a few minutes and can produce quantitative data on about 200 metabolites. Strengths of the NMR metabolomics are simple sample preparation, low costs, and high throughput, which have made the examination of large cohorts possible. The main limitations are the lesser number of quantifiable metabolites and the clearly lower sensitivity compared to MS metabolomics with the main focus on various lipoprotein particles and their lipid concentrations.

MS is usually coupled with liquid chromatography or gas chromatography for initial separation of different molecules before ultimately identifying them on the basis of their mass-to-charge (m/z) ratio. The samples must first be ionized to measure the m/z ratio. The "shotgun" approach, sometimes used for nontargeted lipidomics, simplifies the process by omitting the chromatography

step but this approach is only able to detect and quantify abundant lipid species. These technologies and their applications in medical research have been recently described (Hinterwirth et al., 2014; Lind et al., 2016; Dona et al., 2016; Lei, 2011). In particular, the chromatography step requires careful sample preparation and control of analysis conditions, which makes MS-based methods more laborious and more expensive than the NMR-based methods. The higher costs are compensated by the much higher sensitivity of MS compared to NMR. Metabolites can be detected and quantified down to low nano- or high picomolar concentrations. At the moment, published studies using MS-based technologies have been smaller than those using NMR, but developments in laboratory technology and automation are rapidly reducing the costs and the time needed for a measurement. Thus, it is likely that the throughput of MS-based metabolomics will increase substantially in the near future.

Several freely available online data repositories are designed to help with metabolite identification and characterization as well as interpretation of their biological roles. The list of relevant websites is presented in more detail at the end of the article.

The metabolites quantified by MS and NMR platforms are largely different but some metabolites, for example, certain amino acids, can be obtained from both. It should be noted, however, that there are no broadly accepted standardizations between the different technologies or even between different platforms using the same technology. Thus, biomarker results obtained with different platforms may not be comparable. Despite this, robust biological findings have been found across platforms, such as the associations of aromatic amino acids with different manifestations of atherosclerosis which are consistent in both MS- and NMR-based studies (Würtz et al., 2015; Magnusson et al., 2013).

Sample Requirements, Quality Control, and Data Preprocessing

A schematic overview of metabolomics workflow and aspects to be considered is given in Fig. 1.

The most appropriate sample type for metabolomics studies depends on the study questions. In practice, biofluids such as serum, plasma, urine, and stool are most often used in large-scale studies because of easy availability. As in all laboratory studies, preanalytical conditions like fasting or nonfasting status, hour of blood drawing, storage time and storage temperature of the samples, and the number of freeze-thaw cycles can have a major impact on the results and must be carefully considered at the planning stage of the study. Common problems at the analysis stage are batch-to-batch variation and analytical drift over time,

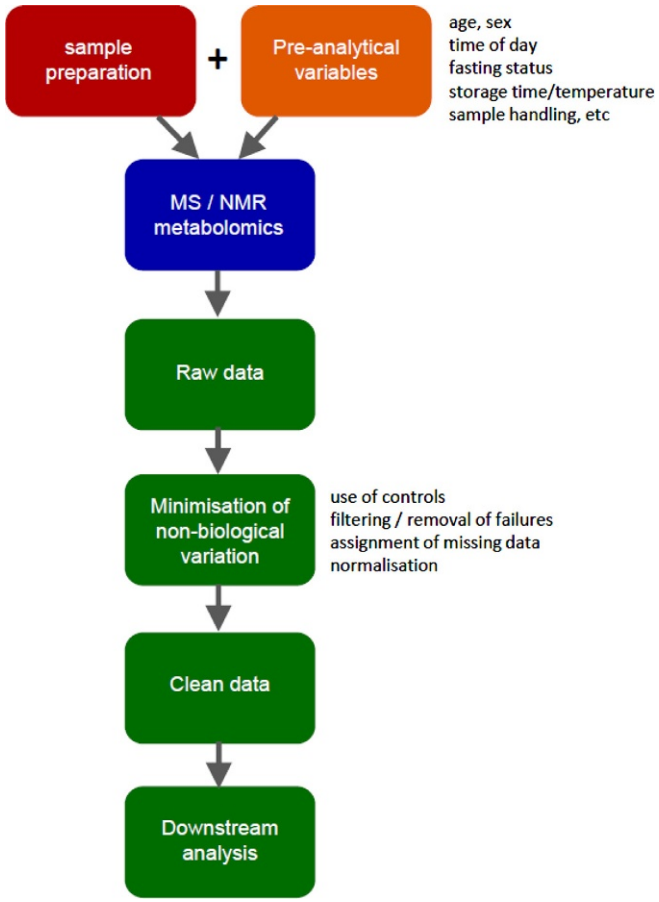

Fig. 1 Schematic overview of metabolomics workflow and aspects to be considered.

which can be controlled using internal standards. After the analysis, data are usually normalized, centered, and scaled to produce a set of metabolites which minimize technical variation and maximize biological variation. Because a large number of metabolites are quantified, some may have unacceptably high coefficients of variation (CV) and may need to be excluded from data analyses. With careful quality control, however, the majority of metabolites will have CVs, which are only slightly larger than those of single biomarker measurements.

Strategies for Analyzing Metabolomics Data

In principle, metabolomics data can be analyzed using linear models similar to other quantitative biomarker data. This can be done on each metabolite separately or multiple metabolites simultaneously using multivariate approaches or metabolic pathway analysis (Xia and Wishart, 2010; Inouye et al., 2012). However, analysis and subsequent interpretation typically must account for complex correlations among metabolites and the large numbers of metabolite variables tested, making statistical control for multiple testing necessary. Several methods can be used to control for multiple testing and to reduce the number of statistical tests needed, including Bonferroni correction, false discovery rate, and principal component analysis. In addition to controlling for multiple testing, the findings should ideally be replicated in an independent cohort, to ascertain their robustness and to avoid false positive results.

A more challenging opportunity is the integration of metabolomics data with genomics and other omics for systems level analyses. These kinds of analyses can be more sensitive in detecting novel biomarkers and give more insights into the underlying biology than association analyses that only consider one type of omic data (Inouye et al., 2010; Kastenmüller et al., 2015; Fearnley and Inouye, 2016). Also, genome-wide association study (GWAS) analyses on metabolomics profiles as interim traits seem to be more powerful than corresponding analyses on traditional cardiovascular risk factors traits (Suhre et al., 2011). Kettunen et al. (2016) reported recently a GWAS on 123 metabolites quantified by NMR and identified 62 associated loci, many of them relevant for cardiovascular traits. The median proportion of variance explained by the genetic variants was 5% with the highest being for glycine (12.5%). An even more extensive exploration of genetic influences on metabolites detected by MS has been published by Shin et al. (2014). In addition to metabolite concentrations, their analysis included relevant metabolite/metabolite ratios, which in principle offered additional reaction rate information within metabolic pathways. Another advantage from combining metabolomics with genomics is the opportunity to assess causality of the metabolite-disease associations using Mendelian randomization techniques as demonstrated in the studies mentioned earlier. Ganna et al. (2014) have applied these techniques for analyzing cardiovascular manifestations in Swedish cohorts and found a weak but probably causal association of monoglyceride 18:2 with incident coronary heart disease.

Associations of Metabolite Profiles With Cardiometabolic Risk Factors

Diabetes, Insulin Resistance, and Obesity

Metabolite profiling may refine traditional cardiovascular risk factors, inform on underlying biology and suggest new targets for therapeutic interventions. Probably the most studied entity is the triad of diabetes, insulin resistance and obesity. Both MS- and NMR-based studies have consistently shown that branched-chain amino acids are associated with increased risk of diabetes (Wang et al., 2011; Stancáková et al., 2012). Similar findings were observed for fasting and 2-h glucose, and for insulin resistance (Würtz et al., 2012) (**Fig. 2**).

In an MS analysis of the Framingham Offspring cohort, body mass index was associated with the same amino acids as diabetes and insulin resistance, as well as with citric acid cycle, the tryptophan pathway and the urea cycle (Ho et al., 2016). In an NMR-based study of Finnish cohorts, body mass index was associated with 66 metabolites in men and 61 in women, and higher body mass index was associated with generally atherogenic metabolite profiles (Würtz et al., 2014). Furthermore, a Mendelian randomization study was performed with a genetic risk score for body mass index as the instrument to assess causality. The results suggested causal adverse effects of adiposity on multiple metabolites, including lipoprotein lipid subclasses and particle size, branched-chain and aromatic amino acids, and inflammation-related glycoprotein acetyls. Consistently, a dramatic weight reduction caused by bariatric surgery was paralleled by declining trends in branched-chain amino acids, phenylalanine, alanine, and choline, and increasing trends in indoxyl-sulfate and trimethylamine-*N*-oxide (TMAO) (Narath et al., 2016).

Lipidomics

Lipid metabolism plays a key role in cardiovascular disease development. For decades, cardiovascular risk estimation has been based on relatively crude measurements of total and HDL cholesterol and triglycerides, complemented with calculated or directly measured LDL cholesterol. Both NMR- and MS-based metabolomics methods can provide much more detailed lipid profiling than the methods currently in clinical use. NMR is mainly focused on measuring lipoprotein particle sizes and their lipid concentrations, while MS measures molecular lipid species with great sensitivity (Soininen et al., 2015; Hinterwirth et al., 2014). Some promising findings have been published (Alshehry et al., 2016; Stegemann et al., 2014) but at the moment the clinical benefit of more precise lipidomics profiling still needs to be demonstrated.

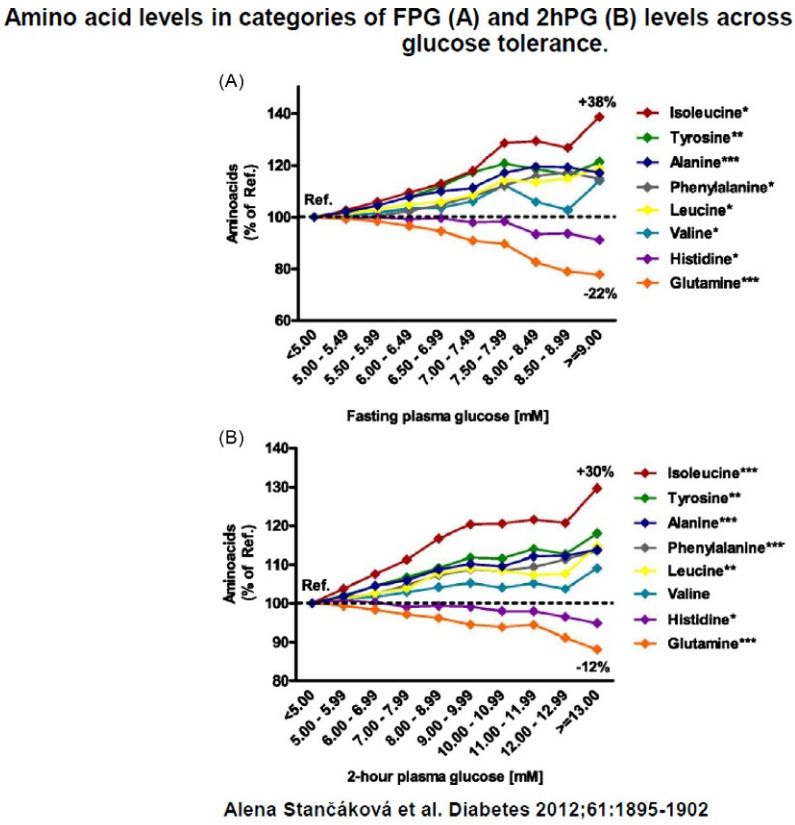

Fig. 2 Amino acid levels in categories of fasting plasma glucose (A) and 2-h plasma glucose (B) levels across the entire range of glucose tolerance. Points represent unadjusted means of amino acid levels in each glucose category. *P* values were calculated using general linear model adjusted for age and BMI. $^{*}P<0.05$, $^{**}P<10^{-10}$, $^{***}P<10^{-30}$. Source: Stancacova et al. *Diabetes* 2012;61:1895–1902. With permission from the American Diabetes Association.

Blood Pressure and Hypertension

Few studies have been published on metabolomics of elevated blood pressure or hypertension. Investigators of the Atherosclerosis Risk in Communities (ARIC) study examined metabolomic predictors of incident hypertension in African Americans with high incidence of hypertension (Zheng et al., 2013). They found that 1 standard deviation difference in 4-hydroxyhippurate, which is a product of gut microbial fermentation, was associated with 17% higher risk of incident hypertension and the association remained significant after adjusting for other risk factors of hypertension, including baseline systolic and diastolic blood pressure. Sex steroid pattern was also associated with incident hypertension and the association was consistent in both sexes.

Menni et al. (2015) analyzed 280 metabolites in 3980 Twins UK females for associations with blood pressure. Only a dicarboxylic acid, hexadecanodioate, showed an independent association with systolic blood pressure which consistently replicated in two other cohorts. In a series of elegant animal experiments the authors showed that hexadecanodioate may have a causal influence on blood pressure. They speculated that hexadecanodioate may be a "low hanging fruit" and that there will be other metabolites associated with blood pressure, which can be detected in larger studies.

Smoking and Physical Activity

Metabolomic studies on smoking have been mainly focused on nicotine metabolism and risk of lung cancer as well as other cancers, but largely have not considered cardiovascular disease outcomes. Gu et al. (2015) carried out an untargeted MS metabolomics study in 892 men and women from four countries. They found 25 metabolites associated with smoking behaviors, 24 with current smoking and 8 with the number of cigarettes per day. Possibly relevant for cardiovascular diseases is that serum bilirubin concentrations were lowered among smokers. Circulating bilirubin is a powerful antioxidant and has been inversely associated with cardiovascular disease. Investigators of the KORA cohort from Germany showed in a longitudinal study with a follow-up of 7 years that metabolite alterations associated with smoking were reversible after smoking cessation (Xu et al., 2013).

Physical activity has consistently been associated with lower levels of branched chain amino acids in multiple metabolomics studies (Fukai et al., 2016; Xiao et al., 2016; Kujala et al., 2013). Glucose and monosaccharides were also lower in persons with high physical activity level (Xiao et al., 2016; Kujala et al., 2013).

Metabolomics and Cardiovascular Outcomes

A few prospective studies have been published on metabolomics and cardiovascular outcomes (**Table 1**). The study of Würtz et al. (2015) is the largest of the listed prospective studies. The investigators first carried out NMR metabolomics profiling in 7256 persons free of cardiovascular disease at baseline. In this discovery cohort, 19 metabolites were associated with cardiovascular disease risk independently of traditional risk factors. In replication cohorts, levels of four metabolites (phenylalanine, monounsaturated fatty acids, omega-6 fatty acids, and docosahexaenoic acid) robustly replicated (**Fig. 3**).

Table 1 Prospective studies on metabolomics and cardiovascular outcomes

Study	N, *design*	*Metabolomics technology*	*Main findings*
Shah et al. (2012)	2023 Patients undergoing cardiac catheterization	Targeted MS	Dicarboxylacylcarnitines, medium-chain acylcarnitines, and fatty acids predictive of future CVD
Magnusson et al. (2013)	506, nested case-control study	Targeted MS	A score consisting of three amino acids is predictive for incident CVD
Stegemann et al. (2014)	685, population-based cohort study	Shotgun lipidomics, MS	Triacylglycerols, cholesterol esters, and phosphatidyletanolamides predictive for incident CVD
Ganna et al. (2014)	3668, population-based cohort study	Untargeted MS	Monoglyceride 18:2 causally associated with incident CHD, three other metabolites also associated
Würtz et al. (2015)	13,441, population-based cohort study	NMR	Phenylalanine and MUFA directly, ω-6 FA and docosahexaenoic acid inversely associated with incident CVD
Laaksonen et al. (2016)	3384, case-control study among patients with CHD	Targeted MS	Ceramides significantly associated with CVD death
Havulinna et al. (2016)	8101, population-based cohort study	Targeted MS	Ceramides significantly associated with incident CVD

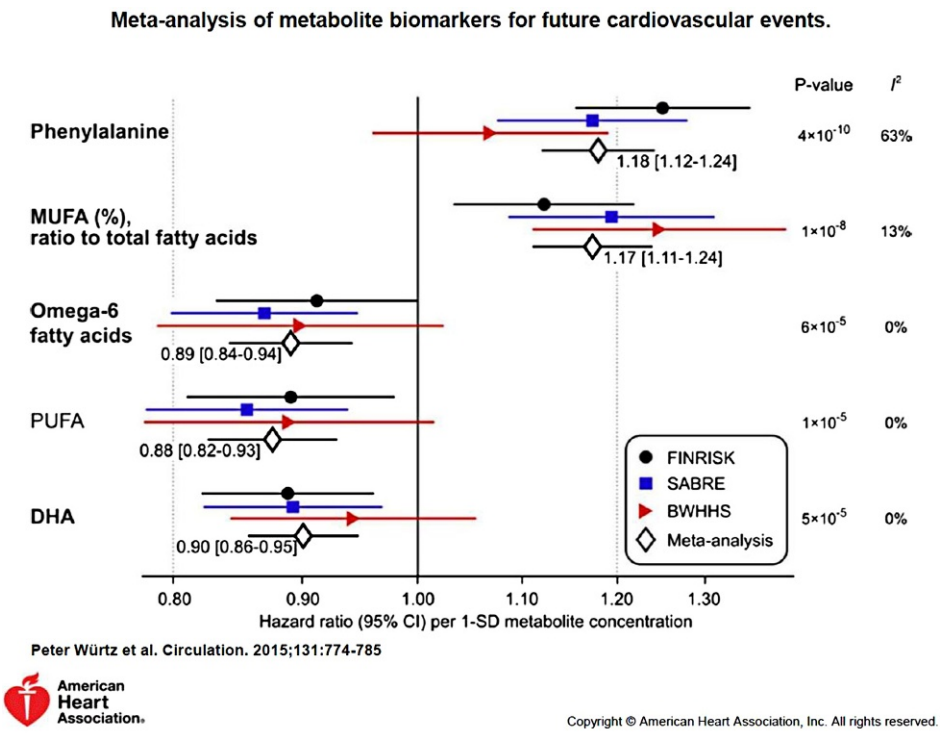

Fig. 3 Meta-analysis of metabolite biomarkers for future cardiovascular events. Source: Würtz et al. *Circulation* 2015;131:774–785. With permission from Wolters Kluwer.

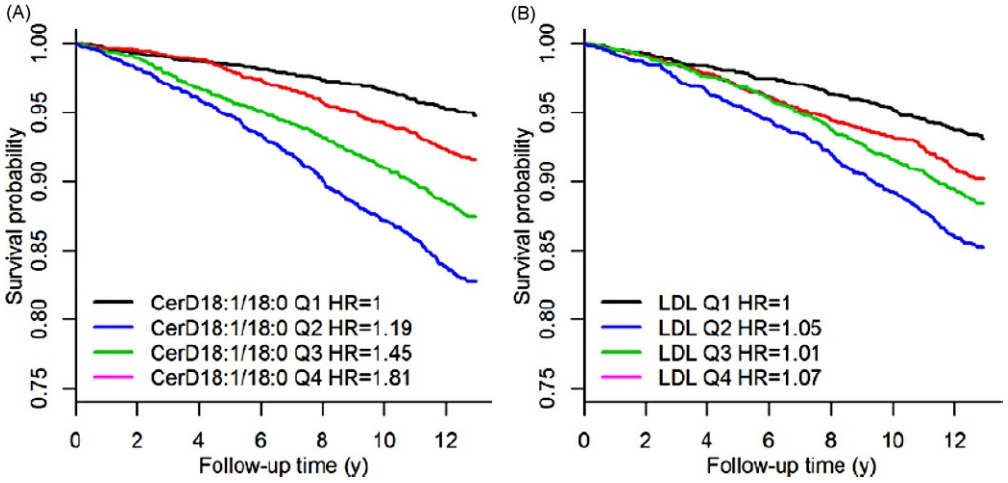

Fig. 4 (A and B) Kaplan–Meier estimates of incident major adverse cardiovascular event (MACE) in Cer(d18:1/18:0) and low-density lipoprotein-cholesterol (LDL-C) quartiles. A hazard ratio in the legend indicates the quartile-specific hazard relative to the first quartile (models stratified for sex). Source: Havulinna et al. *ATVB* 2016. https://doi.org/10.1161/ATVBAHA.116.307497. With permission from Wolters Kluwer.

The metabolite associations were further corroborated with MS in the Framingham Offspring study. A risk score was derived in the discovery cohort using these four metabolites and tested in the replication cohorts. The score improved risk classification, especially in the intermediate risk group ("clinical NRI") but discrimination, assessed using C-statistics, was not improved. Laaksonen et al. (2016) have carried out a targeted MS analysis in three different cohorts of coronary heart disease patients from Finland, Switzerland, and Norway. Ceramides, especially the Cer(d18:1/16:0)/Cer(d18:1/24:0) ratio, were associated with the risk of cardiovascular death in all cohorts. In a follow-up study, Havulinna et al. (2016) investigated the associations of the same ceramides with the risk of incident major cardiovascular events in a general population-based Finnish cohort, FINRISK. In this case cer(d18:1/18:0) showed the strongest association with the outcome. When considered quartile by quartile, the association of cer(d18:1/18:0) with incident major cardiovascular events seemed stronger than that of LDL cholesterol (**Fig. 4**). Reclassification of risk due to ceramides remained, however, modest.

Metabolomic studies on other specific cardiovascular outcomes are so far limited and with small sample sizes, but it has been reported that lysophosphadidylcholine is associated with stroke recurrence in patients with transient ischemic attack (Jové et al., 2015), two conjugated bile acids are associated with incident atrial fibrillation in African Americans (Alonso et al., 2015), and that long-chain acylcarnitine is associated with heart failure with higher levels in patients with reduced ejection fraction compared to those with preserved ejection fraction (Hunter et al., 2016). Cheng et al. (2015) demonstrated recently that metabolomics is a promising tool for the diagnosis and prognosis of heart failure. In their study, a metabolite panel consisting of the asymmetric methytarginine/arginine-ratio, butyrylcarnitine, spermidine, and the total amount of essential amino acids, provided significant prognostic value independent of brain natriuretic peptide and other traditional risk factors.

Pharmacometabolomics

Pharmacometabolomics is the application of metabolomics for the study of drug effects and variation in drug responses, which together with pharmacogenetics has the potential to improve possibilities for selecting each individual patient a more effective drug treatment with fewer side effects. Investigators of the KORA cohort from Germany have reported 41 metabolites, which were significantly associated with various antihypertensive and lipid lowering drugs (Altmaier et al., 2014). In another study, the same group reported that the metabolite differences between users and nonusers of angiotensin convertase inhibitors were dependent on genetic variant at the ACE locus (Altmaier et al., 2016). Two of the observed peptides, aspartylphenylalanine and phenylalanylserine, showed significant association with blood pressure and may thus be functional markers of ACE activity.

Würtz et al. (2016) used NMR profiling to examine the metabolic effects of statin use in four cohorts in the United Kingdom and Finland. Substantial lowering of remnant cholesterol was observed but only modest lowering of triglycerides. Omega-6 fatty acids decreased substantially while the effects on amino acids, glycolysis, and gluconeogenesis-related metabolites were minimal. The metabolic changes closely matched the association pattern of a single nucleotide variant (rs12916) in the HMGCR gene, itself shown to be associated with cholesterol levels (Teslovich et al., 2010).

Gut Microbial Metabolites and Cardiovascular Disease

In the past decade it has become evident that the gut microbiome influences human health, including cardiovascular diseases and their risk factors, through introduction of diverse small molecules into circulation. These molecules can be detected and

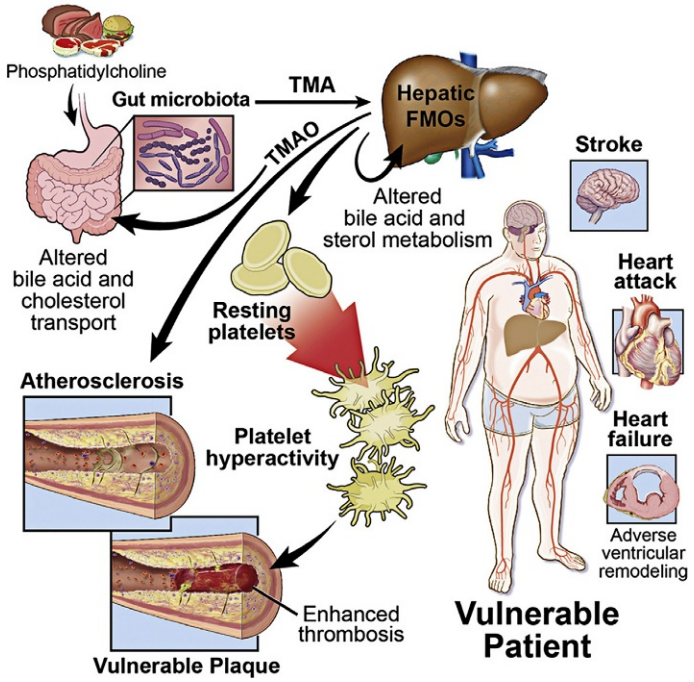

Fig. 5 Pathways linking dietary phosphatidylcholine, intestinal microbiota, and incident adverse cardiovascular events. Source: Zhu et al. *Cell* 2016:165:111–124, with permission from Elsevier.

quantified by plasma and stool metabolomics. At the moment, most evidence exists for choline and carnitine, and for branched-chain amino acids. Tang et al. have shown that intestinal microbiota participates in metabolizing dietary phosphaditylcholine to trimethylamine which is then oxidized to TMAO (Tang et al., 2013; Zhu et al., 2016). They next measured TMAO in 4007 patients undergoing elective diagnostic cardiac catheterization and showed that increased plasma levels of TMAO were associated with increased risk of a major adverse cardiovascular event. Zhu et al. provided more insight into the biological mechanisms by showing that plasma TMAO enhanced platelet hyperreactivity and thrombosis risk (**Fig. 5**) (Zhu et al., 2016; Tilg, 2016).

It should be noted, however, that a smaller European study was unable to corroborate the association of TMAO with angiographically assessed coronary atherosclerosis or with cardiovascular events during 8 years of follow-up (Mueller et al., 2015).

Pedersen et al. (2016) examined untargeted MS metabolomics in serum samples of 277 nondiabetic Danish individuals and in a separate group of 75 patients with type 2 diabetes. Both groups had quantitative gut metagenomics data available. They showed that the serum metabolome of insulin-resistant individuals is characterized by increased levels of branched-chain amino acids, which correlate with a gut microbiome that has an enriched biosynthetic potential for branched-chain amino acids. *Prevotella copri* and *Bacteroides vulgatus* were identified as the main drivers of the association between insulin resistance and the biosynthesis of branched-chain amino acids. The investigators further demonstrated in mice that *Prevotella copri* can induce insulin resistance, aggravate glucose intolerance, and increase circulating levels of branched-chain amino acids.

Short-chain fatty acids produced by gut bacteria have also been implicated in the development of obesity and metabolic syndrome but so far the data have been largely based on animal experiments and the data in humans are limited and findings not entirely consistent (Perry et al., 2016; Trajkovski and Wollheim, 2016).

Summary

Metabolomics is a rapidly developing field and the new technologies have shown great promise in uncovering novel biological pathways leading to cardiovascular disease. The improved understanding of disease pathogenesis suggests new targets for prevention and treatment. However, we are still at an early stage of the development; human data on the emerging novel pathways and biomarkers are still limited and to some extent inconsistent. Sufficiently powered prospective cohort studies and clinical trials are clearly needed for more definitive conclusions. Likewise, standardization of sample processing and laboratory technology would greatly help in comparing and combining results from different studies. Ultimately, metabolomics data must be integrated with genomics, microbiome, and other "omics" data using system level analyses to properly understand the complex functions of human metabolism at the cellular level.

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Relevant Websites

http://www.ebi.ac.uk/metabolights—MetaboLightsMetaboLights contains information on metabolite structure, reference spectra, pathway, localization, and experimental data. It includes individual study information and data (Kale, 2016).
http://www.hmdb.ca/—Human metabolome databaseThe HMDB contains chemical, biochemical, molecular biology, and clinical data on >42,000 metabolites. Information on each metabolite includes links to other external databases (e.g., KEGG, PubChem, GenBank, etc.) as well as structural and pathway applets (Wishart, 2013).
https://metlin.scripps.edu/index.php—METLINMETLIN includes information on >900,000 diverse molecules including metabolites, small peptides, carbohydrates, drugs, and toxicants together. METLIN includes both pathway links (e.g., KEGG) as well as the similarity search algorithm for unknown metabolite identification using tandem mass spectrometry profiles (Smith, 2005).

MicroRNAs in Cardiac Development and Function

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Introduction

The heart is the first functional organ established during embryogenesis. Heart development is characterized by exceptionally rapid transitions in gene activity, with complex integration of molecular information giving way to precise on/off patterns of gene expression. Posttranscriptional repression plays a pivotal role in this process, delineating sharp developmental transitions by suppressing residual transcripts that were specific to the previous stage (Ebert and Sharp, 2012).

One of the landmark discoveries of the 20th century was of small noncoding RNAs, microRNAs (miRNAs). miRNAs repress the expression of genes by accelerating messenger RNA (mRNA) degradation and inhibiting translation, thereby reducing the level of protein (Lim et al., 2005; Guo et al., 2010; Schmiedel et al., 2015). It is now recognized that miRNAs constitute an integral part of the gene regulatory program. They act as rheostats and switches to modulate multiple facets of cardiac development and function by interacting with the network of protein-coding genes, transcription factors, and signaling pathways (Voinnet, 2009; Liu and Olson, 2010). This article presents an overview of the role of miRNAs as key players in cardiac development, and discusses the implications of exploiting miRNAs in regenerative medicine to treat heart diseases.

miRNAs Biogenesis and Function

miRNAs are approximately 22 nucleotides in length and are evolutionarily conserved in metazoans (Bartel, 2004). miRNAs negatively regulate gene expression by accelerating mRNA degradation and inhibiting translation (Lim et al., 2005; Guo et al., 2010). They have emerged as key modulators in many biological processes, including developmental timing, cell proliferation, apoptosis, metabolism, cell differentiation, and morphogenesis (Stark et al., 2005; Ebert and Sharp, 2012). Current insights about miRNA functions in the heart have been influenced by outcomes using animal models with loss- and gain-of-function of miRNAs and their targets and assessing heart development and mature function. These miRNAs have been described as developmental switches to modulate multiple facets of cardiac development and function (Liu and Olson, 2010; Malizia and Wang, 2011; Porrello, 2013; Fuller and Qian, 2014; Piubelli et al., 2014; Hodgkinson et al., 2015; Katz et al., 2016; Yan and Jiao, 2016).

miRNAs can be transcribed either individually or in clusters. Based on their location in the genome, miRNAs are classified as intergenic miRNAs, which are transcribed from their own promoters, or intronic and exonic miRNAs, which are usually cotranscribed and coexpressed with their host genes (Bartel, 2004). The biogenesis of miRNAs starts with initial gene transcription by RNA polymerase II. This results in a primary miRNA transcript (pri-miRNA), which can be several hundreds to several thousands of nucleotides long. Pri-miRNAs are processed by the endonuclease Drosha and its cofactor DiGeorge syndrome critical region 8 (Dgcr8) into 60–100 nucleotide hairpins known as pre-miRNAs. Once exported from the nucleus into the cytoplasm, pre-miRNAs are further processed into the miRNA duplexes by the RNase III endonuclease Dicer. One strand of the duplex becomes a mature miRNA, which is incorporated into the RNA-induced silencing complex (RISC). The mature miRNA directs the RISC to specific mRNA targets usually by binding to the 3′ untranslated region (UTR) of the target mRNA. The miRNA–mRNA interaction causes gene silencing either by promoting mRNA degradation or by inhibiting protein translation (Braun et al., 2011). At the molecular level, each miRNA molecule may target hundreds of mRNAs, and have multiple target sites in the 3′UTRs of a particular mRNA, thereby increasing repression efficiency (John et al., 2004; Krek et al., 2005; Lewis et al., 2005; Stark et al., 2005; Xie et al., 2005; Hornstein and Shomron, 2006). Each mRNA is also predicted to be targets of many distinct miRNAs, suggesting that different miRNAs might work together to regulate mRNA stability and translation. Furthermore, miRNAs are found to act in feedforward and feedback regulations that can reinforce the transcriptional gene expression program (Tsang et al., 2007; Ebert and Sharp, 2012; Rottiers and Näär, 2012).

 https://doi.org/10.1016/B978-0-12-809657-4.99824-7

miRNAs in Cardiac Development

Cardiac development is a well-defined multistep process controlled by an evolutionarily conserved complex of genetic pathways and an extended network of transcriptional and posttranscriptional regulatory programs. The major stages of cardiac development in vertebrates are depicted in **Fig. 1**. The first myocardial cells derive from two major cardiac progenitor cell populations, referred to as heart fields (Buckingham et al., 2005). The first heart field contributes to the left ventricle and parts of the atria. The second heart field contributes to the rest of the heart, including the right ventricle, outflow tract, sinus venosus, and atria (Kelly et al., 2001; Cai et al., 2003; Zaffran et al., 2004; Verzi et al., 2005; Galli et al., 2008; Bruneau, 2013). The development of cardiac progenitors is governed by a core set of transcriptional regulatory networks such as Nkx2.5, Gata, Tbx, Hand, and Mef2 (Olson, 2006). Mouse embryos with mutations in these genes display abnormalities in the heart (Buckingham et al., 2005). In addition to the differentiation of cardiac progenitors into cardiomyocytes, the proliferation of existing cardiomyocytes is responsible for the formation and growth of mature cardiac structures, such as the layered compact muscle comprising the chamber walls and the fingerlike trabeculae that protrude inside the developing chambers. These processes are driven by interactions between the myocardium and adjacent endocardium via molecular signals including neuregulin/ErbB, Notch, Ephrin/Eph, and BMP10 (Gassmann et al., 1995; Meyer and Birchmeier, 1995; Wang et al., 1998; Chen et al., 2004; Grego-Bessa et al., 2007).

miRNAs have a variety of roles in cardiac development and function (**Table 1**). Several studies have provided evidence of differential expression of miRNAs during cardiac development, both during embryogenesis (Chinchilla et al., 2011) and postnatal stages (Porrello et al., 2013). The critical role of miRNAs in cardiac development was identified by deletion of Dicer, the RNase III endonuclease essential for miRNA biogenesis in mammals. Cardiac deletion of Dicer results in embryonic lethality. When Dicer was deleted in cardiac progenitor cells using Cre recombinase under control of Nkx2.5 regulatory elements, embryos exhibit cardiac failure with hypoplastic ventricular myocardium (Zhao et al., 2007; Saxena and Tabin, 2010). The myocardial deletion of Dicer at midgestation stage (E10.5-11.5) using cTnt-Cre leads to embryonic lethality at embryonic day (E) 13.5-E15.5. Mutant hearts display severe myocardial defects including increased cell death and noncompaction of ventricular myocardium (Peng et al., 2014). Deletion of Dicer in cardiomyocytes during early postnatal life using α-myosin heavy chain (MHC)-Cre causes dysregulation of cardiac contractile proteins and sarcomere disarray, resulting in dilated cardiomyopathy, heart failure, and early postnatal lethality (Chen et al., 2008). Similarly, postnatal deletion of Dgcr8, another component of the miRNA biogenesis pathway, in cardiomyocytes using muscle creatine kinase-Cre, leads to dilated cardiomyopathy and premature death within 2 months after birth (Rao et al., 2009). Taken together, these studies demonstrate the importance of miRNA biogenesis in maintaining proper cardiac function and survival during embryonic and postnatal life.

miRNA Regulation of Cardiac Muscle Differentiation

miRNA-1 and miRNA-133 are specifically expressed in cardiac and skeletal muscle cells with expression increasing substantially from E8.5 to adulthood in vertebrates (Lagos-Quintana et al., 2002; Sempere et al., 2004; Chen et al., 2006; Rao et al., 2006). The miRNA-1 family has two members, miRNA-1-1 and miRNA-1-2, each of which is co-transcribed with miRNA-133a-2 and miRNA-133a-1 gene, respectively. The expression of miRNA-1/133a clusters is regulated by upstream and intragenic enhancers controlled by combinations of the basic helix-loop-helix protein MyoD and the MADS-box transcription factors serum response factor (SRF) and myocyte enhancer factor 2 (Zhao et al., 2005; Rao et al., 2006; Liu et al., 2007). In *Drosophila*, deletion of the single miRNA-1 gene (dmiRNA-1) results in a defect in muscle differentiation (Kwon et al., 2005; Sokol and Ambros, 2005). dmiRNA-1 represses the expression of the Notch ligand, Delta, a known regulator of cardiogenesis and myogenesis in fruit flies (Kwon et al., 2005). In *Xenopus* embryos, mi RNA-1 promotes myoblast differentiation as a consequence of its negative regulation of histone deacetylase 4, a transcriptional repressor of muscle gene expression (Chen et al., 2006).

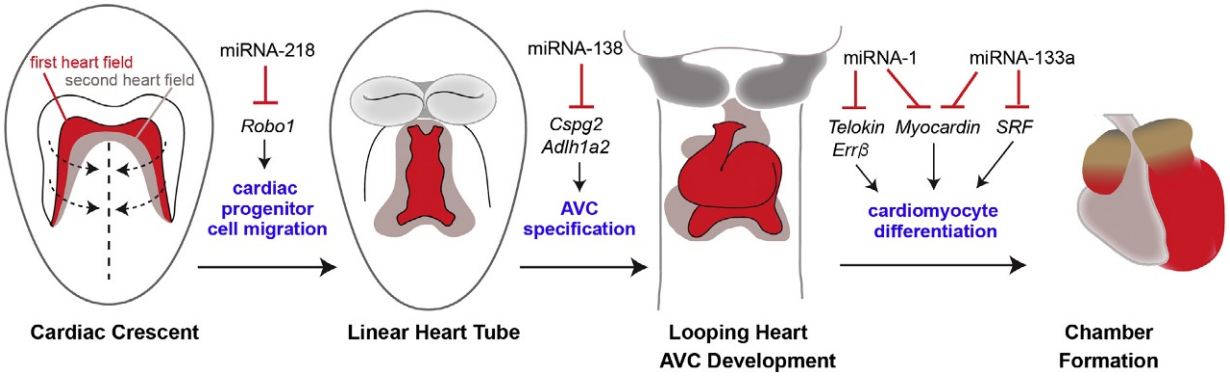

Fig. 1 miRNA regulation of cardiac development. The diverse mechanisms of miRNA activity are presented together with the related cardiac developmental stages in vertebrate animals. *AVC*, atrioventricular canal.

Table 1 Examples of miRNA null mutant mice and cardiac phenotypes during development

miRNA name	*miRNA null mouse phenotype*	*Target genes*	*References*
miRNA-1-2	~50% embryonic lethal, VSD in embryos, increased cardiomyocyte proliferation, cardiac arrhythmias in surviving mutants	Hand2, Irx5	Zhao et al. (2007), Heidersbach et al. (2013)
miRNA-1-2	Viable, no phenotype		Wystub et al. (2013), Wei et al. (2014)
miRNA-1-1	Similar to miRNA-1-2		Heidersbach et al. (2013), Wystub et al. (2013), Wei et al. (2014)
miRNA-1-1/ miRNA-1-2 double null	Lethal within 3 weeks after birth, sarcomere disruption, impaired cardiac function, increased expression of fetal sarcomere-associated genes, increased cardiomyocyte proliferation	Myocardin, Telokin, Errβ	Heidersbach et al. (2013), Wei et al. (2014)
miRNA-133a-1	Viable, no phenotype		Liu et al. (2008), Wystub et al. (2013)
miRNA-133a-2	Viable, no phenotype		Liu et al. (2008), Wystub et al. (2013)
miRNA-133a-1/ miRNA-133a-2 double null	~56% lethal during late embryonic and neonatal stages, VSD and chamber dilation at P1, disrupted sarcomere structure, increased cardiomyocyte proliferation and smooth muscle gene expression at P1	SRF, Myocardin, Cyclin D2	Liu et al. (2008)
miRNA-1-2/133a-1 cluster	Viable, abnormal cardiac conduction triggered by β-adrenergic signaling		Besser et al. (2014)
miRNA-1-1/133a-2 cluster	Similar to miRNA-1-2/133a-1		Besser et al. (2014)
miRNA-1/133a cluster double null	Lethal before E11.5, decreased cardiomyocyte proliferation, hypoplastic ventricular myocardium, increased expression of smooth muscle genes	Myocardin	Wystub et al. (2013)
miRNA-208a	Viable, abnormal sarcomere structure, decreased expression of slow muscle gene β-MHC, abnormal cardiac conduction	Thrap1, Myostatin, Cx40, Hop, Gata4	Van Rooij et al. (2007), Callis et al. (2009)
miRNA-208b	Viable, no phenotype		Van Rooij et al. (2009)
miRNA-499	Viable, no phenotype		Van Rooij et al. (2009)
miRNA-208b/ miRNA-499 double null	Viable, decreased expression of slow muscle gene β-MHC	Thrap1	Van Rooij et al. (2009)
miRNA-17-92 cluster (cardiac specific)	Viable, decreased cardiomyocyte proliferation and hypoplastic ventricular myocardium in embryonic heart	Pten	Chen et al. (2013)
miRNA-302-367 cluster (cardiac specific)	Viable, decreased cardiomyocyte proliferation, and hypoplastic ventricular myocardium in embryonic heart	Mst1, Lats2, Mob1b	Tian et al. (2015)

Studies in embryonic stem (ES) cells reveal the role of miRNA-1 and miRNA-133 in driving cardiac differentiation. The expression of miRNA-1 and miRNA-133 is enriched in ES cell-derived cardiomyocytes but not in undifferentiated ES cells (Ivey et al., 2008). Overexpression of these miRNAs in embryoid body-based culture of ES cells enhances mesodermal lineage commitment and inhibits endoderm or neuroectoderm differentiation. In contrast, miRNA-1 and miRNA-133 seem to exert opposite functions on further differentiation of ES cells toward the cardiac muscle lineage. miRNA-1 promotes differentiation of ES cells toward the cardiac lineage by repressing the Notch ligand Delta-like 1 (Dll-1), whereas miRNA-133 suppresses the cardiac differentiation of ES cells (Ivey et al., 2008). Moreover, in ES cell-derived multipotent cardiovascular progenitors, overexpression of miRNA-1 promotes cardiomyocyte differentiation and inhibits endothelial cell commitment by repressing the Wnt receptor Fzd7 and fibroblast growth factor receptor substrate 2 (Frs2) (Lu et al., 2013). However, the effects of miRNA-1 and miRNA-133 on ES cell cardiac differentiation may be dependent on the model used because behavior of ES cells in 2D is markedly different. In this setting, forced expression of miRNA-1 and miRNA-133 by lentivirus reduces cardiac gene expression (Takaya et al., 2009). miRNA-1 acts by repressing cyclin-dependent kinase 9 (Cdk9), which interacts with p300/Gata4 to active expression of cardiac-specific genes (Takaya et al., 2009). Although there are discrepancies related to miRNA-1 function, these studies have demonstrated the importance of miRNA-1 and miRNA-133 in regulating cardiac muscle differentiation.

miRNA Regulation of Cardiomyocyte Sarcomere Organization

Sarcomeres are the basic contractile units of cardiac muscle. They are composed of thick and thin filaments essential for generation and propagation of mechanical force. Myosin, the major component of the thick filament, is comprised of MHC subunits and

myosin light-chain (MLC) subunits. α-MHC (Myh6) and β-MHC (Myh7) are both expressed in the heart during development and in the adult. In rodents, expression of β-MHC is downregulated after birth so that in the adult, α-MHC is the dominant MHC isoform in the heart (Lyons et al., 1990; England and Loughna, 2013). MHC isoform switches in response to cardiac stress or hypothyroidism. Pathologic hypertrophy is associated with upregulation of β-MHC and downregulation of α-MHC (Krenz and Robbins, 2004; Gupta, 2007).

Expression of α- and β-MHC isoforms is controlled by miRNA-208a, miRNA-208b, and miRNA-499 (van Rooij et al., 2007, 2009; Callis et al., 2009). miRNA-208a and miRNA-208b are encoded in an intron of the α-MHC and β-MHC gene, respectively. Mice null for miRNA-208a are viable but show abnormalities in sarcomere structure and declined cardiac function at 6 months of age (van Rooij et al., 2007). However, miRNA-208a null mice are resistant to cardiac hypertrophy in response to stress induced by transverse aortic banding or calcineurin (van Rooij et al., 2007; Callis et al., 2009). This is concomitant with decreased expression of slow skeletal muscle contractile protein, β-MHC, in the miRNA-208a null heart. The function of miRNA-208a is mediated, in part, by repression of thyroid hormone receptor associated protein 1 (Thrap1), which negatively regulates the expression of β-MHC.

miRNA-208a controls not only the expression of β-MHC in the heart but also that of the closely related slow myosin isoform, Myh7b (van Rooij et al., 2009). Both β-MHC and Myh7b genes encode intronic miRNAs, miRNA-208b, and miRNA-499, respectively (Berezikov et al., 2006; Landgraf et al., 2007). Mice lacking the miRNA-208b or miRNA-499 gene have no obvious developmental defects (van Rooij et al., 2009). However, miRNA-208b/-499 double null mutant mice display reduced expression of slow myofiber β-MHC and increased expression of fast type myosin isoforms. By contrast, overexpression of miRNA-499 leads to increased expression of β-MHC and drives muscle toward a slow myofiber phenotype. Forced cardiac miRNA-499 expression promotes hypertrophy in mice (Shieh et al., 2011; Matkovich et al., 2012). Together, these miRNAs are important in the specification of the identity of muscle fibers by stimulating slow myofiber gene programs at the expense of those that control fast myofiber gene expression (Hodgkinson et al., 2015).

It has been demonstrated that miRNA-1 and miRNA-133 can act as specific activators or suppressors of sarcomere formation and muscle gene expression. Deletion of both miRNA-1-2 and miRNA-1-1 in mice (miRNA-1 null) leads to sarcomere disruption in cardiomyocytes and impaired cardiac function. All the miRNA-1 null mice died before weaning age (Heidersbach et al., 2013; Wei et al., 2014). miRNA-1 functions to negatively regulate myocardin, the major regulator of smooth muscle gene expression, and telokin, the smooth muscle-specific inhibitor of MLC-2 phosphorylation (Heidersbach et al., 2013; Wystub et al., 2013). The upregulation of myocardin and telokin in miRNA-1 null hearts may, in part, contribute to the defect in sarcomere organization. Furthermore, studies from Wei et al. indicated that miRNA-1 directly represses nuclear receptor estrogen-related receptor β (Errβ). The increased level of Errβ in miRNA-1 null heart activates the expression of fetal sarcomere-associated genes (Wei et al., 2014).

miRNA-133a represses smooth muscle gene expression in the heart by directly targeting myocardin and SRF for repression (Liu et al., 2008; Wystub et al., 2013). Deletion of both miRNA-133a-1 and mIRNA-133a-2 (miRNA-133a null) causes late embryonic and neonatal lethality due to ventricular septal defect (VSD) and chamber dilation (Liu et al., 2008). miRNA-133a null mice display sarcomere disorganization and ectopic activation of the smooth muscle gene program (Liu et al., 2008). In addition, mice lacking both miRNA-1 and miRNA-133a displayed severe cardiac dysfunction and died before embryonic day 11.5 (E11.5). Mice with a null mutation in miRNA-1/133a showed increased expression of myocardin and smooth muscle genes in the heart. These studies indicate that miRNA-1 and miRNA-133a clusters are important in cardiomyocyte differentiation and sarcomere formation during embryonic and postnatal life. They act cooperatively to govern the gene transition program from an immature state characterized by expression of smooth muscle genes to a mature phenotype (Wystub et al., 2013).

miRNA Regulation of Cardiomyocyte Proliferation

Mammalian cardiac growth during development is predominantly through cardiomyocyte proliferation (Meilhac et al., 2003). After birth, cardiomyocytes exit the cell cycle, and further increase in myocardial mass is primarily due to hypertrophic increase in cardiomyocyte size (Li et al., 1996; Soonpaa et al., 1996). In mice, cardiomyocyte proliferation peaks between embryonic day 10 (E10) and E12 and again at postnatal day 4 (P4) (Soonpaa et al., 1996). Several studies have highlighted the role of miRNAs in the regulation of cardiomyocyte proliferation during development and early postnatal life (**Fig. 2**).

Mice lacking miRNA-1 exhibit increased cardiomyocyte proliferation and hyperplastic myocardial wall in the developing hearts (Zhao et al., 2007; Wei et al., 2014). miRNA-1 inhibits cardiomyocyte proliferation, in part, by repressing the expression of the heart-and neural crest derivative-expressed (Hand)-2 transcription factor, a positive regulator of cardiac growth (Zhao et al., 2005; Zhao et al., 2007). Similar to miRNA-1, mice lacking miRNA-133a show excessive cardiomyocyte proliferation at postnatal day 1 (Liu et al., 2008). Conversely, overexpression of miRNA-133a in the developing heart reduces cardiomyocyte proliferation. miRNA-133a inhibits cardiomyocyte proliferation by directly repressing cyclin D2 (Liu et al., 2008).

Over the first week of postnatal life in mice, most cardiomyocytes exhibit cell cycle withdrawal (Li et al., 1996; Soonpaa et al., 1996). Cessation of cardiomyocyte proliferation is associated with downregulation of cyclins and cyclin-dependent kinases specific to G1/S and G2/M phases and upregulation of cell cycle inhibitors (Flink et al., 1998; Poolman and Brooks, 1998; Engel et al., 2005). Studies in neonatal hearts have identified the miRNAs that are responsible for the withdrawal of cardiomyocytes from the cell cycle. A microarray analysis for miRNAs differentially regulated during postnatal life identified miRNA-195 as the most highly upregulated miRNA in the mouse heart ventricles at postnatal day 10 (P10), the time point at which mouse cardiomyocytes exit the cell cycle (Porrello et al., 2011). Overexpression of miRNA-195, a member of miRNA-15 family, in the embryonic heart reduces

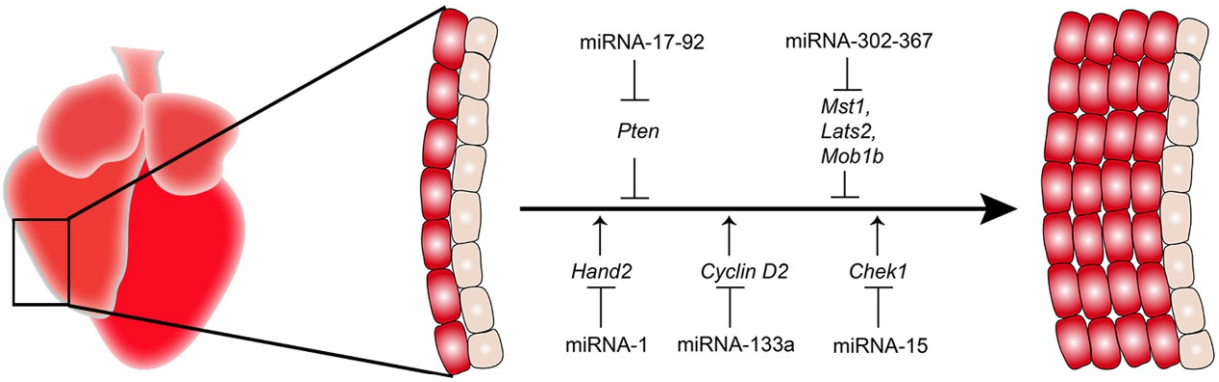

Fig. 2 miRNA regulation of cardiomyocyte proliferation. miRNAs and their target genes in cardiomyocyte proliferation during cardiac development are shown.

cardiomyocyte proliferation and causes ventricular hypoplasia and VSDs (Porrello et al., 2011). Checkpoint kinase 1 (Chek1) was identified as the miRNA-195 target mediating these effects (Porrello et al., 2011, 2013). In contrast, knockdown of the miRNA-15 family in neonatal mice with locked nucleic acid-modified anti-miRNAs leads to increased cardiomyocyte proliferation in both neonates and adult mice (Porrello et al., 2011, 2013).

Other miRNAs of recent interest are the miRNA-17-92 and miRNA-302-367 clusters. Cardiac-specific deletion of either the miRNA-17-92 or miR-302-367 cluster during development leads to reduced cardiomyocyte proliferation and hypoplastic ventricular myocardium (Chen et al., 2013; Tian et al., 2015). Conversely, overexpression of either of these two miRNA clusters causes a profound increase in cardiomyocyte proliferation. The miRNA-17-92 cluster was found to downregulate the tumor suppressor gene, proliferation through repressing phosphatase, and tension homology (Pten) (Chen et al., 2013). The miRNA-302-367 cluster induces cardiomyocyte proliferation by repressing the Hippo signal pathway (Tian et al., 2015). Hippo signaling regulates cell proliferation and organ size in many species (Pan, 2010). Inactivation of this pathway during fetal development in mice leads to marked cardiomegaly (Heallen et al., 2011; Xin et al., 2011; von Gise et al., 2012). miRNA-302-367 represses several Hippo signaling kinases, Mst1, Lats2 and Mob1b, to promote cardiomyocyte proliferation (Tian et al., 2015).

miRNA Function in the Cardiac Conduction System

Cardiomyocytes are excitable cells that can generate and propagate electrical activities. The strict coordination of excitation, impulse propagation, and polarization within cardiomyocytes is important to maintain a normal rhythm in sync with the mechanical actions of the heart. Cardiac excitability is regulated by ion channels, transmembrane proteins that control the movement of ions across the cytoplasmic membrane. Specifically, sodium (Na^+), calcium (Ca^{2+}), and potassium (K^+) channels and gap junction proteins such as connexin43 (Cx43) are critical regulators of cardiomyocyte polarization and depolarization during contraction and relaxation. Several miRNAs, including miRNA-1 and miRNA-133a, target ion channels and gap junction proteins. They might have important pathophysiological functions in the heart, and are potential targets for antiarrhythmic agents.

Ablation of the miRNA-1-2 gene in mice results in arrhythmias, cardiac conduction defects, and sudden cardiac death within 2–3 months of age (Zhao et al., 2007). Cardiac arrhythmias in the miRNA-1-2 mutants may be caused by elevated levels of the transcription factor Iroquois homeobox 5 (Irx5), a direct target of repression by miRNA-1. Irx5 regulates the cardiac ventricular repolarization gradient by negatively regulating the expression of potassium channel genes such as Kcnd2. Similar to miRNA-1-2 null mutants, mice lacking miRNA-1-1 exhibit cardiac conduction abnormalities (Heidersbach et al., 2013). Independent mouse strains with null mutations in miRNA-1-1 and miR-1-2 were engineered by two groups (Wystub et al., 2013; Wei et al., 2014). Cardiac conduction defects were not observed in either of the single knockout strains (Wystub et al., 2013; Wei et al., 2014). The differences between these studies could be due to the methods used to generate the null mice, or genetic background. Interestingly, gain-of-function of miRNA-1 also caused cardiac arrhythmia. miRNA-1 expression is elevated in humans with coronary artery disease and in rats subjected to myocardial infarction (Yang et al., 2007). Overexpression of miRNA-1 in normal or infarcted rat hearts increased the occurrence of arrhythmias. Delivery of an antisense oligonucleotide to suppress miR-1 expression in the heart prevented arrhythmias in the rat infarct model. miRNA-1 regulates cardiac electrophysiology most likely via repressing the expression of KCNJ2 (which encodes the K+ channel subunit Kir2.1) and GJA1 (which encodes connexin 43) (Yang et al., 2007). In addition, miRNA-1 regulates calcium signaling through repressing B56α, the protein phosphatase PP2A regulatory subunit (Terentyev et al., 2009). Overexpression of miRNA-1 results in hyperphosphorylation of L-type calcium channels and ryanodine receptor 2, and promotes arrhythmogenic alterations in myocyte calcium cycling (Terentyev et al., 2009).

It has been further demonstrated that the two miRNA-1/133a clusters are involved in regulation of adrenergic signaling in cardiomyocytes and in the modulation of the electrical properties of the postnatal and adult heart (Besser et al., 2014). The tight regulation of QT duration is essential for general heart function to prevent potentially fatal arrhythmias. Deletion of one or the other miRNA-1/133a cluster leads to longer QT intervals as well as altered calcium currents triggered by β-adrenergic signaling

(Besser et al., 2014). Application of propranolol or verapamil, to block either adrenergic signaling or L-type calcium-channels, abrogated the long QT phenotype in miRNA-1/133a single cluster mutant mice (Besser et al., 2014).

Although muscle myosin-related miRNAs generally act by regulating myofiber identify, miRNA-208a has been shown to control cardiac conduction by regulating expression of gap junction protein connexin 40 (Cx40) and the homeodomain-only protein (Hop) and Gata4 transcription factors (Callis et al., 2009). Functional studies in animal models reveal that mice lacking miRNA-208a display electric conduction defects and cardiac arrhythmias. Overexpression of miRNA-208a in mouse heart induces cardiac hypertrophy and arrhythmias (Callis et al., 2009).

miRNA in Cardiac Patterning

Heart development is a highly organized process. A tight gene regulatory network is required to create stable differences or boundaries between the myocardium of different chambers (Harvey, 2002). Recent studies in zebrafish have indicated that miRNAs control cardiac chamber-specific gene expression patterns. The zebrafish heart contains a single atrium and a single ventricle. During heart development, these two chambers are separated by the atrioventricular canal (AVC), which eventually gives rise to the cardiac valves (Stainier, 2001; Yin and Pacifici, 2001). miRNA-138 is specifically expressed in the ventricular chamber of the zebrafish heart (Morton et al., 2008). Knockdown of miRNA-138 by morpholino or anti-miRNA in zebrafish embryos results in the expansion of AVC gene expression into the ventricular chamber. The mutant hearts display impaired cardiomyocyte maturation and pericardial edema, indicative of cardiac dysfunction. miRNA-138 restricts AVC gene expression in the cardiac ventricle by negative regulation of the cell adhesion molecule versican (Cspg2) and the retinoic acid synthesis enzyme aldehyde dehydrogenase-1a2 (Adlh1a2) (Morton et al., 2008).

Slit ligands and roundabout (Robo) receptors function as crucial regulators of heart tube formation. In zebrafish, Slit-Robo signaling provides a short-range directional cue for the migration of cardiac progenitor cells (Fish et al., 2011). miRNA-218a-1 and miRNA-218a-2 genes are within an intron of the Slit2 and Slit3 genes, respectively. miRNA-218 directly targets Robo1 and Robo2 transcripts for repression. Knockdown of miRNA-218 by morpholinos in zebrafish embryos leads to impaired migration of cardiac progenitor cells and incomplete heart field fusion. The miRNA-218 mutant heart shows increased Robo1 expression and knockdown of Robo1 rescues the miRNA-218 mutant phenotype. This study suggests that miRNA-218 modulates the levels of Robo1 to control cardiac progenitor cell migration and heart tube formation (Fish et al., 2011). In addition, other studies identified different roles for miRNA-218 in zebrafish heart development. Overexpression of miRNA-218 in zebrafish embryos causes heart looping defects and cardiac chamber abnormalities. However, miRNA-218 knockdown does not cause any defects in cardiac development (Chiavacci et al., 2012). The differences between these two studies may reflect that miRNA-218 controls heart development through multiple pathways, or the requirement of miRNA-218 during cardiac development is limited only to a distinct temporal window of early cardiac fusion, and is dispensable thereafter. miRNA-138 and miRNA-218 are conserved from zebrafish to humans. Thus it will be interesting to study whether these miRNAs play similar roles in patterning mammalian heart.

Conclusions and Implications for Regenerative Medicine

Heart development is a paradigm of organogenesis. The study of miRNA in developing heart has yielded important insights into the molecular mechanisms that control cardiac formation and function. miRNAs act to confer accuracy and uniformity to developmental transitions. Loss of miRNAs may result in cardiac abnormalities and dysfunction. Understanding how interactions of miRNAs and targets can drive concerted development during cardiac growth is critical for future prospects in regenerative medicine. For example, the identification of miRNAs that are important in cardiomyocyte proliferation during development has allowed for development of miRNA-based therapeutics that can promote myocardial proliferation and regeneration in adulthood (Chen et al., 2013; Porrello et al., 2013; Tian et al., 2015). With delivery of these miRNA mimics or anti-miRNAs into the ischemic heart of adult mouse, this treatment promotes the regeneration of new myocardial tissues and improved cardiac function (Porrello et al., 2013; Tian et al., 2015).

In addition, many pathologies and abnormalities can occur when miRNAs are dysregulated, including cardiac hypertrophy and heart failure. For excellent reviews on pathological conditions resulting from improper miRNAs, see Small et al. (2010).

miRNA-mediated posttranscriptional gene repression has been recognized as fundamentally important to understand cardiac developmental processes. Changes in miRNAs cause changes in spatial and temporal organizations of tissues, which affect biological function. Heart organogenesis thus requires a greater understanding of the relationships between the posttranscriptional regulatory programs and their physiological effects.

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Microvasculature in Health and Disease

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Introduction

The microvasculature is an integral component to all tissues, necessary for establishing and maintaining tissue health and function via blood perfusion and a dynamic interplay with tissue cells and environment. A microvasculature capable of supporting effective perfusion consists of a properly arranged hierarchical tree of thousands of heterogeneous vessel types generating effective and regulatable blood flow pathways. Comprised of a variety of cell types, the microvasculature is a sophisticated system intimately associated with the tissue environment. As such, it mediates blood:tissue communication important for tissue homeostasis including gas/nutrient exchange, paracrine and endocrine signaling, immune cell dynamics, reparative processes, and specific tissue functions. Because of this central importance of the microvasculature in tissue health, any treatment strategy for a disease state should consider the impact on the microvasculature, including reversing microvascular dysfunction. With this in mind, new treatment strategies targeting specifically the dysfunctional microvasculature are emerging with great promise in managing health and disease.

Microvasculature: Form and Function

The microvasculature, comprised of the most distal aspects of the vasculature, serves as the blood:tissue interface in any tissue. While often considered separately, the microvasculature is an integrated component to any tissue and is necessary for that tissue's function and health. This is highlighted by the many pathologies that either occur due to or are complicated by microcirculatory dysfunction (MCD) (Olsen et al., 1983; Jeremy et al., 1987; Lamah et al., 1999; Georgi et al., 2011; Duncan and Faris, 1986; Futrakul et al., 1995; Claxton and Fruttiger, 2005; Darland et al., 2003; Benjamin et al., 1998; Hallerstam et al., 2004; Cutolo et al., 2010; Faber et al., 2011; Bauer et al., 2005; Humar et al., 2009; Mourad and Levy, 2011; Ryschich et al., 2002; Weidner and Folkman, 1996; Gutterman et al., 2016). In order for a microvasculature to function as an effective circulatory circuit, each microvessel must be properly organized into a microcirculatory network. Native, functional microcirculations, while topologically unique for different tissue

Table 1 Example diseases and conditions linked to microvascular dysfunction

Aging	No-reflow phenomenon
Cancer	Ischemic disease
Hypertension	Reperfusion injury
Pulmonary hypertension	
Atherosclerosis	Peripheral limb ischemia
Cardiomyopathy	
Coronary microvascular disease	Obesity
Cardiac hypertrophy	Metabolic syndrome
Coronary artery disease	Chronic inflammatory disease
Heart failure	Stroke
Atrial fibrillation	Kawasaki disease
Systemic sclerosis	Autoimmune disease
Peripheral neuropathy	Rheumatoid arthritis
Obstructive sleep apnea	Diabetes (Type I & Type II)
Chronic kidney disease	Vascular dementia
Dementia	Radiation injury
Reynaud's syndrome	Amyotrophic lateral sclerosis

types, consist of hierarchically organized microvessels, heterogeneous in size and phenotype, generating a network with short, homogeneous diffusion distances between individual capillaries, low resistances to flow through the network, and no oxygen gradients across the tissue (Pries and Secomb, 2014; Pries et al., 2010). The absence of one or more of these features results in compromised perfusion and/or exchange despite adequate microvessel densities (**Table 1**) (LeBlanc et al., 2012a).

How microvascular networks form and adapt is less well known than how new microvessels form (i.e., angiogenesis). Yet, these network-related dynamics occurring after new vessel segments have grown are critical to the proper regeneration and/or repair of a microcirculation. Questions related to what are the factors promoting and regulating this "postangiogenesis" progression, how do newly formed, immature neovessels integrate into a network, what level of plasticity is required in network formation, and how is microvascular stability maintained during periods of intense neovessel adaptation and remodeling need to be answered before we can develop an effective regenerative strategy for establishing therapeutically competent microcirculations.

Microvascular Networks

As mentioned, a functional microcirculation consists of an ordered arrangement of microvessels with a heterogeneous distribution of diameters in a diameter- and function-based hierarchical topology. With caveats (e.g., in skeletal muscle arcades or arterio-venous shunts), healthy blood flow is unidirectional through this network from arterioles to capillaries to venules. An often described example of a microvascular network in which this diameter-based hierarchical network architecture is disrupted is the tumor microcirculation in which these three microvascular compartments are compromised (Pries et al., 2009, 2010; Secomb et al., 2012). Thus the perfusion circuit in a tumor is disorganized and unregulated, resulting in zones of flow stasis, regional inhomogeneous blood:tissue exchange, and hypoxia. Importantly, many regions of the tumor microvasculature contain higher than typical microvessel densities, highlighting the additional point that more vessels do not necessarily equal more perfusion; effective perfusion requires proper microvascular network organization.

Topology

Branching is a hallmark of a microvascular network and is the means by which blood is distributed throughout the tissue via low resistance pathways (Koller et al., 1987; Ley et al., 1986; Ellsworth et al., 1987). Often, microvascular networks are schematized as a symmetrical hierarchical distribution of progressively branching vessels with the inflow paths located on one side of the network and the outflow paths located on the opposite side. However, most native vascular networks are asymmetrical in organization; feed arteries are often adjacent to draining veins with the hierarchical network distributed around them in the tissue. This asymmetrical topology eliminates tissue-wide oxygen and exchange gradients, which are present in the contrived symmetrical networks (Pries and Secomb, 2014).

Vessel Density and Perfusion

Intuitively, more vessels should provide more perfusion. However, this is not always the case and, in fact, too many vessels within a network can compromise perfusion. For example, in the aforementioned solid tumors, there are high microvessel densities yet tumor perfusion is chaotic and limited (Pries et al., 2009; Dewhirst et al., 1989). Also, perfusion in granulation tissue—which has high vessel density—is abnormal, highlighted by congested flow (Dewhirst et al., 1989; Dudar and Jain, 1983). In the repairing mouse ischemic hindlimb 1–2 weeks following the ischemic insult, vessel densities are almost twice that of normal, yet resting perfusion is roughly half that of the uninjured contralateral hindlimb (Sullivan et al., 2002; Couffinhal et al., 1998). Similarly,

coronary flow reserve (CFR) is compromised postmyocardial infarction, despite an elevated vessel density (Leblanc et al., 2013). Thus, perfusion and vessel density are not necessarily coupled and while it is necessary to have sufficient numbers of microvessel segments in a network, other components related to network form and function are needed to provide sufficient perfusion.

Indeed, skeletal muscle vascularity following chronic exercise increases leading to proportional increases in muscle bed perfusion (Egginton and Hudlicka, 1999; Egginton et al., 1998). Importantly, this increase in vascularity occurs while preserving normal network topology with concomitant increase in arterioles and capillaries (Hansen-Smith et al., 1998, 2001). In tissues of growing juvenile rats, similar proportional changes occur in the network in the growing heart preserving coronary microvascular architecture (Batra and Rakusan, 1992). Yet, in the growing intestine, arteriole density and branching remain constant but microvessel segment lengths and caliber increase (Unthank et al., 1990; Unthank and Bohlen, 1987). The implications from these and other observations related to repair of microvasculatures are profound. Changes in vascular volume and not microvessel numbers appear to be a more productive objective. Of course one way to increase vascular volume, for example, is by promoting the formation of new microvessels (e.g., via angiogenesis). However, without the coordinated integration of these new microvessels into the network, perfusion will not be increased. In this regard, network architecture (as well as flow control) are, perhaps, more important in expanding a perfusion bed than vessel numbers. This argues for a new approach in vascular regeneration; one that targets the determinants of microvascular network form and function.

Microvascular Dysfunction

In a system as complex as the microvasculature, a number of biological problems may arise and contribute to dysfunction and disease, including alterations in vessel density, faulty flow control, prothrombotic conditions, and oxidative stress (**Fig. 1**). The endothelial cell (EC) lining of the microvasculature comprises the majority of ECs within the circulatory system and, as such, EC dysfunction is a significant contributor to the onset and progression of various forms of microvascular dysfunction. In health, the EC integrally regulates angiogenesis, vasomotor tone, vascular permeability, thrombolysis, immune cell targeting/extravasation, and wound healing. Characteristic changes that occur in the transition from healthy to dysfunctional EC signaling include significant metabolic perturbations with increased reactive oxygen species (ROS) generation (Goveia et al., 2014), decreased nitric oxide (NO) bioavailability (Karbach et al., 2014), proinflammatory signaling (Pober and Sessa, 2007), and blunted flow-mediated dilation (Anderson et al., 1995). While the EC is a focus of many investigations into microvascular health and disease, it is important to note that the microvessel wall consists of a system of cell types including the EC. Thus, dysfunction often reflects deficits in control and regulation between the other vascular cells, in particular the vascular smooth muscle cells (Alexander and Owens, 2012), comprising the microvessel.

Vascular Density

As previously mentioned, diameter mismatch and overall network disorganization between vessels within the microcirculation can lead to blood perfusion discrepancies, such as in tumor biology (Pries et al., 2009, 2010; Secomb et al., 2012). Oftentimes, the

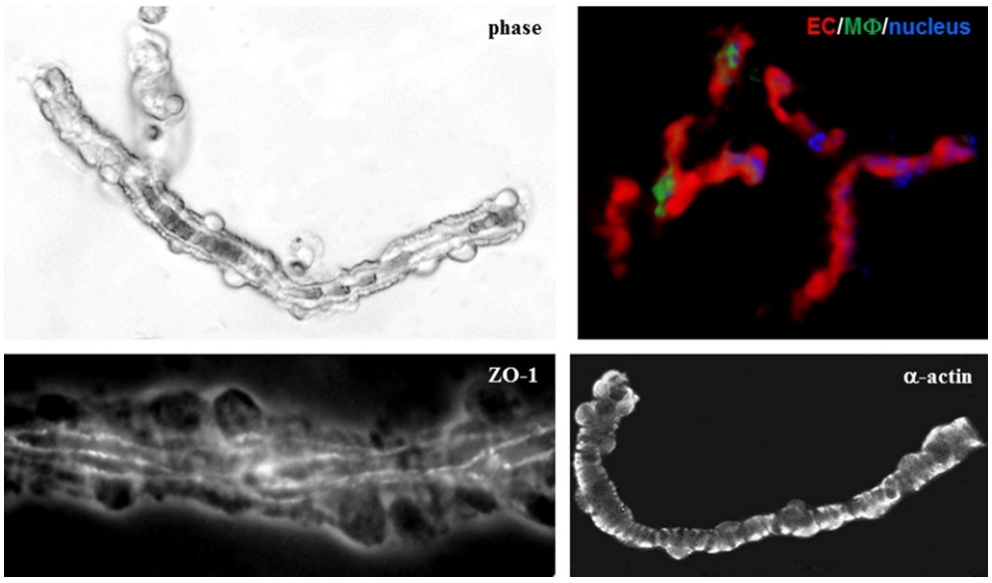

Fig. 1 Example structure of microvessels isolated from adipose tissue (phase panel is a precapillary arteriole) highlighting the complex and multicellular structure of the microvessel wall. Cells within the microvessel are in intimate contact and communication with each and the surrounding tissue environment.

perfusion inefficiency seen in tumors is a result of hypervascularization (Pries et al., 2009), a phenomenon perhaps driven by the overexpression of angiogenic factors within the tumor (Pries et al., 2010). The aberrant vascular conditions within the tumor milieu leads to an environment that is simultaneously hypoxic and flooded with ROS, including hydrogen peroxide (H_2O_2). This environment can lead to gap junction dysfunction and impaired cell-cell communication within the tumor microvasculature (Secomb et al., 2012; Pries et al., 2010).

On the opposite end of the microvascular density spectrum, capillary rarefaction, as seen in hypertension (Humar et al., 2009; Mourad and Levy, 2011; Lubrano and Balzan, 2016) and systemic sclerosis (Cutolo et al., 2010), leads to a reduction in overall capillary number. Indeed, microvascular rarefaction is quite common in hypertensive patients, beginning with loss of endothelium function followed by a narrowing of the vessel lumen. This irregular vasoconstriction of the capillaries ultimately leads to a loss of perfusion; subsequently, the cells within these vessels may undergo apoptosis (Mourad and Levy, 2011). In a deleterious positive feedback loop, this capillary loss causes increased resistance through the remaining peripheral vasculature, resulting in even higher blood pressure (Humar et al., 2009). Interestingly, patients who receive antiangiogenic drugs to treat tumors commonly exhibit increased blood pressure following treatment. VEGF-A is an important target of these drugs; the decrease in VEGF-A following antiangiogenic treatment may decrease the transcription of endothelial nitric oxide synthase (eNOS), thus reducing endogenous levels of the vasodilator NO. Less NO in the system leads to vasoconstriction, thereby inducing hypertension in these patients (Mourad and Levy, 2011).

Flow Control

Compromised tone and flow control is the hallmark of MCD, and may be manifested in several diseases (Gutterman et al., 2016). Indeed, the rarefaction of capillaries previously discussed with regard to hypertension is, at least initially, due to compromised vascular tone in these small vessels (Humar et al., 2009). Additionally, many patients who present with angina but do not exhibit signs of coronary artery disease (CAD) yet are thought to be experiencing ischemic disease likely due to coronary microvascular dysfunction (CMD) brought on by diminished vasodilatation of the coronary arterioles and small arteries. This microvascular tone dysfunction inhibits CFR, leaving the heart with insufficient resources when challenged by stress or exercise (Marinescu et al., 2015). Furthermore, other patients without signs of CAD have presented with angina associated with atrial fibrillation (AF) caused by microvascular flow disturbance. In these patients, oxidized LDL receptor-1 (LOX-1) was upregulated by increased systemic angiotensin II (Ang II) brought on by the AF. This cascade led to an increase in ROS in the blood, resulting in reduced NO levels and therefore decreased vasodilatory ability within the microvessels (Lubrano and Balzan, 2016).

Impaired vascular tone is also seen in both Type I and Type II diabetes. Some diabetic patients exhibit decreased CFR in response to a cardiac challenge, as well as impaired coronary vasodilation (Nahser et al., 1995; Gutterman et al., 2016; Di Carli et al., 2003). In addition, in a rat model of prediabetic metabolic syndrome, microvascular atrophy as well as decreased lumen size and vessel wall thickness were all observed (Stepp et al., 2004). In humans, patients diagnosed with both diabetes and metabolic syndrome exhibited reduced vasoconstriction in resistance arteries following exogenous administration of endothelin (ET-1), likely caused by a downregulation of receptors due to overproduction of endogenous ET-1 (Cardillo et al., 2002). This abundance of endogenous ET-1 in diabetic patients may also be linked to the increased rate of hypertension in this population (Cardillo et al., 2002). Taken together, it is apparent that microvascular tone is an important regulatory component of blood flow, and numerous diseases can have deleterious effects on the system.

Thrombosis

Ischemia arises from perfusion deficits due to failures in the microcirculation often related to EC activation secondary to insult or inflammation. Microvascular ischemia often occurs as clumped leukocytes and/or platelets lodge in the bloodstream (Gutterman et al., 2016); the smaller diameters of the microvessels within the microcirculation make it particularly susceptible to these thrombotic events. Inevitably, the location of the ischemic episode will play a large role in the deficits manifested in the patient. An ischemic microcirculatory event in the heart can lead to angina, cardiovascular disease, and even death, whereas similar incidents in other organs or tissues of the periphery may only lead to localized impairment (Gutterman et al., 2016; Kothawade and Bairey Merz, 2011). Following an ischemic event of the coronary microvasculature, reperfusion injury is a common occurrence. Perivascular edema, cell swelling, increased capillary permeability, and increased levels of ROS within the coronary compartment can lead to further complications including necrotic cell death and destruction of the coronary microcirculation (Heusch and Gersh, 2016). In a prediabetes model of microvascular disease, reduced lumen size and thinner vessel walls in the microcirculation have been observed (Lubrano and Balzan, 2016). These physiological deficits inherently put the system at greater risk for a thrombotic event, especially as the patient progresses into full manifestation of Type II diabetes. Another example is that of capillary leakage. Depending on the location of the compromised vessels within the microcirculation, this can lead to sepsis and/or multiorgan failure (Lubrano and Balzan, 2016).

Shear Stress

EC responses to shear stress are markedly different in health and disease. A notable example is the switch from NO as a primary effector of flow-mediated dilation, to a secondary mediator like H_2O_2 (Gutterman et al., 2016). In the context of vascular disease with an increasingly oxidative milieu, NO is scavenged through reactions with superoxide (O_2^-) radicals and produced at a lower

rate as eNOS is uncoupled through various mechanisms (Chen et al., 2010; Antoniades et al., 2009; Landmesser et al., 2003). Loss of NO availability can result in: vascular smooth muscle cell (VSMC) proliferation (Jeremy et al., 1999), impaired postischemic angiogenesis (Murohara et al., 1998), impaired neovascularization (Aicher et al., 2003) and increased EC expression of leukocyte adhesion molecules (Liao, 2013). For these reasons, restoration of eNOS functionality and NO bioavailability are major focuses in treating EC dysfunction.

Microparticles/Exosomes

Recently, microparticles/exosomes generated by ECs have been investigated for their role in paracrine signaling during the progression of disease, particularly EC-originated microparticles (Zernecke et al., 2009; Wang et al., 2016; Deng et al., 2015; Xue et al., 2015; Diamant et al., 2004). Microparticles and exosomes are vesicular products released from the plasma membranes and endosomal membranes, respectively, of cells in response to a variety of stimuli. They contain a wide spectrum, depending on the originating cell type, of regulatory, signaling, and transcriptional molecules (Mause and Weber, 2010; Raposo and Stoorvogel, 2013). Microparticle-microRNA (miR) delivery has restorative effects in the context of EC inflammation (Ma et al., 2016), though microparticles released from dysfunctional ECs are sufficient to induce pathology in vitro and in vivo when introduced into an otherwise healthy circulatory system (Jansen et al., 2013). Low, disturbed shear stress and other CVD risk factors appear to be physiological regulators of EC microparticle release (Vion et al., 2013). These findings support a hypothesis that localized microvascular EC dysfunction has the capacity to induce dysfunction in otherwise healthy ECs through increased microparticle secretion into the vasculature. For these reasons there is an emerging consensus that early diagnosis of MCD through biomarkers among the lipids, proteins, and microRNAs within EC-released microparticles will improve patient outcomes and lead to identification of unique therapeutic targets in MCD (Gutterman et al., 2016; Gulati et al., 2012). This approach of identifying circulating EC microparticle biomarkers may provide a much needed standard diagnostic criterion for various forms of MCD (Marinescu et al., 2015). Novel therapeutic approaches for EC dysfunction may include specific miR inhibition (Sun et al., 2012; Loyer et al., 2014; Menghini et al., 2014), or pharmacological suppression of microparticle secretion once there is a more complete understanding of their mechanism of release.

Oxidative Stress

The interplay between ROS, reactive nitrogen species (RNS), and the physiological mechanisms of the microvascular network is complicated. Excess levels of ROS and RNS are involved in a number of disease states, including tumor growth (Pries et al., 2010; Secomb et al., 2012), ischemia/reperfusion injury (Lubrano and Balzan, 2014; Montezano and Touyz, 2012), diabetes mellitus (Lubrano and Balzan, 2014, 2016; Montezano and Touyz, 2012; Gutterman et al., 2016), hypertension (Lubrano and Balzan, 2014, 2016; Montezano and Touyz, 2012; Gutterman et al., 2016; Simplicio et al., 2016), atherosclerosis (Beckman and Koppenol, 1996; Lubrano and Balzan, 2014; Montezano and Touyz, 2012), chronic kidney disease, systolic cardiac failure, pulmonary hypertension, dementia, inflammatory disease, and radiation-induced endothelial dysfunction (Montezano and Touyz, 2012). ROS/RNS levels also play an important role in the loss of function due to aging seen in coronary arterioles (Kang et al., 2011; LeBlanc et al., 2008, 2009; Nevitt et al., 2016) and collateral microvessels (Faber et al., 2011). On the other hand, ROS and RNS have also been described as beneficial to the microvasculature in a number of ways. For example, they play a role in inflammation and induction of host defense genes (Montezano and Touyz, 2012; Beckman and Koppenol, 1996), activation of transcription factors, stimulation of ion transport systems, and controlling endothelial function and vascular tone (Montezano and Touyz, 2012). They are also integral to the flow-mediated dilation of the microvasculature (Gutterman et al., 2016; Beyer and Gutterman, 2012; Kang et al., 2011; LeBlanc et al., 2008, 2009). Low levels of ROS are required for the growth of new microvessels (Lubrano and Balzan, 2014), and also help to modulate blood flow, thrombosis, and neural activity in healthy individuals (Beckman and Koppenol, 1996). Thus, it becomes apparent that endogenous levels of ROS and RNS within the microvascular system involves an intricate balance; too much or too little of one or more may lead the system into dysfunction and disease.

Superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are clearly important players and a number of enzymes are involved in the production and elimination of both of these compounds. The primary source of ROS in the vasculature is nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which reduces O_2 to O_2^- using NADPH as an electron donor (Simplicio et al., 2016). The ROS generated from the activity of NADPH oxidase can induce injury and/or disease via mitogen-activated protein kinase pathways, resulting in inflammation, hypertension, and atherosclerosis (Simplicio et al., 2016). In addition, as previously mentioned, oxidized LDL binding to its receptor LOX-1 has been shown to activate NADPH oxidase to produce high levels of both ROS and RNS, including O_2^-, H_2O_2, hydroxyl radical (OH^-), peroxynitrite ($ONOO^-$), and NO in patients with hypercholesterolemia (Lubrano and Balzan, 2016). This system creates a positive feedback loop in which the elevated levels of ROS and RNS stimulate the upregulation of LOX-1 receptors, leading to further binding of oxidized LDL and production of still higher levels of ROS and RNS, ultimately resulting in decreased vasodilation and EC apoptosis (Lubrano and Balzan, 2014, 2016).

The NADPH oxidase pathway is also involved in hypertension; in these patients, Ang II is upregulated, leading to increased NADPH oxidase levels. This in turn produces higher levels of O_2^-, which binds with NO to produce the toxic oxidant $ONOO^-$, thereby causing endothelial dysfunction. This reaction also depletes the bioavailability of NO in the microvasculature, resulting in further vasoconstriction in hypertensive patients (Lubrano and Balzan, 2016). This role of NADPH oxidase-derived ROS is also critical in the development of alcohol-induced hypertension in adult rats, with the potential for similar ROS-driven symptoms in humans (Simplicio et al., 2016). Similarly, excess ROS—especially O_2^-—can be produced by ECs, macrophages, and other immune

cells following an ischemic event in small vessels such as arterioles and capillaries. As in hypertension, the higher levels of O_2^- in the ischemic system reacts with NO to produce $ONOO^-$ and can lead to reperfusion injury, and ROS scavengers may help prevent this reperfusion insult (Lubrano and Balzan, 2014; Escobales et al., 2014).

Notably, the enzyme superoxide dismutase (SOD) acts to deter $ONOO^-$ formation and the subsequent reduction in NO from the microvascular system by rapidly converting the potentially harmful O_2^- into H_2O_2. The role of H_2O_2 within the cardiovascular system is complex; excess levels have been linked with tumor development (Secomb et al., 2012; Pries et al., 2010), ischemia/reperfusion injury (Escobales et al., 2014), inflammation-induced thrombosis (Gutterman et al., 2016), and alcohol-induced hypertension (Simplicio et al., 2016). Given its involvement in these disease models, it is perhaps surprising that H_2O_2 is also an important regulator of vasodilation when NO bioavailability is reduced, as the endothelium can stimulate smooth muscle cells of the microvasculature to generate compensatory H_2O_2 when NO levels drop due to disease or aging (Gutterman et al., 2016). In a healthy cardiovascular environment, SOD can keep pace with spontaneous generation of O_2^- and convert it to H_2O_2; problems arise when SOD becomes inefficient and/or the levels of O_2^- become so high that it becomes toxic to the system. Increasing the levels of SOD in the diseased system—for example, via the antioxidant Resveratrol—helps to reduce the potentially damaging levels of O_2^- radicals (Spanier et al., 2009). There is also evidence that estrogen plays an important role in regulation of SOD; in aged female rats, there is decreased NO-mediated vasodilation of the coronary arterioles due to inadequate buffering of O_2^- radicals (Kang et al., 2011). Estrogen treatment helps to reverse this symptom via upregulation of SOD expression in the microvasculature (Kang et al., 2011) and an increase in phosphorylation of eNOS (LeBlanc et al., 2009).

The primary enzyme for maintaining adequate NO levels in the blood vessel wall, eNOS catalyzes L-arginine to produce NO. In a healthy vascular system, eNOS monomers spontaneously form dimers, allowing eNOS to bind tetrahydrobiopterin (BH_4) and L-arginine to produce NO. When the monomers become uncoupled, they produce O_2^- rather than NO; thus, eNOS uncoupling is another source of potentially harmful ROS within the microvascular system (Zhao et al., 2015). For example, uncoupled eNOS plays a role in cardiac dysfunction and hypertrophy (Gutterman et al., 2016), hypertension (Zhao et al., 2015; Montezano and Touyz, 2012), diabetes mellitus (Montezano and Touyz, 2012; Zhao et al., 2015), and hypercholesterolemia (Zhao et al., 2015; Lubrano and Balzan, 2016; Montezano and Touyz, 2012). NADPH oxidase is also important in the uncoupling of eNOS; as previously discussed, NADPH oxidase can generate increased levels of O_2^-, which binds with NO to form $ONOO^-$. In addition to the deleterious effects of $ONOO^-$ already discussed, it can also oxidize BH_4 to BH_3 radical, resulting in insufficient levels of BH_4 and thus leading to eNOS uncoupling (Zhao et al., 2015).

Finally, inhibition of eNOS via other chemical-dependent pathways can have similar negative effects on NO levels in the microvasculature. For example, in aged rats, increased levels of thrombospondin-1 (Thbs-1) has been shown to inhibit NO production via eNOS, and also decreases the downstream soluble guanylyl cyclase, all of which leads to an increase in O_2^- levels (Nevitt et al., 2016). Similarly, aged mice exhibit decreased phospho-eNOS in the walls of collateral microvessels, a possible player in the reduced collateral remodeling often seen in old age (Faber et al., 2011). The diminished eNOS activity in these older mice leads to increased EC apoptosis, further adding to the complications associated with aging microvasculature (Faber et al., 2011).

Taken together, the complex interplay between ROS, RNS, and their associated enzymes offer important insight into a number of cardiovascular disease models. Therapeutic strategies that reduce harmful oxidants and/or increase levels of antioxidants have shown promising results for ameliorating the deleterious effects of oxidative stress. Cell therapies offer another potential avenue for treatment of microvascular dysfunction. Adipose-derived stromal vascular fraction (SVF) cells injected into normal mice led to enhanced pressure-dependent relaxation in the saphenous artery (Morris et al., 2015). In these vessels, both H_2O_2 and O_2^- were significantly increased within the vessel walls, indicating enhanced production of both (Morris et al., 2015). Thus, as previously mentioned, it is important to consider that, despite extensive literature on the negative effects of ROS and RNS, their roles within the microvasculature are not fully understood. Effective treatment of microvascular dysfunction in the many disease models in which it is implicated will likely depend on a multifaceted strategy involving ROS/RNS attenuation and/or enhancement.

Microvascular Therapeutics

Given the causative role of the microvasculature in disease, it is worthwhile to develop therapies targeting specifically the microcirculation as a means to treat the disease (Hill et al., 2009; Flammer et al., 2012). Indeed, others have championed establishing clinical programs focused on EC medicine similar to the organ-specific programs traditionally in place (Aird, 2007, 2012). There are a number of vascular-targeting medical, surgical, and interventional therapies available to clinicians. However, most of these are focused on manipulating large vessels such as bypass and stenting. The high level of complexity and heterogeneity, in both the cell biology and physiology, intrinsic to the microvasculature has made current macrovascular therapies difficult to translate to the microvasculature. Microvascular-specific therapies currently being explored relate to regenerating a new microvasculature and/or normalizing microvascular function (e.g., flow control). Both approaches offer significant promise, but have yet to be fully realized clinically (**Table 2**).

Microvascular Regeneration

In microvascular regeneration, the objective is to rebuild or reconstitute a microcirculation. Originally explored in the context of ischemic disease in which the objective was/is to expand the microvasculature to improve compromised blood flow to an effective tissue, this is also known as "therapeutic angiogenesis" (Annex, 2013; Silvestre et al., 2013). Based on successful animal studies trials

Table 2 Therapies for microvascular dysfunction[a]

Vasoactivity/tone: compromised flow/reserve	
Antioxidants	Exercise
ACE inhibitors	Weight loss
Angiotensin blockers	Smoking cessation
β-Blockers	
Calcium-channel blockers	*Cell therapy*
Nitric oxide donors	*Exosome/microparticle therapy*
Phosphodiesterase inhibitors	*MicroRNA inhibition*
Sodium current inhibition (ranolazine)	
Statins	
Vessel stability/density: rarefaction, hypervascularity	
Exercise	*Angiogenic factor therapy*
Smoking cessation	*ACE inhibitors*
Weight loss	*Cell therapy*
	MicroRNA inhibition
	Nitric oxide donors
Hemostasis: thrombosis, inflammation	
Antioxidants	Exercise
Statins	Weight loss
Hormone therapy	Smoking cessation
Cell therapy	
Exosome/microparticle therapy	
MicroRNA inhibition	
Nitric oxide donors	
Remodeling/topology: perfusion deficit	
Exercise	*Cell therapy*
Smoking cessation	*MicroRNA therapy*
Weight loss	*Nitric oxide donors*
	Angiogenic factor therapy

[a]Italics indicates those therapies being developed or may have unproven effects.

(Giordano et al., 1996; Baffour et al., 1992; Takeshita et al., 1994; Tsurumi et al., 1996), a number of clinical trials involving the delivery of angiogenic factors intended to grow new vascular elements were performed with limited overall success (Henry et al., 2003, 2007; Losordo et al., 2002; Fortuin et al., 2003; Hedman et al., 2003; Annex, 2013). The poorer than expected outcomes likely reflected an inability to reestablish competent perfusion to the affected tissues, although technical issues related to angiogenic factor delivery and patient selection were likely also relevant. While it is important to promote the generation of new microvessel segments (i.e., via angiogenesis) in these ischemic tissue beds, it is equally if not more important that the regeneration strategy promote the progression of these newly formed neovessels into a mature, stable microvascular network capable of supporting effective tissue perfusion (LeBlanc et al., 2012a). Because the therapies were designed to promote vascular growth and not necessarily postangiogenesis maturation and stabilization of the neovascular network that formed, perfusion posttreatment likely remained compromised. Due to the difficulties related to measuring deep-tissue perfusion in the microvasculature, measurements necessary to confirm this were not possible in these studies, yet there is experimental evidence that functional compromise occurs in newly formed or remodeled microvasculatures. For example, hyperemic flow-reserve in the profunda femoris artery of the postischemic revascularizing mouse hindlimb is reduced (Cardinal et al., 2011). Similarly, in an animal model of myocardial ischemia, coronary endothelium-dependent vasoactivity was compromised in the remodeled small arteries in the affected myocardium (Sellke et al., 1994; Sellke and Quillen, 1992).

Postangiogenesis

Overall, native formation of a microcirculation involves the generation of numerous immature neovessels which interconnect to form a simple, nonhierarchical, and homogeneous network (all via angiogenesis) which then, upon the introduction of blood flow into the network, revises and remodels (via post-angiogenesis) to derive a new, stable microcirculation that is lower in microvessel density (via pruning) than initially present and comprised of hierarchically arranged, heterogeneous microvessels (both in diameter and phenotype) forming effective perfusion paths (Nunes et al., 2010) (**Fig. 2**). This implies that the considerable plasticity and adaptation (and the requisite instability) that is occurring postangiogenesis are necessary aspects of normal postangiogenesis maturation. Furthermore, conditions that do not permit or promote these dynamic changes will result in poorly formed and ineffective microcirculations. Strategies focused on regenerating the microcirculation must permit this dynamic stability and adaptation to occur in order to form a functional microvasculature.

A number of vascular processes impact the evolution of a functional microvascular network. These include maturation, pruning, arterio-venous specification, phenotypic specification, and structural remodeling. During the generation of a new microvascular network in the adult (i.e., neovascularization), new vessel elements are usually formed via sprouting angiogenesis. These newly

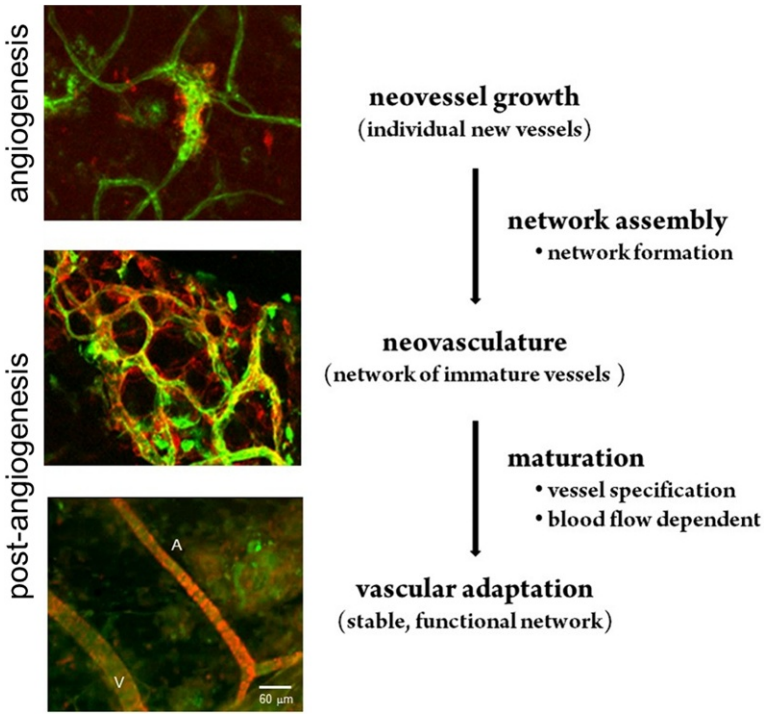

Fig. 2 Progression of a regenerating microvasculature through the different vascular phenotypic phases. Following angiogenesis, the immature and disorganized neovasculature undergoes extensive remodeling and revision in order to eventually form a new, mature microcirculation. Progression through the two postangiogenesis phases are blood flow dependent. *A*, arteriole; *V*, venule.

formed neovessels then integrate into an existing network or assemble into a new network of their own as they simultaneously reacquire an arteriole/capillary/venule identity, revise in caliber, and mature in function, all in the context of an interconnected network. Consequently, we have come to collectively call these processes postangiogenesis activities, because they occur after the initial sprouting angiogenesis event. Thus, while angiogenesis generates new vessel segments, postangiogenesis generates a new, mature microcirculation.

Neovascular plasticity and adaptation

Neovascular adaptation is inherent to the formation of a perfusion-competent microvascular network as the newly formed, immature neovasculature revises and matures into a microcirculation that reflects the specific needs of the tissue (LeBlanc et al., 2012a). Microvascular adaptation reflects considerable plasticity in the individual microvessels but also in the spectrum of topological arrangements of the network. Necessarily, microvessel phenotype and network organization reciprocally interact to determine the final microcirculatory outcome following any regeneration event. Superimposed on this necessarily intrinsic vascular dynamic are external cues such as hemodynamic forces and stromal influences (e.g., tissue biomechanics, stromal cells, etc.). Ultimately, these external cues act to influence the adaptation activities, providing the context for the final microcirculation form and function (Secomb et al., 2012). Important to a successful neovascularization outcome is the generation of microvascular network topologies that (1) have short, homogenous diffusion distances, (2) are comprised of low-resistance perfusion paths, and (3) are free of oxygen gradients across the relevant tissue bed (Pries and Secomb, 2014) (**Fig. 3**). In the absence of satisfying all three criteria, microvascular networks will not fully support a tissue. How these normalized topologies form as an integrated dynamic of multiple vascular activities, such as collateralization, structural adaptation, vasoactive changes, and shear stress and pressure responses, is not fully clear.

Based on work involving an implantation model of neovascularization, progression through the relevant postangiogenesis vascular activities appears to require a necessarily unstable, yet distinct, phenotypic phase (Nunes et al., 2010). Following the generation of neovessel segments and their inosculation with each other to form an immature network during angiogenesis, the neovascular network progresses into a morphologically, transcriptionally, and functionally distinct dynamic phase initiated by intravascular perfusion into the neovessels and characterized by significant cell turnover characteristics of vessel pruning, the reacquisition of perivascular cells to the EC-derived neovessels, and the specification of arterio-venous identity (Nunes et al., 2010, 2011). Importantly, it is during this phase that flow paths, which are initially chaotic and nonproductive, are remodeled and revised into a more effective perfusion circuit (Nunes et al., 2010). As might be expected, proper progression through this phase depends on blood flow; without it, the immature network collapses and regresses, leading to maturation into a more refined, hierarchical, low-resistance network.

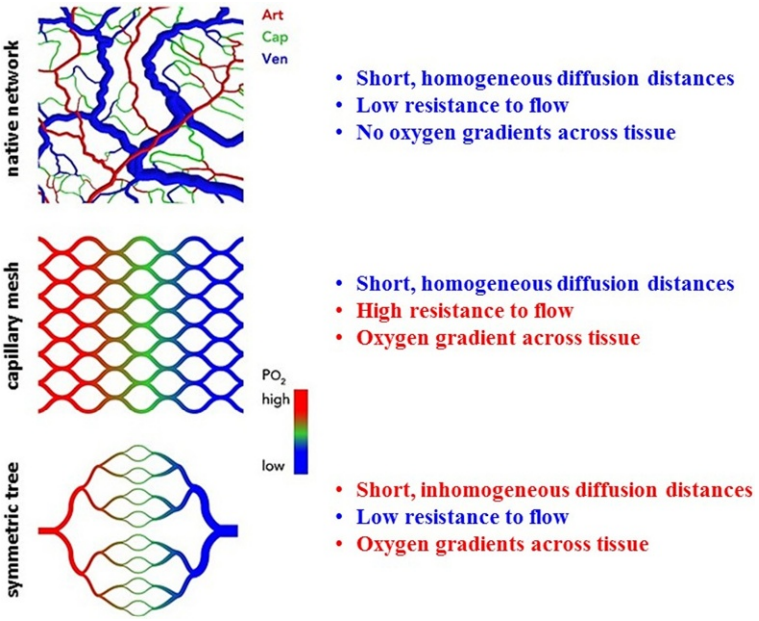

Fig. 3 Comparison of different microvascular network topologies, native and contrived, and the consequences of each to tissue perfusion. In each of the contrived example networks, blood enters the network from the left side. *Art*, arterioles; *Cap*, capillaries; *Ven*, venules. Modified from Pries, A. R. and Secomb, T. W. (2014). Making microvascular networks work: angiogenesis, remodeling, and pruning. *Physiology (Bethesda) 29*, 446–455, with permission.

Restoring Vasoactivity

Typical approaches to treating perfusion deficits involve restoring vasodilation capabilities of the microvasculature, thus targeting the underlying vascular dysfunction which led to the deficits. Given that NO is pivotal in vasoactivity of healthy vessels, organic nitrates are often used as vasodilators. Originally thought to serve as NO donors, these agents are now thought to produce a spectrum of vascular-acting ROS via intracellular enzymes (Munzel et al., 2014). Other therapeutic agents include calcium-channel blockers or, more recently, ranolazine (a late inward sodium current inhibitor), which disrupt the electrical-contraction coupling of the vascular smooth muscle (Ennis et al., 2014; Lamendola et al., 2013). Additional drug classes used include those affecting the angiotensin system either reducing the production of Ang II or blocking its interactions with cellular receptors (Cameron et al., 2016). Finally, phosphodiesterase inhibitors, which block the degradation of cyclic nucleotides such as cGMP, are becoming more commonly used to treat vascular dysfunction. In vascular smooth muscle, cGMP acts to reduce contractility via cGMP-dependent kinases, reducing intracellular Ca^{2+} levels and promoting K^{+} efflux (Sausbier et al., 2000; Bobin et al., 2016). Phosphodiesterase inhibitors reduce the breakdown of cGMP by these enzymes, thereby prolonging turnover of the cGMP and maintaining vasorelaxation (Bobin et al., 2016). Almost invariably, other agents are included in combination therapies to control comorbid conditions, although many of these agents can also influence vascular cell function. For example, statins are concomitantly prescribed to manage lipid dynamics that lead to EC dysfunction (e.g., dyslipidemia). However, in addition to the effects on lipid metabolism, statins also appear to regulate EC function directly, impact inflammation, and alleviate oxidative stress (Agouridis et al., 2016). Similarly, β-blockers for hypertension, antiplatelet therapy to manage thrombosis, and a spectrum of agents for managing diabetes are often prescribed in conditions involving suspected microvascular dysfunction (Thompson and Davis, 2016; Cameron et al., 2016; Mousa, 2010).

Exercise

It is well known that physical activity improves cardiovascular health and outcomes. For example, daily exercise alleviates the pain associated with limb ischemia and improves limb function in peripheral artery disease (Hiatt et al., 1990). Based on experimental studies in animals, the clinical improvement is thought to be due, in part, to improved vascularity and microvascular flow secondary to muscle activity and the increased metabolic demand (Prior et al., 2004; Yang et al., 2008). Concomitant with the increase in distal vessel density is elevated collateral flow secondary to arteriogenesis (Bondke Persson et al., 2011). Muscle activity, such as that associated with exercise, also promotes adaptive changes in vascular cells leading to improved vasoactivity dynamics (Dora, 2016).

Cell-Based Approaches

An emerging therapy for improving vascular function, particularly in the microvasculature, involves the broadly defined class of mesenchymal cell therapies. Generally explored as potential regenerative therapies for improving overall tissue health and repair,

these cells appear to have indirect and direct provascular therapeutic effects. While bone marrow-derived mesenchymal stem cells (BM-MSCs) were among the first cell types explored, a variety of MSC sources are now actively investigated with adipose-derived stromal cells (ASCs) representing another widely used therapeutic cell population. Bone marrow and circulating derivatives of bone marrow consist of a variety of cell types including mesenchymal stem cells, hematopoietic stem cells, endothelial progenitors and others normally involved in hemostasis, homeostasis, inflammation, and tissue repair (Yan et al., 2013). When collected either as a whole or as discreet subpopulations, and almost always involving cell culturing, the BM-MSCs are thought capable of forming new vascular elements and improving tissue perfusion. Some evidence indicates that these cells can differentiate into vascular cells and/or incorporate into existing microvascular structures, possibly normalizing microvessel function (Jackson et al., 2001; Kamihata et al., 2001; Davani et al., 2003; Gojo et al., 2003; Hatzistergos et al., 2010). However, additional evidence indicates that increased vascularity and perfusion in the absence of vascular incorporation by these cells in ischemic muscle suggests that other provascular mechanisms exist (Kinnaird et al., 2004). These findings, as well as evidence that MSCs actively produce a variety of cytokines and growth factors, suggest that MSCs act primarily via a paracrine mechanism, including the production of angiogenic and vascular factors (Kamihata et al., 2001; Tang et al., 2004; Kinnaird et al., 2004; Gnecchi et al., 2006; Tse et al., 2007). As mentioned, ASCs promote similar therapeutic outcomes as BM-MSCs, also via a putative paracrine mechanism (Rigol et al., 2014; Wang et al., 2009; Cai et al., 2007). In the context of cardiac ischemia, ASCs cultured on a degradable patch and delivered to the epicardial surface of the postinfarcted heart arrested lesion progression which was correlated with improved coronary perfusion (LeBlanc et al., 2012b, 2013). While incorporation of the delivered cells into the coronary vasculature occurred, it was not at a level that would explain the increased vascularity and functional improvements (LeBlanc et al., 2012b; Kim et al., 2013). Alternatively, freshly harvested adipose tissue cells representing the SVF of the adipose tissue can be used as is without the subsequent culturing used to derive ASCs. In this form, the adipose SVF retains many of the vascular cell types comprising the adipose microvasculature (Zimmerlin et al., 2010; James et al., 2012). Consequently, this therapeutic preparation has profound vasculogenic potential and can readily form new microvasculatures *de novo* in an EC-dependent manner (Nunes et al., 2013; Koh et al., 2011). The appeal of using cell therapies to promote vascular health is high given that cells are dynamically involved in tissue form and function, potentially using a variety of mechanisms to promote repair and health. Cell therapies are currently being explored for a spectrum of disease conditions in a large number of clinical trials, testing this promise. Interestingly, MSCs that reside within tissues are thought to be intimately associated with the microvasculature. Called the "perivascular niche," many of the cell types collected from tissues, such as adipose, to derive the therapeutic MSC population appear to originate at sites associated with the microvasculature (Caplan, 2008). Presumably, these cells are playing an essential role in endogenous microvascular health analogous to their therapeutic activities.

Example: Microvascular Angina

While there are many disease conditions in which microvascular dysfunction is a cause or complication, microvascular angina, perhaps, represents an ideal example. Traditionally, ischemic heart disease has been viewed as a coronary macrovascular disease in which the major coronary arteries are compromised, ultimately leading to occlusion and arrest of blood flow to the downstream myocardium. In patients presenting with angina symptoms (e.g., chest pains, abnormal stress test, shortness of breath), examination of the coronary arteries often reveals significant reduction in lumen cross-sectional area such that flow is restricted sufficiently to compromise oxygen supply to the heart. In the past, a "clear" angiogram (usually viewed as less than 50% occlusion in one or more coronary arteries) indicated an absence of CAD and that angina symptoms reflected a nonvascular etiology and/or a nonlife-threatening situation. However, there is a growing appreciation for different manifestations of ischemic heart disease that does not necessarily center on coronary artery involvement and instead focuses on CMD as the primary disease etiology.

Building on insights of others, much of our new understanding of coronary microvascular disease has arisen from the Women's Ischemia Syndrome Evaluation (WISE) study established in 1996 (Bairey Merz et al., 2006), which has been systematically characterizing and evaluating patients with symptoms of angina in the absence of occlusive CAD. Importantly, women are inordinately more affected (5+ times more) by this manifestation of ischemic heart disease than men. Traditionally thought to be benign, WISE investigators have found that the majority of the affected women will continue to have symptoms, a declining quality of life, repeated hospitalizations and tests, and an increased risk for a sudden cardiac event (Gulati et al., 2012).

While there remain potentially many etiologies for angina without coronary artery involvement, a growing consensus is that this type of ischemic heart disease is a manifestation of CMD. As such, it is often termed "microvascular angina." There are a number of recent reviews describing microvascular angina, its manifestation, the challenges of diagnosing and treating microvascular angina, and the underlying disease mechanisms (Dean et al., 2015; Gori and Fineschi, 2012; Kothawade and Bairey Merz, 2011; Lim et al., 2009; Marinescu et al., 2015; Scalone and Niccoli, 2015). Importantly, the prevalence of microvascular angina is inordinately high in women; over half of the women with ischemic heart disease will present with microvascular angina as compared to about 6% in men (Kothawade and Bairey Merz, 2011).

Coronary Microvascular Dysfunction

Typically, the coronary microvasculature is considered to be that portion of the coronary circulation comprised of vessels smaller than 200 μm in diameter with a focus on the resistance vessels regulating downstream blood flow. This includes not only the more distal arterioles, but also the distal, smaller feed arteries to a microvascular bed. In CMD, this compartment of the coronary microcirculation is unable to properly maintain blood flow to meet the needs of the working myocardium. Clinically, this manifests

as an inappropriate coronary blood flow response leading to deficient myocardial perfusion (Beltrame et al., 2009). While this dysfunction is often attributed to extension of atherosclerosis (i.e., plaque accumulation) into these smaller vessels (Panza, 2010), it is likely that the CMD reflects a broader spectrum of compromised function arising from many causes leading to EC dysfunction, ROS imbalances within the vessel wall, smooth muscle dysfunction, and undoubtedly decompensating microvascular remodeling (Ahmed, 2014; Widmer et al., 2014; Kothawade and Bairey Merz, 2011; Durand and Gutterman, 2013; Phillips et al., 2007). Highlighting this, others have described both EC dependent and independent vasoactive dysfunction in women with nonobstructive ischemic heart disease (Pepine et al., 2010; Diver et al., 1994).

Diagnosing Microvascular Angina

Accurately diagnosing microvascular dysfunction can be difficult (Bairey Merz and Pepine, 2011). A definitive test, although invasive, is coronary reactivity testing in which microvascular coronary function is assessed by use of a Doppler guide wire in the cardiac catheterization laboratory and pharmacological agents to determine endothelium-dependent and -independent function (Phan et al., 2009; Mehta et al., 2011). Typically, flow responses to intracoronary delivery of adenosine (EC-independent) and acetylcholine (EC-dependent) are used as measures of CFR, as these agents normally induce a flow hyperemia in the distal coronary circulation. Recently, an NHLBI-funded study demonstrated that cardiac MRI-based measurements of myocardial perfusion reserve index was as effective at assessing microvascular dysfunction as coronary reactivity testing (Thomson et al., 2015). Importantly, these assessments are designed to measure the vascular dynamics of the coronary circulation, the very aspect thought compromised in microvascular angina (Thomson et al., 2015). As with most disease, an accurate and informative diagnosis is critical to the formulation of a treatment strategy.

Treatment of Microvascular Angina

Current treatment guidelines for microvascular angina are not focused on targeting the MCD, perhaps reflecting a paucity of microvascular targeting drugs. Most medical therapies reflect atherosclerosis and coronary artery occlusive disease as the primary etiology (Fraker et al., 2007). An emphasis is on managing the atherosclerosis and relieving angina symptoms. Thus, patients are often prescribed statins and life-style changes to manage plasma lipid/cholesterol levels and a spectrum of agents intended to lessen cardiac work load and thus myocardial oxygen demand (Bairey Merz and Pepine, 2011). Additionally, standard of care for microvascular angina involves one or more of nitrates (to promote vasodilation), β-blockers (to reduce cardiomyocyte work), and calcium channel blockers (also to reduce cardiomyocyte work). Recent clinical trials indicate that typical "1st-line" drugs used for obstructive angina can be effective at relieving angina symptoms only in subsets of microvascular angina patients (Bairey Merz and Pepine, 2011). These include the use of β-blockers, ACE inhibitors, nitrates, and calcium channel antagonists. Sildenafil, a phosphodiesterase inhibitor, appears to improve CFR, a measure of microvascular coronary function, in women with microvascular angina (Marinescu et al., 2015; Denardo et al., 2011). Despite nitrates being effective in CAD, they are less effective in microvascular angina, reflecting the microvascular aspect of microvascular angina in that nitrates selectively target larger caliber coronary vessels (Russo et al., 2013). Interestingly, particularly given the close association of microvascular angina with women, low-dose hormone therapy was not effective (Bairey Merz et al., 2006). This reflects, perhaps, that women of premenopausal age who are still producing estrogen also manifest microvascular angina (Gulati et al., 2012). Recently, new generation antiangina drugs are being used. The best example is ranolazine, which is promising in the treatment of microvascular angina, particularly when the more standard, 1st-line agents are ineffective (Mehta et al., 2011; Codolosa et al., 2014; Tagliamonte et al., 2015). Ranolazine is a relatively new antiangina agent that is thought to reduce calcium loading in cardiomyocytes secondary to the inhibition of the late sodium current (Chaitman, 2006; Undrovinas et al., 2006). However, in animal model studies, ranolazine promoted precapillary vasodilation and increased microvascular perfusion in skeletal muscle in rats when administered acutely (Fu et al., 2013). Similarly, ranolazine promoted endothelium-independent vasodilation in rat and rabbit aorta rings (Paredes-Carbajal et al., 2013; Malavaki et al., 2015). This suggests that perhaps ranolazine influences microvascular function directly in addition to moderately improving cardiomyocyte function. While all of these agents can relieve symptoms of angina, none are disease modifying. Furthermore, patient responsiveness to these agents is variable, with women being less responsive than men, and treatment often involves a progression through the spectrum of medical agents until positive results are obtained (i.e., angina symptoms are lessened).

Comorbidities

As with most cardiovascular disease, microvascular angina is associated with a variety of comorbidities. It is well known that diabetes, metabolic syndrome, hypertension, and smoking have profound negative impacts on the cardiovascular system and the microvasculature in particular (Briones et al., 2014; Legein et al., 2013; Durand and Gutterman, 2013; Schleithoff et al., 2012; Clough and Cracowski, 2012; LeBlanc et al., 2012a; Ruiter et al., 2010). In a recent prospective study of women being evaluated for chest pains in the Southeastern United States, ~97% were determined to have microvascular angina. Of the subjects who consented, all were relatively young (mean age 43.8 ± 12.5) and obese (mean body mass index of 30.8 ± 7). The prevalence of diabetes was 10.3%, hypertension was 37.1%, hyperlipidemia was 17.8%, and current tobacco use was 23.5% (Winchester et al., 2015). Interestingly, obesity and hypertension, both of which lead to systemic microvascular complications, were the most prevalent comorbidities in these women with microvascular angina.

Mechanisms of Disease

These comorbidities complicate an already complex process of disease mechanisms leading to ischemic heart disease and CMD. Beyond a generalized understanding that vascular (both endothelial and smooth muscle cell) dysfunction is at the core of microvascular deficits, very little is known about the CMD specific to microvascular angina. In CAD, the coronary microvasculature is altered such that the balance of vasodilators mediating vasoactivity in the coronary microcirculation is, in part, H_2O_2 mediated rather than NO mediated (Freed et al., 2014; Durand and Gutterman, 2013; Phillips et al., 2007). Similar switching of vasomediators is associated with the diminished vasoactivity of coronary microvessels in aging (Miura et al., n.d.; Durand and Gutterman, 2013). In the no-reflow phenomenon, wherein microvascular dysfunction complicates reperfusion of the myocardium following revascularization of an obstructed coronary artery, problems related to microemboli and EC dysfunction secondary to reperfusion injury drive distal vessel-mediated perfusion deficits (Durante and Camici, 2015; Feher et al., 2014; Ito, 2006). Whether all of these mechanisms are involved in microvascular angina is unknown. Furthermore, the impact of antiangina drugs routinely used to treat microvascular angina is unclear; whether these drugs alter microvascular function directly or simply moderate angina symptoms by reducing cardiac work is also unknown at this time.

Summary

Given the ubiquitous and intimate presence of the microvasculature in tissues, it is intuitive that it plays a central role in tissue health and disease. Not only it is essential in distributing blood to the tissues, but also the microvasculature mediates all forms of blood:tissue interface, contributes directly to tissue and organ function, and critically participates in tissue repair. Consequently, deficits in any one of these microvascular functions can lead to tissue dysfunction. Additionally, a limited functional microvasculature in a tissue will undoubtedly compromise healing and likely exacerbate disease. Despite considerable knowledge of microvascular biology and physiology, the microvasculature itself remains a relatively under-treated target in the clinic. Certainly, therapies that target generalized imbalances such as statins and antioxidants will benefit the microvasculature. However, there remain few options available for clinicians to improve specific deficits in microvascular function. This is, in part, due to the challenges related to assessing the microvasculature in the patients; these deficits in diagnostic methods have hampered our understanding of microvascular dysfunction and its contribution to the disease state. Traditional methods of assessment, such as histological vessel density measurements, tell only part of the story and do not assess the functional status of the microvasculature in question. This is particularly relevant given that vessel density is not directly correlated to perfusion capacity. Experimental means of measuring function, such as vasoactivity, are difficult to translate into clinical practice as they involve invasive approaches (e.g., diagnosis of microvascular angina). All of this is compounded by the likelihood of varied causes of microvascular dysfunction leading to perfusion deficits. Nevertheless, the renewed appreciation of the critical roles the microvasculature plays in tissue health and disease is increasing our understanding of the microvasculature to promote health and reverse disease.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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Mitochondrial Bioenergetics in the Heart

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Introduction

The heart is a critical organ. The circulation of blood delivers absolutely vital O_2. The heart beats 60 bpm, = 3600/h, = 86,400/day = 31.536 million/year = 2.207 billion/life span. The engine for beating is the mitochondria, which provide energy in the form of ATP.

Structure

Contractile Apparatus

Cardiac muscle fibers are syncytia of individual cells (cardiomyocytes) joined end-to-end at intercalated discs, which not only provide the structural stability of the fiber, but their content of gap junctions permits the free flow of the action potential down the length of the fiber (Fig. 1). Like skeletal muscle, cardiomyocytes are striated, consisting of A and I bands; this appearance is based on the distribution of actin and myosin filaments. Contraction is based on the sliding of the actin relative to the myosin filament. It is in this action that mitochondrial ATP comes into play (Hernandez et al., 2001).

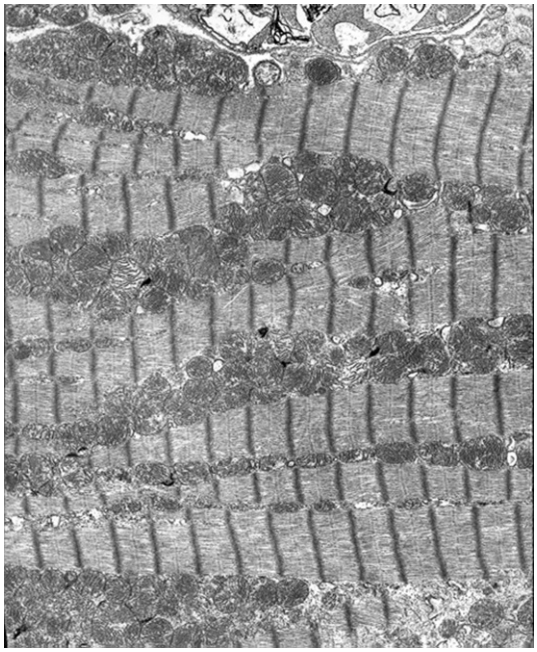

Fig. 1 A survey of a portion of a cardiomyocyte showing a series of apposed sarcomeres that are joined to each other by the densely-stained Z-discs. The parallel myofibrils are separated by rows of interfibrillar mitochondria.

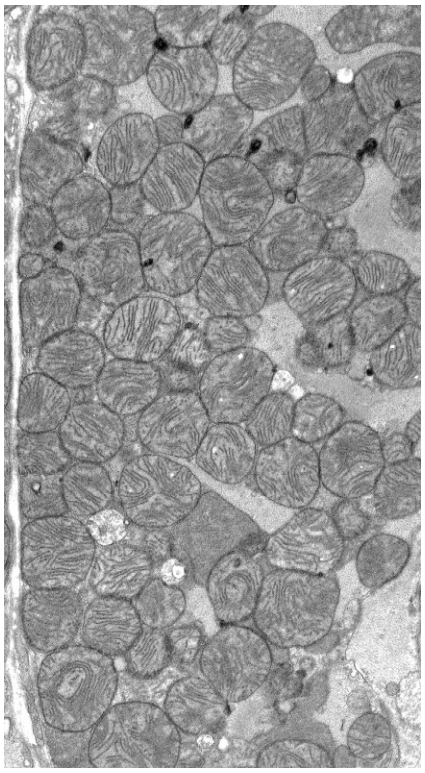

Fig. 2 Electron micrograph of clustered subsarcolemmal mitochondria.

Cardiac Mitochondria

As seen using transmission electron microscopy (TEM), cardiac mitochondria, in general, have a relatively simple silhouette that ranges from ovoid to elliptical, and numerous cristae. The latter structures appear as simple invaginations of the internal mitochondrial membrane and appear to lack any subspecialization (Fig. 2). However, when examined by high-resolution scanning

electron microscopy (HRSEM), differences in the structure of cristae become apparent when the subsarcolemmal mitochondria (SSM) are compared to the interfibrillar mitochondria (IFM) (Riva et al., 2005; Hoppel et al., 2009; **Fig. 3**). Because the SSM are somewhat larger than the IFM, the cristae tend to be longer and are attached to the boundary membrane by means of cristae junctions. Moreover, HRSEM revealed that the overall shape of cristae varies in these two populations of mitochondria in that those in the SSM are largely lamelliform, whereas those in the IFM tend to have a tubular shape (Riva et al., 2005; Hoppel et al., 2009; **Fig. 4**). In the latter, cristae junctions could not be clearly identified. The forgoing description of mitochondrial cristae applies to in situ organelles; interestingly in isolated SSM, the cristae retain their lamelliform structure whereas in IFM the cristae appear to be more plastic and their shape varies. In cardiac mitochondria examined by TEM, the matrix often contains scattered dense granules

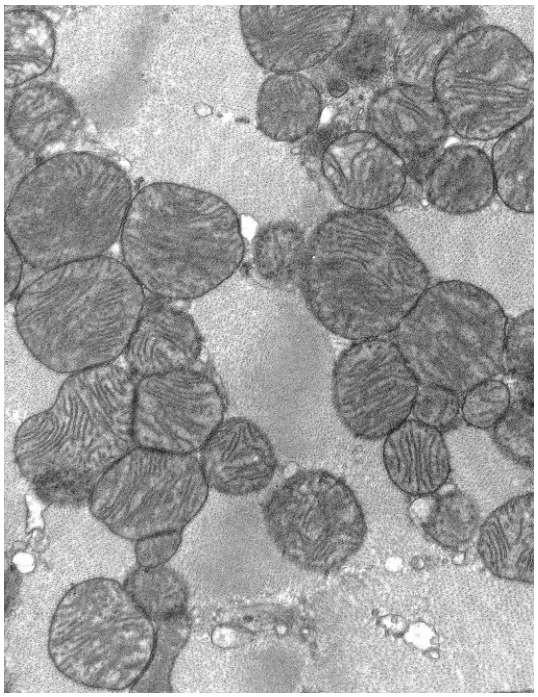

Fig. 3 Electron micrograph of a transversely sectioned cardiomyofiber showing aggregates of interfibrillar mitochondria.

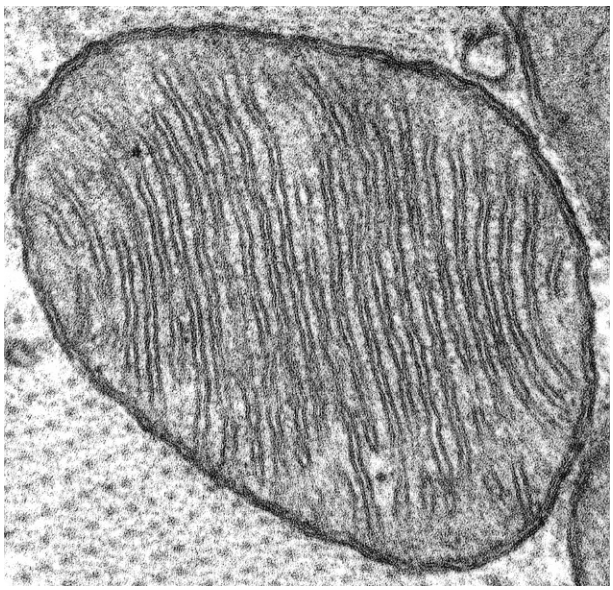

Fig. 4 High-resolution electron micrograph of cardiac mitochondria showing the unit membrane of its component membranes. The same structure prevails in both subsarcolemmal and interfibrillar mitochondria.

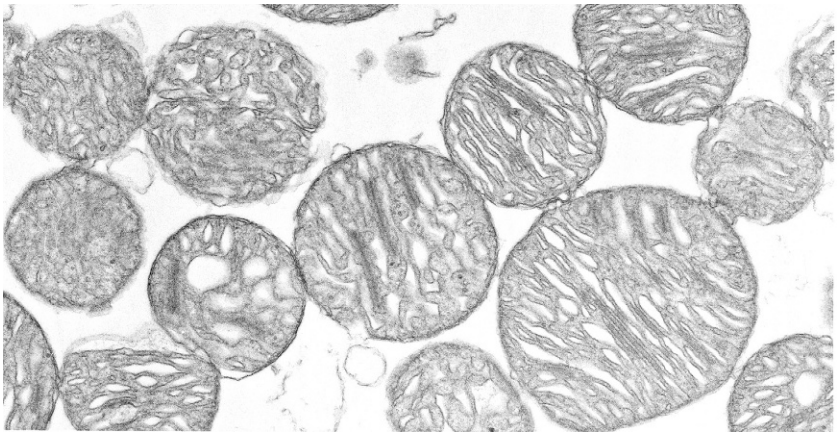

Fig. 5 Electron micrograph of a pellet of isolated cardiac mitochondria. No differences are observable between isolated subsarcolemmal or interfibrillar mitochondria. These organelles retain the same basic structure they exhibit in situ.

that are deposits of divalent cations (Peachey, 1964). The presence of these matrix granules appears to be associated with the modality of specimen preparation for TEM; under some conditions of fixation, the granules are starkly apparent, while with other fixatives the granules are either few in number or lacking altogether.

Earlier studies of cardiac mitochondria function were based on isolation of the total mitochondrial content of cardiomyocytes by exposure to the protease, Nagarse, which solubilized the contractile apparatus and released all mitochondrial irrespective of their intracellular localization (Jacobus et al., 1975). The fortunate circumstance that mitochondria are resistant to the rigors of isolation allows their ready identification (**Fig. 5**). This technique allowed identification of the multifarious metabolic roles played by the mitochondria. These findings became the standard for cardiac mitochondrial metabolic function. With the passage of time, the Polytron, which mechanically disrupts the sarcolemma, became the popular tool for isolation of cardiac mitochondria. An aspect of the use of this methodology that was largely ignored was that the yield of mitochondria was reduced 60%–70%. The reason for this discrepancy was revealed when the mitochondrial fraction and the "debris" fraction were examined ultrastructurally. It turned out that although the Polytron released the SSM, a large portion of the mitochondria remained in their original position between myofibrils, thus unavailable for isolation. We found that after Polytron treatment, exposure of the debris fraction to Nagarse freed the entrapped IFM and made their separate isolation a relatively simple matter (Palmer et al., 1977). The result of this approach was that two distinct populations of mitochondria became available for biochemical investigation (Palmer et al., 1977).

Network

The mitochondria that are abundant in each cardiomyocyte have been established by TEM (Fawcett, 1966). These organelles are situated both beneath the sarcolemma and between the myofibrils. A school of thought holds that all of the mitochondria within a given myocyte are interconnected, in effect forming a continuous network (Skulachev, 2001). If this interpretation is correct, the entire mitochondrion operates as a single organelle and no part of the cell is deficient in ATP. Moreover, this organization would eliminate the necessity for exchange of intramitochondrial components such as substrates and intermediates from one organelle to the other via transfer through the cytosol.

Substrate Selection

In the baseline state, the heart is a metabolic omnivore, utilizing fatty acids, glucose, and lactate (**Fig. 6**). Glucose uptake requires insulin-mediated uptake via the membrane glucose transporter, GLUT4 (Abel, 2004). In the absence of insulin, glucose uptake by the heart is minimal. Fat substrates are provided via long-chain fatty acids with uptake via the CD36 membrane transporter, and by intracellular lipolysis followed by the formation of acyl-CoA esters by long-chain fatty acid CoA synthetase (van der Vusse et al., 2002). The acyl-CoAs are subsequently activated for mitochondrial uptake via the formation of acylcarnitines by carnitine palmitoyltransferase-I (CPT-I) (Lee et al., 2011), as discussed below. The enzymes involved in fatty acid activation and transport are located in the mitochondrial outer membrane and contact sites (Lee et al., 2011).

Glucose

Glucose metabolism via glycolysis generates pyruvate for mitochondrial uptake and subsequent intramitochondrial activation by the pyruvate dehydrogenase complex (**Fig. 7A**). Lactate is taken up by the monocarboxylate transporter and is converted to pyruvate by the enzyme, lactate dehydrogenase, which is located in the cytosol (Lesnefsky et al., 2001c; Dym et al., 2000). Mitochondrial

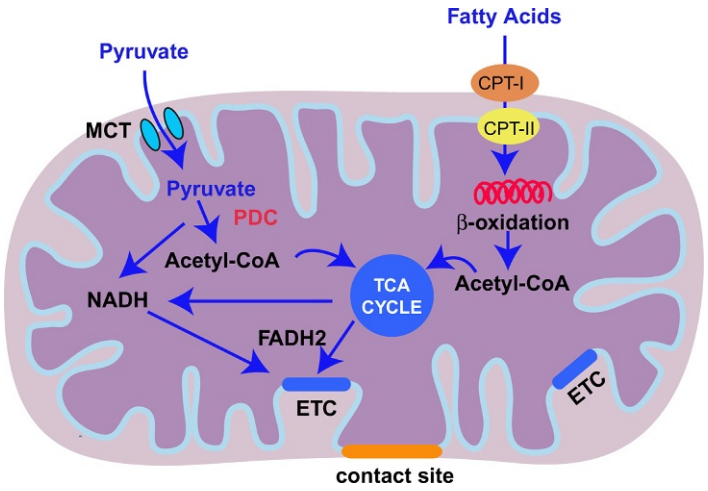

Fig. 6 A diagram showing in a superficial manner the elementary pathways of fatty acid and pyruvate oxidation leading to the intermediate metabolite, acetyl-CoA on its way to the tricarboxylic acid cycle (TCA).

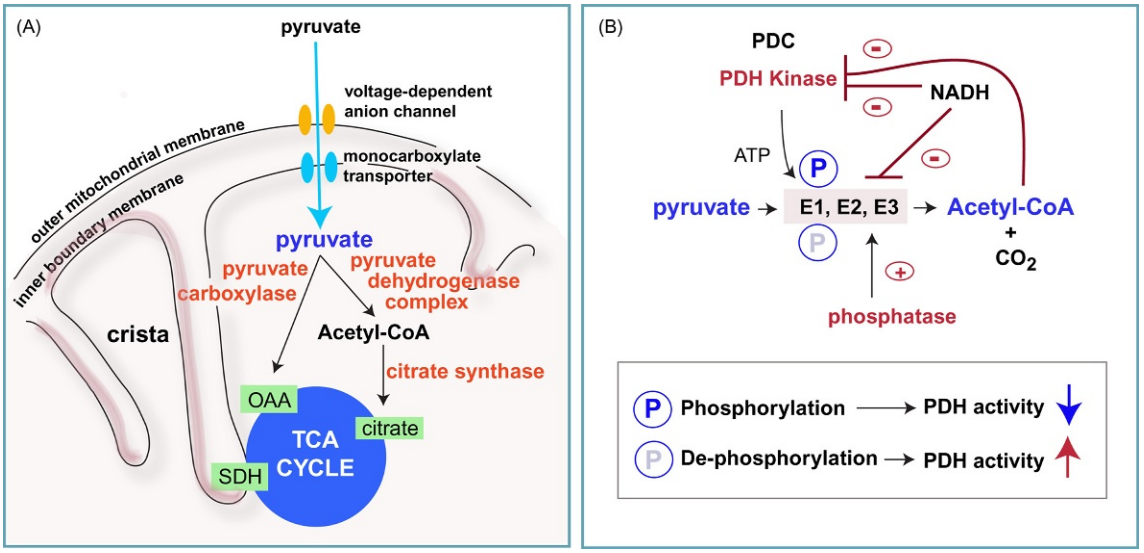

Fig. 7 (A) A diagram showing in detailed fashion the pathways of pyruvate through the outer membrane via the voltage-dependent anion channel and subsequent transport through the inner boundary membrane by the monocarboxylate transporter. In the matrix, pyruvate can be metabolized by pyruvate dehydrogenase complex to produce acetyl-CoA, which enters the TCA after conversion to citrate. Alternatively, pyruvate is carboxylated by pyruvate carboxylase to form oxaloacetate (OAA), which enters the TCA as the other substrate for citrate synthase. The TCA itself is anchored to the crista membrane by means of succinate dehydrogenase (SDH) of complex II. (B) A schematic of the pyruvate dehydrogenase complex by phosphorylation/dephosphorylation and inhibition by the products acetyl-CoA and NADH.

substrate selection is tightly controlled and is affected not only by exogenous substrate availability but also by metabolic regulation in response to differing physiologic conditions (Lesnefsky et al., 2001c). Pyruvate dehydrogenase complex and CPT-I coregulate carbohydrate and fatty acid oxidation in a reciprocal manner via crosstalk mediated by metabolic intermediates and via regulation by intracellular signaling systems (Lesnefsky et al., 2001c, 2016).

Pyruvate Dehydrogenase Complex

The pyruvate dehydrogenase complex catalyzes oxidative decarboxylation of pyruvate to acetyl-CoA, which enters the tricarboxylic acid (TCA) cycle (Folmes et al., 2010; Sugden and Holness, 2006; Arjunan et al., 2002). The pyruvate dehydrogenase complex is composed of three enzymes: pyruvate dehydrogenase (PDH, E1), dihydrolipoamide acetyltransferase (E2), and dihydrolipoamide dehydrogenase (E3) (Folmes et al., 2010; Sugden and Holness, 2006; **Fig. 7B**). In mammalian cells, PDH is found in the mitochondrial matrix and includes four subunits: α_1, α_2, β_1, and β_2. PDH activity is decreased by a reversible phosphorylation of the α (Sugden and Holness, 2006) or β (Folmes et al., 2010) subunits through pyruvate dehydrogenase kinase (PDK). In the heart,

PDH kinase isoform 4 predominates and is rapidly induced by starvation and diabetes, leading to decreased PDH activity (Sin et al., 2015). Overexpression of PDK4 in mice inhibits PDH activity and subsequent glucose oxidation (Chambers et al., 2011). Activation of PDH is achieved by dephosphorylation of the subunits by pyruvate dehydrogenase phosphate phosphatase (PDP) (Sugden and Holness, 2006). Fatty acids directly decrease PDH activity via activation of peroxisome proliferator-activated receptor-α, leading to increased PDH kinase expression (Gao et al., 2014). PDH kinase activity is directly inhibited by pyruvate, leading to increased PDH activity. High rates of fatty acid oxidation inhibit PDH via elevated mitochondrial content of acetyl-CoA and NADH, which directly inhibits PDH as well as activate PDH kinase (Latipaa et al., 1985). The activity of PDH phosphatase is increased by Ca^{2+} and Mg^{2+}, leading to increased PDH activity (Rutter et al., 1989). The Ca^{2+}-mediated activation is in line with activation of electron transport by calcium, the net calcium activation of PDH complex providing increased substrates for the downstream electron transport chain (ETC).

Fatty Acid Oxidation

Increased oxidation of pyruvate from carbohydrates inhibits fatty acid oxidation by increasing the content of malonyl-CoA, leading to inhibition of CPT-I (**Fig. 8A** and **B**). The molecular basis of this inhibition is the extreme sensitivity of the muscle isoform of CPT-I (the predominant isoform in the heart) to malonyl-CoA (Fillmore et al., 2014; Stanley et al., 2005). Although acetyl-CoA produced from the oxidation of pyruvate and that produced from fatty acids are biochemically identical, these molecules are utilized in different ways by the heart, possibly due to compartmentalization. One pool of acetyl-CoA derived from pyruvate oxidation would be amenable to export to the cytosol for malonyl-CoA synthesis and subsequent inhibition of CPT-1, which is located on the outer mitochondrial membrane. In contrast, acetyl-CoA derived from β-oxidation of fatty acids remains contained within mitochondria and likely does not have access to CPT-1 (Lesnefsky et al., 2001c). Consistent with the importance of malonyl-CoA as a regulator of fatty acid oxidation, enzymes for synthesis (acetyl-CoA carboxylase) and degradation (malonyl-CoA decarboxylase) exist (Fillmore et al., 2014; Ussher and Lopaschuk, 2009). These synthetic and degradation enzymes are under control of signaling cascades, providing key regulation of fatty acid oxidation in response to the physiologic state of the myocyte. In addition to malonyl-CoA content, other mechanisms can regulate fatty acid oxidation. These factors include cytoskeletal proteins, as well as palmitoyl-CoA, the latter a major substrate of fatty acid oxidation. Thus, increasing concentrations of palmitoyl-CoA attenuate CPT-I inhibition and increase the flux through fatty acid oxidation (Fillmore et al., 2014). An increased palmitoyl-CoA to malonyl-CoA ratio due to increased uptake and activation of fatty acids and decreased malonyl-CoA content also explains the high rates of fatty acid oxidation observed during cardiac reperfusion following ischemia (Stanley et al., 2005).

Mitochondria

Compartmentalization

The matrix contains the enzymes of the TCA cycle, antioxidant defense enzymes, and biosynthetic enzymes. TCA cycle oxidation of acetyl-CoA generates NADH and $FADH_2$ for oxidation by the ETC. The ETC consists of four multisubunit enzyme complexes, with

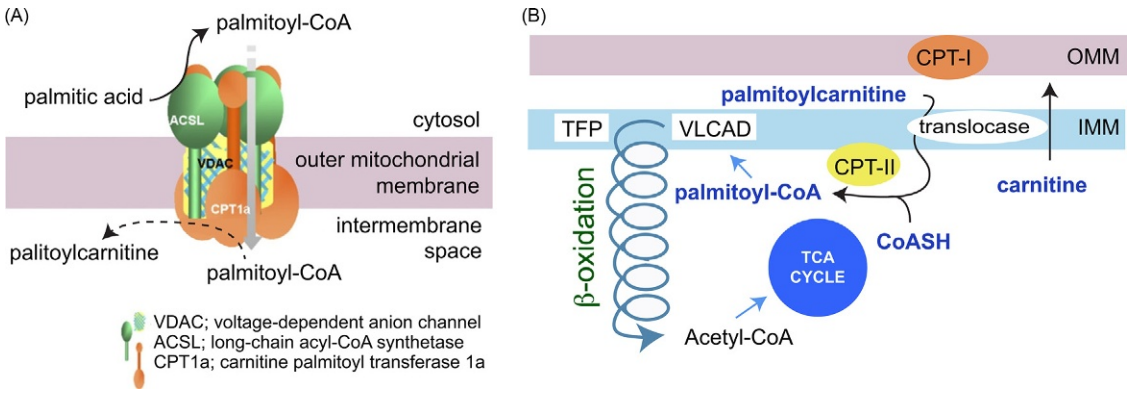

Fig. 8 (A) A model of the mitochondrial outer membrane complex for entry of fatty acyl groups into the intermembrane space. Long-chain fatty acid (using palmitate as an example) is first activated to palmitoyl-CoA by the long-chain acyl-CoA synthetase (ACSL), on the outer membrane. Palmitoyl-CoA passes through the outer membrane through the VDAC and is converted to palmitoylcarnitine by carnitine palmitoyltransferase 1 (CPT-I) on the inner surface of the outer membrane. (B) A diagram tracking the fate of the palmitoylcarnitine formed by CPT-I. Palmitoylcarnitine is transported via the carnitine–acylcarnitine translocase (translocase) into the matrix in exchange for carnitine and the palmitoylcarnitine is converted to palmitoyl-CoA by carnitine palmitoyltransferase 2 (CPT-II) using matrix Coenzyme A (CoASH). Palmitoyl-CoA enters fatty acid oxidation as a substrate for the very-long-chain acyl-CoA dehydrogenase (VLCAD) followed by β-oxidation using the trifunctional protein (TFP), both of which are inner membrane proteins. The subsequent cycles of β-oxidation all form acetyl-CoA, which enters the TCA cycle whose fate is illustrated in **Fig. 6**. From Lee, K., Kerner, J. and Hoppel, C. L. (2011). Mitochondrial carnitine palmitoyltransferase 1a is part of an outer membrane fatty acid transfer complex. *Journal of Biological Chemistry* **286**, 25655–25662. The American Society for Biochemistry and Molecular Biology.

the mobile electron carriers, coenzyme Q (complexes I and III as well as II and III), and cytochrome *c* (III and IV), interspersed between specific complexes (Lesnefsky et al., 2001c). Electron transfer down the redox potential gradient from NADH or $FADH_2$ to oxygen is coupled to the active transport of hydrogen ions from the matrix to the cytosolic side of the inner membrane, as described by the chemiosmotic theory of Mitchell (Mitchell, 1976). Complexes I, III, and IV translocate protons from matrix to cytosolic aspect of the inner membrane, coupled to electron flux through the complex. The multisubunit complexes of the ETC are organized into higher levels organization of supercomplexes that maximize electron transfer via substrate channeling (Rosca et al., 2008; Nubel et al., 2009; Schagger et al., 2004; Schagger and Pfeiffer, 2000).

Inner Membrane

The inner membrane is impermeable to ions and small molecules. The chemiosmotic theory of Mitchell (Mitchell, 1976) features the active transport of hydrogen ions from the matrix side to the cytosolic side of the inner membrane (vectorial proton transfer) during the flow of electrons down their free energy gradient through the redox centers of complexes I, III, and IV. The inner membrane consists in part of specific transporters that regulate the uptake of small molecules and ions, such as inorganic phosphate and ADP into the matrix.

Coupling

These forgoing components work in concert to harness the stored energy of the inner membrane electrochemical gradient to phosphorylate ADP as protons flow down the electrochemical gradient from cytosol to the matrix, providing energy that drives complex V to phosphorylate ADP (Mitchell, 1976). This ATP synthesis couples mitochondrial oxidation to energy demand. In energy-replete states, a decrease in the availability of ADP leads to a slower rate of phosphorylation that in turn results in a greater electrochemical gradient across the inner membrane and a lessened rate of electron transport to oxygen, leading to decreased oxygen consumption. An increase in ADP content secondary to increased energy demand and consumption will increase phosphorylation and utilization of the proton gradient that consequently stimulates the rate of electron transport and oxygen consumption (Lesnefsky et al., 2001c; Lesnefsky and Hoppel, 2006). The coupling of respiration to phosphorylation is the key concept in integrated mitochondrial respiration of oxidative phosphorylation.

Uncoupling

The uncoupling of respiration from phosphorylation can occur via multiple mechanisms. First, the presence of naturally occurring uncoupling proteins that allow proton movement down the electrochemical gradient without ATP synthesis can dissipate membrane potential (Fink et al., 2005). Second, there is regulated or pathologic lack of vectorial proton movement within an ETC complex [as seen for complex IV (Papa et al., 1997) or complex V (Yagi and Hatefi, 1987)] despite electron flux through the complex, so-called proton slip. Last, inner membrane damage will decrease the integrity of the inner membrane, allowing unregulated back-diffusion of protons and dissipating electrochemical gradient.

The Electron Transport Chain

The ETC is composed of four multisubunit enzyme complexes. NADH is oxidized by complex I, reducing the lipid soluble mobile electron carrier, coenzyme Q. Complex III oxidizes reduced coenzyme Q and in turn reduces the mobile carrier protein, cytochrome *c*, that donates its electron to cytochrome oxidase (complex IV) for the reduction of oxygen to water. Complexes of the ETC have two degrees of translational freedom within the inner membrane (Chazotte and Hackenbrock, 1988; Gupte and Hackenbrock, 1988), leading to complexes that are organized into larger supercomplexes to form a respirasome (Schagger and Pfeiffer, 2000; Schagger et al., 2004) in order to optimize channeling of reducing equivalents.

Complex I

Complex I (NADH:quinone oxidoreductase) is the largest respiratory complex of the ETC (Fiedorczuk et al., 2016). Complex I is an L-shaped molecular cluster that crosses the mitochondrial inner membrane and consists of 45 protein subunits. A portion of complex I is situated within the matrix and includes the NADH dehydrogenase, flavin mononucleotide (FMN), and eight iron–sulfur clusters that are responsible for electron transfer, whereas the membrane portion of the complex is involved in proton translocation across the inner membrane. Complex I activity is decreased when its protein subunits are degraded. Alteration of phospholipids, especially cardiolipin, also decreases complex I activity (Fiedorczuk et al., 2016; Paradies et al., 2004). Complex I activity also is affected by its conformational change—a switch from the normal "active" A-form to the "deactive" dormant D-form leads to decreased complex I activity (Galkin et al., 2009).

Complex II

Complex II (succinate:quinone oxidoreductase) oxidizes succinate to fumarate with coupled reduction of ubiquinone to ubiquinol (Kim et al., 2012). Unlike complex I, there is no net proton translocation across the inner membrane during succinate oxidation.

Complex III

Complex III (ubiquinol:cytochrome *c* oxidoreductase, *bc*1 complex) is a dimer embedded in the inner membrane (Kim et al., 2012; Lesnefsky et al., 2016). Electron transfer through complex III leads to the translocation of protons across the inner membrane to the intermembrane space. There are 11 protein subunits in complex III. Cytochrome *b*, c_1, and iron–sulfur protein (ISP) are the three catalytic subunits. Complex III is a key site of reactive oxygen species (ROS) generation within mitochondria. Blockade of electron transport at cytochrome *b* using antimycin A dramatically increases ROS generation from complex III (Turrens, 2003; Chen et al., 2003; Moghaddas et al., 2003). A dysfunction of its catalytic domain in ISP blocks electron transport to cytochrome c_1 and subsequently decreases complex III activity in ischemia-damaged mitochondria (Lesnefsky et al., 2001a).

Complex IV

Complex IV (cytochrome oxidase) is the terminal enzyme of the ETC. Cytochrome oxidase reduces molecular oxygen to water, coupled with the oxidation of cytochrome *c*. Like complex I and complex III, proton translocation across the inner mitochondrial membrane occurs during the redox reactions within complex IV. There are 13 subunits in complex IV. Three subunits (Cox1, Cox2, and Cox3) are encoded by mitochondrial DNA, and other structural subunits are encoded by the nuclear DNA (Kim et al., 2012). Complex I, III, and IV form supercomplexes to optimize electron transportation within respiratory substrates (Schagger et al., 2004; Schagger and Pfeiffer, 2000).

Mitochondrial Dynamics

Mitochondrial Remodeling

Metabolic homeostasis requires that damaged mitochondria must eventually undergo repair or removal (Gustafsson and Gottlieb, 2008). Genetic mouse models have been generated to study proteins involved in the process of mitochondrial remodeling. These studies have shown that remodeling plays a key role in preserving cellular homeostasis by the timely and appropriate clearance of damaged mitochondria. Important contributors to the mechanics of mitochondrial remodeling are fusion proteins (mitofusin-1, 2, and Opa1) and fission proteins (Fis1, Drp1) (Gottlieb and Gustafsson, 2011; Gottlieb et al., 2011; Youle and van der Bliek, 2012; Papanicolaou et al., 2012). Fission leads to increased number of mitochondria, whereas fusion produces larger and sometimes interconnected organelles (Youle and van der Bliek, 2012). There is a dynamic homeostasis between fission and fusion (Chen et al., 2011). Fission can eliminate damaged portions of mitochondria—the latter are removed by mitophagy (Gottlieb and Gustafsson, 2011; Gottlieb et al., 2011). Thus, mitochondrial integrity is maintained at a high level. Genetic deletion of mitofusin-1 and mitofusion-2 in adult mouse hearts results in mitochondrial individualization and cardiomyocyte respiratory dysfunction accompanied by the early onset of dilated cardiomyopathy (Chen et al., 2011; Papanicolaou et al., 2012). Thus, lack of mitochondrial fusion during normal metabolic states is detrimental to the heart. Following coronary ligation, fusion is attenuated with a decreased content of Opa-1 and mitochondrial fragmentation (Chen et al., 2009).

Mitophagy has an important role in the removal of damaged mitochondria, leading to the basal turnover of organelles (Gottlieb and Gustafsson, 2011). However, excessive mitophagy in response to stress may result in increased cardiomyocyte death (Lee et al., 2012). Damaged mitochondria generated during either the ischemic insult or the stress of reperfusion can eventually be cleared by mitophagy (Narendra and Youle, 2011; Youle and van der Bliek, 2012). The collapse of the inner membrane electrochemical gradient is a critical physiologic step that points mitochondria toward the pathway to destruction. The loss of $\Delta\Psi$ triggers a kinase PINK1-dependent translocation of the E3 ubiquitin ligase Parkin from cytosol to the surface of mitochondria (Narendra et al., 2009; Narendra and Youle, 2011; Youle and van der Bliek, 2012). Thus, the PINK1–Parkin system is continuously monitoring mitochondria for quality control, with preservation of inner membrane electrochemical gradient, a key readout of mitochondrial health. The PINK1 content of mitochondria with intact $\Delta\Psi$ is low, owing to its rapid import and cleavage by mitochondrial proteases (Ziviani et al., 2010). Upon $\Delta\Psi$ depolarization, the import and degradation of PINK1 are inhibited, leading to PINK1 accumulation on the outer membrane, initiating a mitochondrial signal for its removal. The localization of PINK1 on the mitochondrial outer membrane is necessary for Parkin recruitment to the depolarized mitochondria. The precise mechanism of interaction between PINK1 and Parkin remains unclear. In addition to the recruitment of Parkin with potential phosphorylation of Parkin, PINK1 probably phosphorylates other targets, including mitofusin-2, converting mitofusin-2 to participation in Parkin recruitment to depolarized mitochondria. Parkin subsequently ubiquitinates proteins on the outer mitochondrial membrane (Gegg et al., 2010; Geisler et al., 2010), favoring removal of the damaged mitochondrion (Narendra and Youle, 2011). Ubiquitinated mitochondrial proteins are recognized by the autophagy adaptor protein, p62/SQSTM1 (Seibenhener et al., 2004), which favors interaction with the LC3 protein on the mitophagosome (Pankiv et al., 2007), with subsequent engulfment of the damaged mitochondria by an LC3-positive mitophagosome.

Transgenic mouse models of overexpression or knockout of PINK1 and Parkin clearly support the notion that these key proteins mediate mitophagy in response to cardiac stress (Siddall et al., 2013; Kubli et al., 2013a,b). However, the in situ regulation of these key mediators in the cardiac myocyte remains less clear. Of interest, and perhaps leading to an integrated cell biology response, one response of cardiac mitochondria to severe metabolic stress—including oxidative damage and calcium overload—is to undergo abrupt permeation of the inner mitochondrial membrane with catastrophic loss of the electrochemical gradient across the inner

membrane, so-called mitochondrial permeability transition (Weiss et al., 2003). Such a loss of inner membrane potential as a marker of substantial mitochondrial damage recruits PINK1 and Parkin, activating mitophagy and favoring removal of severely damaged mitochondria that pose a threat to cardiomyocyte survival. Intervention to protect cardiac mitochondria during cell stress, including the early reperfusion period of the treatment of myocardial infarction, leads to preservation of membrane potential, attenuating loss of the cardiac mitochondrial pool (Chen et al., 2012; Xu et al., 2013).

Caveolae and Subsarcolemmal Mitochondria

Caveolae are small inpouchings of the plasma membrane that are involved in the uptake of molecules that are too large to pass through the sarcolemma. This uptake may be of the bulk variety or receptor-mediated. A second important function of these structures involves cell signaling, particularly cardiomyocyte stress responses (Head et al., 2014; Fridolfsson et al., 2014). Caveolae are closely apposed to SSM (Fridolfsson et al., 2012). Ischemic stress increases this association that is accompanied by the increased mitochondrial localization of the caveolae protein, caveolin-3 (Fridolfsson et al., 2012). Transgenic mice that overexpress caveolin-3 exhibit increased mitochondrial content of caveolin-3 concomitant with an increase in respiration rate, decreased production of ROS, and enhanced resistance to calcium-overload damage. Increased localization of the protein caveolin-3 to SSM favors enhanced stress tolerance of the cardiac myocyte, including a relative resistance to cell death induced by cardiac ischemia–reperfusion injury (Fridolfsson et al., 2012). Crosstalk between plasma membrane microdomains and mitochondria is a novel adaptation of the jeopardized cardiomyocyte to dynamically alter mitochondrial metabolism and enhance cardiac stress response.

Mitochondria-Associated Membranes and Mitochondria

There are particular sites in cardiac muscle cells where endoplasmic reticulum (ER) membranes are linked to mitochondria; these associations, which persist during isolation of the mitochondria, are known as mitochondria-associated membranes (MAM) (Wieckowski et al., 2009). MAMs are involved in directing calcium to mitochondria to regulate metabolism. They also act in concert with the mitochondrial outer membrane proteins to regulate mitochondrial fission and fusion (Bravo-Sagua et al., 2013). It is by interaction with MAM that lipid is transferred from ER to mitochondria (Bravo-Sagua et al., 2013). MAM consist, in part, of the outer membrane including VDAC (Paillard et al., 2013; Wieckowski et al., 2009). Contact sites facilitate the flow of metabolites from the cytosol and perhaps ER into the mitochondrial matrix, a key aspect of ER-MITO crosstalk via MAM. Interaction of VDAC with ER proteins IP3R1 and Grp75 occurs (Paillard et al., 2013) and is involved in regulation of calcium flux from ER to mitochondria (Gomez et al., 2016). An area of emerging study is the role of dysfunction of the MAM in cardiac injury including myocardial stress due to ischemia–reperfusion (Gomez et al., 2016) or congestive heart failure.

Cardiolipin: Role in Mitochondrial Structure and Physiology

Cardiolipin is a phospholipid unique to mitochondria that is critical for the optimal activity of the ETC. Cardiolipin contains two phosphatidic acid groups linked by a glycerol with the majority of cardiolipin species containing four linoleic acid groups (C18:2). The four C18:2 acyls are essential for normal cardiolipin function in the inner mitochondrial membrane (Sparagna and Lesnefsky, 2009). Cardiolipin interacts with cytochrome *c* to retain this mobile electron carrier on the outer surface of the inner membrane. The delocalization and release of cytochrome *c* from mitochondria, a key signal of the irreversible loss of mitochondrial integrity, activates cell death programs. Cardiolipin is required for the optimal activity of complex I, complex III, and complex IV of the ETC (Lesnefsky and Hoppel, 2006; Lesnefsky et al., 2001d; Paradies et al., 2004).

Oxidatively-sensitive cardiolipin is a major target of ETC-mediated damage in pathologic cardiac stress settings, including ischemia–reperfusion, heart failure, and the comorbid condition of aging (Sparagna and Lesnefsky, 2009). During ischemia–reperfusion, cardiolipin is oxidatively damaged (Lesnefsky and Hoppel, 2008; Paradies et al., 1999) and depleted (Lesnefsky et al., 2001d). Cardiolipin is oxidatively sensitive due to the presence of the C18:2 residues that contain conjugated double bonds. Oxidative damage to cardiolipin occurs via both random chemical oxidation and a specific cardiolipin peroxidase (Kagan et al., 2005). Oxidation of a sensitive methionine of cytochrome *c* creates a pathologic function for cytochrome *c* to oxidize and deplete cardiolipin, activating programmed cell death (Kagan et al., 2005); this occurs during ischemia–reperfusion (Aluri et al., 2014).

Oxidized cardiolipin is remodeled with the net replacement of the oxidized residue. In contrast to its canonical role in the remodeling of newly synthesized cardiolipin, it appears that the enzyme, tafazzin, does not substantially contribute to the remodeling of cardiolipin in response to ischemia–reperfusion stress (Schlame, 2013; Szczepanek et al., 2016). In contrast, it appears that ALCAT-1, located in the ER (Baile et al., 2013), is a major enzyme in cardiolipin remodeling in response to disease. ALCAT-1 is a nonselective acyltransferase (Zhao et al., 2009; Cao et al., 2009) and replaces the lost C18:2 group with a different acyl group, unsaturated and often of longer chain length (Sparagna et al., 2007; Sparagna and Lesnefsky, 2009). The change in the composition of cardiolipin observed following acute ischemia–reperfusion and subsequently in models of congestive heart failure is consistent with this mechanism.

Conditions Associated With Mitochondrial Dysfunction

Diabetes

Mitochondrial dysfunction is manifested in the diabetic db/db mouse heart by decreased rates of oxidative phosphorylation, greater ROS generation, and increased fatty acid-driven uncoupling of mitochondrial respiration (Boudina et al., 2007; Boudina and Abel, 2006). The mitochondrial defect in the db/db mouse is localized to SSM (Dabkowski et al., 2010). In contrast, the mitochondrial defect in type I diabetic mouse is limited to IFM (Dabkowski et al., 2009; Hollander et al., 2014). The previously observed defects in oxidative phosphorylation in respiration with complex I substrates in the db/db heart was attenuated by activation of protein kinase G, achieved by long-term treatment with the phosphodiesterase inhibitor, tadalafil (Koka et al., 2014).

Aging

Aging leads to a decrease in the number of cardiomyocytes, increases the size of individual myocytes due to myocyte hypertrophy, and increases the number of small focal areas of myocardial fibrosis, probably through myocyte dropout due to inappropriate activation of programmed cell death (Wei, 1992). Mitochondrial dysfunction is a key contributor to the aging cardiac phenotype with enhanced ROS production due to the presence of age-related ETC defects (Suh et al., 2003), enhanced susceptibility to mitochondrial permeability transition (Hafner et al., 2010), and a greater predisposition to release cytochrome *c* (Phaneuf and Leeuwenburgh, 2002). The age-related mitochondrial phenotype predisposes to increased cardiomyocyte injury in the aged heart during the tissue stress of ischemia–reperfusion (Lesnefsky et al., 1994), where ischemic damage to mitochondria is superimposed upon the age-related changes in mitochondrial metabolism (Lesnefsky et al., 2016).

The initial studies of age-related changes in mitochondrial function yielded conflicting results, latter rescinded (Lesnefsky et al., 2001c; Lesnefsky and Hoppel, 2003; Fannin et al., 1999). The rate of oxidative phosphorylation in mitochondria (SSM) obtained by the use of the Polytron was similar in mitochondria isolated from adult and aged hearts (Takasawa et al., 1993). When a combined mitochondrial population of SSM and IFM was obtained by the use of protease digestion, some studies reported a decrease in the rate of oxidative phosphorylation (Chen et al., 1972; Hansford, 1978), whereas a single one did not (Manzelmann and Harmon, 1987), due to differences in isolation technique that resulted in variable admixtures of SSM and IFM. These divergent findings were reconciled by the subsequent study of the two individual subpopulations of cardiac mitochondria (Fannin et al., 1999).

The protein content of IFM is decreased in the aged heart (Fannin et al., 1999). The enzyme cytology approach of DeDuve (1967) conclusively showed that the recovery of mitochondria did not differ in adult and aged hearts (Fannin et al., 1999). Thus, supported by marker enzyme studies, the decreased protein yield of IFM from aged hearts reflects a true decreased content of mitochondria in the myocardium (Fannin et al., 1999).

Oxidative phosphorylation was studied in SSM and IFM isolated from 6-month adult and from 24 and 28 months aged Fischer 344 rat hearts (Fannin et al., 1999). Respiration was tightly coupled in both adult and aged heart mitochondria (Fannin et al., 1999). The maximal rate of oxidative phosphorylation was decreased only in IFM in aged hearts compared to adult controls, whereas rates in SSM were unaltered by age (Fannin et al., 1999).

The decrease in uncoupled respiration in IFM localized the age-related defect to the ETC rather than to the phosphorylation apparatus (Fannin et al., 1999). Substrates that donate reducing equivalents to specific electron transport complexes were employed to localize the aging defect in IFM to specific sites in the ETC. The decrease in the oxidation rate of TMPD-ascorbate, an electron donor to cytochrome oxidase via the reduction of cytochrome *c*, localized a defect to cytochrome oxidase (Fannin et al., 1999). Measurement of the enzyme activities of the individual ETC complexes also disclosed an aging defect in complex III due to alteration of the quinol binding site, with potential involvement of a regulatory subunit of complex III (subunit VII, "hinge protein") as well (Moghaddas et al., 2003; Lesnefsky et al., 2001b).

Age-induced reductions in mitochondrial content and respiration occur in IFM, whereas SSM are unaffected. In addition to respiration, further studies of SSM found that ROS production (Muscari et al., 1990) and protein import (Craig and Hood, 1997) were unaltered by age. The ability to separate and study SSM and IFM separately permits the uncovering of key pathogenic mitochondrial defects (Lesnefsky and Hoppel, 2003; Hoppel et al., 2002; Lesnefsky et al., 2016; Judge et al., 2005).

Ischemia

Following coronary occlusion with the cessation of myocardial perfusion, the oxygen content of myocardium declines precipitously, leading to a marked slowing in the rate of respiration. As a consequence, ischemia does not immediately lead to anoxia and during the evolution of ischemic injury, some oxygen remains available in the tissue (Ilangovan et al., 2004). The slowed respiration favors the leak of electrons from complexes I and III directly to the remaining oxygen to form ROS, as shown by experimental studies in cardiomyocytes (Becker et al., 1999; Vanden Hoek et al., 1997). Consistent with the presence of some remaining oxygen in this pathologic milieu, ROS production increases during stop-flow ischemia in the intact heart (Kevin et al., 2003; Lee et al., 2000; Riess et al., 2004) until all the oxygen is consumed (Levraut et al., 2003).

Ischemia progressively damages the ETC (Lesnefsky et al., 2001c), in part due to this ROS production. The first target of the electron transport driven damage is the mitochondria themselves (Chen et al., 2006a, 2007; Lesnefsky et al., 2004a). The catalytic centers of complexes I and III sustain damage (Chen et al., 2007; Lesnefsky et al., 2001c). Ischemia damages complex I at sensitive iron–sulfur centers and the flavin-containing center (Chen et al., 2008; Ohnishi et al., 2005). Complex III suffers deactivation of the

Rieske iron–sulfur protein (Lesnefsky et al., 2001a). The ischemic damage decreases electron transfer capacity and favors electron leak to generate ROS. Thus, complex I and complex III become key sites that generate ROS during reperfusion (Ambrosio et al., 1993; Chen et al., 2003; Han et al., 2003; St-Pierre et al., 2002). The initial damage from ischemia predisposes to increased oxidant production during reperfusion.

Complex IV requires the inner membrane phospholipid, cardiolipin, for optimal activity (Vik and Capaldi, 1977). Ischemia decreases enzyme activity via a decrease in cardiolipin content (Lesnefsky et al., 2001d). The proximity to the ETC makes cardiolipin, containing oxidatively sensitive linoleic acid (C18:2) groups, a prime target for oxidative damage. Rather than random oxidative damage, the decrease in cardiolipin during ischemia probably occurs via targeted oxidative damage from the cytochrome *c* cardiolipin peroxidase discussed above (Kagan et al., 2005). The neopathologic function of cytochrome *c* as a cardiolipin peroxidase directs ROS-mediated oxidation of cardiolipin to lipid peroxide formation (Kagan et al., 2005). Furthermore, the methionine-oxidized cytochrome *c* is no longer effective in normal electron transport (Kagan et al., 2005). An increase in oxidized cardiolipin delocalizes cytochrome *c* from the inner membrane into the intermembrane space (Kagan et al., 2005), in turn activating cell death programs (Kagan et al., 2005; Ott et al., 2002).

Reperfusion

Mitochondria are a major source of oxidative damage during reperfusion (Chen et al., 2006b, 2007). The decrease in complex IV activity and the oxidation and loss of cytochrome *c* (Borutaite et al., 2001; Lesnefsky et al., 1997; Kagan et al., 2005) results in impaired electron flow through the distal ETC. The blockade of electron flux downstream of the sites of electron leak at complexes I and III enhances ROS production from these proximal sites by favoring greater reduction of their catalytic centers and enhanced electron leak to oxygen (Chen et al., 2007). The block in the distal ETC at complex III and cytochrome c also may favor reverse electron transport with succinate as a complex II substrate from complex II through complex I to NAD+ as a source of ROS production (Chouchani et al., 2013; Pell et al., 2016). The extent of reformation of the inner membrane electrochemical potential during early reperfusion dictates the relative contribution of forward and reverse electron transport to oxidative injury (Ross et al., 2013). In the presence of only moderate ischemic damage to mitochondria that allows the ETC to generate an electrochemical potential, reverse electron transport is favored. In contrast, in the absence of a significant inner membrane potential, electrons will flow down their free energy gradient from complex I or complex II into complex III, with ROS generation (Chen et al., 2003).

Blockade of electron transport at or proximal to the sites of electron leak attenuates ROS production (Chen et al., 2003, 2007). The blockade of electron transport at the quinone-binding site of complex I will block ROS from forward electron transport (complex I forward into complex III with III as the ROS source) and reverse electron transport (complex II retrograde into complex I with I as the ROS source). Not surprisingly, this intervention is highly protective of mitochondria (Chen et al., 2006b, 2007; Chouchani et al., 2013; Lesnefsky et al., 2004a), whereas chemical blockade of the distal ETC at complex IV during ischemia is not protective (Chen et al., 2010). Thus, strategic blockade of the ETC at specific sites is critical to prevent flow into more distal sites of the chain that generates the damage (Chen et al., 2007). As a result of these studies, complex III and the modified cytochrome *c* peroxidase are the likely mediators of damage to the ETC during ischemia and probably early reperfusion as well (Chen et al., 2006a, 2010; Lesnefsky et al., 2004a).

Damage to the ETC clearly begins during ischemia (Borutaite et al., 2001; Lesnefsky et al., 1997, 2004b). Although the bulk of the damage to electron transport occurs during ischemia (Lesnefsky et al., 2004b), reperfusion results in additional damage at complexes I and III (Flameng et al., 1991). The critical structural damage to mitochondrial membranes that jeopardizes cell survival occurs mainly during reperfusion (Halestrap et al., 2004; McCully et al., 2004; Murphy and Steenbergen, 2008; Weiss et al., 2003). Although other sites of ROS generation during early reperfusion include monoamine oxidase (Di Lisa et al., 2009), NADPH oxidase (Sciarretta et al., 2013), and possibly xanthine oxidase (Cantu-Medellin and Kelley, 2013), and lipoxgenases (Li et al., 2009), impaired electron transport remains the predominant source of intramitochondrial and extramitochondrial oxidative damage to the cardiomyocyte.

Cytosolic calcium content increases during ischemia, especially during reperfusion. Calcium accumulation by mitochondria occurs via the inner membrane calcium uniporter (Murphy and Steenbergen, 2008). ER-driven calcium overload of mitochondria during reperfusion exacerbates injury (Paillard et al., 2013). Increased matrix calcium content leads to cycles of mitochondrial calcium release and reuptake, generating futile energy-consuming cycles. Elevated matrix calcium activates proteases and phospholipases, degrading inner membrane phospholipids and matrix proteins (De Windt et al., 1998; Chen and Lesnefsky, 2015). Calcium overload is a key stimulant for opening of the mitochondrial permeability transition pore (MPTP), leading to permeation of the inner mitochondrial membrane with collapse of the inner membrane electrochemical gradient with subsequent mitochondrial swelling and outer membrane rupture that favors release of cytochrome *c*, apoptosis-inducing factor, and other intermembrane space proteins that activate programmed cell death (Halestrap et al., 2004; Weiss et al., 2003; Rasola and Bernardi, 2014).

Congestive Heart Failure

Reduction of bio-energy generation due to impaired mitochondrial function is a major factor in the development of heart failure. Impaired ETC leads to decreased generation of ATP that is a general feature of heart failure (Dolinsky et al., 2016; Parihar and Parihar, 2017; Rosca and Hoppel, 2010, 2013; Rosca et al., 2013). Cardiac energy levels are decreased in patients with cardiomyopathies and in animal models of heart failure (Fosslien, 2003). In addition to ischemia-induced mitochondrial dysfunction,

defective mitochondrial metabolism occurs in nonischemic models of cardiomyopathy (Griffiths, 2012). Complex IV activity is decreased in the failing heart due to dysregulation of respirasome formation (Quigley et al., 2000; Rosca and Hoppel, 2013; Rosca et al., 2013). Mitochondrial dysfunction not only decreases energy production, but also activates signaling pathways to trigger myocyte death. Loss of cardiomyocytes is an important step in the development of heart failure. Cardiomyocyte loss can be due to apoptosis, necrosis, and autophagy. Therapeutic approaches to improve metabolic energy generation would be beneficial for the treatment of heart failure.

Mitochondria are the major source of ROS in cardiomyocytes (Chen et al., 2003). An impaired ETC markedly augments the production of ROS, contributing to the development of heart failure. Increased ROS generation leads to oxidation of cardiolipin, which decreases cytochrome oxidase activity and favors release of cytochrome *c* from mitochondria into the cytosol. Cardiolipin oxidation results in a reduction of energy generation and an increase in apoptosis (Dolinsky et al., 2016).

The opening of the MPTP is a final step in triggering necrotic cell death (Weiss et al., 2003). MPTP is activated by mitochondrial calcium overload or by enhanced oxidative stress, leading to a depolarized inner mitochondrial membrane potential that contributes to decreased ATP synthesis. MPTP opening is involved in the progression of heart failure through decreased energy production and enhanced cell death.

In addition to apoptotic and necrotic cells, autophagic cells also are found in failing human hearts with dilated cardiomyopathy (Tong and Sadoshima, 2016). Autophagy, an adaptive response designed to preserve cardiomyocytes during stress, removes damaged macromolecules as well as damaged mitochondria. However, extensive autophagy can lead to cell injury (Shires and Gustafsson, 2015). It is still unclear whether autophagy, including mitophagy, is an adaptive response to keep cardiomyocytes healthy or is a means to eliminate injured cardiomyocytes during the development of heart failure.

Conclusion

Cardiac performance is critically dependent on mitochondrial function in health and disease. The heart is able to use a variety of fuel sources to support metabolism and can change fuel preference in response to changing conditions at the organismic and cardiac levels. Damage to mitochondria is a critical mechanism underpinning many cardiac diseases, with resultant deficits in energy production and enhanced generation of ROS contributing to cardiac injury. Defective mitochondria need to be repaired or eliminated and replaced. Mitochondria are critical checkpoints for cardiac myocyte survival versus death, with indicators of severe, unrepairable mitochondrial injury including oxidation of cardiolipin and the loss of select mitochondrial proteins activating pathways of cardiomyocyte suicide. Comorbid conditions such as aging and diabetes independently cause mitochondrial dysfunction, which exacerbates disease-mediated mitochondrial-driven injury. Cardiac dysfunction and cell death lead ultimately to cardiomyopathy and heart failure. Mitochondria present intriguing targets for new therapies to reduce cardiac cell injury in acute and chronic disease settings. When all is said and done, it is the mitochondria, minuscule inhabitants of the cardiomyocyte, that by their incessant bioenergetic activity enable the heart to beat without surcease, a sine qua non of life itself.

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Modern Considerations in ICD Therapy

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Introduction

The first implantable cardioverter defibrillators (ICD) were implanted by cardiac surgeons through lateral thoracotomy, with a large 250–300 cc abdominal generator with battery life lasting 1.5 years, no programmable features, and no pacing capability. Modern ICDs have a 30 cc generator, are often implanted in less than an hour, can include single- or dual-coil ICD leads, VVI, DDD, or CRT pacing including use of quadripolar and multisite pacing capability. ICDs commonly can deliver in excess of 35 J shocks following several attempts at ATP (antitachycardia pacing), which is often effective at painless termination of ventricular arrhythmias (VA). "Leadless" defibrillation is also available with an entirely subcutaneous ICD system. ICD implantation has grown exponentially after a series of randomized trials demonstrating the lifesaving benefits of ICDs in well-selected patients with cardiomyopathy, prior MI, or prior cardiac arrest (Moss et al., 1996, 2002; Bardy et al., 2005). CRT (cardiac resynchronization) pacing benefits in patients with LBBB and QRS duration >130 ms have demonstrated improved morbidity and reduced mortality independent of the lifesaving effect of ICD on VAs. Along with a massive growth in implantation has come a greater number of complications, lead fractures, lead recalls, ICD generator recalls, battery failures, and device-related infections, all of which demand more careful attention to strategies to reduce these risks.

In the modern era, the choice of whether or not to implant an ICD is the first of many for the electrophysiologist. There are now a variety of device types and programming schema available to individualize device therapy for a particular patient, and considerations to reduce the risk of complications for our ICD recipients.

Choice of Device

Single- Versus Dual-Chamber ICDs

There is no expert consensus recommendation regarding the choice of a single- or a dual-chamber ICD as the data available to guide the electrophysiologist in making this decision is quite inconclusive.

First, patients with an indication for atrial pacing such as a history of symptomatic sinus node dysfunction (sick sinus syndrome), high degree A–V block, sinus arrest with syncope, or a history of Torsades de Pointes should likely receive dual-chamber devices based on the indication for atrial pacing or the benefit of maintaining AV synchrony alone. Additionally, if atrial fibrillation diagnostics are needed to guide decisions on anticoagulation, a dual-chamber device may be considered. The high rate of inappropriate ICD shocks for patients with rapidly conducted AF gives another rationale for dual-chamber systems; however, formal studies have been disappointing on its ability to actually reduce ICD shocks.

What is less clear is whether the addition of atrial sensing can decrease the rate of inappropriate therapies due to supraventricular tachycardias. A recent randomized controlled trial (the RAPTURE Trial) found no reduction in inappropriate shocks with a dual-chamber ICD as compared to a single-chamber device when used for primary prevention and modern shock reduction programming with SVT discrimination was utilized (Friedman et al., 2014). Another recent randomized trial (the OPTION study) did find a

7% absolute risk reduction of inappropriate shocks when dual-chamber programming was utilized in a population of both primary and secondary prevention ICD patients (Kolb et al., 2014). Several prospective and retrospective cohort studies as well as one meta-analysis have failed to show any reduction in inappropriate shocks in patients with dual-chamber devices compared to those with single-chamber devices (Theuns et al., 2008; Ruwald et al., 2013; Deisenhofer et al., 2001; Van Rees et al., 2011). Finally, the randomized detect supraventricular tachycardia study found a small 8% absolute risk reduction for inappropriate detection of SVT when a dual-chamber system was used (Friedman et al., 2006).

The disadvantages of a dual versus single-chamber ICD include the potential for atrial lead complications, increased cost, and decreased device longevity. Upgrade or revision of a single-chamber system to add an atrial pacing lead has been associated with at least a 7% complication rate related to hematoma, pneumothorax, and lead dislodgement (Dewland et al., 2011; Poole et al., 2010). One appealing ICD lead choice for atrial diagnostics in a single-chamber MRI conditional device is using a DX lead (Biotronik, Germany) which provides a large bipolar sensing ring in place of an SVC coil, however this does not provide atrial or AV synchronous pacing.

Cardiac Resynchronization

The addition of left ventricular pacing, or cardiac resynchronization therapy, is another consideration when implanting an ICD. CRT is most commonly accomplished by placement of a pacing lead into a posterolateral branch of the coronary sinus, thus reaching the epicardial surface of the left ventricle. Unlike ICDs, CRT devices are not only beneficial in the case of malignant arrhythmias but have the potential to improve both symptoms and prognosis in patients with heart failure and left ventricular dyssynchrony. The premise of CRT is to improve the efficiency of cardiac function by allowing for simultaneous contraction of the septal and free walls of the left ventricle and avoiding wasted energy moving the blood-pool back and forth laterally. Invasive and echocardiographic studies have shown that CRT can dramatically improve myocardial performance within a single heart beat when active. The randomized controlled CARE-HF trial demonstrated that patients with class III or IV heart failure, LVEF less than 35%, and a QRS duration greater than 120 ms had a 10% reduction in the absolute risk of death at 2 years follow-up when treated with CRT compared to standard pharmacologic therapy alone (Cleland et al., 2005). Similarly, the randomized COMPANION trial demonstrated a reduction in mortality in patients with class III or IV CHF, QRS duration >120 ms, and LVEF <35% when treated with either a CRT pacemaker or defibrillator compared to pharmacologic therapy alone (Bristow et al., 2004). Finally, the randomized controlled MADIT–CRT trial enrolled patients with class I or II CHF, LVEF <30%, and QRS duration greater than 130 ms and compared outcomes after randomization to either CRT and ICD or ICD alone. This trial demonstrated a significant 41% reduction in the risk of heart failure events (Moss et al., 2009). Subsequent subgroup analyses of the MADIT–CRT trial as well as a long-term follow-up examination of randomized patients have demonstrated that this benefit appears to be restricted to patients with a true LBBB morphology with the optimal response in those with QRS duration >150 ms (Zareba et al., 2011). Ideally, for optimal resynchronization, CRT lead position should be targeted to the latest site of left ventricular activation. As such, patients with LBBB are expected to benefit more from CRT compared to those with RBBB as activation of their posterolateral left ventricle, the most common site of LV lead implantation, should be delayed by their conduction system disease. The advent of quadripolar LV leads has helped to facilitate higher rates of success delivering CRT. These leads have resulted in a lower incidence of phrenic capture or lead dislodgment, and provide an option for multipoint LV pacing which may further augment CRT response by targeting sites of late activation for pacing.

Single Versus Dual-Coil Defibrillation Leads

When implanting a transvenous ICD system, one of the intraoperative decisions an implanting physician must make is whether or not to utilize a single- or dual-coil defibrillation lead. The vast majority of implanted systems today utilize a dual-coil system (Neuzner and Carlsson, 2012). This preference is attributed to studies demonstrating that the use of a dual-coil system reduces the defibrillation threshold (DFT) by 2–3 J at the time of system implantation (Bardy et al., 1994; Gold et al., 1998); however in several retrospective analyses of large cohorts of patients with ICDs, the use of a dual-coil system has not been associated with improved clinical outcomes nor with higher first shock efficacy (Aoukar et al., 2013; Kutyifa et al., 2013; Mokabberi et al., 2011; Ellis and Hurt, 2012). Specifically, patients who have a right-sided ICD system implantation derive no benefit on DFT from an SVC coil. Since the shock vector an ICD delivers includes the pulse generator as part of the circuit ("hot-can"), a right-sided implant necessarily directs energy toward the right hemithorax making the position of the SVC coil within the dominant path of the defibrillation vector, lowering shock impedance. Right-sided systems tend to have higher DFTs; however, conversion efficacy for both spontaneous VA (VAs; i.e., VT/VF) and induced ventricular fibrillation has been found to be no different between right- and left-sided systems (Gold et al., 2007). In prospective randomized trials of ICD implantation and lead surveillance registries, the dominant clinically treated VA is sustained ventricular tachycardia not ventricular fibrillation (~90% of ICD shocks delivered for VT, not VF) (Ellis and Hurt, 2012). Antitachycardia pacing therapy is at least 75% effective to terminate sustained VT and ATP is independent of the presence of an SVC coil (Wathen et al., 2004; Wilkoff et al., 2008a).

While there are few apparent clinical benefits of a dual-coil system, the risk of extraction of these leads, should it become necessary, is significantly higher. The major risk of lead extraction is vascular injury including SVC tears. The most common site of major vascular injury during transvenous extraction is in the SVC or SVC right atrial junction. It is felt that the presence of a defibrillation coil in the SVC increases the fibrotic response of the vasculature at this site, increasing the likelihood of injury during

extraction (Epstein et al., 2013). This is particularly problematic for nonsilicone back-filled ICD coils (i.e., St. Jude Medical, Riata), and may be somewhat mitigated with the use of Gore-ePTFE coated coils (Boston Scientific, Reliance) (Kohut et al., 2013). Given the availability of modern ICD generators capable of delivering 40 J maximum output shocks, the added 2–3 J DFT reduction from an SVC coil is unlikely to be clinically relevant. An exception are patients with a significantly elevated DFT where consideration of dual-coil leads should be made, HOCM, LV EDD dilation >6 cm, male gender, African-American race, and use of chronic Amiodarone (Shih et al., 2016).

In summary, in current transvenous ICD systems, despite a 2–3 J improvement in DFT there appears to be no demonstrable clinical benefit to an SVC coil on the efficacy of termination VT or VF. The default choice of dual-coil ICD leads appears unwarranted apart from unique circumstances, and may pose future harm long term, conferred through the risk of lead extraction and SVC tear.

The Subcutaneous ICD

While ICDs are widely accepted as an established therapy for the prevention of sudden cardiac death, these devices have traditionally required the implantation of either epicardial patches or transvenous leads for cardiac sensing and defibrillation. Unfortunately, despite decades of engineering innovations in lead design, defibrillator leads truly remain the "weakest link" in the ICD system. Lead failure can lead to inappropriate shocks, or worse, ICD generator short circuit or failure to deliver appropriate therapy. Furthermore, the presence of an intravascular lead can allow a portal of entry to the circulation if local infection occurs around an ICD generator, allowing a soft tissue infection to become a potentially life-threatening intravascular or endocardial infection. With this in mind the subcutaneous ICD system was created.

Hardware

The subcutaneous ICD consists of a generator and single electrode. The generator is implanted in the left mid-axillary line, typically tucked under the anterior edge of the latissimus dorsi muscle and overlying the cardiac apex and the single electrode is tunneled subcutaneously from the generator to the lower sternum near the xiphoid process and then extending to the superior sternum near the manubriosternal junction (**Fig. 1**).

Patient selection

The obvious advantage of a subcutaneous system is the lack of any intravascular component, obviating the need for vascular access and significantly decreasing the chances of device-related endovascular infection. As such, this device is considered preferable in patients with poor vascular access and those at high risk for infectious complications (prior history of endocarditis, hemodialysis, mechanical valves, etc.; see **Table 1** and **2**). This type of device is also potentially advantageous in younger patients with an indication for ICD protection as it avoids the long-term risk of lead-related complications. The greatest disadvantage of a subcutaneous ICD system is its lack of ability to pace. As such, patients who require pacing have an absolute contraindication to a subcutaneous system and those who would benefit from antitachycardia pacing have a relative contraindication. The advent of transcatheter pacemakers may allow a "leadless" communication with an S-ICD, and potentially provide pacing therapy in the future for those patients who would benefit from ATP.

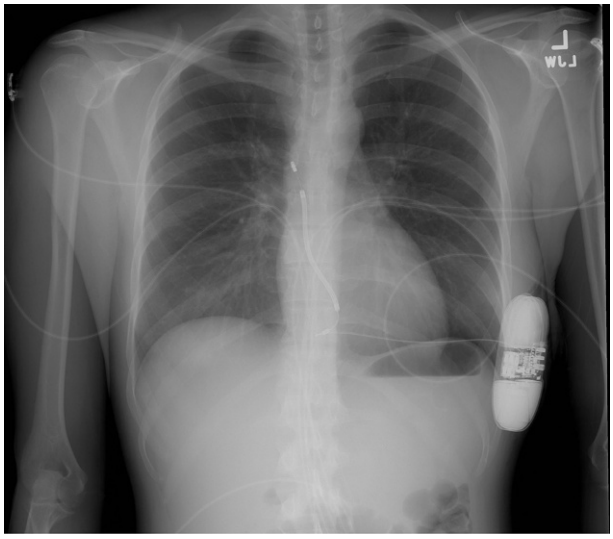

Fig. 1 Typical position of a subcutaneous ICD with generator in the mid-axillary line and electrode tunneled to the sternum.

Table 1 Patient selection for S-ICD

Patient selection for S-ICD		
Preferred	*Strongly considered*	*Avoid*
• Lack of venous access (occluded veins or congenital anomalies) • High risk of TV-complications (dialysis, pediatric, and immunocompromised) • Channelopathies (LQT, Brugada, and HCM) • Previous device infections/lead failures • History of endocarditis	• Young patients • Life expectancy >10 years • Primary prevention indicated patients with ischemic/nonischemic heart failure • Prosthetic valves • Cosmetic consideration (preferred generator placement lateral wall) • Secondary prevention with no evidence of monomorphic VT	• CRT indicated • Symptomatic bradycardia • Recurrent sustained MVT

Table 2 Comparison of outcomes and complications between a transvenous ICD and subcutaneous ICDs

Study	*Mortality (at 2 years)*	*Average age*	*1^0 prevention*	*Ischemic*	*NYHA*	*LVEF*
S-ICD pooled (Burke et al., 2015)	3.2%	50	70%	38%	37.5% classes II–IV	39%
MADIT-RIT (Moss et al., 2012)	5%–7% High rate and delayed therapy arms	63	100%	53%	98% class II or III	26%

Acute major complications (% of patients)	S-ICD (Burke et al., 2015)	TV-ICD
	Pooled data	NCDR analysis (Peterson et al., 2013) Meta-analysis (Van Rees et al., 2011)
	2%	3%–5%
	(Hematoma, lead or device malposition or displacement, pneumothorax)	

Sensing and defibrillation

Sensing is performed in one of three configurations, either from the electrode tip (at the upper sternum) to the generator, the mid electrode (which is located at the lower sternum) to the generator, or from the tip to mid electrode. In order for this device to properly discriminate tachyarrhythmias from sinus rhythm, a patient must be "screened" with electrocardiographic measurements in these three sensing vectors to ensure that their QRS complex fits into the devices template (Groh et al., 2014). Thus, patients with unusual QRS morphologies, hypertrophic cardiomyopathy, or symptomatic bradycardia may not be appropriate for this device. Indeed, inappropriate shocks have proven to be the biggest potential pitfall of the S-ICD system with rates on inappropriate shocks as high as 13%–15% in initial clinical experience with up to 80% of these shocks being attributed to T-wave oversensing (Olde Nordkamp et al., 2012, 2015; Jarman and Todd, 2013). The addition of dual-zone programming and discrimination algorithms has reduced this rate significantly, in the most recent data available the rate of inappropriate shocks has been reported as low as 4.5%–6.9% (Burke et al., 2015).

Programming Considerations

Shock Reduction

While ICDs can provide lifesaving antitachycardia therapies, the counterpoint to this benefit is the possibility of delivering unneeded, painful shocks. These inappropriate therapies have not only been found to be quite common (15% of patients in MADIT II and 17% of SCD-HeFT patients received at least one inappropriate shock) but are also frequently associated with depression and anxiety resulting in both immediate and long-term morbidity for patients affected. Multiple studies have demonstrated that careful device programming can significantly reduce the burden of inappropriate therapies.

PREPARE

The PREPARE study was a prospective cohort study comparing patients with a primary prevention biventricular ICD programmed with high rate detection limits, prolonged detection intervals, initial ATP therapy, and initial high energy shocks to (see **Table 3** for particular of programming) control patients from two other trials, the MIRACLE-ICD and EMPIRIC studies. This trial demonstrated a statistically significant reduction in overall shocks as well as inappropriate shocks (3.6% versus 7.5%) after controlling for baseline differences in the cohort (Wilkoff et al., 2008b).

Table 3 PREPARE tachycardia programming parameters

Detection		*Threshold*	*Time to detection*	*Therapies*
VF	On	250 BPM	30 of 40	30–35 J (max output) × 6
FVT	Via VF	182 BPM	30 of 40	Burst × 1, 30–35 J (max output) × 5
VT	Monitor only	167 BPM	32	Off

Table 4 RELEVANT tachycardia programming parameters

Detection		*Threshold*	*Time to detection*	*Therapies*
VF	On	250 BPM	30 of 40	30 J (max output) × 6
FVT	Via VF	181 BPM	30 of 40	Burst × 1, 30–35 J (max output) × 5
VT	Monitor only	167 BPM	32	Off

Table 5 MADIT-RIT tachycardia programming parameters

Detection		*Threshold*	*Time to detection*	*Therapies*
Standard programming				
VF	On	200 BPM	1 s delay	Quick convert ATP + shock
VT	On	170 BPM	2.5 s delay	ATP + shock
High rate cutoff programming				
VF	On	200 BPM	2.5 s delay	Quick convert ATP + shock
VT	Monitor only	170 BPM	Off	Off
Long delay programming				
VF	On	250 BPM	2.5 s delay	Quick convert ATP + shock
VT	On	200 BPM	12 s delay	ATP + shock
VT-1	On	170 BPM	60 s delay	ATP + shock

RELEVANT

The RELEVANT trial was a prospective, nonrandomized trial enrolling patients with a primary prevention indication for a biventricular ICD. Patients received either a simplified BiV ICD with a monitor only VT zone and a fixed long detection interval in the FVT/VF zones with a single round of ATP prior to shocks in the VF zone, or a standard programmable BiV ICD (see **Table 4**). The main difference in the trial was that patients in the control arm had a much shorter time to detection requiring only 12 of 16 intervals rather than 30 of 40 in the intervention arm. This trial demonstrated a significant reduction in overall as well as inappropriate device therapies. Patients in the control arm experienced 289 treated episodes versus only 36 in the intervention. There were 48 inappropriate treatments in the control arm versus 10 in the intervention arm (Gasparini et al., 2009).

MADIT-RIT

The MADIT-RIT study was a multicenter randomized controlled trial enrolling patients with a primary prevention indication for an ICD. Patients were randomized to one of three programming schema as outlined in **Table 5**. Both a high rate cutoff and long delay strategy led to a significant reduction in both total shocks and inappropriate shocks at an average of 1.4 years follow up. In the control group, 5% of patients received appropriate and 6% of patients inappropriate shocks compared with 5% and 3% in the high rate group and 4% and 3% in the long delay group. Interestingly, a statistically significant reduction in mortality was also observed in both the long delay and high rate groups (0.12% vs. 0.09% vs. 0.05%) (Moss et al., 2012).

While the details of these studies differ slightly they at least establish that in a primary prevention cohort the use of more conservative programming measures such as long detection delays and high rate cutoffs can significantly reduce the morbidity of ICD therapy without any change, and possibly improvement in mortality. The generalizability of these results to a secondary prevention population is not known and an individual patient's risk profile should be carefully considered when making programming decisions in this group. Other groups of patients such as those with catecholaminergic polymorphic ventricular tachycardia (CPVT) or an implanted left ventricular assist device may also benefit from a conservative programming strategy.

CPVT

The available data regarding defibrillator therapy in patients with CPVT is somewhat conflicting. While these patients are at high risk for life-threatening tachyarrhythmias, ICD therapy can be ineffective in a significant portion of these patients and potentially even

harmful. Retrospective cohort studies have demonstrated that arrhythmia mechanism appears to be a major determinant of the likelihood of success of defibrillation. In one cohort no episodes of observed polymorphic or bidirectional ventricular tachycardia (PMVT, BDVT) successfully terminated with ICD therapy, while 100% of episodes of sustained VF terminated with shock (Miyake et al., 2013). With these results in mind the authors recommended device programming with a single VF zone with rates as high as 250–300 BPM. A second retrospective cohort found similar results with only 3% of shocks delivered for PMVT or BDVT being successful and 83% of shocks for VF being successful (Roses-Noguer et al., 2014).

LVAD

While patients with implanted left ventricular devices obviously have severe cardiomyopathy and high risk of ventricular tachyarrhythmias, the clinical implications of these arrhythmias and the benefits of ICD therapy remain unknown. Additionally, the effects of CRT in this population have also not been defined. It has been well described that patients with LVAD in place may hemodynamically tolerate what are typically malignant VAs but potentially experience a reduction in right ventricular function leading to eventual decompensation. Retrospective studies have demonstrated that increased rates of VAs are associated with poor outcomes and that the presence of an ICD may be associated with improved outcomes. However, these studies are subject to potential confounding as all retrospective analyses are. Prospective, randomized controlled trials of ICD programming and therapies are needed in this population.

Battery Life

Device longevity is a vital component of ICD performance. Current drain in a pacemaker generator is fairly low output, and typically can be accomplished with a traditional lithium-Iodide battery. ICD shocks require up to 830 V of stored charge per full output ICD shock and require large capacitors. Shorter pulse generator battery life leads to more frequent replacement of ICD generators particularly with a CRT system, exposing patients to increased risk for lead damage, hematoma formation, infection, and even death. Many factors play a role in ICD longevity, including both device factors and patient factors. For example, patients who require pacing support from their ICD, or those who receive defibrillation will predictably experience a lower expected device longevity. A typical single full capacitor charge from an ICD battery will reduce the usable device capacity by roughly 1 month. In contrast, capture thresholds will have a variable effect on longevity depending on the frequency of pacing support required, that is, if pacing never occurs stimulation threshold has little to no effect on longevity.

Recent analyses of patients with CRT devices, which ideally pace both ventricles 100% to provide heart failure therapy through CRT, have additionally demonstrated that not only did a lower pacing threshold on left ventricular leads improve longevity but so did high LV impedance values (Ellis et al., 2016; Zanon et al., 2016). A rudimentary understanding of the principles of electric circuits clarifies this finding as circuits with higher impedance will conduct less total current at a given voltage. As such, higher impedance circuits will require less power (current multiplied by voltage) leading to longer battery life. Additionally, the particulars of device circuitry including AF diagnostics and wireless monitoring vary greatly between manufacturers, and can play a significant role in determining ICD longevity. Features such as automatic remote transmissions and automated capture threshold management will lead to variable rates of energy consumption depending on the manufacturer's design. These studies imply that physicians should carefully consider manufacturer's battery technology, whether certain device features are truly necessary, choice of lead type and programmed vector to optimize lead impedance and threshold, and ICD and LV lead position at the time of implantation to ensure maximal device longevity for their patients. Avoidance of an additional ICD generator change may reduce morbidity, mortality, and overall health care cost utilization.

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MR Imaging of the Heart and the Great Vessels

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Introduction

MRI Physics

Magnetic resonance imaging (MRI) generates images by harnessing the properties of hydrogen atoms, which are ubiquitously concentrated throughout the human body, particularly in water and fat. The main principle of MRI relies on using perturbations of the hydrogen atoms to create differential signals between unique tissue types, with generation of images. Initially, the stable magnetic field (B_0) of the MRI aligns hydrogen nuclei with its magnetic axis, a process called precession. Small additional magnetic fields are then applied in a controlled fashion (pulse sequence) to disturb this baseline precession. The shift in precession of the hydrogen atoms and relaxation properties of the atoms within each tissue are detected and processed to create visual images of tissue characteristics. The pulse sequences can be varied to investigate different aspects of the cardiovascular system and the large number of clinical relevant pulse sequences leads to the immense versatility of MRI.

The strength of a scanner's magnetic field is measured in tesla (T). Field strengths of 1.0, 1.5, and 3.0 T are commercially available for clinical application, and greater strengths are advantageous for animal studies. An increase in field strength offers improvement in spatial resolution leading to a better delineation of the heart and great vessels, but also to more artifacts.

Advantages of CMR as a Noninvasive Imaging Modality

Cardiac magnetic resonance (CMR) imaging has several advantages over other noninvasive cardiovascular imaging modalities. First, it achieves high spatial and temporal resolution. At field strength of 1.5 T, a spatial resolution of $1 \times 1 \times 3$ mm can be obtained with most pulse sequence strategies. Routinely, frame rates of 20–40 ms are acquired with cine sequences. CMR permits the comprehensive assessment of multiple different parameters of cardiovascular anatomy and function, integrating parameters that cannot be assessed by other techniques. In a single study, CMR can characterize anatomy and structure of the cardiac chambers, myocardium,

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and coronary arteries, visualize and quantify myocardial perfusion, assess myocardial viability and scarring, characterize myocardial tissue composition, and evaluate metabolism with spectroscopic techniques. CMR is able to acquire images in any tomographic plane, allowing manipulation of three-dimensional images. Though CMR may be susceptible to artifacts with greater body habitus, image quality remains significantly improved and may overcome suboptimal acoustic windows with transthoracic echocardiography or attenuation artifacts with radionuclide scintigraphy. Additionally, CMR is well suited for serial examinations in the clinical and research setting. It is able to differentiate tissue characteristics without need for ionizing radiation or administration of iodinated contrast or radioactive isotopes. Given that patients frequently require serial monitoring for cardiovascular disease, CMR eliminates the cumulative radiation exposure of other techniques such as myocardial perfusion imaging and computed tomography. The high reproducibility and low measurement variance in repeated samples underscores the high accuracy of CMR (Gandy et al., 2008) and is ideal for comparison of change in measured parameters over time.

MRI Safety

Though CMR has several outlined advantages, MRI safety is paramount. Two major considerations include implanted metallic cardiovascular devices and intravenous contrast administration. Regarding the former, the magnetic field of the CMR may theoretically cause shift in the position of ferromagnetic objects in the body. Additionally, the magnetic resonance pulse sequences can produce electric currents in leads, with associated arrhythmias shown in a small number of patients (Russo et al., 2017). The MRI radiofrequency pulses have been shown to generate a small amount of heat ($<1°C$) in implanted devices, resulting in possible interference with electronic components and wires (Russo et al., 2017).

Understanding of the risks and benefits of Gadolinium contrast agents in CMR is important. Intravenous gadolinium chelates are routinely used in contrast-enhanced MR angiography, myocardial perfusion, late gadolinium enhancement to identify scarring and viability, and the evaluation of cardiac masses. Adverse effects associated with gadolinium contrast agents range from mild allergic reactions (most commonly nausea, vomiting, cough, rash, or hives), moderate allergic reactions (e.g., tachycardia, hypertension, generalized erythema, or dyspnea), severe anaphylactic reactions (e.g., convulsion, unresponsiveness, cardiopulmonary arrest, or profound hypotension), and nephrogenic systemic fibrosis. In an international multicenter registry with 37,788 patients, the occurrence rate for mild, moderate, and severe allergic reactions was 0.11%, 0%, and 0.005%, respectively (Bruder et al., 2015). Nephrogenic systemic fibrosis is very rare but an important complication of gadolinium administration in patients with severe chronic or acute renal failure. As a result, screening for a reduction in renal function prior to a CMR study is recommended and gadolinium is contraindicated in patients with an estimated glomerular filtration rate <30 mL/min per 1.73 m^2. The doses of contrast agent given should be adjusted in patients with mild to moderate renal dysfunction.

Assessment of Vascular Anatomy

A variety of CMR methods exist to visualize vascular anatomy. T1- and T2-weighted dark blood techniques allow proper delineation of vessel walls (Krinsky et al., 1997) and bright blood imaging techniques make the assessment of blood flow possible.

Imaging the Coronary Arteries

Coronary magnetic resonance angiography (MRA) can detect the extent and severity of obstructive native coronary artery disease with high accuracy. Due to tortuosity, motion, and the small caliber of the coronary arteries (3–6 mm in diameter), as well as signals from adjacent epicardial fat and myocardium (Bunce et al., 2003), it is more technically challenging to image the coronary arteries than the great vessels and systemic vasculature. The diagnostic accuracy of coronary MRA depends on the scan technology used as well (Di Leo et al., 2016). For instance, scanning at 1.5 compared with 3 T yields lower sensitivity (1.5 vs. 3 T, 78%–88% vs. 82%–94%) and specificity (1.5 T vs. 3 T, 68%–91%; vs. 77%–89%) (Schuijf et al., 2006; Yang et al., 2009; Kato et al., 2010; Hamdan et al., 2011; Nagata et al., 2011; Di Leo et al., 2016). Also, 1.5 T CMR has been reported to have lower accuracy than cardiac computed tomography angiography when compared with quantitative invasive angiography as the reference standard (Schuijf et al., 2006), upon which 3 T CMR may improve (Hamdan et al., 2011). In an international multicenter study, coronary MRA had an accuracy of 87% and a negative predictive value of 100% for the diagnosis of left main or three-vessel disease, whereas the accuracy for the diagnosis of CAD in general, including single vessel disease, was only 72% (Kim et al., 2001). Accordingly, the negative predictive value of coronary CMR is valuable in patients with low to intermediate pretest probability to exclude significant CAD. Coronary MRA is also able to assess the anatomy and patency of coronary artery bypass grafts (Engelmann et al., 2000), though the advantage of CMR is evaluation of the proximal, rather than distal, vessels.

Other applications of coronary MRA are the identification of coronary anomalies, for example, suspected anomalous origins (**Fig. 1**) (McConnell et al., 1995) or coronary aneurysms (Mavrogeni et al., 2004).

CMR of the Thoracic Aorta

CMR is a versatile tool to image the aorta and aorta-related pathologies. It is used to assess anatomic abnormalities or to identify the location and extent of aortic disease as well as predisposing or resulting changes (Russo et al., 2006). This includes the identification

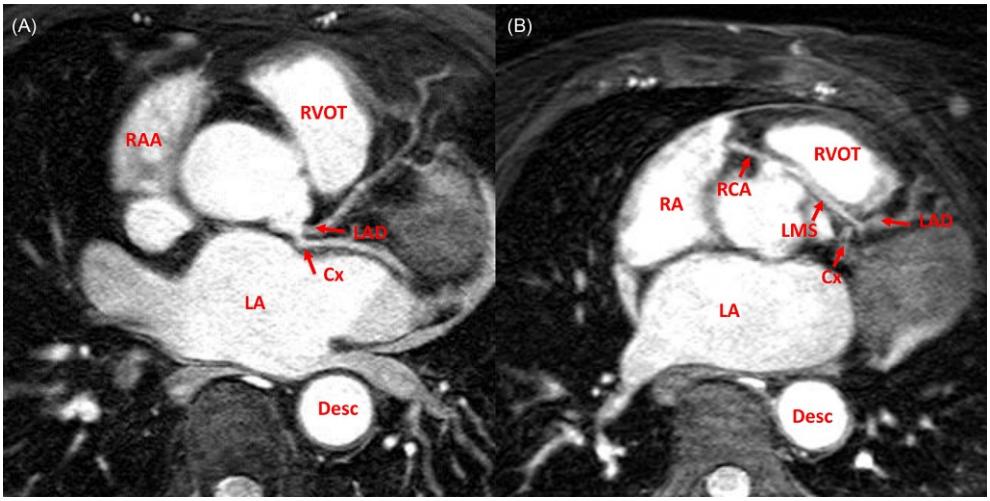

Fig. 1 Coronary MRA of a patient with anomalous origin of the coronary arteries. Submillimeter resolution free-breathing navigator gated/corrected T2 prep 3D turbo field echo coronary MR angiography of the aortic root and proximal coronary arteries were obtained in the transverse imaging planes. Shown are separate ostia of the left anterior and left circumflex coronary artery (A) and an anomalous origin of the left main stem arising anteriorly from the right coronary cusp with continuation between aorta and pulmonary artery in an interarterial course (B). In both the cases, the arteries have normal signal characteristics and caliber. *LMS*, left main stem; *LAD*, left anterior descending coronary artery; *RCA*, right coronary artery; *Cx*, circumflex coronary artery; *Desc*, descending aorta; *LA*, left atrium; *RA*, right atrium; *RAA*, right atrial appendage; *RVOT*, right ventricular outflow tract.

of aortic pathology such as aortic atherosclerotic plaque, aneurysm, ulcer, intramural hematoma, aortitis, or dissection (**Movie 1**) (Gebker et al., 2007). Due to three-dimensional imaging and the ability to reconstruct images in any plane, CMR can also be used to accurately measure the caliber of the aortic root, aortic arch, and supra-aortic branches. Similarly, CMR is able to delineate the location and degree of stenosis secondary to coarctation with accuracy similar to conventional angiography (**Fig. 2**). In a large cohort of children undergoing evaluation for congenital obstructive aortic abnormalities, such as coarctation or interruption, the diagnostic sensitivity and specificity of MR angiography in comparison with conventional angiography and/or surgery were 98% and 99%, respectively (Ming et al., 2006). However, CMR is superior to other imaging modalities with integration of anatomical and physiological assessment (e.g., trans-coarctation pressure gradient determination, collateral flow measurements) (Oshinski et al., 1996). Accordingly, CMR is able to distinguish pseudocoarctation (lack of hemodynamically significant narrowing, or collateral vessels) from true coarctation of the aorta.

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The accuracy of CMR in the diagnosis of thoracic aortic dissection is at least equal to computed tomography and transesophageal echocardiography (Sommer et al., 1996). However, the greater scan time for CMR as compared with computed tomography and limited acute clinical care available in the scanner area limit the use of CMR in emergent or clinically unstable situations.

CMR is able to detect and evaluate aortic atherosclerosis including information about plaque characteristics such as regions with lipid components, calcification, or plaque thrombosis (Fayad et al., 2000). An example of a patient with significant complex aortic atheroma is shown in **Fig. 3**. However, CMR has other limitations in comparison with alternate imaging modalities: limited temporal resolution to detect small mobile aspects of atherosclerotic plaque, for example, plaque itself or associated thrombi; lower spatial resolution in comparison with computed tomography; and greater estimation of plaque thickness in comparison with transesophageal echocardiography (Saam et al., 2007) leading to possible overestimation of cardiovascular risk.

CMR imaging of the aorta is useful for serial evaluation of pathology and to assess progression or regression of the underlying disease process. It is particularly favorable in younger individuals and women, in whom avoidance of exposure to ionizing radiation

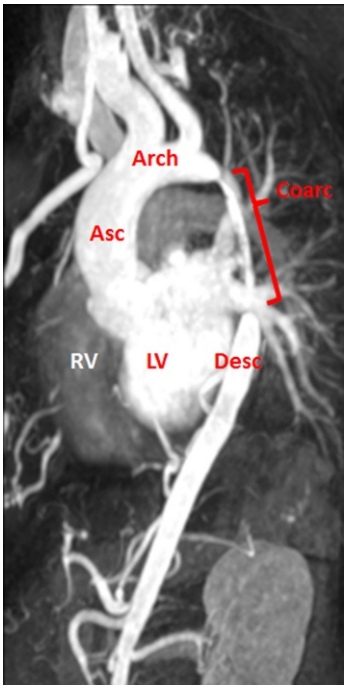

Fig. 2 MR angiography of a coarctation of the descending aorta after take-off of the left subclavian artery. First pass angiography of the aorta was obtained after administration of a bolus of gadopentetate dimeglumine 0.2 mmol/kg (40 mL Magnevist® solution). Shown is a three-dimensional reconstruction of the thoracic and abdominal vasculature. The diameter of the ascending aorta and arch was normal. The origin of the coarctation is very narrow and the coarctation extends for a total of 64 mm. *Asc*, ascending aorta; *arch*, aortic arch; *Desc*, descending aorta; *Coarc*, coarctation; *RV*, right ventricle; *LV*, left ventricle.

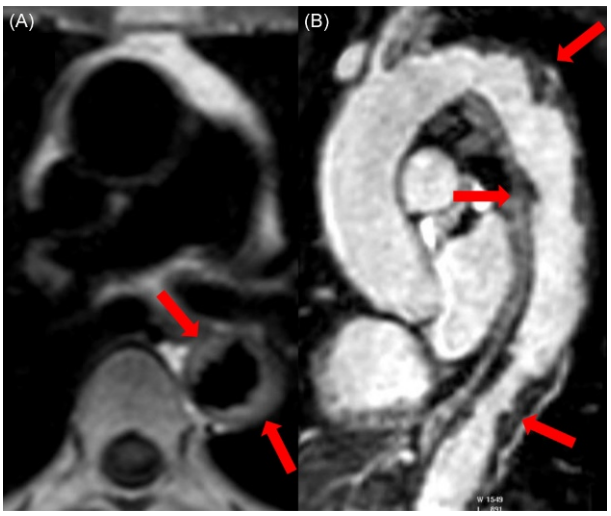

Fig. 3 Diffuse extensive atherosclerotic plaque within the thoracic aorta (*arrows*). Shown are a T1-weighted (*black blood*) dual-inversion T1-weighted fast spin echo images in axial orientation (A) and a 3D SSFP thoracic MRA reconstructed in a candy-cane orientation (B).

is preferred. CMR evaluation may also assist in pre- and postoperative assessment, for example, the identification of complications of surgery such as graft infections (Gaubert et al., 1995; Kawamoto et al., 1997). Additionally, CMR can provide functional data, including assessment of aortic wall stiffness and compliance, and quantification of forward and reverse aortic flow (Whitlock and Hundley, 2015). This information can be clinically relevant. For instance, in 2122 participants of the Dallas Heart Study, ascending aortic distensibility and aortic arch pulse wave velocity predicted nonfatal cardiac events and nonfatal extracardiac events, respectively (Maroules et al., 2014).

CMR Evaluation of the Pulmonary Vasculature

CMR angiography can be used to assess the pulmonary arteries and veins.

CMR is an effective method to image the anatomy of pulmonary veins. It is an accurate method to delineate congenital pulmonary venous abnormalities including an abnormal course (Fig. 4), to quantify pulmonary vein anatomy and size prior to electrophysiological studies and pulmonary vein isolation, and to visualize scar formation or pulmonary vein narrowing after ablation procedures (Fig. 5). A role for CMR in assessment of the pulmonary arteries and their pathologies is less established.

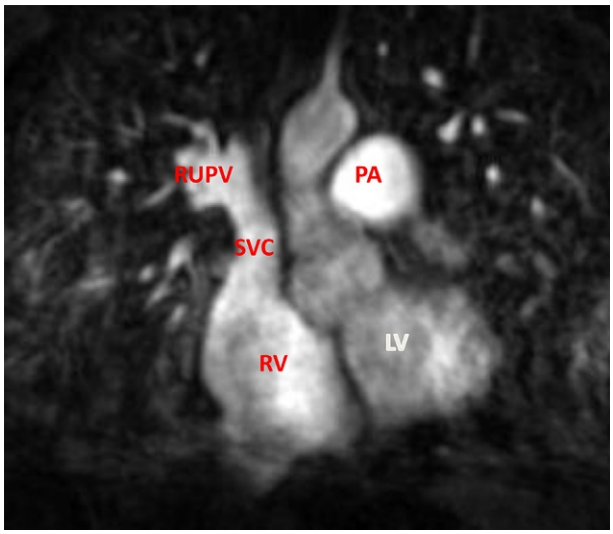

Fig. 4 MR angiography showing the right upper pulmonary vein draining into the superior vena cava. This image is acquired by first pass angiography of the pulmonary veins after administration of a bolus of gadopentetate dimeglumine 0.2 mmol/kg (33 mL Magnevist® solution). *RUPV*, right upper pulmonary vein; *SVC*, superior vena cava; *RV*, right ventricle; *LV*, left ventricle.

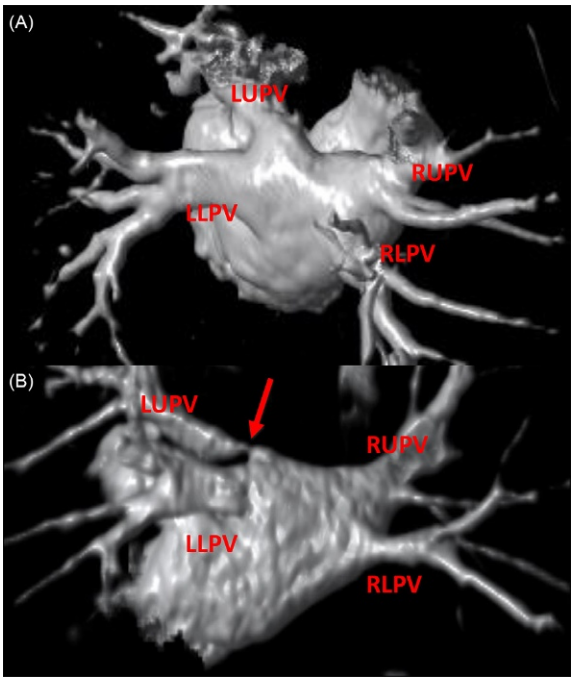

Fig. 5 Three-dimensional reconstruction of the pulmonary veins pre- (A) and post- (B) pulmonary vein ablation. The reconstruction is based on a first pass pulmonary vein angiography obtained after administration of a bolus of gadopentetate dimeglumine 0.2 mmol/kg (34 mL Magnevist® solution). Shown are a normal pulmonary vein anatomy with two left and two right pulmonary veins (A) and a focal stenosis (arrow) at the ostium of the left upper pulmonary vein after pulmonary vein ablation (B). *RUPV*, right upper pulmonary vein; *RLPV*, right lower pulmonary vein; *LUPV*, left upper pulmonary vein; *LLPV*, left lower pulmonary vein.

Imaging of Cardiac Anatomy

Congenital Heart Disease

Several features of CMR provide significant advantages in the evaluation of congenital heart disease in both the pediatric and adult populations: high spatial and temporal resolution, three-dimensional image acquisition, tissue characterization, blood flow quantification, and the lack of ionizing radiation. The latter in combination with the high reproducibility of CMR is very useful in a pediatric or a relative young population where repetition of scans pre- or postsurgical and/or during follow-up is crucial for clinical management. Supported by the listed features, CMR is used to accurately describe the anatomy of lesions (including the atria, ventricles, and great vessels) (**Movie 2**), their physiology (including conduit and great vessel blood flow) (**Movie 3**), and their effect on ventricular function. When validated against other gold standard techniques, anatomical assessment of congenital heart disease by CMR is more accurate than echocardiography (Fogel et al., 1994; Geva et al., 1994). Functionally, CMR can be used to quantify biventricular function (Markiewicz et al., 1987), to assess intracardiac shunts with pulmonary to systemic flow ratios (Beerbaum et al., 2001), and to measure regurgitation fractions after tetralogy of Fallot repair (van den Berg et al., 2007). Also, the identification of fibrosis and scar tissue by late gadolinium enhancement can guide electrophysiological procedures, pacemaker lead positioning, or risk stratification (Kilner et al., 2010; Fratz et al., 2013).

Pericardial Disease

CMR provides excellent visualization of the entire pericardium, allowing assessment of pericardial thickness, effusion, and abnormal structures, such as cysts (Sechtem et al., 1986). Also, CMR can detect abnormal cardiovascular pathophysiology to identify pericardial disease. Hepatic vein distension as well as flattening and paradoxical motion of the interventricular septum are signs of increased right heart pressures and seen in constrictive physiology. Real-time CMR of respirophasic changes in ventricular septal motion (Francone et al., 2005) and ventricular inflow (Thavendiranathan et al., 2012) can be used to assess ventricular coupling. These findings and pericardial contrast enhancement (Zurick et al., 2011) contribute to the differentiation of constrictive pericarditis from restrictive cardiomyopathy. CMR contrast enhancement of the pericardium (**Fig. 6**) by itself is, however, a relative nonspecific finding which may represent inflammation, infection, or tumor infiltration (Wang et al., 2003). Myocardial tagging, a technique applying a grid pattern to moving structures and allowing assessment of deformation, can be used to visualize the lack of systolic myocardial distortion adjacent to the pericardial cavity, a finding representing pericardial adhesion (Kojima et al., 1999). When present, the lack of myocardial movement adjacent to the pericardium, known as tethering, can be observed.

Valvular Function

CMR can be used to evaluate stenotic or regurgitant valve lesions. CMR sequences with high temporal resolution (20–40 ms) provide accurate visualization of the aortic cusp anatomy and measures of systolic valve opening area in aortic stenosis (**Movie 4**) (Kupfahl et al., 2004). However, as a consequence of longitudinal cardiac movement CMR likely overestimates the diastolic valve opening area of mitral stenosis (Djavidani et al., 2005). CMR provides the gold standard assessment of right and left ventricular volumes and systolic function, measured traditionally by ejection fraction (Bellenger et al., 2000). This allows accurate assessment of single-valve regurgitation by comparison of right and left ventricular stroke volumes (Didier et al., 2000). Quantitative calculation of degree of valve insufficiency, regurgitant volume and fraction, is facilitated by direct measurements of blood flow

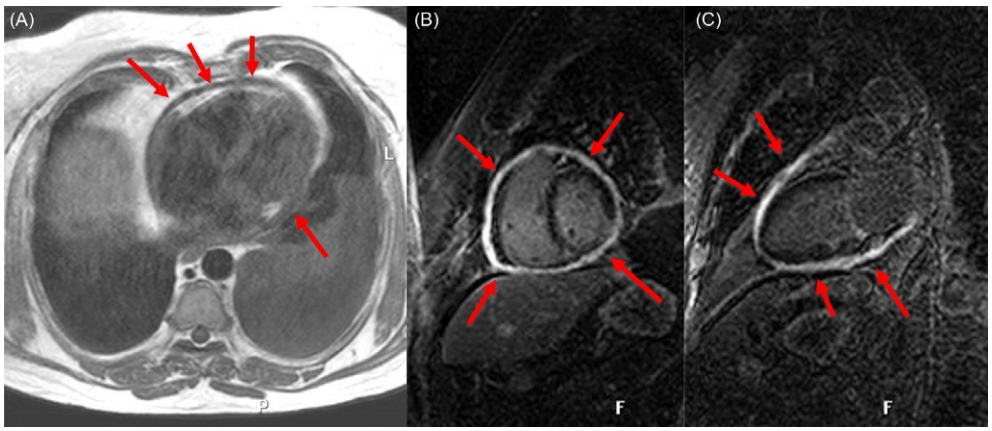

Fig. 6 CMR of pericardial thickening (*arrows*) without (A) and with late gadolinium enhancement (B and C). Shown are T1 weighted images of the pericardium (*black*) in a four-chamber orientation and late gadolinium enhancement images obtained with a segmented inversion-recovery TFE acquisition with a spectral fat saturation prepulse. The short-axis (B) and two-chamber long-axis (C) images were obtained 15 min after injection of a total of 0.1 mmol/kg gadopentate dimeglumine (10 mL Magnevist® solution).

(Didier et al., 2000; Dall'Armellina et al., 2007) or the combination of flow and stroke volumes (Hundley et al., 1995). CMR can also be used to quantify the regurgitant orifice area directly (Buchner et al., 2011). Compared with transthoracic echocardiography for evaluation of mitral regurgitation, CMR offers improved reproducibility (Cawley et al., 2013), improved prediction of left ventricular remodeling after surgery (Uretsky et al., 2015), and identification of myocardial fibrosis and scarring (Fernandez-Golfin et al., 2011). Due to accurate assessment of the proximal thoracic aorta, CMR has also advantages in assessment of aortic regurgitation if aortic root enlargement is suspected or established (e.g., Marfan syndrome, bicuspid aortic valve). In patients with severe aortic stenosis, mid-wall fibrosis also predicts worse improvement in left ventricular function after aortic valve replacement and is an independent predictor of long-term survival (Azevedo et al., 2010).

Cardiac Masses

CMR is an excellent method to characterize masses. Features such as size, location, mobility, impact on surrounding structures (e.g., infiltration or compression), and hemodynamic effects of cardiac and extracardiac adjacent masses are readily imaged. In addition to its superior accuracy when compared with echocardiography (Gulati et al., 2004), CMR has the advantage of tissue characterization. Quantification of water content allows the differentiation of edematous tissue or cystic structures from others (Dawson and Mohiaddin, 2011). The vascularity of a mass can be evaluated with first pass perfusion CMR (Fussen et al., 2011). Late gadolinium enhancement establishes the presence of extracellular matrix, its absence can be used to diagnose thrombus (Srichai et al., 2006; Pazos-Lopez et al., 2014), and in a small cohort study it had the accuracy of 79% for the differentiation between 17 benign and 25 malignant tumors (Pazos-Lopez et al., 2014). Also, the ability of specific CMR sequences to suppress the signal of fatty tissue enables the differentiation of lipomatous septal hypertrophy, lipomas, and liposarcomas from other cystic or solid cardiac tumors (Fussen et al., 2011). This technique was applied in **Fig. 7**, where the lack of suppression with a fat saturation prepulse excluded a lipoma/liposarcoma. Shown is a myxoma with low signal intensity with T1-weighted imaging and minimal enhancement after contrast administration suggesting low extracellular matrix content and low vascularity, respectively.

Evaluation of the Ventricular Myocardium

Heart Failure

Being the gold standard for volume assessment (Bellenger et al., 2000), CMR provides an accurate assessment of cardiac function (**Movie 5**) and morphology, such as left and right ventricular ejection fraction, left ventricular mass or wall thickness, in almost all patients. Regional wall motion abnormalities are well demarcated with cine SSFP sequences or with myocardial tagging (Castillo et al., 2006). For visualization of the advantages of CMR in anatomical and functional assessment of heart failure, **Movie 5** depicts the heart of a patient with dilated cardiomyopathy including severe left ventricular systolic impairment. Additionally, CMR can be used to identify tissue abnormalities indicative for specific pathologies (see "Ischemic Heart Disease" to "Nonischemic Cardiomyopathy" sections). For example, information about myocardial perfusion, viability, and fibrosis helps to identify an ischemic etiology in patients with new-onset heart failure (Valle-Munoz et al., 2009). Patterns of subendocardial or transmural in comparison with mid-wall fibrosis can also be used to differentiate between ischemic and nonischemic cardiomyopathies, respectively (McCrohon et al., 2003). The etiology of nonischemic cardiomyopathies can also be investigated with CMR: inflammation in acute myocarditis can be detected with T2-weighted pulse sequences (Skouri et al., 2006a), iron overload cardiomyopathy can be established with T2* relaxation times (Meloni et al., 2014), or late gadolinium enhancement following a noncoronary distribution is frequently seen in cardiac sarcoidosis (Greulich et al., 2016). Also, the quantity of late gadolinium-enhanced myocardium has a prognostic value in different nonischemic cardiomyopathies (Adabag et al., 2008; Green et al., 2012).

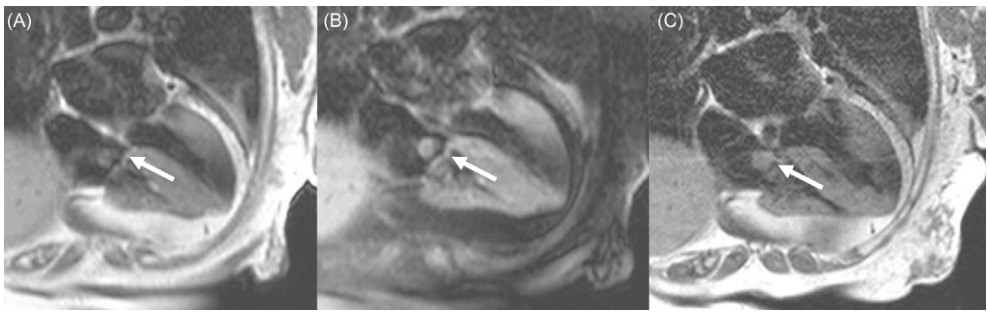

Fig. 7 CMR of a right atrial myxoma (*arrow*). Axial dual-inversion T1-weighted images of the myocardium were obtained in the four-chamber orientations with (A) and without (B) spectral fat saturation prepulses. C represents an image after the administration of gadopentate demeglumine 0.1 mmol/kg (8 mL Magnevist® solution). Shown is an 8 mm by 11 mm mass in the right atrium with low signal intensity with T1-weighted imaging (A), lack of suppress with fat saturation prepulse imaging (B), and minimal enhancement after the administration of contrast (C).

Ischemic Heart Disease

In addition to anatomic assessment of the coronary arteries (see "Imaging of Coronary Arteries" section), CMR is useful to detect the physiologic consequences of ischemia such as wall motion, perfusion, and metabolic abnormalities. Myocardial perfusion imaging and functional assessment with pharmacological stress are well-established for clinical practice, while CMR spectroscopy is a developing technique mostly limited to research. These will be explained in more detail in the following sections.

Myocardial perfusion imaging

CMR perfusion imaging visualizes the first passage of a gadolinium-based contrast agent through the myocardium. After contrast injection in the peripheral circulation, the appearance of contrast in the heart can be noticed as a rapid brightening of dark (nulled) myocardium during vasodilation stress. The signal intensity correlates with contrast concentration and the intensity mismatch between hypoperfused and normally perfused, brighter myocardium allows next to CMR reader dependent, qualitative also quantitative assessment. In a meta-analysis of 24 independent studies, the sensitivity and specificity of stress perfusion CMR for the detection of coronary artery disease were 0.91 and 0.81, respectively (Nandalur et al., 2007). When used to triage patients with suspected coronary artery disease in comparison with guideline-directed standard care, stress perfusion CMR significantly reduced the number of unnecessary invasive coronary angiographies (Greenwood et al., 2016). A negative perfusion CMR result in patients with established or suspected coronary artery disease has also a very good prognostic value (Jahnke et al., 2007).

Stress CMR: Evaluation of ischemia and viability

Stress imaging of ventricular function may be performed with CMR, similar to other stress imaging methods. As with pharmacologic stress echocardiography, dobutamine, in gradually increasing doses, may be administered to evaluate wall motion. Dobutamine infusion for CMR has a small risk (1:1000) of complications such as onset of sustained ventricular tachycardia (Wahl et al., 2004). In a meta-analysis of 14 independent studies, stress-functional CMR detected coronary artery disease with a sensitivity of 83% and a specificity of 86% (Nandalur et al., 2007). Another clinical question that can be answered with stress CMR is myocardial viability. In patients having LV wall motion abnormalities at rest, stress-functional CMR with low dosages of dobutamine allows to identify the contractile reserve, an improvement of wall motion under pharmacological influence. The presence of a contractile reserve indicates a recovery of systolic thickening after coronary arterial revascularization (Sayad et al., 1998; Trent et al., 2000). Stress-functional CMR, similar to perfusion imaging, has also prognostic value with a very low risk of cardiovascular death and myocardial infarction in patients with normal studies (Jahnke et al., 2007; Lipinski et al., 2013).

Magnetic resonance spectroscopy

A CMR technique with future promise in the diagnostic process of ischemic heart disease is magnetic resonance spectroscopy. Its strength is the assessment of myocardial metabolism without the application of contrast agents or radionuclides. For instance, phosphorus-31 nuclear magnetic resonance spectroscopy is able to measure directly high-energy phosphates in the myocardium and to identify metabolic evidence of ischemia (Bottomley et al., 1987; Weiss et al., 1990; Neubauer et al., 1992; Buchthal et al., 2000; Johnson et al., 2004). The technique measures the phosphocreatine to ATP ratio which is for instance reduced in myocardial infarction (Bottomley et al., 1987) or in the myocardium supplied by coronary arteries with flow limiting stenosis after isometric exercise (Weiss et al., 1990). Also, patients with dilated cardiomyopathy, where the ratio correlates with myocardial dysfunction (Neubauer et al., 1992), and women after isometric exercise with normal coronary arteries and chest pain syndrome (Buchthal et al., 2000) were shown to have abnormal values. In the latter, a decreased ratio predicted recurrent symptoms and hospitalizations (Johnson et al., 2004).

Myocardial Infarction

CMR in combination with gadolinium-based contrast agents can be used to image the extent and location of infarcted myocardium in patients with chronic or acute ischemic heart disease. The visualization of late gadolinium enhancement is based on the agent's ability to alter myocardial T1 values and to enter healthy myocardium from the blood pool within 1–3 min, the wash-in phase. Due to renal excretion, the myocardial concentrations decline 10–30 min after contrast administration, the washout phase. The large molecular size of gadolinium prevents the contrast agent from crossing cell membranes. Therefore, gadolinium effectively labels the extracellular space and, secondary to a delayed washout, accumulates for instance in regions of replacement fibrosis (Weber et al., 1995). These areas can be detected using T1-weighted sequences 15–20 min after contrast injection.

In myocardial infarction, late gadolinium enhancement is the gold standard technique to quantify infarct size. During different postinfarction stages, the area of delayed hyperenhancement mirrors closely the region of reduced perfusion, and overlaps with areas of necrotic or fibrotic histological changes (Fieno et al., 2000). The distribution of enhancement follows a subendocardial to transmural pattern and covers the effected vessel territory (**Fig. 8**). The presence of hypo-enhanced regions at the core of delayed hyperenhancement reflects on postinfarct microvascular obstruction (Judd et al., 1995) and predicts poor functional recovery of the infarcted territory (Rogers et al., 1999) as well as an increased risk of future cardiovascular events (Homback et al., 2005). Also, the transmural extent of late gadolinium enhancement relates to functional recovery of contractile function. In a study examining patients shortly after reperfusion, late gadolinium enhancement in no or less than 25% left ventricular wall thickness was a good predictor of global improvement (Choi et al., 2001). In patients without history of myocardial infarction but clinical suspicion for

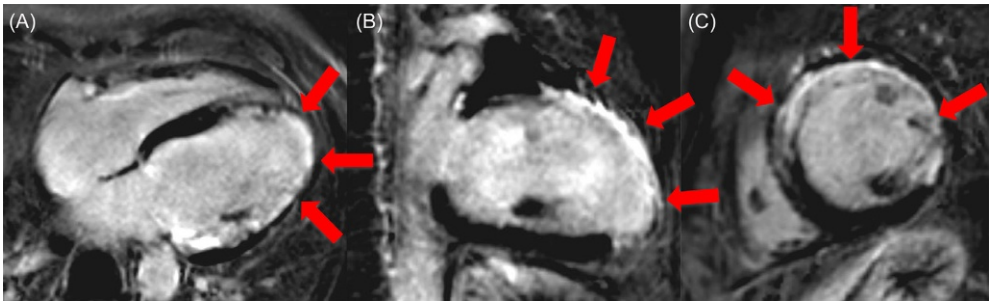

Fig. 8 Subendocardial to transmural late gadolinium enhancement (*arrows*) after a myocardial infarct in the mid segment of the left anterior descending artery. Late gadolinium images were acquired using a navigator-gated 3D phase sensitive inversion-recovery (PSIR) sequence with spectral fat saturation prepulse 15 min after injection of a total of 0.1 mmol/kg (18 mL) Gd-BOPTA (Multihance). Shown are transmural late gadolinium enhancement of the basal anterior and basal anterolateral wall segments in long-axis four-chamber (A), two-chamber (B), and short-axis orientation (C).

ischemic heart disease, the detection of a small area of late gadolinium enhancement carries a high risk for future cardiovascular events (Kwong et al., 2006). The prevalence of delayed enhancement with subendocardial distribution in a US-community based multiethnic cohort was 3.8% (Turkbey et al., 2015). If other distribution patterns were included, the number increased to 7.9% of which 78% were undetected by electrocardiography or clinical evaluation. In a small cohort of 70-year olds, undetected myocardial infarctions, defined as the presence of delayed hyperenhancement with subendocardial distribution and lack of prior diagnosis, doubled the risk of major adverse cardiac events (Barbier et al., 2016).

Nonischemic Cardiomyopathies

The term nonischemic cardiomyopathy covers a large variety of diseases which include genetic forms (arrhythmogenic right ventricular cardiomyopathy, hypertrophic cardiomyopathy, left ventricular noncompaction, and others), mixed forms (restricted, and dilated cardiomyopathy), and acquired forms (stress-induced cardiomyopathy, peripartum cardiomyopathy, myocarditis, and others). Diagnostic, therapeutic, and prognostic implications make the identification of a particular etiology important. CMR provides a noninvasive technique to distinct mentioned etiologies from each other through a combination of the following features: chamber size and structure assessment, left and right ventricular regional and global function quantification, perfusion imaging, and tissue characterization.

Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy is characterized by inappropriate myocardial hypertrophy ($\geq$15 mm), possible dynamic systolic obstruction of the left ventricular outflow tract, loss of diastolic function, and development of intramyocardial fibrosis. Of these, CMR is able to accurately quantify myocardial mass and regional wall thickness, to visualize increased flow velocities in the left ventricular outflow tract as well as the related systolic anterior motion of the mitral valve, and to examine for fibrosis.

Late gadolinium enhancement, as illustrated in Fig. 9, is present in 65% of patients with hypertrophic cardiomyopathy (range 33%–84%). It is typically distributed in a patchy mid-wall pattern in areas of hypertrophy and at the anterior and posterior right ventricular insertion points (Rudolph et al., 2009). Delayed enhancement is unusual in areas without hypertrophy with the exception of advanced stages in which transmural late gadolinium enhancement in regions of wall thinning is common (Rudolph et al., 2009).

The prognostic value of late gadolinium enhancement in patients with hypertrophic cardiomyopathy has been investigated in several studies, which were limited by incomplete risk assessment, selection, and referral bias. In a meta-analysis of four studies covering 1063 patients, a relationship between late gadolinium enhancement and all-cause death, cardiovascular mortality, and heart failure death was seen, while only a trend toward an increased risk of sudden cardiac death was noted (Green et al., 2012). In a more recent study of 1293 patients, the presence of late gadolinium enhancement in $\geq$15% the left ventricular mass was however associated with a twofold increase in sudden cardiac death event risk (Sado et al., 2013).

CMR rarely differentiates the causes of cardiac hypertrophy by their magnetic properties alone. The pattern and quantity of interstitial expansion can however indicate a specific diagnosis. For instance, Anderson-Fabry disease frequently features a reduced noncontrast T1 signal (Chan et al., 2014) and late gadolinium enhancement in inferolateral wall segments (Deva et al., 2016). Cardiac amyloidosis, on the other hand, often presents with similar myocardial and blood T1 signals (Vogelsberg et al., 2008) and with circumferential, subendocardial to transmural late gadolinium enhancement (Fontana et al., 2015a).

Nonischemic dilated cardiomyopathy

The majority of patients with heart failure and cavity enlargement in the absence of coronary artery disease have no late gadolinium enhancement (McCrohon et al., 2003). In a small cohort study, 28% of patients with dilated cardiomyopathy had late gadolinium enhancement in the mid-wall (McCrohon et al., 2003), which corresponds to fibrotic tissue on histology of explanted

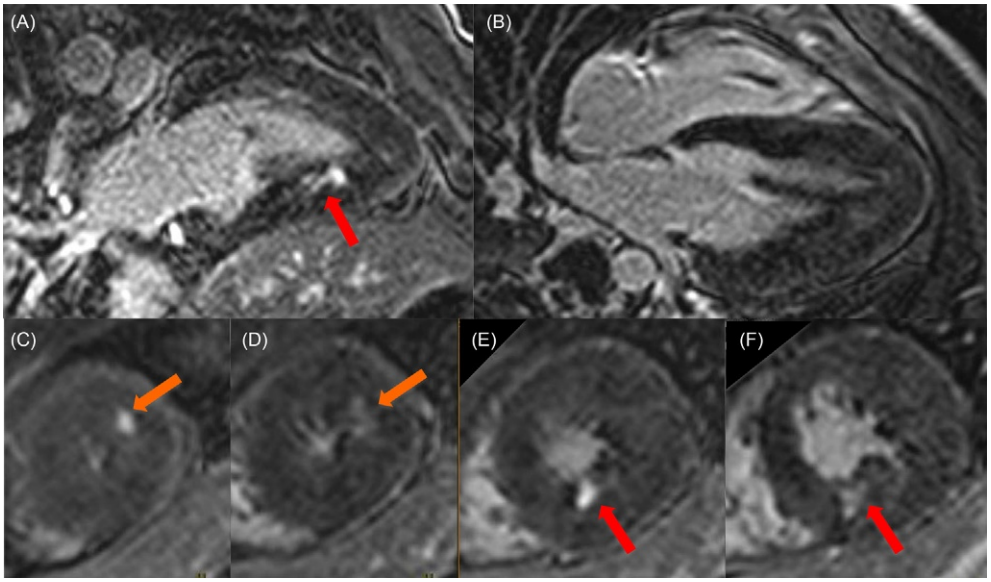

Fig. 9 Late gadolinium enhancement of the myocardium in a patient with hypertrophic cardiomyopathy. Using a segmented inversion-recovery TFE acquisition, images in long-axis two-chamber (A), four-chamber (B), and short-axis (apical C and E, mid E and F) orientation were obtained 15 min after injection of the total of 0.2 mmol/kg gadopentetate dimeglumin (24 mL Magnevist solution) with spectral fat prepulses. *Arrows* point toward areas of mid-wall late gadolinium enhancement in the inferior (*red*) and anterolateral wall (*orange*).

hearts (Gulati et al., 2013) and potentially represents the chronic healing phase of myocarditis. In a single-center observation study of 472 patients with dilated cardiomyopathy, detection of mid-wall fibrosis by CMR was associated with higher all-cause mortality and an increase risk for sudden cardiac death (Gulati et al., 2013). In patients undergoing cardiac resynchronization, the presence of fibrosis in dilated cardiomyopathy had a similar predictive value as its presence in ischemic cardiomyopathy patients (Leyva et al., 2012).

Myocarditis

CMR plays an important role in the diagnosis of myocarditis (Skouri et al., 2006b). Late gadolinium enhancement in the mid-wall and subepicardium, predominantly in the lateral wall of the left ventricle, is a typical finding in viral myocarditis (Mahrholdt et al., 2004). Also, early postcontrast T1-weighted enhancement of the myocardium (Friedrich et al., 1998) and T2-weighted imaging demonstrating myocardial edema are potential marker of inflammation in myocarditis (**Fig. 10**). In this context, the Lake Louise Consortium suggested in 2009 to base the diagnosis of myocarditis on the presence of at least two positive results from these three techniques (Friedrich et al., 2009). Nowadays other approaches might be more feasible, as the early enhancement ratio is difficult to reproduce and the more recently developing quantitative T2 mapping has several advantages over T2-weighted imaging alone. Also, quantitative T1 mapping has been shown to correlate with histologic inflammation in a rat model (Jeuthe and Wassilew, 2016) and, using the cut-off of 990 msec at 1.5 T, was able to distinguish patients with suspected myocarditis from controls with a sensitivity, specificity, and accuracy of 90%, 91%, and 91%, respectively (Ferreira et al., 2013). In another study, T1 and T2 mapping were also able to differentiate between patients with clinically suspected myocarditis and control subjects (Luetkens et al., 2016). The combination of these newer techniques with late gadolinium enhancement may be the best approach to a CMR diagnosis of myocarditis. For example, the diagnostic yield of the combination of native T1 and delayed enhancement was much better than the individual techniques in a small study comparing chronic myocarditis patients with control subjects (Hinojar et al., 2015).

Sarcoidosis

A large proportion (~50%) of patients with pulmonary have coexisting cardiac sarcoidosis, despite the lack of cardiac symptoms in the majority of cases. As myocardial involvement contributes largely to mortality, a proper diagnosis is clinically warranted. CMR's capacity for tissue characterization in addition to the accurate identification of typical features such as biventricular dilation and dysfunction is hereby helpful. The pattern of late gadolinium enhancement with cardiac sarcoid is variable. Classically, mid-wall or epicardial late gadolinium enhancement can be observed. Other distributions such as subendocardial or transmural enhancement in any location are however also possible (**Fig. 11**). In a study investigating 58 patients with biopsy-proven pulmonary sarcoid, one-third had evidence of late gadolinium enhancement, which occurred predominantly in the lateral and basal myocardium (Smedema et al., 2005). In another small cohort study of 81 patients with proven extracardiac sarcoidosis, patients with late gadolinium enhancement had a ninefold higher incidence of adverse events than those without (Patel et al., 2009). In both studies, CMR was able to identify more patients with cardiac sarcoidosis than standard assessment including a 12-lead ECG and at least one non-CMR cardiac imaging study (Standard Japanese Ministry of Health guidelines, Smedema et al., 2005).

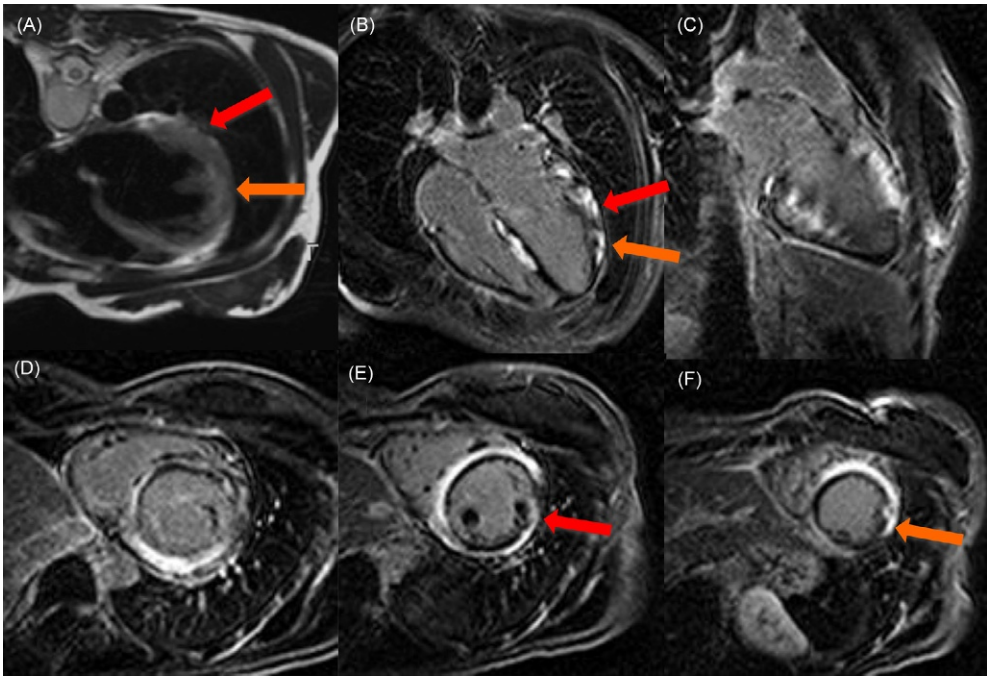

Fig. 10 CMR in a patient with acute myocarditis. Shown are a T2-weighted four-chamber long-axis image (A) and late gadolinium enhancement images obtained using a segmented inversion-recovery TFE acquisition (B–F). Short-axis (basal D, mid E, apical F), four-chamber (B), and two-chamber (C) long-axis images were obtained 15 min after injection of the total of 0.2 mmol/kg gadopentetate dimeglumin (24 mL Magnevist solution) with spectral fat prepulses. There is increased signal intensity of the left ventricular myocardium in comparison with the skeletal musculature on the T2-weighted image (A). There is late gadolinium enhancement with epicardial distribution of the basal and mid left ventricle and relative sparing of the apex (B–F). Color-coded arrows correspond to the basal lateral wall segment (*red*) and the mid lateral wall segment (*orange*). The overlap between increased intensity on T2 images (A) with late gadolinium enhancement images (B) suggests oedema/inflammation instead of fibrosis.

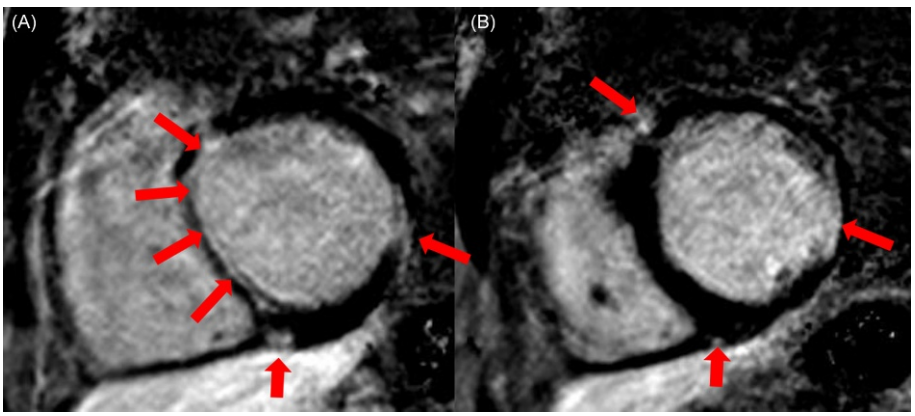

Fig. 11 Short-axis CMR at the basal-(A) and mid-(B) left ventricle in a patient with cardiac sarcoidosis. There is a moderate enlargement of the left ventricular cavity size and late gadolinium enhancement of the basal septum (endocardial), the basal to mid lateral wall (transmural), and the anterior and inferior right ventricular insertion points (*arrows*).

Arrthythmogenic right ventricular cardiomyopathy

CMR is the gold standard imaging technique for the diagnosis of arrthythmogenic right ventricular cardiomyopathy. The technique allows assessment of focal or global dilatation or dysfunction of the right ventricle (e.g., right ventricular bulging or aneurysms) and identification of fatty and/or fibrous replacement of the myocardium (**Fig. 12**). In up to 61% of cases these changes also involve the left ventricle (Santangeli et al., 2010; Marra et al., 2012). It is important to note that tissue characterization is not incorporated in current diagnostic criteria and that the diagnosis of arrthythmogenic right ventricular cardiomyopathy is based on a multimodality approach and not CMR alone (Marcus et al., 2010). Several reports showed that the detection of intramyocardial fat with CMR suffers from a high interobserver variability (Tandri et al., 2006; Macedo et al., 2007). Also, the detection of late gadolinium enhancement can be difficult due to disease-related right ventricular thinning and potential confusion with fat (Tandri et al., 2005).

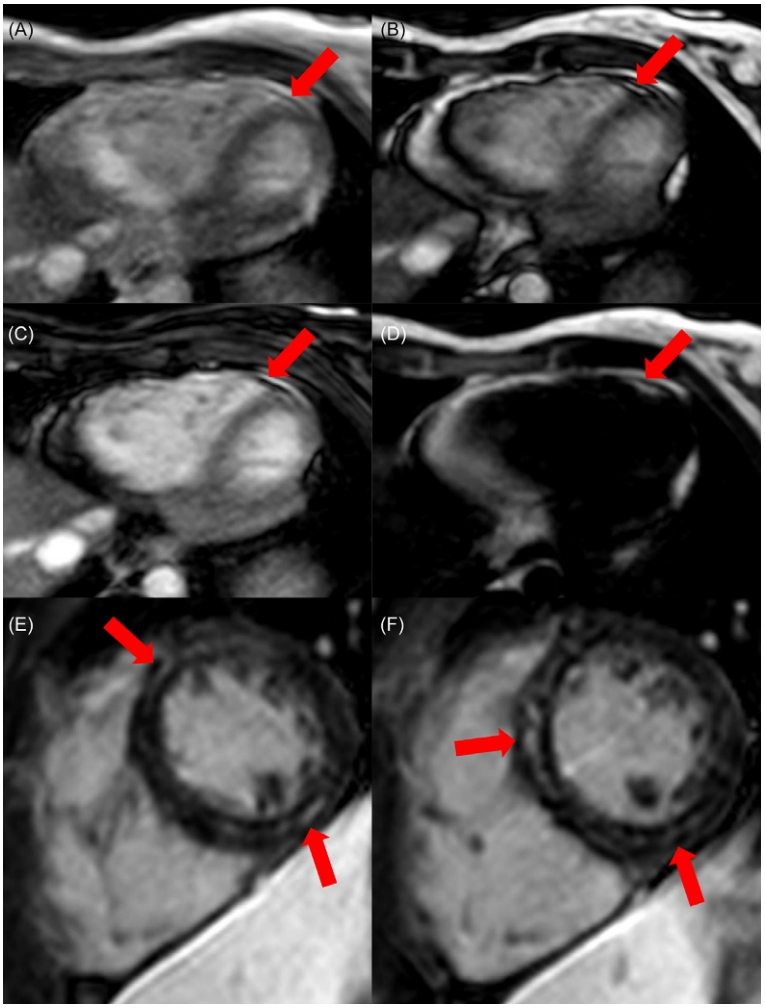

Fig. 12 CMR images of a patient with arrhythmogenic right ventricular cardiomyopathy. Shown are a Dixon-type pulse sequences (A, in-phase; B, out-of-phase; C, water only; D, fat only) in axial orientation and late gadolinium enhancement images acquired using a 3D phase sensitive inversion-recovery sequence with spectral fat saturation prepulses 15 min after contrast injection in short-axis orientation. There is fatty replacement of the distal right ventricular free wall (A–D, *arrows*) and late gadolinium enhancement in the septum, anterior, and inferior wall of the mid left ventricle (E–F, *arrows*).

Left ventricular noncompaction

Left ventricular noncompaction is a relative rare congenital abnormality that results from the failure of the myocardial development during embryogenesis. The diagnosis of noncompaction cardiomyopathy (**Movie 6**) with CMR is based on the end-diastolic ratio of noncompacted to compacted myocardium $\geq$2.3 on long-axis views (Peterson et al., 2005). Additional criteria, such as a ratio $\geq$3.0 on short-axis views (Stacey et al., 2013) or a noncompacted mass >20% of the global left ventricular mass (Jacquier et al., 2010), both at the end of diastole, have been suggested. In a study with 1480 asymptomatic participants free of cardiovascular disease, approximately 15% were positive for at least one of the mentioned CMR markers (Weir-McCall et al., 2016). These findings suggest that the established criteria are either quite unspecific for noncompaction cardiomyopathy or that a positive result might represent an anatomical phenotype instead of a distinct cardiomyopathy (Weir-McCall et al., 2016).

Amyloidosis

Amyloid deposition in the myocardium occurs frequently in patients with systemic amyloidosis. This leads to hypertrophy with reduced ventricular function. As mentioned (see 4.4.1), amyloid leads to abnormally T1 relaxations times (Vogelsberg et al., 2008), to an accelerate gadolinium clearance from the blood pool, and to a significant accumulation of gadolinium within the affected tissue (Am, 2005). CMR, with the characteristic pattern of global subendocardial late enhancement coupled with abnormal myocardial and blood-pool gadolinium kinetics, is therefore highly accurate to make the diagnosis (**Fig. 13**). The presence of subendocardial circumferential late gadolinium enhancement, for instance, provided a sensitivity of 80% and a specificity of 94% in 33 patients with cardiac amyloid on endomyocardial biopsy (Vogelsberg et al., 2008). In progressive disease stages, transmural late gadolinium enhancement can also occur and was shown to have a poor prognostic value in a study including 250 amyloidosis patients (Fontana et al., 2015b).

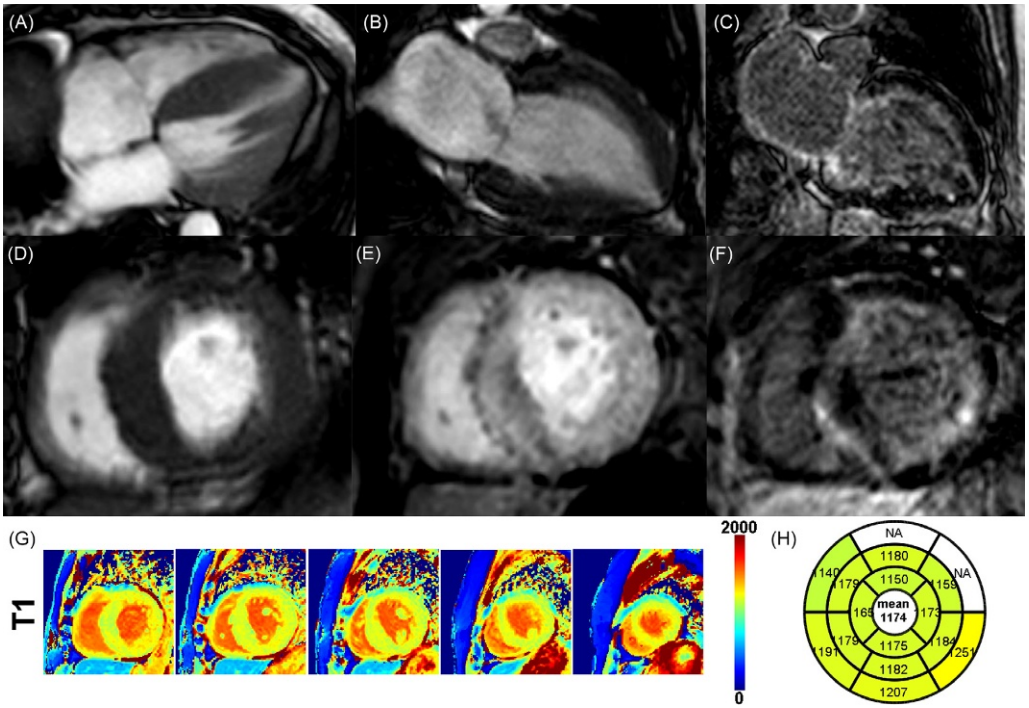

Fig. 13 CMR findings in a patient with cardiac amyloid. Depicted are the following pulse sequences: breath-hold bSSFP cine (A, B, D), early gadolinium enhancement with ultrafast 3D GRE inversion-recovery (E), late gadolinium enhancement with 2D/3D phase sensitivity inversion-recovery sequence (C, F), and free-breathing slice-interleaved T1 (STONE). The latter was used to generate T1 maps (G). Relevant findings were: asymmetric severe septal hypertrophy, atrial enlargement, diffuse circumferential early (E) and late gadolinium enhancement (C, F) with predominant subendocardial distribution, hypointense blood signal on late gadolinium enhancement images suggestive of abnormal blood-pool gadolinium (C, F), and an elevated mean T1 signal of $\geq$1157 ms as listed in the center of 16-segment model (H). *NA*, not applicable.

Conclusions

CMR is an advancing, noninvasive technique for comprehensive evaluation of the heart and great vessels. With sequences that can evaluate anatomy, function, and tissue characteristics at high spatial resolution and reproducibility, CMR has wide applications in the diagnosis and prognosis of patients with diverse cardiovascular pathologies and in research. With ongoing research designed to improve image quality, reduce acquisition time, develop noncontrast sequences to minimize risk, create novel methods for analyzing tissue characteristics and function, CMR will continue to remain a powerful modality for the clinician and medical researcher.

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Myocardial Perfusion Imaging

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Introduction

Myocardial perfusion imaging (MPI) is an important clinical tool that allows the noninvasive assessment of myocardial blood supply. It is a useful tool for patient diagnosis and prognostication. Frequently ordered for perioperative cardiovascular evaluation and chest pain, it allows the detection of flow-limiting coronary artery disease (CAD) without the risks of diagnostic coronary angiography.

There are various methods of performing MPI. The most widely used is single-photon emission computed tomography (SPECT) MPI, described in detail later. Other methods, depending on institution preference and technology availability, include positron emission tomography (PET) MPI, cardiac MRI perfusion, myocardial contrast echo, and cardiac computed tomography (CT). Each of these methods will be discussed in detail in this article, outlining the sensitivity and specificity of each, the technology involved, and the radiation risk, all important considerations for the clinician when deciding which modality to choose.

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SPECT MPI

The history of nuclear cardiology dates back to 1973 when the exercise stress-test myocardial scan was introduced (Zaret et al., 1973). Since that time, it has been increasingly used in clinical cardiology, to the current day where it is a widely available imaging modality used in the assessment of patients with suspected or known myocardial ischemia. Technical developments that have fueled this increase are single-photon emission computed tomographic (SPECT) imaging, pharmacological stress, and electrocardiogram (ECG)-gated imaging.

SPECT MPI is a nuclear medicine topographic imaging technique that uses gamma rays and involves flow-dependent and/or metabolism-dependent selective myocardial uptake of a radioactive tracer. Under conditions of stress, diseased myocardium receives less blood flow than normal myocardium. Poststress SPECT imaging reveals the distribution of the radiopharmaceutical and therefore the relative blood flow to the different myocardial regions. Comparing stress images with a set of images obtained at rest aids in diagnosis of reversible myocardial ischemia. A small amount of radioactive tracer (gamma-emitting radioisotope) injected intravenously is avidly taken up by normally functioning cardiomyocytes. Images acquired during dynamic exercise or pharmacologically induced stress and rest injections enable independent assessment of myocardial perfusion and viability.

To acquire SPECT images, the gamma camera rotates around the patient. Projections are acquired at defined points during the rotation, typically every 3–6 degrees. Typically, a full 360-degree rotation is used to obtain an optimal reconstruction. The time taken to obtain each projection is also variable, but 15–20 s is typical, giving a total scan time of 15–20 min. Multiheaded gamma cameras can provide accelerated acquisition. Conventional Anger cameras (Anger, 1953) typically have two large thallium-doped sodium iodide (NaI(Tl)) crystal detectors coupled to arrays of photomultiplier vacuum tubes, allowing two projections to be acquired simultaneously, with each head requiring 180 degrees of rotation.

Cardiac electrocardiogram (EKG)-gated acquisitions are possible with SPECT. Triggered by EKG to obtain differential information about the heart in various parts of the cardiac cycle, gated myocardial SPECT can obtain quantitative information about myocardial perfusion, thickness, and contractility and also allows the calculation of left ventricular ejection fraction (LVEF), stroke volume, and cardiac output. The D-SPECT camera was designed to increase sensitivity and spatial and energy resolution compared with A-SPECT.

Radiopharmaceuticals Commonly Used in Clinical Practice

Many choices exist for a myocardial perfusion agent. The ideal properties of a nuclear perfusion agent include the following:

1. A linear relationship between myocardial perfusion agent uptake and blood flow
2. High first-pass extraction over a wide range of blood flow rates
3. Low extracardiac radiotracer uptake
4. Minimal myocardial redistribution
5. Intrinsic chemical stability
6. Relatively straightforward radiochemical synthesis and purification

For SPECT, a family of technetium-99m-based radiotracers was developed in the 1990s including sestamibi, tetrofosmin, and teboroxime. Of these tracers, sestamibi and tetrofosmin are used widely in clinical practice and meet the majority of the earlier criteria (**Table 1**). 99mTc-teboroxime, despite its initial FDA approval, is not in widespread clinical use. Due to the high initial uptake and rapid washout, image acquisition must occur within 2 min of injection, which is technically challenging.

Thallium-201, first introduced in the early 1970s and having many favorable characteristics, has been extensively used as a perfusion radiotracer for this reason. It has a high first-pass extraction rate of 85%, and a better relationship is observed between blood flow and myocardial uptake at higher flow rates during stress testing (Krahwinkel et al., 1988). Often used as an agent to establish underlying myocardial viability in addition to ischemia, it has excellent myocardial first-pass extraction. However, its relatively low photon energy means that myocardial perfusion images with 201Tl are more prone to attenuation artifacts, especially seen affecting the inferior wall, as compared with 99mTc-labeled tracers, which have a higher-energy (140 keV) photopeak.

Table 1 Radiopharmaceuticals used in routine clinical practice

Radioisotope	*Chemical symbol*	*Chemical form*	*Emission energy (keV)*	*Half-life*	*Trade name*	*Diagnostic use*
Technetium-99M	99m Tc	Technetium 99m-pyrophosphate	140	6 h	Technescan PYP	SPECT
Technetium-99M	99m Tc	Technetium 99m-sestamibi	140	6 h	Cardiolite	SPECT
Technetium-99M	99m Tc	Technetium 99m-tetrofosmin	140	6 h	Myoview	SPECT
Thallium-201	201 Tl	Thallium-201 chloride	60–80	72.9 h	Amersham	SPECT
Rubidium-82	223 Ra	Rubidium-82 chloride		1.27 min	Cardiogen-82	PET
N13 ammonia	13 N	Nitrogen-13 ammonia		9.97 min		PET

Protocols for MPI

Imaging protocols are set out for each individual patient depending on certain factors such as the clinical question being asked, patient age and gender, ability to exercise, and body mass index. Small doses of radiotracer are injected at rest and peak stress, and subsequent images are acquired after the adequate circulation of radiotracer. As most Tc-based radiotracers are predominantly excreted through the gut, offering the patient food and plenty of liquids to assist with the passage of radiotracer through the gut is an important step prior to image acquisition. Failure to do so leads to attenuation artifact that can affect the perceived radiotracer uptake in the inferior wall and thus lead to erroneous results. Because the radiotracer is trapped at the time of injection, the images provide a view of myocardial perfusion at that precise moment, even in the case of delayed acquisition. If the patient is having ongoing chest pain at rest, a rest-only scan can provide important information, without stress, as to the underlying myocardial blood flow (MBF) at that precise moment.

Contemporary SPECT scanners are often combined with a CT scanner. The CT portion of the exam is used to guide patient positioning in the field of view and to correct inhomogeneities in radiotracer distribution from attenuation by soft tissue (known as attenuation correction (AC)). It can also be used for obtaining information regarding coronary calcium score +/− CT coronary angiography.

Detection of Ischemia and Viability

SPECT imaging performed post stress reveals the distribution of the radiopharmaceutical used and therefore the relative blood flow to different regions of the myocardium. Comparing stress images with a set of images obtained at rest aids in the diagnosis of myocardial ischemia by detecting reversibility (consider inserting example).

The presence of LV cavity dilation at rest or during exercise is noted upon the review of the rest and stress images. Dilation on both the stress and resting studies suggests LV dysfunction but may occur in volume overload states with normal ventricular function. Transient ischemic dilation (TID) is a marker for multivessel CAD. It is typically described qualitatively but may be quantified (Weiss et al., 1987).

The detection and localization of perfusion defects is typically performed by the use of visual analysis of the unprocessed images. Perfusion defects are assigned to a particular myocardial segment or segments according to the 17-segment model. Standardized nomenclature is used. Perfusion defect severity is typically expressed qualitatively with terms such as mild, moderate, or severe. Defect extent may be characterized qualitatively as small, medium, or large. A semiquantitative scoring approach standardizes the visual interpretation of scans, reduces the likelihood of overlooking significant defects, and provides an important semiquantitative index that is applicable to diagnostic and prognostic assessments. Furthermore, semiquantitative scoring can be used to reproducibly and objectively designate segments as normal or abnormal, as outlined later.

Category Score

- Normal perfusion 0
- Mild reduction in counts – not definitely abnormal 1
- Moderate reduction in counts – definitely abnormal 2
- Severe reduction in counts 3
- Absent uptake 4

Summed scores are calculated by adding all segment scores together to provide an integrated index of severity and extent of myocardial ischemia and infarction. This is done on the initial and resting or delayed images, and a summed difference score is then used to represent the amount of defect reversibility (Porenta et al., 1994), that is, the calculated difference between the score at stress and rest. Reversibility of perfusion defects is categorized qualitatively as minimal, partial, or complete. Reversibility can be defined quantitatively as defects in which pixels improve to fewer than 2.5 SDs from the normal reference distribution at that location. Through the use of SPECT imaging, it is now possible to view ventricular perfusion in any plane, to map the systolic function of the LV myocardium into single images, and to estimate chamber volumes (Garcia et al., 1981; Loong and Anagnostopoulos, 2004).

The use of polar maps allows a standardized 2-D representation of the 3-D myocardium for reproducible pixel-by-pixel comparisons of rest and stress studies. In addition, they allow follow-up of a patient over time or comparison between patients. Several different polar map generation algorithms are in widespread use. All have been clinically validated for regional perfusion, wall motion, and wall thickening (Kaul et al., 1987; Lin et al., 2006) (**Figs. 1** and **2**).

ECG Gating

ECG gating requires a stable and consistent heart rhythm in addition to sufficient temporal resolution to correctly characterize the cardiac cycle. A stable heart rate and rhythm are achieved by rejecting heartbeats that fall out of range at the expense of a longer

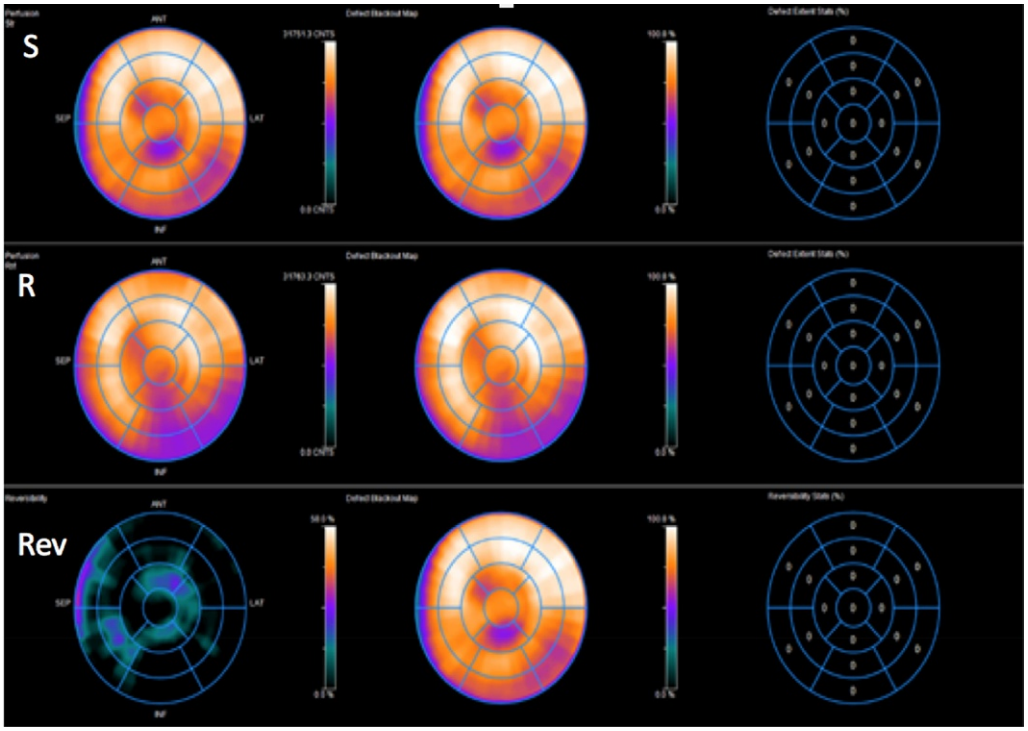

Fig. 1 Normal polar map showing normal perfusion with no defect identified. S, stress; R, rest; Rev, reversibility. The *left panel* represents perfusion, the *middle panel* represents a blackout map that would reveal the areas of perfusion defect present, and the *right panel* reveals a scoring chart laid out in a fashion similar to the 17-segment model, indicative of defect extent. The presence of zeros here indicated normal perfusion, that is, 0% abnormality.

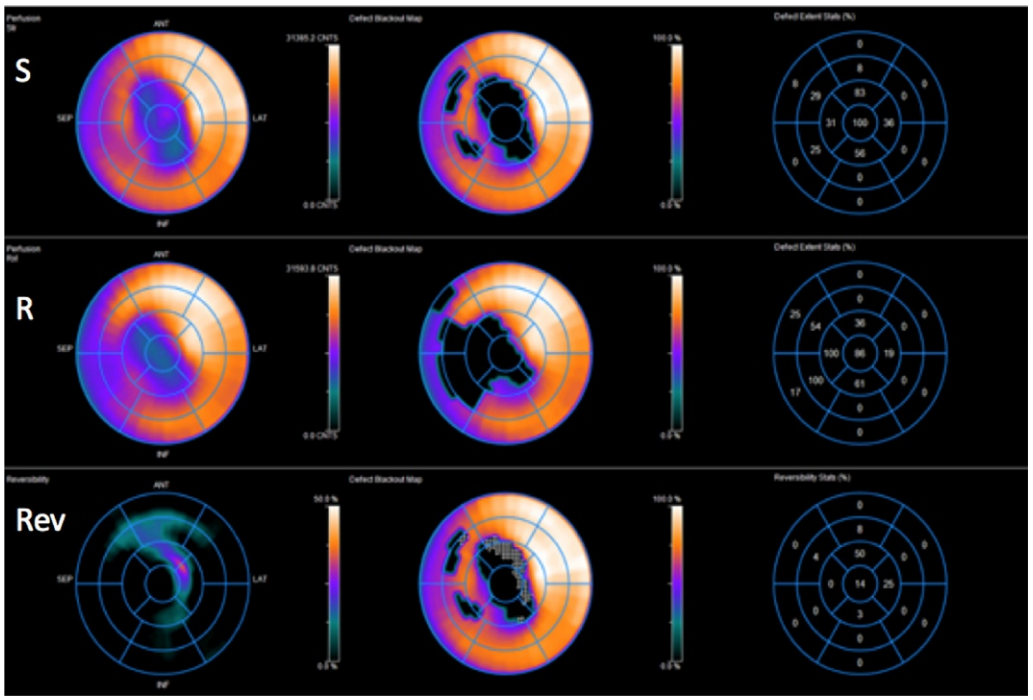

Fig. 2 Abnormal polar map with a medium to large area of scar in the region of the mid LAD, with partial reversibility: S, stress; R, rest; Rev, reversibility. In a similar fashion to the polar map earlier, the *left panel* represents perfusion, the *middle panel* represents a blackout map indicating the distribution of the perfusion defect, and the *right hand panel* indicates the defect extent (0%–100%) as set out by the 17-segment model.

image acquisition time. This "beat length acceptance window" can vary from 20% to 100% of the expected R-to-R duration. The recommended value is 20% if an "extra frame" is provided that allows the accumulation of rejected counts. Most stress laboratories gate the heart for eight frames per cycle. An increasing number of laboratories have reported good results with 16 frames per cycle and have used the increased temporal sampling to derive more accurate estimates of LVEF and parameters of diastolic function.

Sensitivity and Specificity of SPECT MPI

The value of SPECT in the diagnosis of CAD was highlighted in a meta-analysis (Loong and Anagnostopoulos, 2004), which demonstrated it to be a highly effective method of assessing myocardial perfusion with a sensitivity of 86%, a specificity of 74%, and a normalcy rate of 89%. The negative predictive value of the test is up to 98%, thus offering excellent prognostic value. A normal technetium-201 or technetium-99m scan is typically associated with a low risk of future cardiac events (less than 1% per year) (Hachamovitch et al., 1998). High-risk features on SPECT that have been shown to predict an increased risk of cardiac events include the following:

1. Extensive ischemia involving more than 20% of the left ventricle
2. Defects in more than one coronary vascular supply region
3. Reverse ischemia in multiple segments
4. Transient or persistent left ventricular cavity dilatation
5. Left ventricular ejection fraction <45% (Hachamovitch et al., 1998)
6. Increased lung uptake of thallium or sestamibi (Gill et al., 1987)

These factors predict an annual mortality rate of >3%. Normal, mildly abnormal, moderately abnormal, or severely abnormal perfusion defects have been reported to carry an annual rate of cardiac death of 0.5%, 2.7%, 2.9%, and 4.2%, respectively (Hachamovitch et al., 1998).

Case Examples

Case 1: Use of MPI to guide management in patient presenting with resting abnormal EKG. A 60-year-old female with hypertension, family history of CAD, and atypical chest pain presented with nonspecific T-wave changes on her EKG. Exercise SPECT was carried out. She achieved a moderately high workload on a Bruce treadmill protocol, and the following images were obtained (**Fig. 3**).

Case 2: Preoperative risk stratification in a patient with cardiac risk factors (high CHD risk) undergoing surgery. A 68-year-old male with a past history of coronary artery bypass graft surgery and severe peripheral vascular disease presented with an acutely ischemic limb. A preoperative risk stratification evaluation was sought. Given some recent symptoms of dyspnea on exertion and the inability to exercise, a pharmacological SPECT MPI was performed (**Fig. 4**).

The gated images revealed akinesis of the basal and mid inferolateral segment and hypokinesis of the basal anteroseptum. These findings were overall consistent with a prior transmural infarction in the distribution of the left circumflex coronary artery and a small area of ischemia in the distribution of the LAD/LIMA graft.

The patient was managed medically and proceeded with vascular surgery without cardiac adverse effects.

Case 3: SPECT MPI and cardiac catheterization correlation. A 69-year-old patient with a history of paroxysmal atrial fibrillation, hypertension, type 2 diabetes, and obesity presented to the office complaining of worsening dyspnea. A SPECT MPI was scheduled for further evaluation (**Figs. 5** and **6**).

Overall, the consensus from these images was that the inferior/inferolateral reversible perfusion defect seen on the SPECT was a result of the most severe coronary lesion in the setting of three-vessel disease in a diabetic with diffuse coronary disease. The high-risk findings from the SPECT included increased RV tracer uptake and TID. The patient underwent coronary artery bypass grafting (CABG) and did well post-op.

Myocardial Perfusion PET Imaging

Fundamentals

PET is a nuclear medicine technique that utilizes positron-emitting radionuclides to generate images of myocardial perfusion and metabolism. Through the use of positron-emitting radionuclides, unique quantitative information of important biological processes, such as myocardial perfusion, glucose metabolism, and fatty acid metabolism, can be obtained in vivo (Le Meunier et al., 2006). The fundamental principle is that positron-emitting radionuclides (e.g., carbon-11, nitrogen-13, oxygen-15, and fluorine-18) decay by emitting "positively" charged electrons called positrons. Once released from the nucleus, positrons travel short distances in tissue and annihilate when they encounter nearby electrons. Annihilation releases energy in the form of two gamma rays or photons that are emitted in opposite directions, typically 180 degree from each other. The opposite high-energy photons (511 keV) are captured externally by an array of detector elements (scintillators) located in the PET gantry. The PET system is designed to facilitate the detection of 511 keV photons arriving at opposite detectors within a specific narrow temporal window and

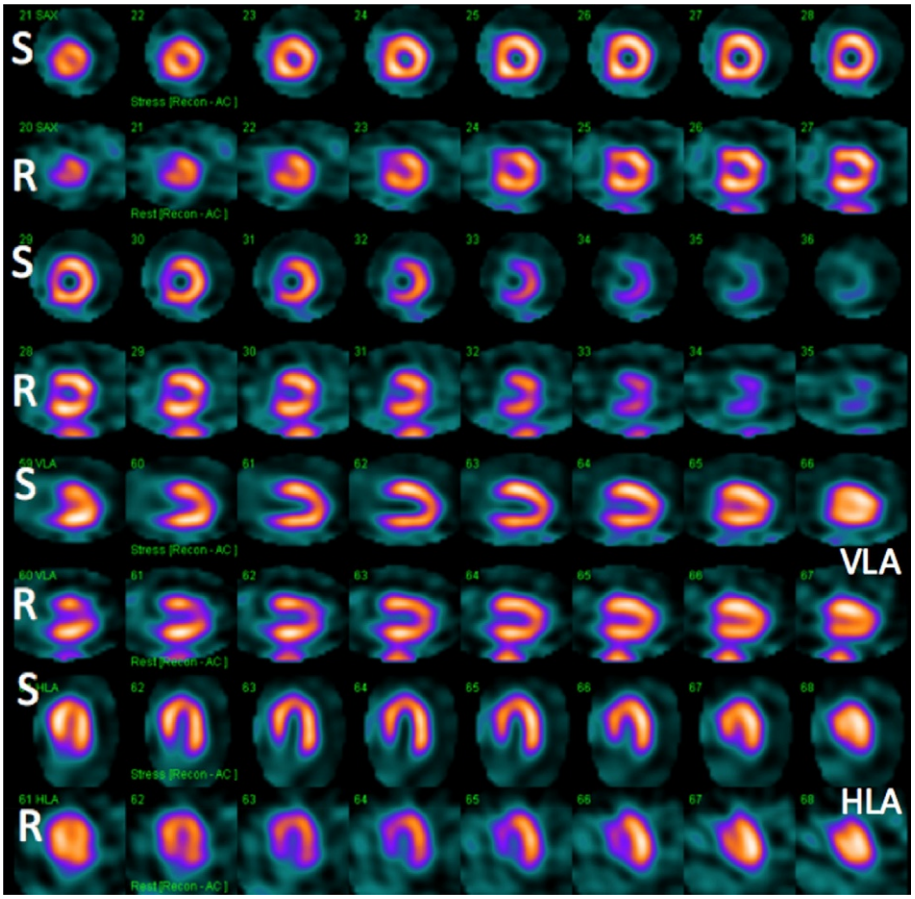

Fig. 3 Short-axis (top four rows, apex to base from left), vertical long-axis (VLA), and horizontal long-axis (HLA) images depict homogeneous radiotracer uptake at stress (S) and rest (R) with no significant perfusion defect identified. Gated images revealed normal LV volumes and ejection fraction (EF).

reject scattered photons arriving outside the preset temporal window, thus enhancing the spatial and contrast resolution of the images obtained.

The technical advantages that account for the improved image quality and diagnostic ability of PET compared with SPECT include the following:

(a) Routine measured AC, which decreases the number of false positives and thus increases specificity.
(b) High spatial and contrast resolution allowing for improved detection of small perfusion defects.
(c) High temporal resolution allowing fast dynamic imaging of tracer kinetics. This makes the absolute quantification of myocardial perfusion (in milliliters per minute per gram of tissue) possible.
(d) The use of short-lived radiopharmaceuticals allowing fast, sequential assessment of regional myocardial perfusion (e.g., rest mand stress), thus improving laboratory efficiency and patient throughput.

Despite the earlier factors, SPECT scanners and imaging radiotracers (e.g., 99mTc agents and 201Tl) remain more widely available and less expensive than PET scanners and positron-emitting radiotracers (e.g., 82Rb and 13N-ammonia).

Patient Preparation

Similar to that for stress and rest myocardial SPECT imaging, it includes an overnight fast of 6 h or more. Patients should avoid caffeinated beverages for at least 12 h, and avoid theophylline-containing medications for at least 48 h.

Radiation Dose

Because of the use of short-lived radiopharmaceuticals, radiation doses to patients are lower with PET than SPECT (Knuuti, 2009). Also, PET imaging offers true-stress (rather than poststress) functional measurements and allows time-efficient (<30 min) stress–rest protocols (Di Carli et al., 2007) (**Table 2**).

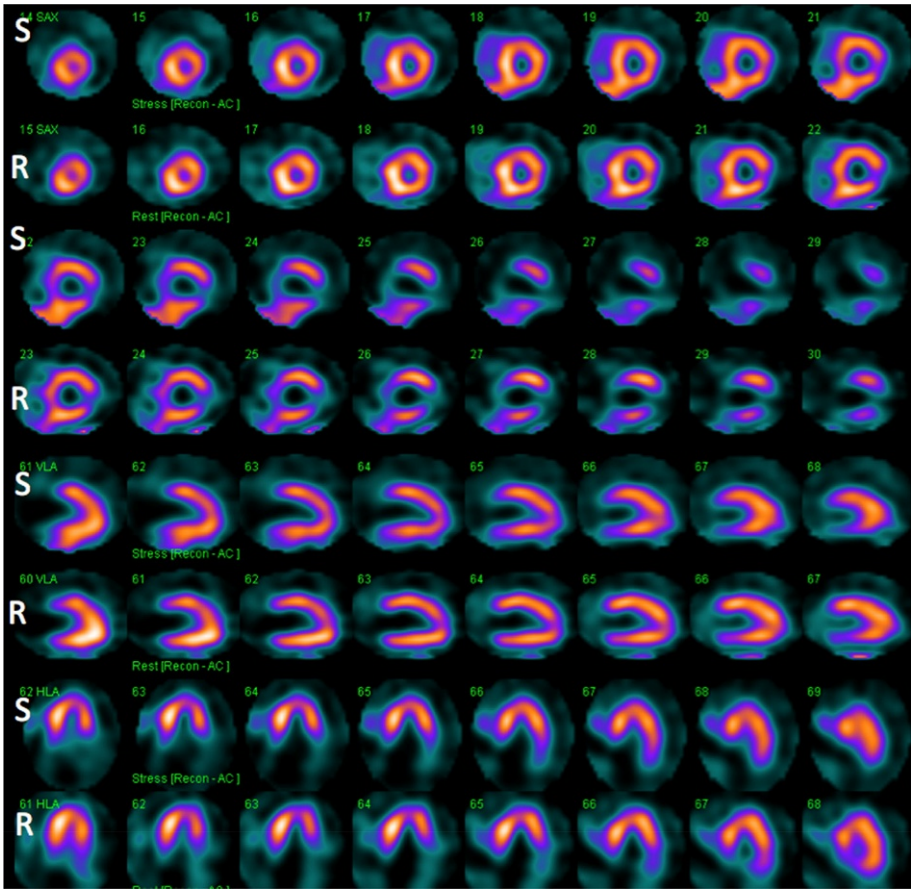

Fig. 4 This reveals the stress (S) and rest (R) perfusion images in short-axis (top four rows), VLA, and HLA projections. These reveal a large perfusion defect of severe intensity that is predominantly fixed affecting the basal and mid inferolateral segment. In addition, there is a small defect of moderately severe intensity with reversibility affecting the basal anteroseptum. The gated images revealed akinesis of the basal and mid inferolateral segment and hypokinesis of the basal anteroseptum. These findings were overall consistent with a prior transmural infarction in the distribution of the left circumflex coronary artery and a small area of ischemia in the distribution of the LAD/LIMA graft.

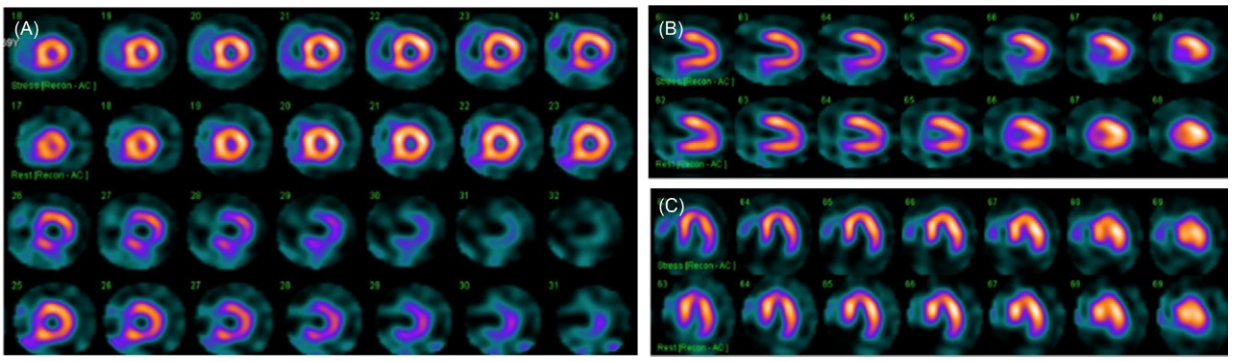

Fig. 5 (A) Short-axis slices, stress on top row, apex (*left*) through base (lower rows). (B) Vertical long-axis, anterior and inferior walls, stress on top row. (C) Horizontal long-axis, stress on top row, septal and lateral walls. These images demonstrate prominent RV radiotracer uptake at stress in addition to a medium in size, mild–moderate in intensity reversible perfusion defect involving the entire inferior and inferolateral walls, consistent with ischemia in the circumflex/PDA territory. The percentage of abnormal myocardium was 10%.

Comparative Studies of PET MPI Versus SPECT

The superior diagnostic accuracy and risk stratification of obstructive coronary disease of PET when compared with SPECT perfusion imaging has been demonstrated in several publications including a systematic review by Mc Ardle et al. (2012), which demonstrated that the sensitivity and specificity utilizing Rb-82 was 90% and 88%, respectively, higher than for comparable SPECT

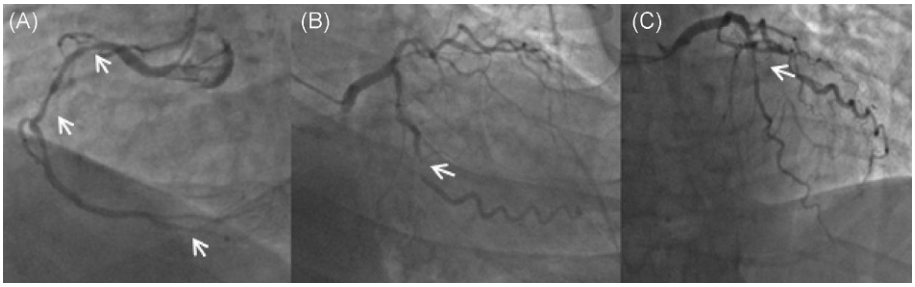

Fig. 6 (A–C) Still images of cardiac catheterization reveal significant disease involving the RCA (A), left circumflex (B), and LAD (C), *arrows*. Moderate-to-severe diffuse disease is seen in the RCA with a proximal 70%–80% stenosis. In the circumflex (panel B), the *arrow* indicated a severe stenosis in the midcircumflex. In the LAD (panel C), there is a proximal 80% stenosis (*arrow*).

Table 2 Common PET radiotracers in clinical use

Radiotracer	*Physical half-life (min)*	*Availability*	*Application*	*Advantages*	*Disadvantages*
Rubidium-82 (82Rb)	1.27	Strontium 82 generator	MPI	Simplicity of use	Relatively poor resolution
Nitrogen13 ammonia	9.97	Cyclotron	MPI	High extraction simplifies quantitation	Requires cyclotron Heterogeneous uptake
18F-FDG	110	Cyclotron	Myocardial viability imaging	Simplicity of radiotracer use	Oral glucose load can pose a problem for diabetic patients, requiring insulin use

data. Similarly, Parker et al. reported how PET MPI demonstrated a higher sensitivity for CAD than SPECT MPI (Parker et al., 2012). Myocardial PET also plays an important role in risk stratification. Dorbala et al. recently described how the extent and severity of ischemia and scar on PET MPI can provide powerful and incremental risk estimates of cardiac death and all-cause death compared with traditional coronary risk factors (Dorbala et al., 2013).

PET also has the capability to accurately evaluate regional and global MBF in addition to blood flow reserve. MBF (in milliliters per minute per gram of myocardium) is quantified during both rest and stress perfusion, and the ratio between peak stress and rest blood myocardial flow, the so-called coronary flow reserve (CFR), offers an enhanced ability to delineate the extent and severity of CAD to the level of coronary circulation (Schelbert, 2012). The ability to quantify MBF has been shown to have an incremental prognostic value for predicting adverse cardiac events (Ziadi et al., 2011; Taqueti et al., 2015).

Attenuation Correction

Positron-emitting tracers are more sensitive to attenuation artifacts than single photons. Coincidence detection systems must detect both simultaneously emitted photons. As each of the two photons is susceptible to tissue attenuation, attenuation artifacts are generally greater. Therefore, only attenuation-corrected images should be used for clinical interpretation (Dilsizian et al., 2016). Potential sources of errors include misalignment between emission and transmission data resulting from patient motion. Misalignments of 1.5–2 cm, for example, can lead to as much as a 30% change in the observed regional myocardial radioactivity (Le Meunier et al., 2006; Gould et al., 2007; Loghin et al., 2004).

Myocardial Viability Assessment Using PET

Stunned myocardium is viable myocardium that has been reperfused but exhibits prolonged postischemic dysfunction following reperfusion. On the other hand, hibernating myocardium is ischemic myocardium supplied by a narrowed coronary artery in which ischemic cells remain viable but contraction is chronically depressed. Stunned myocardium usually returns to normal after a prolonged period of time (hours to weeks). Hibernating myocardium returns to normal function quickly if the cause is removed. Cardiac PET is useful for the evaluation of patients with known CAD, in whom the amount of viable myocardium is unclear. In clinical practice, 18F-FDG is the radiotracer used to assess myocardial viability. Accompanying rest perfusion images may be obtained using either ammonia or Rb PET.

Myocardial viability assessment using PET consists of injecting radiotracer in order to acquire resting images and comparing these with a set of stress images as well as often using FDG in order to assess myocardial cell viability.

FDG imaging is performed following a 6–12 h fast followed by a glucose load. Under glucose-loaded conditions, FDG uptake by normal myocardium is maximized. A common approach includes using 25–100 g of oral glucose followed by supplemental IV

insulin as needed. Use of IV insulin promotes maximum uptake of FDG by the normal myocardium and optimal image quality. Once the patient has been injected with FDG, monitoring of the patient's glucose levels is continued until a stable glucose level is reached and the patient is asymptomatic.

Procedure

Rest images are acquired first (SPECT or PET) so that the areas of hypoperfusion are identified. LV volume assessment can be carried out by assessing LV size and function. A severely dilated left ventricle is unlikely to recover global function even if FDG uptake is present in segments with contractile dysfunction. Unless contraindications for stress testing exist, stress MPI is then performed to identify the presence and amount of reversible perfusion defects. This is because patients with moderate-to-severe (>10%) reversible perfusion defects will likely benefit from revascularization. Where possible, the rest/stress perfusion images should be interpreted prior to the commencement of the FDG study because if there is a moderate-to-large reversible perfusion defect or normal rest perfusion, then there may be no need to proceed with FDG PET imaging.

Four distinct resting perfusion–metabolism patterns may be observed in dysfunctional myocardium (Dilsizian et al., 2016; Marshall et al., 1983; Tillisch et al., 1986; vom Dahl et al., 1996; Beanlands et al., 1998; Gerber et al., 2001):

1. Normal blood flow associated with normal FDG uptake
2. Reduced blood flow associated with preserved or enhanced FDG uptake (perfusion–metabolism mismatch)
3. Normal or near-normal blood flow with reduced FDG uptake (reversed perfusion–metabolism mismatch)
4. Proportionally reduced blood flow and FDG uptake (perfusion–metabolism match)

The patterns 1–3 are all indicative of viable myocardium, whereas pattern 4 represents nonviable tissue.

PET MPI Protocols

Typically, clinical imaging protocols are sequential with stress following rest MPI, with time in between the tests for myocardial radiotracer activity to be decayed or diminish by biological pathways. Unlike SPECT, PET images are always corrected for attenuation. Conventional dedicated PET scanners typically use radionuclide transmission imaging in contrast to hybrid scanners that use CT transmission imaging for the measurement and correction of soft-tissue attenuation (Al-Mallah et al., 2010). The most commonly used CT protocol is a nongated low-dose CT in shallow tidal breathing. AC is important because inaccurate registration of transmission and emission images from respiratory or patient motion can lead to artifactual defects in up to 40% of cases (Kennedy et al., 2009; Lang et al., 2006; Lautamaki et al., 2008). Most commercial PET/CT systems now include software tools that correct for transmission–emission misalignments from respiratory motion or patient movement between the transmission and emission images (Al-Mallah et al., 2010). However, patient motion during either the transmission or the emission imaging will limit image quality and cannot be easily corrected using software (Naum et al., 2005).

Stress Protocols

Exercise stress with treadmill or bicycle and pharmacological stress with adenosine, dipyridamole, or dobutamine have all been used since the 1970s. In practice, due to the short half-life of most of the PET radiotracers, pharmacological stress is preferred. Adenosine produces maximal hyperemia and is short-lived, but it necessitates two intravenous lines for use with 82Rb and is hence somewhat cumbersome to use. More recently, regadenoson, an adenosine A2A receptor agonist, has been approved for SPECT MPI (Al Jaroudi and Iskandrian, 2009). Advantages of use include bolus administration with standardized dosing regardless of body weight and better tolerability. Regadenoson as the pharmacological stress agent, when used in conjunction with 82Rb PET, allows ultrashort imaging protocols with complete stress and rest MPI completed in approximately 17 min. It is also possible to use dobutamine as a stress agent, in patients with contraindications to the earlier agents. This agent requires more than one IV access site, careful timing is necessary especially when used in conjunction with ammonia as timing of dose arrival from the cyclotron, and peak stress needs to be coordinated carefully.

Quantification of Left Ventricular Function

ECG-gated PET imaging has the capability to assess LV function, both at rest and during stress. The addition of stress LVEF measurement to clinical and perfusion information has significantly enhanced the value of the 82Rb MPI to predict all-cause mortality (Lertsburapa et al., 2008). A large body of evidence supports the prognostic value of PET MPI-derived EF in intermediate- to high-risk subjects, in female patients, in obese individuals, and in post CABG individuals (Dorbala et al., 2007). A normal perfusion study indicates low risk (<1% annualized rate of cardiac events), while an abnormal study indicates high risk (Dorbala et al., 2007). Dorbala et al. (2007) demonstrated that LVEF at rest increases during peak vasodilator stress in patients with normal MPI. Patients with severe ischemia however and those with three-vessel/left main disease demonstrate a significantly lower LVEF during peak vasodilator stress and a lower LVEF reserve. They found that an LVEF reserve <+5% is a highly sensitive marker of left main/three-vessel CAD (sensitivity of 94% and a negative predictive value (NPV) of 97%) (Dorbala et al., 2007). Rest and peak stress LVEF is therefore routinely incorporated into the clinical practice of PET MPI.

MBF and CFR Assessment

Measurement of MBF (in milliliters per minute per gram of myocardium) is validated for most PET perfusion tracers (Camici and Rimoldi, 2009). In recent years, accurate AC and simultaneous tomographic acquisition of counts in list mode (with newer PET scanners) in addition to quick quantitation using commercial software have made MBF and CFR assessment the mainstream clinical practice rather than a predominantly research tool. These absolute measurements of tissue perfusion are accurate and reproducible (Dorbala et al., 2013). A relatively normal CFR (>1.9) essentially excludes the possibility of significant coronary stenosis (Naya et al., 2014). CFR is also a useful tool in the prognostication of patients with suspected CAD (Dorbala et al., 2013).

Coronary Artery Calcification

Myocardial PET/CT imaging also has the capability to screen for and measure coronary artery calcification. A high calcium score is associated with a greater burden of coronary atherosclerosis (Leber et al., 2001) and ischemia (Berman et al., 2004). This additional information is helpful in cases of small mild perfusion defects or where artifacts are suspected, causing falsely abnormal perfusion. In such cases, the absence of coronary artery calcification makes significant CAD (Bellasi et al., 2007) highly unlikely.

TID of the Left Ventricle

TID of the left ventricle with exercise and vasodilator SPECT MPI is a risk marker for multivessel severe obstructive CAD (Mazzanti et al., 1996; Chouraqui et al., 1990). It suggests underlying true ischemic cavity dilation or global subendocardial ischemia resulting in apparent cavity dilation (Dorbala et al., 2013).

Recent study data indicate that abnormal TID in 82Rb PET is associated with more extensive left ventricular dysfunction, ischemic compromise, and reduced global flow reserve. Preliminary outcome analysis suggests that TID-positive subjects have a lower overall survival probability and a lower likelihood of recovery of overall myocardial function post successful revascularization (Rischpler et al., 2012).

RV Tracer Uptake

Increased RV tracer uptake may be seen on both perfusion and metabolism images in the presence of pulmonary hypertension with or without significant RV hypertrophy (Schulman et al., 1993). Assessment of increased RV uptake is usually assessed in relation to the radiotracer uptake in the LV myocardium. Since the septum is shared by both ventricles, assessment of increased RV uptake should be made in relation to nonseptal regions of the LV myocardium. Abnormally increased RV tracer uptake is a qualitative assessment.

Lung Uptake

Increased lung uptake on the perfusion images especially when severe may reflect severe LV dysfunction and is a marker of increased LV end-diastolic and capillary wedge pressures. It can also reflect infiltrative diseases of the lungs and can be seen in smokers (Harper et al., 1973).

Clinical Case Examples

Case 4: A 71-year-old female with a femoral neck fracture who developed chest pain and a mild troponin rise postoperatively. Peak troponin was 0.05 ng/mL. She underwent myocardial PET perfusion with the measurement of CFR to assess for demand ischemia postoperatively (**Fig. 7** and **Table 3**).

Given the patient's comorbidities, her flat troponin trend, and how well her symptoms responded to medical therapy, the decision was made to manage her acute cardiac event medically. She thus avoided an invasive coronary catheterization, the rationale being that despite the reduced CFR, the normal perfusion images were reassuring. She was however treated with dual antiplatelet therapy, statin, and beta-blockade as a result of the PET findings. In addition, the CT for AC revealed moderate three-vessel coronary calcification that supported the decision for medical therapy.

Case 5: A 61-year-old female developed chest discomfort and ECG changes post mastectomy. She had a background history of CABG 10 years prior. Cardiac risk factors include hypertension, dyslipidemia, diabetes, and obesity. Coronary angiography revealed multivessel disease, and it was not clear which vessel was the culprit lesion for the NSTEMI. She underwent myocardial perfusion PET imaging to determine regional ischemia and flow and to further guide revascularization (**Figs. 8** and **9**).

Following these results, the patient underwent revascularization of the left main stem, first diagonal branch, and left circumflex arteries (**Table 4**).

Case 6: A 58-year-old man with a background of type 2 diabetes mellitus and hypertension presented with clinical evidence of a stroke and was found to have ECG changes consistent with a late presentation STEMI. Given his acute CVA with hemorrhagic transformation on initial brain imaging, emergent coronary angiography and PCI were deferred. Subsequent coronary angiography

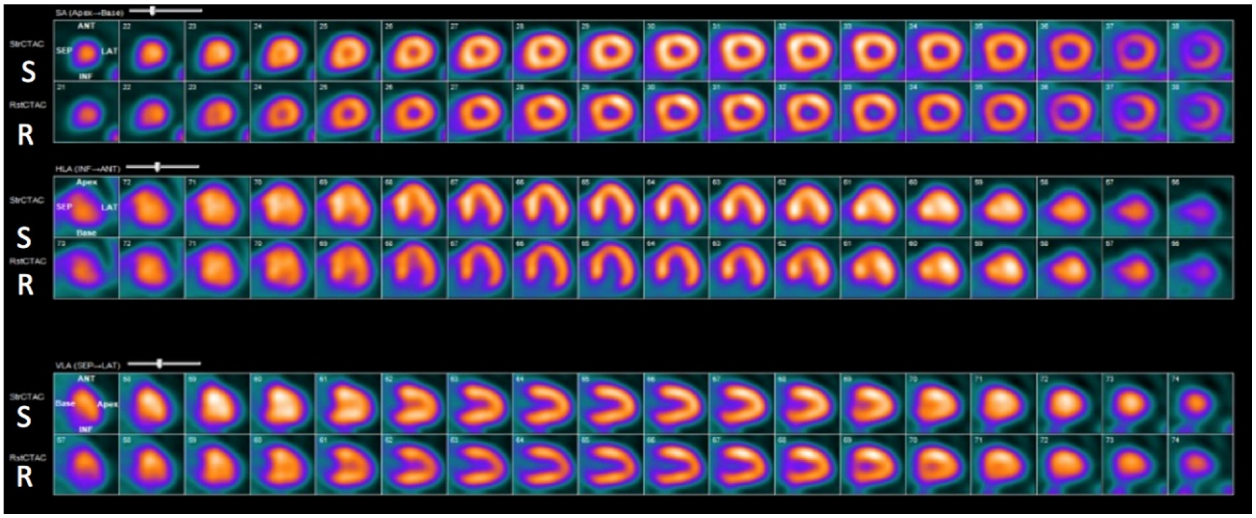

Fig. 7 PET perfusion images reveal stress (S) and rest (R) perfusion with short-axis slices represented in the *top row*, horizontal long-axis (HLA) view in the *middle row* and vertical long-axis (VLA) view in the *bottom row*. Images reveal normal myocardial perfusion. Gated images in this case revealed normal LV systolic function (LVEF 72% at rest and 82% with stress). Coronary flow reserve was also measured and was particularly useful in this case given the normal perfusion and function in the setting of an elevated troponin (see later). Images courtesy of Marcelo Di Carli MD, Brigham and Women's Hospital.

Table 3 Coronary flow reserve indicates globally reduced flow in all three coronary artery territories

Region	*Stress (mL/min/kg)*	*Rest (mL/min/kg)*	*Reserve (mL/min/kg)*
LAD	2.22	2.02	1.1
LCX	1.79	1.35	1.33
RCA	2.43	1.5	1.62
Total	2.13	1.67	1.27

Normal flow reserve is usually indicated by a value >2 mL/min/kg. Flow in this case was reduced to an overall value of 1.27 mL/min/kg

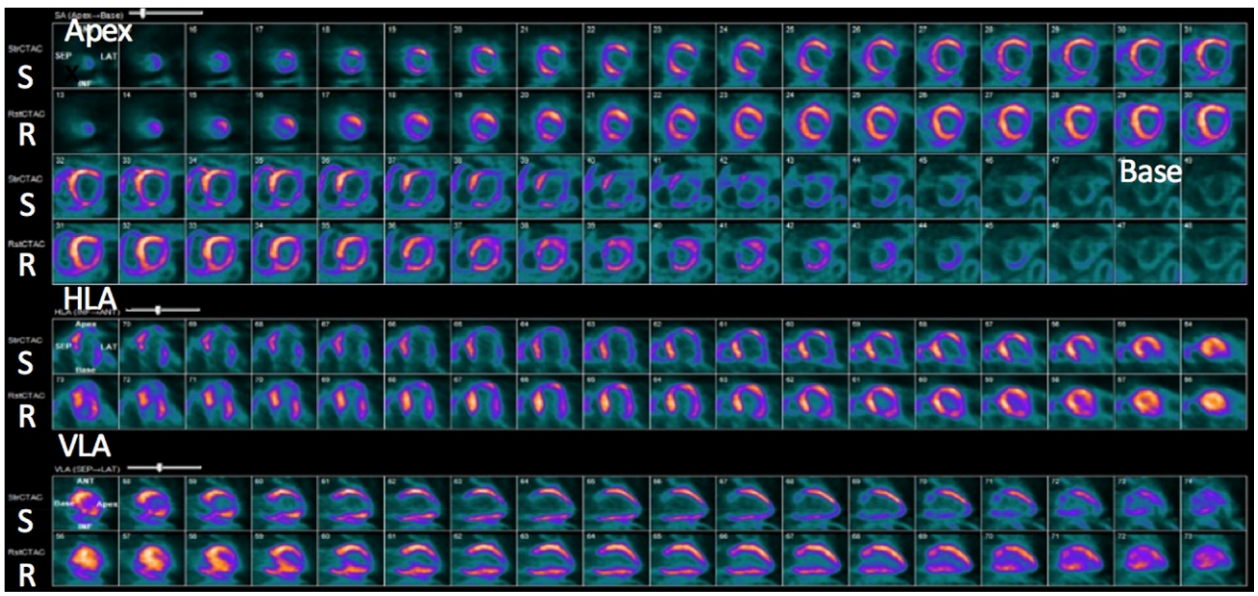

Fig. 8 Myocardial perfusion PET images reveal a large area of ischemia involving the apical, mid, and basal inferolateral segments (LCx territory); a medium sized area of ischemia in the apical, mid, and basal inferior and inferolateral segments (PDA territory); and a small amount of ischemia affecting the true apex on the HLA and VLA images (LAD territory). ECG-gated functional imaging reveals abnormal global LV systolic function. Images courtesy of Marcelo Di Carli MD, Brigham and Women's Hospital.

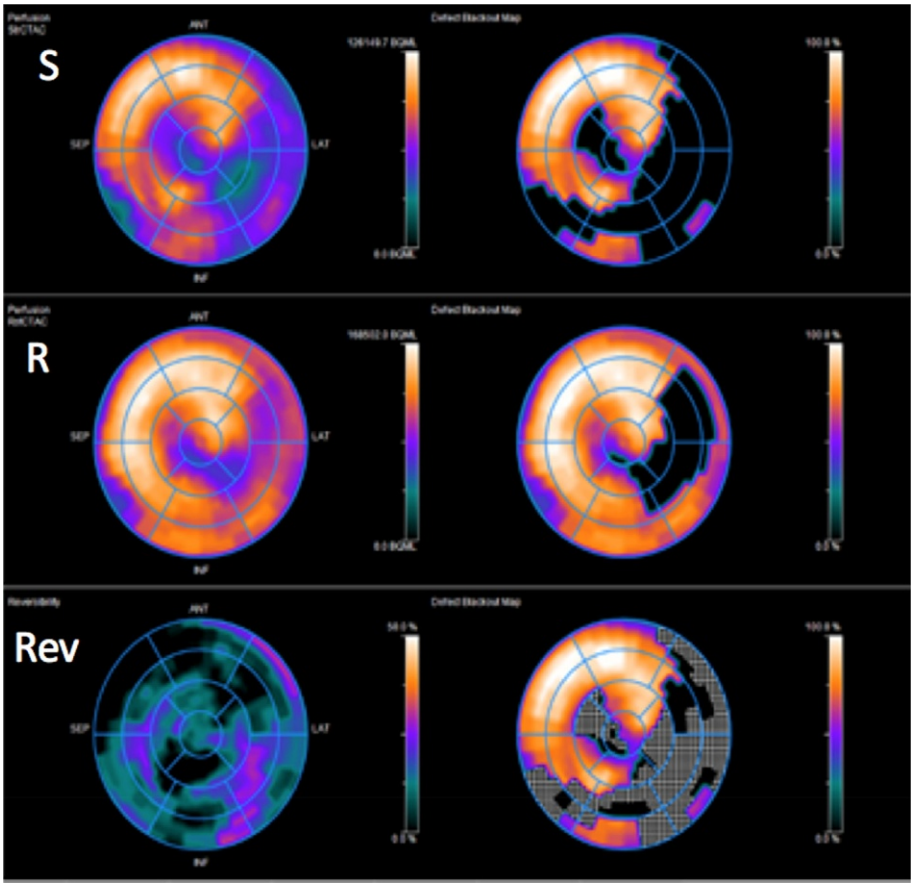

Fig. 9 Polar maps reveal the distribution of the perfusion defect (*left panel*), worse at stress (S, *top*) than rest (R, *middle*), and the blackout map on the right panel confirms the presence of reversible perfusion abnormalities in the distribution of LCX, PDA, and LAD, as described earlier. The reversibility is seen in the lower row. Images courtesy of Marcelo Di Carli MD, Brigham and Women's Hospital.

Table 4 Coronary flow reserve revealed severely reduced flow in all three coronary territories and overall severely reduced coronary flow

Region	*Stress (mL/min/g)*	*Rest(mL/min/g)*	*Reserve (mL/min/g)*
LAD	1.28	1.07	1.19
LCX	0.87	0.87	0.94
RCA	0.97	0.97	1.05
Total	0.99	0.99	1.09

revealed significant three-vessel disease. He underwent rest-only PET MPI to assess for ischemia/infarct burden and FDG imaging for viability assessment and to risk stratify prior to revascularization. Bedside echo revealed LVEF 30%–35% with anterior, anteroseptal, and apical hypokinesis to akinesis (Figs. 10–12).

The gated images revealed the presence of a dilated LV with severely reduced LV systolic function and an EF of 32%. The patient had an LV thrombus detected during contrast echocardiography, and therefore, intervention was deferred at that time. He was commenced on single-antiplatelet therapy and a novel oral anticoagulant in view of the LV thrombus. Four months later, he returned for repeat PET MPI, which is displayed in comparison with the initial rest imaging later (Figs. 13 and 14).

These findings support the hypothesis that in this particular case, the initial abnormal myocardium was likely stunned rather than hibernating, and with medical therapy, there was interval improvement in perfusion. Also, the LV remodeled with smaller LV volumes and an improvement in LVEF from 32% to 44%. The patient opted to continue with medical therapy rather than undergo high-risk PCI/CABG, given his recent stroke and multiple comorbidities.

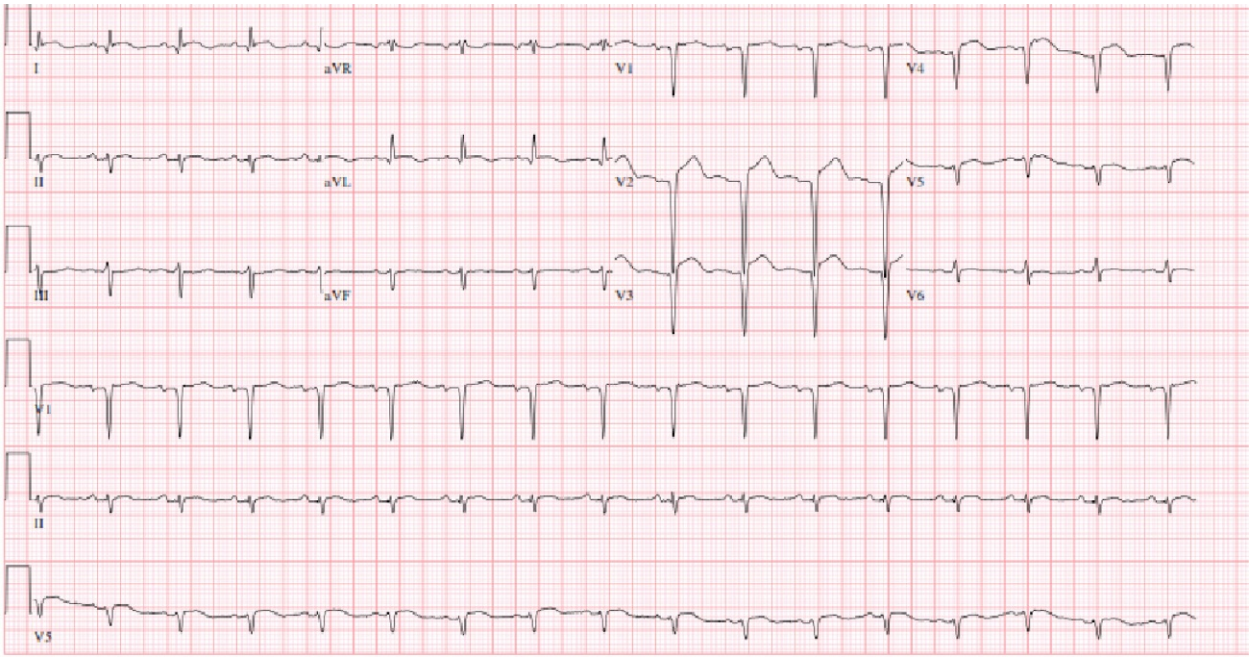

Fig. 10 EKG reveals anterior ST elevation and q waves. The patient denied chest pain.

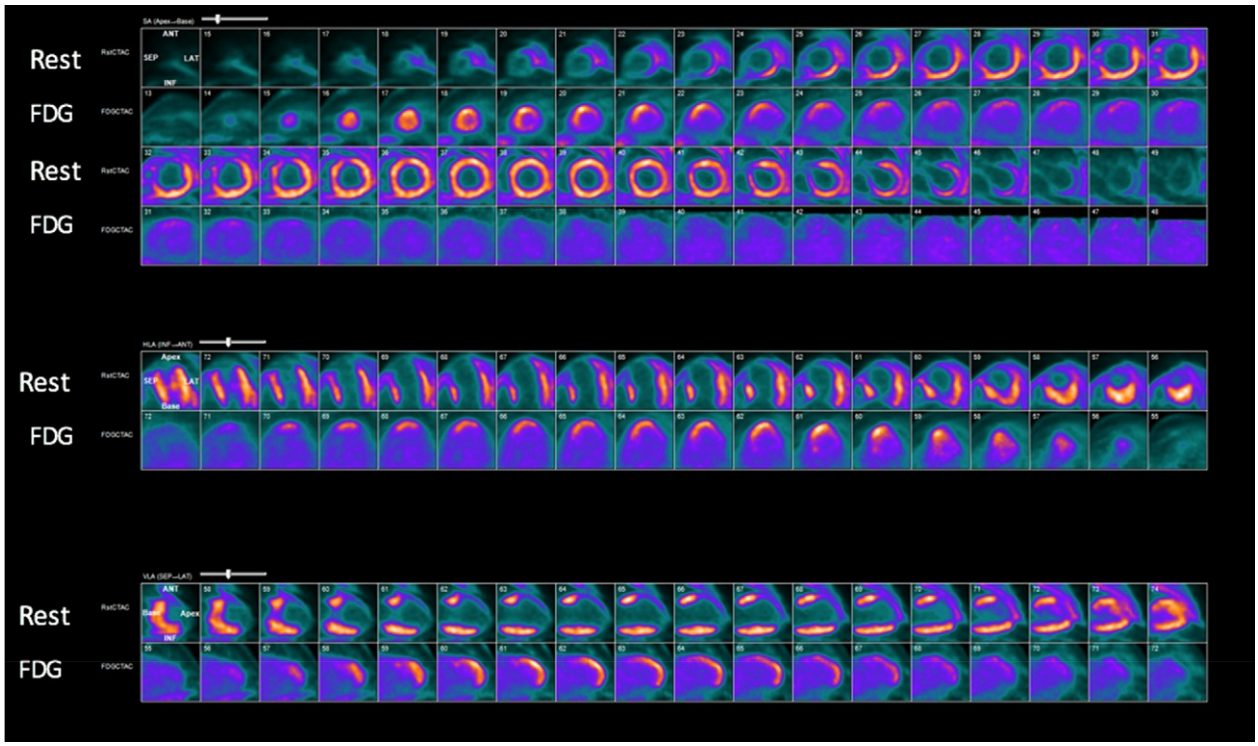

Fig. 11 Rest ammonia PET (*top row*) and FDG PET in short-axis (*top*), HLA (*middle*), and VLA (*bottom*) views reveal a matching perfusion defect and corresponding FDG avidity in the distribution of the LAD indicating a large area of hibernating myocardium in the LAD territory. This finding was confirmed by the polar maps later. Images courtesy of Marcelo Di Carli MD, Brigham and Women's Hospital.

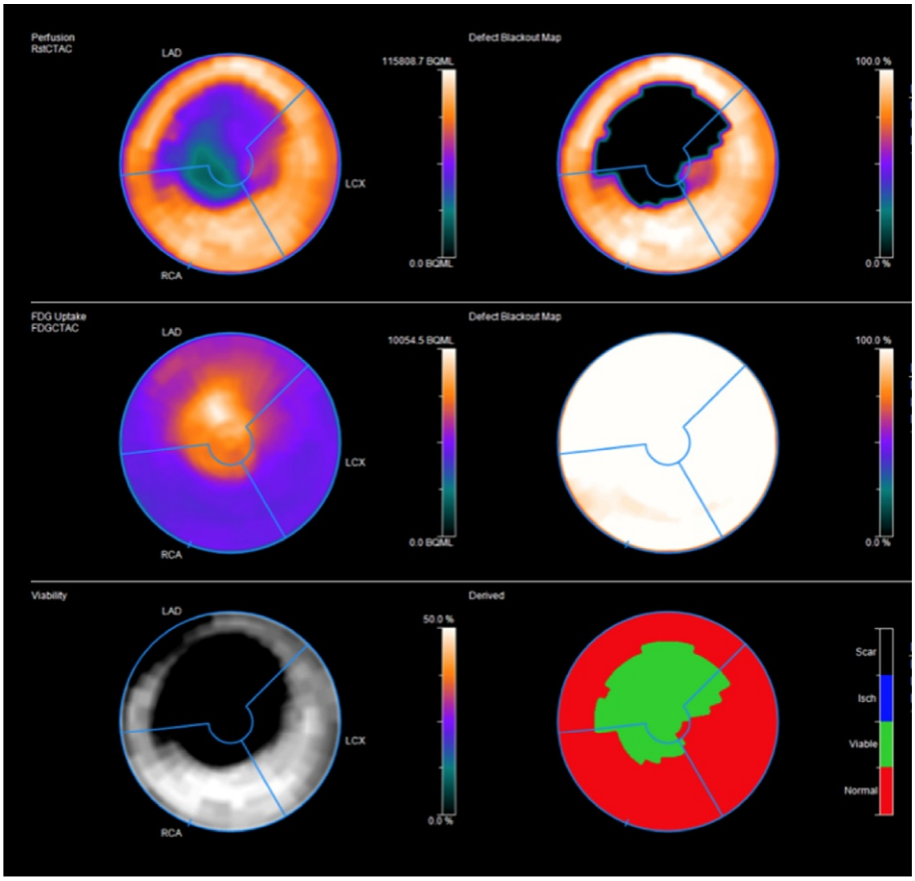

Fig. 12 This reveals the perfusion defect (*top*), FDG uptake (*middle*), and viability as indicated by the *green region* (*bottom*). In total, 42% of the myocardium was abnormal and viable. Images courtesy of Marcelo Di Carli MD, Brigham and Women's Hospital.

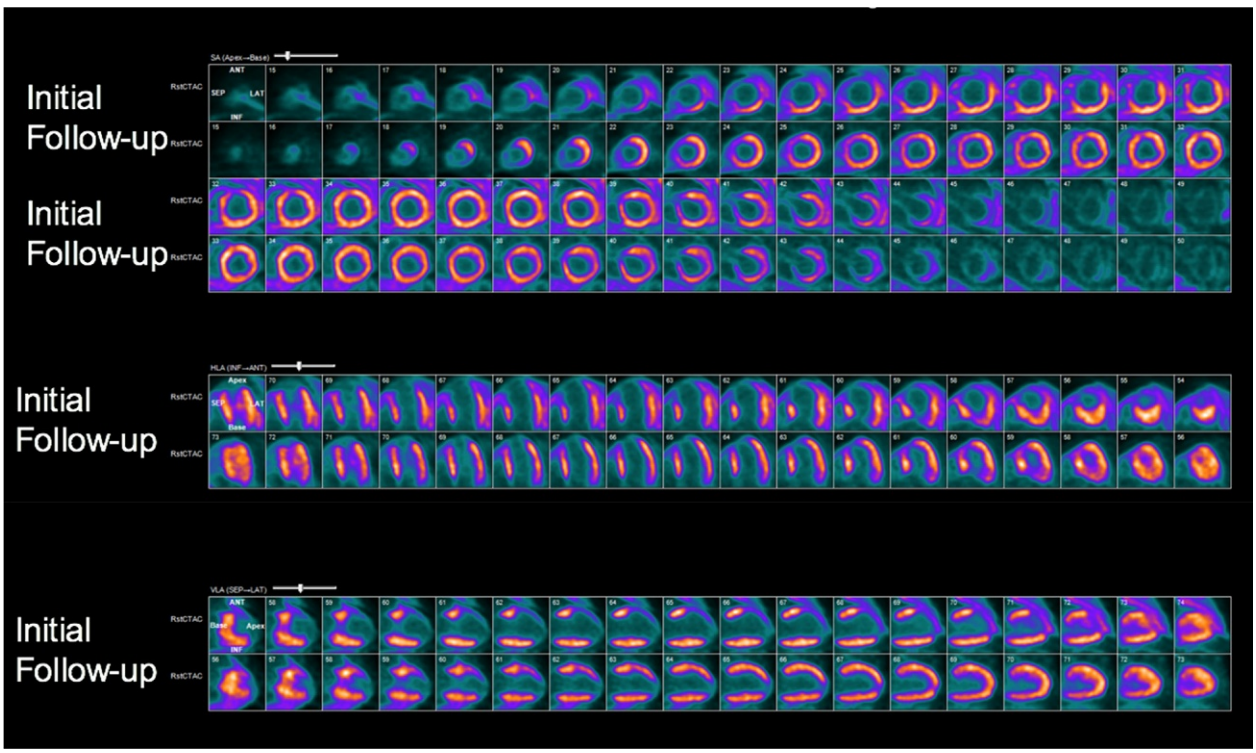

Fig. 13 The initial NH4 rest perfusion (*top row*) and follow-up NH4 rest perfusion (*bottom row*) images reveal an interval improvement in rest perfusion in all coronary artery territories, with a reduction in total abnormal myocardium from 42% to 9% (see polar maps later). Images courtesy of Marcelo Di Carli MD, Brigham and Women's Hospital.

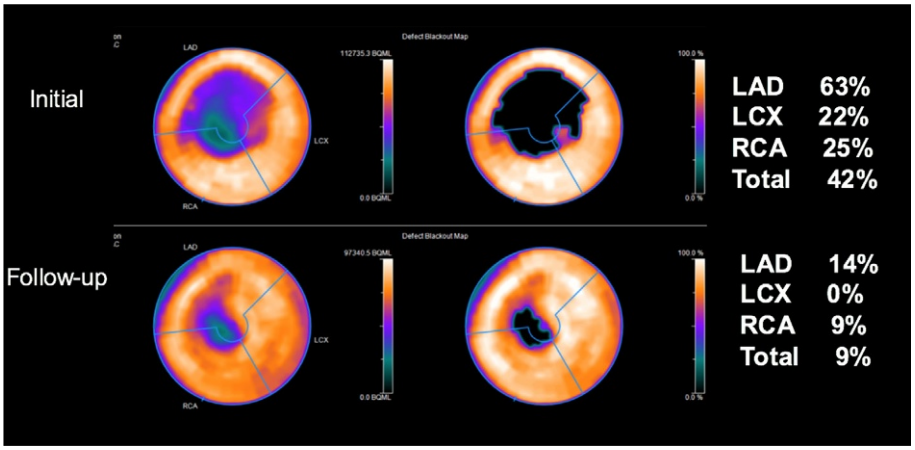

Fig. 14 Polar maps of initial rest perfusion (*top*) and follow-up rest perfusion (*bottom*) and corresponding blackout map with coronary artery distribution abnormality as a percentage of myocardium on the right. Images courtesy of Marcelo Di Carli MD, Brigham and Women's Hospital.

Cardiac Magnetic Resonance Imaging (MRI) Perfusion

With significant improvements in cardiac MRI scanning protocols and reduced scanning duration, the availability and feasibility of CMR perfusion has improved in recent years. The physiological basis for stress perfusion imaging rests in the concept of CFR (Klocke, 1987). CFR measures the capacity of the coronary circulation to increase blood flow through the coronary tree, under conditions of maximal vasodilation. This is typically achieved using pharmacological vasodilators such as adenosine or regadenoson. In healthy adult subjects, these agents cause an up to fourfold increase in coronary flow, and they are particularly effective in minimizing the resistance of the distal coronary perfusion bed. This process can reveal the presence of flow-limiting lesions in the epicardial coronary arteries. When the patient is at rest, an epicardial lesion will become flow limiting only then the luminal narrowing becomes approximately 85% or higher. In contrast, with maximal vasodilation, this threshold is lowered to approximately 50% or higher (Coelho-Filho et al., 2013). Vigorous exercise used as a stressor will produce the same CFR response as maximal vasodilation achieved with adenosine (Duncker and Bache, 2008).

CFR is typically measured during PET MPI and is described in earlier. It can also be measured by CMR but is more cumbersome. It is measured using direct visualization of the lumen of the proximal coronary artery and with the quantification of blood flow velocity in the vessel lumen by using the phase-contrast technique (Nagel et al., 2003). This technique can prove challenging due to cardiac and respiratory motion and the relatively small coronary lumen dimensions (approximately 1.5–3 mm).

In contrast, myocardial perfusion reserve (MPR) is typically measured by CMR. In this case, myocardial perfusion refers to the blood flow through the coronary microcirculation, that is, the volume of blood flowing through a volume, or mass unit of the myocardium, per unit of time (Coelho-Filho et al., 2013). Perfusion is quantified independently at rest and during maximal vasodilation, and thus, one can calculate the MPR, analogous to the CFR, as the ratio of hyperemic flow divided by the resting flow. In the normal coronary circulation, the CFR and the MPR agree in magnitude; however, in the case of epicardial disease, the presence of coronary collaterals can result in an epicardial flow reserve, which is lower than the MPR measured downstream from the lesion (Geldof et al., 1995). Thus, one can argue that the MPR, rather than CFR, is, perhaps, a closer and potentially more accurate measurement of the capacity of the coronary circulation to deliver sufficient oxygen to the myocardium. An abnormal MPR is thus considered a useful surrogate marker for ischemia, although the threshold below which it needs to decrease to cause clinically significant ischemia is not well-defined (Coelho-Filho et al., 2013).

Myocardial First-Pass Perfusion

It is only possible to measure flow through the coronary microcirculation indirectly. This is done either by labeling the blood and observing signal intensity changes resulting from the transit of the labeled blood or alternatively by introducing a blood-borne MR-detectable tracer and observing the injected tracer through the myocardium (Coelho-Filho et al., 2013). The so-called myocardial first-pass perfusion or first-pass imaging is currently the most widely used and reliable technique to assess myocardial perfusion by CMR. It is performed by measuring contrast enhancement during the first pass of a contrast agent bolus through the cardiac chambers and the myocardium. Cardiac MR perfusion studies rely primarily on the use of T1-weighted techniques, and during frozen cardiac motion, one applies dynamic imaging techniques that allow the acquisition of an image during a fraction of a heartbeat, which is repeated every heartbeat during the first pass and ideally covers a stack of sections through the heart (Coelho-Filho et al., 2013). Typically, a 0.05 mmol/kg bolus of gadolinium-based contrast agent is injected during three heartbeats after the start of image acquisition by using a power injector at a rate of 4 mL/s. Three short-axis LV slices at base, mid, and apical LV

level are selected, along with a four-chamber view prior to scanning. These are the views thus obtained for the evaluation of first-pass perfusion. Analysis of post perfusion images can be performed using either qualitative or quantitative techniques. In research CMR labs, quantitative measures are used. For clinical purposes, qualitative assessment is typically performed.

Stress Perfusion Imaging

Typically, stress perfusion images are obtained before rest, following localizing images and using a standard dose of intravenous regadenoson (0.4 mg). The images are acquired during approximately 60 heartbeats to cover a precontrast phase, the first pass of the contrast agent after its injection, and the recirculation of contrast agent (Coelho-Filho et al., 2013). Breath holding is typically performed for the initial phase of the study, and the patient resumes breathing when necessary. Breathing motion is sufficiently slow that it typically does not cause motion artifacts, but it is possible using post processing techniques to correct for breathing motion if required. Heart rate and blood pressure are monitored throughout and recorded. Adverse effects of regadenoson are infrequent but can include severe dyspnea, chest discomfort, headache, and GI upset due to the vasodilation of the vessels in the gut; thus, IV aminophylline is typically held in close proximity for reversal where required.

Rest Perfusion Imaging

Usually acquired at the end of the study, allowing sufficient time for the effect of the stress agent to have dissipated. A further contrast bolus is given, and similar slices are obtained in the manner described earlier. Rest and stress perfusion images can thus be compared side by side to assess for reversible or fixed perfusion defects using the standard 17-segment model.

Image Interpretation

Visual interpretation of myocardial contrast enhancement displaying the perfusion images in cine mode is the standard used in routine clinical practice. Myocardial regions with perfusion defects are identified by a reduced rate of contrast enhancement. Such regions appear hypointense during early myocardial contrast enhancement. Playing the images in cine mode is essential for differentiating between image artifacts (in particular, so-called dark-rim artifacts at the endocardial border) and true perfusion defects (Di Bella et al., 2005). The key to distinguish between dark-rim artifacts and true perfusion defects is the number of frames during which the signal hypointensity can be observed. Artifacts typically only appear in a couple of frames during peak contrast enhancement in the blood pool and before peak contrast enhancement in the myocardial tissue (Coelho-Filho et al., 2013).

Sensitivity and Specificity of CMR Perfusion Imaging for the Detection of Underlying CAD

Multiple single- and multicenter trials have shown that CMR perfusion has excellent sensitivity and specificity in the detection of underlying CAD (Panting et al., 2001; Klem et al., 2006; Schwitter et al., 2001; Al-Saadi et al., 2000; Wolff et al., 2004; Giang et al., 2004), including comparison with alternative methods of MPI such as SPECT and PET. In 2001, Schwitter et al. described a sensitivity and specificity for the detection of underlying coronary stenosis of >50% of 0.86 and 0.8, respectively, using dipyridamole as a stress agent. More recently, Manka et al. described a sensitivity and specificity of 0.92 and 0.75, respectively, for lesions >50% (Manka et al., 2011) using adenosine compared with dobutamine as the stress agent. Such data have led to the increasing utilization of stress cardiac MRI in recent years for the detection of underlying CAD, particularly in young patients where radiation dose is a concern and a noninvasive means of assessment is desirable. Compared with well-established nuclear techniques and the more recently developed stress multidetector CT, stress perfusion CMR studies can also provide additional information about LV and RV size and systolic function, regional function (wall thickening or strain imaging), and edema (T2 SPAIR) and infarction imaging, all at high spatial resolution and tissue contrast.

Limitations of Stress CMR

At present, the commonly used gadolinium-based cardiac MR contrast agents such as Dotarem or Gadavist do not have regulatory approval for any cardiac indication in the United States, and their use is therefore off-label. From a practical standpoint, the administration of vasodilator stress agents (e.g., adenosine) in the MR imaging environment can be more challenging than when other imaging modalities (e.g., PET or SPECT) are used. Adenosine and dobutamine require the use of MRI compatible infusion pumps and tubing; however, a new FDA-approved vasodilator stress agent, regadenoson (Al Jaroudi and Iskandrian, 2009), has reduced symptom intensity and is associated with better patient tolerance. It is delivered as a single bolus, which allows easier administration without the use of a pump.

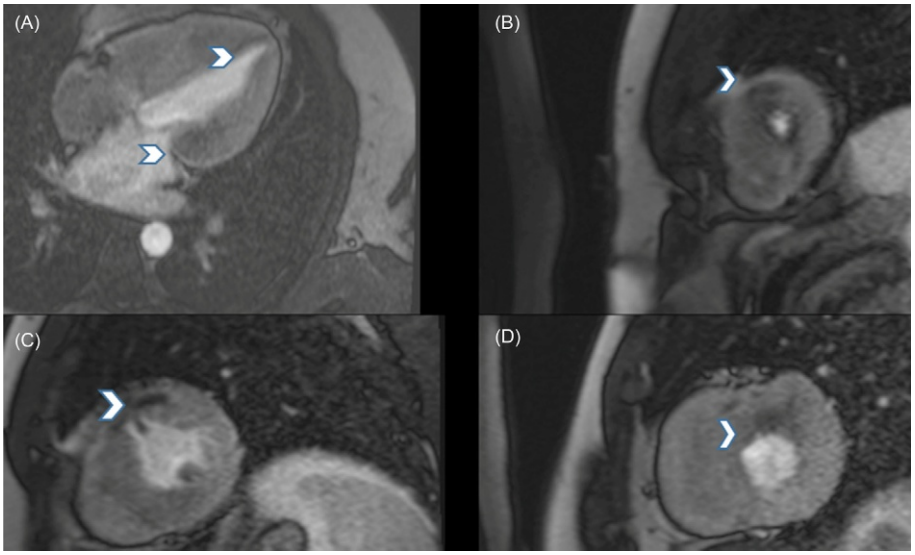

Fig. 15 Panels A–D: four-chamber view (A), short-axis apical (B), mid (C), and basal (D) LV slice in still frame indicate the presence of subendocardial perfusion defects (*black area* marked by *arrows*) affecting the apical anterior, mid anteroseptum, and basal anterior segments. Subsequent coronary angiography revealed significant LAD stenosis that was amenable to percutaneous coronary intervention (PCI). Rest perfusion in this case was normal. Images courtesy of Raymond Kwong MD, Brigham and Women's hospital.

Gadolinium use is typically avoided in patients with EGFR <30 mL/min/m^2. The incidence of the described gadolinium-associated risk of nephrogenic systemic fibrosis (NSF) (Marckmann et al., 2006) is extremely rare but given its severity care is taken in this subgroup of patients. Newer gadolinium-based agents have lower reported rates of NSF than older ones (Bruce et al., 2016).

Clinical Example

A 47-year-old patient with recently diagnosed hypertrophic cardiomyopathy complained of chest pain. He underwent a cardiac MRI to evaluate the degree of LV hypertrophy in addition to the assessment of late gadolinium enhancement as a marker of underlying myocardial fibrosis. A stress perfusion sequence was carried out to evaluate for potential underlying CAD (**Fig. 15**).

MPI Utilizing Echocardiography (Myocardial Contrast Echocardiography (MCE))

Myocardial perfusion can be readily assessed at the bedside using contrast echocardiography. Echo-contrast agents consist of tiny microbubbles (approximately 1–8 μm in diameter), which persist in the systemic circulation for ~3–5 min after intravenous injection. Low-intensity ultrasound imaging is required to prevent the destruction of the microbubbles. Myocardial perfusion is assessed by a process of the destruction of the microbubbles with a "flash" of higher-intensity ultrasound and then analyzing their replenishment rate as the microbubbles seep back into the myocardial circulation.

Echo-contrast agents consist of microbubbles that contain a hemodynamically inert gaseous core (e.g., octafluoropropane and sulfur hexafluoride) and a stabilizing outer shell (e.g., lipid, albumin, or biopolymer), which oscillate when under the influence of ultrasound waves (Postema et al., 2005). These contrast echo microbubbles form multiple small liquid–air interfaces whose boundaries have a high acoustic impedance mismatch resulting in enhanced ultrasound reflection (Orde and McLean, 2016). Contrast microbubbles are small enough to traverse the pulmonary capillaries to enter the systemic circulation. When ultrasound interacts with the microbubbles, they oscillate, and this effect is dependent on the ultrasound acoustic pressure in addition to the shell and core gas properties of the contrast agent. Ultrasound acoustic pressure is described as the "mechanical index" and corresponds to the power output of the scanner (Pathan and Marwick, 2015). With standard 2-D echo, the mechanical index is typically ~1.4. At this level, the microspheres would oscillate to burst and be destroyed. Thus, when performing contrast echo imaging, the mechanical index is set much lower (<0.2) to avoid this phenomenon. Microbubbles reflect ultrasound in a nonlinear format compared to body tissues, which reflect ultrasound in a linear manner. Thus, microbubble reflection can also be detected using higher harmonic frequencies, producing a contrast between highly vascular and less vascular tissues.

Initial animal studies, subsequently validated in humans, investigated the hypothesis that MBF could be assessed using contrast echocardiography by destroying the contrast microbubbles with a "flash" of high-diagnostic-intensity ultrasound and subsequently assessing the rate of replenishment of the microbubbles into the myocardium (Jayaweera et al., 1994; Wei et al., 1998; Vogel et al., 2005). This is assessed by the change in intensity or brightness in a "region of interest" (ROI). Microbubbles behave like RBCs. Therefore, any change in signal intensity indicates a change in MBF.

Under normal conditions, that is, normal MBF, 90% of the coronary circulation resides within myocardial capillaries, and red blood cells travel at approximately 1 mm/s at rest (Orde and McLean, 2016). After the destruction of the echo contrast, signal intensity usually returns to normal after approximately five to seven cardiac cycles (Wei et al., 1998). Under stress, where vasodilation and increased capillary blood flow are present, the rate of return of signal intensity is approximately two to three cardiac cycles. Qualitative assessment of the rate of microbubble replenishment is possible, but it is also possible to assess it quantitatively by reviewing the change in signal intensity over time in a specific ROI. MBF is considered the product of plateau signal intensity and rate of replenishment. The slower the rate of replenishment and the lower the plateau signal intensity, the poorer the MBF.

Side effects of intravenous echo-contrast administration are rare and include a headache, flushing, or back pain. Symptoms are usually relieved on the cessation of contrast agent administration. There is a reported 1:10,000 chance of an anaphylaxis-type reaction (Orde and McLean, 2016).

Quantification during MCE has not become widespread due to a number of factors including (a) attenuation, (b) underlying tissue signals, (c) incomplete microbubble destruction with high MI impulses, and (d) difficulties with software quantification techniques (Porter and Xie, 2010). MCE compares well with other myocardial perfusion techniques. Kaul et al. (1997) described a 92% concordance between segmental perfusion scores by MCE and 99mTc-sestamibi SPECT at rest and during dipyridamole stress. In another study, Heinle et al. (2000) compared MCE perfusion with 99mTc-sestamibi SPECT in 123 patients with suspected CAD and found an overall concordance between both techniques of 83%; concordance was higher in patients with no or multivessel CAD. Despite these data, the use of MCE in routine clinical practice is low, and most centers prefer the use of nuclear MPI.

Myocardial CT Perfusion

In a similar fashion to CMR perfusion, CT assessment of myocardial perfusion is based on the distribution of contrast (iodinated in the case of CT) material during its first pass through the myocardium. The contrast material's distribution is affected by the underlying arterial blood supply. Myocardial perfusion defects can be identified as hypoattenuating areas containing reduced amounts of contrast material. Static CT perfusion imaging allows the visual qualitative assessment of a single snapshot of myocardial iodine contrast attenuation. This snapshot is acquired during the early arterial phase of first-pass contrast enhancement. The optimal time frame for stress perfusion acquisition is 8–16 s after contrast enhancement in the aorta reaches 100 HU (Bischoff et al., 2013). Static single-shot myocardial CT perfusion can be performed using any CT scanner capable of acquiring diagnostic-quality coronary CTA (Vliegenthart et al., 2012).

Static rest myocardial perfusion evaluation is generally performed using coronary CTA image sets already obtained for the morphological assessment of the coronary arteries. If the assessment of myocardial perfusion at stress is desired, an additional acquisition during pharmacological stress should be obtained (Varga-Szemes et al., 2015).

Assessment of static myocardial CT perfusion is performed qualitatively by the visual evaluation of LV myocardial contrast enhancement. To improve discrimination between artifacts and true perfusion defects, the recommendation is that the evaluation of myocardial perfusion takes place in multiphase reconstructions (Varga-Szemes et al., 2015). Artifacts typically affect only a single cardiac phase, whereas true defects will typically persist throughout the entire cardiac cycle (Vliegenthart et al., 2012). It is also possible to carry out a semiquantitative assessment of myocardial perfusion using the transmural perfusion ratio, that is, the ratio between subendocardial and subepicardial attenuations (Cury et al., 2011). Further, advanced image processing methods, including color-coding myocardial attenuation and indexing signal density, are also available and may enhance diagnostic accuracy (George et al., 2006; Mahnken et al., 2008; Kachenoura et al., 2009).

When compared with SPECT or SPECT plus invasive coronary angiography, most of the static single-energy studies showed high specificity and sensitivity for the detection of hemodynamically significant CAD (Cury et al., 2011; George et al., 2006; Kachenoura et al., 2009; Blankstein et al., 2009; Ko et al., 2012). Sensitivity for the detection of reversible perfusion defects significantly improves under pharmacological stress (Gould and Lipscomb, 1974). As with other methods of MPI, various agents are available to induce pharmacological stress with the vasodilator adenosine most commonly used. Adenosine is administered in a continuous IV infusion at a dose of 140 μg/kg/min for at least 120 s to induce an increase in the heart rate of 10–20 beats above the resting heart rate. Regadenoson is advantageous because it can be administered in a single dose, making stress CT studies using regadenoson more time-efficient (Kurata et al., 2005).

Conclusion

MPI is an important tool that adds much information in the assessment of cardiac patients, and it can take many forms, most commonly involving nuclear imaging. However, depending on the institutional availability of cardiac MRI, contrast echo, and perfusion CT, the preferred technique may vary. Patient characteristics can also affect this decision such as the age and presenting characteristics of the patient, whether radiation should be avoided at all costs and whether iodinated contrast can be used depending on renal function. This article explores the use of each modality and highlights the pros and cons of each method. In addition, clinical case examples in the case of nuclear cardiology and cardiac MRI highlight the clinical use of such techniques in everyday clinical practice.

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Myocardial Repair

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Approaches to Myocardial Repair

The human heart has very limited regenerative capacity. The estimated rate of cardiomyogenesis of 0.5%–1% per year (Bergmann et al., 2009, 2015) is not sufficient to compensate for the loss of cells after injury such as myocardial infarction. Therefore, regenerating an injured heart requires stimulation of the low endogenous cardiomyocyte proliferation, addition of new cardiomyocytes, or reprogramming fibroblasts (in the scar) to cardiomyocytes (**Fig. 1**).

Stimulation of Endogenous Cardiomyocyte Proliferation

During murine development cardiomyocyte DNA synthesis occurs in two temporally distinct phases. The first phase occurs during fetal development and is associated solely with cardiomyocyte proliferation. The second phase takes place during early neonatal life and is associated with binucleation (Soonpaa et al., 1996). A recent study in mice reported a second proliferative burst during preadolescence, leading to an increase in cardiomyocyte number by ~40% (Naqvi et al., 2014). However, another group analyzing cardiomyocyte DNA synthesis and cell-cycle activity could not confirm this finding (Soonpaa et al., 2015), leaving this an open question. In the adult zebrafish heart cardiomyocyte proliferation occurs at a low rate but is increased after cardiac injury (Poss et al., 2002; Gemberling et al., 2013; Arnaout et al., 2014). Regeneration was also observed in newborn rat (Robledo, 1956) and mice (Porrello et al., 2011). The mechanisms are not entirely clear. Genetic fate mapping experiments in adult mice concluded that myocardial infarction led to the formation of new cardiomyocytes from a noncardiac (=non-α-myosin heavy chain-positive) population of progenitor cells (Ellison et al., 2013; Loffredo et al., 2011; Hsieh et al., 2007). In contrast, other studies showed that progenitor cells do not contribute to the low renewal rate of cardiomyocytes in adult mammals but that cardiomyocytes can reenter the cell cycle at a low rate (Malliaras et al., 2013; Senyo et al., 2013; Porrello et al., 2013).

Despite these partially opposing results, stimulation of cardiomyocyte proliferation in vivo is a possible therapeutic avenue for cardiac regeneration without the in vitro culture, invasive intervention, and immunosuppression likely needed for cell-based therapies. But unrestricted proliferation might promote tumor formation and cause arrhythmias. Different strategies have been developed, whose efficiency, safety, and feasibility regarding a clinical application remain to be investigated, especially because most studies have used transgenic approaches to stimulate cardiomyocyte proliferation.

One possibility to increase the proliferative activity is to overexpress proteins that naturally promote the progression of the cell cycle, or knockdown their respective regulatory factors. Overexpression of cyclin B1-CDC2 (Bicknell et al., 2004) or knockdown of p21/p27 (Di Stefano et al., 2011) was shown to increase cell-cycle activity of cardiomyocytes in vitro. After myocardial infarction in mice, overexpression of cyclin D2 (Pasumarthi et al., 2005) or A2 (Chaudhry et al., 2004; Woo et al., 2006) led to an increased proliferation of cardiomyocytes, regeneration of the scar, and improved cardiac function. Similarly, the overexpression of the transcription factor E2F2 (Ebelt et al., 2008) or knock-out of its regulators Rb and p107 led to elevated proliferation of cardiomyocytes (Sdek et al., 2011; MacLellan et al., 2005).

Another approach is the administration of growth factors or activation of their receptors. Periostin (Kuhn et al., 2007) and fibroblast growth factor (Engel et al., 2005) were described to promote cardiomyocyte proliferation, but both were also shown to cause hypertrophy (Baliga et al., 1999; House et al., 2010; Oka et al., 2007). Others showed that periostin is not expressed in cardiomyocytes, but within noncardiomyocytes throughout the rest of the heart, where it enables normal valvular remodeling and

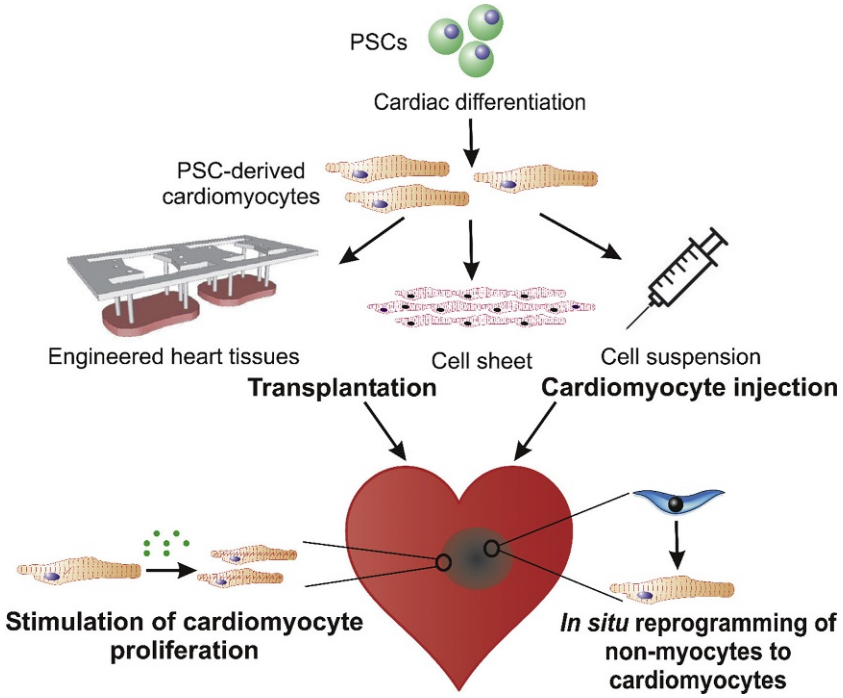

Fig. 1 Approaches to cardiac regeneration.

establishment of a mature cardiac skeleton (Snider et al., 2008). Neuregulin stimulated cardiomyocyte proliferation in some (Bersell et al., 2009; Polizzotti et al., 2015), but not in other studies (Reuter et al., 2014). Overexpression of the receptors for neuregulin, ErbB2 (D'Uva et al., 2015) and ErbB4 (Bersell et al., 2009) induced cardiomyocyte proliferation. While the induction of a constitutively active ErbB2 in neonatal, juvenile, or adult cardiomyocytes resulted in cardiomegaly, transient induction of ErbB2 following myocardial infarction triggered cardiomyocyte dedifferentiation and proliferation followed by redifferentiation and regeneration (D'Uva et al., 2015).

A third possibility to stimulate proliferation is to recapitulate developmental pathways such as the Wnt-signaling pathway. Knock-out of GSK3β was associated with increased cardiomyocyte cycling (Tseng et al., 2006; Kerkela et al., 2008). Several cross-regulations occur between the Wnt/β-catenin and Hippo signaling pathways (**Fig. 2**). Inhibition of Wnt signaling by the Hippo pathway limits cardiomyocyte proliferation and fetal heart growth (von Gise et al., 2012; Xin et al., 2013; Heallen et al., 2011; Heallen et al., 2013) (reviewed in Zhou et al., 2015; Zhou et al., 2015). Mst1/2 (homologues of hippo), Lats1/2 (homologues of Wts), Sav1, and YAP/TAZ (Yki homologues) are the core proteins of the Hippo/Yap pathway in mammals. Activation of Mst1/2 leads to the phosphorylation of Sav1, which in turn activates Lats1/2, which phosphorylates and thereby inhibits the transcriptional coactivators YAP/TAZ. Cardiomyocyte-specific deletion of YAP led to decreased cardiomyocyte cell-cycle activity during embryonic development (von Gise et al., 2012). Conversely, Yap gain of function increased proliferation of neonatal cardiomyocytes in vivo (von Gise et al., 2012; Xin et al., 2013; Xin et al., 2011). Furthermore, inactivation of Lats1/2 led to a moderate stimulation of adult cardiomyocyte proliferation (Xin et al., 2013; Heallen et al., 2013).

miRNAs are another promising tool to increase proliferation of cardiomyocytes, both in in vitro culture and in vivo. After myocardial infarction in mice, administration of miR-590 or miR-199a stimulated cardiac regeneration and improved cardiac function (Eulalio et al., 2012). There is evidence that the mechanism of miRNA-induced proliferation is associated with a suppression of the expression of sarcomeric genes, disassembly of the strict sarcomeric organization of myocytes, and inhibition of the Hippo pathway (Tian et al., 2015). Long noncoding RNAs contribute to cell-cycle regulation and cardiac development (Klattenhoff et al., 2013; Werber et al., 2014; Ounzain et al., 2015), but their possible role in cardiac regeneration has to be further investigated.

Taken together, multifold experimental evidence suggests that cardiomyocyte quiescence in the adult mammalian heart can be therapeutically addressed. Future studies need to address the following issues. (i) To which extent do findings in (cultured) neonatal cardiomyocytes translate to the adult heart? (ii) Do the reported rates of cardiomyocyte proliferation match the functional recovery of injured hearts? In other words, are improvements of contractile function fully explained by new myocardium? Answering this question requires thorough absolute quantification of new myocardium. (iii) How can a potentially tumorigenic concept of cell-cycle activation be translated in a therapeutic concept without causing harm? This will likely require the design of short-term, pulsed interventions early after myocardial infarction as shown, for example, in studies overexpressing ErbB2 receptors (D'Uva et al., 2015).

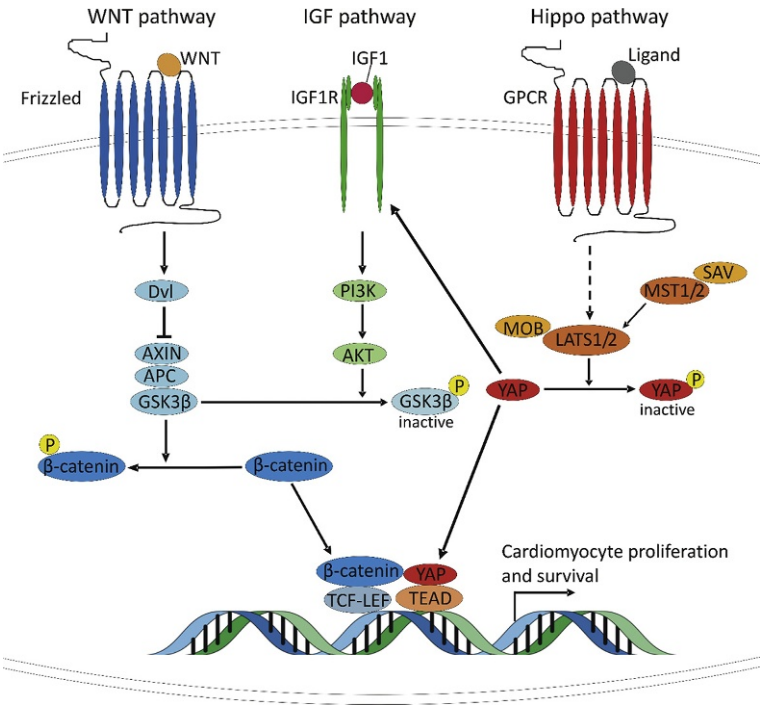

Fig. 2 Cross-regulation between the Wnt/β-catenin, IGF, and Hippo signaling pathways. Activation of the Hippo pathway results in the phosphorylation and nuclear exclusion of the transcriptional coactivator Yes-associated protein (YAP) and thereby impedes cardiomyocyte proliferation. Deletion of the upstream scaffold protein Salvador (SAV) or the inhibitory Hippo kinases large tumor suppressor 2 (LATS2), the kinases mammalian STE20-like protein kinase 1 (MST1) or MST2 promotes cardiomyocyte proliferation, as unphosphorylated YAP can activate the expression of genes in the WNT-signaling pathway by interacting with β-catenin. YAP also promotes cardiomyocyte proliferation through the activation of the insulin-like growth factor 1 (IGF1) pathway. *APC*, adenomatosis polyposis coli; *AXIN*, axis inhibition protein; *GPCR*, G protein-coupled receptor; *DVL*, Dishevelled; *GSK3β*, glycogen synthase kinase 3 β; *IGF1R*, IGF1 receptor; *LEF*, lymphoid enhancer-binding factor; *MOB*, Mps one binder; *TCF*, T cell factor.

Cellular Therapies

In this article we focus on cellular therapies aiming to replace damaged or necrotic heart muscle cells by new cardiomyocytes or to add new myocytes/muscle tissue to the injured heart. Therapies using other cell types addressing paracrine factors are described in reviews with a wider scope (Garbern and Lee, 2013; Eschenhagen et al., 2012). The prerequisite for cellular therapies aiming at the addition of new heart muscle is the generation of human cardiomyocytes in vitro at a high scale. The concept is principally straightforward, namely to transplant preformed cardiomyocytes into or onto the injured heart, expecting them to integrate and couple to the remaining host myocardium, and overtake the contractile function of the lost tissue.

Cell type

Human pluripotent stem cells (hPSC), that is, human embryonic stem cells (hESC), human-induced pluripotent stem cells (hiPSC), human parthenogenetic stem cells (hPGSC), and human nuclear transfer-mediated ESC (hNT-ESC), can differentiate into all cell types of the three germ layers ectoderm, endoderm, and mesoderm. They are the only cell source that can be differentiated to *bona fide* cardiomyocytes at high efficiency.

Human embryonic stem cells

hESCs were for the first time derived from the inner cell mass of a blastocyst in 1998 (Thomson et al., 1998). They show high levels of telomerase activity (Thomson et al., 1998), which prolongs the replicative life-span (Harley, 1991; Harley et al., 1992) and allows the completion of embryonic development. Differentiation protocols have been established that mimick embryonic development (Yang et al., 2008) and yield principally unlimited numbers of cardiomyocytes with a purity of up to >90% (Lian et al., 2013; Zhang et al., 2015; Kempf et al., 2015; Palpant et al., 2017; Lin et al., 2016; Burridge et al., 2011). The transplantation of hESC-derived cardiomyocytes into injured myocardium showed positive effects after infarction in different species (Klug et al., 1996; Behfar et al., 2007; Laflamme et al., 2007; van Laake et al., 2007; Shiba et al., 2012; Chong et al., 2014). Human grafts showed cell–cell contacts and electrical coupling and were associated with improved left-ventricular function in the damaged tissue (Behfar et al., 2007). Early studies injected hESC-derived cardiomyocytes in mouse and rat and observed reduced infarct area, increased vascularization, and at least transiently improved heart function (Laflamme et al., 2007; van Laake et al., 2007). Injection of large amounts of hESC-derived cardiomyocytes into cryo-injured guinea-pig hearts generated relatively large human grafts, improved heart function, and reduced arrhythmia susceptibility (Shiba et al., 2012). In a follow-up study in nonhuman primates significant graft formation was observed

that were proven to electrically couple to the host myocardium when applied shortly after injury (Chong et al., 2014). Given that infarct size in the monkeys was small and contractile function was not compromised by the infarct, grafting had no beneficial effect on heart function, but was associated with the transient induction of ventricular arrhythmias in all of the five experimental animals. In chronic injury models, hESC-derived cardiomyocytes failed to electrically integrate into the host myocardium after injection in suspension (Shiba et al., 2014) or to improve heart function after transplantation of engineered heart muscle (Riegler et al., 2015).

Human-induced pluripotent stem cells

The first iPSCs were reprogrammed from mouse fibroblasts by retroviral transduction with the four transcription factors c-MYC, OCT3/4, SOX2, and KLF4 in 2006 (Takahashi and Yamanaka, 2006). One year later, human iPSCs were generated by two similar approaches in parallel (Takahashi et al., 2007; Yu et al., 2007). Like hESCs, iPSCs can differentiate into cells of all three germ layers in vitro and in vivo (Takahashi et al., 2007). As the random genetic integration of retro- or lentivirus into the genome could have unpredictable effects, nonintegrating virus-based protocols (e.g., Sendai virus, Fusaki et al., 2009) are now widely used as a better alternative. The permanent presence of the oncogene c-*MYC* in the genome bears tumorigenic risks, which can nowadays be circumvented by plasmid-, RNA-, or protein-based reprogramming methods (Fusaki et al., 2009; Okita et al., 2008; Stadtfeld et al., 2008; Kaji et al., 2009; Woltjen et al., 2009; Kim et al., 2009; Warren et al., 2010). Interestingly, the reprogramming factors are only transiently induced and not measurable after 10-15 passages, but the endogenous pluripotency factors OCT4 and NANOG are permanently increased, indicating that the process of reprogramming stably induces the endogenous pluripotency program. Depending on the method used, reprogramming efficiencies are between 0.001% and 4.4% (Robinton and Daley, 2012).

By recapitulating the most important stages of embryonic cardiac development, hiPSCs can reproducibly and efficiently be differentiated to >90% pure cardiomyocytes (Lian et al., 2013; Kempf et al., 2015; Palpant et al., 2017; Lin et al., 2016; Burridge et al., 2011, 2014; Breckwoldt et al., 2017). Different transplantation studies showed positive effects of hiPSC-derived cardiomyocytes on heart function after myocardial infarction. In a guinea-pig model of myocardial infarction human engineered heart tissue (hEHT) strips from hiPSC-derived cardiomyocytes and hiPSC-derived endothelial cells formed large, vascularized grafts in the scar, improved left-ventricular function and were, at least in a subset of experiments, able to electrically couple to the host myocardium (Weinberger et al., 2016) (**Fig. 3**). Allogeneic, cynomolgus monkey iPSC-derived cardiomyocytes injected into infarcted primate hearts survived, showed electrical coupling with host cardiomyocytes and improved cardiac contractile function (Shiba et al., 2016). In a study with human hiPSC-derived cardiomyocyte cell sheets transplanted into infarcted minipig hearts, only few cells survived but cardiac function was improved (Kawamura et al., 2012), suggesting that paracrine mechanisms contributed to the beneficial effect.

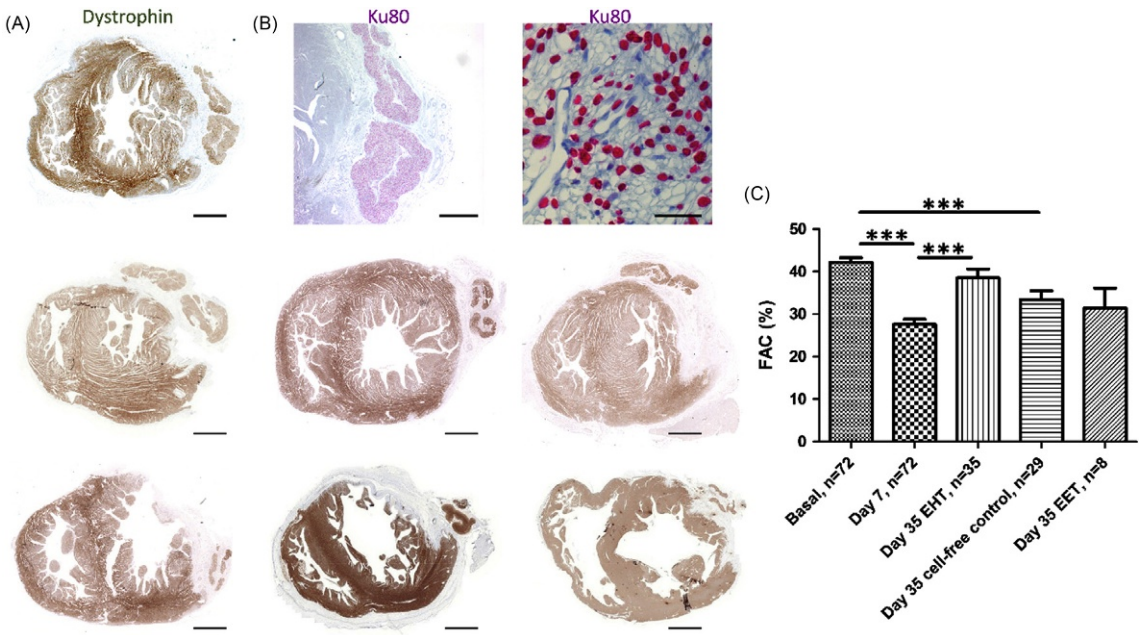

Fig. 3 EHTs partially remuscularize the scar after injury and improve left-ventricular function. (A) Short axis sections of seven different guinea-pig hearts 28 days after hEHT transplantation. (A) Staining for dystrophin. Scale, 2 mm. (B) Staining for human Ku80 in low (scale, 1 mm) and high magnification (scale, 40 μm) of the graft in the corresponding section in (A). (C) Fractional area change (FAC) before cryoinjury (basal, $n=72$), 7 days after cryoinjury prior to transplantation ($n=72$), and 35 days after cryoinjury (hEHT $n=35$, cell-free control $n=29$, hEET $n=8$). Data are individual animals with means ± SEM (A). $^{***}P<0.001$ determined by one-way ANOVA analysis, followed by Bonferroni test. Figures are taken from Weinberger, F., et al. (2016). Cardiac repair in guinea pigs with human engineered heart tissue from induced pluripotent stem cells. *Science Translational Medicine* 8, 363ra148, doi:10.1126/scitranslmed.aaf8781 with permission.

Human parthenogenetic stem cells

Parthenogenesis is a naturally occurring form of uniparental, asexual reproduction, leading to different meiosis and zygosity outcomes (Chapman et al., 2007). Although the mechanism underlying this phenomenon is still not fully understood, different protocols aiming to imitate parthenogenesis have been described (reviewed by Daughtry and Mitalipov, 2014). Parthenogenesis does not normally occur in mammals, but still PGSC-derived embryos of most mammalian species are developing to blastocysts. After transfer into recipients, mouse parthenotes can implant and reach day E9.5 of development (Kaufman et al., 1977). Further development failed. This may be partially due to impaired placental formation (Tarkowski et al., 1970) but also to defective genomic imprinting, because many imprinted genes are directly involved in fetal growth pathways (Kono, 2006). PGSC derivation efficiencies (Paull et al., 2013; Mai et al., 2007), morphological, growth and culture characteristics, transcriptional profile (Sritanaudomchai et al., 2010) and in vitro and in vivo differentiation potential (Paull et al., 2013; Mai et al., 2007; Sritanaudomchai et al., 2010) are similar to ESCs. hPGSCs were differentiated to fibroblasts, cardiomyocytes, neurons, and β cells (Paull et al., 2013; Gonzalez et al., 2013). After transplantation of mouse PGSC-derived cardiomyocytes in MHC-matched allogeneic or syngeneic mice, the engraftment was as efficient as that of ESC-derived cells (Kim et al., 2007; Didie et al., 2013; Liu et al., 2013). PGSC-derived cardiomyocytes promoted regeneration after cardiac infarction (Didie et al., 2013; Liu et al., 2013) and formed a functional syncytium with the healthy host myocardium when transplanted as PGSC-derived engineered heart tissues (EHTs) (Didie et al., 2013) or injected as suspended cells (Yang et al., 2015).

Human nuclear transfer-mediated ESC

Somatic cells can be reprogrammed to pluripotent ESCs by somatic cell nuclear transfer (SCNT) into a previously enucleated human oocyte. Since sheep Dolly in 1997 (Wilmut et al., 1997), cells from different mammalian species have been generated by SCNT. The technology is now routinely used to generate transgenic pigs or other farm animals (Fischer et al., 2016). A recent advancement was the derivation of hNT-ESC lines (Tachibana et al., 2013), whose nuclear genome resulted exclusively from parental somatic cells. These hNT-ESCs had normal diploid karyotypes and gene expression profiles comparable to ESCs derived from blastocysts. Further progress was the generation of hNT-ESCs using nuclei from adult cells (Chung et al., 2014) and not from fetal and infant somatic cells, as aging-related diseases are the main application of regenerative approaches.

Application procedure

Cell injection

The most straightforward way to deliver PSC-derived cardiomyocytes into an injured heart is to inject them directly into the infarcted area and its border zone (Gerbin and Murry, 2015a), where successful integration into healthy tissue and electrical coupling to the host myocardium has been demonstrated (Shiba et al., 2012; Chong et al., 2014). On the other hand, low cell retention and high cell death rates (partly pressure-induced) lead to less than 10% of cells remaining a few days after injection into animal hearts (Dow et al., 2005; Qiao et al., 2009; Zhang et al., 2001; Hofmann et al., 2005). Therefore, high cell numbers are needed and at least some cells will be distributed into other organs, thereby increasing the risk of tumor formation due to remaining pluripotent cells in a differentiated cell population (Cao et al., 2007). Studies that achieved relevant remuscularization, that is, a graft size of 1%–8% of scar size, injected 100 Mio hESC-derived cardiomyocytes in a 2.5 g guinea-pig heart (Shiba et al., 2012) or 1000 Mio in a 40 g nonhuman primate heart (Chong et al., 2014). This number has to be compared with the normal 10–15 Mio cardiomyocytes/g native heart tissue (Bergmann et al., 2015; Adler and Friedburg, 1986). To enhance engraftment the cells were delivered in a prosurvival cocktail (Laflamme et al., 2007; Shiba et al., 2012; Chong et al., 2014) and surgical techniques were used to reduce washout (Chong et al., 2014).

Cell sheets

An elegant approach is the application of cell sheets, which are based on the propensity of cardiomyocytes to detach from their growth surface as a cell monolayer in long-term culture. Temperature-sensitive culture dish surfaces facilitate the detachment of cell monolayers from the culture dish at room temperature (Okano et al., 1995; Shimizu et al., 2002). These scaffold-free thin sheets can be transplanted to the heart without sutures or glue, which has been shown with different cell types in rat (Masumoto et al., 2014), minipig (Kawamura et al., 2012), and even humans (skeletal myoblasts, Sawa et al., 2012). Compared to single cell injections the cell retention rate is higher due to cell–cell contacts within the sheet at the time of transplantation.

Cell sheets containing hiPSC-derived cardiomyocytes engrafted, were vascularized, and significantly improved cardiac function of infarcted rat hearts (Masumoto et al., 2014). The limitation in tissue thickness, which is due to the natural diffusion limits, has been dealt with by repeated surgeries to allow progressive vascularization (Shimizu et al., 2006). In a porcine model of ischemic cardiomyopathy the transplantation of cell sheets led to a significantly improved cardiac function although only few cells survived a period of 8 weeks after transplantation (Kawamura et al., 2012). The discrepancy between few surviving cells and improved heart function was observed in several studies (Kawamura et al., 2012; Ye et al., 2014) and is usually explained by paracrine effects and mechanical stabilization of the injured myocardium.

Cell–matrix injection

Cell retention can also be increased by injecting cells suspended in extracellular matrices that form hydrogels and immobilize the cells at the injection site, while preventing direct contact to the infarcted muscle (Anderl et al., 2009; Danoviz et al., 2010;

Kofidis et al., 2005). Hydrogels provide a microenvironment, which could facilitate cell survival and engraftment. On the other hand, pressure-induced cell death and the number/volume of maximally applicable cells remain a current limitation. Despite initial positive results (Kofidis et al., 2005), it was shown later that significant fractions of injected microspheres were lost from the injection site and that the long-term retention rate was low (Anderl et al., 2009).

Heart muscle patches

Another approach is to generate 3-D artificial heart muscle tissues in vitro and transplant them onto injured hearts. Several techniques for generating such tissues have been developed (Schaaf et al., 2011; Tulloch et al., 2011; Kensah et al., 2013; Nunes et al., 2013; Thavandiran et al., 2013; Turnbull et al., 2014; Radisic et al., 2008; Radisic et al., 2006; 2007; Zhang et al., 2013) and differ in construct size, extracellular matrix, and the mode of tissue anchoring. One possibility is to generate EHTs from liquid hydrogels such as collagen I (Bian et al., 2009; Eschenhagen et al., 1997; Zimmermann et al., 2002a), matrigel (Morritt et al., 2007), or fibrin (Hansen et al., 2010) to immobilize cells in a 3-D form. In this tissue format the cells align, form intercellular connections, produce their own extracellular matrix, and show improved sarcomeric organization compared to single cell cultures. Exposure to auxotonic stretching (the physiological mode of stretching within the heart) by attaching the ends of the tissue to flexible PDMS posts is essential for the formation of force generating tissue with parallel orientation of the cardiomyocytes (Bian et al., 2009; Eschenhagen et al., 1997; Zimmermann et al., 2002a; Fink et al., 2000). EHTs from hPSC-derived cardiomyocytes beat spontaneously and exhibit canonical reactions to changes in extracellular calcium-concentrations, sympathetic (isoprenaline) and parasympathetic (carbachol) stimulation, and changes in preload/length (Schaaf et al., 2011; Eschenhagen et al., 1997; Zimmermann et al., 2002a; Mannhardt et al., 2016).

Allogeneic transplantation of EHTs with neonatal rat (Zimmermann et al., 2006) or PGSC-derived mouse cardiomyocytes (Didie et al., 2013) onto infarcted hearts led to the formation of new myocardium on the scar, which electrically coupled to the recipient hearts, increased the conduction velocity in the scar and were associated with improved left-ventricular function. The transplantation of EHTs generated from hiPSC-derived cardiomyocytes and hiPSC-derived endothelial cells onto injured guinea-pig hearts led to a profound remuscularization of the scar (mean 12% of infarct scar size), accompanied by improved left-ventricular function. The grafts showed cardiomyocyte proliferation, vascularization, and evidence for electrical coupling to the intact heart tissue in a subset of tissues (Weinberger et al., 2016). While these positive results were observed in a subacute injury setting (transplantation 7 days after infarction), transplantation of EHTs from hESC-derived cardiomyocytes did not improve heart function in a chronic injury rat model (Riegler et al., 2015). It is not clear whether the lack of functional impact in the latter study was due to the chronic model and/or the choice of the rat as recipient which may impede coupling of the human cardiomyocytes. Data with hESC-cardiomyocytes in a chronic guinea-pig infarct model indicate that coupling may be less efficient than in a more acute model (Shiba et al., 2014).

Direct Cardiac Reprogramming

Instead of reprogramming somatic cells to iPSCs and subsequently differentiating them to different cell types, somatic cells can be directly transdifferentiated into another cell type. In 2010 Ieda et al. showed that fibroblasts can be transdifferentiated to cardiomyocyte-like cells with the three transcription factors GATA4, MEF2C, and TBX5 in vitro (Ieda et al., 2010). Intramyocardial retroviral delivery of the same factors led to the formation of cardiomyocyte-like cells from resident fibroblasts and improved myocardial function after infarction in young mice (Qian et al., 2012). Similar results were obtained with the transcription factors GATA4, HAND2, MEF2C, and TBX5 (Song et al., 2012); the transcription factors ETS2 and MESP1 (Islas et al., 2012); and with micro RNAs (Jayawardena et al., 2012). Despite these encouraging results, the reprogramming efficiency is currently still low, the phenotype of the generated cardiomyocytes appears incomplete (Chen et al., 2012), and the mechanisms of transdifferentiation are poorly understood. Furthermore, the in vivo delivery of reprogramming factors could have an influence on adjacent noncardiac tissue and uncontrolled reprogramming could lead to cardiomegalies and cause arrhythmias. Delivery of reprogramming factors could be realized by intramyocardial injections of lentiviruses, but their random integration in the genome constitute a significant safety concern. Nonintegrating vectors like adeno-associated virus (AAV) could circumvent this problem. AAVs (esp. AAV serotypes 1, 6, 8, and 9) efficiently transduce nondividing cells including cardiomyocytes (Bish et al., 2008; Wang et al., 2008; Palomeque et al., 2007). Cardiomyocyte-specific promoters further enhance organ specificity (Muller et al., 2006). So far AAVs have only been applied in preclinical models to stimulate cardiomyocyte proliferation by intramyocardial (Eulalio et al., 2012) or intravenous injections. A clinical application likely requires intracoronary application (reviewed in Katz et al., 2010 and Ishikawa et al., 2011). Clinical trials with AAVs have been conducted for several diseases. Alipogene tiparvovec (Glybera), an AAV 1 vector, was approved in 2012 by the European Medicines Agency to treat patients with familial LPL deficiency, and an AAV 1 coding for the sarcoplasmatic reticulum ATPase has been used in clinical trials for patients with heart failure (Greenberg et al., 2014). Although clinically relevant immune reactions and preexisting neutralizing antibodies have been described, translation toward the clinic seems feasible. Advantages of direct cardiac reprogramming are that it would circumvent time-consuming in vitro culture to generate a sufficient number of cardiomyocytes and avoid the need for immunosuppression. It would also be a simpler product in term of regulatory requirements and would be easier to administer.

Clinical Perspectives of Cellular Therapies

The Right Approach

As described earlier, each cell application technique has advantages and disadvantages regarding simplicity, required cell number, cell survival, biodistribution, accuracy of application, and efficiency of electrical integration. A direct comparison of the efficacy of cell injections, matrix-assisted cell injection, cell sheets, and muscle patches would be desirable but requires large group numbers in a relevant animal model to statistically demonstrate superiority or noninferiority of one over the other technique. An important additional limitation is that such a direct head-to-head comparison required high expertise in all application techniques while currently most laboratories specialize in one or the other technique.

Theoretically, endocardial or intramural injection of cells might provide better electromechanical coupling and effects on contractile function than the epicardial application of cell sheets or muscle patches (Gerbin et al., 2015). Especially because epicardially grafted tissue is often separated from the remaining subendocardial muscle layers of the host heart by a fibrous layer. However, endocardial application of cell sheets or muscle patches would require a larger surgical intervention with extracorporal circulation and would expose the transplanted tissue to the high intraventricular pressure. Thus, the vast majority of studies used the epicardial approach (Weinberger et al., 2016; Kawamura et al., 2012; Masumoto et al., 2014; Sawa et al., 2012; Zimmermann et al., 2006).

Independent of the application method and electromechanical coupling, the transplanted tissue has to be large enough to cover the scar and support contractile function. But the size of a nonperfused transplant is limited to a thickness of 100–200 μm in vitro (Shimizu et al., 2006; Hansen et al., 2010; Radisic et al., 2004,2005; Zimmermann et al., 2002b) by the diffusion limits of oxygen and nutrients. One possibility to minimize hypoxia-induced cell death is to implant thin tissue constructs composed of a loose network of immature and thereby hypoxia-resistant and proliferating cardiomyocytes. After host-derived vascularization of the transplant, cardiomyocytes in the graft can proliferate, likely explaining why some studies have observed compact muscle layers of 0.5–5.5 mm thickness (Weinberger et al., 2016; Zimmermann et al., 2006; Thompson et al., 1988; Wake et al., 1994; Mikos et al., 1993; Peters et al., 2002). An alternative strategy is to engineer prevascularized thicker tissues with a dense network of cardiomyocytes. Cocultures of cardiomyocytes, endothelial cells, and smooth muscle were shown to improve the formation of myocardial tissue in vitro and in vivo (Sekine et al., 2008; Stevens et al., 2009; Narmoneva et al., 2004; Levenberg et al., 2005). Artificial or biological vessels have been integrated into growing tissues (Masumoto et al., 2014; Tulloch et al., 2011; Chiu et al., 2012; Vollert et al., 2014), and heart constructs were grown on arterial-venous loops (Morritt et al., 2007; Tee et al., 1992–1999). While most studies showed improvement of cardiac tissue structure and function, the connection of the preformed vasculature to host vessels directly after transplantation remains a conceptional problem (for detailed review, see Coulombe et al., 2014). Furthermore, prevascularization increases the complexity of the tissue-engineered product in regulatory terms.

The next difficulty is the correct integration of the graft into the complex tissue structure of the heart, in which muscle strands wind around the heart lumen and align in several layers perpendicular to each other. Immature cardiomyocytes orient to the direction of mechanical load in a hydrogel (Zimmermann et al., 2002a; Hansen et al., 2010). Whether this holds true for injected cell suspensions or tissue-engineered muscle patches remains to be elucidated. A radically different approach is to use entire decellularized hearts (Ott et al., 2008) as a scaffold that comes with the full complexity of normal myocardial tissue and the entire organ. However, reintroducing cells into these matrices at sufficient cell density remains challenging (Guyette et al., 2016).

The Right Cell Type

The different types of PSCs have advantages and disadvantages (**Table 1**), which have to be considered for a therapeutic approach. The therapeutic application of PSCs could be realized by using cells from stem cells banks. Disadvantages of all PSC types are the risk of teratoma formation (Ohnishi et al., 2014; Blin et al., 2010; Riegler et al., 2016), immaturity of PSC-derived cardiomyocytes (reviewed in Yang et al., 2014), and the risk of increased arrhythmia susceptibility (Chong et al., 2014) (see also "Risks" section).

hESC are considered to be the most native hPSC cell source, as their genetic material has not been manipulated during isolation. But most hESC lines are being used for more than a decade and it is known that long-term cell culture itself may lead to genetic and epigenetic changes (Silva et al., 2008; Mitalipov et al., 2007). Therapy with hESC-derived cardiomyocytes could be realized by allogeneic transplantation of optimally MHC-matched cells from stem cells banks. Nevertheless, life-long immunosuppression with its severe adverse effects is inevitable. Up to date, ESC therapies are restricted to organs like the eye and the central nervous system (Drukker and Benvenisty, 2004), which are protected from immune response, to minimize rejection of transplanted cells. But due to their isolation from blastocysts, ESCs are ethical controversial and their use is restricted in many countries.

Advantages of human iPSCs are the lack of ethical concerns and their potential for autologous transplantation. But generating a stable patient-specific hiPSC line and differentiating sufficient numbers of high-purity cardiomyocytes (100–1000 Mio) is very time-consuming. Additionally, each single cell line would constitute an own "therapeutic product," requiring sufficient safety testing before a therapeutic application gets approved. This includes screening for genetic variations due to reprogramming, age-acquired mutations in coding regions (Young et al., 2012; Abyzov et al., 2012), and copy number variations (Hussein et al., 2011; Martins-Taylor et al., 2011). It is questionable if such time-consuming and expensive procedures can be carried out for each individual patient. Furthermore, even autologous iPSCs provoked low-level immune responses in mice (Zhao et al., 2011).

Table 1 Advantages and disadvantages of pluripotent stem cells for cardiac regeneration

Cell type	*Advantages*	*Disadvantages*
hESC	● High levels of telomerase activity (Thomson et al., 1998) ● No genetic modification ● High differentiation efficiency (Lian et al., 2013; Zhang et al., 2015; Kempf et al., 2015; Palpant et al., 2017; Lin et al., 2016; Burridge et al., 2011)	● Genetic and epigenetic consequences due to long-term culture (Silva et al., 2008; Mitalipov et al., 2007) ● Ethically controversial and currently restricted/not allowed in many countries ● Allogenic approach, life-long immunosuppression
hiPSC	● No ethical concerns ● High differentiation efficiency (Lian et al., 2013; Kempf et al., 2015; Palpant et al., 2017; Lin et al., 2016; Burridge et al., 2011, 2014; Breckwoldt et al., 2017)	● Inefficient reprogramming (0.001%–4.4%, Robinton and Daley, 2012) ● Heterogeneity in the levels of telomerase and telomere length (Wang et al., 2012) ● Tumorigenic risks of the permanent presence of the oncogene c-*MYC* in the genome ● Relatively high risk of chromosomal aberrations, mutations, and gene duplications (Gore et al., 2011; Hussein et al., 2011; Young et al., 2012; Abyzov et al., 2012; Martins-Taylor et al., 2011)
hPGSC	● No ethical concerns (some countries) ● Efficient parthenogenetic activation protocols (16.6%–28.6%) (Rogers et al., 2004; Lin et al., 2003) ● Less immunogenic than hESCs ● Higher heterozygosity compared to their oocyte donor (Dighe et al., 2008), which prevents the activation of cytotoxic natural killer cells (Kim et al., 2007) ● Homozygous HLA-loci allow for a coverage of the majority of a population with a limited amount of cell lines	● Ethical concerns (other countries including Germany) ● Loss of heterozygosity might cause immune rejection by cytotoxic killer cells (Kim et al., 2007) ● Loss of heterozygosity might be associated with cancer (Cavenee, 1989)
hNT-ESC	● No ethical concerns (some countries)	● Ethical concerns (other countries incl. Germany) ● Age-acquired mutations (Young et al., 2012; Abyzov et al., 2012) ● Less efficient generation than hPGSC (2.3%–20%, Paull et al., 2013; Tachibana et al., 2013) ● Mismatched mitochondria in NT-ESCs induce adaptive immune rejection (Deuse et al., 2015)

Although potentially occurring immune reactions will likely be lower than with HLA-matched allogeneic cells, thereby lowering the need for immunosuppression, the use of cells from stem cells banks seems more realistic.

The use of hPGSCs has advantages. Parthenogenetic activation protocols are rather simple and at the same time more efficient (16.6%–28.6%) (Rogers et al., 2004; Lin et al., 2003) than those resulting in NT-ESCs (2.3%–20%, Paull et al., 2013; Tachibana et al., 2013) or hiPSCs (0.001%%–4.4%, Robinton and Daley, 2012). On the other hand, PGSCs show aberrant genomic imprinting. Disruption or inappropriate expression of imprinted genes is associated with severe clinical syndromes and carcinogenesis in humans (Nakagawa et al., 2001). Another safety issue is genomic homozygosity anticipated in diploid, monoparental PGSCs, since extensive loss of heterozygosity is the most common genetic alteration in human cancers (Cavenee, 1989). However, PGSCs showed high heterozygosity compared to their oocyte donor (Dighe et al., 2008), which is due to the shuffling of alleles between parental chromosomes in prophase I. This is also an immunological advantage. Next to non-self-alleles, the immune system recognizes missing alleles within the major histocompatibility complex (or human leucocyte antigen, HLA). Thereby, heterozygosity is pivotal to prevent the activation of cytotoxic natural killer cells (Kim et al., 2007). To implement histocompatible transplantations, banked PGSC lines should be analyzed for heterozygosity in HLA loci to avoid transplant rejections and to reduce the need for immunosuppressive therapy (Bray et al., 2008).

NT-ESCs share some advantages and disadvantages with iPSCs. NT-ESCs theoretically allow an autologous therapeutic approach. Indeed, SCNT was thought to allow for the production of patient-matched stem cells, even in patients with hereditary mitochondrial diseases. But mismatched mitochondria in NT-ESCs were observed to induce adaptive immune rejection (Deuse et al., 2015), thereby necessitating the development of strategies to produce mitochondrial-matched NT-ESCs. Possible disadvantages are age-acquired mutations (Young et al., 2012; Abyzov et al., 2012) and the associated screening for coding region mutations before clinical use.

Cell Number

The minimum effective cell number for functional improvement is an important parameter in terms of feasibility, safety, and therapy costs. Studies utilized 0.03 (Ye et al., 2014) to 25 (Chong et al., 2014) Mio PSC-derived cardiomyocytes/g heart tissue and observed similar, albeit not identical improvements of cardiac function, sometimes even in the absence of substantial formation of new myocardium. This discrepancy is usually explained by poorly defined paracrine effects (Loffredo et al., 2011; Kawamura et al., 2012; Ye et al., 2014; Gnecchi et al., 2005; Iso et al., 2007; Williams and Hare, 2011; Li et al., 2012). Dose–response studies are

difficult due to multifold reasons like varying infarct size, variability of echocardiography or magnetic resonance imaging to measure cardiac function, and considerable graft size differences even with a constant dose of cells (Weinberger et al., 2016; Laflamme et al., 2005). In some studies the induced injury was not sufficient to significantly decrease heart function, partly due to high mortality rates associated with large injuries (Chong et al., 2014; Blin et al., 2010). Systematic trials with large study cohorts have to further investigate the effect of different cell numbers on therapeutic outcome.

Risks

Risks have to be carefully considered before clinical application of PSC-derived cardiomyocytes becomes a real therapeutic option. As differentiation protocols do not completely eliminate pluripotent stem cells, PSC-based cell therapies inevitably bear the risk of teratoma formation. The problem is further aggravated by biodistribution of cells after transplantation (Laflamme et al., 2007; Weinberger et al., 2016; Dow et al., 2005). Teratoma formation has been observed after application of ESC-derived cardiac progenitor cells into immunosuppressed Rhesus monkeys or of hiPSC-derived cardiomyocytes into immunocompromised rat hearts (Blin et al., 2010; Riegler et al., 2016). The problem may be circumvented by further improvement of purification protocols for differentiated cardiomyocytes (Laflamme et al., 2007; Dubois et al., 2011; Kim et al., 2010; Hattori et al., 2010; Uosaki et al., 2011; Tohyama et al., 2013) or elimination of remaining pluripotent cells with selection media (Matsuura et al., 2015). Nevertheless, long-term experiments in sufficient numbers of immunodeficient animals are needed to quantify the risk. For such experiments, a combination of serum biomarkers with MRI screening could be useful (Riegler et al., 2016).

Another problem of hiPSCs is their relatively high risk of chromosomal aberrations, mutations, and gene duplications (Gore et al., 2011; Hussein et al., 2011; Young et al., 2012; Abyzov et al., 2012; Martins-Taylor et al., 2011). All of these could affect not only the function of iPSC-derived cardiomyocytes and the functional outcome of transplantation studies but also safety. Whole-genome sequencing of a therapeutic cell line is probably required before clinical application. The same holds true for PGSCs, which show expression of mutated genes associated with cancer (Knudson, 1971), thereby bearing the risk of tumor formation.

It is well known that PSC-derived cardiomyocytes are rather immature. hiPSC-CMs, for example, beat spontaneously; show proliferation (Weinberger et al., 2016); and exhibit fetal-like ion channel patterns (Beqqali et al., 1956–1967), electrophysiological signals (Davis et al., 2011), and gene expression profiles (Cao et al., 2008). Long-term cultivation, mechanical loading, pattern of extracellular matrix, and electrical stimulation can improve maturation (Tulloch et al., 2011; Hirt et al., 2014; Domian et al., 2009; Piccini et al., 2015; Ruan et al., 2016; Weber et al., 2016; Dai et al., 2017). While immaturity is a disadvantage for in vitro testing, transplanting immature cardiomyocytes could also have advantages. Due to their ability of anaerobic metabolism, they are hypoxia-resistant and thereby able to survive without sufficient oxygen and nutrient support before vascularization occurs. Their proliferative activity could compensate cell loss after transplantation and increase graft size. On the other hand, unrestricted proliferation might promote tumor formation and cause arrhythmias.

The latter complication could also occur if transplanted cardiomyocytes continued to beat spontaneously and autonomically. Next to the functional contribution of transplanted cells to contraction force of the recipient's heart, this is why electrical coupling of transplanted to host cardiomyocytes is crucial for a successful and safe therapy. The hitherto published studies give opposing results. While an antiarrhythmic effect of ESC-derived cardiomyocytes was described after injection into injured guinea-pig hearts (Shiba et al., 2012), the follow-up study in nonhuman primates showed the opposite (Chong et al., 2014).

Important Questions to be Answered on the Way to Cardiac Regeneration in Patients

Recent progress in regenerative therapies is exciting and the first hPSC-derived product has already been applied in a patient (Menasche et al., 2015). Yet, important questions remain to be answered before these therapies can be translated to patients at a larger scale.

1. Can the promising results of proproliferative intervention (e.g., with miRNAs or overexpression of growth factor receptors) in mice be transferred to large animal models?
2. To which extent can the functional recovery observed after such therapies be explained by new heart muscle formation?
3. How can the low efficiency of direct reprogramming of (scar) fibroblasts into cardiomyocytes be improved without causing unwanted effects?
4. How can such therapy be restricted in terms of time and space to prevent unwanted effects?
5. Can the promising results of PSC-cardiomyocyte transplantation (as cell suspension or tissue) in small animal models be transferred to large animal models?
6. To which extent can the functional recovery after PSC-cardiomyocyte transplantation (as cell suspension or tissue) be explained by remuscularization and new contractile mass participating in overall heart function?
7. How can cell retention after injection be improved?
8. How many cells (in suspension or as tissue) are needed to repair an injured (human) heart?
9. How can electrical coupling be improved to allow robust functional integration of hPSC-cardiomyocytes and prevent arrhythmias?
10. Is coupling truly a more relevant issue in chronic injury models that better mimic the situation in patients than acute injury models?

11. How to overcome hPSC-associated immunological issues and the need for immunosuppression?
12. What is the ideal patient population for the first clinical studies of the different approaches?

Answering these questions will need time, but the ground is laid to make these approaches a realistic scenario in the foreseeable future.

Further Reading

As described earlier, encouraging progress was achieved in cell transplantation approaches, but there is still no consensus on the best cell type to use. Clinical trials aiming to regenerate the heart used bone-marrow-derived cells (Assmus et al., 2010; Marban and Malliaras, 2012; Traverse et al., 2012), mesenchymal stromal cells (Hare et al., 2012; Guijarro et al., 2016; Lee et al., 2014; Trachtenberg et al., 2011; Gao et al., 2015; Can et al., 2015; Mathiasen et al., 2015), or cardiac progenitor cells (Bolli et al., 2011; Makkar et al., 2012). Although signals of improved cardiac function were observed, the effect on morbidity and mortality remains unclear (Clifford et al., 2012), and regeneration of the injured myocardium was not observed. Detailed information on this topic can be found in reviews with a wider scope (Singh and Sen, 2016; Madonna et al., 2016; Gouadon et al., 2016; Gerbin and Murry, 2015b; Lin and Pu, 2014; Behfar et al., 2014).

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Myocarditis

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Introduction

Myocarditis does not have a typical clinical presentation and may mimic other noninflammatory diseases. Thus, in current classifications from the World Health Organisation/International Society and Federation of Cardiology (WHO/ISFC) (Richardson et al., 1996), the European Society of Cardiology (ESC) (Elliott et al., 2007), the American Heart Association (AHA) (Maron et al., 2006) as well as in ESC expert consensus statements (Caforio et al., 2013a), myocarditis is defined as an inflammatory myocardial disease, diagnosed on endomyocardial biopsy (EMB) using established histological, immunological, immunohistochemical, and molecular criteria (to identify possible infectious agents, mainly cardiotropic viruses by gene amplification techniques). The definition of "inflammatory cardiomyopathy" is applied to biopsy-proven myocarditis in patients with associated ventricular dysfunction. The current nomenclature is summarized in **Table 1**. Although EMB is the gold standard for myocarditis and is the only tool which allows distinction of its different etiologies, it is mainly used in tertiary expert referral centers (Cooper et al., 2007; Leone et al., 2012). Thus, there is a relative paucity of unbiased epidemiology data. Biopsy-proven myocarditis may heal spontaneously, relapse, or become chronic leading in about one third of cases to dilated cardiomyopathy (DCM), death, or heart transplantation (Caforio et al., 2007; Schultheiss et al., 2011). Historically, when the diagnosis was based upon the Dallas criteria, myocarditis was considered a relatively rare cause of heart failure or sudden cardiac death (Aretz et al., 1987). Nowadays, the application of immunohistochemistry and highly sensitive molecular tools to EMB (Leone et al., 2012; Schultheiss et al., 2011) as well as new noninvasive tissue imaging techniques, in particular cardiovascular magnetic resonance (CMR) (Friedrich et al., 2009; Francone et al., 2014) suggest that myocarditis prevalence in the population may be substantially underestimated.

Pathology

The pathological picture is described by the Dallas definition of inflammatory cell infiltrate with associated cardiomyocyte necrosis, not typical of ischemic necrosis, observed on EMB or at autopsy (**Table 1**) (Aretz et al., 1987). Myocarditis is histologically classified

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Table 1 Definitions

Myocarditis
According to WHO /ISFC (Richardson et al., 1996), myocarditis is defined as:
Inflammatory disease of the myocardium diagnosed by established histological*, immunohistochemical** and immunological criteria***
*Established histological Dallas criteria defined as follows (Aretz et al., 1987):
"histological evidence of inflammatory infiltrates within the myocardium associated with myocyte degeneration and necrosis of non-ischemic origin"
**Immunohistochemical criteria, abnormal inflammatory infiltrate defined as follows (Leone et al., 2012; Caforio et al., 2013a):
"$\geq$14 leucocytes/mm^2 including up to 4 monocytes/mm^2 with the presence of CD 3 positive T-lymphocytes $\geq$7 cells/mm^2"
***Immunological criteria and myocarditis etiology defined as follows (Caforio et al., 2013a):
–Viral: histology (Hx) and immunoHx positive (pos), polymerase chain reaction (PCR) pos for $\geq$virus
–Autoimmune: Hx and immunoHx pos; viral PCR negative (neg); with or without pos cardiac autoantibodies (aabs); exclusion of other known inflammatory causes
–Viral and immune ^: Hx and immunoHx pos; viral PCR pos; cardiac aabs pos
^N.B. a follow-up EMB may identify persistent viral myocarditis, resolved myocarditis (Hx and virological), or persistent virus-negative myocarditis, e.g. post-infectious autoimmune
Inflammatory Cardiomyopathy and Dilated Cardiomyopathy (DCM)
According to WHO /ISFC (Richardson et al., 1996), inflammatory cardiomyopathy is defined as: Myocarditis in association with cardiac dysfunction
Inflammatory cardiomyopathy is involved in the pathogenesis of DCM and includes idiopathic, autoimmune and infectious subtypes
According to WHO /ISFC (Richardson et al., 1996) and ESC (Elliott et al., 2007) DCM is defined as:
DCM is a clinical diagnosis characterized by dilation and impaired contraction of the left or both ventricles that is not explained by abnormal loading conditions or coronary artery disease
DCM includes idiopathic, familial/genetic, viral and/or immune, alcoholic/toxic subtypes

according to the predominant cellular infiltrate as lymphocytic, eosinophilic, polymorphic, giant cell myocarditis, or cardiac sarcoidosis. Although the Dallas criteria represent an important first attempt to standardize histological diagnosis, in the subsequent decades several limitations have been pinpointed: (a) inflammatory cell characterization by immunohistochemistry and fibrosis amount are not considered; (b) the type and extent of myocyte damage are not specified; (c) the term borderline myocarditis, including also chronic forms, remains equivocal and does not help in the clinical setting; (d) any reference to etiological agents is lacking (Leone et al., 2012). Thus, more recently the Dallas criteria have been implemented by adding immunohistochemical and molecular criteria (Leone et al., 2012; Caforio et al., 2013a) (**Table 1**). In particular, an international Task Force of the Association for European CardioVascular Pathology (AECVP) and the Society for CardioVascular Pathology (SCVP) (Leone et al., 2012) and the European Society of Cardiology (ESC) Working Group on Myocardial and Pericardial Disease Myocarditis Task Force (Caforio et al., 2013a) published consensus papers concerning the current role of EMB in myocarditis. In these documents, it has been emphasized that pathological analysis of EMB is complex and requires standard protocols. Optimal specimen procurement and triage indicates at least three, preferably four, EMB fragments, each 1–2 mm in size, that should immediately be fixed in 10% buffered formalin at room temperature for light microscopic examination. In expected focal myocarditis additional sampling is recommended. Moreover, in this clinical setting, one or two specimens should be snap-frozen in liquid nitrogen and stored at −80°C for possible molecular tests or specific stains. Otherwise, fragments may be stored in RNAlater (a solution preventing RNA degradation). Infectious myocarditis is diagnosed by a positive result for one or more infectious agents, mainly viruses, using molecular techniques, mainly reverse transcriptase polymerase chain reaction (R-T PCR) on EMB (**Table 2**) (Leone et al., 2012; Pankuweit and Klingel, 2012; Caforio et al., 2007; Schultheiss et al., 2011).

Etiology and Pathogenesis

Etiopathogenetic agents in myocarditis are listed in **Table 2**.

Viral Myocarditis

Viral agents are presumed to be the most frequent causes of myocarditis in North America and in Europe. Viral genomes are detected to a variable extent on EMB by molecular techniques, mainly R-T PCR (Pankuweit and Klingel, 2012; Caforio et al., 2007; Schultheiss et al., 2011; Bock et al., 2010; Martin et al., 1994; Bowles et al., 2003; Kühl et al., 2005; Kindermann et al., 2008). In recent studies the most commonly detected viruses, particularly in Germany, are Parvovirus B-19 (PVB-19) and Human Herpes virus 6 (HHV6) and these two agents are both present in a sizable proportion of patients (Pankuweit and Klingel, 2012; Schultheiss et al., 2011; Bock et al., 2010; Kühl et al., 2005; Kindermann et al., 2008). The mechanism of cardiomyocyte damage in myocarditis associated with PVB-19 and/or HHV6 is still poorly understood. Some, but not all studies suggest that PVB-19 infection may be pathogenic only in a selected patient group (Pankuweit and Klingel, 2012; Bock et al., 2010, 2014). The frequency of biopsy-proven viral myocarditis may vary as a function of the disease stage, as well as the diverse epidemiology of viral infection in distinct populations and ethnic groups (Pankuweit and Klingel, 2012; Caforio et al., 2007; Schultheiss et al., 2011; Bock et al., 2010, 2014; Martin et al., 1994; Bowles et al., 2003; Kühl et al., 2005; Kindermann et al., 2008).

Table 2 Etiopathogenesis of myocarditis

1. *Infectious agents*	
Bacterial	Haemophilus influenzae, Mycobacterium (tuberculosis), Mycoplasma pneumoniae, others (rare)
Spirochetal	Borrelia (Lyme disease), Leptospira (Weil disease)
Fungal	Uncommon, mainly immunocompromised patients
Protozoal	Trypanosoma cruzi (common in South America), others (rare)
Parasitic	Rare
Rickettsial	Rare
Viral (common)	*RNA viruses*: coxsackievirus A and B, echovirus, influenza A and B virus, respiratory syncytial virus, human immunodeficiency virus-1, others (rare)
	DNA viruses: parvovirus B19 (most common in recent German series), adenovirus (mainly pediatric cases) cytomegalovirus (immunocompromised patients), herpes simplex virus, human herpes virus-6, common in recent German series, often in association with parvovirus B19), Epstein-Barr virus, others (rare)
2. *Immune-mediated (infection-negative)*	
Allergic	Tetanus toxoid, vaccines, serum sickness
	Drugs: penicillin, cefaclor, colchicine, furosemide, isoniazid, lidocaine, tetracycline, sulfonamides, phenytoin, phenylbutazone, methyldopa, thiazide diuretics, amitriptyline
Alloantigenic	Heart transplant rejection
Autoimmune	Organ-specific: lymphocytic (common), giant cell (rare)
	Associated with autoimmune or immune-oriented disorders: systemic lupus erythematosus, rheumatoid arthritis, Churg-Strauss syndrome, Kawasaki's disease, inflammatory bowel disease, scleroderma, polymyositis, myasthenia gravis, insulin-dependent diabetes mellitus, thyrotoxicosis, sarcoidosis, Wegener's granulomatosis
3. *Toxic*	
Drugs	Amphetamines, anthracyclines, cocaine, cyclophosphamide, ethanol, fluorouracil, lithium, catecholamines, hemetine, interleukin-2, trastuzumab, clozapine
Heavy metals	Copper, iron, lead
Miscellaneous	Scorpion sting, snake, and spider bites, bee and wasp stings, carbon monoxide, inhalants, phosphorus, arsenic, sodium azide
Hormones	Pheochromocytoma, vitamins: beri-beri
Physical agents	Radiation, electric shock

Adapted from Caforio, A., Pankuweit, S., Arbustini, E., et al. (2013) Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *European Heart Journal* **34**(33), 2636–2648.

Postinfectious autoimmune myocarditis

Postinfectious myocarditis has been best studied in experimentally enterovirus-induced murine models (Pankuweit and Klingel, 2012). Several strains show persistence of enteroviral RNA and myocardial inflammation for several weeks, with subsequent autoimmune reaction. In addition, it is clinically observed that patients with previous biopsy-proven viral myocarditis develop persistent virus-negative disease with production of serum anti-heart autoantibodies (AHAs), for example, postinfectious autoimmune myocarditis (Caforio et al., 2007). Thus, the clinical diagnosis of postinfectious autoimmune myocarditis requires a follow-up EMB (**Table 1**). The same murine strains susceptible to postinfectious myocarditis develop autoimmune myocarditis following immunization with cardiac myosin, or spontaneously (Pankuweit and Klingel, 2012; Caforio et al., 2013b), under control of major histocompatibility complex (MHC) and non MHC genes, similar to other autoimmune conditions (Caforio et al., 2013b). The human equivalent of the murine MHC is the human histocompatibility complex (HLA) and it has been shown that HLA is involved in genetic predisposition to inflammatory DCM (Meder et al., 2014).

Primarily autoimmune myocarditis

The diagnosis of primarily autoimmune myocarditis requires exclusion of cardiotropic virus genome on EMB and of other causes, for example, toxic (**Table 1**). The detection of serum organ-specific AHAs is additional evidence in support of autoimmunity, although, as for other autoimmune conditions, the absence of these markers does not rule out an autoimmune process, since there may be predominant cell-mediated forms (Caforio et al., 2013a,b). In addition, AHAs titers may be reduced in advanced stages of the disease (Caforio et al., 2013b). Autoimmune myocarditis is most commonly an organ-specific disease, e.g. confined to the heart, or takes place in the context of systemic immune-mediated diseases (SIDs) (**Table 2**). In these cases, the heart may host self-antigens or neoantigens against which specific autoreactive clones are expanded and directed (autoimmune organ-specific myocarditis and autoimmune myocarditis in the context of multisystem autoimmune diseases). Alternatively, the myocardium may be passively infiltrated and damaged in the course of localized or generalized proliferation of immunocompetent cells or during systemic hyper-inflammatory reactions. This mechanism may be involved in eosinophilic myocarditis in the setting of drug-induced reaction with eosinophilia and systemic symptoms syndrome (DRESS syndrome) (Caforio et al., 2013b).

Table 3 Fulfilled Rose-Witebsky autoimmune features in myocarditis and inflammatory cardiomyopathy

Major
- Mononuclear cell infiltrates and abnormal HLA expression on EMB in the absence of infectious agents (by PCR) or known inflammatory causes
- Serum autoantibodies in patients and in unaffected family members
- Autoantibody and/or autoreactive lymphocytes in situ within the affected tissue
- Identification and isolation of autoantigen (s) involved
- Disease induced in animals by immunization with relevant autoantigen, and/or passive transfer of serum, purified autoantibody and/or lymphocytes
- Efficacy of immunosuppressive therapy and of immunoadsorption in patients

Minor

(a) Common to all autoimmune disorders
  - Familial aggregation
  - HLA association
  - Clinical course characterized by exacerbations and remissions
  - Autoimmune diseases associated in the same patient or in family members

(b) Typical of organ-specific autoimmune disorders
  - Autoantibodies directly against organ-specific autoantigens
  - Induction of antibodies induces an organ-specific disease/phenotype
  - Transfer of autoantibodies also transfers the disease/phenotype

Adapted from Caforio, A., Marcolongo, R., Jahns, R., et al. (2013). Immune-mediated and autoimmune myocarditis: clinical presentation, diagnosis and management. *Heart Failure Reviews* **18**(6), 715–732.

In human autoimmune diseases the diagnosis requires fulfillment of at least two major Rose-Witebsky criteria (Table 3). Autoimmune features in myocarditis or cardiomyopathy include: familial aggregations and HLA associations; detection of lymphomonocytic cellular infiltrates in the myocardium on EMB tissue, in conjunction with enhanced HLA and adhesion molecule expression of cardiac endothelial cells, in the absence of infectious agents in patients and affected family members; abnormal serum cytokine and cardiac autoantibodies (aabs) in patients and affected family members (Fig. 1) and efficacy of immunosuppression/immunomodulation. Using different immunological techniques and antigenic substrates, many cardiac aabs have been detected and for some aabs a direct pathogenic role has been demonstrated. Proofs of their role include: (1) functional effects of aabs in vitro; (2) demonstration of myocarditis, or cardiomyopathy features in genetically predisposed experimental animals following immunization with specific autoantigens, for example, myosin or troponin; (3) transfer of myocarditis, or cardiomyopathy features from immunized to healthy animals following injection of aabs or immune cells, for example, passive transfer experiments; (4) improvement of cardiac chamber dimension and pump function following removal of aabs by immunoadsorption (IA) both in animals and in patients (Table 3).

Pathophysiology

The myocarditis process can result in: (1) cardiomyocyte necrosis, with an increase in troponin levels; (2) systolic and diastolic dysfunction, which can be transient, as a result of spontaneous or drug-induced healing, or persistent with reactivations or a more chronic subacute downhill course leading, if untreated, to interstitial fibrosis and end-stage burned-out DCM; (3) arrhythmias, which may be associated with the acute inflammatory and/or the irreversible post-myocarditic phases. These three features may be variably intermingled in single patients.

Clinical Presentation

In myocarditis cardiac signs and symptoms are heterogeneous and unspecific, depending upon the extent of inflammation and of cardiac dysfunction; they may be subtle and the disease may be unrecognized for months or years. The disease should be suspected especially in young patients with unexplained heart failure signs and/or symptoms, unexplained arrhythmias, and/or chest pain with unobstructed coronary arteries with or without troponin release, fever, and associated respiratory or gastrointestinal viral syndromes. However, although less frequently, myocarditis may occur at any age. The spectrum of arrhythmia in myocarditis is wide, including syncope and sudden cardiac death. Similarly, the heart failure spectrum includes acute, subacute, chronic presentations, also in the peri-partum period, mimicking a peri-partum cardiomyopathy as well as unexplained cardiogenic shock. The so-called "fulminant" myocarditis is defined by the following features: a viral prodrome within 4 weeks from symptom onset, new-onset heart failure, hemodynamic instability, and a favorable outcome, provided that giant cell or eosinophilic forms are excluded as well as myocarditis associated with SIDs. Since myocarditis may mimic other noninflammatory disease (valvulopathy, coronary artery disease, Takotsubo, peri-partum, or arrhythmogenic cardiomyopathy) it is a diagnosis of exclusion.

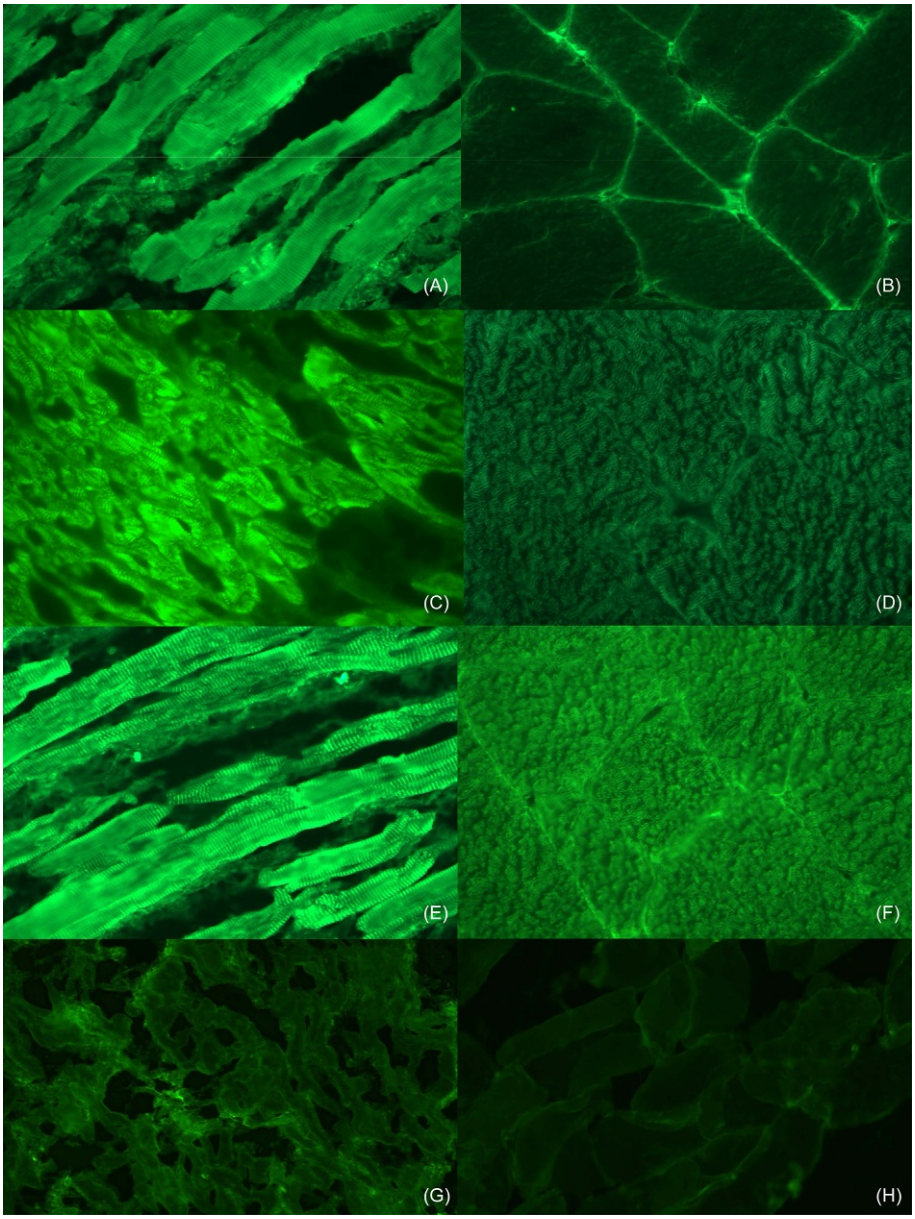

Fig. 1 Anti-heart aabs (AHA) patterns by indirect immunofluorescence test. *Organ-specific AHA pattern: panel A* on human heart tissue: cytoplasmic diffuse staining of cardiac myocytes (organ-specific AHA pattern) (×400); *panel B* (×400) on human skeletal muscle tissue: negative. *Partially organ-specific (or cross-reactive 1) AHA pattern: panel C* on human heart tissue: positive fine striational pattern (×400), and *panel D* on human skeletal muscle: weak positive fine striational pattern (×400). *Other example of cross-reactive 1 AHA pattern: panel E* on human heart tissue: strong positive fine striational pattern (×400), and *panel F* on human skeletal muscle: positive pattern (×400). *Negative AHA control serum pattern: panel G* on human heart tissue: negative (×400), and *panel H* on human skeletal muscle: negative (×400).

Diagnostic Work-Up

Electrocardiography and Echocardiography

Electrocardiography (ECG) features in myocarditis are unspecific and may include any kind of idiopathic supraventricular or ventricular brady or tachyarrhythmias, as well as ST-T segment abnormalities. Some suggestive ECG findings include: diffuse ST segment elevation without reciprocal changes, PR depression in myocarditis associated with pericarditis. Pathological Q waves are uncommon in myocarditis. T wave inversion generally takes place after ST-T segment normalization.

Echocardiography is useful to study morphology and function of the cardiac chambers and valves, but it is unspecific, may be normal, with segmental wall motion abnormalities or diffuse hypokinesis similar to DCM. Pericardial effusion is not common, although may be present and suggest associated pericarditis.

Left ventricular aneurisms may be present in Chagas disease or in cardiac sarcoidosis. In fulminant myocarditis ventricular walls may be thickened and ventricular chambers are most often mildly dilated and severely hypokinetic. Echocardiography helps in excluding associated causes of heart dysfunction, such as valve disease. In addition, it is key in monitoring cardiac function noninvasively at follow-up, since biventricular function may change as a result of resolution or reactivation of the inflammatory process.

Nuclear Imaging and Cardiovascular Nuclear Magnetic Resonance

Nuclear imaging is currently rarely used, except for Gallium-67 scintigraphy and positron emission tomography using 18 fluorodeoxyglucose in clinically suspected cardiac sarcoidosis. CMR gives morpho-functional information similar to echocardiography, but provides tissue characterization data using contrast sequences (gadolinium).

An International CMR consensus report (Friedrich et al., 2009) in clinically suspected myocarditis recommends the combined use of three techniques (the so-called Lake-Louise criteria), which include: regional or global myocardial signal intensity increase in T2-weighted edema images (myocardial edema); increased global myocardial early gadolinium enhancement ratio between myocardium and skeletal muscle in gadolinium-enhanced T1-weighted image (early myocardial hyperemia); at least one focal lesion with nonischemic regional distribution in inversion recovery-prepared gadolinium-enhanced T1-weighted images, for example, late gadolinium enhancement (LGE) (myocardial necrosis/fibrosis). In myocarditis LGE location is typically subepicardial, often located at the infero-lateral or septal left ventricular wall segments. The combination of these three parameters carries the highest diagnostic accuracy (78%), although correlative data with EMB are still scarce. CMR is not specific for a particular type of myocardial infiltrate (e.g., eosinophilic, lymphocytic) or for a specific etiology (viral vs. autoimmune). In hemodynamically stable patients, CMR may strengthen the diagnostic suspicion of myocarditis before EMB is performed, but, in life-threatening conditions, EMB should be performed as soon as possible. During follow-up CMR can be a valid tool to monitor evolution of myocardial inflammation, especially in virus-negative forms. Conversely a follow-up EMB can proof viral clearance in previously diagnosed viral myocarditis.

Biomarkers

Erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) serum levels are not increased in the majority of myocarditis patients, unless associated pericarditis is present. Cardiac troponins are unspecific markers of cardiomyocyte necrosis and, if normal, do not exclude myocarditis. The same applies to cardiac hormones, for example, natriuretic peptides, and circulating cytokines.

Viral Serology

Serological positivity does not imply an active myocardial infection. In the general population IgG seroprevalence against common cardiotropic viruses is high in the absence of myocardial infection and inflammation. Moreover, noncardiotropic enteroviral infection can induce reactive antibodies indistinguishable from those induced by cardiotropic strains and major studies demonstrated no correlation between viral serology and EMB viral genome positivity. Therefore, the ESC 2013 Task Force did not recommend viral serology in myocarditis patients, except when hepatitis C virus, Rickettsia, Borrelia Burgdorferi, or HIV infections are suspected, especially in high risk groups (Caforio et al., 2013a).

Serum AHAs

In myocarditis/idiopathic DCM patients and relatives, AHAs are directed against various antigens, some of which are cardiac-specific (e.g., organ-specific), others expressed both in myocardium and skeletal muscle (e.g., muscle-specific), as shown in **Fig. 1**. Different aabs have different prevalence between patients and healthy controls (e.g., organ-specific and type 1 cross-reactive AHAs are specific for myocarditis/DCM, whereas some other muscle aabs are not) (Caforio et al., 2013a,b). IgG class aabs, which are specific for heart tissues and myocarditis/DCM, can be used as autoimmunity markers to identify patients candidate to immunosuppressive therapy and their at risk relatives. Some aabs have a demonstrated functional pathogenic role and may have a therapeutic and prognostic potential. The 2013 ESC Task Force recommends AHA testing in patients with clinically suspected or biopsy-proven myocarditis, using one or more of the validated tests, and preferably testing for those AHAs that have a demonstrated good diagnostic accuracy for myocardial inflammatory disease (Caforio et al., 2013a).

Clinically Suspected Myocarditis: 2013 ESC Task Force Criteria

The 2013 ESC Task Force on myocarditis introduced new criteria for clinically suspected myocarditis, to help clinicians in identifying myocarditis, even at centers in which EMB is not routinely performed. In the setting of a compatible clinical presentation, additional criteria from other categories are required to establish a strong clinical suspicion, provided noninflammatory causes of cardiac disease have been excluded.

Clinically suspected myocarditis is defined in the presence of at least one of the compatible clinical presentations (with or without ancillary features) and at least one diagnostic criteria from the four diagnostic categories summarized in Fig. 2, in the absence of:

- Coronary artery disease (stenosis $\geq$50%);
- Previously diagnosed cardiovascular disease or extra-cardiac causes explaining the clinical syndrome (e.g., valvulopathy, congenital diseases, etc.)

The suspicion is higher when an elevated number of criteria is fulfilled; in asymptomatic patients the diagnosis can be established if at least two criteria from the four diagnostic categories have been satisfied, for example ECG abnormalities and increased myocytolysis makers.

In clinically suspected myocarditis, the following anamnestic clues should be carefully reviewed:

- Family history of: DCM, other cardiomyopathies, sudden cardiac death, autoimmune disease;
- Personal history of: a recent (within 2 weeks) viral syndrome of the upper respiratory airways or of the gastrointestinal tract, allergies, extra-cardiac autoimmune diseases, previous clinically suspected or biopsy-proven myocarditis, alcoholism, exposure to toxics or recreational drugs (e.g., cocaine), vaccination, travels to countries at high risk for cardiotropic microbiological agents (e.g., Brazil, Argentina for Chagas cardiomyopathy), domestic animals, classical risk factors for coronary disease, etc. The aim is to identify potentially curable causes of cardiac disease.

Regarding the clinical presentations shown in Fig. 2:

- Infarct-like disease can present with or without global or regional right and/or left ventricular dysfunction, and troponin release pattern can simulate ischemic necrosis or follow a more prolonged course with possible increases in the case of myocarditis reactivation.
- Life-threatening presentations include malignant arrhythmia and aborted sudden cardiac death, cardiogenic shock, and severely depressed left ventricular systolic function.

Biopsy-Proven Myocarditis: Task Force Criteria 2013

In patients with clinically suspected myocarditis, the 2013 ESC Task Force recommends to perform coronary angiography and EMB with histological and immunohistochemical analysis as well as the search of viral genome by polymerase chain reaction (PCR) (Fig. 2). To increase the diagnostic sensitivity of histological analysis and to characterize the inflammatory infiltrate, immunohistochemical analysis uses a panel of monoclonal and polyclonal antibodies, which include antibodies against CD3, a pan-T lymphocyte marker, CD68, a macrophage marker. Anti-HLA-DR staining may identify its increased expression on myocardial tissue, which, in the absence of infection, may identify reversible immune-mediated myocarditis. These recommendations also apply to patients with infarct-like presentation although this specific scenario has not been included in the list of EMB indications listed by the American Heart Association/American College of Cardiology/European Heart Society consensus statement (Cooper et al., 2007). The 2013 ESC Task Force has developed these recommendations based upon the following considerations: (a) EMB complication rate is very low and comparable to that of standard coronary angiography (0%–0.8%); (b) EMB confirms the diagnosis and actually is the only tool that allows etiological diagnosis and etiology-driven therapy; (c) EMB is key for safe and efficacious immunosuppressive therapy in EMB-proven absence of viral genome. Thus, main contributions of EMB are summarized in Table 4.

Clinical Features of Specific Forms of Myocarditis

Infectious, Nonviral, Myocarditis

Lyme disease, caused by Borrelia Burgdorferi, has a wide spectrum of organ involvement and clinical presentations, ranging from asymptomatic cardiac disease to grade I to III atrioventricular block and/or transient or permanent severe heart failure. Advanced atrio-ventricular block may require a temporary pacemaker implantation since, in the majority of cases, it resolves within 1 week from onset.

Trypanosoma Cruzi is a common cause of myocarditis/DCM in central and south America (Chagas cardiomyopathy), characterized by an acute infectious febrile phase followed by a chronic asymptomatic autoimmune phase, lasting till 30 years. This agent is transmitted to humans through a tick's bite. Clinically, the acute phase manifests with heart failure and arrhythmia and usually lasts some months. In about 20% of patients who survive the acute phase, a more chronic disease ensues, leading to DCM, usually after a symptom-free interval of 20 years. The main clinical features are biventricular dilatation with systo-diastolic dysfunction, apical aneurysms of the left ventricle, severe hypo- or hyper-kinetic arrhythmia, partially due to abnormal autonomic nervous system heart firing, intracavitary thrombus formation with cardio-embolization and finally cardiac death.

Toxoplasma gondii myocarditis, often in the setting of fungal coinfection, is typical of seronegative transplanted recipients of seropositive donors, and of immunosuppressed patients, particularly HIV-infected patients, who are at risk of serious life-threatening opportunistic infections.

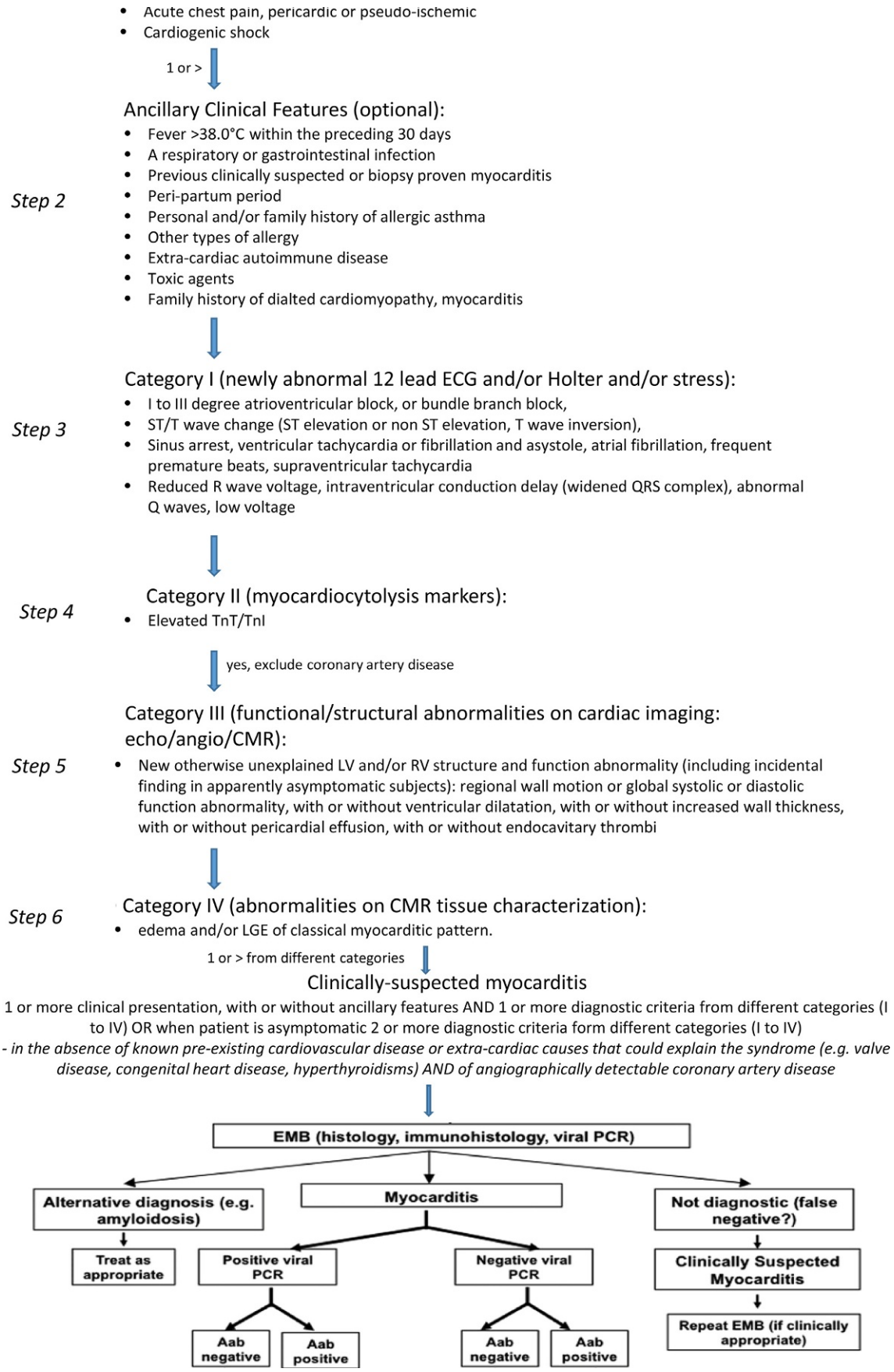

Fig. 2 Diagnostic work-up and etiology-based management in myocarditis according to ESC 2013 Task Force criteria (adapted from Caforio, A., Pankuweit, S., Arbustini, E., et al. (2013) Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *European Heart Journal* **34**(33), 2636–2648). Myocarditis (acute or chronic, lymphocytic or other inflammatory infiltrate). Dallas criteria positive (active or borderline myocarditis) and/or immunohistology positive (see text), with positive or negative viral polymerase chain reaction (PCR) on endomyocardial biopsy (EMB), with or without a DCM clinical phenotype, with normal or depressed biventricular function. Specific myocarditis types would also be included in this definition according to standard histopathological diagnosis (e.g., giant cell, eosinophilic, polymorphic, granulomatous myocarditis).

Table 4 Contributions of EMB to the diagnosis of myocarditis

- EMB is the gold standard for the diagnosis of myocarditis, provided that traditional Dallas histologic criteria (table) immunological and immunohistochemical criteria are fulfilled
- Absence of infectious agents on EMB, including viral genome detection by PCR, identifies immune-mediated myocarditis, whether primary or post-infectious variant, and supports the use of immunosuppressive agents
- EMB allows definition and identification of specific histopathological patterns of myocarditis (e.g., giant cells, eosinophils, etc.) which may require disease-specific therapeutic approaches and follow-up
- EMB helps ruling out cardiac diseases that can simulate myocarditis (Takotsubo cardiomyopathy, peri-partum cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy (ARVC), infiltrative/storage diseases, cardiac neoplasms)
- EMB complication rate is comparable to that of coronary angiography, if performed by expert operators
- EMB samples can be obtained from left and/or right ventricles according to operator's expertise and preferences
- Immunosuppressive treatment is recommended, in the absence of contraindications, in biopsy-proven infection-negative autoimmune myocarditis (giant-cell, lymphocytic and eosinophilic forms, cardiac sarcoidosis, myocarditis in the context of systemic autoimmune diseases)
- EMB may be repeated during the follow-up to guide posology adjustments and immunosuppression withdrawal

Giant Cell Myocarditis, Sarcoidosis, and SID Associated Myocarditis

Giant cell myocarditis, considered the prototype of autoimmune myocarditis, is a very rare but devastating disease, presenting similarly to other forms of myocarditis, but characterized by an aggressive rapidly progressing course and refractoriness to standard medical therapy. It can be associated with extra cardiac or systemic autoimmune diseases and can relapse in a considerable proportion of transplanted recipients (25%).

Sarcoid cardiac involvement, whether isolated or in the setting of multisystem sarcoidosis, may mimic ARVC. The interventricular septum is preferentially affected resulting in atrio-ventricular conduction abnormalities and/or hypo- or hyper-kinetic arrhythmias. The clinical suspicion of myocarditis in the setting of connective tissue disease or other immune-mediated diseases is based on the same criteria proposed for isolated forms.

Myocarditis and Rheumatic Fever

Myocardial involvement in the setting of acute rheumatic fever (RF) is usually associated with valvular and/or pericardial disease. RF is an inflammatory multisystem disease, occurring few weeks to 6 months following group A streptococcal (GAS) infection of the tonsillopharynx. Its diagnosis is still based upon the 2015 revised Jones' criteria (Dajani et al., 1992; Gewitz et al., 2015) (**Table 5**). Major criteria include: (1) carditis; (2) polyarthritis; (3) chorea; (4) erythema marginatum; (5) subcutaneous nodules. Minor criteria are represented by: (1) fever; (2) arthralgia; (3) elevated ESR or CRP; (4) prolonged P-R interval on standard 12-lead ECG. If supported by evidence of GAS, for example, positive throat culture or rapid streptococcal antigen test, high or rising antistreptococcal antibody titer, the presence of two major criteria (or one major and two minor) indicates a high probability of acute RF. The diagnosis of clinical carditis during acute RF is based upon unequivocal fulfillment of any of the following findings: (1) new onset of nonfunctional cardiac murmurs, (2) cardiac enlargement, (3) sign and symptoms of heart failure, (4) pericardial friction rubs or accumulation of pericardial fluid. Importantly, in the 2015 revised criteria a greater use of echocardiography with Doppler analysis is recommended to confirm clinically suspected carditis, in particular mitral and aortic valvular regurgitation, typical of acute rheumatic carditis (Gewitz et al., 2015). Although RF remains a leading world cause of acquired cardiac disease, EMB-based studies are lacking since RF is rare in Europe and the North America, most of the literature dating back to the 1950s. The histological finding of Aschoff bodies is the hallmark of rheumatic inflammation in the heart (Leone et al., 2012). Rheumatic myocarditis is thought to be a component of acute rheumatic carditis, which also includes pericarditis and valvulitis. However, in acute rheumatic myocarditis, histopathology reveals no necrosis and for this feature there are authors who have questioned that isolated rheumatic myocarditis exists (Leone et al., 2012). When myocarditis is present, high dose steroid therapy should be considered.

Toxic and Hypersensitivity Myocarditis

Hypersensitivity myocarditis, the most prevalent form of drug-induced myocardial toxicity, is unpredictable and dose independent. It can be accompanied by unspecific cutaneous rash, fever, peripheral blood eosinophilia, and general malaise. On the other hand, direct cardiac toxicity is dose dependent, totally or partially reversible and favored by concomitant oncological treatment, for example radiotherapy.

Pediatric Myocarditis

Myocarditis is considered a frequent cause of new-onset DCM in the pediatric age range; some small observational cohort studies have documented a high rate of myocardial recovery in this setting. Most epidemiological data are retrospective, observational, and often based on administrative records. Moreover, the diagnosis of myocarditis is biopsy-proven in a minority of patients. The most

Table 5 Revised 2015 Jones criteria for acute rheumatic fever (ARF)

Criteria	*Low risk populations*[a]	*Moderate-high risk populations*
Major	Clinical or subclinical[b] carditis Arthritis ● Polyarthritis Chorea Erythema marginatum Subcutaneous nodules	Clinical or subclinical[b] carditis Arthritis ● Mono or polyarthritis ● Polyarthralgia Chorea Erythema marginatum Subcutaneous nodules
Minor	Polyarthralgia Fever >38.5°C *ESR* >60 mm in the first hour and/or *CRP* >6 mg/L[c] *Prolonged P-R interval*, age-corrected (unless carditis is a major criterion)	Monoarthralgia Fever >38°C *ESR* >30 mm in the first hour and/or *CRP* >6 mg/L[c] *Prolonged P-R interval*, age-corrected (unless carditis is a major criterion)

Initial ARF diagnosis = evidence of preceding GAS infection and 2 major criteria or 1 major plus 2 minor.

Recurrent ARF diagnosis = evidence of preceding GAS infection and 2 major criteria or 1 major plus 2 minor or 3 minor.

[a]Low risk populations = ARF incidence ≤2 per 100,000 school-aged children or all-age rheumatic heart disease prevalence of ≤1 per 1000 population per year.

[b]Subclinical carditis is defined as presence of valvulitis on echocardiographic and doppler analysis (detailed by Gewitz et al., 2015).

[c]CRP should be higher than the upper limit of normal for laboratory.

Adapted from Gewitz, M., Baltimore, R., Tani, L., et al. (2015). Revision of the Jones criteria for the diagnosis of acute rheumatic fever in the era of Doppler echocardiography: a scientific statement from the American Heart Association. *Circulation* **131**(20), 1806–1818.

prevalent clinical presentations include heart failure, refractory arrhythmia, and syncope. Some high-risk patients may benefit from mechanical ventricular assist protocols, as a bridge to cardiac transplantation or to ventricular recovery, with a survival rate of 73% and complete remission without sequelae in 2/3 of patients. In pediatric myocarditis new longitudinal studies are needed to clarify the prognostic impact of etiological factors and disease severity.

Prognosis

Myocarditis clinical outcome depends on its cause, clinical presentation, and clinical severity at the time of diagnosis. Acute myocarditis spontaneously resolves in about 50% of patients without sequelae in the first 2–4 weeks, in about 25% of patients a persistent ventricular dysfunction ensues, and in 12%–25% of cases worsening cardiac function leads to end-stage DCM with heart failure and need for heart transplant listing. Biventricular dysfunction at presentation represents the main predictor of adverse prognosis. The prognostic value of viral genome detection on EMB has provided controversial results in different studies, with mainly enterovirus persistence being associated with progressive deterioration, viral clearance (spontaneous or drug-induced) with improvement in pump function and 10 years' prognosis (Schultheiss et al., 2011). Conversely, other studies describe a negative prognostic value for chronic immunohistochemical inflammatory findings, not for a positive viral PCR (Kindermann et al., 2008). These discrepancies may relate to different frequency of viral agents in different populations.

Therapy

Standard Cardiological Management

Standard myocarditis treatment includes symptomatic management of arrhythmias and heart failure. Hemodynamically unstable patients are treated in keeping with international heart failure guidelines, and, if necessary, providing advanced respiratory and cardiac support in Intensive Care Units. In refractory cardiogenic shock, with severely depressed ventricular pump function, implantation of an extracorporeal membrane oxygenation system (ECMO) or other mechanical assist devices may be required as a bridge to transplant or to recovery. In hemodynamically stable asymptomatic or mildly symptomatic patients, meeting criteria for clinically suspected myocarditis, admission to the cardiological ward is recommended until a final diagnosis is reached. During the acute phase ergometric testing for inducible ischemia is contraindicated due to the high arrhythmic risk. When heart failure is present, diuretics, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, and then beta-blockers are used until symptoms are well controlled; if symptoms persist, mineralocorticoid receptor antagonists are indicated. Nonsteroidal antiinflammatory drugs are not strictly indicated in myocarditis, since experimental animal studies have demonstrated an increased mortality rate in treated animals. In advanced atrio-ventricular block a temporary pacing may be necessary. Implantable cardioverter defibrillator (ICD) implantation is debatable since myocarditis-induced ventricular pump dysfunction may undergo spontaneous, though unpredictable, recovery. A wearable defibrillator may be considered in young patients with perceived high arrhythmic risk as

a bridge to recovery or definitive ICD implantation. Physical activity, a potential arrhythmia trigger, is contraindicated during the acute phase and until complete resolution of the inflammatory substrate. Athletes and nonathletes should be temporarily excluded from any kind of amatorial or competitive sport activity for at least 6 months. After 6 months, physical activity may be gradually resumed, provided an exercise test is negative for inducible arrhythmia. Athletes should be monitored every 6 months.

Immunomodulating Therapy

Antiviral therapy with acyclovir, ganciclovir, or valaciclovir may be considered in patients with biopsy-proven active herpesvirus myocardial infection, although its efficacy is unproven in myocarditis (Caforio et al., 2013a,b). Preliminary data are available for interferon beta treatment of enteroviral or adenoviral myocarditis, showing improved NYHA class and prognosis (Kühl et al., 2012). In general terms, an interdisciplinary approach including infectious disease specialists and clinical immunologists is advisable. High dose intravenous immunoglobulin (IVIG) treatment is a standard therapeutic approach in some systemic autoimmune diseases and has shown some therapeutic advantage in terms of improving ventricular function and prognosis in chronic symptomatic heart failure of various causes (Gullestad et al., 2001). Conversely, IVIG had a neutral effect in the IMAC trial of recent-onset DCM where only 15% of patients had biopsy-proven myocarditis of unspecified etiology (McNamara et al., 2001), but therapy was well tolerated. IVIG may be used in biopsy-proven infectious or autoimmune, particularly autoantibody-mediated, myocarditis, refractory to standard management (Caforio et al., 2013a). IA, by removing pathogenic serum aabs, might represent a promising therapeutic option in autoimmune myocarditis/DCM. Small randomized trials in selected DCM patients have demonstrated improvement of pump function and reduced inflammatory myocardial infiltration in treated patients (Felix et al., 2000). A larger multicentric trial is ongoing to confirm these preliminary results and justify the routinary use of such a highly expansive technique.

Immunosuppressive Treatment

The results of immunosuppressive treatment in biopsy-proven virus-negative myocarditis and inflammatory cardiomyopathy are extremely promising, both in terms of efficacy and safety. Immunosuppression efficacy has been proven in virus-negative, giant-cell and lymphocytic autoimmune myocarditis. The most frequently adopted regimen in clinical trials includes the use of prednisone 1 mg kg^{-1} day^{-1} for 4 weeks, then tapered to 0.33 mg kg^{-1} day^{-1} for 5 months, and azathioprine 2 mg kg^{-1} day^{-1} for 6 months (Frustaci et al., 2009). In giant-cell myocarditis, in light of its particularly aggressive course, intensified immunosuppressive regimens are usually adopted, including, beside prednisone and azathioprine, cyclosporine, anti-thymocyte globulins, and biologics (alemtuzimab, rituximab) (Cooper et al., 2008). Both careful selection of patients and exclusion of potential contraindications and comorbidities are key points in the therapeutic strategy. A constant dialog between cardiologists and clinical immunologist is essential for this purpose. In this emerging field of cardiology, EMB reveals its important diagnostic and therapeutical role (**Table 4**).

For specific myocarditis forms, immunosuppression is actually an established therapy, but further data from multicentric randomized trials are needed to optimize therapeutic regimens and duration. Moreover, the same approach will pave the way to test new antiviral and immunomodulatory drugs in virus-positive patients that, at present, are not candidates to immunosuppression.

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Myocarditis in Heart Failure

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Myocarditis is a specific phenotype of cardiac disease, characterized by inflammatory involvement of the myocardium and, sometimes, pericardium. Though the diagnosis is strictly defined by established histological (Aretz et al., 1987), immunological, and immunohistochemical criteria (Caforio et al., 2013; Leone et al., 2012), clinical presentation is highly variable, ranging from asymptomatic myocarditis to over heart failure or even sudden death (Sinagra et al., 2016; Anzini et al., 2013, 2016). Modern techniques of cardiovascular imaging and the exhaustive molecular evaluation of the myocardium with endomyocardial biopsy have provided valuable insight into the pathophysiology of this disease. Moreover patient-tailored diagnostic and therapeutic management appear pivotal to improve outcomes of these patients. Nevertheless, many gaps of knowledge persist in crucial issues of the practical management of patients with suspected myocarditis. In particular the prompt management, the early accurate prognostic stratification of patients with myocarditis, the indications, and the role of endomyocardial biopsy and cardiac magnetic resonance are currently challenging tasks for clinical cardiologists.

Epidemiology and Etiology

The incidence of myocarditis in the real world is very difficult to establish because of the large heterogeneity of clinical presentation of the disease and challenging diagnosis. Its prevalence changes considerably in relationship to the population under study and to the adopted diagnostic criteria. A recent epidemiological study identified myocarditis as the final diagnosis for 0.5% of all hospital admissions for cardiovascular reasons, frequently affecting a young population of mainly male patients (Kyto et al., 2013). Previous studies detected myocarditis on endomyocardial biopsy (EMB) in 10%–17% of patients with otherwise unexplained cardiomyopathy (Hufnagel et al., 2000; Felker et al., 2000). Similarly, myocarditis was found in 5% of individuals in a series of unselected and consecutive autopsies, but the disease was considered the main cause of death in only a minority (Carniel et al., 2004). In this sense, it appears that myocarditis is underdiagnosed.

Myocarditis can be triggered by different causes: infections (i.e., viruses, bacteria, parasites), autoimmune diseases, hypersensitivity, high catecholamine states, drugs, toxic substances, or physical agents (Dennert et al., 2008). Autoimmune myocarditis may occur with exclusive cardiac involvement or in the context of autoimmune disorders with extracardiac manifestations, most frequently in sarcoidosis, hypereosinophilic syndrome, scleroderma, and systemic lupus erythematosus. Hypersensitivity myocarditis may result from drug- or vaccine-related heart-specific autoimmunity. Methyldopa, hydrochlorothiazide, furosemide, ampicillin, tetracycline, azithromycin, aminophylline, phenytoin, benzodiazepines, tricyclic antidepressants, and tumor necrosis factor antagonists all have a low rate of myocarditis (Burke et al., 1991). Although most cases of hypersensitivity myocarditis develop early in the course of drug use, up to 15% of clozapine-induced myocarditis develops later, up to 2 years after initiation of drug therapy (Cook et al., 2015; Youssef et al., 2016; Haas et al., 2007). Bacteria, such as diphtheria and Borrelia burgdorferi (Lyme disease), or parasites, such as Trypanosoma cruzi (Chagas disease), are very important in specific regions (Marin-Neto et al., 2007; Karatolios et al., 2015).

Although the several causes listed above most of cases of myocarditis observed in clinical practice are attributable to viral infections. In particular, even when no viruses are detected by serologic and polymerase chain reaction (PCR) analyses, an unrecognized viral infection remains the most probable cause of the so-called idiopathic myocarditis. These cases are presumably observed in an advanced phase (usually 3 or 4 weeks after the infection) when the immune system has already achieved a complete clearance of the virus (Anzini et al., 2013). In the past 2 decades, polymerase chain reaction and in situ hybridization have identified a range of cardiotropic viruses, including enterovis, adenovirus, influenza viruses, human herpes virus-6 (HHV-6), Epstein–Barr virus, cytomegalovirus, Hepatitis C virus, and parvovirus B19 in heart biopsy samples. Even if the etiologic role of the viruses is undoubtable, their role in maintaining the chronic inflammatory process is controversial.

Pathophysiology

Several above-mentioned noxae may damage the myocardium, directly and indirectly. Although the viruses are the most frequent (i.e., about 90%) cause of myocarditis encountered in clinical practice, the pathophysiology of viral myocarditis remains widely

unknown. This is the main reason of the lack of specific and reliable therapies for this polymorphic disease. The time course of the myocarditic process consequent to viral infection may be resembled in three distinct phases (Pollack et al., 2015; Dennert et al., 2008; Sinagra et al., 2016):

1. *The acute phase*. In predisposed subjects, acute viral infection may harm the myocardium. Viruses are internalized leading to activation of innate immune response, viral myocytolysis, virus-mediated apoptosis/necrosis, or production of autoantibodies against the infected myocyte. In this phase the viral titer is generally high. Usually this phase takes up to 7 days and the diagnosis is done often later to this phase.
2. *The intermediate phase*. Innate immunity stoops to acquired immune response, thus infiltration of T-cells and macrophage may be found in the injured myocardium. Also in this phase elevated viral titers may be found. This phase typically lasts from 7 days to 1 month, depending on the clinical presentation. This phase, indeed, is generally characterized by severe symptoms/arrhythmias.
3. *The chronic phase*. This phase is characterized by either complete resolution or evolution to end-stage postinflammatory cardiomyopathy (Anzini et al., 2013). Indeed, as viral titers decrease, inflammation usually subsides leading to complete resolution of myocardial injury and normalization of cardiac function. However, chronic inflammatory and host innate immunity activation may lead to the development of dilated cardiomyopathy and end-stage heart failure with possible need for heart transplantation (Pollack et al., 2015). The role of viral genome persistence within the myocytes in the chronic inflammatory myocardial process remains extremely controversial (Kuhl et al., 2005). Indeed some of those viruses (i.e., Parvovirus B19) has been found also in nonmyocarditic patients (Moimas et al., 2012); similarly the prognostic role of persistent viral genome in cardiomyocytes is still debated (Kindermann et al., 2008).

Although pathophysiology appears to be straightforward, clinical presentation and natural history of myocarditis is extremely polymorphic and unpredictable. Both the nature of the infectious agent (i.e., virus or bacteria) and individual predisposition may contribute to the wide range of clinical presentations and several human–host factor interactions, such as the formation of the inflammasome (Toldo et al., 2014), might favor myocarditis, influence the severity of process, or protect from myocardial inflammatory injury. However, the gap of knowledge linking pathophysiology to the different clinical presentations remains still unfilled.

Clinical Features and Diagnosis

As stated earlier, the clinical presentation of myocarditis is extremely variable. Indeed it ranges from subclinical and asymptomatic forms to severe heart failure, life-threatening ventricular arrhythmias, and ultimately to sudden death (Anzini et al., 2013).

According to the most important studies and recent statement (Caforio et al., 2007, 2013; Anzini et al., 2013), clinical presentation of myocarditis is grouped into three main categories:

- recent-onset heart failure (usually within 6 months)
- arrhythmic events
- infarct-like syndrome (the so-called myopericarditis or perimyocarditis).

Clinical suspicion of inflammatory heart disease is crucial in the evaluation of patients admitted with one of the above-mentioned scenarios. Indeed, exclusion of other etiologies, such as ischemic heart diseases, valvular heart diseases, congenital heart diseases, and hypertensive cardiomyopathy, should be made rapidly and possibly precede the diagnosis of myocarditis (Caforio et al., 2013).

Recently, practical and clinically oriented classification of myocarditis has been proposed based on event-risk derived by clinical and laboratory presentation and short-term evolution (Sinagra et al., 2016). This classification has important implications in management and prognostic stratification of those patients. Indeed, patients with myocarditis can be divided into three different subcategories (**Fig. 1**):

1. *Low risk myocarditis*: usually presenting with chest pain and/or supraventricular arrhythmias with preserved left ventricular function. This clinical scenario often has a rapid (1–4 weeks) and complete resolution of the electrocardiographic and echocardiographic abnormalities with good long-term prognosis.
2. *Intermediate risk myocarditis*: characterized by mild-to-moderate ventricular dysfunction with persistent wall motion abnormalities, presence of LGE, electrocardiographic changes, and nonsustained ventricular arrhythmias.
3. *High risk myocarditis*: usually presenting with recent-onset severe left ventricular dysfunction and heart failure and/or life-threatening arrhythmias. Typically prognosis is highly dependent on the short-term response to therapy.

Common diagnostic tools, such as personal history, biochemical, ECG, and Echocardiographic features, are the cornerstones for the clinical suspicion of myocarditis. Each test has specific indications and some hurdles, and their potential applications in this clinical setting are summarized in **Table 1**. Those tests should drive clinicians to further assessment with cardiac magnetic resonance and/or endomyocardial biopsy.

Cardiac magnetic resonance imaging (MRI) represents the gold standard for morphofunctional evaluation of cardiac structures and characterization of the myocardium, providing useful diagnostic and prognostic information in various clinical settings (Francone et al., 2014; Caforio et al., 2007). In patients with low risk myocarditis, particularly in patients with infarct-like onset, cardiac MRI has a good accuracy and allows a distinction between myocarditis and acute coronary syndromes (subepicardial vs

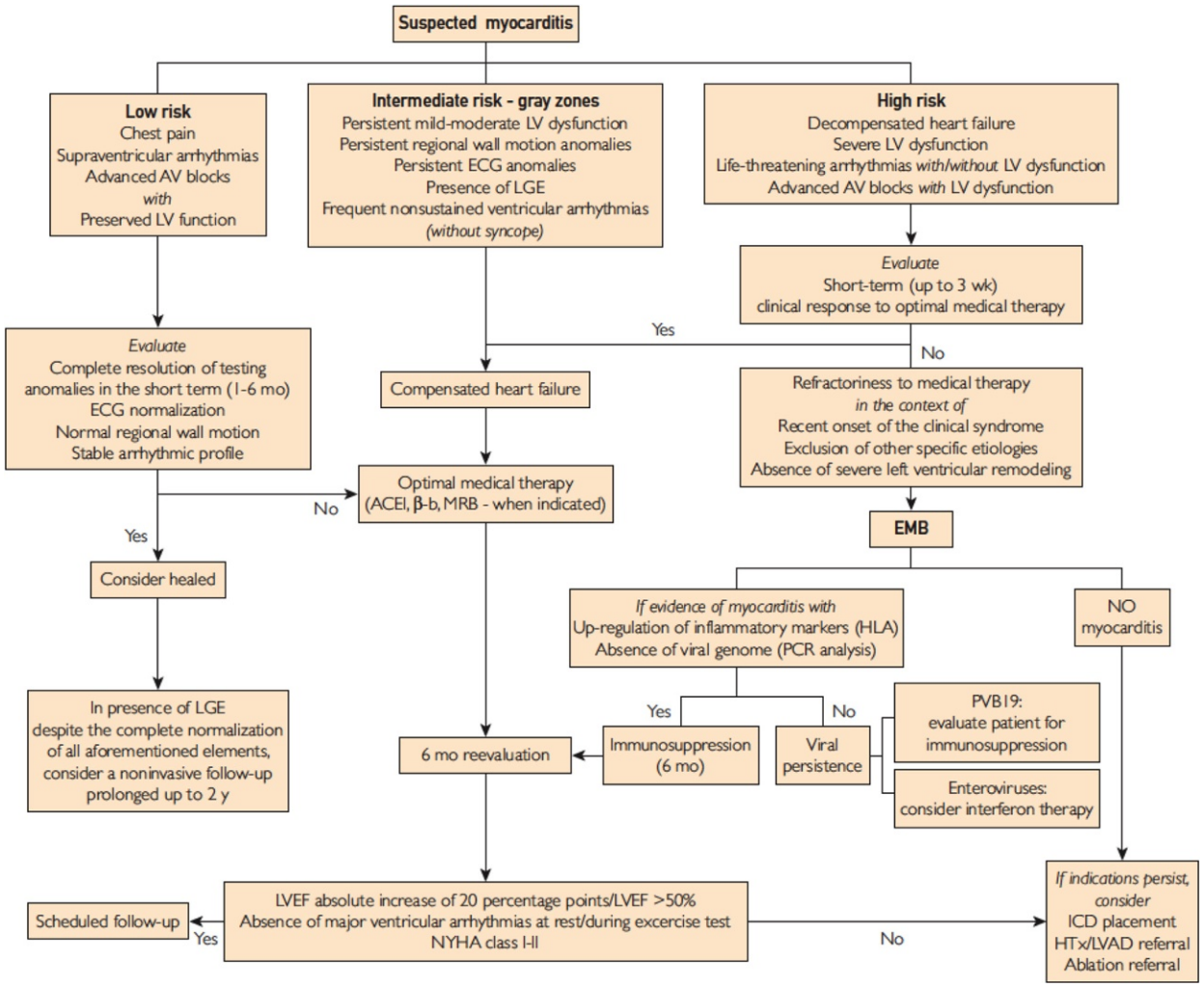

Fig. 1 Clinical work-up for suspected myocarditis. *ACE-i*, angiotensin-converting enzyme inhibitors; *AV*, atrio-ventricular; *β-b*, beta-blockers; *EMB*, endomyocardial biopsy; *HTx*, heart transplantation; *ICD*, implantable cardioverter-defibrillator; *LGE*, late gadolinium enhancement; *LV*, left ventricular; *LVAD*, left ventricular assit device; *LVEF*, left ventricular ejection fraction; *MRB*, mineralcorticoid receptor blockers; *PVB19*, Parvovirus B19. Adapted from Sinagra, G., Anzini, M., Pereira, N. L., et al. (2016). Myocarditis in clinical practice. *Mayo Clinic Proceedings* **91** (9), 1256–1266.

subendocardial/transmural LGE distribution) (Lurz et al., 2012). MRI should be performed on the right time in order to be accurate, and the low accessibility of this technique currently still represents a concern. In high- and intermediate-risk forms cardiac MRI has modest diagnostic accuracy, in fact the edema in T2-weighted images may be absent, its presence highly depends on the time of MRI performance. In patients with biopsy-proven viral myocarditis the presence of myocardial scar, indicated by LGE, is the best independent predictor of all-cause mortality and cardiac mortality; no data are available about the prognostic value of additional cardiac MRI-related parameters, such as the pattern of distribution and the extension of LGE (Grun et al., 2012). These data support the need for future prospective studies to establish the prognostic role of LGE in viral myocarditis, as well as to evaluate the incremental value of additional MRI parameters.

Noteworthy, the role of endomyocardial biopsy (EMB) is still controversial (**Fig. 1**). Though this test is the gold standard for the diagnosis of myocarditis, it remains associated with a nonnegligible rate of major complications, even in specialized Centers (around 1% of the cases) (Chimenti and Frustaci, 2013; Yilmaz et al., 2010). Furthermore, diagnostic accuracy and yield of EMB is still debated, highly depending on the operator experience who performs the EMB and who analyze the samples obtained, on the number and the location of tissue samples and, finally, on the timing of the EMB. Although no clear recommendations are available, biventricular approach is preferred (Yilmaz et al., 2010; Sinagra et al., 2016). Besides, the possible usefulness of cardiac magnetic resonance imaging in selecting the site of biopsy is still not demonstrated (Yilmaz et al., 2010). Therefore, EMB should be performed only in selected life-threatening scenarios, such as heart failure with severe ventricular dysfunction and/or life-threatening arrhythmias refractory to optimized medical therapy in the short term (usually 3 weeks) (Heymans et al., 2016; Sinagra et al., 2016). In low-risk myocarditis, typically characterized by a good long-term prognosis, EMB could not provide useful information and be potentially harmful for the patients (Imazio et al., 2013; Buiatti et al., 2013; Pollack et al., 2015; Sinagra

Table 1 Role and typical findings of noninvasive testing and endomyocardial biopsy in the diagnostic work-up of myocarditis

	Low risk	*Intermediate Risk*	*High risk*
Personal history	Flu-like symptoms: 35%–65% of cases; insect bite (for borrelia or ricketsiae suspicion). Not to be considered in the diagnostic work-up.		
Biomarkers	Troponin tipically elevated (without prognostic value). *Antimicrobial serology*: low accuracy for detection of myocardial infection. Not to be routinely considered in the diagnostic work-up. It may be useful to detect infections by Borrelia or Rickettsia in the presence of bradyarrhythmias, advanced AV blocks, and clinical suspicion. *Serologic analyses for autoimmune diseases*: should be considered only in presence of clinical suspicion.	Troponin and acute phase reactants: low accuracy for detection of myocardial inflammation.	
Electrocardiography	Diffuse and saddle-shapes ST segment elevation. Bradyarrhythmias or advanced AV conduction defects in the absence of LV dysfunction may be suggestive for infections by borrelia or rickettsia.	Low voltages. Discordance between the severity of the clinical scenario and the "scarcity" of electrocardiographic alterations (absence of left atrial dilation and left intraventricular conduction delay). Bradyarrhythmias or advanced AV conduction defects in the presence of LV dysfunction may be suggestive for sarcoidosis or giant cell myocarditis.	
Echocardiography	Normal ventricular dimensions, morphology,wall motion and global function. Transient wall motion anomalies. Transient mild ventricular dysfunction. Pericardial effusion.	Global impairment of left or biventricular function without significant remodeling. Diastolic dysfunction. Patchy alterations of the wall motion not respondent to coronary distribution or electrocardiographic alterations. Pericardial effusion. Transient pseudohypertrophy of the ventricular wall. Alteration of the echocardiographic myocardial texture.	
Cardiac magnetic resonance	Good accuracy. Distinction between myocarditis and acute coronary syndromes (subepicardial vs subendocardial/ transmural LGE distribution).	Modest accuracy. May be considered a conclusive diagnostic test.	Modest accuracy. Cannot be considered a conclusive diagnostic test in the context of a major clinical syndrome.
Coronary angiography	Evaluate risk factors for coronary artery disease.	Evaluate risk factors for coronary artery disease.	Indicated.
Endomyocardial byopsy	Not indicated.	Controversial.	Indicated in presence of refractoriness to medical therapy*in order to*evaluate potential reversibilityand plan eventual specific therapy.

Adapted from: Sinagra, G., Anzini, M., Pereira, N. L. et al. (2016). Myocarditis in clinical practice. *Mayo Clinic Proceedings* **91** (9), 1256–1266.

et al., 2016). In intermediate-risk syndromes, EMB may be considered and a case-by-case decision is mandatory in these patients (Sinagra et al., 2016).

Natural History and Prognostic Stratification

According to their initial presentation, patients with myocarditis appear to have different prognosis (Pollack et al., 2015; Anzini et al., 2013). For a long time, clinical presentation was considered a major predictor of worse outcomes (Caforio et al., 2007). Indeed, prognosis of patients presenting with overt heart failure is significantly worse than those presenting with chest pain and patients presenting with life-threatening arrhythmias show an intermediate outcome (Mason et al., 1995; Magnani and Dec, 2006; Anzini et al., 2013; Caforio et al., 2007). Actually, recently it is emerged the independent role of LV dysfunction and derangement. Patients with preserved LV function at diagnosis have better prognosis compared to patients with severe LV and biventricular dysfunction. Similarly the presence of late gadolinium enhancement at cardiac magnetic resonance, expression of myocardial fibrosis, is associated with worse outcome. Other important markers, such as syncopes, prolonged QRS duration, left bundle branch block, pulmonary hypertension, have to be taken into account and are expression of myocardial involvement (Kindermann et al., 2012). Finally even in the prognostication of the most challenging myocarditis patients (i.e., those presenting with life-threatening arrhythmias) left ventricular ejection fraction (LVEF) <35%, the presence of 2 or more contiguous akinetic segments or aneurysmatic deformation at the baseline echocardiography, the presence of Late Gadolinium Enhancement (LGE) at the baseline CMR, may predict worse outcomes (Anzini et al., 2013, 2016). Extensive ventricular remodeling at the time of admission may be the expression of a severe and irreversible structural derangement predicting worse prognosis during the follow-up. An exception is represented by patients with aggressive clinical presentation, such as cardiogenic shock and fulminant myocarditis, that appear to

have better outcomes than acute nonfulminant myocarditis once the acute phase of the disease has been resolved (McCarthy et al., 2000). Specific subtypes, such as giant cell myocarditis, necrotizing eosinophilic myocarditis, or cardiac sarcoidosis, have worse 5-year prognosis without immunosuppressive treatment or advanced mechanical support (Baughman, 2006; Vaideeswar and Cooper, 2013; Chaudhry et al., 2015).

Management and Treatment

Therapeutic regimens and intensity of treatment change according to initial presentation, severity of symptoms, and LV involvement. Currently, therapy for myocarditis is focused on supportive care and on heart failure/arrhythmias management (Caforio et al., 2013).

In the setting of myopericarditis (i.e., concomitant myocardial involvement in pericarditis), management is similar to that recommended for pericarditis (Adler et al., 2015). Prognosis of myopericarditis, with absent or mild left ventricular dysfunction, and without symptoms of heart failure, is benign. Therefore endomyocardial biopsy is not indicated for definite diagnosis (Adler et al., 2015).

Long-term arrhythmic risk of patients with myocarditis presenting with sustained ventricular tachyarrhythmias without LV dysfunction is still unclear, and current guidelines do not provide clear indications for Implantable Cardioverter Defibrillator (ICD) for these patients (Priori et al., 2015). However, clinical and instrumental characteristics at baseline may be helpful to predict the risk of arrhythmic events in this setting of patients (Anzini et al., 2016). Therefore indications for ICD implantation should be individually evaluated according to clinical presentation (i.e., aborted sudden cardiac death or syncope), the magnitude of structural–functional ventricular derangement (i.e., ventricular remodeling, presence of akinetic segments or aneurysmal deformation, extent of LGE), histopathological substrate (i.e., cardiac sarcoidosis or giant cell myocarditis), and therapeutic response to standard care (Anzini et al., 2013, 2016). In patients with active myocarditis and severe left ventricular dysfunction, major clinical decisions like the indication for a prophylactic ICD should be deferred for at least 3–6 months, and in this intermediate phase a LifeVest wearable defibrillator could be considered for sudden cardiac death prevention.

Hemodynamically unstable patients presenting with fulminant or severe myocarditis and cardiogenic shock should be immediately referred to intensive care units possibly with availability of invasive hemodynamic support, such as left ventricular assist device (LVAD) or extracorporeal membrane oxygenator (ECMO) (Ponikowski et al., 2016; Hsu et al., 2011).

Patients presenting with acute myocarditis and severe left ventricular dysfunction without cardiogenic shock should be avoided in the short term (3–6 months) from aggressive invasive managements, such as heart transplantation, left ventricular assist device, or prophylactic implantable cardioverter defibrillator, unless refractoriness to standard therapy in the short term (Anzini et al., 2013). Likewise, inotropic support should be considered in cardiogenic shock. Treatment with angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin receptor blockers (ARBs), beta-blockers, and additional treatment with mineralocorticoid receptor antagonists (MRAs) and diuretics should be promptly initiated and uptitrated to the maximum tolerated dosage (Ponikowski et al., 2016; Pollack et al., 2015; Sinagra et al., 2016; Anzini et al., 2013).

As stated earlier, baseline left ventricular function is an important prognostic marker regardless of clinical presentation, the reassessment of the ejection fraction after 6 months appears to be also more useful for predicting long-term outcomes. Patients who experience an increase of LVEF $>20\%$ or have LVEF $\geq 50\%$ and a low NYHA functional class at 6 months demonstrated an excellent long-term prognosis (Anzini et al., 2013). Also in this setting, LifeVest wearable defibrillator can be considered during the reassessment time period of 3–6 months.

Treatment with nonsteroidal antiinflammatory drugs (NSAIDs) should be avoided in this setting because they have been associated with higher mortality rate in animal models of acute viral myocarditis (Costanzo-Nordin et al., 1985; Feldman and McNamara, 2000).

Treatment with immunomodulating agents, such as steroids, immunosuppression, antiviral drugs, and intravenous immunoglobulin, is still controversial. Though some trials showed improvement of left ventricular function (Frustaci et al., 2009; Wojnicz et al., 2001), large randomized studies on the use of immunosuppression are not available and recent meta-analysis showed that immunomodulation did not have positive impact on outcomes of patients with myocarditis (Caldeira et al., 2015). Immunosuppressive therapy with prednisone and azathioprine appears to be suitable for patients with EMB-proven active myocarditis with major clinical symptoms such as HF with severe ventricular dysfunction and/or life-threatening ventricular arrhythmias in whom conventional treatments have failed in the short term of 7–10 days. In lymphocytic myocarditis, immunosuppressive treatment should be considered only in the absence of the viral genome on EMB, whereas giant cell myocarditis, necrotizing eosinophilic myocarditis, and cardiac sarcoidosis represent clear indications for immunosuppressive therapy (Frustaci et al., 2009; Pollack et al., 2015; Sinagra et al., 2016). However the role of persistent viral genome in cardiomyocytes is still unclear. In particular the role of parvovirus B19, recently emerged as an endemic virus encountered with similar frequency in almost half of patients with dilated cardiomyopathy (Moimas et al., 2012) or other noninflammatory heart diseases (Kubanek et al., 2012), is still controversial. Therefore, careful and comprehensive evaluation is a priority to guide the selection of patients at higher risk who could benefit from a comprehensive molecular evaluation of the myocardial substrate with EMB (Pollack et al., 2015; Sinagra et al., 2016).

IFN-beta-1b treatment given over 24 weeks to patients with chronic viral cardiomyopathy and moderate heart failure is safe, can eliminate or reduce the virus burden, ameliorates symptoms, and diminishes physical disability; however the role of interferon

treatment in acute myocarditis is still debated. Currently it seems to be limited to the presence of entero-virus and its efficacy on hard end-point is not yet demonstrated (Schultheiss et al., 2016; Sinagra et al., 2016).

Conclusions

Myocarditis remains a challenging clinical scenario. Often under-diagnosed, essentially due to variable clinical presentation, prompt diagnosis and tailored therapy often lead to favorable outcomes and to almost complete *restitutio ad integrum*. However still much has to be done to deeply investigate pathophysiology and to improve treatment of this condition.

Risk stratification is, at the moment, the most important tool for the management of this disease. Conservative managements should be reserved to low-risk patients while progressively more intensive treatments should be pursued for life-threatening situations.

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Neurohormonal Blockade

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Neurohormonal Blockade

Overview

Chronic activation of neurohormonal pathways has deleterious effects on the cardiovascular system and has been the focus of considerable study in heart failure (HF). Following a cardiac insult such as a myocardial infarction (MI) or exposure to a myocardial toxin or stress there is activation of neurohormonal compensatory mechanisms. The goal of these compensatory mechanisms is to maintain cardiac output and end-organ perfusion in the setting of myocardial function compromise. In acute and subacute states, following an initial drop in cardiac function, these mechanisms are of benefit to the host; however, chronically they play a role in the progressive decline of myocardial function and ventricular remodeling (Jessup and Brozena, 2003).

Immediately following a myocardial insult, baroreceptors within the aorta and the myocardium recruit the sympathetic nervous system (SNS) to maintain homeostasis. The SNS primarily mediates its effects via catecholamines and provides signaling within the heart, kidneys, brain, and musculature. Subsequently, stimulation from the SNS as well as intrinsic renal mechanisms activates the renin-angiotensin and aldosterone system collectively referred to as the RAAS. The combined effect of the two systems leads to increased myocardial inotropy, chronotropy, sodium and water retention (leading to an increase in pre-load), and vasoconstriction (causing an increase in afterload). These effects over the short term help maintain cardiac output (CO) and blood pressure (BP) (**Fig. 1**). However, chronic activation of these neurohormonal systems results in increased hemodynamic stress and deleterious effects on the heart and vasculature which cause disease progression in HF. This is referred to as the *neurohormonal hypothesis* (Packer, 1992).

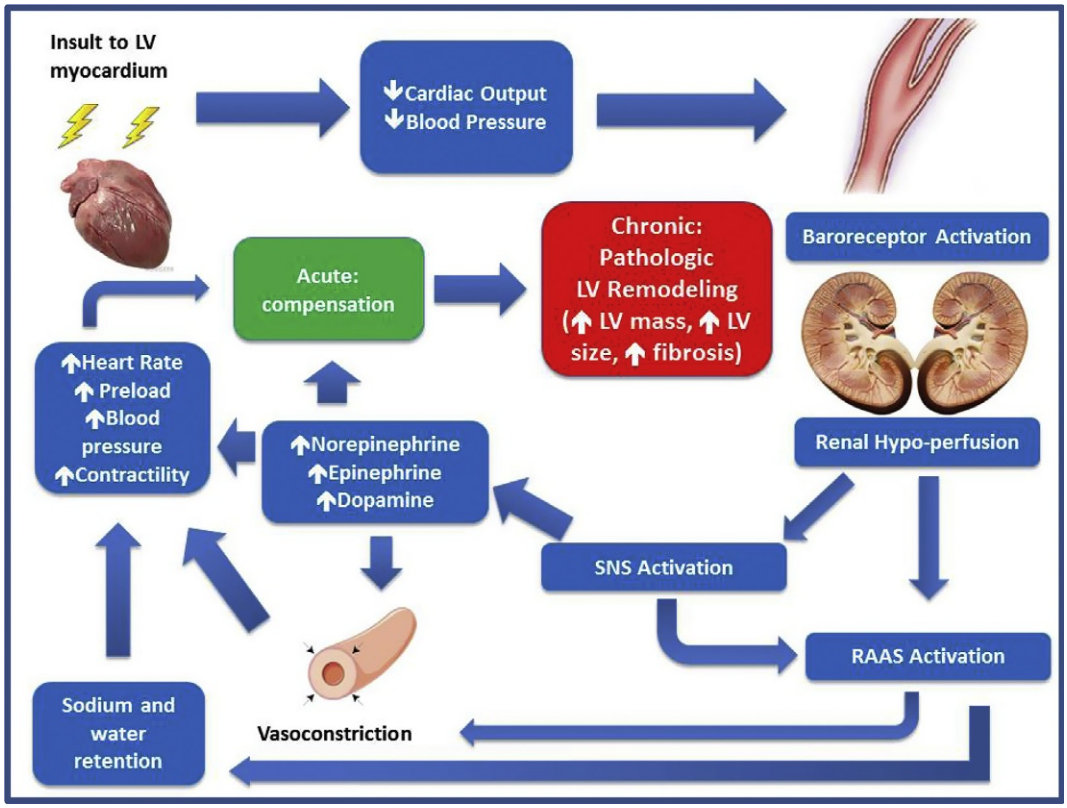

Fig. 1 The pathophysiology of HF with activation of the renin-angiotensin-aldosterone system and sympathetic nervous system. *LV*, left ventricle; *RAAS*, renin-angiotensin-aldosterone system; *SNS*, sympathetic nervous system.

Normally, several feedback pathways are activated to offset the actions of RAAS. For example, the natriuretic peptides cause vasodilation and natriuresis, and have antifibrotic effects that can counterbalance the effects of the RAAS. However, in HF there is increased peripheral resistance to natriuretic peptides thus potentiating the effects of the RAAS (Volpe et al., 2016).

Currently, medical therapies that have demonstrated benefit in the treatment of HF with reduced ejection fraction (HFrEF) block the RAAS and the SNS, and augment the beneficial effects of the natriuretic system. These agents have incremental benefit through reduction of clinical symptoms, morbidity, and mortality. While these therapies do have hemodynamic effects, their alteration of neurohormonal signaling appears to dictate their beneficial effects on long-term clinical improvements and outcomes (Packer, 1992). Although much better studied in HFrEF, regardless of the etiology, some of these mechanisms may play a role in HF with preserved ejection fraction (HFpEF).

Renin-Angiotensin-Aldosterone Receptor Blockade

The RAAS has been characterized in detail through investigation of HF treatment. The cascade begins with renin causing cleavage of angiotensinogen, produced by the liver, to form the peptide angiotensin I. Renin is produced in the kidneys as a result of increased sympathetic tone, decreased systolic BP perceived at the juxtaglomerular cells, or decreased sodium or chloride ion delivery to the macula densa. Angiotensin I is an inactive peptide which is cleaved by angiotensin converting enzyme (ACE) to angiotensin II, which is the end product of the cascade. Angiotensin II produces its long-term deleterious effects predominantly through the angiotensin II receptor-type 1 (AT1) receptors which have been described in the heart, brain, kidneys, adrenal tissue, adipocytes, and vascular smooth muscle.

The RAAS causes vasoconstriction, and sodium and water retention (Packer, 1992; Burnier and Brunner, 2000; Hartupee and Mann, 2016). The AT_1 receptor stimulation causes cellular migration, proliferation, hypertrophy, and hyperplasia of fibroblasts with increased extracellular matrix deposition in the myocardium and vasculature. Finally, these systems lead to myocardial fibrosis, remodeling of the left ventricle (LV), and alterations in the vasculature resulting in endothelial dysfunction. LV remodeling describes the changes in LV mass, volume, and shape and in the composition of the heart after injury or abnormal hemodynamic loading (Mann and Bristow, 2005; Shah and Mann, 2011; van Berlo et al., 2013) (**Figs. 1** and **2**). Direct myocyte stimulation leads to cellular hypertrophy and an increase in cellular volume. Grossly the LV increases in volume (dilates), develops thinned walls, and becomes more spherical in shape due to the increased volume.

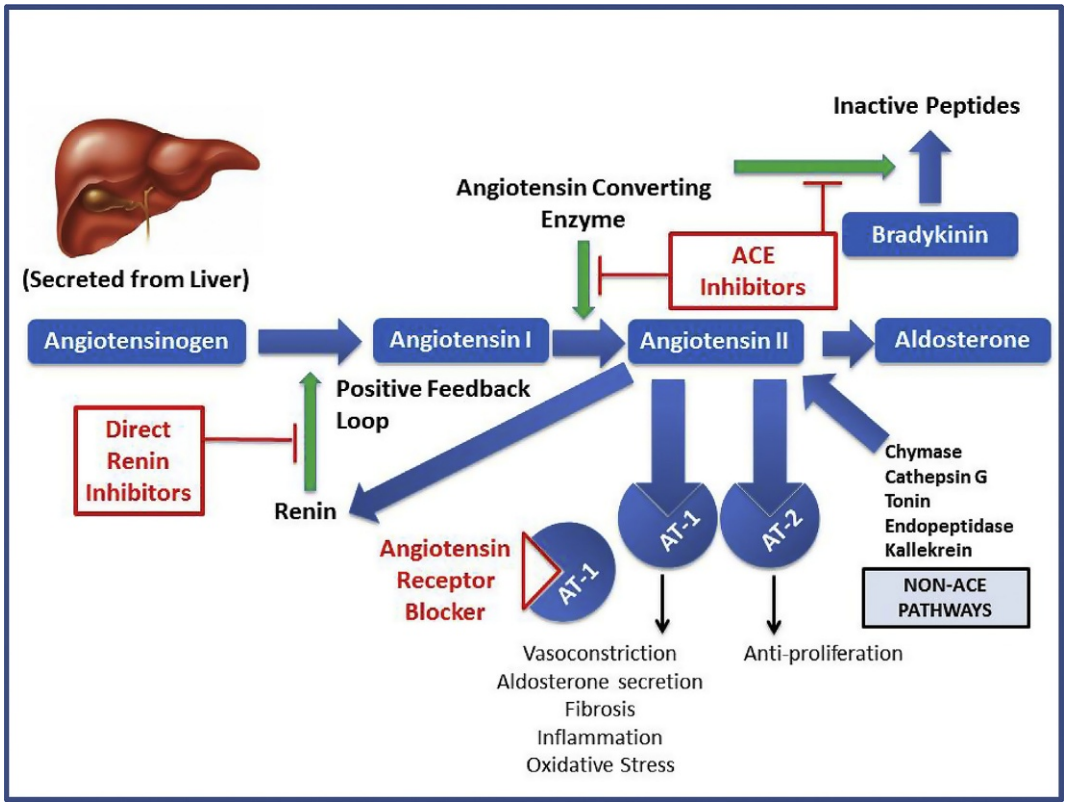

Fig. 2 The renin angiotensin aldosterone system and sites of action of ACE inhibitors and angiotensin receptor blockers (ARBs). Angiotensinogen is secreted from the liver and cleaved by renin (produced in the kidneys) to produce Angiotensin I. Angiotensin I is then cleaved by Angiotensin Converting Enzyme (ACE) to produce Angiotensin II. Angiotensin II can also be produced via non-ACE pathways. Angiotensin II acts on Angiotensin II Type 1 (AT1) and Type 2 (AT2) receptors. When activated the AT1 receptor produces vasoconstriction and augments aldosterone secretion. Angiotensin II produces a positive feedback loop to increase renin production.

The beneficial effects of vasodilation have been documented in the early years of HF research and were found to provide improvement in HF symptoms and mortality. Interest in ACE inhibitors in HF therapy initially stemmed from their vasodilator properties and renal effect of improved natriuresis. The ACE enzyme, in addition to its role in converting angiotensin I to angiotensin II, also facilitates the degradation of bradykinin and substance P, both of which are potent vasodilators (Fig. 2). All of these effects of ACE inhibitors lead to increased vasodilation and facilitate natriuresis and diuresis.

ACE inhibitors have side effects which manifest due to the effects on the RAAS pathway. One common side effect of ACE inhibitors is a medication-induced cough which is due to accumulation of upstream byproducts like bradykinin. The resultant dry cough can be responsible for discontinuation of up to 19% of ACE inhibitors. Accumulation of these byproducts can also lead to more serious complications such as angioedema which has been reported to occur in up to 0.1%–1% of those on ACE inhibitors. Importantly, angioedema can occur at any time point while a patient is on therapy and should prompt expeditious evaluation if any patient on therapy develops lip, tongue, or throat swelling (Mahmoudpour et al., 2013).

In addition, clinicians must also use caution with initiation of these agents in HF patients with low blood pressure or renal failure as their vasodilatory effects may lead to hypotension and exacerbation of symptoms. ACE inhibitors are avoided or used very carefully in patients with severe renal dysfunction and a creatinine of over 3 mg per deciliter. ACE inhibitors have the specific mechanism to vasodilate the efferent arteriole of the kidney which mildly decreases glomerular filtration rate (Denton et al., 1992). In patients with severe HF and renal dysfunction, this can lead to worsening of renal function and further fluid retention.

ACE Inhibitors in Heart Failure with Reduced Ejection Fraction (HFrEF)

Chronologically, investigation of ACE inhibitors in HF was completed prior to evaluation of beta-blockers and other neurohormonal blocking agents. There has also been a great diversity of HF patients in which ACE inhibitors have been evaluated including those with mild to severe symptomatic HF, in patients with asymptomatic left ventricular systolic dysfunction and in post-MI HF, with improvement in symptoms and outcomes. The predominant proportions of these patients were in the category of those with heart failure with reduced ejection fraction (HFrEF). Enalapril and captopril are the best studied ACE inhibitors; however, their effects are thought to be class effects and there are several more guideline-recommended ACE inhibitors available.

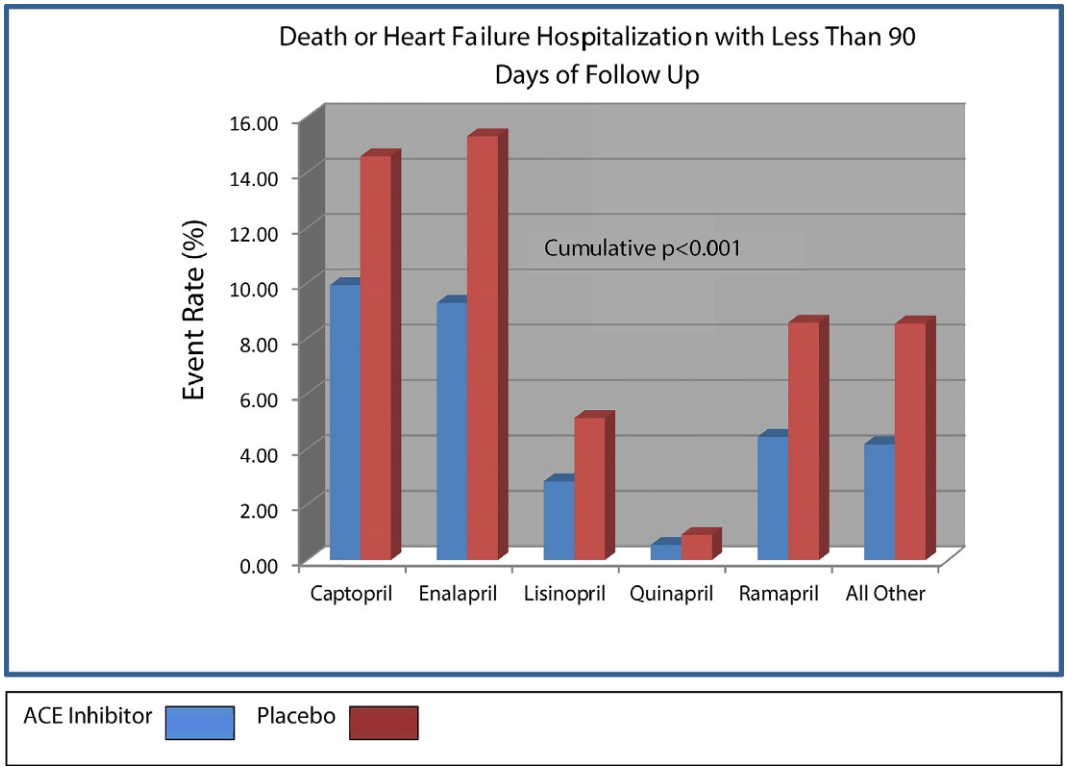

Fig. 3 Summary of ACE inhibitor therapy compared to placebo in patients with HFrEF with the combined end point of death or heart failure hospitalization with less than 90 days of follow-up. Data from Garg, R., Yusuf, S. (1995). Overview of randomized trials of angiotensin-converting enzyme inhibitors on mortality and morbidity in patients with heart failure. *Journal of American Medical Association* 273, 1450–1456.

Enalapril was initially studied in 253 patients with NYHA Class IV HF who were randomly assigned to maximally tolerated enalapril versus placebo in the Effects on Enalapril in Severe Congestive Heart Failure: Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) (Swedberg and Kjekshus, 1988). Concomitant use of diuretic and/or digitalis was allowed among enrolled patients and diagnosis of HF was made based on clinical evaluation and radiologic evaluation of heart size (>600 mL/m^2 for men, >550 mL/m^2 for women). Among the patients who received enalapril there was an improvement in clinical symptoms and a reduction in mortality of 31% at 1 year compared to placebo (HR 1.44; $P=0.001$).

Further studies found symptomatic and clinical outcome benefit translated to patients with mild or moderate congestive HF symptoms (Magnani and Magelli, 1986; Lewis, 1989; Lechat et al., 1993) and included the landmark Studies of Left Ventricular Dysfunction (SOLVD) Prevention Trial and the SOLVD Treatment Trial, which evaluated the effect of enalapril on mortality and HF progression in patients with asymptomatic and symptomatic HFrEF, respectively. The SOLVD prevention trial enrolled 4228 predominantly NYHA Class I patients randomly assigned to either enalapril 2.5–20 mg daily (mean daily dose 12.7 mg) or placebo. While the risk of death was not significantly reduced, there was a 20% risk reduction in heart failure hospitalization (HR 0.8; 95% CI 0.9–0.7, $P<0.001$) and a 29% reduction in the development of HF symptoms (HR 0.71; 95% CI 0.64–0.79, $P<0.001$) in those receiving enalapril (The SOLVD Investigators, 1992). The SOLVD Treatment Trial randomized 2569 symptomatic patients with HFrEF to enalapril or placebo. The use of enalapril was associated with a significant risk reduction of 16% in mortality and 26% in mortality or HF hospitalization over an average follow-up of 41 months (The SOLVD Investigators, 1991).

A meta-analysis reviewing 32 trials comparing various ACE inhibitors versus placebo in 7105 symptomatic HFrEF patients of ischemic and non-ischemic etiology showed an overall 23% reduction in time to death among the pooled cohort (OR 0.77, 95% CI 0.67–0.88). The effect was demonstrated among 8 ACE inhibitors; however, the majority of the effect was noted from early trials using enalapril. Importantly, reduction in mortality predominantly came from a decrease in death due to worsening HF (**Figs. 3** and 4) (Garg and Yusuf, 1995).

ACE Inhibitors in Heart Failure with Preserved Ejection Fraction

To date no single trial has provided definitive evidence that ACE inhibitors offer similar benefits to patients with HFpEF as have been demonstrated in those with HFrEF. The largest trial in those with HFpEF was the Perindopril in Elderly People with Chronic Heart Failure (PEP-CHF) trial which randomized 850 patients with HFpEF to the ACE inhibitor perindopril versus placebo. This study failed to show a significant improvement in the primary outcome (composite of all-cause mortality and unplanned HF-related hospitalization) with the ACE inhibitor. However, significant improvements were observed favoring ACE inhibitor

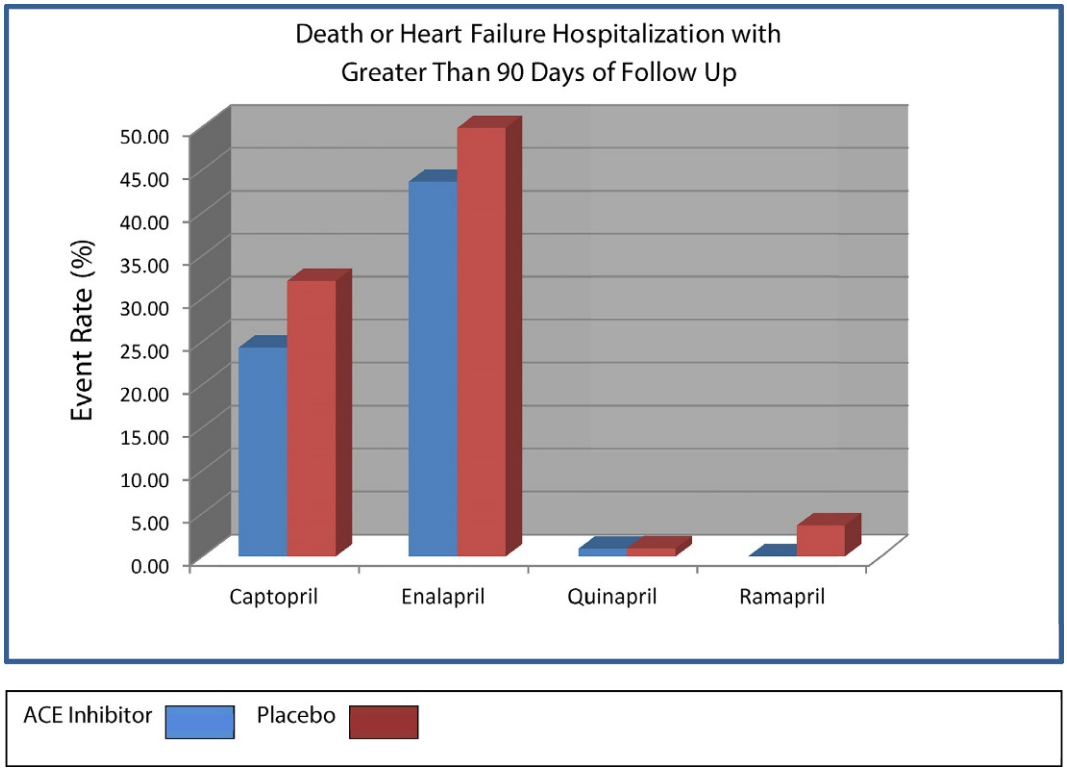

Fig. 4 Summary of ACE inhibitor therapy compared to placebo in patients with HFrEF with the combined end point of death or heart failure hospitalization greater than 90 days of follow-up. Data from Garg, R., Yusuf, S. (1995). Overview of randomized trials of angiotensin-converting enzyme inhibitors on mortality and morbidity in patients with heart failure. *Journal of American Medical Association* 273, 1450–1456.

therapy in some secondary end points, including improving the NYHA functional class and improvement in the six-minute-walk distance at one year of follow-up in the trial (Cleland et al., 2006).

A meta-analysis of 7 trials reviewing the outcomes of 1309 patients with HFpEF receiving ACE inhibitors also failed to show improvement in mortality due to worsening HF, rehospitalization due to HF, or all-cause rehospitalization. This study included the outcomes of the PEP-CHF trial and in subgroup analysis demonstrated a reduction in rehospitalization for HF in a subgroup of patients over the age of 75 years (Fu et al., 2012).

Angiotensin Receptor Blockers

As previously described angiotensin II is an end product of the RAAS pathway and has important effects including sodium and water retention, vasoconstriction, cardiomyocyte hypertrophy, cardiomyocyte inotropy, and vascular/myocardial fibrosis. While ACE inhibitors have upstream effects on the RAAS pathway it has been shown that they do not fully suppress conversion of angiotensin I to angiotensin II. Other enzymatic substances are also capable of producing angiotensin II, including, trypsin, cathepsin, and the heart chymase; however, it is unclear how much an effect these enzymes have on production (**Fig. 2**) (Burnier and Brunner, 2000). This provided the rationale for the development of angiotensin receptor blockers (ARBs) that could theoretically offer more complete protection against angiotensin II by directly blocking the AT1 receptor. In addition, during chronic AT1 receptor blockade, the increased levels of angiotensin II that occur as a result of interruption of the normal negative feedback mechanism, can stimulate the AT2 receptors, and therefore could theoretically be a beneficial target in patients with HF; however, this remains controversial (Burnier and Brunner, 2000). On the other hand, the beneficial effects of ACE inhibitors are also thought in part to be due to their augmentation of the effects of bradykinin, which would not be affected by ARBs. Therefore, several trials evaluated the effects of ARBs compared with ACE inhibitors in HFrEF, as well as the effects of both ACE inhibitors and ARBs used in combination.

Angiotensin Receptor Blockers in HFrEF

A direct comparison of ARBs and ACE inhibitors was completed in the Evaluation of Losartan In The Elderly I and II trials (Pitt et al., 1997, 2000) and in high risk patients after acute myocardial infarction with HF and/or LV dysfunction in the Optimal Trial In Myocardial Infarction with the Angiotensin II Antagonist Losartan (OPTIMAAL) (Dickstein et al., 2002) trials. These trials compared losartan 50 mg daily with captopril 50 mg three times daily in patients with NYHA Class II-IV HF and those patients post MI,

respectively. There was no evidence of superiority of the ARB over the ACE inhibitor as mortality was similar in the ELITE II trial (11.7% vs. 10.4%), but there was greater improvement in LV systolic function and remodeling in patients receiving captopril in the OPTIMAAL trial. However, there were fewer patients who discontinued the study medication in the losartan group (9.7% vs. 14.7%, $P<0.001$) suggesting better tolerability. It has been argued that the dose of losartan (50 mg daily) may have been inadequate in these trials (Gring and Francis, 2004).

Going one step further, the Valsartan in Acute Myocardial Infarction (VALIANT) trial compared valsartan 160 mg daily, captopril 50 mg three times a day, and valsartan 160 mg daily plus captopril 50 mg thrice daily in a 1:1:1 fashion in patients within 10 days post MI with LV systolic dysfunction and HF. Outcomes in the ACE inhibitor and ARB study groups were similar in terms of overall mortality as well as fatal and nonfatal cardiovascular outcomes. The VALIANT trial showed that combined therapy of ACE inhibitor and ARB after myocardial infarction resulted in increased incidence of adverse events without improving overall survival. Adverse events were less common for monotherapy, with hypotension and renal dysfunction being more common in the ARB group and cough, skin rash, and taste disturbance more common in the ACE inhibitor group (Pfeffer et al., 2003).

To determine if ARBs had a benefit in addition to ACE inhibitors in chronic HF patients, the Valsartan in Chronic Heart Failure Trial (Val-HeFT) randomized 5010 patients with stable NYHA class II-IV HF and a left ventricular ejection fraction of $\leq$40% to either maximally tolerated valsartan or placebo in addition to currently prescribed therapies. Of note, in this study 93% of patients were already receiving ACE inhibitors prior to the addition of valsartan. Compared to placebo there was a 13.2% reduction in the primary end point of combined mortality, cardiac arrest with resuscitation, hospitalization for worsening HF, or new requirement of inotropic medications. However, mortality was similar in the two groups, and the benefit was primarily because of a 24% reduction in the rate of HF hospitalizations in patients in the valsartan arm. Also, subgroup analysis demonstrated that the small subgroup of 366 patients (7%) who were not receiving ACE inhibitors received maximal benefit with a 33% reduction in mortality and a 49% decrease in mortality and morbidity with valsartan compared to placebo, whereas the morbidity and mortality benefit of valsartan observed in the overall trial was no longer significant in patients already receiving background ACE inhibitor therapy (HR 0.92; $P=0.0965$); this modest favorable trend in the group receiving an ACE inhibitor was largely driven by the patients receiving less than the recommended dose of an ACE inhibitor (Konstam, 2002).

Similarly, the Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM-Alternative) trial provided the definitive answer to whether ARBs are an effective alternative to ACE inhibitors in patients with HFrEF. In this trial, patients with symptomatic HFrEF and history of intolerance to ACE inhibitors were randomized to either the ARB, candesartan, or to placebo (history of ACE intolerance included cough in 72%, symptomatic hypotension in 13%, and renal dysfunction in 12% of patients). The use of candesartan was associated with a significant 23% reduction in the primary composite outcome of cardiovascular mortality or HF hospitalization with benefits on both cardiovascular death and HF hospitalization (Granger et al., 2003).

A second large study that evaluated the combined effects of ACE inhibitors and ARBs in patients with chronic HFrEF was the Effect of Candesartan in Patients with Chronic Heart Failure and Reduced Left-Ventricular Systolic Function Taking Angiotensin Converting Enzyme Inhibitors (CHARM-Added Trial). This study evaluated 2548 patients with NYHA Class II-IV HF, LV ejection fraction $\leq$40%, and receiving an ACE inhibitor, who were randomized to either candesartan once daily or placebo and monitored for a combined clinical end point of death and/or hospitalization for HF. There were a higher proportion of patients also prescribed additional medical therapy including 55% on beta-blockers and 17% on aldosterone receptor blockers (McMurray et al., 2003). In this trial a modest benefit was seen with the use of combination therapy with ACE inhibitor and ARB versus ACE inhibitors alone for both outcomes that were included in the primary outcome of cardiovascular death or HF hospitalization (overall HR 0.85; 95% CI 0.75–0.96: $P=0.01$).

One important end point seen in Val-HeFT and the CHARM-Added trials, as well as other studies reviewing concomitant use of ACE inhibitors and ARBs, is the higher incidence of hypotension, hyperkalemia, and renal dysfunction. Using the trial data, the recommendations for the use of ACE inhibitors and ARBs in HFrEF in the AHA/ACC HF guidelines are detailed in **Table 1**.

Table 1 Summary of ACE inhibitor and ARB treatment guidelines

Therapy	*Recommendation*	*Treatment effect*[a]
ACE inhibitors		
	(1) ACE inhibitors are recommended for all patients with HFrEF	Class I
ARBs		
	(1) ARBs are recommended in patients with HFrEF who are ACE inhibitor intolerant	Class I
	(2) ARBs are reasonable as alternative to ACE inhibitors as first-line therapy in HFrEF	Class IIa
	(3) Addition of an ARB may be considered in persistently symptomatic patients with HFrEF on guideline directed therapy	Class IIb
	(4) Routine combined use of an ACE inhibitor, ARB, and aldosterone antagonist is potentially harmful	Class III: Harm

[a]Class I: indicates treatment SHOULD be performed, Class IIa: indicates therapy is REASONABLE, Class IIb: indicates therapy MAY BE CONSIDERED, Class III: indicates therapy is HARMFUL.

Angiotensin Receptor Blockers in HFpEF

There is no compelling evidence for the use of ARBs specifically for patients with HFpEF. In the Effects of Candesartan in Patients with Chronic Heart Failure and Preserved Left Ventricular Ejection Fraction (CHARM-Preserved) study, 3023 patients with NYHA Class II-IV HF with a left ventricular ejection fraction $\geq$40% were randomized to either candesartan or matched placebo. While there was no significant difference in cardiovascular death between the Candesartan and placebo groups, there was a modest reduction in patients with HF hospitalization (15.1% vs. 18.5%, $P=0.017$) and a marginal reduction in risk for HF hospitalization (HR 0.85, 95% CI 0.72–1.01, $P=0.072$) (Yusuf et al., 2003). However, the subsequent and larger Irbesartan in Heart Failure with Preserved Ejection Fraction (I-PRESERVE) study randomized a larger number and better ascertained patients with HFpEF and left ventricular ejection fraction of $\geq$45% to either irbesartan or placebo. In total 4128 patients were randomized and followed for a duration of 49.5 months. At the end of the trial there were no significant differences in mortality or the rate of cardiovascular hospitalization between the two groups (Massie et al., 2008).

Due to lack of sufficient evidence for benefit of both ACE inhibitors and ARBs in patients with HFpEF the ACC/AHA guidelines on heart failure recommend these agents as a Class IIb therapy for reduction of hospitalization. In addition, both can be used in patients with HFpEF if a non-HF indication is present such as in patients with hypertension or diabetes (Yancy et al., 2013).

Aldosterone Receptor Antagonists

Further evaluation of the influence of the neurohormonal axis revealed that plasma volume regulation also is influenced by the adrenal hormone, aldosterone. Aldosterone is a short-lived plasma hormone which is synthesized in the zona glomerulosa of the adrenal cortex. It exerts its effects predominantly on the distal collecting tubule of the renal nephron through the mineralocorticoid receptors which upregulate and promote expression of the $3Na^+/2K^+$ pump. Secretion of aldosterone is promoted by elevated levels of potassium, angiotensin II, angiotensin III and through stretch receptors of the SNS within the myocardium (Dzau et al., 1981; Weber and Villarreal, 1993).

Stimulation of the mineralocorticoid receptor promotes water retention as activation of the Na/K pump drives sodium into the interstitium and potassium is secreted into the collecting duct. The resultant osmotic gradient promotes volume retention in HF patients and leads to the clinical syndrome of volume overload. Aldosterone also leads to loss of magnesium through secretion in the nephron and has been associated with a greater incidence of arrhythmias. Moreover, chronically elevated aldosterone levels increase myocardial and vascular fibrosis as well as left ventricular hypertrophy (Dzau et al., 1981; Swedberg et al., 1990).

As the use of ACE inhibitors and ARBs has increased in HF, "aldosterone escape" or "aldosterone breakthrough," a phenomenon resulting from only partial inhibition of aldosterone by inhibitors of the renin–angiotensin system (RAS) due to non-RAS activation of aldosterone has been described (Schrier, 2010). Following these observations, the safety and efficacy of add-on therapy with aldosterone receptor antagonists including spironolactone and eplerenone has been evaluated in HF patients.

Aldosterone Receptor Antagonists in Heart Failure with Reduced Ejection Fraction

The aldosterone receptor antagonist, spironolactone, was initially evaluated in patients with severe HFrEF already receiving an ACE inhibitor in the Randomized Aldactone Evaluation Study (RALES). In this study 1663 patients with left ventricular ejection fraction of $\leq$35% and NYHA Class III or IV HF symptoms were randomly assigned to either 25 mg of spironolactone daily or placebo. At least 94% of enrolled patients were receiving ACE inhibitors but only ~10% were receiving beta-blockers at the time of enrollment. Among those receiving spironolactone there was a 30% reduction in mortality (RR = 0.70, 95% CI 0.60–0.82; $P<0.001$) compared to those receiving placebo, after a mean follow-up of 24 months. Additionally, there was a 35% decrease in the frequency of hospitalization for worsening HF and a significant improvement in symptoms of HF based on NYHA classification (Pitt et al., 1999).

In the RALES trial there were approximately 50% of patients designated with HF due to ischemic causes; however, animal models had also demonstrated efficacy among those with HFrEF and coronary artery disease. Therefore, the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) was conducted to evaluate treatment in humans, specifically post-MI with a reduction in left ventricular ejection fraction and HF symptoms. The study randomized 6642 patients to either eplerenone 25 mg daily or placebo at least 3–14 days following an MI and documented left ventricular ejection fraction of $\leq$40%. Among those treated, at least 86% were already receiving ACE inhibitors and 75% were receiving beta-blockers. After a mean follow-up of 16 months, compared to placebo, there was a modest 13% reduction in the primary end point of combined death or hospitalization for HF among those assigned to eplerenone (RR 0.87, 95% CI 0.79–0.95; $P=0.002$) (Pitt et al., 2003).

Most recently, the Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF) further evaluated the effect of aldosterone antagonism in those patients with HF and milder symptoms. Patients enrolled had a documented left ventricular ejection fraction of no more than 30% and NYHA class II HF symptoms. Ultimately, 2737 patients were enrolled to either 25 mg eplerenone (with titration to 50 mg at 4 weeks if tolerated) or placebo with an evaluated primary end point of death or HF hospitalization. At a median follow-up of 21 months there was a 37% reduction in the primary end point (HR 0.63, 95% CI 0.54–0.74; $P<0.001$) as well as a 24% reduction in cardiovascular death (HR 0.76, 95% CI 0.61–0.94; $P=0.01$) (Zannad et al., 2011). In contrast to the RALES trial where only ~10% of patients were on beta-blockers at baseline, most patients in the EMPHASIS-HF trial (87%) were on baseline beta-blocker therapy. This trial solidified the significant incremental benefit of aldosterone antagonists on outcomes in HFrEF patients already on guideline-directed therapy of ACE inhibitors and beta-blockers.

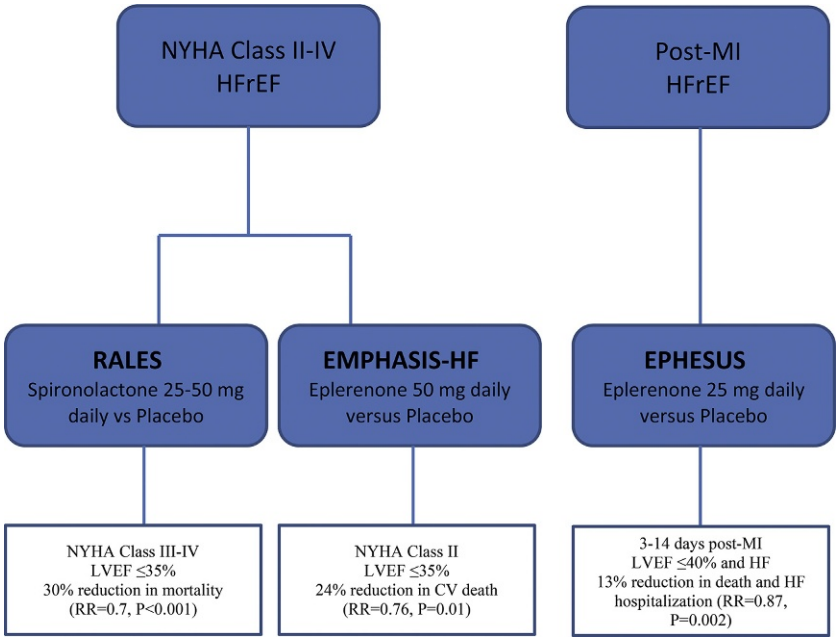

Fig. 5 Trials of aldosterone receptor blockers (eplerenone and spironolactone) in HFrEF with differing severity of HF. *RALES*, Randomized Aldactone Evaluation Study (Pitt et al., 1999); *EPHESUS*, Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (Pitt et al., 2003); *EMPHASIS*, Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (Zannad et al., 2011).

Based on the above evidence, the 2013 ACC/AHA guidelines for the management of HF provide a class I recommendation for the use of aldosterone receptor antagonists in patients with NYHA Class II-IV HF symptoms and a left ventricular ejection fraction ≤35% or ≤40% in those with recent MI (**Fig. 5**). Caution is advised in the initiation of these agents in patients with renal insufficiency or a history of hyperkalemia (Yancy et al., 2013). In the trials patients were excluded from analysis that had a creatinine level greater than 2.5 mg per deciliter, an estimated glomerular filtration rate <30 mL/min/1.73 m^2 of body-surface area, or a serum potassium level of greater than 5.0 mmol per liter prior to randomization. Rates of hyperkalemia ranged from 2% to 8% among those receiving aldosterone receptor antagonists; however, definitions varied between studies.

Aldosterone Receptor Antagonist in Heart Failure with Preserved Ejection Fraction

Use of aldosterone receptor antagonists has also been evaluated in HFpEF without definitive evidence of improvement of outcomes. The Spironolactone for Heart Failure with Preserved Ejection Fraction (TOPCAT) study randomized 3445 patients with symptomatic HF and a left ventricular ejection fraction of 45% or higher to receive either spironolactone (15–45 mg daily) or placebo. After a mean follow-up of 3.3 years there was no difference in the primary end point of death from cardiovascular causes, aborted cardiac arrest, or hospitalization for HF among those receiving spironolactone versus placebo group (hazard ratio, 0.89; 95% confidence interval, 0.77–1.04; $P=0.14$). Notably in the spironolactone group a small reduction in hospitalizations for HF was observed (hazard ratio, 0.83; 95% CI, 0.69–0.99, $P=0.04$); however, total hospitalizations were not different between the two arms (Pitt et al., 2014).

To further evaluate this effect a post hoc analysis of TOPCAT patients enrolled in the Americas versus Russia/Georgia (from where almost 50% of patients were enrolled) was completed and demonstrated significant heterogeneity in underlying patient risk and clinical response to spironolactone. In the subgroup enrolled in the Americas, the primary composite end point in the placebo arms occurred at ~4 times higher rate in the placebo arm compared with the subgroup enrolled from Russia/Georgia. In the higher risk subgroup in the Americas, the primary end point was reduced by spironolactone compared to placebo (HR 0.82, 95% CI 0.69–0.98), but there was no effect in the subgroup enrolled in Russia/Georgia (HR 1.10, 95% CI 0.79–1.51) (**Fig. 6**) (Pfeffer et al., 2015).

Similar investigations have also been completed evaluating the effect of aldosterone antagonists on exercise capacity in patients with HFpEF including the Effect of Spironolactone on Diastolic Function and Exercise Capacity in Patients with Heart Failure with Preserved Ejection Fraction (ALDO-HF) (Edelmann et al., 2013) and Randomized Aldosterone Antagonism in Heart Failure with Preserved Ejection Fraction (RAAM-PEF) (Deswal et al., 2011) trials. In both there was no significant change in maximal oxygen consumption and six minute walk test after treatment with spironolactone 25 mg daily (with an increase to 50 mg daily if tolerated in the RAAM-PEF study) for 12 and 6 months, respectively. Given the lack of clear benefit of aldosterone antagonism in HFpEF on mortality, hospitalization, or symptoms, the use across all patients with HFpEF cannot be advocated. However, given the paucity of

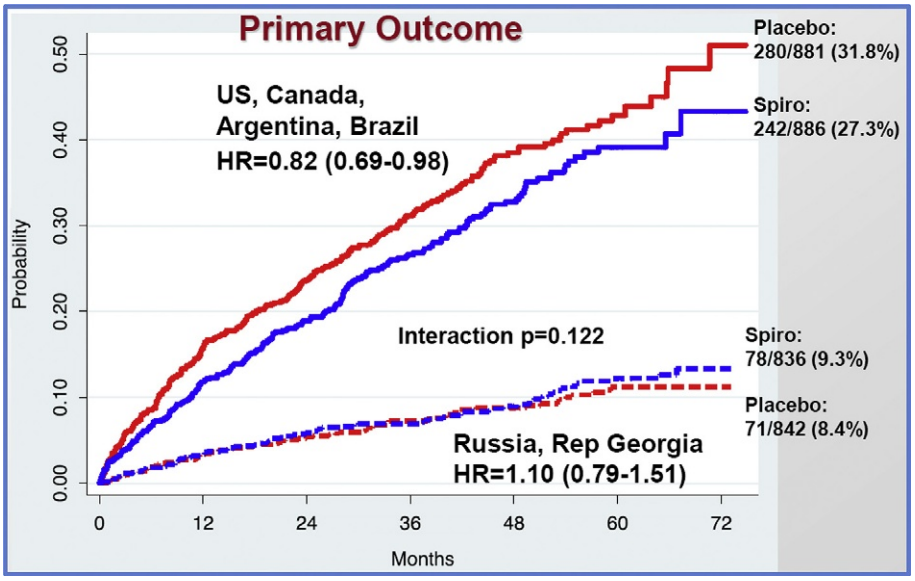

Fig. 6 Kaplan-Meier curves for the post hoc analysis in Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) trial of spironolactone in HFpEF delineating significant differences in those patients treated in the Americas versus those in Russia/Georgia (Adapted from Pfeffer et al., 2015). The primary outcome was time to cardiovascular death, heart failure hospitalization, or resuscitated cardiac arrest.

clearly beneficial treatment in this group of patients and the heterogeneity of data across regions, the 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure has provided a weak Class IIb recommendation for its use in HFpEF as follows:

> In appropriately selected patients with HF*p*EF (with EF ≥45%, elevated BNP levels or HF admission within 1 year, estimated glomerular filtration rate >30 mL/min, creatinine <2.5 mg/dL, potassium <5.0 mEq/L), aldosterone receptor antagonists might be considered to decrease hospitalizations (Yancy et al., 2017).

Direct Renin Inhibitors

Feedback loops within the RAAS cascade provide regulation of the signaling peptides and therapies such as ACE inhibitors and ARBs disrupt these signals. It has been demonstrated in early trials that elevated levels of plasma renin confer a higher risk of mortality. The use of ACE inhibitors or ARBs leads to an increase in renin despite their benefits of decreased symptoms and improved mortality as they target downstream substrates (Fig. 2). This is thought to occur by competitive saturation of ACE due to high levels of ACE inhibition. Additionally, high levels of renin directly act at the renin receptor of the myocardium causing hypertrophic, apoptotic activity, and extracellular matrix remodeling. Therefore use of direct renin inhibitors (DRI) was evaluated as a treatment in HF as it may produce similar effects as an ACE inhibitor and may overcome the effects of renin in conjunction with ACE inhibitor use (Nguyen, 2011; Schroten et al., 2012).

Aliskiren was the most evaluated DRI in HFrEF. However, studies have not demonstrated any incremental benefit of DRI in addition to an ACE inhibitor or with DRI in comparison with an ACE inhibitor in HFrEF (McMurray et al., 2008, 2016; Solomon et al., 2011). No studies have been published to date to evaluate DRIs in patients with HFpEF.

Sympathetic Nervous System (SNS) Blockade

Cardiac insults such as myocardial infarction or necrosis impair cardiac output or contractility leading to activation of the sympathetic nervous system (SNS). There are multiple complex interactions that play a role in increased sympathetic output including input from arterial baroreceptors, cardiopulmonary baroreceptors, muscle metaboreceptors, and renal nerves (Fig. 7). As left ventricular function decreases in patients with HF, a proportionate dysfunction between sympathetic and parasympathetic regulation occurs (Floras and Ponikowski, 2015).

Baroreceptors within the carotid sinus and aortic arch as well as mechanoreceptors within the myocardium provide an inhibitory signal for sympathetic output from the central nervous system in healthy individuals. A decrease in cardiac output or effective arterial volume will result in decreased output from these baroreceptors and increase signaling with sympathetic hormones, including norepinephrine and epinephrine (Hartupee and Mann, 2016; Floras and Ponikowski, 2015). Early in the development of HF, these neurohormonal changes are seen due to decreased effective cardiac output. The sympathetic signaling increases as a compensatory mechanism to improve end-organ perfusion through inotropic and chronotropic effects.

Specifically, increased sympathetic output will lead to increased heart rate through stimulation of beta-1-adrenergic receptors and increased peripheral vasoconstriction through alpha-1-adrenergic receptors. In addition, there is crossover in the RAAS pathway

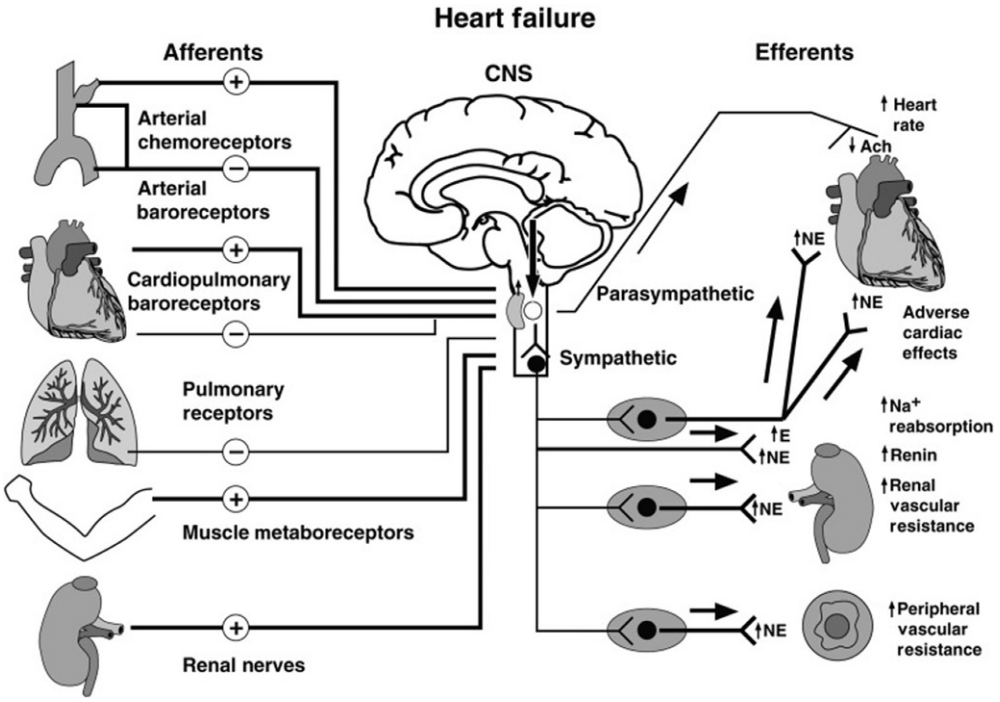

Fig. 7 Autonomic mechanisms of HFrEF. Outflow from cardiac and vascular mechanoreceptors, pulmonary stretch receptors, muscle metaboreceptors, and the renal afferent arterioles activate central sympathetic signaling through norepinephrine (NE) and epinephrine (E). NE and E have end organ effects of vasoconstriction, fibrosis, increased renin secretion, increased sodium reabsorption, and increased peripheral vascular resistance. There is also parasympathetic inhibition leading to decreased acetylcholine (Ach) secretion and thus increased heart rate (Floras and Ponikowski, 2015).

as sympathetic output stimulates the juxtaglomerular apparatus in the kidney (Floras and Ponikowski, 2015). Increased sympathetic tone, therefore, will also lead to increased fluid and sodium retention through increased levels of renin and thus angiotensin II.

Levels of norepinephrine are not routinely checked in patients with HF; however, elevated levels have been directly correlated with mortality in patients with HFrEF (Anand et al., 2003; Cohn et al., 1984). Despite this correlation hormonal measurement does not seem to correlate with left ventricular function or quality of life measurements. During evaluation of patients in the Vasodilator-Heart Failure II Trial (V-HeFT II) it was found that those with higher levels of neuroendocrine activation had a greater benefit from the vasodilator and RAS blocker, enalapril (Francis et al., 1993).

In addition to the direct physical changes seen due to SNS activation, chronically heightened sympathetic tone has intracellular effects. As described previously sympathetic neuroendocrine hormones, norepinephrine and epinephrine, act through the G-protein-coupled beta-adrenergic receptors which increase inotropy, heart rate, and peripheral vascular resistance. This compensatory mechanism leads to increased myocardial stress through upregulation of cyclic adenosine monophosphate which produces elevated levels of intracellular calcium. Ultimately, the rise in cellular calcium and oxidative stress leads to cellular death. In addition, the cellular shifts in calcium appear to promote after depolarizations and ventricular arrhythmias (Floras and Ponikowski, 2015; Sackner-Bernstein JaM, 1995; Ponikowski et al., 2001; Lown and Verrier, 1976).

Chronic activation of the SNS leads to deleterious effects in the heart including desensitization of beta adrenergic receptors, myocyte hypertrophy, necrosis, apoptosis, and fibrosis; in the kidney including activation of the RAAS, increase in salt and water retention, and an attenuated response to natriuretic peptides; and in the peripheral vessels including neurogenic vasoconstriction and vascular hypertrophy (Hartupee and Mann, 2016).

Beta-blockers in Heart Failure with Reduced Ejection Fraction

Beta-blockers have become a Class I guideline-recommended medical therapy in addition to ACE inhibitor or ARB therapy in patients with HFrEF. Unlike ACE inhibitors, which demonstrate a class effect in HF therapy, mortality benefit has only been seen with three specific beta-blocking agents, including metoprolol succinate (MERIT-HF-Investigators, 1999), carvedilol (Packer et al., 2001), and bisoprolol (CIBIS II-Authors, 1999). Bisoprolol and sustained-release metoprolol (succinate) selectively block beta-1–receptors and carvedilol blocks alpha-1-, beta-1-, and beta-2-receptors. Beta-blockers have been demonstrated to ameliorate the effects of chronic catecholamine activation and lead to improvement in symptoms and clinical outcomes of chronic HF. Several trials have shown that beta-blocker use can lead to improvement in survival, left ventricular function (Groenning et al., 2000;

Bristow, 2000), reduction in left ventricular size (reverse remodeling) (Colucci et al., 2007; Lowes et al., 1999), and reduced HF hospitalization (CIBIS II-Authors, 1999) among those with HFrEF, across all NYHA classes.

Despite initial reports demonstrating improvement in symptoms in patients with HF (Bristow et al., 1994; Eichborn et al., 1994; Fisher et al., 1994; Woodley et al., 1991), enthusiasm initially remained limited over initiation of these medications due to the potential early risk of adverse effects after initiation of this class of negative inotropic medications, especially in patients with decompensated HF (Epstein and Braunwald, 1966). One early trial aimed at detecting improvement in mortality was the Cardiac Insufficiency Bisoprolol Study (CIBIS) in which 641 patients with predominantly NYHA Class III CHF (95% of enrollees) and a left ventricular ejection fraction of ≤40% were randomized to either the beta-blocker bisoprolol or matched placebo. Many of the patients were already receiving vasodilators, digitalis, and ACE inhibitors with 90% receiving the latter therapy. After a mean follow-up of 1.9 years despite significant improvements seen in functional class, the 20% mortality reduction that was seen among those receiving bisoprolol was not statistically significant (RR 0.8, 95% CI 0.56–1.15; $P=0.22$). Of note, there were no forced titration protocols for bisoprolol dosing among participating clinicians with only 50% of the enrolled patients reaching the target dose of 5 mg daily (The CIBIS Investigators, 1994).

However, the U.S. Carvedilol Heart Failure trial helped beta-blocker therapy gain traction. In this study patients with NYHA Class II-IV symptoms for at least 3 months were randomized to maximally tolerated doses of carvedilol (starting with 6.25 mg twice daily) or placebo. Therapy with vasodilators, digitalis, and ACE inhibitors was allowed with 95% of patients already receiving the latter. Among the 1094 patients enrolled in the trial, a 65% relative reduction in mortality was seen after a median follow-up time of 6.5 months (95% CI 39%–80%; $P<0.001$), leading to early termination of the study (Packer et al., 1996).

Based on results of the studies it was suggested that the beneficial effect of carvedilol was seen due to its nonselective blockade of beta-1 and beta-2 receptors. However, a follow-up study to CIBIS was completed in the Cardiac Insufficiency Bisoprolol Study II (CIBIS-II) which demonstrated beneficial effects of bisoprolol in 2647 NYHA Class III and IV patients on a background of diuretic and ACE inhibitor therapy with a 34% reduction in all-cause mortality in the bisoprolol group compared to placebo (OR 0.66, 95% CI 0.54–0.81; $P<0.0001$). Notably, follow-up analysis showed a significant reduction in hospitalization for ventricular fibrillation and ventricular tachycardia ($P=0.006$), along with a 44% reduction in sudden cardiac death (CIBIS II-Authors, 1999). Subsequent improvement in mortality was also demonstrated with another selective beta-1 adrenergic antagonists metoprolol succinate in the MERIT-HF trial (MERIT-HF-Investigators, 1999) (**Table 2**). Analysis of benefits in mode of death in the MERIT-HF trial, which enrolled patients in NYHA class II-IV functional class, showed similar results, with a 41% reduction in sudden cardiac death as well as 49% reduction in the HF death. Taking results of both trials together, it suggests that beta-blockers demonstrate benefit in both sudden cardiac death as well as HF death. When mode of death was evaluated overall in the MERIT-HF trial, consistent with prior literature, sudden death was more common in patients with less severe HF (NYHA class II) and death from worsening HF was more common in patients with increased severity of HF (NYHA class III and IV) (**Fig. 8**).

Initiation of beta-blocker therapy should be considered in patients who clinically are not currently exhibiting signs of volume overload, cardiogenic shock, symptomatic bradycardia, or with reactive airway disease exacerbation. In patients with an ongoing signs of severe acute HF, initiation of beta-blockers may blunt the compensatory response of increased heart rate and vascular tone which could lead to precipitation of cardiogenic shock. However, it has been demonstrated that introduction of beta-blocker therapy is safe in patients with stable advanced heart failure symptoms. In a study evaluating patients with an estimated left ventricular ejection fraction of less than 25% and with relatively stable, euvolemic NYHA Class IV HF symptoms, a reduction in mortality of 35% was seen in patients receiving maximally tolerated carvedilol versus placebo (Packer et al., 2001). While there were higher incidences of hypotension, edema, and bradycardia among the carvedilol group than placebo, the most common severe

Table 2 Summary of beta-blocker therapy in HFrEF

Study	*CIBIS (The CIBIS Investigators, 1994)*	*Carvedilol Heart Failure Study Group (Packer et al., 1996)*	*CIBIS II (CIBIS II-Authors, 1999)*	*MERIT-HF (MERIT-HF-Investigators, 1999)*
Medication	Bisoprolol 5 mg daily versus Placebo (51% reached max dose)	Carvedilol 25–50 mg twice daily[a] versus Placebo (MDD = 45 mg)	Bisoprolol 10 mg daily versus Placebo (43% reached max dose)	Metoprolol CR/XL (Succinate) 200 mg daily versus Placebo (MDD = 159 mg daily)
Study subjects	641 Patients with LVEF ≤40% and NYHA Class III or IV heart failure	1094 Patients with LVEF ≤35% and 3 months of heart failure symptoms	2647 Patients with LVEF ≤35% and NYHA Class III or IV heart failure	3991 Patients with LVEF ≤40% and NYHA Class III-IV heart failure
Key points	• Reduced CHF Hospitalization ($P<0.01$) • Nonsignificant reduction in mortality (RRR = 20%, $P=0.22$) • Improvement in NYHA Class ($P=0.03$)	• 65% RRR in death (95% CI 0.2–0.61, $P<0.001$) • Reduced CHF hospitalization (RRR = 27%, $P=0.036$)	• 44% RRR in death (95% CI 0.54–0.81, $P<0.001$) • Reduced all cause and CHF hospitalization ($P=0.0049$ and $P=0.001$, respectively) • Reduced hospitalization for VT and VF	• 44% RRR in death (95% CI 0.53–0.81, $P<0.001$) • Reduction in progression of heart failure at 18 months ($P=0.0023$)

MDD, Mean daily dose; *LVEF*, Left ventricular ejection fraction; *CHF*, congestive heart failure; *RRR*, relative risk reduction; *VT*, ventricular tachycardia; *VF*, ventricular fibrillation.
[a]There was a run-in phase of carvedilol 6.25 mg BID for all patients.
Adapted from the 2013 ACCF/AHA guideline for the management of heart failure.

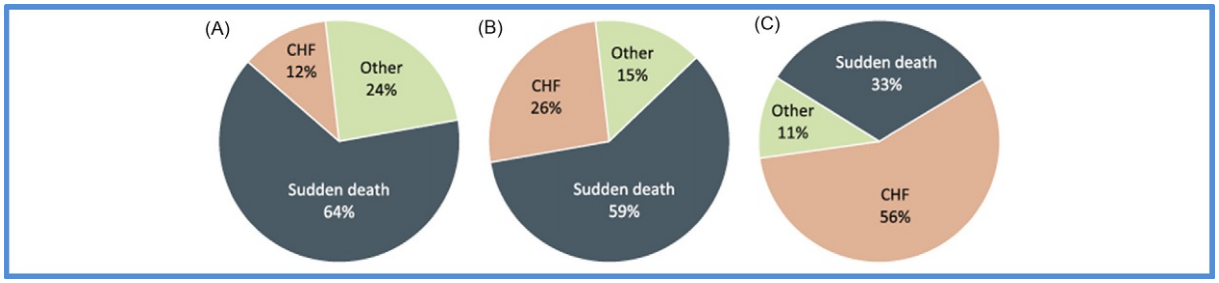

Fig. 8 Mode of cardiovascular death based on severity of HF at baseline in the MERIT-HF trial. Panels A, B, and C demonstrate NYHA Class II, Class III, and Class IV patients, respectively (MERIT-HF-Investigators, 1999). *CHF*, chronic heart failure.

adverse events were worsening HF which occurred at similar rates among both randomized groups. All major trials of beta-blockers in HFrEF have been carried out on a background of ACE inhibitors which had previously been proven of benefit in these patients. It has been suggested that initiation of beta-blockers prior to achieving higher doses of ACE inhibitors or ARB may lead to a greater improvement in symptoms and mortality in patients with HFrEF (Packer et al., 1999).

Based on the strong evidence, the AHA/ACC HF guidelines recommend use of one of the three beta-blockers proven to reduce mortality (e.g., bisoprolol, carvedilol, and sustained-release metoprolol succinate) for all patients (hemodynamically stable) with current or prior symptoms of HFrEF, unless contraindicated, to reduce morbidity and mortality (Yancy et al., 2013).

Beta-blockers in HFpEF

The same clinical benefits of beta-blockers in HFrEF have not been proven in patients with HFpEF. In HFpEF the underlying pathology seen is increased filling pressures of the left ventricle which leads to increased pulmonary arterial pressures which clinically presents as pulmonary edema and decreased exercise tolerance. Elevated filling pressures are measured on echocardiography during diastole and occur typically due to increased viscoelastic properties, increased wall thickness, or changes in left ventricular size and shape leading to a compromised contribution of pre-load to cardiac output (Bhuyian and Mauer, 2011; Bourlaug and Paulus, 2010). These physiologic derangements lead intuitively to thinking that beta-blockers would be of benefit by decreasing beta-adrenergic drive, lowering heart rate, and prolonging the diastolic filling phase allowing improved filling of the left ventricle (Bourlaug and Paulus, 2010). However, current data does not support the routine use of beta-blocker therapy in patients with HFpEF to primarily improve clinical outcomes (Aronow et al., 1997; Farasat et al., 2010; Flather et al., 2005). Therefore, beta-blockers are indicated only for use in the treatment of comorbidities in HFpEF, such as atrial fibrillation, angina, postmyocardial infarction, and hypertension.

Natriuretic Peptide System Potentiation

Natriuretic peptides have ameliorating effects on manifestations of HF including increased natriuresis, diuresis, and vasodilation. Atrial natriuretic peptide and B-type natriuretic peptide are released from the atrial and ventricular myocardium, respectively, in response to chamber distention, wall stress, or increased activation of the SNS or RAAS. They exert their effects by counter-regulation including decreasing renin production and increasing cyclic GMP production (Levin et al., 1998). However, in HF there is increased peripheral resistance to natriuretic peptides, thus potentiating the effects of the RAAS (Volpe et al., 2016). In addition, the half-life of natriuretic peptides is short as they are degraded by neutral endopeptidases, including neprilysin. Neprilysin is involved in breakdown of vasoactive peptides like angiotensin I, angiotensin II, endothelin-1, kinins, adrenomedullin, and atrial and B- type natriuretic peptides. Studies have demonstrated that through augmentation of active natriuretic peptides through neprilysin inhibition, an increase in myocardial cyclic guanosine 3′5 mono phosphate can be achieved, which improves myocardial relaxation, reduces hypertrophy, and stimulates diuresis, natriuresis, and vasodilation (Mangiafico et al., 2013). Neprilysin inhibition may be a useful target in patients with HF. However, neprilysin is a nonspecific enzyme which has a variety of nonvasoactive peptide targets such as opioid peptides, encephalin, gastrin, and amyloid beta (Aβ). Importantly, angiotensin II breakdown is also decreased which may potentiate RAAS effects; therefore, in HF, neprilysin inhibitors need to be combined with inhibitors of RAS (**Fig. 9**).

Angiotensin Receptor Blocker-Neprilysin Inhibitors (ARNI) in HFrEF

Combined angiotensin receptor blocker–neprilysin inhibitors (ARNI) have recently been validated as a mortality-reducing therapy in patients with HFrEF. LCZ696 or Valsartan/Sacubitril is the first angiotensin receptor-neprilysin inhibitor (ARNI) that combines the molecular moieties of the neprilysin inhibitor prodrug AHU377 and the ARB, valsartan in one compound. The Prospective comparison of ARNI with ACE inhibitor to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) trial studied the efficacy and safety of LCZ696 200 mg twice daily compared to enalapril 10 mg twice daily in 8458 patients with chronic HF (NYHA Class II–IV and EF <35%). The drug was evaluated for the primary end point of combined cardiovascular

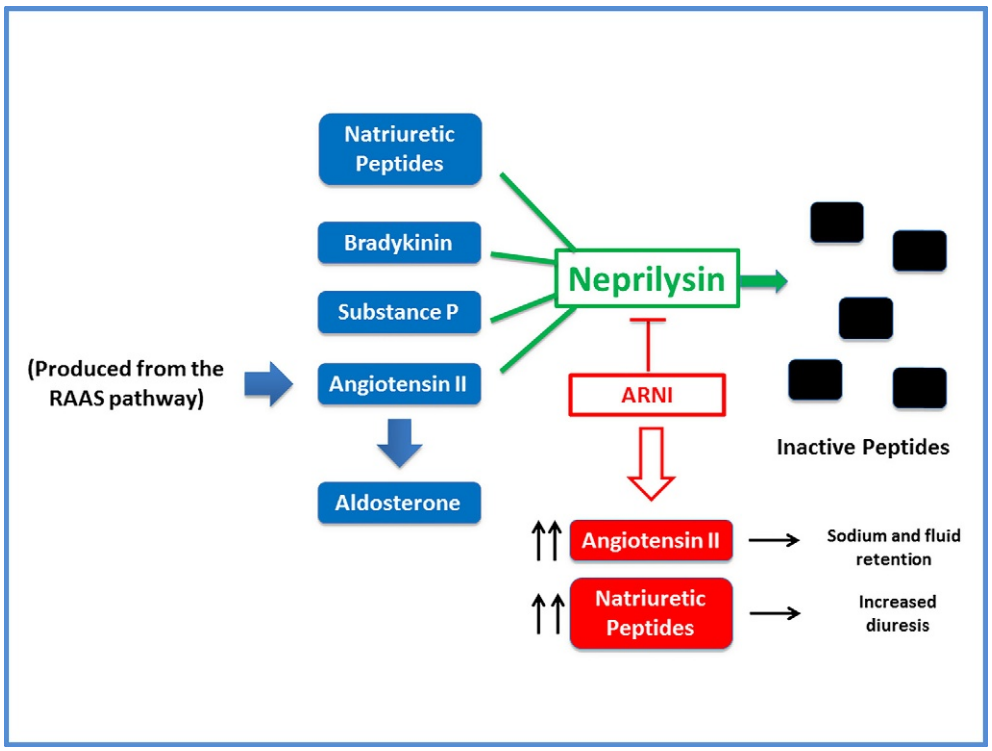

Fig. 9 Mechanism of ARNI in HF. Under normal conditions neprilysin is responsible for enzymatically degrading natriuretic peptides, bradykinin, substance P, and angiotensin II. When ARNI is used there is a downstream increase in angiotensin II and natriuretic peptides which have competing actions.

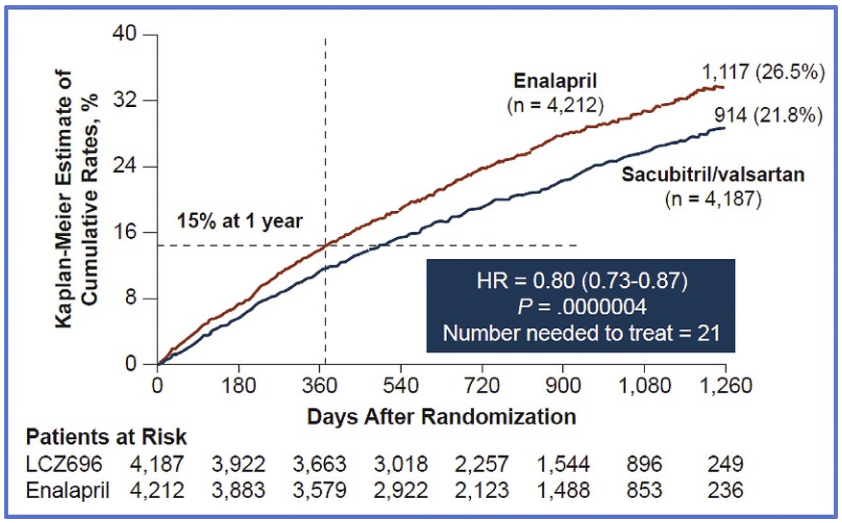

Fig. 10 Kaplan-Meier curves detailing the outcomes in the PARADIGM trial. After a median follow-up of 27 months, an absolute reduction of 4.8% was seen in the primary end point of cardiovascular death and HF hospitalization (Adapted from McMurray et al., 2014).

death and HF hospitalization. After a median follow-up of 27 months, an absolute reduction of 4.8% was seen in the primary end point (HR 0.80, 95% CI 0.73–0.87; $P < 0.001$) (Fig. 10). A similar beneficial effect was seen for cardiovascular death (HR 0.8, 95% CI 0.71–0.89), hospitalization for HF (HR 0.79, 95% CI 0.71–0.89), as well as death from any cause (HR 0.84, 95% CI 0.76–0.93) (McMurray et al., 2014). Subgroup analysis revealed that the significant reduction in cardiovascular death was driven primarily due to the reduction in NYHA Class II patients rather than Class III and IV.

The LCZ696 group had a higher incidence of hypotension and nonserious angioedema but lower incidence of renal impairment, hyperkalemia, and cough than the enalapril group. In a more recent report, the PARADIGM investigators indicated that, in comparison with enalapril, LCZ696 exhibited additional evidence of clinical benefit, including a reduced need for intensification

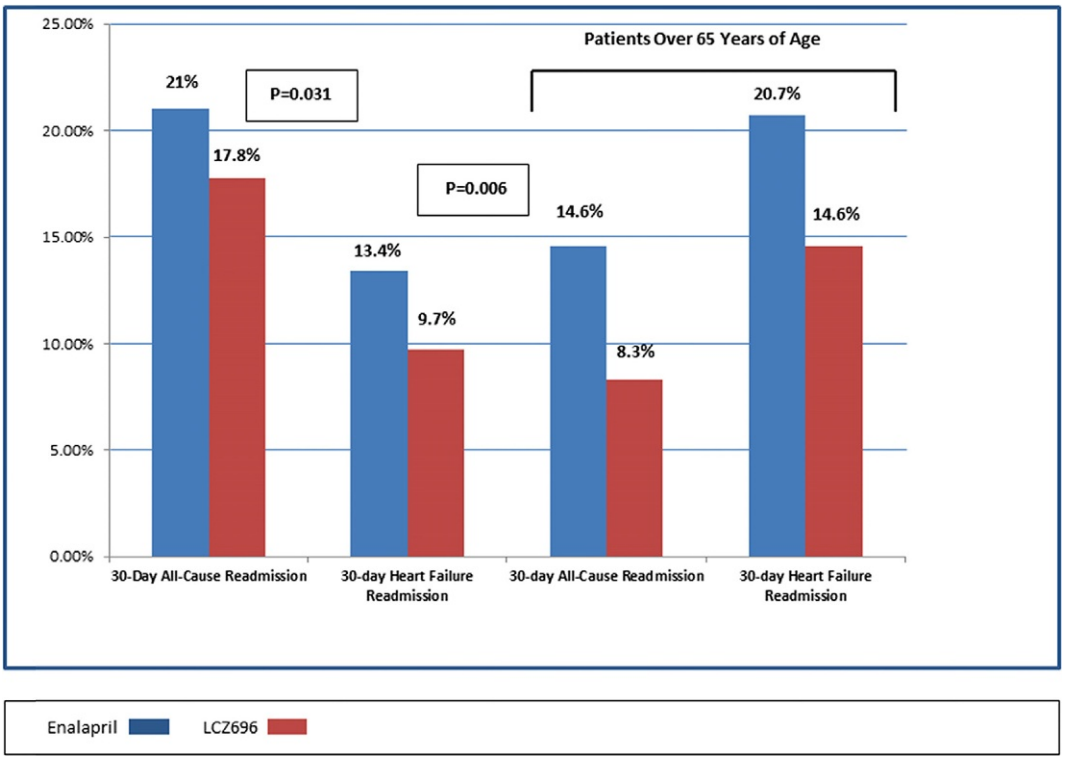

Fig. 11 Post hoc analysis of the PARADIGM-HF Trial is reviewed showing 30-day all-cause readmission and heart failure readmission is shown with applicable p-values. Subgroup analysis of patients over 65 years of age is also shown (Adapted from Desai et al., 2016).

of the treatment for HF, fewer visits to an emergency department for HF, and a lower requirement for intensive care or need for inotropic agents, an HF device, or cardiac transplantation (Packer et al., 2015).

Following completion of the PARADIGM-HF trial further review was completed to assess the effect of LCZ696 on 30 day rehospitalization rates. Given the increase in mortality of HF patients after readmission (Bello et al., 2014; Setoguchi et al., 2007) and reimbursement driven incentives, interest has grown in agents that can decrease rehospitalization in this group of patients. In their analysis it was found that at 30 days after initiation of LCZ696 there was a 38% reduction in HF hospitalization (OR 0.62, 95% CI 0.45–0.87; $P=0.006$) as well as a 26% decrease in hospitalization for any cause (OR 0.74, 95% CI 0.56–0.97; $P=0.031$) (Fig. 11).

It should be noted that in the PARADIGM trial there was a 2-week run-in phase where patients had an open label period in which they demonstrated tolerability to enalapril and then to LCZ696 before they were randomized. Therefore, only patients who tolerated both drugs were randomized in the trial and in practice clinicians will need to get experience with the tolerability and titration of this medication. Nevertheless, the benefits seen with this medication prompted an update of the ACC/AHA HF guidelines in 2016 with the following class I indications (Yancy et al., 2016):

> The clinical strategy of inhibition of the renin-angiotensin system with ACE inhibitors OR ARBs, OR ARNI in conjunction with evidence-based beta blockers, and aldosterone antagonists in selected patients is recommended for patients with chronic HFrEF to reduce morbidity and mortality.
>
> In patients with chronic symptomatic HFrEF NYHA class II or III who tolerate an ACE inhibitor or ARB, replacement by an ARNI is recommended to further reduce morbidity and mortality.

Due to the higher risk of angioedema, ARNI should not be administered concomitantly with ACE inhibitors or within 36 h of the last dose of an ACE inhibitor. Furthermore, in the PARADIGM trial, patients with stable HF were started on this medication. The safety and tolerability of ARNI, when initiated at the time of HF hospitalization, is being examined in the PIONEER trial (ClinicalTrials, 2015).

Of note, with the use of ARNI serum BNP levels will be elevated due to their inhibitory elevated effect on neprilysin (Fig. 9). This was seen among enrollees in the PARADIGM trial where serum levels of BNP were significantly reduced among those receiving LCZ696 versus those receiving enalapril. This reduces the usefulness of serum BNP for monitoring of volume status and prognosis. Additionally, enhanced diuresis is noted as a side effect and may lead to orthostatic hypotension. This should prompt clinicians to adjust diuretics as indicated.

Some concern remains about the safety of ARNI due to the nonselective nature of blockade, specifically the inhibition of amyloid β degradation. Amyloid β (Aβ) accumulation has been demonstrated to play a primary role in the etiology of Alzheimer's

disease (AD). Similar mechanisms also appear to play a role in age-related macular degeneration (AMD) (Ohno-Matsui, 2011). In AD the accumulation of Aβ leads to synaptic loss and clinically neurologic decline. Neprilysin has been identified as one of the predominant amyloid-degrading enzymes of Aβ (Leissring et al., 2003; Marr et al., 2003; Spencer et al., 2014) and levels of neprilysin have been shown to decrease with age as well as following a hypoxic event or ischemic stroke all which are precursors of AD. Additionally, mice studies have shown that with neprilysin inhibition and neprilysin-deficient mice there is a rise of specific peptide targets, Aβ1-40 and Aβ1-42 (Iwata et al., 2001; Shirotani et al., 2001).

The PARADIGM-HF trial investigators attempted to allay fears about Aβ deposition by citing a 2-week study in which healthy volunteers received sacubitril/valsartan and there was no significant increase in cerebrospinal fluid (CSF) concentration of Aβ1-40 and Aβ1-42, but an increase in Aβ1-38 (Langenickel et al., 2016). It was also noted that other enzymes have been shown to contribute to the degradation of Aβ, including insulin-degrading enzyme, endothelin-converting enzyme, angiotensin-converting enzyme, thimet oligopeptidase, and plasmin (Carson and Turner, 2002; Wang et al., 2006; Baranello et al., 2015). However, despite this evidence questions still remain about the influence of sacubitril/valsartan on the possible development of Aβ deposition and neurocognitive deficits. Given subjects used in the CSF study were healthy volunteers critics suggest this model does not represent the more permeable blood-brain barrier that is present in more elderly and ill HF patients. Therefore, the Food and Drug Administration is requiring the sponsor of the PARADIGM-HF study to complete a follow-up study entitled "multicenter, randomized, double-blind, active-controlled trial to evaluate the effects of Entresto compared to valsartan on cognitive function as assessed by comprehensive neurocognitive battery and [positron emission tomography] imaging in patients with chronic heart failure with preserved ejection fraction" (US Food and Drug Administration, 2015). Until results are available, clinicians should remain aware of the theoretical neurodegenerative and visual deficits that could occur with neprilysin inhibition.

Combined Angiotensin Blocker-Neprilysin Inhibitor (ARNI) in HFpEF

LCZ696 was tested in HFpEF patients in the Prospective comparison of ARNI with ARB on Management Of heart failUre with preserved ejectioN fracTion (PARAMOUNT) trial, a phase II clinical trial. Three hundred and one patients with LVEF $\geq$45%, NYHA II-III, and NT-pro BNP >400 ng/mL were randomized to LCZ696 or valsartan. The primary outcome, NT-proBNP, was significantly reduced at 12 weeks in the LCZ696 group compared with the valsartan group. Moreover, a significant improvement was seen in NYHA class and an improvement in echocardiographic parameters like left atrial size with LCZ696 at 36 weeks compared with valsartan. However, left ventricular ejection fraction, volume, and Doppler parameters of diastolic function did not improve with LCZ696 (Solomon et al., 2012). Given the overall encouraging results, the PARAGON (Prospective Comparison of LCZ696 with ARB Global Outcome in HF with Preserved Ejection Fraction), phase III trial has begun. It is intended to enroll 4300 patients with LVEF >45% and will evaluate the effects of LCZ696 on morbidity and mortality end points in HFpEF (ClinicalTrials, 2016).

Incremental Therapies in Heart Failure with Reduced Ejection Fraction (HFrEF)

Each of the neurohormonal blockade therapies discussed have individually been shown to be of benefit in patients with HFrEF. However, current guidelines recommend addition of therapies in a stepwise fashion in appropriately selected patients. Additionally, many of the trials completed selected patients already on treatment for HF as therapies were gaining evidence for benefit. Based on the clinical trials completed thus far, there is evidence that each drug class has an incremental benefit with its addition including ACE inhibitor/ARB, β-blockers, and aldosterone antagonists. In a review of each added therapy based on prior randomized controlled trials a calculated relative reduction in mortality of 17% for ACE inhibitor/ARB therapy, 34% for β-blocker therapy, and 30% for aldosterone antagonists (**Table 3**) was estimated (Fonarow et al., 2011). Use of the ARNI provided an incremental reduction of 16% in mortality relative to ACE inhibitors, in patients already on β-blockers +/- aldosterone antagonists.

Table 3 Incremental benefit of neurohormonal blockade in HFrEF (Yancy et al., 2013; Desai et al., 2016)

Therapy	*RR in mortality (%)*	*RR in HF hospitalization (%)*
ACE Inhibitor or ARB	17	31
Beta-Blocker	34	41
Aldosterone Antagonist	30	35
ARNI	26	28

RR, relative reduction; *ACE*, angiotensin converting enzyme; *ARB*, angiotensin receptor blocker; *ARNI*, angiotensin receptor neprilysin-inhibitor.

The estimated benefit of ARNI is based on its use instead of ACE inhibitors or ARBs.

Adapted from Yancy et al. (2013). ACCF/AHA guideline for the management of heart failure—A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation 138*, e270.

Conclusions

HF is a chronic, progressive disease which leads to reduced quality of life due to volume overload, dyspnea, and recurrent hospitalization as well as reduction in longevity when compared to healthy patients. These pathologic changes have been linked to volume and sodium retention, myocardial remodeling, and increased vasoconstriction through neurohormonal mechanisms in patients with HFrEF and HFpEF. Evidence has shown reduction in mortality, hospitalization for HF, and improvement in myocardial function with blockade of the neurohormonal systems using ACE inhibitors, ARBs, beta-blockers, aldosterone antagonists, and ARNI, specifically among those HFrEF. Clinicians should strive to initiate these agents and gradually increase the doses toward target doses, as tolerated, and based on the published AHA/ACC HF guidelines to modify clinical outcomes in patients with HF.

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Neurological Regulation of the Circulation

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Introduction

The autonomic nervous system (ANS) is an organized compendium of afferent and efferent neurons that enable the central nervous system to communicate with organs and tissues throughout the body. The ANS functions constantly and involuntarily and is regulated by centers located in the spinal cord, brain stem, and hypothalamus. It is comprised of two differentially regulated cholinergic preganglionic pathways, named the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS), which innervate ganglia (e.g., paravertebral or prevertebral sympathetic ganglia), glands (e.g., adrenal and mammary glands), and neural networks servicing the cardiovascular system. As such, the ANS serves as a key player in blood pressure (BP) regulation and blood flow/hematocrit redistribution.

The SNS and PNS exert antagonistic effects on the heart (see section "**Neurological Regulation of the Heart**"), which regulate BP via alterations in cardiac output (CO). In contrast, in a majority of tissues, the SNS provides the primary efferent input regulating vascular resistance (in arteries and arterioles) and blood volume (in veins and venules) through alterations in vascular tone.

This article aims to give an overview of major neurological inputs and receptor ligand interactions that contribute to cardiovascular homeostasis. The discussion begins with neurological regulation of the heart, moves chronologically through the vascular system, and ends with a discussion of modifiers of neurological regulation of the circulation (see **Fig. 1** for overview).

Neurological Regulation of the Heart

The heart serves as the main energy source of the circulation and is responsible for the movement of blood throughout the periphery with every beat, where the ANS serves to modify function and energy output of the heart. In light of discussing neural control of the circulation, it is important to address the effects of the ANS on heart function, as its efferent control is integrated into the afferent circulatory reflex regulated at the level of the brainstem. The heart is innervated by both parasympathetic and sympathetic postganglionic fibers arising from the vagus, stellate, and middle cervical ganglion, respectively (Gourine et al., 2016; Coote, 2013; Coote and Chauhan, 2016). Together, parasympathetic and sympathetic neurons modulate chronotropy (heart rate, HR), dromotropy, (conduction of cardiac impulse), lusitropy (myocyte relaxation), and inotropy (contraction force) of the heart.

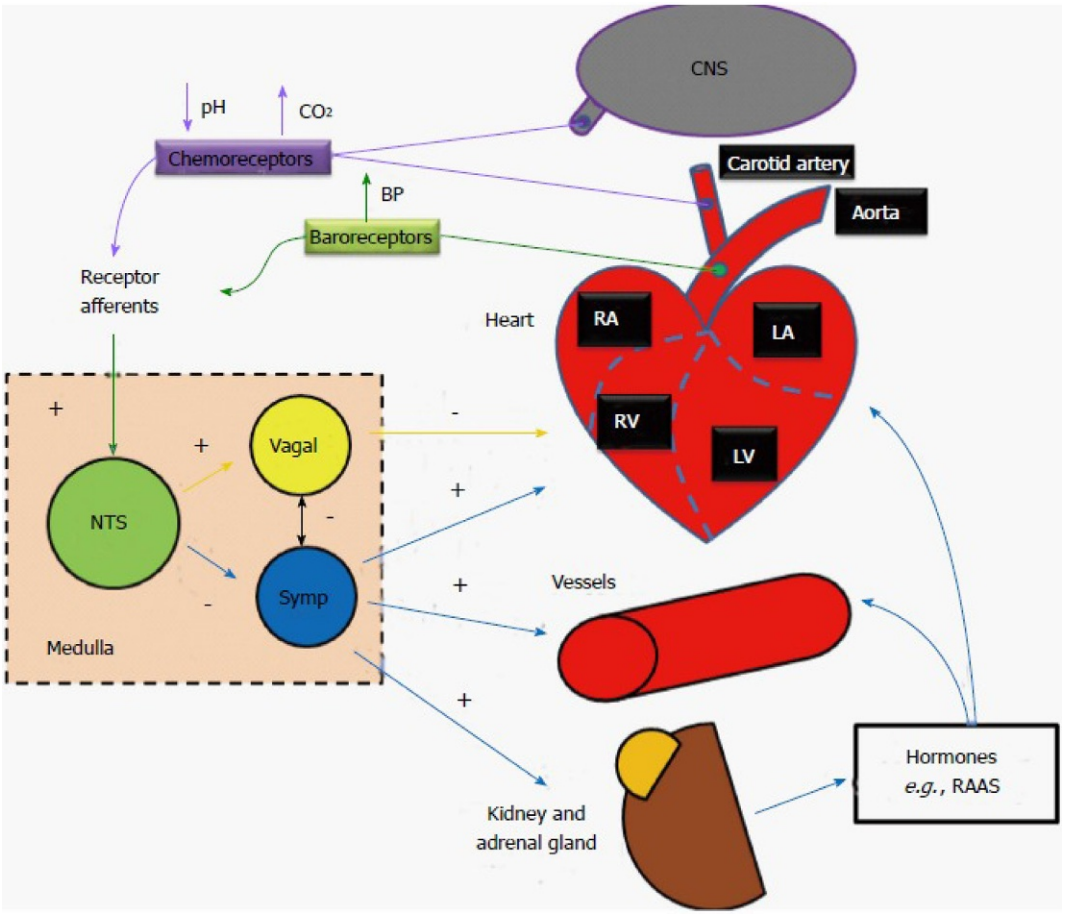

Fig. 1 Schematic of cardiovascular reflexes and their influences on heart and vessel functions. *NTS*, nucleus tractus solitarii; *Symp*, sympathetic; *CNS*, central nervous system; *RAAS*, renin-angiotensin-aldosterone system. From *World Journal of Cardiology* 2015, **7**(4), 204–214. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4404375/figure/F3.

The sinoatrial (SA) and atrioventricular (AV) nodes, and the atria receive dense neural innervation from the PNS and SNS. In contrast, the ventricular myocardium receives dense neural innervation from mostly sympathetic fibers and less from parasympathetic nerve fibers (Levy et al., 1981). It was not until recently that anatomical and functional evidence have highlighted a putative role of parasympathetic neural control on ventricular contractility (Coote, 2013).

Parasympathetic postganglionic nerve fibers release acetylcholine upon activation. Acetylcholine binds to cholinergic M_2 muscarinic receptors expressed on nodal cells (G_i-protein-coupled receptors) which activate the opening of G-protein-coupled inward rectifying potassium channels, causing hyperpolarization (Harvey and Belevych, 2003). This leads to a decrease of the intrinsic firing rate of the SA node, as well as the slowing of impulse conduction through the AV node, thus slowing HR. In addition, activation of the cholinergic M_2 receptor also leads to the inhibition of adenylyl cyclase (AC). This inhibits the formation of cyclic adenosine monophosphate (cAMP), and depending on the effector cardiac tissue, can inhibit the pacemaker channel (funny current) of the SA and AV nodes and decrease calcium channel activity (membrane-bound L-type- and ryanodine receptor-mediated changes in cellular calcium) necessary for cardiac muscle contraction (Harvey and Belevych, 2003; Gordan et al., 2015).

Sympathetic postganglionic nerve fibers are adrenergic and release norepinephrine (NE) to activate β_1- and α_2-, and β_1-, β_2, and β_3 adrenergic receptors expressed in nodal cells and cardiomyocytes, respectively (Harvey and Belevych, 2003; Gordan et al., 2015). However, the effects of NE on cardiac function are mediated primarily by β_1-adrenergic receptor (β_1R) activation and are the predominant receptor subtypes found in healthy mammalian hearts (Kaumann, 1997; Brodde and Michel, 1999). The β_1R is a G_s-protein-coupled receptor and elicits downstream responses via activation of AC, generation of cAMP, and activation of protein kinase A (PKA). Within the cells of the SA node, β_1R-mediated elevation of cAMP activates the pacemaker current (funny current), increasing the steepness of diastolic depolarization, and increasing HR (Difrancesco, 2010). β_1R activation also increases the lusitropic and inotropic effects of cardiomyocytes via heightened activation of PKA and downstream phosphorylation of cardiac cell regulatory proteins. PKA decreases myofilament calcium sensitivity and accelerates cross bridge cycling, leading to enhanced relaxation of cardiomyocytes (positive lusitropic effect). In addition, PKA can interact with sarcoplasmic reticulum (SR) proteins, accelerating the uptake of calcium into the SR to enhance relaxation. This also sequesters greater amounts of calcium within the SR that becomes available for release upon the next contraction (positive inotropic effect). Further contributing to the inotropic effects

of β-adrenergic receptor activation, PKA activates cardiac cell L-type calcium channels, increasing calcium influx during the action potential and activating calcium-mediated calcium release via SR ryanodine receptors, ultimately increasing contraction force of the cardiomyocytes (Najafi et al., 2016). Both the positive lusitropic and inotropic effects of sympathetic stimulation of the heart therefore increase stoke volume.

Recently, the β_3R's role in the heart has been described as "brakes" for the SNS, as they are activated at high catecholamine concentrations, which elicits a negative inotropic effect. It is postulated that this system antagonizes the actions of β_1R and β_2R to buffer sympathetic overstimulation of the heart. β_3R stimulation has been shown to produce nitric oxide (NO), which essentially prevents pressure overload-induced myocardial nitric oxide synthase (NOS) uncoupling (Moens et al., 2010).

In addition to NE, the sympathetic cotransmitter neuropeptide Y (NPY) plays a role in mediating cardiac function; however, its actions are complex and must often be assayed in the presence of β-blockers. NPY is found in the peripheral nerves of the heart, the intracardiac ganglia, endocardium, and myocardium. It is costored with NE and on its own within large dense-cored secretory vesicles of postganglionic neurons, and its release is often associated with elevated levels of sympathetic drive to the heart (Horackova et al., 1999; Dalsgaard et al., 1986; Kennedy et al., 1998; Malmstrom, 2002). NPY interacts with its respective Y1 (Y1R) and Y2 (Y2R) receptors to elicit its effects on heart function, and recently the Y3 receptor (Y3R) has also been suggested to contribute to NPY's actions on the heart (Balasubramaniam, 1997; Smith-White et al., 2002; Protas and Robinson, 1999). Interestingly, NPY can modify prejunctional sympathetic and vagal neurotransmitter release, contractility of the ventricles, and vascular resistance of coronary blood vessels (Herring et al., 2008). NPY can act on presynaptic Y2Rs to inhibit its own release, as well as on Y2Rs of prejunctional vagal cholinergic neurons to modify acetylcholine release and reduce the magnitude of vagal bradycardia (Malmstrom, 2002; Herring, 2015; Awad et al., 1991; Potter et al., 1989). In addition to its cross-talk with vagal afferents, NPY can also act on Y1Rs and Y2Rs of the ventricular myocytes to modify myocardial contractility and has recently been implicated in contributing to arrhythmias occurring with heart disease (Herring, 2015; Awad et al., 1991). NPY has been shown to modify transient outward potassium currents (Heredia et al., 2002), L-type calcium currents (Bryant and Hart, 1996; Millar et al., 1991), and pacemaker currents within the right ventricles (Qu et al., 2000), as well as increase cell shortening and calcium transients of venticular myocytes (Heredia Mdel et al., 2005).

Based on the dynamic blood flow requirements of the body, the ANS modifies HR and stroke volume to increase or decrease CO accordingly. Under basal conditions, there is still a need for active autonomic drive to the heart and a balance between parasympathetic and sympathetic discharge to maintain resting HR and CO. Due to the intrinsic spontaneity of the SA node, HR would be 90–100 beats/min in the absence of extrinsic neural influences. Thus, elevated vagal activation maintains resting HR 30% lower than the intrinsic rate of the heart and moderate tonic sympathetic activation maintains CO 30% higher than in the absence of sympathetic discharge (Thomas, 2011). The required changes in CO can therefore be governed by the amount of vagal and sympathetic withdrawal and/or activation.

Neurological Regulation of Arteries and Arterioles

Due to the high resistance offered by the arteriolar microcirculation, blood moves through the arterial system under relatively high pressure and velocity. As such, the arterial side of the cardiovascular system is considered a pressure reservoir. Sympathetic nerves servicing arteriolar beds provide the chief extrinsic input regulating resistance (and blood flow) to organs. Postganglionic sympathetic nerves are localized to the adventitial-medial border of most arteries, arterioles, and veins throughout the body (Thomas, 2011). Postganglionic sympathetic neurotransmitters are released based on neuronal firing frequency and amplitude (i.e., sympathetic nerve activity, SNA), with increases in SNA associated with potent and prolonged vasoconstriction. Like the autonomic nerves that innervate the heart, the sympathetic nerves innervating the vasculature also display ongoing tonic activity. This background of SNA sets baseline tone (i.e., level of vasoconstriction) in resistance vessels. As such, modulating sympathetic outflow via increased SNA or sympathetic withdrawal promotes rapid changes in contractile state thereby increasing or decreasing resistance as needed. In the majority of peripheral vascular beds (particularly skeletal muscle), sympathetic neurotransmission is adrenergic (via the catecholamine NE), purinergic (via adenosine triphosphate, ATP), and peptidergic (via NPY).

Isolated in situ and in vivo stimulation of sympathetic nerves has demonstrated the corelease of NE, ATP, and NPY from axon terminals (Morris, 1999; Lundberg et al., 1990). However, NE, ATP, and NPY have different potencies for vasoconstriction and are not released in the same relative concentrations. For example NPY's potent vasoconstrictor effects are realized at concentrations $\sim$1000 $\times$ lower than similar responses elicited by NE and ATP. The release of each transmitter is dependent on the intensity and the pattern of SNA, where ATP and NE concentrations peak under relatively low SNA and NPY concentration peaks at much higher SNA intensities (Hirsch and Zukowska, 2012). Although our knowledge of SNS-mediated vasoconstriction has expanded in the past decades, the roles of NPY and ATP in skeletal muscle blood flow regulation remain to be characterized to the level of NE.

Adrenergic Control

SNS-mediated adrenergic regulation of small arteries and arterioles occurs when NE is released from sympathetic neurons to interact with adrenergic receptors located on the abluminal surface of vascular smooth muscle. Currently, at least nine adrenergic receptor subtypes have been cloned (α_{1A}, α_{1B}, α_{1D}, $\alpha_{2A/D}$, α_{2B}, α_{2C}, β_1, β_2, and β_3); however, their functional roles are yet to be fully described (Guimaraes and Moura, 2001). In terms of SNS-mediated adrenergic vasoconstriction, postjunctional α_1R and α_2-adrenergic

receptors (α_2R) predominate, albeit, the specific receptor subtypes that are involved vary among different vascular beds (Thomas, 2011). Currently, α_{1A}-, α_{1D}-, $\alpha_{2A/D}$-, and α_{2B}-receptor subtypes are most commonly associated with vascular regulation. The α_1R belongs to the $G_{q/11}$ family of heterotrimeric G proteins. Upon activation, β-isoforms of phospholipase C are stimulated, which leads to an IP3-induced release of intracellular calcium (Ca^{2+}) and activation of protein kinase C (PKC; to phosphorylate and activate a downstream cascade of protein signaling). This release of Ca^{2+} binds onto calmodulin, which activates myosin light chain kinase to phosphorylate myosin heads, increasing myosin ATPase activity and promoting contraction of vascular smooth muscle. In contrast, postjunctional α_2Rs belong to the G_i family of the heterotrimeric G proteins. Activation of this receptor promotes AC inhibition, ultimately attenuating cAMP production from ATP. The drop in cAMP increases the activity of myosin light chain kinase, thereby phosphorylating myosin light chain and resulting in overall vasoconstriction. In skeletal muscle arterioles, α_1R- and α_2R-mediated constriction occurs with greatest potency in lager diameter proximal arterioles, suggesting that they are primarily involved in redistributing bulk blood flow to tissues (Al-Khazraji et al., 2015; Jackson et al., 2004, 2010).

Most small arteries and arterioles also express β-adrenergic receptor subtypes (β_1, β_2, and β_3), with the β_2-receptor (β_2R) subtype being the best described. Specific activation of vascular β-adrenergic receptors promotes vasodilation; however, since β-adrenergic receptors are most commonly exposed to the lumen of vessels, they are activated by interactions with circulating catecholamines (see section "Neurohumoral Regulation of the Circulation" below) as opposed to direct activation by the SNS. Of note, although β_3-adrenergic receptors (β_3R) belong to the superfamily of G-protein-coupled receptors (like the β_1R and β_2R), β_3R can interchangeably couple to both Gs and Gi without the requisite for receptor phosphorylation. β_3R activation promotes vasodilation via the production of NO and occurs at catecholamine concentrations that are higher than those required for β_1R and β_2R activation (Conti et al., 2013). Taken together, the β_3R likely provides protective "dilatory escape" under hyper-adrenergic conditions.

Purinergic Control

Sympathetic neurons release ATP to act on purinergic P2X1 receptors (P2X1R) located on the abluminal surface of arteries and arterioles. The P2X1R is an inotropic receptor and a member of the ligand-gated ion channel P2X receptor family (Valera et al., 1994). Upon binding of ATP, the P2X1R changes conformation and allows for Ca^{2+} to enter the cell, depolarizing smooth muscle cells and resulting in overall vasoconstriction of the arteriole. The role of ATP-mediated arteriolar constriction in microvascular networks was recently evaluated and, in contrast to adrenergic control, ATP elicits its greatest constrictor effects in small diameter, distal arterioles, suggesting a role for the P2X1R in red blood cell/hematocrit distribution to capillary beds (Al-Khazraji et al., 2015).

Peptidergic Control

NPY is a vasoconstrictive neuropeptide released from neuronal varicosities that act on NPY receptors located abluminally on vascular smooth muscle and prejuctionally on axon terminals. NPY Y1Rs are abundant throughout mammalian systems and peripherally located Y1Rs are expressed mainly in arteries, arterioles, and veins, where they are associated with vasoconstriction and potentiation of other vasoconstrictors of neurogenic origin. The NPY Y1R is a G-protein-coupled receptor located mainly postjunctionally on vascular smooth muscle cells. When activated, there is inhibition of AC and via cAMP/Phospholipase C coupling, which elicits increases in intracellular Ca^{2+} leading to potent and long-lasting vasoconstriction.

Neuronally released NPY can be modified by proteolytic processing via enzymes that reside throughout mammalian systems. The exopeptidase dipeptidyl peptidase IV (DPPIV) is a membrane-bound metalloprotease, which cleaves the Tyr-Pro dipeptide (in the penultimate position) from the N-terminus of the complete 36 amino acid NPY_{1-36}. The resulting fragment, NPY_{3-36}, has a low affinity for the Y1R, but favorably binds to the Y2R (Mentlein and Roos, 1996). In blood vessels, DPPIV is abundant on endothelial cells and a soluble form of DPPIV is found in plasma. Another proteolytic enzyme that targets NPY is Aminopeptidase P (APP). APP cleaves the N-terminal Tyr residue from NPY_{1-36} resulting in the NPY_{2-36} fragment that has low affinity for Y1R, but high affinity for Y2R. APP is present on neurons, smooth muscle, and endothelial cells; furthermore, a soluble form is present in the circulation.

The NPY Y2R was first cloned in 1995 (Gerald et al., 1995). Current data indicates that Y2Rs are located mostly presynaptically; however, past research has shown that postjunctional Y2Rs can exist at low levels in pig spleen, causing vasoconstriction (Modin et al., 1991). Such postjunctional effects have not yet been observed in other tissues; as such, Y2R activation is generally associated with inhibition of neurotransmitter release. This effect is manifested though inhibition of AC and voltage-dependent Ca^{2+} channels via Y2R linked G proteins. Research has shown that endogenous, neuronally released NPY activates prejunctional Y2R to exert autoinhibition of its own release (Malmstrom et al., 2002a,b). This effect has also been exhibited using exogenous NPY or its truncated analogs (Malmstrom et al., 2002b). Cleavage of NPY at the ultimate N-terminus residue Tyr_1 or penultimate Tyr_1-Pro_2 residues results in NPY analogs (NPY_{2-36} and NPY_{3-36}, respectively), these analogs are equipotent to NPY with respect to Y2R binding affinity (Beck-Sickinger and Jung, 1995).

Ligand Potentiation

As stated above, sympathetic postganglionic neurons release NE, ATP, and NPY, with release of these contents being more prevalent at high nerve stimulation frequencies (Lundberg et al., 1994). This cocktail of neurochemical messengers enables a number of

biological effects and interactions (i.e., slow, intermediary, and quick sympathetic signaling) (Lundberg, 1996). For example, NPY produces a slow acting, potent, and persistent increase in vascular tone. In contrast, NE-induced vasoconstriction occurs more quickly and disappears at a similar rate. Moreover, ATP-induced vasoconstriction has been shown to be immediate and very short acting. It has been postulated that the duration of each transmitter's effects relies on each one's unique mode of deactivation/removal. For example, NE's vascular effects are removed quickly (via NE reuptake) compared to the prolonged duration of NPY-induced vasoconstriction that is eliminated by enzymatic degradation of "free" NPY (Lundberg, 1996).

In several early investigations, NPY was shown to potentiate α-adrenergic vasoconstriction in vivo (Dahlof et al., 1985; Revington and McCloskey, 1988; Linder et al., 1996; Jackson et al., 2005) and in vitro (Edvinsson et al., 1984; Ekblad et al., 1984; Glover, 1985). The synergistic interaction between NPY and NE is receptor-mediated, as NPY also enhanced the vasoconstrictive response to sympathetic nerve stimulation and phenylephrine (specific α1R agonist)-induced vasoconstriction (Dahlof et al., 1985). The interactive effects are not a result of general changes in the reactivity of smooth muscle cell to increased Ca^{2+} concentration, because in some tissues/preparations NPY does not modify the effects of other neurotransmitters (Edvinsson et al., 1984). Thus, the interactive effects should not only be tissue specific, but these effects must also be due to consequent modification of specific receptor properties and/or second messengers.

Neurological Regulation of the Venous System

Once blood has made its way out of the heart, through the arteries, and into the microcirculation it is collected by the venous system, where it has lost most of the energy (i.e., pressure) originally imparted on it by the heart. As such, venous pressure represents the lowest pressure in the circulation, and since approximately 70% of the total blood volume is stored by the venous system (Rothe, 1983), it is regarded as a capacitor or volume reservoir. Based on this ideology, any alteration in venous capacity has profound effects on venous return and CO.

Similar to arteries and arterioles, the SNS is the primary regulator of venomotor tone (Mellander and Lewis, 1963; Tabrizchi and Pang, 1992), with sympathetic nerve fibers directly innervating vascular smooth muscle cells of the venous system. However, in contrast to arterioles, increased SNA to small veins and venules results in alterations in venous compliance, thus altering venous volume (capacitance) with little impact on systemic resistance. Notably, compared to arteries and arterioles, there is a relative dearth of literature describing neurogenic regulation of the venous system.

Adrenergic Control

SNS influence on the venous circulation occurs primarily through the α-adrenoceptors that are found on vascular smooth muscle cells (De Mey and Vanhoutte, 1981). Like arterioles, α_1 and α_2 adrenoceptors expressed on the smooth muscle of the vasculature induce venoconstriction upon activation (van Brummelen et al., 1986), which increases central venous pressure and venous return to the heart (de Souza Rossignoli et al., 2015). Overall, both α_1R and α_2R are involved in regulation of venous resistance, but magnitude of responses varies among vascular beds and between species.

Evidence of β_2R involvement in venous circulation is relatively scant; however, stimulation of β_2Rs results in venodilation (Schindler et al., 2004). For example, studies using isoproterenol (β_2R agonist) observed a venodilation in the veins of the hand (Schindler et al., 2004; Harada et al., 1996), but only in conditions of elevated venous tone induced by vasoactive drugs. Furthermore, a study which blocked β_2Rs with propranolol in the splanchnic vasculature showed evidence of β-adrenergic involvement in venous circulation (Mastai et al., 1987). Overall, there is evidence that β-adrenoceptors are involved in regulation of the venous circulation, but only under experimental conditions where venoconstriction is induced or sympathetic tone is impaired. It has been postulated that stimulation of venous β_2Rs is most likely due to interactions with circulating catecholamines (described below: section "Neurohumoral Control"), instead of direct SNS stimulation (Thomas, 2011).

Peptidergic Control

Like arterioles, the SNS also exhibits peptidergic control in the venous system, as NPY is colocalized with NE in sympathetic neurons servicing most venous and venular networks (Ekblad et al., 1984). As well, NPY containing nerve fibers have been shown to innervate hepatic veins in several species (Pongor et al., 2010). In support, a study by Luu et al. reported NPY-induced constriction in the saphenous vein of humans (Luu et al., 1992) illustrating that, in addition to α_1- and α_2R control, sympathetic activity can modify venous tone through NPY Y1R activation.

Modifiers of Cardiovascular Neurological Regulation

Neurogenic regulation of the circulation is modulated by reflex alterations in autonomic activity on a moment-to-moment basis responsible for maintaining CO, mean arterial BP, and adequate tissue perfusion. Importantly, the greatest contributor to the central neurological regulation of the circulation is the modulation of SNA due to its potent effects on peripheral vascular

resistance, that is vasoconstrictive, and thus the ability to impact changes in BP. The most fascinating phenomenon highlighting the activity of reflex neurogenic control of the circulation is the profound cardiovascular and hemodynamic modifications encountered from rest to dynamic exercise. Such reflexes exist to meet the increased metabolic demand of active skeletal muscle. In addition to exercise, postural changes, temperature, altitude, and microgravity have also been associated with cardiovascular reflex activation (Mitchell and Victor, 1996). In light of exercise, precise cardiovascular and hemodynamic tuning occurs in an intensity-dependent manner via the integrated nature of several neural mechanisms in place to support skeletal muscle perfusion, sufficient oxygen (O_2) delivery, and waste removal over time. From as early as the late 1800's, the notion of descending signals from higher brain centers and the presence of neural afferent feedback from skeletal muscle, the carotid artery, and aortic arch, and pulmonary circulation have been studied in depth and demonstrate their involvement in effecting cardiovascular and hemodynamic responses to exercise (Zuntz and Geppert, 1886; Fadel, 2015; Krogh and Lindhard, 1913; Johansson, 1895). Such important neural mechanisms have been primarily identified as: (1) central command (feedforward mechanism associated with perception of exercise), (2) the exercise pressor reflex (encompasses feedback mechano- and chemoreflexes), (3) arterial baroreflex (negative feedback from carotid sinus and aortic arch), and (4) cardiopulmonary baroreflex (negative feedback from the chambers of the heart and pulmonary circulation).

Together, the integrated compliment of these cardiovascular control mechanisms has been best described by Mitchell in 1990, where responses to exercise are dependent upon the "type of exercise (static or dynamic), the intensity of exercise, the time after onset of exercise (immediate, steady state, exhaustion, etc.), and the effectiveness of blood flow in meeting the metabolic needs of the contracting muscles (Mitchell, 1990)." However, present-day knowledge lends itself to the numerous human and animal studies allowing for the discrete identification of each mechanism in modulating cardiovascular and hemodynamic parameters. The neural modulation reflexes are discussed in further detail below.

Central Command

It is often stated that central command encompasses the descending neural signals from higher brain centers that can influence sympathetic- and parasympathetic-mediated cardiovascular responses to exercise even before the onset of activity, that is vagal withdrawal and elevated BP (Williamson et al., 1995; Nobrega and Araujo, 1993). The original concept of higher brain centers was introduced when Zuntz and Zeppert suggested a feed-forward "central control mechanism" responsible for simultaneous activation of respiratory centers and locomotor pathways that caused the increased breath rate at the onset of exercise in conjunction with voluntary activity (Zuntz and Geppert, 1886; Fadel, 2015). Building on this hypothesis, Nobel Prize Winners Krogh and Lindhard coined "cortical irradiation" to describe the signals responsible for activation of not only respiratory neural circuits but also neural reflex control of the circulation during the initial stages of exercise (Fadel, 2015; Krogh and Lindhard, 1913; Paterson, 2014). Now known as "central command," this concept is commonly defined as a "feedforward mechanism" involving parallel descending neural signals from motor centers in the brain that activate somatomotor, respiratory, and cardiovascular centers (Goodwin et al., 1972).

In effort to identify these "higher brain centers" associated with cardiovascular control, animal and human studies have attempted to isolate the specific role of "central command" amidst various intricate and often redundant central and peripheral neural contributions. Both animal and human studies have demonstrated the role for the subthalamic locomotor region of the posterior hypothalamus and mesencephalic locomotor region as key structures in cardiorespiratory neurocircuits (Paterson, 2014; Pitts et al., 1941; Smith et al., 1960; Eldridge et al., 1981). In addition, accumulating evidence has demonstrated the periaqueductal gray as an important structure associated with the neural circuitry involved in "central command" as it is activated with exercise and is an area associated with cardiorespiratory control during stress and emotional states (Paterson, 2014; Bandler and Shipley, 1994; Basnayake et al., 2011, 2012; Dampney et al., 2013; Green et al., 2007; Green and Paterson, 2008). Albeit a lack of direct neural measurements from these brain regions in humans, muscle SNA, HR, and arterial BP changes associated with "central command" input have been identified in studies using neuromuscular blockade, tendon stimulation, and electrically stimulated muscle contraction (Goodwin et al., 1972; Victor et al., 1995; Secher et al., 1988; Leonard et al., 1985; Boulton et al., 2016). The magnitude of the "central command" on cardiovascular and hemodynamic variables have also been demonstrated by altering one's perception of effort or exertion, thus removing the direct effect of workload on cardiovascular responses to exercise (Mitchell, 1990; Borg, 1973; Williamson et al., 2001; Thornton et al., 2001). It must be noted that other factors may also modify cardiovascular responses to perception of effort, such as somatosensory signals from muscle and cardiorespiratory changes (Amann et al., 2008), neurocognitive factors (environment, trained or experienced cues, anticipation of work end point) (Faulkner and Easton, 2008), general discomfort (pain, heat exhaustion) (Cabanac, 2006), and attitude/emotion toward activity performance (Morgan, 1994). To further complicate identification of the cardiovascular and hemodynamic changes elicited by "central command," the traditional feed-forward component of the response may also be accompanied by a feedback component (Fadel, 2015; Amann et al., 2008; Williamson et al., 2006; Williamson, 2010). Indeed, cardiovascular and hemodynamic changes during exercise may be a result of input signals modifying the effects of "central command." In theory, this would correct any "error" in output activity from "central command" between perceived and actual level of effort necessary to meet and maintain cardiovascular and hemodynamic parameters sufficient to the level of activity (Williamson, 2010). Further studies are needed to ascertain the integration of feed-forward and feedback function of "central command," as well as identify the involvement of sensory signals modifying the neural control of cardiovascular and hemodynamic responses to exercise.

Exercise Pressor Reflex

Pioneering work by Alam and Smirk established the involvement of neural afferent feedback from skeletal muscle that evoked adjustments of cardiovascular and hemodynamic variables in response to exercise (Alam and Smirk, 1937, 1938). Two main feedback reflexes originating within skeletal muscle have been identified and are known as the mechanoreflex and metaboreflex (i.e., chemoreflex), which together compose the exercise pressor reflex. These afferent components relay both mechanical and chemical sensory information to the cardiovascular centers within the medulla of the brainstem, which becomes integrated within corresponding efferent signals of central command to modify BP, HR, and muscle SNA in response to exercise (Fadel, 2015; Mitchell, 1990; Rowell and O'Leary, 1990; Smith et al., 2006; Crisafulli et al., 2015; Kaufman et al., 1984; McCloskey and Mitchell, 1972; Potts, 2001).

The afferent fibers of the muscle mechanoreflex and metaboreflex are primarily comprised of thin slow-conducting unmyelinated type III and IV neurons, respectively. Their receptors are activated by intramuscular mechanical deformation via changes in pressure or stretch, and by skeletal muscle metabolites produced during muscle contraction. The rapid onset and recovery of group III afferent activity corresponds with mechanoreflex function and the initial event of muscle contraction at exercise onset. In contrast, group IV afferent activity increases gradually after exercise onset, characterizing the function of the metaboreflex with the production and accumulation of metabolites by contracting skeletal muscle. Of note, the functional classification of group III and IV afferents is indefinite as both fiber types can act dually as mechano- and metaboreceptors. In addition, the responsiveness of both fiber types can be sensitized by changes in local metabolites (Fadel, 2015; Rowell and O'Leary, 1990; Crisafulli et al., 2015; Kaufman et al., 1983, 1988; Cui et al., 2008). Regardless of fiber type activation, afferent feedback from skeletal muscle is essential for the increase in BP associated with the exercise pressor reflex and for modulation of efferent sympathetic and parasympathetic activity mediated by central command at the onset of exercise (Rowell and O'Leary, 1990; McCloskey and Mitchell, 1972; Strange et al., 1990; Nobrega et al., 2014).

The array of evidence defining the muscle mechanoreflex and its role in cardiovascular modification with exercise is rather limited, and often can only be characterized at the onset of exercise with initial skeletal muscle contraction (Kaufman et al., 1983; Cui et al., 2006; Gladwell and Coote, 2002; Gladwell et al., 2005). This is simply due to the difficulties encountered upon isolating mechanoreceptor activity in the midst of central command and metaboreflex activation accompanying skeletal muscle contraction. In addition, skeletal muscle metabolites can sensitize mechanoreceptor afferents, further complicating the identification of discrete effects of the mechanoreflex compared to the metaboreflex during muscle contraction. The metaboreflex, however, has been identified to evoke significant neural effects on cardiovascular and hemodynamic variables in response to exercise, specifically by postcontraction occlusion in effort to maintain interstitial levels of metabolites after skeletal muscle contraction has ceased. This isolates the metaboreflex from both the neural effects of central command and the mechanoreflex once physical activity has ceased. Using this technique, the metaboreflex has been identified through its ability to increase BP and muscle SNA (i.e., increased systemic vascular resistance) during moderate and intense exercise (Rowell and O'Leary, 1990; Crisafulli et al., 2015; Strange et al., 1993; Piepoli et al., 1995; Mark et al., 1985; Amann et al., 2010, 2011). Although initially observed as insignificant, the effects of the metaboreflex on HR and CO are also apparent. Using postexercise ischemia does not implicate the metaboreflex in HR changes; however, initiating muscle ischemia during exercise can indeed elicit a HR response (Crisafulli et al., 2011; O'Leary, 1993, 1996). In addition to HR, there is evidence supporting the metaboreflex as a mediator of cardiac contractility, preload, and stroke volume during exercise (Crisafulli et al., 2003, 2011, 2015; Nobrega et al., 2014; Bastos et al., 2000). Reports have also identified a role of the metaboreflex in modulating venoconstriction and splanchnic vasoconstriction to increase venous return and right ventricular filling pressure. This is imperative during exercise as adequate diastolic function is important for the metaboreflex modifications of stroke volume (Crisafulli et al., 2009, 2015; Piepoli et al., 2008).

Multiple metabolic/chemical substances have been associated with activation of the metaboreceptors and the reciprocal changes in cardiovascular, hemodynamic, and cardiac function have been associated with exercise. There is no consensus on a particular substance per se, but rather an accumulation of evidence for multiple contributors to this response. Several metabolites produced by muscle contraction have been identified in contributing to the metaboreflex, such as lactic acid, potassium, bradykinin, arachidonic acid, deprotonated phosphate, ATP, adenosine, and hydrogen ion (Mitchell, 1990; Smith et al., 2006; Murphy et al., 2011; Mortensen et al., 2013; Kaufman, 2012). Among such studies there are conclusions that both oppose and support the involvement of certain metabolites involved, and thus it is likely that the metaboreflex can be activated by a number of interstitial substances.

Arterial Baroreceptors

The arterial baroreflex is a crucial negative feedback mechanism that serves to rapidly adjust autonomic activity in effort to maintain arterial BP on a moment-to-moment basis. The arterial baroreceptors are sensitive to stretch and are located in the carotid sinus and the aortic arch (Sheehan et al., 1941; Sagawa, 1983). A branch of the glossopharyngeal nerve and small vagal branches carry baroreflex afferents from the carotid and aortic baroreceptors, respectively, to the nucleus tract solitarus in the medulla oblongata of the brain stem (Aicher and Randich, 1990). When changes in pressure are sensed via baroreceptor stretch or compression, activated afferents reflexively elicit alterations in sympathetic and parasympathetic nerve activity to the heart and blood vessels, modifying CO and peripheral vascular resistance to maintain arterial BP at its operating pressure point (Melcher and Donald, 1981; Potts et al., 1993; Raven et al., 1997, 2006; Fadel et al., 2003). Under basal conditions, a rise in BP increases baroreceptor activity leading to

decreased SNA, activation of parasympathetic nerve activity, and corresponding decreases in vascular resistance and HR. Conversely, a fall in BP decreases baroreceptor activity, leading to increased SNA, inhibition of parasympathetic nerve activity, and increased vascular resistance and HR. Under conditions of exercise however, a shift in the operating pressure point of the baroreflex occurs as BP is regulated within a higher range and with the same level of reflex sensitivity, continuing to rise with increasing exercise intensity (Potts et al., 1993; Raven et al., 2006; Fadel et al., 2003; Papelier et al., 1994; Norton, 1985; Ogoh et al., 2003). Studies have also demonstrated that such "resetting" of the arterial baroreflex during exercise can be influenced by central command, the exercise pressor reflex, and cardiopulmonary baroreflex (Ogoh et al., 2002, 2006, 2007; Smith et al., 2003; Fisher et al., 2008; Drew et al., 2008a,b; Potts and Mitchell, 1998). With exercise, blood flow is directed to active skeletal muscle by means of metabolic vasodilation/active hyperaemia, which in theory would promote a drop in BP. Thus the baroreflex functions to buffer these drops in pressure via increased SNA to generate greater vascular resistance. Of importance, changes in CO and vascular conductance that are mediated by baroreflex modulation of BP are primarily a consequence of its ability to alter total vascular resistance at rest and during exercise. Therefore, the ability of the baroreflex to regulate SNA during exercise provides a means of tuning skeletal muscle perfusion while maintaining BP, and it also buffers the BP effects of central command and exercise pressor reflex (Crisafulli et al., 2015).

Cardiopulmonary Baroreflex

The cardiopulmonary baroreceptors are low-pressure sensory receptors sensitive to mechanical stretch that respond to changes in central venous volume and pressure, and left atrial pressure. They reside in the cardiac chambers of the heart, the great veins, and within the pulmonary circulation. Despite the limited availability of literature, the cardiopulmonary baroreflex can modulate BP, vagal afferent nerve firing, muscle SNA, and is involved in the resetting of the arterial baroreflex operating point during exercise (Fadel, 2015; Fadel and Raven, 2010; Oren et al., 1993; Desai et al., 1997; Karim et al., 1972; Hainsworth, 1991). The most seminal findings identifying a role for the cardiopulmonary baroreflex in modulating muscle SNA during exercise come from the studies of Ray et al. (1993) and Saito et al. (1985), where humans performed one-legged kicking exercise in different postures in effort to modify cardiopulmonary load. Muscle SNA decreased significantly when one-legged kicking was performed in the upright posture and central venous pressure was increased due to greater venous return. In contrast, no change in muscle SNA was observed from baseline when exercise was performed in the supine position, as no change in central venous pressure was induced. In a separate study, light-intensity cycling in the upright position also caused a decrease in muscle SNA. Together, these studies concluded that increasing central blood volume via the muscle pump activates cardiorespiratory baroreflexes and decreases muscle SNA (Fadel, 2015; Fadel and Raven, 2010). With increasing intensity of exercise however, a rise in muscle SNA would correspond with the activated skeletal muscle afferents associated with the exercise pressor reflex and the change in baroreflex operating point of modulating BP.

In addition to increasing vagal drive and decreasing SNA with increased venous pressure and volume, the cardiopulmonary baroreceptors can decrease SNA to the kidneys and vasopressin release from the pituitary. This causes a decrease in renal sodium and water reabsorption, increasing urine production and decreasing blood volume. Modification of blood volume provides another mechanism by which the cardiopulmonary baroreflex can affect BP and CO (Thomas, 2011).

Sympatholysis

Sympatholysis describes the intrinsic ability of the vasculature to overcome the vasoconstrictive effects of elevated SNA accompanying muscle contraction (i.e., exercise), whereby the vasculature maintains the ability to increase blood flow via vasodilation despite the presence of heightened sympathetic drive for vasoconstriction (Buckwalter and Clifford, 2001; Remensnyder et al., 1962). In contracting muscle, some level of sympathetic vascular tone must be conserved since the maintenance of BP is a key component of baroreflex regulation (Joyner and Casey, 2015). Thus, sympatholysis is an optimal mechanism for maintaining blood flow to active skeletal muscle, demonstrating increased effectiveness at higher intensities of exercise (Buckwalter et al., 2001; Ruble et al., 2002; Tschakovsky et al., 2002). The production of vasoactive metabolites has been suggested as a potential mechanism for functional sympatholysis during muscle contraction. Substances such as NO (Thomas and Victor, 1998; Sander et al., 2000; Chavoshan et al., 2002), adenosine, prostacyclin (Quayle and Standen, 1994; Nelson and Quayle, 1995; Keller et al., 2004), and interstitial hydrogen (Mcgillivray-Anderson and Faber, 1990) have been identified as potential sympatholytics as they have been shown to reduce NE release from sympathetic nerves, or inhibit vasoconstriction caused by sympathetic nerve stimulation or αR agonists. Most recently, ATP has received much attention for its ability to alter sympathetically mediated increases in vascular resistance, and is also favored as a key player in exercise hyperaemia. It has also been shown to alter αR sensitivity, thus decreasing the vasoconstrictor effectiveness of sympathetic activation (Mortensen et al., 2009) (**Fig. 2**).

Neurohumoral Regulation of the Circulation

Direct SNS innervation of the vasculature will induce a rapid response at the vascular level, but the SNS can also upregulate humoral responses which have a more prolonged effect on the vasculature. The two main neurohumoral systems are (1) the sympathoadrenal system and (2) the renin-angiotensin-aldosterone system (RAAS).

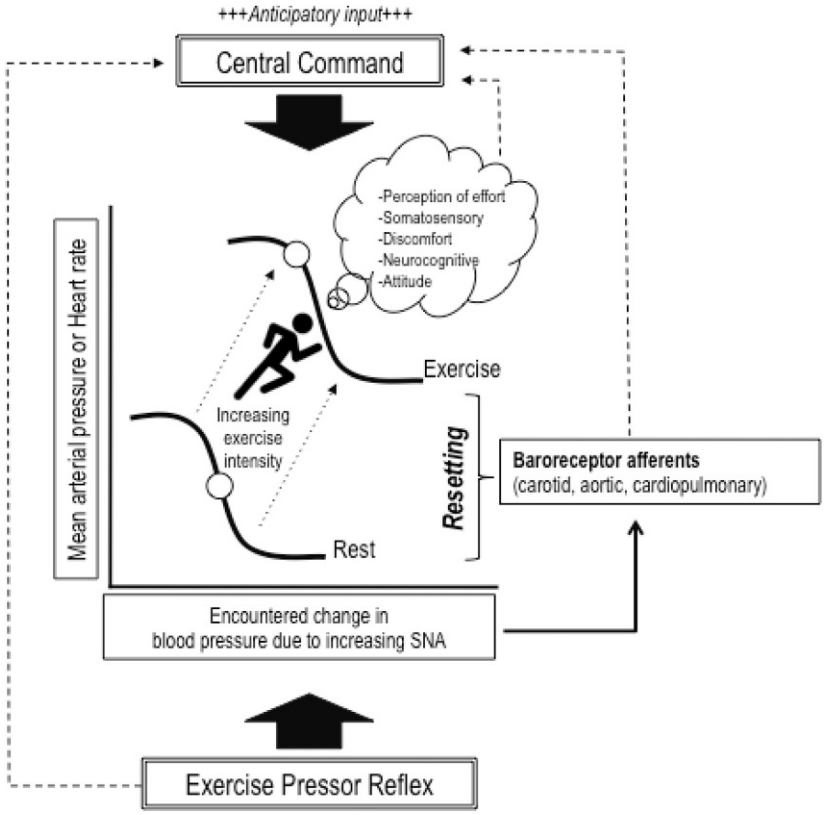

Fig. 2 The integration of multiple cardiovascular reflex components modifies baroreceptor resetting of operating blood pressure from rest to exercising conditions. Under resting conditions, the baroreflex negative feedback loop functions within an operating blood pressure range (curved tracing). Any encountered changes in blood pressure sensed by the baroreceptors reflexively modify sympathetic and parasympathetic outflow to the heart and blood vessels in an effort to maintain blood pressure (and heart rate) at a basal operating point *(white circle)*. Under exercising conditions, a shift in the operating pressure point of the baroreflex occurs as BP (and heart rate) is regulated within a higher range, continuing to rise with increasing exercise intensity. The "resetting" phenomenon of the arterial baroreflex is mediated by central command (higher cardiovascular brain centers), which continues to receive afferent information from the exercise pressor reflex, the cardiopulmonary baroreflex, and determinants of psychological perception, thus continuously modifying the "resetting" of the operating pressure point and range of baroreceptor activity. As the need for skeletal muscle perfusion increases, and arterial vasodilation and hyperemia increases, the baroreflex buffers the potential drop in blood pressure by increasing sympathetic nerve activity and promoting greater vascular resistance. Adapted from Raven, P. B., Fadel, P. J. and Ogoh, S. (2006). Arterial baroreflex resetting during exercise: a current perspective. *Experimental Physiology* **91**, 37–49.

Sympathoadrenal System

Preganglionic cholinergic sympathetic nerve fibers release acetylcholine to activate chromaffin cells of the adrenal medulla of the kidney. Chromaffin cell activation promotes the release of catecholamines, epinephrine (E) and NE, into the blood stream where they circulate as hormones. Typically, more E (80%) than NE (20%) is released and consequently the effects of circulating E have been a primary area of study (Thomas, 2011). The original studies in 1960, which have since been validated, demonstrate vasodilation in conditions of elevated circulating E (Richardson et al., 1967; Weisbrod et al., 2001).

Epinephrine preferentially binds to β_2R, a G_S-protein-coupled adrenoceptor predominately found on vascular smooth muscle cells, upregulating AC leading to the formation of cAMP which activates PKA. PKA phosphorylates, and inactivates, myosin light chain kinase leading to vascular smooth muscle relaxation. There is also evidence of β_2R on endothelial cells which play a role in NO-mediated vasodilation (Guimaraes and Moura, 2001). However, the involvement of NO in β_2R-mediated dilation is debated as most studies used in vitro preconstricted models for evidence. Epinephrine also has affinity for αRs and can induce vasoconstriction, but this is only observed at supraphysiological levels of E (Thomas, 2011). In contrast, NE targets αRs to induce vasoconstriction, but the effects are more potent with NE as a neurotransmitter versus hormone. However, the combined release of E and NE suggests that E vasodilation offsets NE vasoconstriction to prevent BP overshoot during elevated SNA (Ziegler et al., 2012).

Renin-Angiotensin-Aldosterone System

Sympathetic fibers innervate the juxtaglomerular cells, specialized smooth muscle cells of the afferent arterioles of the kidney, and activate β_1Rs resulting in renin release into the circulation (Thomas, 2011). Renin converts circulating plasma angiotensinogen, produced by the liver, to angiotensin I, then angiotensin converting enzyme, located on endothelial cells of the pulmonary

capillaries and renal arterioles, converts angiotensin I to angiotensin II (AII). AII binds to a heterogeneous population of AII receptors, identified as AT1 and AT2. AT1 receptors are well understood and play a role in vasoconstriction, aldosterone (ALDO) release, increase in SNS transmission, and cellular growth (Dinh et al., 2001). AT1 is a G-protein-coupled receptor and upregulates five different transduction mechanisms, involving activation of phospholipase A_2, phospholipase C, phospholipase D, L-type calcium channels, and inhibition of adenylate cyclase. Activated phospholipase C, D, and L-type calcium channels lead to vasoconstriction, while activated phospholipase A_2 is associated with production of arachidonic acid and induces a vasodilation which is generally overpowered by the vasoconstriction and unnoticed overall (Dinh et al., 2001). In contrast, the AT2 receptor has recently been identified to oppose functions of the AT1 receptor through antiproliferation functions, apoptosis, and vasodilation (Carey et al., 2000). Signal transduction of AT2 receptors is still not well defined but NO, bradykinin, and cyclic GMP are thought to play a role in its vasodilatory function (Carey et al., 2000). Overall, AT2 receptors are less expressed than AT1 receptors, with the expression being greatest during fetal development and then rapidly decreases at birth to undetectable levels in most adult tissues (Carey et al., 2000). However, expression of both AT1 and AT2 is not static and can be enhanced or suppressed in response to certain stimuli (Chopra et al., 2011; Carey and Siragy, 2003; Kaschina and Unger, 2003). AII has several cardiovascular functions, each of which will be discussed below.

Enhancement of Sympathetic Neurotransmission

SNS stimulated production of AII is supported via a positive feedback loop. Circulating AII further enhances sympathetic neurotransmission by directly augmenting sympathetic outflow and increasing neurotransmission at the nerve endings by increasing NE release into the synaptic cleft (Ebert and Stowe, 1996; Peach, 1977). The primary sites of AII action are CNS, adrenal medulla, adrenergic nerve terminals, and postsynaptic effector cells (Saxena, 1992). At the CNS level, AII attenuates the baroreceptor reflex-mediated decrease in SNA. At the adrenal medulla, AII liberates adrenomedullary catecholamines by directly depolarizing chromaffin cells. AII may also inhibit the reuptake of NE by the nerve terminal and augment biosynthesis of NE at the adrenergic nerve terminals (Peach, 1977; Khairallah, 1972; Zimmerman et al., 1984; Roth, 1972; Boadle et al., 1969; Davila and Khairallah, 1971; Khairallah et al., 1971; Panisset and Bourdois, 1968). Finally, at high concentrations, AII enhances the vasoconstrictor effects of NE on the effector cells by facilitating the vasoconstrictory effects of α1R and α2R (Van Zwieten and De Jonge, 1986).

Vasoconstriction

AII binds to AT1 receptors on the vascular smooth muscle to induce a powerful vasoconstriction. Stimulated AT1 receptor works via similar mechanisms as α2R by coupling to $G_{q/11}$ protein, stimulating phospholipase C, leading to increased cytosolic calcium concentration via opening of L-type calcium channels or release of calcium from intracellular stores leading to contraction of arteriolar smooth muscle. Systemically produced AII at the lung results in an overall increase in TPR and BP. The increase in systemic resistance would have a decreasing effect on glomerular filtration rate (GFR) at the kidney; however, the endogenous production of AII at the kidney counteracts this decrease in GFR by constricting efferent arterioles more than the afferent arterioles. This difference in level of constriction at the efferent and afferent renal arterioles is likely due to the AT1 receptor population being higher in efferent compared to afferent arterioles (Razga and Nyengaard, 2008). Overall, the greater constriction of the efferent versus afferent arterioles maintains pressure through the glomerulus during decreased TPR and thus maintains GFR during elevated AII levels.

In contrast, at supraphysiological concentrations, endogenous AII has an opposing effect on renal medullary circulation by acting on AT1 receptors on the smooth muscle to induce a vasodilation of kidney medullary arterioles. This dilation works primarily by stimulating NOS to increase NO and cyclooxygenase activity to increase prostaglandin production (Evans et al., 2010). The cellular sources of NO and prostaglandin must be from the endothelial cells; however, the mechanisms of how AII leads to increases of NO and prostaglandins remain to be elucidated.

Aldosterone

Circulating AII stimulates the AT1 receptor in the adrenal zona glomerulosa cells of the adrenal cortex resulting in ALDO secretion into the blood stream (Wang et al., 1998). The primary role of ALDO is to maintain body sodium homeostasis. It acts at the kidney to increase renal sodium and water reabsorption. However, ALDO's actions go beyond its role as a renal regulator of sodium reabsorption and has been identified to play a role on vascular reactivity, vascular growth, and development. At the vascular level, ALDO binds to the mineralocorticoid receptor (MR) located on endothelial cells and induces a vasodilation regulated via NO, prostacyclin, and endothelium-derived hyperpolarizing factor (Feldman and Gros, 2013; Heylen et al., 2009; Palmer et al., 1987). Conversely, ALDO induces constrictor responses if it binds to MR on the smooth muscle cell (Liu et al., 2003). The constrictor response works by increasing endothelin-1 (ET-1) and vasoconstrictor prostanoids (Yanagisawa et al., 1988).

Endothelin-1

ET-1 is the most potent, long-lasting vasoconstrictor ever discovered and serves to increase peripheral resistance. ET-1 binds to class A G-protein-coupled receptors, ET_A or ET_B located on vascular smooth muscle, in quasi-irreversible manner (Shihoya et al., 2016). ET_A and ET_B exert opposing actions on several physiological processes. For example, activation of ET_A activates phospholipase

C leading to increases in inositol triphosphate (IP_3) and diacylglycerol (DAG). These increases in IP_3 and DAG levels result in the release of calcium from intracellular stores, specifically the SR, and activation of PKC, respectively, leading to vasoconstriction (Kedzierski and Yanagisawa, 2001; La and Reid, 1995; Resink et al., 1988; Van Renterghem et al., 1988; Griendling et al., 1989; Marsden et al., 1989). Calcium is released in a biphasic fashion, with the initial increase related to calcium released from intracellular stores, and the second and sustained increased related to calcium from extracellular stores likely through the opening of L-type Ca^{2+} channels (Yanagisawa et al., 1988). In contrast, activation of ET_B leads to an NO-mediated vasodilation but can further act as a "clearance receptor" by scavenging the circulating ET-1 (Shihoya et al., 2016). The pathway by which ET_B functions is largely unknown, but it is thought that intracellular degradation of ET-1 is the primary means of removing ET-1 from the circulation via ET_B (Bremnes et al., 2000).

Vasopressin

AII stimulates the release of antidiuretic hormone (ADH), also known as vasopressin, from the posterior pituitary gland. The primary role of ADH is to stimulate reabsorption of water by the kidneys by acting on their vasopressin V_2 receptors. However, ADH can bind to vasopressin V_{1a} G-protein-coupled receptors on the vascular smooth muscle to induce vasoconstriction (Henderson and Byron, 2007). Activation of V_{1a} leads to increases in IP_3 and consequently increases in Ca^{2+} release from intracellular stores as well as extracellular stores through L-type Ca^{2+} channels (Henderson and Byron, 2007). The vasoconstrictor response to ADH is tissue dependent, where the highest sensitivity is observed in skin, muscle, and splanchnic arteriolar beds, possibly to shunt blood to more vital organs during elevated levels which occur in response to decreased blood volume or increased plasma osmolality.

Atrial Natriuretic Peptide

Lastly, atrial natriuretic peptide (ANP) acts in a neurohumoral fashion as it is released into the circulation in response to stretch at the atria (e.g., under conditions of increased blood volume). Its primary role is to regulate normostatic BP (Potter et al., 2009). In the vasculature, ANP acts as a vasodilator by binding to natriuretic peptide receptor-A on vascular smooth muscle and upregulates a NO pathway (Potter et al., 2009; Edvinsson et al., 2016). Finally, ANP also antagonizes the RAAS, consequently affecting BP and circulation (Brandt et al., 1993).

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Noninfective Inflammatory Disorders of the Pericardium

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Introduction

The pericardium is a fibroserous sac composed of a serous visceral layer and a fibrous parietal layer with a small amount of pericardial fluid between the two layers. The fibrous parietal layer helps anchor the heart in place, and the pericardial fluid helps provide lubrication and reduce friction during cardiac activity. The pericardium is also an important barrier to infection. Perhaps most importantly, however, the pericardium has a restraining effect on cardiac volume. Although the pericardium can adapt to accommodate chronic increases in cardiac volume, more rapid dilatation of the cardiac chambers is often not well tolerated due to this effect.

Diseases of the pericardium can occur in isolation or in conjunction with more systemic disease processes. The leading manifestations of pericardial disease include pericarditis, which can present acutely, subacutely, or as a chronic fibrinous process, and pericardial effusion. Although pericarditis is the most common pericardial disease encountered in clinical practice, epidemiologic data on it is lacking. It has been estimated that acute pericarditis is diagnosed in 1 of every 1000 hospital admissions and comprises 1% of emergency room visits in patients with ST-segment elevation (Spangler, 2014). The etiology of pericardial diseases can be divided into infectious and noninfectious causes. Noninfectious causes can be further classified into idiopathic, inflammatory, neoplastic, metabolic, and traumatic causes, among others. This article will focus on the noninfective, inflammatory disorders of the pericardium, which are shown in **Table 1**.

Acute pericarditis, the most common of the pericardial diseases, typically presents with pleuritic chest pain. A pericardial friction rub may be present, and diffuse ST-segment elevation with PR depression is classically seen on an electrocardiogram. A new or worsening pericardial effusion may be seen on echocardiography. Diagnosis of pericarditis requires that at least two of the aforementioned features are present (Adler et al., 2015). Elevation of inflammatory markers or evidence of pericardial inflammation on other imaging modalities can help support the diagnosis in atypical cases but are not part of the diagnostic criteria. An electrocardiogram, transthoracic echocardiography, chest X-ray, and assessment of inflammatory markers (e.g., C-reactive protein) and myocardial injury (e.g., creatinine kinase and troponin) are recommended in patients with suspected acute pericarditis (Adler et al., 2015).

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Table 1 Etiology of noninfective, inflammatory pericardial diseases

Idiopathic pericarditis
Pericarditis associated with inflammatory diseases
• Connective tissue diseases
• Systemic vasculitidies
• Granulomatous diseases
• Autoinflammatory disesases
• Miscellaneous diseases
Post-MI and post-cardiac injury pericarditis
• Early post-MI pericarditis
• Late post-MI pericarditis (Dressler syndrome)
• Postpericardiotomy syndrome
Drugs and toxins

An extensive evaluation to elucidate the etiology of pericarditis is not always necessary, especially given the low yield of a diagnostic work up. Patients who have manifestations of an underlying systemic process, such as an inflammatory disease, or those with high-risk features (i.e., fever >38°C, subacute course, treatment failure, suspected myopericarditis, large pericardial effusion, or cardiac tamponade) typically warrant hospitalization and further work up. Patients who present with a pericardial effusion have a higher likelihood of having an etiology discovered, especially when pericardial fluid is examined and bacterial or neoplastic pericarditis is suspected (Zayas et al., 1995).

Treatment of most cases of acute pericarditis consists of nonsteroidal antiinflammatory drugs (NSAIDs) or aspirin (ASA). Colchicine is often added for its efficacy in symptom reduction, improving remission rates, and reducing the incidence of recurrent pericarditis. Corticosteroids should generally be avoided as a first line treatment agent due to an increase in recurrent pericarditis with its use. Patients presenting with pericardial effusions and echocardiographic evidence of cardiac tamponade may require pericardiocentesis. Treatment options related to specific etiologies will be further discussed in the appropriate sections to follow.

Idiopathic Pericarditis

In the vast majority of cases, the etiology of pericardial disease cannot be determined. Traditionally, 85%–90% of the cases of acute pericarditis have been deemed idiopathic. Attempting to reach a diagnosis is frequently limited by the lack of or inaccessibility to pericardial fluid. While these cases are sometimes presumed to be of infectious etiology, the lack of cost effective, accurate, and timely diagnostic viral testing frequently prohibits further attempts at a diagnosis. However, a recent large prospective cohort demonstrated that using a systematic prescription of tests for the different etiological causes of pericarditis can increase diagnostic yield and reduce the number of idiopathic cases (Gouriet et al., 2015). In this study, the authors used a number of noninvasive tests, such as blood cultures, viral serologies, antinuclear antibodies, and thyroid stimulating hormone, as well as invasive studies to test pericardial fluid, if available. If an etiology was not reached from these studies, but the patient had a history of an underlying disease process associated with pericardial disease, the etiology was presumed to be secondary to that disease process. If the patient's history was unremarkable, the etiology was classified as idiopathic. Using this method, an etiology was established in 45% of patients, which is significantly higher than that previously reported. Similarly, other studies have shown that a specific etiology can often be determined in those patients with pericardial effusions when clinical history and inflammatory signs are considered (Sagrista-Sauleda et al., 2000).

First line treatment for most patients with idiopathic pericarditis consists of combination therapy with colchicine plus NSAIDs/ASA, with ibuprofen or indomethacin being the most common NSAIDs used. While most patients have resolution of symptoms with the use of NSAIDs alone, colchicine has been shown to decrease the rate of recurrent pericarditis. The COPE (COlchicine for acute PEricarditis) trial was a randomized, prospective trial that sought to evaluate the safety and efficacy of colchicine as adjunctive therapy for patients with a first episode of pericarditis. The addition of colchicine to ASA led to a statistically significant reduction in symptom persistence at 72 h (11.7% vs. 36.7%) as well as a significant reduction in recurrence rate at 18 months (10.7% vs. 32.3%) (Imazio et al., 2005b). In the ICAP (Investigation on Colchicine for Acute Pericarditis) trial, the use of colchicine versus placebo, in addition to antiinflammatory therapy alone, significantly reduced the risk of recurrence (17% with colchicine vs. 38% with placebo). This trial also showed significantly better remission rates and fewer hospitalizations with the addition of colchicine (Imazio et al., 2013). Colchicine is generally administered for 3 months, while duration of therapy with NSAIDs is based upon duration of symptoms. Patients with contraindications to NSAID/ASA therapy or those with symptoms refractory to colchicine and NSAIDs may be treated with systemic glucocorticoids, although this practice is associated with an increase in recurrent pericarditis (Lilly, 2013). Prophylaxis against gastrointestinal bleeding with histamine-2 antagonists or proton pump inhibitors is warranted, particularly in those at high risk or who require longer durations of treatment.

Recurrent Pericarditis

Approximately one-third of patients with idiopathic pericarditis develop recurrent disease, sometimes with a pericardial effusion. Cases with persistent symptoms within 6 weeks of the acute attack are considered "incessant." In most cases, recurrent pericarditis is thought to be autoimmune mediated, but some cases may represent reactivation of viral pericarditis or a new infection. Recurrence can occur any time after the index event but usually occurs weeks to months later. Presenting symptoms are similar to symptoms of the initial presentation, with pleuritic chest pain being the most prominent manifestation; however, symptoms usually are not as severe and are rarely life threatening. Nonetheless, these recurrent symptoms can affect one's quality of life. Importantly, symptom severity does not predict the risk of recurrence. Patients treated with glucocorticoids during the index event have a higher rate of recurrence, while patients who respond well to NSAIDs/ASA and colchicine during the index event tend to have a lower risk of recurrence.

Colchicine can also help in reducing the risk of further recurrent episodes in patients who have experienced a recurrent episode of pericarditis. The CORE (COlchicine for REcurrent pericarditis) trial compared use of colchicine plus ASA to ASA alone for treatment of first recurrence of pericarditis. Use of colchicine significantly decreased the recurrence rate at 18 months (24.0% vs. 50.6%) as well as persistence of symptoms at 72 h (10% vs. 31%) (Imazio et al., 2005a). Therefore, treatment for recurrent pericarditis should consist of combination therapy with colchicine plus NSAIDs or ASA. For patients with contraindications to NSAIDs/ASA therapy or those who fail to respond to colchicine and NSAIDs/ASA, a slow taper of systemic glucocorticoids is an option. Intrapericardial steroid therapy and immunosuppression agents such as azathioprine (Vianello et al., 2011), human intravenous immune globulin (Imazio et al., 2016), and anakinra (Jain et al., 2015) have also been shown to help prevent recurrent episodes. Although the disease can be prolonged and frustrating, the overall prognosis for patients with recurrent pericarditis is good. Effective communication with patients is critical. Restriction of activity until there is resolution of symptoms, and in some instances (e.g., athletes) inflammatory markers, is warranted. Patients who have failed repeated attempts at medical therapy may be considered for pericardiectomy.

Pericarditis Associated With Inflammatory Diseases

Pericardial disease can be associated with numerous systemic inflammatory diseases, such as connective tissue diseases, systemic vasculitides, inflammatory bowel diseases (IBD), and a variety of other systemic processes. The degree of pericardial involvement usually correlates with the severity of the underlying disease and can range from small, asymptomatic pericardial effusions that are found incidentally to severely symptomatic pericarditis with more significant effusions. Likewise, progression of the pericardial involvement can range from subtle to rapid. Pericardial effusions are more prevalent and tend to be larger in these patients compared to patients with idiopathic pericarditis. While pericardial involvement is most often seen later in the disease course, it can also be a presenting symptom of the disease. A definitive diagnosis of pericardial disease related to inflammatory diseases requires specific findings in the pericardium or pericardial fluid, but a clinical diagnosis is often made presumptively in patients with systemic inflammatory diseases.

Treatment of pericardial involvement in inflammatory diseases typically depends on the level of symptoms, and collaboration with a rheumatologist is often needed. Treatment of the underlying systemic disease should be the focus for asymptomatic patients. On the other hand, patients with a pericardial effusion and cardiac tamponade require pericardiocentesis. Most patients with symptoms of acute pericarditis should be treated with a combination of NSAIDs and colchicine. The overall prognosis for patients with pericardial involvement from inflammatory diseases is good. While some patients may have recurrent episodes of pericarditis that can affect their quality of life, serious long-term complications are rare (Imazio, 2011).

Connective Tissue Diseases

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is one of the most common autoimmune diseases to involve the pericardium. Patients with SLE can have a number of cardiac abnormalities, but presence of a pericardial effusion and pericarditis are the most common (Doria et al., 2005). In fact, over half of all patients with SLE reportedly have a pericardial effusion, although the vast majority are asymptomatic and detected incidentally by echocardiography (Roman and Salmon, 2007). Pericardial and myocardial involvement is seen frequently at autopsy (Doherty et al., 1988). Large pericardial effusions or effusions that cause tamponade physiology are rare, but tamponade may be the first manifestation of SLE. An example of a pericardial effusion in a patient with newly diagnosed SLE is shown in **Fig. 1**. **Figs. 2–4** demonstrate indicators of cardiac tamponade, including right ventricle diastolic collapse, respiratory variation of mitral valve inflow velocities, and inferior vena cava plethora, respectively. An example of a pericardial effusion on CT scan is demonstrated in **Fig. 5**. Pericardial fluid analysis in these patients, although rarely of clinical value, typically shows high protein content, normal or slightly low glucose content, low complement levels, and may contain antinuclear antibodies and phagocytic cells with nuclei. Pericarditis in patients with SLE is less common than pericardial effusions, but can be seen, especially in those patients with other types of serositis, such as ascites or pleural effusions, and often accompanies flare-ups. On rare occasions, patients with SLE develop long-term complications such as recurrent pericarditis or constrictive pericarditis.

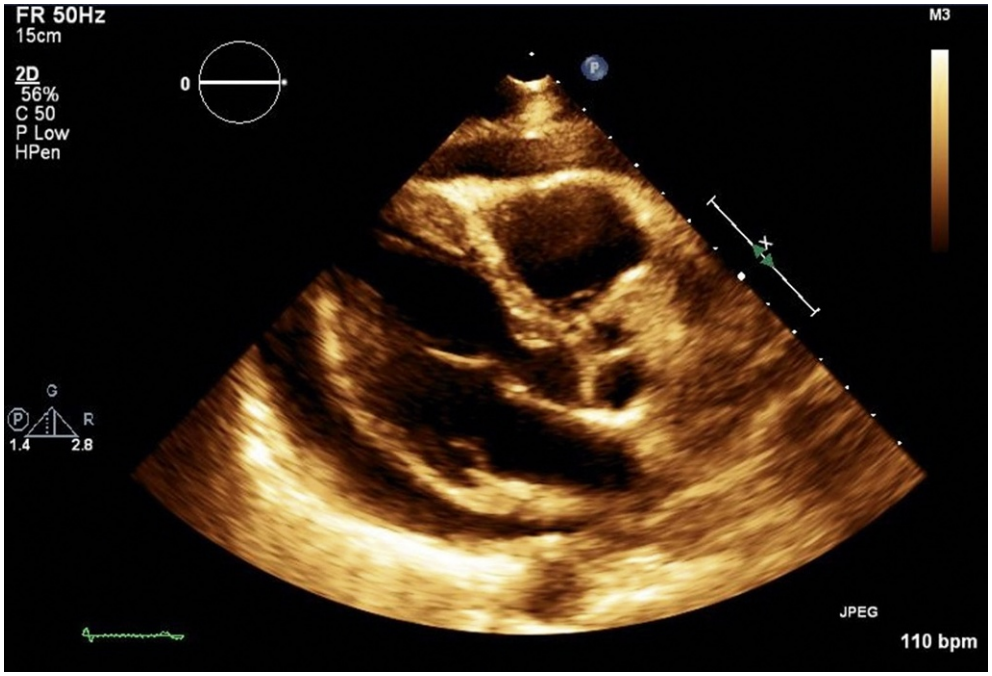

Fig. 1 Parasternal long axis view of a 26-year-old female with a circumferential pericardial effusion due to newly diagnosed systemic lupus erythematosus seen on a transthoracic echocardiogram.

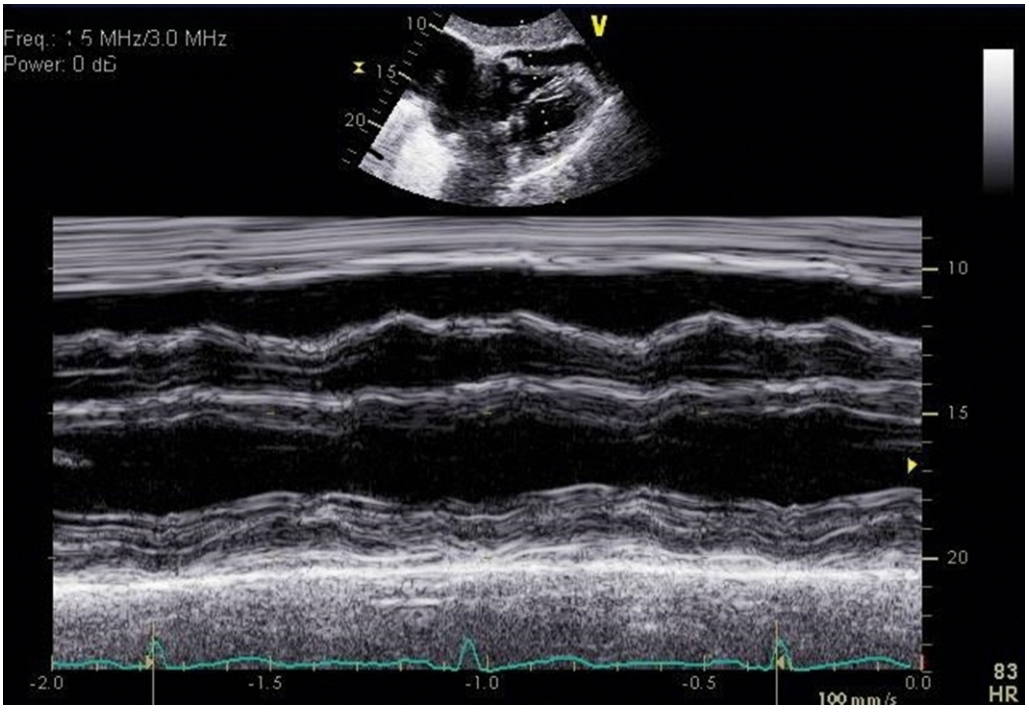

Fig. 2 M-mode echocardiography showing evidence of right ventricle diastolic collapse, suggestive of cardiac tamponade.

Treatment for symptomatic pericarditis, in addition to treatment of the underlying SLE, consists of NSAIDs in combination with colchicine to reduce the risk of recurrence. Glucocorticoids are an option for patients with contraindications to NSAID therapy or those who fail to respond to colchicine and NSAIDs. Very rarely, patients may require drainage for hemodynamically significant pericardial effusions, but case reports suggest that even these patients do well over time with medical therapy (Castier et al., 2000).

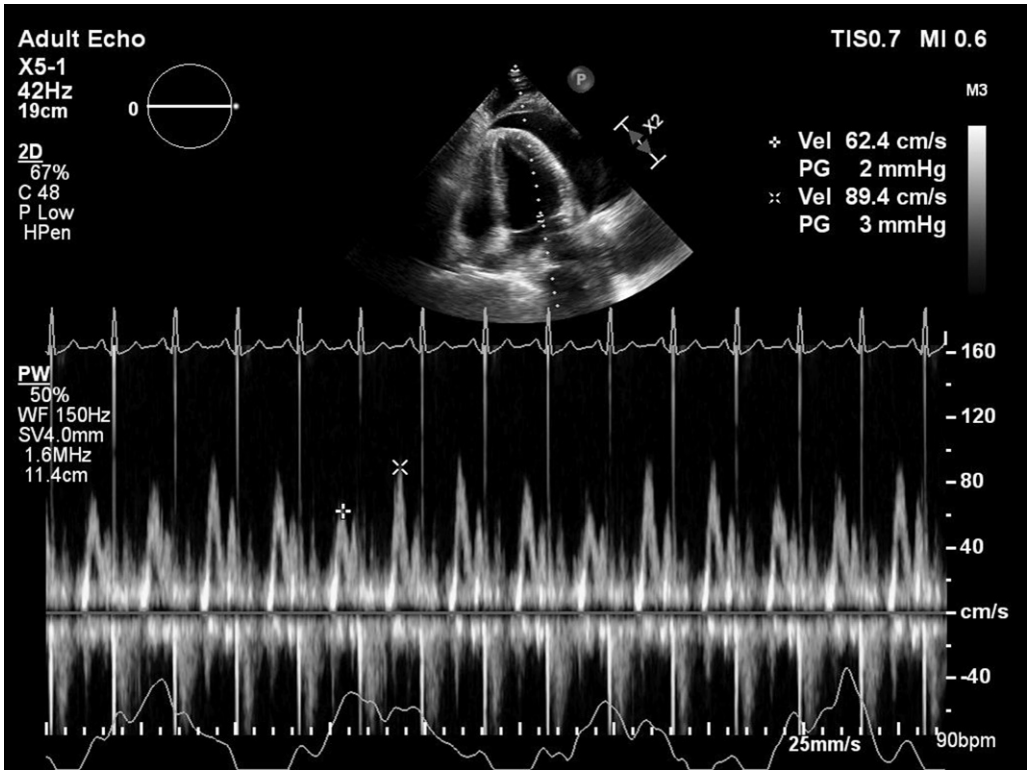

Fig. 3 Significant respiratory variation during mitral valve inflow suggestive of cardiac tamponade.

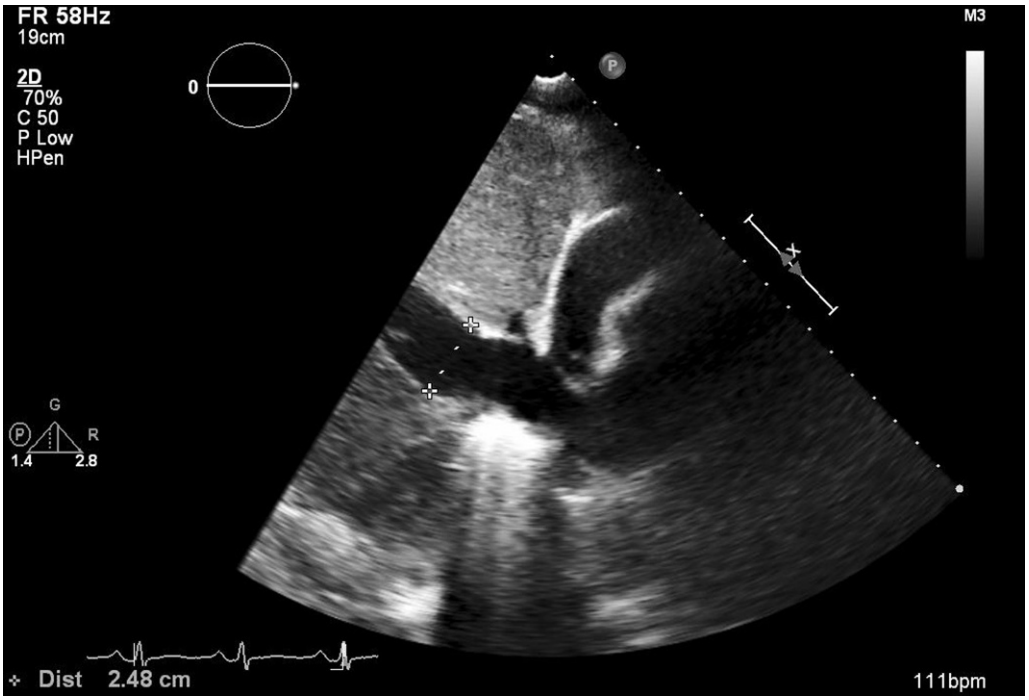

Fig. 4 Plethoirc inferior vena cava seen on a subcostal view of a transthoracic echocardiogram, which can be seen in cardiac tamponade.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic, inflammatory disease that primarily involves the joints, although a number of other organ systems can be involved. The pathology is usually a fibrous obliterative pericarditis with pericardial deposits of calcium, fibrin, and



Fig. 5 Pericardial effusion on CT scan for the same patient shown in **Fig. 1**.

cholesterol (Ferrans and Rodriguez, 1985); acute fibrinous pericarditis is seen in the remainder. RA has a slightly lower incidence of pericardial disease than does SLE; nearly one-third of patients have a pericardial effusion, which is usually asymptomatic (Imazio, 2011). Pericardial fluid tends to contain low glucose, neutrophilic leukocytosis, high titers of rheumatoid factor, and low complement levels. Rarely, fluid with high cholesterol concentration can be found. As is the case with SLE, pericarditis is less common in patients with RA than are pericardial effusions. When pericarditis does occur, it most commonly affects patients with active rheumatoid disease and positive serum testing for rheumatoid factor, especially middle-age men with acute onset of arthritis (Hoit, 2011). Some studies have also suggested a correlation between pericardial involvement, especially the development of cardiac tamponade, and duration of disease with RA (Escalante et al., 1990). Notably, patients with RA can occasionally develop constrictive pericarditis, which may develop with surprising rapidity, as a result of long-standing inflammation. Treatment for rheumatoid pericarditis is the same as that for pericarditis in SLE, with treatment of the underlying disease process being essential. Pericardial disease also complicates systemic juvenile RA (Still's disease) (Jamieson, 1983).

Systemic sclerosis

Systemic sclerosis, also known as scleroderma, is a disease most notably known to cause hardening of the skin and can cause fibrosis of other organs as well. Over half of patients with systemic sclerosis show evidence of pericardial involvement at the time of autopsy, but symptomatic pericarditis occurs only in approximately 10% of patients (Imazio, 2011). Pericardial involvement in patients with scleroderma is often associated with a cardiomyopathy (Hoit, 2011). Pericardial effusions, if present, are usually small and asymptomatic, but large or rapidly occurring effusions leading to tamponade can occur. Cardiac complications in patients with systemic sclerosis are most commonly due to pulmonary vascular disease. This can make the diagnosis of symptomatic pericardial disease more problematic, as it can be difficult to distinguish the etiology of symptoms such as dyspnea. Response to treatment for pericarditis with NSAIDs or ASA in patients with systemic sclerosis is often suboptimal. Furthermore, patients with symptomatic cardiac involvement tend to have a poor prognosis, with 2-year mortality estimated at 60% (Imazio, 2011).

Miscellaneous connective tissue diseases

A number of other connective tissue diseases may have pericardial involvement at a lower incidence rate. Dermatomyositis and polymyositis are inflammatory myopathies known for causing muscle weakness. Pericarditis or pericardial effusion is less common but can be associated with cardiac tamponade. Mixed connective tissue disease (MCTD) is a generalized connective tissue disorder that often presents with nonspecific symptoms. Pericarditis is the most common cardiac abnormality in MCTD, affecting up to 30% of patients, but significant pericardial effusions causing tamponade are exceedingly rare (Lundberg, 2005). Sjogren's syndrome is an autoimmune disease that results in dry mouth and dry eyes. Pericarditis is the most common form of cardiac involvement in patients with Sjogren's syndrome but is still rare nonetheless.

Systemic Vasculitides

The vasculitides are defined by the presence of inflammatory leukocytes in vessel walls with reactive damage to mural structures. Classification has traditionally been based on the size of the vessels involved. Pericardial involvement in the vasculitides is less common than in the connective tissue diseases, and pericardial involvement in large vessel vasculitis, such as Takayasu arteritis and giant cell arteritis, is especially rare. Among the medium vessel vasculitides, Kawasaki disease more commonly has pericardial involvement than does polyarteritis nodosa. Estimates suggest that up to 30% of patients with Kawasaki disease have pericardial involvement (Knockaert, 2007). However, a study of 198 patients with Kawasaki disease documented that less than 5% had a pericardial effusion >1 mm (Printz et al., 2011). Of the small vessel vasculitides, granulomatosis with polyangiitis (Wegener's) and eosinophilic granulomatosis with polyangiitis (Churg–Strauss) have been most frequently implicated.

Behcet's disease is a rare inflammatory disease that is unique in that it can affect arterial and venous vessels of all sizes. The most typical manifestations include recurrent aphthous stomatitis, genital ulcerations, and uveitis, but a number of cardiac manifestations can occur as well. Acute or recurrent pericarditis, although rare, has been reported (Geri et al., 2012).

Granulomatous Diseases

Sarcoidosis, likely the most recognized granulomatous disease, is characterized by noncaseating granulomas in multiple organ systems, with the lungs being most commonly involved. The etiology of this disorder remains unclear. Clinically, cardiac involvement in sarcoidosis is seen rarely, but subclinical cardiac sarcoidosis seen on autopsy is not uncommon. Signs and symptoms of cardiac sarcoidosis are broad and depend upon the location and extent of granulomatous inflammation. Pericarditis due to sarcoidosis is rare but has been reported (Wyplosz et al., 2010). Small pericardial effusions are much more commonly seen.

Autoinflammatory Diseases

Autoinflammatory diseases are a group of clinical conditions caused by dysregulation of innate immunity without any apparent involvement of antigen-specific T cells or significant production of autoantibodies. They result in intense episodes of inflammation that result in fever and other intermittent symptoms such as rash or joint swelling. These disorders usually present in the pediatric population, although some patients do not present until adulthood. While there has been an increase in prevalence of these diseases recently, they remain rare. The two most prevalent diseases in this group include familial Mediterranean fever (FMF) and tumor necrosis factor receptor-1 associated periodic syndrome (TRAPS).

Familial Mediterranean fever

FMF, as the name suggests, occurs more commonly in ethnic groups of the Mediterranean region. It is an autosomal recessive disease characterized by paroxysmal attacks of fever and serosal inflammation. Pericarditis is not uncommon in patients with FMF but is usually self-limiting when it occurs. Colchicine should be given as prophylactic therapy in order to prevent recurrent attacks and the development of secondary (AA) amyloidosis, a major complication of FMF (Zemer et al., 1986). Patients resistant or intolerant to colchicine should receive therapy with an antiinterleukin (IL-1) agent, such as anakinra, cankinumab, or rilonacept.

Tumor necrosis factor receptor-1 associated periodic syndrome

TRAPS, an autosomal dominant disorder caused by mutations in the TNFRSF1A gene encoding the 55-kD receptor for TNF-alpha, is characterized by recurrent febrile attacks with serosal inflammation that lasts at least 5 days to weeks. Recurrent pericarditis is seen commonly in patients with TRAPS, and family history is often positive, suggesting the possibility of a genetic predisposition to pericardial involvement. Unlike FMF, TRAPS does not respond well to colchicine (Cantarini et al., 2009), and researchers are still investigating treatment options.

Miscellaneous

Acute rheumatic fever

Acute rheumatic fever can occur 2–4 weeks after group A streptococcus pharyngitis. The incidence of rheumatic fever has significantly decreased in developed countries due to treatment of group A streptococcus pharyngitis with antibiotics. Classic symptoms include fever, arthritis, chorea, erythema marginatum, subcutaneous nodules, and sometimes carditis, including acute pericarditis. NSAIDs and colchicine are typically first line treatment options for pericarditis associated with rheumatic fever.

Immunoglobulin G4-related systemic disease

Immunoglobulin G4-related systemic disease (IgG4-RD) is an increasingly reported syndrome of unknown etiology. It most commonly occurs in middle-aged and older men and can involve one organ or multiple organs, with autoimmune pancreatitis being one of the most frequent manifestations in early observations of the disease. Constrictive pericarditis has been associated with IgG4-RD, and case reports have demonstrated that glucocorticoids, which are typically a first-line therapy for active IgG4-RD, can be effective in treatment of constrictive pericarditis in these patients (Sekiguchi et al., 2012).

Erdheim–Chester disease

Erdheim–Chester disease (ECD) is a rare disorder most commonly affecting middle-aged adults that is classified as a form of non-Langerhans cell histiocytosis. Histiocytosis in these patients leads to inflammation and scarring that typically involves long bones as well as at least one nonosseous site. Cardiovascular manifestations of the disease are underdiagnosed. An analysis of 178 cases of ECD from 2004 showed that 54 patients had heart involvement, and 32 of these patients had pericardial infiltration (Haroche et al., 2004). Five of those patients developed cardiac tamponade. There is no cure or standard treatment regimen for ECD, and the prognosis tends to be poor.

Inflammatory bowel diseases

Cardiac involvement in IBD is uncommon, but pericardial involvement seems to be the most frequent complication when heart involvement does occur (Katsanos and Tsianos, 2012). Cases of Crohn's disease with an initial presentation of pericarditis have been reported, with these patients presumably having had longstanding, virtually asymptomatic intestinal disease prior to presentation. Pericardial involvement may be associated with the degree of bowel activity, but this is not always the case. Additionally, it may be difficult to ascertain whether pericarditis in these patients is due to IBD itself or a side effect of IBD therapy, such as with sulfasalazine or 5-aminosalicylic acid. Patients with severe cases of IBD may have pericardial involvement due to a pericardio-colonic fistula. Cases of pericarditis in patients with IBD tend to respond well to therapy with corticosteroids.

Post-MI and Post-Cardiac Injury Pericarditis

Early Post-MI Pericarditis

Early post-MI pericarditis, or peri-infarct pericarditis (PIP), occurs during the first few days after myocardial infarction. It is caused by transmural myocardial necrosis with inflammation affecting the adjacent pericardium. Pericardial involvement is related to infarct size and is associated with a poor prognosis. The incidence of PIP has decreased dramatically in the era of improved early revascularization. When it occurs, early PIP is often asymptomatic and is identified only by the presence of a friction rub on physical examination. When patients are symptomatic, it is essential to distinguish between recurrent ischemic pain and pericardial pain. This can be done clinically and with ECG guidance. However, patients with PIP often do not have the classic ECG changes associated with pericarditis. Instead, the presence of atypical T wave evolution consisting of persistent upright T waves or early normalization of inverted T waves can be very useful in diagnosing pericarditis in these patients (Adler et al., 2015).

Treatment for PIP is generally supportive, as most cases are self-limiting. Symptomatic patients may be treated with high dose ASA for 1–2 weeks. Addition of colchicine may be helpful in reducing inflammation and reducing the risk for recurrent pericarditis. NSAIDs and glucocorticoids should be avoided due to the possibility of harmful effects in the early post-MI setting. Patients with an associated pericardial effusion generally do not need to have antiplatelet therapy or anticoagulation held. However, those with large or enlarging pericardial effusions, or signs of tamponade, may need cessation of anticoagulation to avoid hemorrhagic conversion of the pericardial effusion.

Late Post-MI Pericarditis (Dressler's Syndrome)

Late post-MI pericarditis, or Dressler's syndrome, one of the postcardiac injury syndromes, occurs from 10 days to a few months after myocardial infarction. Improved early revascularization has also led to a significant decrease in Dressler's syndrome. Dressler's syndrome is believed to be autoimmune in etiology with myocardial antibodies causing an inflammatory reaction that tends to be diffuse (Imazio and Hoit, 2013). Patients may present with pleuritic chest pain, friction rub, fever, leukocytosis, and sometimes pleural effusion or pulmonary infiltrates. The diagnosis is clinical, although objective findings, such as elevated inflammatory markers, electrocardiographic changes, and pericardial effusion on echocardiogram, can be helpful.

Like PIP, late post-MI pericarditis may also be treated with high dose ASA. NSAIDs may be considered, but the risk of NSAID use in patients with ischemic heart disease should be considered. Colchicine may be helpful in this population, although data are lacking. Glucocorticoids can be considered if necessary, but the benefit must be weighed against the risk of delayed myocardial healing. Pericardial effusions in late post-MI pericarditis have a higher tendency for hemorrhagic conversion than in early post-MI pericarditis, and anticoagulation, if absolutely necessary, should therefore be used with caution in this population.

Postpericardiotomy Syndrome

Postpericardiotomy syndrome (PPS), like all of the postcardiac injury syndromes, is initiated by the combination of damage to mesothelial pericardial cells and blood in the pericardial space, which releases cardiac antigens and stimulates an immune response. An inflammatory response is later generated by immune complexes that deposit in the pericardium, pleura, and lungs. PPS occurs several days to weeks after cardiothoracic surgery. It most commonly occurs after surgery involving the pericardium, but it can also occur after pacemaker lead implantation, coronary stenting, and other therapeutic misadventures. Like late post-MI pericarditis, symptoms may include chest pain and fever. Physical examination may disclose a pericardial friction rub. Leukocytosis and elevated

inflammatory markers are common laboratory findings. Pericardial effusions are commonly seen, but tamponade physiology is rare. Pleural effusions or pulmonary infiltrates may also be seen.

Two large randomized, placebo-controlled control trials, COlchicine for the Prevention of Post-pericardiotomy Syndrome (COPPS) and COPPS-2, have suggested a benefit to prophylactic use of colchicine after cardiac surgery in preventing PPS. In the COPPS trial, postoperative cardiac surgery patients were randomly assigned to receive colchicine or placebo. Colchicine significantly reduced the occurrence of PPS at 12 months (8.9% vs. 21.1%) and postoperative atrial fibrillation (0.6% vs. 5.0%) without a significant increase in side effects (Imazio et al., 2010). The COPPS-2 trial again showed a reduction in PPS in postoperative cardiac surgery patients treated with colchicine (19.4% vs. 29.4%). However, colchicine did not reduce the incidence of postoperative pleural/pericardial effusions or the incidence of postoperative atrial fibrillation. Furthermore, there was an increase in adverse effects, primarily gastrointestinal, in patients on colchicine (Imazio et al., 2014). Nonetheless, colchicine may be recommended after cardiac surgery, especially in those patients deemed at high risk for the development of PPS. ASA or NSAIDs are again the treatment of choice. Combination therapy with colchicine may be helpful, but data in this population is limited. Glucocorticoids are an alternative treatment, and intrapericardial triamcinolone has also been proposed as a possible treatment option, although further research is needed regarding its efficacy.

Drugs and Toxins

Drugs and toxins can cause pericardial disease by a variety of mechanisms, including through inflammatory pathways. A number of drugs can induce a lupus-like syndrome; procainamide, tocainide, hydralazine, isoniazid, methyldopa, and phenytoin are most commonly responsible, but over forty drugs have been implicated. Clinical and laboratory features are similar to SLE, but patients generally make a full recovery after the offending agent is discontinued.

Drug-induced lupus differs from typical drug hypersensitivity reactions in a few ways. Drug-induced lupus usually takes months to years of drug exposure and is dose dependent. It generally does not result in immune sensitization to the drug. Drug hypersensitivity has a number of possible adverse reactions, including pericarditis, particularly with penicillins. Mesalamine and sulfasalazine have also been associated with hypersensitivity reactions causing pericarditis. As these drugs are often used in the treatment of IBD, it can be challenging to determine if the development of pericarditis in this population should prompt discontinuation of the drug or rather be attributed to the underlying disease process (Kupferschmidt et al., 1996).

Conclusion

Pericardial diseases can occur in isolation or in the setting of systemic disease processes. Although diseases of the pericardium tend to manifest in only a limited number of ways, the causes of pericardial disease are vast. This chapter focused on the noninfective, inflammatory causes of pericardial disease, including idiopathic pericarditis, which are frequently seen in clinical practice. While the diagnosis and treatment of pericarditis is often straightforward, it can be frustrating and challenging to diagnose and manage, and long-term sequelae may occur. Fortunately, good medical therapy exists, and advances in diagnostic tools and treatment options have helped maintain a generally benign prognosis for patients with noninfective, inflammatory pericardial diseases.

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Non-ST-Elevation Acute Coronary Syndrome Prognosis

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Introduction

Ischemic heart disease remains the primary cause of mortality worldwide and represents the leading cause of years of life lost in developed nations (Lozano et al., 2012). Patients presenting with acute coronary syndrome (ACS), the acute manifestation of atherosclerotic heart disease, have a continuum of disease severity shown to benefit from prognosis-guided treatment strategies. Within ACS, contemporary management algorithms for patients with ST-segment elevation myocardial infarction (STEMI) have become highly standardized and protocol-driven to favor primary percutaneous coronary intervention (PCI) or fibrinolysis given the established benefit of immediate reperfusion therapy in these patients (O'Gara et al., 2013). In contrast, non-ST-segment elevation ACS (NSTE-ACS), a term encompassing unstable angina (UA) and non-ST-segment elevation myocardial infarction (NSTEMI), represents a significantly more heterogeneous disease with a wider variability in prognosis. As the absolute benefit of aggressive management and early invasive strategies is greater in patients with high-risk NSTE-ACS (Fanning et al., 2016), accurate assessment of the prognosis and risk of complications in NSTE-ACS is vital to reliably identify such patients and direct them toward appropriate streams of care.

In the emergency department setting, this risk stratification should be performed immediately for suspected NSTE-ACS (Amsterdam et al., 2014; Roffi et al., 2016). This may be challenging to accomplish accurately in practice as current literature suggests that NSTE-ACS patients are presenting with increasingly complex risk profiles. As discussed in greater detail later, the patient demographics and associated risk profiles constituting NSTE-ACS have shifted considerably over the last three decades (Yeh et al., 2010; Rogers et al., 2008). Notably, the widespread use of high-sensitivity troponin assays has led to an increasing re-classification of UA to NSTEMI (Costa et al., 2012), coupled to epidemiological trends producing reductions in the incidence of STEMI and, consequently, NSTEMI emerging as the predominant presentation of ACS (Rogers et al., 2008). These trends have had the net effect of considerably widening of the risk profiles of patients diagnosed as NSTEMI, resulting in greater heterogeneity of their potential prognoses. Subjective risk assessment by physicians in the context of these ongoing shifts in patient characteristics is

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challenging and may be subject to cognitive bias (Yan et al., 2009). Several studies have demonstrated that physicians systematically underestimate risk in patients with high-risk NSTE-ACS (Lee et al., 2008), and that their assessments only weakly correlate with formally validated risk scores (Yan et al., 2009). This may be in part responsible for an observed treatment-risk paradox, with associated worse outcome (Yan et al., 2007a; Fox et al., 2007a).

Early, individualized risk-stratification of patients with NSTE-ACS at their time of presentation serves to empirically guide physicians in their choice of therapy, optimize resource allocation, and inform patients of their anticipated likelihood of recovery or complication. In the acute phase of care, a major consideration is determining which patients will benefit from an early invasive strategy during their index hospitalization. Closer to the time of discharge, subsequent risk assessment should be aimed at determining which patients are at high long-term risk of recurrent adverse cardiovascular events. Following a brief survey of the evolution of prognosis in NSTE-ACS over the past three decades, this article will review the individual prognostic factors generally available to physicians upon patient presentation, compare validated bedside clinical prognostic models, and consider the role of additional testing during hospitalization. We will conclude with a discussion of the application of these prognosticators and risk models in NSTE-ACS, as well as emerging future avenues of individualized risk determination.

Temporal Trends in Prognosis

Several notable trends have changed the frequency distribution and underlying characteristics of NSTE-ACS in developed countries which merit consideration when evaluating the prognosis of contemporary patients diagnosed with NSTEMI or UA. Broad implementation of primary preventative strategies may have resulted in fewer cases of STEMI (Go et al., 2006; Mukamal et al., 1999) while the concurrent aging of the population over time, increasing rates of comorbid diabetes, hypertension, and dyslipidemia, and recent trends in smokers presenting with NSTE-ACS have contributed to an increase in the proportion of NSTEMI among patients hospitalized with MI (Yeh et al., 2010; Fox et al., 2007b; Hall et al., 2016; Arbel et al., 2016). From a diagnostic standpoint, considering that acute MI is now defined by positivity in biomarkers of myocardial necrosis (Thygesen et al., 2012), ongoing improvements in the test characteristics of these diagnostic tools have also led to a shift in the classification of patients presenting with ACS (Costa et al., 2012). The wide adoption of high-sensitivity troponin in particular has moved the diagnosis of NSTE-ACS away from UA to NSTEMI (Rogers et al., 2008), likely due to increased detection of small infarcts. The net consequence of these trends has resulted in NSTEMI becoming the predominant presentation of MI, representing nearly 60% of all cases in 2006, compared to just 14% in 1990 (Rogers et al., 2008). Concomitantly, there has been a gradual increase in the incidence rates of NSTE-ACS since 1999 despite falling rates of STEMI and MI overall (Yeh et al., 2010; Rogers et al., 2008).

Large population studies in NSTE-ACS during this time period have found an 18%–23% relative decrease in risk-adjusted odds of short-term mortality (Yeh et al., 2010; Rogers et al., 2008; Fox et al., 2007b; Hall et al., 2016; Krumholz et al., 2009) (Figure). Data from the National Registry of Myocardial Infarction in the United States demonstrated an in-hospital mortality of 5.2% in 2006 for NSTEMI, down from 7.1% in 1994 (Rogers et al., 2008), while 30-day mortality similarly decreased from 10.0% in 1999 to 7.6% in 2008 (Yeh et al., 2010). In the Global Registry of Acute Coronary Events (GRACE) which grouped NSTE-ACS data from 14 developed countries, risk-adjusted in-hospital mortality in 2005 was 2.2%, down from 2.9% in 1999 (Fox et al., 2007b) and 6-month mortality was similarly decreased to 3.3% from 4.9% during the same time period. Further data from the Myocardial Ischemia National Audit Project (MINAP) in England and Wales demonstrated the continuation of this trend between 2003 and 2013, with 30-day mortality decreasing from 2.6% to 2.0% and 6-month mortality from 10.8% to 7.6% over the 10-year period (Hall et al., 2016). Previous studies comparing long-term outcome between STEMI and NSTEMI have established that, while short-term mortality in STEMI is higher, rates of death in NSTEMI beyond 6-month follow-up approach or exceed that of STEMI (Armstrong et al., 1998; McManus et al., 2011; Yan et al., 2004). This phenomenon still persists in contemporary cohorts of ACS despite advances in pharmacotherapy and high rates of revascularization (Alnasser et al., 2015). Beyond the 6-month mark, case fatality among patients with NSTEMI in GRACE exceeded that of STEMI, with a significantly higher age- and sex-adjusted odds of mortality for the former at 2 years following MI and the absolute mortality reaching 9.5% among NSTEMI patients and 4.9% in UA patients (Alnasser et al., 2015). In addition to reductions in case fatality rates, hospital-level analyses in the United States have also observed decreasing variability in mortality among institutions (Krumholz et al., 2009), suggesting improved standardization of care. Rates of complications have similarly decreased, with patients less likely to develop heart failure, cardiogenic shock or reinfarction during their initial hospitalization (Fox et al., 2007b).

These ameliorations in patient outcome result from the substantial advancements in prevention, diagnosis, and management of ACS that have been accomplished over the past three decades as a product of intense clinical research efforts. The increasingly robust body of evidence for the implementation of pharmacotherapeutic and interventional treatments has led to modifications in the clinical guidelines on NSTE-ACS management (Amsterdam et al., 2014; Roffi et al., 2016). Increases in the adoption of these recommendations over the course of a 5- to 10-year period following their publication have been demonstrated in large, representative, observational studies (Yeh et al., 2010; Fox et al., 2007b; Hall et al., 2016; Yan et al., 2007b; Rao et al., 2009). Notably, evidence-based pharmacological therapies (aspirin, adenosine diphosphate receptor inhibitors, β-blockers, statins, angiotensin receptor inhibitors/angiotensin II-receptor blockers, low-molecular weight heparins) are now routinely employed in the majority of patients with NSTE-ACS, while the frequency of angiography and PCI in NSTE-ACS have risen considerably (Fox et al., 2007b; Hall et al., 2016; Yan et al., 2007b). Improvements in clinical outcome thus likely reflect a combination of improved adherence to evidence-based

management strategies (Fox et al., 2007b; Yan et al., 2007b), increased use of in-hospital revascularization (Hall et al., 2016; Gale et al., 2014), and, in part, the success of quality improvement initiatives (Jha et al., 2005; Jencks & Wilensky, 1992) addressing early concerns of disparities in the uptake of guideline recommendations (Fox et al., 2003; Rogers et al., 1994).

Consequent to these trends, the underlying risk profiles of contemporary patients classified as NSTEMI have also likely changed, although the net effect of these changes is not readily apparent in the context of multiple tendencies, some with opposing effects on risk. Data from GRACE have demonstrated a significant increase in the GRACE risk scores of NSTE-ACS patients enrolled over an 8-year period; all other factors kept constant, this represents an expected in-hospital mortality rise from 2.5% to 3.2% between 1999 and 2006 (Fox et al., 2007b). This contrasts with later data from MINAP demonstrating a significant decrease from 2003 to 2013 in the proportion of cases with intermediate to high GRACE risk scores (Hall et al., 2016). However, these net changes in baseline risk likely do not fully reflect the underlying distribution of risk scores, considering that the advances in NSTE-ACS diagnosis and management have allowed both detection of small infarcts earlier in their course of presentation, in addition to tendencies toward higher-risk, more comorbid, presentations of NSTEMI (Hall et al., 2016). This ongoing dual trend is likely responsible for increases in the heterogeneity of risk profiles underlying NSTEMI, and heightens the difficulty in clinically assessing prognosis or risk of complications in contemporary patient populations.

Clinical History and Exam

The initial clinical assessment of patients presenting with NSTE-ACS offers an opportunity to rapidly identify factors predictive of subsequent complicated clinical course or unfavorable outcome. Both baseline patient characteristics and mode of presentation are highly prognostic and contribute information to this assessment. Age is by far one of the most important prognostic factors in NSTE-ACS; patients over the age of 75 presenting with NSTE-ACS have more diffuse coronary atherosclerosis and more severe angiographic disease compared with those below 75 (Stone et al., 1996). In multivariable models such as GRACE and PURSUIT, age carries the strongest independent predictive value for mortality (Granger et al., 2003; Fox et al., 2014; Boersma et al., 2000). Despite this, underappreciation of the impact of age has been identified as one of the main reasons physicians misclassify patients with NSTE-ACS during risk assessment (Yan et al., 2009) and may explain why older patients are less likely to receive aggressive therapy despite high risk (Stone et al., 1996; Yan et al., 2006a). Current guidelines do not recommend the decision to pursue conservative management be solely based on age (Alexander et al., 2007), as age-stratified subgroup analyses from trials such as TACTICS-TIMI 18 suggest elderly patients ($\geq$75 years) may still benefit significantly from an early invasive strategy (Bach et al., 2004).

History of prior atherosclerotic disease predicts worse outcome in NSTE-ACS and has been established in the case of past history of MI, stroke, transient ischemic attack, and symptomatic peripheral artery disease (Cotter et al., 2003; Januzzi et al., 2005; Lee et al., 2010; Mohareb et al., 2013). Studies based on observational registries have demonstrated that these patients paradoxically tend to receive less intensive treatments and less frequently undergo in-hospital angiography due to an underappreciation of risk by physicians (Lee et al., 2010; Mohareb et al., 2013). Similarly, NSTE-ACS patients with a history of chronic heart failure have more high-risk presentations and greater rates of acute decompensated heart failure, cardiogenic shock, renal failure, and reinfarction complicating their hospital course (Zhang et al., 2016; Ranasinghe et al., 2012). Prior heart failure is an independent predictor of both in-hospital and long-term mortality and readmission following discharge (Zhang et al., 2016; Ranasinghe et al., 2012). Large trials (Anavekar et al., 2004; Al Suwaidi et al., 2002) and observational studies (Fox et al., 2014; Yan et al., 2006b; Wong et al., 2009) have recognized renal dysfunction as an independent predictor of adverse prognosis in NSTE-ACS (Fox et al., 2014). Despite being underrepresented in randomized controlled trials (Coca et al., 2006), subgroup analyses in FRISC and TACTICS-TIMI 18 have suggested a potential role for early invasive therapy in selected NSTE-ACS patients with renal dysfunction (Johnston et al., 2006; Januzzi et al., 2002). Among patients presenting with UA, the character and time course of anginal pain have prognostic value. Braunwald's classification of UA is based on the presenting angina provoking factor and relationship with exertion (Braunwald, 1989; Hamm & Braunwald, 2000); this categorization correlates with angiographic disease and independently discriminates mortality at 6 months, with the greatest risk in patients with recent rest pain (class III) and postinfarction angina (class C) (van Miltenburg-van Zijl et al., 1995).

On physical examination, initial hemodynamic parameters are highly predictive of mortality and early clinical course. Tachycardia and hypotension are powerful independent predictors of short- and long-term mortality in NSTE-ACS, and both heart rate and systolic blood pressure are central components of several risk models (Granger et al., 2003; Fox et al., 2014; Boersma et al., 2000). This association remains valid in patients with preexisting hypertension and is independent of prior antihypertensive medication use (Lee et al., 2013). A simple risk index calculated from heart rate, systolic pressure, and age alone is a strong predictor of 30-day mortality and allows rapid bedside risk assessment (Morrow et al., 2001a). These continuous variables have nonlinear relationships with outcome, and the GRACE 2.0 model demonstrated improved discrimination by employing nonlinear models for these indicators (Fox et al., 2014). New mitral regurgitation may complicate the presentation of NSTE-ACS as a result of ischemic damage to papillary muscles or left ventricle dilatation. Besides causing left ventricular dysfunction, the presence of mitral regurgitation itself is an independent prognostic factor for mortality and heart failure at 10-year follow-up even in patients with preserved left ventricular ejection fraction (LVEF) at presentation (Persson et al., 2010).

Overt heart failure is a feature associated with very high short-term risk and complicates the presentation of approximately one-tenth of contemporary NSTE-ACS admissions (Bahit et al., 2013). Its presence on admission is associated with older age, diabetes, prior MI (Khot et al., 2003), and a markedly higher 30-day mortality (Bahit et al., 2013; Steg et al., 2004; van Diepen et al., 2014). The Killip classification is a convenient clinical score for categorizing the severity of heart failure in acute MI based on clinical

exam (Killip & Kimball, 1967). Higher Killip classes on presentation predict greater short- and long-term mortality in contemporary international cohorts (Bahit et al., 2013; Khot et al., 2003; Steg et al., 2004). Paradoxically, despite this substantially higher risk of poor outcome, NSTE-ACS patients with heart failure on admission have lower rates of invasive management with coronary catheterization and PCI than those without heart failure, and are less likely to receive evidence-based pharmacotherapy such as β-blockers or statins upon discharge (Steg et al., 2004, 2008; Spencer et al., 2002; Shah et al., 2012). In NSTE-ACS patients presenting with heart failure, revascularization during index-hospitalization is associated with substantially decreased mortality at 6 months and is likely underutilized compared to patients without heart failure (Steg et al., 2008).

Electrocardiogram

The resting 12-lead electrocardiogram (ECG) remains essential in the immediate evaluation of NSTE-ACS and is one of the core components of clinical risk models. The first ECG should be acquired and screened for ischemic changes within 10 minutes of patient presentation, considered in parallel to initial clinical assessment, and repeated at any instance of change in symptomatology or clinical status (Amsterdam et al., 2014; Roffi et al., 2016). If initial ECG is nondiagnostic despite ongoing symptoms, current guidelines suggest serial ECGs be acquired in 15- to 30-minute intervals to screen for dynamic changes suggestive of evolving ischemia; continuous 12-lead ECG monitoring may also be considered in such patients (Amsterdam et al., 2014; Roffi et al., 2016). Continuous cardiac monitoring following admission to hospital permits further screening for occurrence of reinfarction or arrhythmia.

Beyond the evaluation of ST-segment deviation, assessing abnormalities on the initial ECG or evolving changes on subsequent repeat ECGs in the acute setting is important for dynamic risk assessment which may guide management. Several findings on ECG are recognized to be predictive of unfavorable outcome at short and long term, and novel analyses using repeat or continuous ECG data have emerged which may enhance precision in risk assessment.

ST-Segment Depression

ST-segment depression on initial ECG is strongly associated with unfavorable outcomes in NSTE-ACS and is a major independent prognostic factor in most risk prediction models. Its presence at admission has been shown to significantly correlate with short-term (Savonitto et al., 1999) and long-term ($\geq$1 year) (Kaul et al., 2001; Cannon et al., 1997) mortality. While ST depression as minimal as 0.5 mV has been demonstrated to reflect myocardial ischemia and unfavorable prognosis (Cannon et al., 1997), greater magnitudes of depression predict higher short-term mortality. Both the depth of the ST depression within a single lead (Kaul et al., 2001) and the cumulative sum of the depressions in all leads (Savonitto et al., 2005) carry independent prognostic weight. In an analysis from GUSTO-IIb, the latter was demonstrated to have a graded association with 30-day mortality and to predict the presence of three-vessel or left main coronary disease on angiography (Savonitto et al., 2005).

As patients may present at variable time points during the course of NSTE-ACS evolution, repeat ECGs are valuable for tracking dynamic ST-segment changes reflecting the progression or development of myocardial ischemia. The persistence of ST depression from initial to early repeat ECG performed at 12–24 or 24–36 h after presentation is strongly predictive of increased mortality at 30 days and 6 months, whereas the development of new ST depression on follow-up ECG also identifies patients at higher risk than on the basis of their admission ECG alone (Yan et al., 2010; Alkaabi et al., 2008). Similar associations with outcome were found when repeat ECG was undertaken at the time of discharge; persistent or new ST depression on late repeat ECG despite in-hospital therapies conveys significantly increased long-term risk of death and reinfarction (Hersi et al., 2003).

In the acute management of NSTE-ACS, the presence of ST-segment depression identifies patients most likely to benefit from an early invasive strategy. As opposed to patients with no acute ECG findings or T-wave inversion (TWI) only, those with ST-segment depression have significantly lower mortality if they undergo early invasive management (Cannon et al., 2001; Diderholm et al., 2002a). Furthermore, the extent of this survival benefit increases as the number of affected leads or the cumulative sum of ST depression increases (Holmvang et al., 2003). Assessing ST depression on initial and repeat ECG as a dynamic marker of ischemic burden is thus valuable for guiding the decision to pursue early invasive management.

T-Wave Inversion

Compared with ST depression, the presence of isolated TWI on admission ECG in NSTE-ACS is associated with lower prevalence of atherosclerotic disease on coronary angiography and lower rates of short- or long-term mortality or reinfarction (Savonitto et al., 1999; Collinson et al., 2006; Mueller et al., 2004). Neither TWI on the admission (Tan et al., 2013) nor early repeat (Sarak et al., 2016) ECG was a significant predictor of poor outcome beyond known prognostic factors.

Conduction Abnormalities

In patients with NSTE-ACS, the presence of left bundle branch block (LBBB) at presentation is independently associated with increased severity of angiographic disease, especially in the right coronary artery distribution, and nearly twofold higher cumulative mortality at 1-year follow-up (Rose et al., 2013; Damman et al., 2012). Similarly, new right bundle branch block (RBBB) is associated with higher-risk presentations, more extensive comorbidities, and multivessel disease (Chan et al., 2016). Following adjustment for these variables, RBBB also retains a strong association with early mortality (Chan et al., 2016), comparable in

magnitude to LBBB and twice that of STEMI (Widimsky et al., 2012). Paradoxically, observational studies of unselected patient populations have demonstrated patients with left or right bundle branch block to be less likely to receive evidence-based pharmacotherapy (Go et al., 1998) or to undergo coronary angiography or PCI than those with normal conduction, despite a much higher likelihood of significant stenosis of major epicardial arteries (Chan et al., 2016; Widimsky et al., 2012).

High-grade atrioventricular block (HAVB), comprising either type II Mobitz second-degree or third-degree atrioventricular block, occurs in less than 2% of contemporary cases of NSTE-ACS (Singh et al., 2015). Its presence is associated with higher GRACE risk scores and predicts a complicated hospital course, with significantly higher rates of reinfarction, heart failure, cardiogenic shock, and ventricular arrhythmias (Singh et al., 2015; Haim et al., 1997). Odds of in-hospital death are consequently over sixfold higher in patients with HAVB; however, among those surviving discharge, subsequent long-term mortality is comparable to patients without HAVB (Singh et al., 2015; Haim et al., 1997).

While left anterior hemi-block on admission ECG is associated with greater frequency of comorbidity and high-risk presentations among patients with ACS, this finding is not an independent predictor of mortality in contemporary cohorts (Zhang et al., 2014).

Left Ventricular Hypertrophy

Presence of electrocardiographic criteria for left ventricular hypertrophy (LVH) on initial ECG is associated with higher-risk clinical presentations (Ali et al., 2011; Westerhout et al., 2007). While a substudy of NSTE-ACS patients in the GUSTO IV ACS trial found that women with LVH had increased long-term mortality independent of NT-pro-BNP levels (Westerhout et al., 2007), a significant relationship with adverse outcome was not found in less selected patients from GRACE following multivariable adjustment with the GRACE risk score (Ali et al., 2011). The latter study demonstrated that ST depression, however, remains a strong prognostic indicator of mortality, regardless of the presence of LVH.

Continuous ECG Monitoring

Recurrent ischemia is common in NSTE-ACS patients following their initial event and frequently occurs without any accompanying symptoms (Cohn et al., 2003). Beyond the admission 12-lead ECG, continuous ECG ST-segment monitoring (CSTM) during index hospitalization provides further risk stratification (Scirica et al., 2009; Akkerhuis et al., 2001; Goodman et al., 2000). In the MERLIN-TIMI 36 trial, a single episode of recurrent ST depression lasting at least 1 minute in the week following admission for NSTE-ACS was independently associated with heightened short- and long-term risk of reinfarction and cardiovascular death, improving risk assessment beyond clinical and biomarker data alone (Scirica et al., 2009). Furthermore, ischemic events on CSTM during just the first 24 h of presentation can predict the occurrence of death or MI at as early as 5 days following admission (Akkerhuis et al., 2001). When considered in addition to risk models used for risk stratification at presentation, CSTM adds significant incremental prognostic information beyond that provided by the TIMI, PURSUIT, and GRACE risk scores (Carmo et al., 2011; Yan et al., 2007c). Use of CSTM may guide invasive management decisions as the presence of ST shifts on continuous ECG identifies high-risk patients who would benefit from in-hospital revascularization (Yan et al., 2007c).

Other ECG Findings

While lead aVR is generally viewed as providing reciprocal information to the left lateral distribution of leads (aVL, II, V_5, and V_6) (Gorgels et al., 2001), angiographic studies have demonstrated ST-segment elevations in aVR to also be a marker of left main coronary artery disease (CAD), possibly mediated via ischemia of the basal interventricular septum (Gorgels et al., 2001; Yamaji et al., 2001). In NSTE-ACS, the presence of ST-segment elevation in aVR has been strongly associated with high-risk angiographic disease, with increasing magnitudes of ST elevation independently predicting progressively higher prevalence of significant left main or three-vessel disease (Barrabes et al., 2003; Kosuge et al., 2006, 2008; Yan et al., 2007d; Taglieri et al., 2011). While small studies have associated this ECG finding to in-hospital (Barrabes et al., 2003; Taglieri et al., 2011) and long-term mortality (Kosuge et al., 2006; Taglieri et al., 2011), the incremental prognostic value of ST elevation in aVR beyond known prognosticators was not confirmed following comprehensive multivariable adjustment in a large 5064-patient cohort from GRACE (Yan et al., 2007d).

Low QRS voltage is an infrequent finding on admission ECG in patients presenting with NSTE-ACS. It is defined by a peak-to-peak QRS amplitude of <0.5 mV in all limb leads and <1.0 mV in all precordial leads. In addition to obesity or pericardial effusion, this abnormality may be a result of globally reduced action potentials due to diffuse myocardial injury (Isobe et al., 2002, 2006). In a large observational cohort from GRACE, the presence of low QRS voltage on admission ECG in NSTE-ACS independently predicted increased in-hospital mortality, but this association did not persist at 6 months (Tan et al., 2015). Further studies in contemporary populations are required to confirm the role of low QRS voltage as a routine prognostic indicator.

Heart rate turbulence (HRT) is a novel Holter monitor-based electrocardiographic marker of baroreflex (Bauer et al., 2008). The measurement is made by assessing heart rate patterns following isolated premature ventricular contractions (PVC). In healthy patients, the occurrence of a hemodynamically inefficient PVC results in low cardiac output causing vagal inhibition and a period of brief sinus rate acceleration. This is followed by baroreceptor trigger and a subsequent overcorrection via vagal stimulus, causing gradual heart rate deceleration prior to a return to the normal pre-PVC rate (Bauer et al., 2008). This normal pattern of HRT reflects integrity of the autonomic arc and its blunting in post-MI has been strongly associated with long-term unfavorable outcomes (Exner et al., 2007; Barthel et al., 2003). The detection of early HRT blunting during index hospitalization following acute MI has been validated as a powerful independent predictor of all-cause mortality (Sade et al., 2003) and a complement to LVEF for predicting risk of cardiac arrest or sudden cardiac death (Exner et al., 2007).

Biomarkers

Troponin

Acute MI is defined by the presence of an elevated concentration of serum cardiac troponin in the context of clinical history compatible with myocardial ischemia (Thygesen et al., 2012). Both cardiac troponin I (*c*TnI) (Adams et al., 1993; Cummins & Perry, 1978) and T (*c*TnT) (Perry, 1998; Katus et al., 1991) are highly specific to the myocardium and their presence in the peripheral circulation in elevated quantities is a very sensitive marker of myocardial injury (Saenger & Jaffe, 2008). Its superiority in diagnostic accuracy compared to creatine kinase MB isoenzyme (CK-MB) has led to troponin supplanting CK-MB as the preferred biomarker of myocardial necrosis (Morrow et al., 2007). The Third Universal Definition of Myocardial Infarction recommends a troponin value above the 99th percentile of the upper reference level as a cutoff for diagnostic positivity (Amsterdam et al., 2014; Roffi et al., 2016; Thygesen et al., 2012); studies of prognosis in troponin have also shown this to be the useful cutoff for risk prediction (James et al., 2003a).

The prognostic value of elevated troponin has been clearly demonstrated in NSTEMI, with both *c*TnI and *c*TnT carrying similar predictive value (Olatidoye et al., 1998). A metaanalysis of 21 studies of patients with NSTEMI found that patients with elevated troponin had increased mortality at 30 days and long-term follow-up (5 months to 3 years) (Ottani et al., 2000). In large head-to-head comparisons with CK-MB, analyses from CRUSADE (Newby et al., 2006) and GRACE (Goodman et al., 2006) found troponin superior to CK-MB in predicting mortality and enhancing overall prognostication. Major trials such as GUSTO IV ACS (James et al., 2003b), GUSTO IIa (Ohman et al., 1996), TIMI IIIB (Antman et al., 1996), and FRISC (Lindahl et al., 2000) have demonstrated a graded relationship between the degree of troponin elevation and short-term mortality; furthermore, any detectable elevation in presenting troponin level negatively impacts short-term outcome. Similarly, this mortality gradient was also observed when troponin was analyzed in an unselected, real-world series of 16,318 NSTE-ACS patients from GRACE (Jolly et al., 2011). The magnitude of peak troponin in the first 24 h following presentation had a powerful independent relationship with the incidence of life-threatening arrhythmia, cardiogenic shock, and new heart failure; the degree of troponin elevation modeled as a continuous variable independently predicted both short- and longer-term mortality (Jolly et al., 2011) and is a component of several validated risk scores (Granger et al., 2003; Fox et al., 2014; Antman et al., 2000).

The increased mortality associated with troponin elevation is likely mediated by greater severity or extent of underlying CAD impairment of coronary flow and complex lesion characteristics such as thrombus formation or downstream microembolization (Lindahl et al., 2001; Heeschen et al., 1999a). Peak *c*TnT and *c*TnI levels have also been correlated with infarct size determined by both magnetic resonance imaging (Vasile et al., 2008; Steen et al., 2006) and scintigraphy (Licka et al., 2002; Panteghini et al., 2002).

NSTE-ACS patients with troponin elevation should be managed more aggressively in the acute phase owing to their higher risk of complications and short-term mortality (Amsterdam et al., 2014; Roffi et al., 2016). These patients derive greater benefit from pharmacotherapy with antithrombotic agents such as low-molecular weight heparin (Morrow et al., 2000) and glycoprotein IIb/IIIa inhibitor (Heeschen et al., 1999b). Furthermore, patients with elevated troponins at presentation are more likely to benefit from an early invasive strategy (Diderholm et al., 2002b; Kleiman et al., 2002); this benefit can be substantial even if the magnitude of the troponin elevation is relatively minor (Morrow et al., 2001b). In a metaanalysis of seven randomized trials, patients with elevated troponin had lower short- and long-term adverse outcomes when managed with an early invasive strategy compared to a conservative strategy during index hospitalization (Bavry et al., 2006).

C-Reactive Protein

C-reactive protein (CRP) is an acute-phase reactant and marker of inflammation (Armstrong et al., 2006). Increased levels of CRP are detectable acutely following ACS and have been hypothesized to represent focal and systemic inflammatory processes underlying the destabilization and eventual rupture of vulnerable coronary plaques (Morrow et al., 2007). Several studies in patients presenting with NSTE-ACS have shown early high-sensitivity (hs)-CRP to predict short- and long-mortality in multivariable analyses (He et al., 2010). Importantly, studies have found that hs-CRP yields prognostic information complementary to cardiac troponin results (James et al., 2003b), and may supply supplemental prognostic value in patients without rise in their troponin levels (Lindahl et al., 2000; Morrow et al., 1998). Following ACS, monitoring CRP levels in patients on statin therapy allows improved prognostication of cardiac events beyond low density lipoprotein monitoring alone. The role of a combined biomarker approach to prognostication of NSTE-ACS is a subject of active investigation but not yet validated for routine clinical use (Tello-Montoliu et al., 2007).

BNP and NT-pro-BNP

Brain natriuretic peptide (BNP) is a hormone synthesized predominantly in the myocardium of the left ventricle and secreted secondary to myocyte stretch from pressure overload or ventricular dilatation. Rapid assays have been developed to detect BNP or the N-terminal fragment of its prohormone precursor (NT-pro-BNP) and are now widely available. Elevated levels of both BNP and NT-pro-BNP measured in NSTE-ACS early following symptom onset are independent predictors of mortality (Jernberg et al., 2003; Bazzino et al., 2004; Heeschen et al., 2004; Eggers et al., 2009). Furthermore, an analysis of 6809 patients from the GUSTO IV ACS trial found that increasing N-pro-BNP levels had a gradient relationship with 1-year mortality independent of troponin or CRP

(James et al., 2003c). Serial measurements of BNP following discharge after index hospitalization for NSTE-ACS may further evaluate risk during follow-up (Morrow et al., 2005). Additional research is required to conclusively determine if the addition of BNP or NT-pro-BNP to existing risk stratification methods offers incremental predictive information beyond known prognosticators and results in improved patient management.

Other Emerging Biomarkers

Heart-type fatty acid binding protein (H-FABP) is a cytosolic protein found in, and relatively specific to, cardiomyocytes (Van Nieuwenhoven et al., 1995); it is detected peripherally following myocardial insult and is currently under investigation as a potential biomarker in ACS (Glatz et al., 1994). Small preliminary studies suggest that early measurement of H-FABP may carry prognostic ability to predict long-term mortality independent of troponin, or in troponin-negative NSTE-ACS (Viswanathan et al., 2010; Kilcullen et al., 2007; O'Donoghue et al., 2006). However, larger and more rigorous studies with contemporary troponin assays are required to adequately assess the incremental predictive value of this biomarker prior to clinical use.

Copeptin is the C-terminal segment of the precursor peptide to arginine vasopressin; this molecule is secreted from the pituitary gland early during acute MI (Khan et al., 2007). As a potential emerging biomarker of MI, copeptin's role in diagnosis and prognostication is currently under investigation (Nickel et al., 2012). Initial studies in NSTE-ACS, however, have failed to demonstrate an independent relationship with outcome following adjustment with patient characteristics and troponin (Sanchez et al., 2014).

Additional emerging biomarkers are under investigation in NSTE-ACS. Growth differentiation factor (GDF) 15 (Widera et al., 2012), ST2 (Eggers et al., 2010), corin (Zhou et al., 2016), midregional proadrenomedullin (MR-proADM) and pro-atrial natriuretic peptide (MR-proANP) (O'Malley et al., 2014), and circulating microRNAs (miRNAs) (Navickas et al., 2016), among others, have been associated with clinical outcome in NSTE-ACS and may allow novel avenues of prognostication in the future.

Clinical Prognostic Models

Comprehensive risk assessment in NSTE-ACS should take a wide number of prognostic factors into account to allow an overall assessment of the probability of unfavorable outcome (Steg et al., 2009). While a rare number of clinical predictors are so strong that they may be used individually to guide management decisions, adequate prognostication in most cases of NSTE-ACS at their presentation requires evaluation of multiple clinical parameters simultaneously. For clinicians to effectively perform these assessments at the time of initial patient encounter, current clinical practice guidelines strongly recommend the use of well-validated clinical risk scores to determine prognosis (class I recommendation) and guide management decisions (class IIa recommendation) (Amsterdam et al., 2014; Roffi et al., 2016). While most of these tools share similar core elements, they differ in their structure, setting, and predicted outcomes (Table).

Clinical risk prediction models for non-ST-elevation acute coronary syndrome

	GRACE (admission)	*GRACE (discharge)*	*TIMI*	*PURSUIT*	*OASIS*	*FRISC*	*TRI*	*EMMACE*	*C-ACS*
Age	●	●	●	●	●	●	●	●	●
Sex				●	●	●			
Prior MI		●			●	●			
History of ≥50% coronary stenosis on angiography[a]			●						
≥3 risk factors for CAD			●						
Angina history			●	●					
Aspirin use in past 7 days			●						
Diabetes						●			
Prior HF		●							
Systolic blood pressure	●	●		●			●	●	●
Heart rate	●	●		●			●	●	●
Killip class/signs of HF	●			●					●
Cardiac arrest	●								
ST deviation	●	●	●	●	●	●			
Troponin/CK-MB	●	●	●		●	●			
Creatinine/renal dysfunction	●	●							
NT-proBNP					●				
HbA1c					●				
Biomarker of inflammation						●			

(Continued)

	GRACE (admission)	*GRACE (discharge)*	*TIMI*	*PURSUIT*	*OASIS*	*FRISC*	*TRI*	*EMMACE*	*C-ACS*
PCI during hospitalization		●							
Derivation Study	(Granger et al., 2003; Fox et al., 2006, 2014)	(Eagle et al., 2004)	(Antman et al., 2000)	(Boersma et al., 2000)	(Mehta et al., 2016)	(Lagerqvist et al., 2005)	(Morrow et al., 2001a)	(Dorsch et al., 2001)	(Huynh et al., 2013)
Outcome Measure	D/MI	D	D/MI/ Revasc	D/MI	CV death/ MI/ Stroke	D/MI	D	D	D
Timing	In-hosp, 6-month, 1-year, 3-year	6-month (from postdischarge)	14-day	30-day	1-year	1-year	In-hosp	30-day, 1-year	In- hosp, 30-day, 1-year, 5-year

GRACE, Global Registry of Acute Coronary Events; *TIMI*, Thrombolysis in Myocardial Infarction; *PURSUIT*, Platelet Glycoprotein IIb/IIIa in Unstable Angina Receptor Suppression Using Integrilin; *OASIS*, Organization to Assess Strategies in Ischemic Syndromes; *FRISC*, Fast Revascularization in Instability in Coronary Disease; *TRI*, Thrombolysis in Myocardial Infarction Risk Index; *EMMACE*, Evaluation of Methods and Management of Acute Coronary Events; *C-ACS*, Canada Acute Coronary Syndrome; *MI*, myocardial infarction; *CAD*, coronary artery disease; *PCI*, percutaneous coronary intervention; *CABG*, coronary artery bypass graft; *HF*, heart failure; *CK-MB*, creatine kinase MB isoenzyme; *NT-proBNP*, N-terminal pro b-type natriuretic peptide; *HbA1c*, hemoglobin A1c; *D*, death; *Revasc*, revascularization; *CV*, cardiovascular; *In-hosp*, in-hospital.
[a]May be substituted by prior history of MI, PCI, or CABG.

GRACE

The Global Registry of Acute Coronary Events (GRACE) risk model is a tool derived from a multinational registry of less selected ACS patients (Granger et al., 2003). Spanning 30 countries, the registry enrolled patients diagnosed with ACS from nearly 250 hospitals; recruitment was aimed at minimizing selection bias and ensuring representation of the entire spectrum of patients with ACS. While the majority of other risk models are based on data originating from trial databases, GRACE had minimal exclusion criteria and encouraged consecutive enrollment of cases in its participating hospitals.

The original GRACE risk score is an eight-item multivariable model estimating the risk of in-hospital mortality in ACS based on data from 11,389 patients (Granger et al., 2003) and 6-month mortality derived from 21,688 patients (Fox et al., 2006). This risk model was updated in 2014 (Fox et al., 2014) as GRACE 2.0, derived from 32,037 patients, and further extended to also predict mortality at 1 and 3 years, with simplification of some variables and improvement in discrimination. While the underlying regression models differed, the same eight clinical variables were independently responsible for nearly 90% of the predictive accuracy of the model: age, systolic blood pressure, heart rate, creatinine, Killip class, cardiac arrest on presentation, positive biomarkers of myocardial necrosis, and ST deviation on ECG.

The GRACE risk score may be employed early as it is based on information generally available at initial assessment in the emergency department. In the original GRACE risk score, points are assigned via a nomogram for each of the model predictors and totaled to provide an estimate of the absolute risk of in-hospital mortality. In this model, a score of $\leq$108 represents a $<$1% probability of in-hospital death and may be considered low risk, whereas a score of $\geq$141 is indicative of high ($>$3%) mortality risk (Hamm et al., 2011). GRACE 2.0 simplifies assessment of the Killip and creatinine variables as these may be interchanged with the presence of diuretic use and renal dysfunction, respectively (Fox et al., 2014; Simms et al., 2013). Rather than a points-based system, absolute risk in V2.0 is calculated directly by an electronic application to benefit from nonlinear modeling of some of the continuous variables (Fox et al., 2014).

For patients surviving hospitalization, a separate GRACE risk score was developed to determine late prognosis in ACS. This score was derived in a cohort of 15,007 GRACE patients who survived index hospitalization; nine variables independently predicted all-cause mortality at 6 months postdischarge: age, history of MI, history of heart failure, tachycardia at presentation, hypotension at presentation, elevated creatinine at presentation, positive biomarkers of myocardial necrosis, ST depression on initial ECG, and not undergoing in-hospital PCI (Eagle et al., 2004).

The GRACE risk score has been demonstrated to have excellent discrimination in its validation studies, with a c-statistic of 0.83 for in-hospital mortality in the original GRACE study (Granger et al., 2003) and 0.81, 0.83, and 0.82 for 6-month, 1-year, and 3-year morality, respectively, in the subsequent follow-up studies (Fox et al., 2006, 2014). It has since also been extensively validated in independent datasets, including cohorts from multiple continents (Bradshaw et al., 2006, 2015; Elbarouni et al., 2009; Kelly et al., 2012; Yusufali et al., 2011; Thalib et al., 2016; Prabhudesai et al., 2012; Shaikh et al., 2014). When compared to alternative NSTE-ACS prediction models, a metaanalysis pooling validation studies found the GRACE risk score to have significantly higher discrimination compared to the TIMI risk score (D'Ascenzo et al., 2012). Furthermore, a National Institute for Health and Care Excellence (NICE) analysis testing eight ACS risk prediction scores head-to-head in a 64,312 patient database from the MINAP registry found the GRACE risk score to outperform all other models with a c-statistic of 0.825. Similar results were found in the

Canadian ACS II registry and other patient populations (Yan et al., 2007e; Aragam et al., 2009). Given this overall robustness, it is one of the preferred risk stratification tools recommended in contemporary guidelines (Amsterdam et al., 2014; Roffi et al., 2016). In addition to prognostication, the GRACE risk score may also aid management decisions (Amsterdam et al., 2014; Roffi et al., 2016; de Araujo et al., 2005; Mehta et al., 2009; Yan et al., 2005). A prespecified subgroup analysis of the TIMACS trial showed that an early invasive strategy in NSTE-ACS within 24 h of presentation improves outcomes in high-risk patients (GRACE risk score >140), but not those at low or intermediate risk (Mehta et al., 2009); similar discriminatory value was demonstrated in a less-selected ACS cohort (Yan et al., 2005).

TIMI

The Thrombolysis in Myocardial Ischemia (TIMI) risk score is a simple seven-variable prediction model derived from two large trials of NSTE-ACS patients (TIMI 11B and ESSENCE) (Antman et al., 2000). Multivariable analyses from these datasets demonstrated seven dichotomous variables which independently predicted major adverse events at 14 days (mortality, new or recurrent MI, or severe ischemia requiring revascularization). The number of variables present (age $\geq$65 years, presence of three or more risk factors for CAD, prior evidence of coronary stenosis, ST deviation at presentation, positive cardiac biomarkers, two or more angina episodes in the 24 h before presentation, and aspirin use in the 7 days prior to presentation) is summated to determine the total score and the corresponding absolute risk of major adverse outcome.

The TIMI risk score is simple to calculate at bedside and has been validated in unselected cases of NSTE-ACS (Scirica et al., 2002; Soiza et al., 2006). Increasing TIMI scores have been well correlated with severity of angiographic disease, predicting both overall burden of atherosclerotic CAD (Garcia et al., 2004) and presence of high-risk features, such as impaired flow or coronary thrombus (Mega et al., 2005). Stratification by TIMI risk categories may help predict which NSTE-ACS patients are most likely to benefit from an early invasive management approach; subgroup analyses in the TACTICS-TIMI 18 trial, for example, found that only intermediate and high-risk patients (TIMI score $\geq$ 3) derived clinical benefit from this strategy (Cannon et al., 2001).

PURSUIT

The PURSUIT risk score was created from a trial cohort of 9461 NSTE-ACS patients recruited from 28 hospitals across North and South America and Europe to predict 30-day mortality or reinfarction (Boersma et al., 2000). Multivariable regression analysis established seven variables as predictive of unfavorable outcome: age, male sex, heart rate, systolic blood pressure, ST-segment depression, signs of heart failure, and worse Canadian Cardiovascular Society Angina class in the 6 weeks prior to presentation. By assigning points for each variable, risk of death at 30 days may be calculated from a nomogram; this risk score predicted mortality with good discrimination in the original validation study (c-statistic = 0.81) and when applied in an independent dataset (Gale et al., 2009). Patients deemed high-risk by a PURSUIT score of >14 have been demonstrated to benefit from early myocardial revascularization during index hospitalization, although its accuracy for predicting this benefit was inferior to that of the GRACE risk score (de Araujo et al., 2005).

Other Risk Scores

In contrast to the more exhaustive prediction models described previously, several simple risk scores designed to incorporate only age and hemodynamic parameters have been demonstrated to maintain strong predictive value (Morrow et al., 2001a; Dorsch et al., 2001; Huynh et al., 2013). The TIMI risk index (TRI) (Morrow et al., 2001a) is a simple calculation relating together the absolute values of the patient's age, systolic blood pressure, and heart rate. In NSTEMI patients, the resulting score has a graded association with 30-day mortality, spanning a 30-fold difference between the highest and the lowest deciles (Wiviott et al., 2006). Similar to the TRI, The Evaluation of the Methods and Management of Acute Coronary Events (EMMACE) risk score (Dorsch et al., 2001) is based on age, systolic blood pressure, and heart rate modeled via logistic regression to predict 30-day mortality. This score was demonstrated to have high discrimination in validation cohorts across the ACS spectrum (Gale et al., 2009). The Canadian Acute Coronary Syndrome risk score (C-ACS) (Huynh et al., 2013) dichotomizes four variables, with one point assigned for the presence of each: age $\geq$75, Killip class >1, systolic blood pressure <100 mmHg, and heart rate >100 beats/min. This score is a highly sensitive prognosticator for both short- and long-term mortality, with negative predictive values of a C-ACS score of $\geq$1 at 98% for 30-day mortality and 93% for 1- or 5-year mortality.

In patients with acute chest pain but absence of troponin elevation or ST-segment deviation, a simple risk score taking into account age, chest pain severity score, chest pain frequency, history of insulin-dependent diabetes, and prior PCI predicts 1-year mortality with better discrimination than the TIMI risk score (Sanchis et al., 2005). The OASIS risk score (Mehta et al., 2016) incorporates two novel biomarkers, NT-proBNP and hemoglobin A1c, to five traditional variables (age, sex, prior history of MI or stroke, ST-segment deviation, and cTnT) to create a 20-point risk stratification tool strongly predicting 1-year mortality. The FRISC score (Lagerqvist et al., 2005) is determined by the number of factors present among: age >70 years, male sex, diabetes, previous MI, ST depression, troponin elevation, and increase in markers of inflammation; a score of $\geq$3 predicts significant reductions in death and MI at 1-year for patients who undergo an early invasive management strategy. The Clinical SYNTAX Score (CSS) (Farooq et al., 2013) incorporates angiographic lesion information with baseline clinical variables to predict 1-year mortality, with validation studies in NSTE-ACS patients demonstrating good discrimination.

Noninvasive Cardiac Imaging

Echocardiography

Point-of-care ultrasound is increasingly available in most emergency rooms and use of early resting two-dimensional transthoracic echocardiography (TTE) is feasible at initial assessment of patients with NSTE-ACS. In this context, rapid, targeted bedside TTE may be performed as an extension of the physical examination in the prognostic evaluation of such patients. The presence of wall motion abnormalities on early TTE in patients with NSTE-ACS is associated with severity of angiographic disease (Di Pasquale et al., 2004). A more detailed assessment of regional systolic function with the wall motion score index (WMSI) (Nishimura et al., 1984) early after admission strongly predicts long-term mortality and future admission for heart failure, offering incremental value over LVEF alone (Moller et al., 2006). Novel predictors assessed with speckle-tracking echocardiography may further enhance early risk stratification, as both the evaluation of myocardial strain (Dahlslett et al., 2014) and the duration of systolic lengthening of ischemic myocardial segments during isovolemic contraction (Zahid et al., 2014) in patients with NSTE-ACS have been found to be predictive of coronary stenosis or occlusion on angiography.

Evaluation of resting LVEF following NSTE-ACS offers critical prognostic information and should be undertaken routinely in all patients prior to discharge (Amsterdam et al., 2014; Roffi et al., 2016). Left ventricular systolic dysfunction is a powerful predictor of long-term mortality, arrhythmia, and heart failure (Mukherjee et al., 2016; Bauer et al., 2009; Solomon et al., 2005; Nicolosi et al., 1996; St John Sutton et al., 1994; Van Reet et al., 1984; The Multicenter Postinfarction Research Group, 1983). While several modalities may accurately assess LVEF, TTE is often the most routinely accessible imaging tool and simultaneously permits the detection of other indicators of poor outcome; echocardiographic evidence of restrictive left ventricular filling (Moller et al., 2008), myocardial strain (Milewska et al., 2016), right ventricular dysfunction (Zornoff et al., 2002), left atrial enlargement (Moller et al., 2003), and ischemic MR (Persson et al., 2010; Grigioni et al., 2001) are all associated with unfavorable long-term prognosis independent of left ventricular dysfunction.

In low- to intermediate-risk NSTE-ACS patients treated with an ischemia-guided strategy, noninvasive stress testing is recommended prior to discharge following absence of ischemia for 12–24 h (Amsterdam et al., 2014; Roffi et al., 2016). This may be accomplished via an ECG exercise tolerance test in patients with resting ECGs allowing ST-segment interpretation (Nyman et al., 1992). In patients with baseline ECG abnormalities precluding dynamic interpretation during ECG stress testing, use of an imaging modality such as stress echocardiography is an accurate alternative for identifying patients at higher risk of having angiographically significant disease who may benefit from revascularization (Marwick et al., 1995).

Nuclear Myocardial Perfusion Scintigraphy

Although NSTE-ACS patients with high-risk presenting characteristics have been shown to benefit from an early invasive strategy (Fanning et al., 2016), their low- or intermediate-risk counterparts may be adequately managed with an ischemia-guided approach (Amsterdam et al., 2014; Roffi et al., 2016). Following initial medical stabilization, early stress myocardial perfusion scintigraphy (MPS) has been shown to have significant prognostic value in further risk-stratifying such patients (Brown, 1997). The presence and extent of inducible perfusion defect on MPS is highly predicative of short- and long-term mortality and adverse cardiac events (Mahmarian et al., 1995, 2006; Stratmann et al., 1995a,b; Zhu et al., 1991; Younis et al., 1989) and can guide the decision to pursue in-hospital cardiac catheterization. Conversely, the absence of significant reversible defects may reliably identify patients at low risk who can be considered for conservative management and rapid discharge (Mahmarian et al., 2006).

Hybrid imaging combines a functional myocardial perfusion test (such as single photon emission computed tomography (SPECT) or positron emission tomography (PET)) with anatomical imaging (such as computed tomography coronary angiography (CTCA)). Early hybrid imaging identifies the presence of angiographically significant CAD (Gaemperli et al., 2011) and thus could be employed as a potential noninvasive prognosticator in NSTE-ACS for identifying low- or intermediate-risk patients who may benefit from coronary angiography during initial hospitalization (Rispler et al., 2011). However, hybrid imaging currently has limited availability and further studies should be awaited to establish its incremental prognostic value over clinical risk-stratification prior to routine clinical application (Gaemperli et al., 2011).

Computed Tomography Coronary Angiography

The use of computed tomography coronary angiography (CTCA) in the acute setting of suspected ACS is rapidly expanding as multidetector scanners capable of acquiring ≥ 64 slice images are becoming widely available (Hoffmann & Bamberg, 2009). However, the utility of CTCA in the risk stratification of NSTE-ACS has not yet been clearly demonstrated and is currently of limited clinical application. Preliminary studies have found characterization of culprit (Thomsen & Abdulla, 2016) and nonculprit plaques (Dedic et al., 2014) on CTCA performed during index hospitalization for ACS to predict long-term major adverse cardiovascular events; however, further studies are required to determine the incremental prognostic value of these findings and their role in guiding the acute management of NSTE-ACS.

Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance (CMR) is an emerging imaging modality in NSTE-ACS capable of undertaking detailed assessment of myocardial structure, function, viability and ischemia. Evaluation of myocardial edema, microvascular obstruction, and

intramyocardial hemorrhage are further capabilities unique to this technique (Lockie et al., 2009). Minimal data are available on the role of early CMR in ACS; however, in a study of 68 patients diagnosed with NSTE-ACS who underwent CMR within 72 h of presentation, CMR was found to be highly accurate in predicting flow-limiting coronary stenosis on invasive coronary angiography (Plein et al., 2004). With a sensitivity of 96% and specificity of 83%, acute phase CMR outperformed the TIMI risk score for predicting angiographically significant CAD (Plein et al., 2004). Similarly, the presence of myocardial edema on CMR performed early following NSTE-ACS was found to predict need for in-hospital revascularization and prognosticate risk of long-term cardiac events (Raman et al., 2010). Infarct size is most accurately assessed by CMR, and its measurement early after acute MI has been found to be both a predictor of left ventricular remodeling and a strong independent prognosticator of long-term adverse cardiac events independent of LVEF (Wu et al., 2008). Other specific CMR findings following ACS or primary PCI have been shown to be predictive of clinical outcome (Lockie et al., 2009); however, their prognostic value in early risk stratification soon after NSTE-ACS and role in management has not yet been established.

Coronary Angiography

Coronary angiography is the gold standard for assessing acute manifestations of CAD and provides incremental prognostic information in patients with NSTE-ACS. In ACS patients treated with an early invasive strategy, the presence on angiography of high disease burden, plaque calcification, lesion eccentricity, postprocedural TIMI flow grade, and left ventricular dysfunction measured by left ventriculogram are all independently predictive of unfavorable short- and long-term prognosis (Lansky et al., 2010; Song et al., 2010); however, culprit vessel (Halim et al., 2016) and myocardial blush grade (Ng et al., 2016) have limited predictive value.

The Synergy Between PCI with Taxus and Cardiac Surgery (SYNTAX) score is a system for grading the complexity of coronary anatomy via angiography (Sianos et al., 2005) and has been validated in patients with NSTE-ACS for discriminating risk of death and MI at 30 days, 1 year, and 3 years follow-up when used in concert with clinical variables (Farooq et al., 2013; Palmerini et al., 2011; Iqbal et al., 2014). In a head-to-head comparison with other angiographic risk scores, the SYNTAX score maintained superior model characteristics (Palmerini et al., 2012).

The absence of culprit lesion on coronary angiography is seen in 9%–14% of patients with NSTE-ACS undergoing an invasive management strategy (Agewall et al., 2016; Bugiardini et al., 2006; Patel et al., 2006; Roe et al., 2000; Diver et al., 1994). The finding of absent or mild coronary disease (<50% stenosis of a major epicardial artery) predicts more favorable clinical course; in both the PURUSIT trial (Roe et al., 2000) and the CRUSADE registry (Patel et al., 2006), patients with such insignificant coronary disease had significantly lower short-term mortality. This favorable prognosis was also seen at 1-year follow-up in a study pooling 7656 patients from three TIMI trials where the outcome of death or MI occurred in only 2.1% of such patients, compared to 11.2% in those with obstructive disease (Bugiardini et al., 2006).

Summary

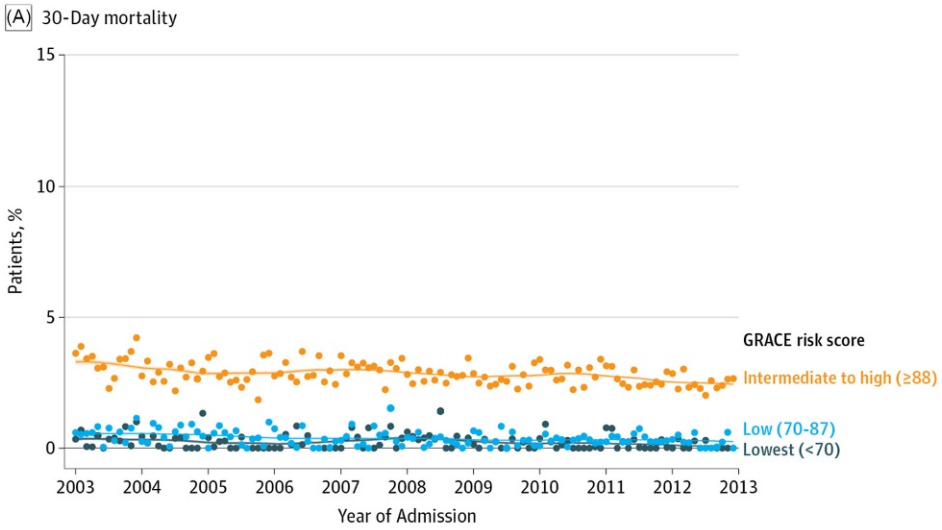
The last four decades have witnessed significant advances in the treatment of NSTE-ACS with the emergence of evidence-based pharmacological agents and the increased availability of cardiac catheterization and early revascularization. While rapid improvements have been achieved in both short- and long-term outcomes, including reductions in death, heart failure, and life-threatening arrhythmias, gaps persist in the use of evidence-based therapies and NSTE-ACS continues to be a major global cause of morbidity and mortality. Ongoing epidemiological trends have resulted in a significantly higher proportion of patients presenting with NSTE-ACS as opposed to STEMI among contemporary cohorts of ACS, with increasingly heterogeneous risk profiles. Systematic underappreciation of patient risk likely in part accounts for gaps in the application of guideline-recommended management, and may perpetuate the ongoing treatment-risk paradox.

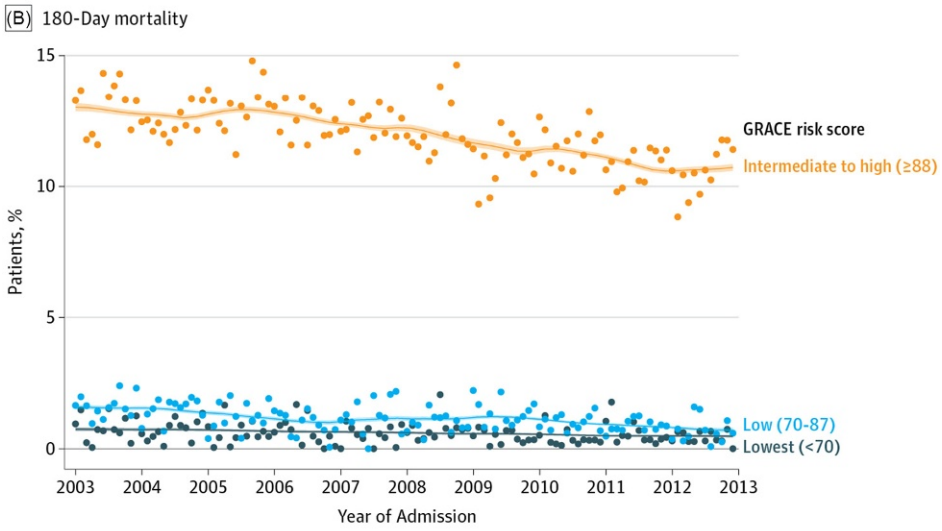
Comprehensive early risk assessment entails systematically integrating information from the initial clinical history, hemodynamic profile, physical exam, ECG, and biomarkers of cardiac injury to estimate prognosis. While risk assessment should always be tailored and interpreted in the context of each unique clinical scenario, an unstructured approach to risk stratification may lead to overemphasis of certain individual indicators and the neglect of others. Indeed, studies investigating the treatment-risk paradox have found that certain well-established and common prognosticators such as age, renal dysfunction, heart failure, and hemodynamic variables are systematically underrecognized. An integrated approach using validated risk scores can significantly improve risk stratification of NSTE-ACS and identify high-risk patients most likely to benefit from intensive therapies.

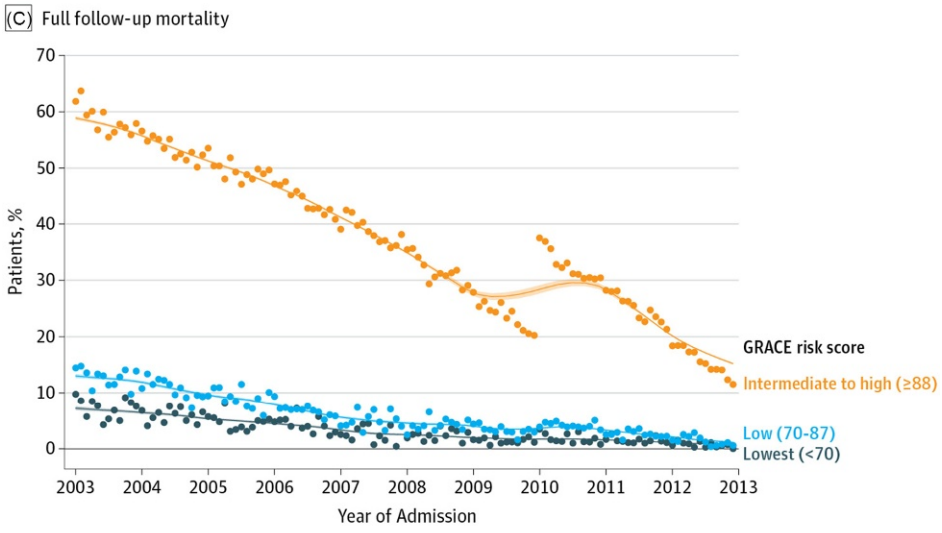
Moving forward, additional investigation is required to further elucidate physicians' attitudes toward risk assessment and their real-world application of standardized prognostic models. Current gaps in matching patient risk to appropriate intensity of treatment may be at least partly responsible for poor outcomes in NSTE-ACS. Quality improvement programs aimed at standardizing risk assessment at patient presentation may consequently improve decision-making and long-term outcome.

Appendix

Figure. Crude all-cause mortality for NSTE-ACS at 30 days, 180 days, and full follow-up (8.4 years) in the Myocardial Ischemia National Audit Project (MINAP) per month by GRACE risk score, 2003–2013







Median No. of patients per mo

Lowest	1	1	4	4	136	191	224	262	255	260	243
Low	2	2	5	4	169	242	270	317	347	350	324
Intermediate to high	18	15	34	48	1362	1709	1940	2292	2203	2175	1967

Legend: NSTE-ACS, non-ST elevation acute coronary syndrome; GRACE, Global Registry of Acute Coronary Events.

Curves were fitted using local polynomial smoothing. Median and range of patients per month for each year are based on complete case data for GRACE risk score only. Numbers of patients per month with lowest, low, and intermediate to high GRACE risk scores ranged from 0 to 301, 0 to 395, and 1 to 2406, respectively, across all time periods.

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Relevant website

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Non-ST-Elevation Myocardial Infarction: Management

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Patient Evaluation and Risk Assessment

The evaluation of patients with suspected acute coronary syndromes (ACS) is based on medical history—including chest pain characteristic evaluation and cardiovascular risk factor assessment—clinical presentation, electrocardiogram (ECG), and biomarkers (preferably high-sensitivity cardiac troponin) (Roffi et al., 2016). While an assessment of left ventricular (LV) function is indicated in all patients with ACS during hospitalization to guide management and assess prognosis, urgent echocardiography is indicated in case of hemodynamic instability or if life-threatening alternative pathologies are suspected, such as pulmonary embolism, aortic dissection, or cardiac tamponade (Roffi et al., 2016).

Based on this first assessment of noncardiac chest pain, unstable angina, non-ST-elevation myocardial infarction (NSTEMI) and ST-elevation myocardial infarction (STEMI) can be differentiated. After the diagnosis of NSTEMI is established, management includes medical therapy (as acute treatment and as secondary prevention) and invasive strategy with coronary angiography and, if appropriate, revascularization. The mainstays of medical therapy are antithrombotic treatment and control of cardiovascular risk factors. Guidelines recommend performing coronary angiography within 24 h of admission in patients with NSTEMI (Roffi et al., 2016). A subset of NSTEMI patients at very high risk, such as individuals with ongoing ischemic chest pain refractory to medical therapy, heart failure secondary to ACS, life-threatening arrhythmias, or cardiogenic shock, require immediate (i.e., within 2 h) coronary angiography (Roffi et al., 2016). The acronyms of the trials mentioned in the manuscript are spelled in **Table 1**.

Medical Therapy

General Measures

The main goal of initial medical therapy is the containment of the coronary thrombotic process and of myocardial ischemia, with subsequent relief of chest pain. In addition to the antithrombotic treatment, pharmacological management of NSTEMI aims to increase myocardial oxygen supply and to decrease myocardial oxygen demand. Oxygen supplement should be limited to patients whose hemoglobin saturation in arterial blood is $<90\%$ or in patients with respiratory distress or failure (Roffi et al., 2016). While no dedicated oxygen-supplementation trial has been performed in the NSTEMI setting, the AVOID trial randomized 441 STEMI patients with arterial blood oxygen saturation $\geq 94\%$ to routine oxygen supply and no oxygen. The study showed an increase in the rate of recurrent myocardial infarction (MI) in the oxygen group compared with the no oxygen group (5.5% vs. 0.9%, $p=0.006$) and increase in the incidence of cardiac arrhythmias, defined as sustained and nonsustained ventricular and atrial tachyarrhythmia requiring medical intervention in the oxygen group (40.4% vs. 31.4%, $p=0.05$). Finally, in a subgroup of 139 AVOID patients investigated with cardiac magnetic resonance at 6 months, an increase in myocardial infarct size (20.3 g vs. 13.1 g, $p=0.04$) was detected in patients receiving oxygen treatment (Stub et al., 2015).

Nitrates are coronary vasodilators that can be used to control the pain/ischemia in the absence of contraindications such as hypotension or recent intake of phosphodiesterase type 5 inhibitors (Schwartz and Kloner, 2010). While opiates may mask ongoing ischemia, their administration is appropriate to relieve chest pain in patients awaiting coronary angiography (Roffi et al., 2016),

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Table 1 Acronyms of clinical trials

ACCOAST	Comparison of prasugrel at the time of percutaneous coronary intervention or as pretreatment at the time of diagnosis in patients with non-ST-elevation myocardial infarction
AFTER EIGHTY	Invasive versus conservative strategy in patients aged 80 years or older with non-ST-elevation myocardial infarction or unstable angina pectoris
AVOID	Air versus oxygen in ST-segment-elevation myocardial infarction
COMFORTABLE AMI	Comparison of biolimus eluted from an erodible stent coating with bare metal stents in acute ST-elevation myocardial infarction
CURE	Clopidogrel in unstable angina to prevent recurrent events
FRISC-II	Fragmin and fast revascularization during instability in coronary artery disease—II
LEADERS FREE	Prospective randomized comparison of the BioFreedom biolimus A9 drug-coated stent versus the Gazelle bare-metal stent in patients at high bleeding risk
MATRIX	Minimizing adverse hemorrhagic events by transradial access site and systemic implementation of angiox
PEGASUS-TIMI 54	Prevention of cardiovascular events in patients with prior heart attack using ticagrelor compared with placebo on a background of aspirin-thrombolysis in myocardial infarction 54
PIONEER AF-PCI	Open-label, randomized, controlled, multicenter study exploring two treatment strategies of rivaroxaban and a dose-adjusted oral vitamin K antagonist treatment strategy in subjects with atrial fibrillation who undergo percutaneous coronary intervention
PLATO	Platelet inhibition and patient outcomes
PRODIGY	Prolonging dual-antiplatelet treatment after grading stent-induced intimal hyperplasia
TIMACS	Timing of intervention in patients with acute coronary syndromes
TRILOGY ACS	Targeted platelet inhibition to clarify the optimal strategy to medically manage acute coronary syndromes
TRITON-TIMI 38	Trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel–thrombolysis in myocardial infarction 38

though morphine can delay the onset of action of $P2Y_{12}$ inhibitors (Kubica et al., 2016; Parodi et al., 2015). Early beta-blocker therapy is recommended in NSTEMI patients who do not have contraindications (Chatterjee et al., 2013). A registry study including 34 661 patients with STEMI or NSTEMI identified patient characteristics associated with at higher risk of developing cardiogenic shock or death within 24 h of hospital admission if treated with beta-blockers: age >70 years, systolic blood pressure <120 mmHg, and heart rate >110 bpm (Kontos et al., 2011). Thus, early administration of beta-blockers should be avoided in these subgroups of patients prior to an assessment of LV function (Roffi et al., 2016). In patients with suspected vasospastic angina or chest pain/ischemia related to cocaine use, calcium channel blockers and nitrates should be used instead of beta-blockers (Roffi et al., 2016; Yasue et al., 1988).

Antiplatelet Therapy

Aspirin

Acetylsalicylic acid (aspirin) is an irreversible inhibitor of cyclooxygenase-1 activity. A meta-analysis of trials comparing aspirin with no aspirin in unstable angina patients showed that aspirin administration up to 2 years was associated with a significant 46% odds reduction in major vascular events (Antithrombotic Trialists' Collaboration, 2002). A nonenteric-coated formulation of aspirin as loading dose of 150–300 mg as soon as the diagnosis is made and a maintenance dose of 75–100 mg/day is recommended in all non-ST-elevation (NSTE)-ACS patients without contraindications such as documented severe allergy or ongoing bleeding (Antithrombotic Trialists' Collaboration, 2002; Lewis et al., 1983; Theroux et al., 1988).

Oral $P2Y_{12}$ inhibitors

Clopidogrel is an oral prodrug, a thienopyridine derivative and an irreversible inhibitor of the platelet $P2Y_{12}$ receptor (**Table 2**). In the CURE trial with 12 562 NSTE-ACS patients treated mainly conservatively—and in the subgroup of patients undergoing PCI (PCI-CURE, $N = 2658$),—the combination of clopidogrel and aspirin significantly reduced cardiovascular events compared with aspirin alone in patients with NSTE-ACS, independently of revascularization strategy, at a follow-up of 3–12 months (mean duration, 9 months) (Mehta et al., 2001; Yusuf et al., 2001). Ticagrelor (administered as 180 mg loading dose followed by 90 mg twice daily as maintenance) is an oral, reversible, inhibitor of the $P2Y_{12}$ receptor. The PLATO trial, including 18 624 patients with NSTE-ACS or STEMI, demonstrated the superiority of ticagrelor over clopidogrel in the overall study population and in the 11 080

Table 2 Pharmacological properties of $P2Y_{12}$ inhibitors

	Clopidogrel	*Prasugrel*	*Ticagrelor*	*Cangrelor*
Chemical class	Thienopyridine	Thienopyridine	Cyclopentyl-triazolopyrimidine	Stabilized ATP analogue
Administration	Oral	Oral	Oral	Intravenous
Dose	300–600 mg orally then 75 mg a day	60 mg orally then 10 mg a day	180 mg orally then 90 mg twice a day	30 μg/kg bolus and 4 μg/kg/min infusion
Dosing in CKD				
Stage 3 (eGFR 30–59 mL/min/1.73 m^2)	No dose adjustment	No dose adjustment	No dose adjustment	No dose adjustment
Stage 4 (eGFR 15–29 mL/min/1.73 m^2)	No dose adjustment	No dose adjustment	No dose adjustment	No dose adjustment
Stage 5 (eGFR <15 mL/min/1.73 m^2)	Use only for selected indications (e.g., stent thrombosis prevention)	Not recommended	Not recommended	No dose adjustment
Binding reversibility	Irreversible	Irreversible	Reversible	Reversible
Activation	Prodrug, with variable liver metabolism	Prodrug, with predictable liver metabolism	Active drug, with additional active metabolite	Active drug
Onset of loading dose effect[a]	2–6 hours[b]	30 min[b]	30 min[b]	2 min
Duration of effect	3–10 days	7–10 days	3–5 days	1–2 h
Withdrawal before surgery	5 days[c]	7 days[c]	5 days[c]	1 h
Plasma half-life of active $P2Y_{12}$ inhibitor[d]	30–60 min	30–60 min[e]	6–12 h	5–10 min
Inhibition of adenosine reuptake	No	No	Yes	Yes ("inactive" metabolite only)

ADP, adenosine diphosphate; *ATP*, adenosine triphosphate; *CKD*, chronic kidney disease; *eGFR*, estimated glomerular filtration rate.

[a]50% inhibition of ADP-induced platelet aggregation.

[b]Onset of effect may be delayed if intestinal absorption is delayed (e.g.,\ by opiate).

[c]Shortening may be considered if indicated by platelet function tests and low bleeding risk.

[d]Affecting the response to platelet transfusion.

[e]The distribution phase half-life is reported since it most likely reflects duration of clinically relevant plasma levels, while the corresponding elimination phase half-life is approximately 7 h.

Reproduced with permission from Roffi et al. (2016).

NSTE-ACS patients, enrolled regardless of the revascularization strategy, with a significant reduction of the primary endpoint of death from cardiovascular reasons, MI or stroke at 1 year. In the NSTE-ACS group, the hazard ratio [HR] for the primary endpoint was 0.83 (95% confidence interval [CI] 0.74, 0.93), $p=0.0013$ (Wallentin et al., 2009; Lindholm et al., 2014). Paralleling the overall study results, in the NSTE-ACS subgroup there was an increased risk of noncoronary artery bypass grafting (CABG)-related thrombolysis in myocardial infarction (TIMI) major bleeds in the ticagrelor group compared with the clopidogrel group (2.9% vs. 2.2%; HR 1.39 [95% CI 1.07, 1.80], $p=0.0131$), while no difference in life-threatening or fatal bleeds was observed (Lindholm et al., 2014). Prasugrel is a prodrug inhibiting the $P2Y_{12}$ receptor irreversibly. The TRITON-TIMI 38 trial, including 13 608 patients with NSTE-ACS or STEMI scheduled for PCI, demonstrated the superiority of prasugrel (60 mg loading dose followed by 10 mg once a day) over clopidogrel with respect to the composite endpoint of death from cardiovascular reasons, MI and stroke in the overall study populations and in the 10 074 NSTE-ACS patients at 15-month follow-up (relative risk [RR]:0.82 [95% CI 0.73, 0.93], $p=0.002$) (Wiviott et al., 2007; De Servi et al., 2014). Severe bleeding complications were significantly higher in the prasugrel NSTE-ACS subgroup both in terms of TIMI non-CABG major bleeds (2.4% vs. 1.8%; HR 1.40 [95% CI 1.05, 1.88], $p=0.02$) and fatal bleeds (0.4% vs. 0.1%; HR 4.19 [95% CI 1.58, 11.11], $p=0.002$) (De Servi et al., 2014). Prasugrel should not be used in patients with ongoing bleeding or with previous cerebrovascular event due to more frequent bleeding rates in this group of patients. Furthermore, no benefit was observed in patients >75 years of age or with low bodyweight: <60 kg (Wiviott et al., 2007). Finally, prasugrel was not superior to clopidogrel in NSTE-ACS patients treated conservatively in the TRILOGY ACS trial (Roe et al., 2012).

In all NSTEMI patients without increased risk of bleeding, ticagrelor and prasugrel are recommended in addition to aspirin for 12 months. While prasugrel administration is limited to patients undergoing PCI, ticagrelor can be administered independently of revascularization strategy (Wallentin et al., 2009; Wiviott et al., 2007). While the superiority of ticagrelor over clopidogrel is established, the timing of ticagrelor administration [i.e., prior to coronary angiography (pretreatment) or at the time of PCI] in patients undergoing coronary angiography still remains to be determined. Pretreatment with prasugrel is not recommended because the ACCOAST study—the only trial one testing $P2Y_{12}$ pretreatment versus no pretreatment in NSTE-ACS—showed no reduction in

ischemic events but an increase in major bleeding associated with prasugrel pretreatment compared with prasugrel administered at the time of PCI (Montalescot et al., 2013).

While standard duration of dual-antiplatelet therapy (DAPT), consisting of aspirin and a $P2Y_{12}$ inhibitor, is 12 months in NSTE-ACS, a shorter (i.e., 3–6 months) DAPT period may be applied in patients at high bleeding risk, while prolonged DAPT beyond 12 months may be considered in patients who did not have bleeding complications in the first year and who remain at high ischemic risk (e.g., patients with multiple stents, multivessel diffuse disease, or advanced diabetes-related coronary artery disease) (see section "Long Term Prevention" after NSTEMI).

Intravenous platelet inhibitors

Glycoprotein IIb/IIIa inhibitors (GPIIb/IIIa) (e.g., tirofiban, eptifibatide and abciximab) block the contact between fibrinogen and the glycoprotein IIb/IIIa receptor on the surface of platelets (Patrono et al., 2011). In a meta-analysis of randomized controlled trial (RCT) allocating 29 570 patients with NSTE-ACS to GPIIb/IIIa inhibitors or placebo, the overall benefit of this agents over placebo was modest and confined to patients undergoing PCI (Roffi et al., 2002). As the benefit of these agents in the era of potent $P2Y_{12}$ inhibitors (ticagrelor or prasugrel) has not been tested and the association is associated with an increased bleeding risk, recommended usage of GPIIb/IIIa inhibitors is currently limited to bailout of thrombotic complications during PCI (Roffi et al., 2016).

Cangrelor is an intravenous (i.v.) adenosine triphosphate (ATP) reversible analogue that binds with high affinity to the platelet $P2Y_{12}$ receptor and has a short plasma half-life (<10 min) (**Table 2**). It produces a highly effective inhibition of platelet aggregation immediately after i.v. bolus administration and allows for restoration of platelet function within 1–2 h of infusion discontinuation (Roffi et al., 2016). Although the benefit of cangrelor over placebo has been demonstrated in NSTE-ACS patients treated with aspirin and clopidogrel, its benefit in addition to a ticagrelor or prasugrel-based regimen remains to be demonstrated (Steg et al., 2013).

Anticoagulation

Several studies demonstrated the benefit of the anticoagulant therapy in patients with NSTEMI (Eikelboom et al., 2000). The major therapeutic targets in this setting are thrombin, factor Va and factor Xa. Parenteral anticoagulation is recommended in the initial management of patients with NSTEMI, in addition to antiplatelet therapy, irrespective of management strategy (Roffi et al., 2016). Recommended drugs include unfractionated heparin, enoxaparin, fondaparinux, and bivalirudin. Dosages according to renal function are detailed in **Table 3**.

Antithrombotic treatment in patients requiring oral anticoagulation

Management of patients requiring oral anticoagulation presenting with NSTEMI is a challenging condition due to the increased bleeding risk and the limited data from the RCT. Triple therapy is defined oral anticoagulant (OAC)—either a vitamin K antagonist (VKA) or a nonvitamin K antagonist oral anticoagulant (NOAC)—plus aspirin and a $P2Y_{12}$ inhibitor. Due to the prohibitive risk of bleeding if prasugrel and ticagrelor are used as part of the triple therapy, clopidogrel should be the only $P2Y_{12}$ inhibitor used in this setting (Roffi et al., 2016). For patients with NSTEMI at a low bleeding risk (e.g., HAS-BLED score ≤2), the 2015 ESC NSTE-ACS guidelines recommend triple therapy for 6 months, followed by dual therapy including OAC (VKA or NOAC) and aspirin (75–100 mg/day) or clopidogrel (75 mg/day) up to 12 months (Roffi et al., 2016). For patients with NSTEMI and high bleeding risk (e.g., HAS-BLED score ≥3), the same guidelines recommend triple therapy for 1 month and dual therapy up to 12 months or dual therapy from the very beginning and continued for 12 months, regardless of the stent type. Beyond 12 months, monotherapy with OAC is recommended for the majority of patients, while in individuals deemed at high risk of coronary events, dual therapy including OAC and clopidogrel or aspirin may be continued beyond 12 months. In association with antiplatelet agents, NOAC should be administered at the minimum approved dose for stroke prevention (Roffi et al., 2016).

Table 3 Dosing of anticoagulants in patients with normal and impaired renal function Reproduced with permission from Roffi et al. (2016)

	Recommendations		
Drug	*Normal renal function or stage 1–3 CKD (eGFR ≥ 30 mL/min/1.73 m²)*	*Stage 4 CKD (eGFR 15–29 mL/min/1.73²)*	*Stage 5 CKD (eGFR <15 mL/min/1.73 m²)*
Unfractionated heparin	• Prior to coronary angiography: 60–70 IU/kg i.v. (max 5000 IU) and infusion (12–15 IU/kg/h) (max 1000 IU/h), target aPTT 1.5–2.5 × control • During PCI according to ACT or 70–100 IU/kg i.v. in patients not anticoagulated (50–70 IU/kg if concomitant with GP IIb/IIIa inhibitors)	No dose adjustment	No dose adjustment
Enoxaparin	1 mg/kg s.c. twice a day	1 mg/kg s.c. once a day	Not recommended
Fondaparinux	2.5 mg s.c. once a day	Not recommended if eGFR <20 mL/min/1.73 m²	Not recommended
Bivalirudin	Bolus 0.75 mg/kg i.v., infusion 1.75 mg/kg/h[a]	Not recommended	Not recommended

ACT, activated clotting time; *aPTT*, activation partial thromboplastin time; *CKD*, chronic kidney disease; *eGFR*, estimated glomerular filtration rate; *IU*, international units; *i.v.*, intravenous; *kg*, kilograms bodyweight; *s.c.*, subcutaneous.

Recommendations for the use of drugs listed in this table may vary depending on the exact labeling of each drug in the country where it is used.

[a]Infusion dose 1.4 mg/kg/h if eGFR ≥30 and ≤60 mL/min/1.73 m².

Following publication of the 2015 ESC NSTE-ACS guidelines, two trials have been published that are of relevance for the management of patients requiring oral anticoagulation. First, the LEADERS FREE trial showed that among 2466 patients at high bleeding risk (879 requiring OAC) and receiving only 4 weeks of DAPT, those allocated to the drug-coated stent BioFreedom had better outcomes in terms of both efficacy and safety at 1 year compared with those receiving the bare-metal stent version of the stent (Urban et al., 2015). Of note, patients with ACS derived an even greater benefit from the drug-coated stents, as demonstrated by a statistical significant interaction between ACS status and benefit from device. These results may open the way for shorter triple therapy (e.g., 1 month) for all NSTEMI patients requiring oral anticoagulation. The PIONEER AF-PCI trial randomized 2124 patients with nonvalvular atrial fibrillation following successful PCI in a 1:1:1 ratio to rivaroxaban 15 mg once a day plus a $P2Y_{12}$ inhibitor for 12 months (group 1), rivaroxaban 2.5 mg twice a daily plus DAPT with aspirin and clopidogrel for 1, 6, or 12 months followed by rivaroxaban 15 mg and aspirin (group 2) or dose-adjusted VKA plus DAPT for 1, 6, or 12 months (group 3) followed by VKA and aspirin (Gibson et al., 2016). Clopidogrel was chosen for the majority of patients in all groups. The investigators had to state upfront (prior to randomization) the duration of triple therapy they recommended for each individual patient. The rates of clinically significant bleeding were lower in the two groups receiving rivaroxaban than in the group receiving VKA (16.8% in group 1, 18.0% in group 2 and 26.7% in group 3; HR for group 1 vs. group 3, 0.59; [95% CI 0.47–0.76], $p < 0.001$; HR for group 2 vs. group 3, 0.63; [95% CI 0.50–0.80], $p < 0.001$). The rates of death from cardiovascular causes, MI or stroke were not significantly different, but the trial was not appropriately powered to address ischemic events (Gibson et al., 2016). In particular, the trial was not powered to prove that patients in the rivaroxaban arms were adequately protected from stroke. Additional trials combining non-VKA or NOACS and antiplatelet agents are ongoing and may shed light on the best management of patients requiring both antiplatelet agents and oral anticoagulation.

In NSTEMI patients treated conservatively or undergoing CABG, the guidelines recommend dual therapy (i.e., OAC plus aspirin or clopidogrel) up to 1 year. Lastly, evidence based therapy should be administered also to elderly patients with NSTEMI but taking into account comorbidities, frailty and quality of life and by adapting of the dosage of antithrombotic agents according to patient's renal function and weight, when appropriate (Roffi et al., 2016; Andreotti et al., 2015).

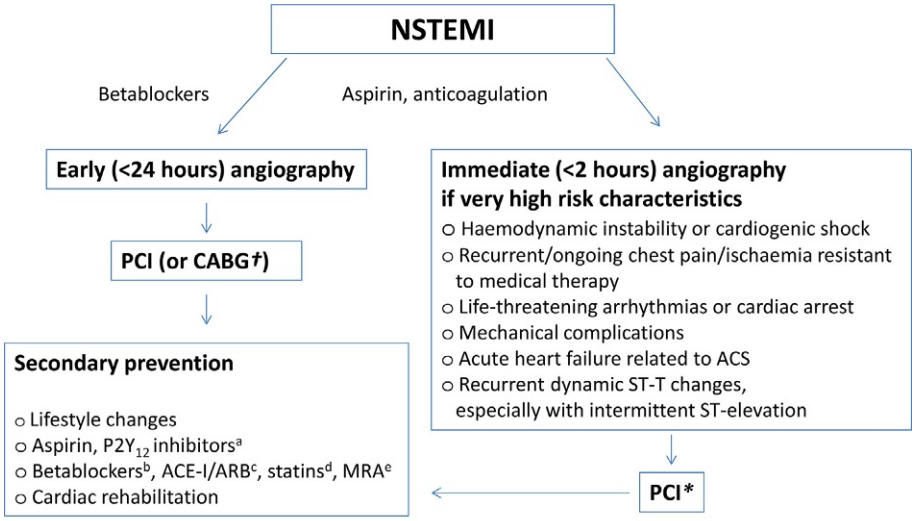
Invasive strategy

Benefit and Timing

Invasive strategy is defined by coronary angiography and, if appropriate, coronary revascularization by either PCI or CABG. Compared with a conservative approach, an invasive strategy was associated with a benefit in terms of ischemic event reduction in NSTEMI patients (Fox et al., 2010). The long-term follow-up of the FRISC-II study, including 2457 patients with NSTE-ACS allocated to either a conservative or an invasive strategy, showed the benefit of invasive management in terms of a difference in time to death or next nonfatal event at 15 years follow-up (Wallentin et al., 2016). Accordingly, the invasive strategy was associated with the postponement of death or recurrence of MI by a mean of 18 months (95% CI 6.8–29.6, $p = 0.002$) compared with the noninvasive treatment strategy. The benefit was mainly driven by postponement of new MI (Wallentin et al., 2016).

According to the 2015 ESC NST-ACS guidelines, NSTEMI patients are considered to be either at high risk or at very high risk. An immediate coronary angiography (<2 h) is recommended for very-high-risk patients, i.e., for individuals with either hemodynamic instability, cardiogenic shock, recurrent or continuous chest pain resistant to medical therapy, life-threatening arrhythmias or cardiac arrest, mechanical complications, acute heart failure related to ongoing ischemia or recurrent dynamic ST-T changes, especially with intermittent ST-elevation (**Fig. 1**). Due to poor prognosis in the absence of prompt revascularization, management of those patients should parallel to one of the STEMI patients (Roffi et al., 2016). Patients with out-of-hospital cardiac arrest and no ST elevation on ECG should be evaluated by a multidisciplinary team in the emergency room or intensive care unit. Conscious survivors with suspected NSTE-ACS should undergo immediate coronary angiography; comatose survivors without any definite noncoronary etiology for the cardiac arrest should also benefit from coronary angiography in the absence of contraindications and if the resuscitation conditions were favorable (Noc et al., 2014).

Early invasive strategy (<24 h) is recommended for all NSTEMI patients (Navarese et al., 2013; Mehta et al., 2009; Katritsis et al., 2011) (**Fig. 1**). This recommendation is based on several studies that compared early and delayed invasive strategy in patients with NSTE-ACS. A meta-analysis including 4013 patients with NSTE-ACS demonstrated that there was a reduction of recurrent ischemia (RR 0.59 [95% CI 0.38, 0.92], $p = 0.02$) and reduction of hospital stay (by 28% [95% CI 22, 35], $p < 0.001$) in favor of early invasive strategy (admission to angiography: 1.16–14 h) versus delayed invasive strategy (admission to angiography: 20.8–86 h) (Katritsis et al., 2011). However, there was no benefit with respect to death or MI (Katritsis et al., 2011). A more recent meta-analysis of 5370 patients with NSTE-ACS enrolled in seven RCTs and 77 499 patients followed in four observational studies compared early invasive strategy (<24 h) and delayed invasive strategy, data from RCTs showed a reduction of refractory ischemia (odds ratio [OR] 0.55 [95% CI 0.35–0.86]; $p = 0.008$) in favor of early invasive strategy (Navarese et al., 2013). Again, there was no significant difference in terms of MI and death (Navarese et al., 2013). The TIMACS study enrolling 3031 NSTE-ACS patients demonstrated the benefit of early invasive management (<24 h) versus delayed invasive strategy (median time 50 h) in prespecified analysis of high-risk patients with the GRACE score >140 at 6 months; early invasive strategy reduced the risk of death, MI, and stroke (HR 0.65 [95% CI 0.48–0.89], $p = 0.006$) in this group while there was no benefit in patients with the GRACE score ≤140 (HR 1.12 [95% CI, 0.81–1.56], $p = 0.48$; $p = 0.01$ for interaction) (Mehta et al., 2009). Real-life data suggests that the elderly population, defined as age



†In patients with advanced multivessel disease PCI vs. CABG discussed within the Heart Team based on: MI extension, LV function, lesions complexity (SYNTAX score), comorbidities, life expectancy.
**Indication for CABG in this setting limited to PCI failures or lesions non amenables to PCI.*

Fig. 1 Management of patients with non-ST-elevation myocardial infarction (NSTEMI). *ACE-I*, angiotensin-converting enzyme inhibitors; *ACS*, acute coronary syndromes; *ARB*, angiotensin receptor blockers; *CABG*, coronary artery bypass grafting; *LV*, left ventricular; *MI*, myocardial infarction; *MRA*, mineralocorticoid receptor antagonist; *NSTEMI*, non-ST-elevation myocardial infarction; *PCI*, percutaneous coronary intervention; *SYNTAX score*, synergy between percutaneous coronary intervention with Taxus and cardiac surgery score.[a]Dual antiplatelet therapy beyond 1 year is discussed in the text. [b]In the long term management, beta-blocker therapy is recommended in patiens with ejection fraction $\leq$40% in the absence of contraindications. [c]ACE inhibitors should be initiated to the patients with ejection fraction $\leq$40% or heart failure, hypertension or diabetes in the absence of contraindication. ARBs can be administered to patients who cannot tolerate ACE inhibitors. [d]In patients with NSTEMI should be administered a high-intensive statin therapy as early as possible unless contraindicated. Ezetimibe can be initiated to patients whose LDL level is $\geq$70 mg/dL ($\geq$1.8 mmol/L) despite the highest tolerated statin therapy. [e]MRAs (especially eplerenone) are recommended in NSTEMI patients with ejection fraction $\leq$35% and either heart failure or diabetes, unless contraindicated.

$\geq$75 years old, have less coronary angiography and revascularization compared with a younger population (Devlin et al., 2008). The AFTER EIGHTY study tested the invasive strategy (mean time 3 days) against the medical treatment alone in 457 patients aged 80 years or older with NSTEMI or unstable angina (Tegn et al., 2016). The primary outcome was a composite of MI, need for urgent revascularization, stroke and death. During a median follow-up of 1.5 years, the primary outcome occurred 40.6% in the invasive strategy group versus 61.4% in the conservative group (HR 0.53 [95% CI, 0.41, 0.69], $p=0.0001$). The difference in MI was also significant (HR 0.52 [95% CI, 0.35, 0.76], $p=0.001$). There was no difference of bleeding complications (Tegn et al., 2016). An early invasive strategy is recommended also in elderly patients, obviously taking into account comorbidities, frailty, mental status, estimated life expectancy and patient wishes (Roffi et al., 2016).

Percutaneous Coronary Intervention

PCI is the preferred strategy of revascularization for the majority of NSTEMI patients, particularly in the setting of ongoing ischemia (Windecker et al., 2014). In patients with advanced multivessel disease and no ongoing ischemia, the revascularization strategy (PCI vs. CABG) should be discussed within the heart team taking into account MI size, LV function, comorbidities, life expectancy and patient's preference (Roffi et al., 2016). New generation drug-eluting stents should be preferred in patients planned for PCI (Roffi et al., 2016). A prespecified analysis from the PRODIGY study compared everolimus-eluting stents (EES), zotarolimus-eluting endeavor sprint stents (ZES-S), paclitaxel-eluting stents (PES), and bare-metal stents (BMS) in 2 013 patients undergoing elective, urgent, and emergent coronary angioplasty (Valgimigli et al., 2014). At 30 days, each stent group received up to 6 or 24 months of clopidogrel therapy. The key efficacy endpoint was the 2-year major adverse cardiovascular events (MACE) (death, MI, or target-vessel revascularization), and the cumulative rate of definite or probable stent thrombosis was the key safety endpoint (Valgimigli et al., 2014). After 2 years of follow-up, the MACE was lowest in EES (19.2%; 95% CI 16.0–22.8), highest in BMS (32.1%; 95% CI 28.1–36.3) and intermediate in PES (26.2%; 95% CI 22.5–30.2) and ZES-S (27.8%; 95% CI 24.1–31.9) groups ($p=0.00029$) (Valgimigli et al., 2014). The 2 year incidence of stent thrombosis in the EES group (1%; 95% CI 0.4–2.2) was similar to that in the ZES-S group (1.4%; 95% CI 0.7–2.8), and it was low compared with the PES (4.6%, 95% CI 3.1–6.8) and BMS (3.6%; 95% CI 2.4–5.6) groups ($p=0.0001$) (Valgimigli et al., 2014).

The COMFORTABLE-AMI trial randomized 1161 patients with STEMI to biolimus-eluting stent (BES) versus BMS, and at 2 years, primary end point (cardiac death, target vessel, and target lesion revascularization) was in favor of BES-treated patients compared

with BMS-treated patients (HR: 0.48 [95% CI 0.31–0.72], $p<0.001$) (Raber et al., 2014). Differences in the primary end point were driven by a decline in target lesion revascularization (3.1% vs. 8.2%; $p<0.001$) and target-vessel reinfarction (1.3% vs. 3.4%; $p=0.023$) (Raber et al., 2014).

Radial access should be preferred instead of femoral approach in ACS (Roffi et al., 2016). In the MATRIX trial, 8404 ACS patients were randomized to radial or femoral access (Valgimigli et al., 2015). Two coprimary outcomes were defined: 30-day MACE (including death, MI, or stroke) and 30-day net adverse clinical events [including MACE or non-CABG Bleeding Academic Research Consortium (BARC) major bleeding]. The first coprimary outcome was (RR 0.85 [95% CI 0.74–0.99] and a two-sided $p=0.0307$) formally nonsignificant at the prespecified α of 0.025 (Valgimigli et al., 2015). The second coprimary endpoint was in favor of radial access (RR 0.83 [95% CI 0.73–0.96], $p=0.0092$) (Valgimigli et al., 2015). Also, radial access was associated with a lower all-cause mortality (RR 0.72 [95% CI 0.53–0.99], $p=0.045$). Rates of cardiac mortality, stroke and MI were not significantly different. Major BARC 3 or 5 bleeding was significantly reduced in the radial group (RR 0.67 [95% CI 0.49–0.92], $p=0.013$) (Valgimigli et al., 2015).

Lastly, for patients on OAC, PCI should be performed without cessation of VKA or NOAC. In patients on VKA, if the international normalized ratio (INR) is more than 2.5, no parenteral anticoagulant is needed at the time of PCI (Roffi et al., 2016). In patients on NOAC, during PCI a low-dose parenteral anticoagulant should be added regardless of timing of last dose (e.g., UFH 60 IU/kg i.v. or enoxaparin 0.5 mg/kg i.v.) (Roffi et al., 2016). Aspirin is recommended in patients under OAC with NSTEMI in the acute settings but a $P2Y_{12}$ inhibitor should not be used as a pretreatment therapy (i.e., prior to coronary angiography) in this group of patients (Roffi et al., 2016).

Coronary Artery Bypass Grafting

Except emergent cases, the management of NSTEMI patients with advanced multivessel disease should be discussed in a heart team (Roffi et al., 2016). Once heart team decision has proposed CABG, timing of surgery should be individualized according to patient's clinical condition. When there is an emergent situation (e.g., hemodynamic instability, ongoing ischemia or major arrhythmias) and PCI is not appropriate, CABG should be performed urgently (Roffi et al., 2016; Windecker et al., 2014; Parikh et al., 2010). Perioperative bleeding is an additional dilemma. Except urgent conditions, CABG should be performed after cessation of ticagrelor and clopidogrel for 5 days and cessation of prasugrel for 7 days, respectively (Held et al., 2011; Nijjer et al., 2011). Due to patient's bleeding risk, aspirin should be continued until CABG and, in the absence of contraindications from a surgical perspective, resumed within 6 h after the surgery (Roffi et al., 2016). RCTs are needed to compare PCI and CABG in patients with NSTEMI. While the SYNTAX score may be beneficial to assess the degree of complexity of coronary lesions, it is important to remember that the SYNTAX trial enrolled mainly patients with stable coronary artery disease (Palmerini et al., 2011).

Long-Term Prevention after NSTEMI

Intensive risk factor adjustment to improve the prognosis is the main goal of secondary prevention of patients with NSTEMI. Secondary prevention comprises medical therapy, lifestyle changes, and cardiac rehabilitation. As mentioned previously, guidelines recommended up to 1-year duration of DAPT in the majority of patients with NSTEMI. DAPT beyond 1 year was also investigated in multiple trials. PEGASUS-TIMI 54 trial including 21 162 patients with the history of MI 1–3 years earlier, compared ticagrelor at a dose of 90 mg twice daily, ticagrelor at a dose of 60 mg twice daily, or placebo at a median follow-up of 33 months. Nearly all the patients (99.9%) received aspirin (Bonaca et al., 2015). The study showed a reduced rate of cardiovascular death, MI or stroke with ticagrelor; 90 mg of ticagrelor group versus placebo group (HR 0.85 [95% CI 0.75, 0.96], $p=0.008$) and 60 mg of ticagrelor group versus placebo group (HR 0.84 [95% CI 0.74, 0.95], $p=0.004$). There were increased rates of major bleeding events with ticagrelor (2.60% with 90 mg, 2.30% with 60 mg, and 1.06% with placebo, $p<0.001$). All-cause mortality was not different significantly between the groups (Bonaca et al., 2015). A recent meta-analysis of 33 435 high-risk patients with prior MI compared more than a year of DAPT and aspirin alone, over a mean of 31 months of follow-up (Udell et al., 2016). DAPT beyond a year decreased the risk of MACE defined as a composite of cardiovascular death, nonfatal MI and nonfatal stroke (6.4 vs. 7.5%; RR 0.78 [95% CI 0.67, 0.90], $p=0.001$) and reduced cardiovascular death (2.3 vs. 2.6%; RR 0.85 [95% CI 0.74, 0.98], $p=0.03$) compared with aspirin alone. All-cause mortality did not differ significantly between the groups. In extended DAPT group, there was an increase risk of major bleeding (1.85 vs. 1.09%; RR 1.73 [95% CI 1.19, 2.50]; $p=0.004$) but not of fatal bleeding (0.14 vs. 0.17%; RR 0.91 [95% CI 0.53, 1.58]; $p=0.75$) (Udell et al., 2016). Due to contemporary evidences, $P2Y_{12}$ inhibitor administration in addition to aspirin beyond 1 year may be considered after careful assessment of the ischemic and bleeding risks of the patients (Roffi et al., 2016).

In patients with NSTEMI should be administered a high-intensive statin therapy as early as possible unless contraindicated (Roffi et al., 2016). Ezetimibe can be initiated to patients whose LDL level is $\geq$70 mg/dL ($\geq$1.8 mmol/L) despite highest tolerated statin therapy (Roffi et al., 2016; Cannon et al., 2015). An angiotensin-converting enzyme inhibitor should be initiated to the patients with ejection fraction (EF) $\leq$40% or heart failure, hypertension, or diabetes if there is no contraindication. Angiotensin receptor inhibitors can be administered to patients who cannot tolerate ACE inhibitors. Beta-blocker therapy should be administered to patients with EF $\leq$40% in the absence of contraindications in the long-term management. Smoking cessation, physical activity, and appropriate diet should be recommended to all patients with NSTEMI (Roffi et al., 2016). Cardiac rehabilitation should be advised to patients after discharge in order to regulate the lifestyle and promote the exercise capacity (Heran et al., 2011).

Key Messages

- Aspirin is indicated in all NSTEMI patients as soon as the diagnosis is made and continued indefinitely.
- The $P2Y_{12}$ inhibitors ticagrelor and prasugrel are preferred over clopidogrel and should be continued for 12 months in absence of contraindications. While ticagrelor can be administered independently of the revascularization strategy, prasugrel should be administered only at the time of PCI.
- Parenteral anticoagulation should be administered in the acute phase of NSTEMI in all patients in addition to antiplatelet therapy, irrespective of management strategy.
- Early (i.e., within 24 h) coronary angiography and, if appropriate, revascularization are indicated in NSTEMI patients, while in the presence of ongoing/refractory ischemia, hemodynamic instability, or life-threatening arrhythmias immediate invasive strategy (i.e., within 2 h) is indicated.
- Radial access is recommended over of the femoral one for coronary angiography and PCI.
- NSTEMI patients with advanced multivessel disease and no ongoing ischemia should be discussed within the heart team to propose the most appropriate revascularization strategy (PCI or CABG).
- Elderly patients should be offered early invasive strategy. However, comorbidities, frailty, mental status, estimated life expectancy and patient wishes should be taken into account.
- Secondary prevention, including drug therapy (and notably statins), life style changes, and cardiac rehabilitation, is critical in long-term prognosis of NSTEMI patients.

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Nutrition—Macronutrients

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Introduction

Cardiovascular disease (CVD) is a chronic inflammatory disease mediated by endothelial damage and circulating blood lipids. Atherosclerosis is affected by abnormal blood lipid and lipoprotein levels, elevated blood pressure, and smoking as well as other risk factors (Glass and Witztum, 2001). Recommended nutritional interventions influence modifiable risk factors and can significantly reduce the progression of atherosclerosis and CVD. These modifiable and nonmodifiable risk factors are shown in **Table 1** (Glass and Witztum, 2001; Sacco, 2011).

High levels of low-density lipoprotein-cholesterol(LDL-C), as well as high levels total cholesterol concentrations, are strong contributors to atherogenesis (Glass and Witztum, 2001). The risk of atherosclerosis is inversely related to high-density lipoprotein-cholesterol (HDL-C) levels that are due to the mediation of reverse cholesterol transport (Gordon et al., 2016). Smoking is a major risk factor for the development of atherosclerosis and is associated with increased triglyceride and decreased HDL-C concentrations (Campbell e,t al., 2008). Another major risk factor for atherosclerosis is high blood pressure, which causes endothelial injury in the artery wall (Campbell e,t al., 2008). Other risk factors, such as overweight, high blood glucose levels, low physical activity, and not healthy diet, play a major role in the progression of atherosclerosis (Falk, 2006). A chronic state of inflammation presented as elevated levels of C-reactive protein (CRP), and other proinflammatory cytokines also has been found to promote atherosclerotic plaque development. It is important that targeting these risk factors is to reduce coronary artery disease. Beneficial effects on reducing mortality risk are also achievable within the general population via combined dietary change (−45%), incorporation of moderate-intensity physical activity at 40%–60% VO_2 max for at least 30 min per day on at least 5 days per week (−25%), moderation of alcohol intake to two drinks per day of women and three drinks per day or men (−20%), and cessation of smoking (−45%) (Iestra et al., 2005).

Role of the Macronutrients

The macronutrient composition of dietary patterns affects lipids and lipoproteins and, consequently, CVD risk. A key question that has been addressed by many researchers concerns which macronutrient should replace calories provided by saturated fat. A metaanalysis of 60 controlled trials by Mensink (Mensink et al., 2003) shown that lipid profile were affected when carbohydrates constituting 1% of energy are replaced isoenergetically with saturated, *cis* monounsaturated, polyunsaturated, or trans monounsatureded fatty acids. Saturated fatty acids (SFAs) increased LDL-C relative to carbohydrates. *cis* monounsaturated fatty acids (MUFAs) were decreasing but trans MUFAs were increasing LDL-C relative to carbohydrate. Polyunsaturated fatty acids (PUFAs) resulted in the greatest LDL-C reduction with slight but significant increase in HDL-C. The total cholesterol: HDL-C ratio did not change when SFA was replaced with carbohydrate; however, the ratio decreased when carbohydrate was replaced with *cis* MUFA or PUFA. In addition, replacing carbohydrates with any type of fatty acid resulted in a lower triglyceride levels. Additional each macronutrients affect CVD risk through a variety of mechanisms that will be discussed in the following section.

CVD and Carbohydrate

In clinical studies, an increase in dietary energy from carbohydrates usually is associated with a moderate increase in fasting plasma triglycerides (TG) levels, a reduction in HDL-C levels, and increased LDL-C levels, which impact adversely on CVD risk. However, no clear evidence has shown that the quantity of dietary carbohydrates alters the risk of CVD independently. According to a

Table 1 Modifiable and nonmodifiable risk factors for atherosclerosis

Nonmodifiable	*Modifiable*	*Goals for modifiable risk factors*
• Age	• Weight	BMI: 18.5–24.9 kg/m^2
• Sex	• Lipid profile	Total cholesterol: <200 mg/dL (5.2 mmol/L)
• Family history/genetics		*LDL-C*:
• Race/ethnicity		Very high risk individuals, <70 mg/dL (1.8 mmol/L)
		High-risk individuals, <100 mg/dL (2.6 mmol/L)
		With optional goal of <70 mg/dL (1.8 mmol/L)
		Moderate-risk individuals, <130 mg/dL (3.4 mmol/L)
		HDL-C:
		Men: ≥40 mg/dL (1.0 mmol/L)
		Women: ≥50 mg/dL (1.3 mmol/L)
		Triglycerides: <150 mg/dL (1.7 mmol/L)
	• Blood pressure	Systolic: <120 mmHg
		Diastolic: <80 mmHg
	• Glycemic control	Fasting blood glucose: <100 mg/dL (5.6 mmol/L)
	• Physical activity	150 min of moderate activity or 75 min of vigorous activity per week
	• Smoking	Stop smoking
	• Diet	Follow a healthy diet
	• Stress	Keep stress as low as possible



Fig. 1 Weighted mean differences in high-density lipoprotein level after 6 (A) and 12 (B) months of follow-up. *Carb*, carbohydrates; *CI*, confidence interval; *UI*, uncertainty interval. To convert cholesterol levels to millimoles per liter, multiply by 0.0259 (Nordmann et al., 2006).

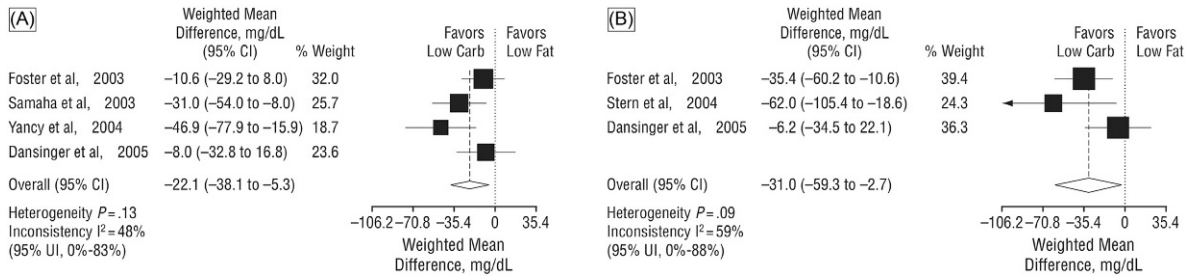

Fig. 2 Weighted mean differences in triglyceride level after 6 (A) and 12 (B) months of follow-up. *Carb*, carbohydrates; *CI*, confidence interval; *UI*, uncertainty interval. To convert triglyceride levels to millimoles per liter, multiply by 0.0113 (Nordmann et al., 2006).

metaanalysis of five randomized controlled trials, it is found that TG and HDL-C values changed more favorably in individuals assigned to low-carbohydrate diets (after 6 months, for triglycerides, weighted mean difference, −22.1 mg/dL (−0.25 mmol/L); and for HDL-C, weighted mean difference, 4.6 mg/dL (0.12 mmol/L) (**Figs. 1** and **2**), but total cholesterol and LDL-C values changed more favorably in individuals assigned to low-fat (LF) diets (Nordmann et al., 2006). Generally, very low-carbohydrate diets restrict energy from carbohydrate to <20–30 g/day, while very LF diets restrict total fat intake to <10%–20% of energy.

Although low-carbohydrate diets have risen in popularity, evidence from intervention trials on the benefit and risks of low-carbohydrate diet to achieve weight loss and modify CVD factors is limited. Furthermore, little is known about the long-term health effects on these diets; micronutrient inadequacy may occur when carbohydrate intake is very low (Bolton-Smith and Woodward, 1995). In conclusion, consuming a diet too high or too low in either fat or carbohydrate may result in adverse health effect, and the relative proportions of the two within the diet should target specific CVD risk factors and personal preferences within current dietary

recommendations. According to 2015–20 Dietary Guidelines for Americans, the acceptable macronutrient distribution range for carbohydrate is 45%–65% of energy.

Glycemic index (GI) and glycemic load (GL) quantify the postprandial blood glucose response to food containing carbohydrate (Jenkins et al., 1981). Specifically, GI is used to compare to increase blood glucose levels (Ludwig, 2002). A high-GI diet may lead to postprandial hyperglycemia and hyperinsulinemia, which are found to increase CVD risk by producing oxidative stress (Lefebvre and Scheen, 1998). However, the evidence for an association between high GI or GL and CVD risk remains limited and inconclusive (Kurotani et al., 2013).

CVD and Fat

Saturated Fatty Acid

Strong evidence indicates a positive association between dietary SFA intake and increased total cholesterol and LDL-C, as well as increased CVD risk (Sanders, 2013). Therefore, reducing SFA intake is a primary focus of dietary interventions to lower LDL-C. Several study indicated that for every 1% increase in energy from SFAs, LDL-C increases by 0.033–0.045 mmol/L and HDL-C by 0.011–0.013 mmol/L. Reducing dietary SFAs typically is associated with an increase in some other macronutrient. The nutrient(s) replacing SFA(s) will differentially affect blood lipid levels (Clarke, et al., 1997; Hegsted et al., 1993; Mensink and Katan, 1992).

Monounsaturated Fatty Acid

MUFAs replacement of SFA for an improvement in blood lipids is reported (Miller et al., 2016). Clinical evidence has shown that MUFA substitution for SFA decreases LDL-C (Berglund et al., 2007). Both MUFA and carbohydrate lower LDL-C levels when replacing SFA, but MUFA can increase HDL-C levels versus carbohydrate, lower the TC:HDL-C ratio, and potentially decrease apolipoprotein B, the main apolipoprotein in LDL-C (Garg, 1998; Mensink et al., 2003).

Primate studies have shown that MUFAs are more thermogenic than PUFAs, eliciting effects similar to SFAs (Rudel et al., 1998, 1995). According a metaanalysis by Cao et al. (2009), it is reported that moderate-fat (MF; average 23.6% of total kcal as MUFA) and LF (average containing 11.4% of total kcal as MUFA) diets both lowered TC and LDL similarly. However, MF diets increased HDL-C and decreased TG compared with LF diets. Thus, the TC: HDL-C ratio was significantly reduced after MF diets. Predicted change in CVD risk estimated that MF diets led to greater reduction in CVD risk compared with LF diets for both men and women. Additional research is needed to explain why animal studies and human studies differ in how MUFAs affect CVD risk.

Polyunsaturated Fatty Acids

It is shown that PUFAs improve in blood lipids, particularly when PUFAs replace SFAs in the diet (Bjermo et al., 2012). PUFAs have multiple double bonds and consequently are more fluid than SFA (Akinshina et al., 2016). Omega-3 (*n*-3) and omega-6 (*n*-6) RUFAs have their first double bond at three and six carbons from the omega end of the fatty acid, respectively. These are essential nutrients that cannot be synthesized by human due to the lack of an enzyme required to insert a *cis* double bond into a carbon backbone. Human can synthesize long-chain (LC) *n*-3 or *n*-6 fatty acids from the essential, parent fatty acids obtained from the diet, albeit at a low rate. The parent fatty acid for the *n*-3 series is α-linolenic acid (ALA), and the parent fatty acid for the *n*-6 series is linoleic acid (LA). **Fig. 3** displays the metabolism of both *n*-3 and *n*-6 FAs (Holligan et al., 2012).

The cardioprotective benefits of marine *n*-3 fatty acids were discovered in the 1970s by Danish researchers assessing the fish-rich diet and CVD occurrence of Greenland Inuits. Since this ecological observation, population, clinical, and basic investigations have confirmed the prospective effect of both marine- and plant-derived *n*-3 FAs against CVD. Flaxseed, walnuts, flaxseed oil, walnut oil, and canola oil are sources of ALA, and cold water fish such as salmon, mackerel, herring, and sardines are the species of seafood that are the richest sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Harris et al., 2008). The evidence for plant-based *n*-3 FAs (rich in ALA) is less conclusive than that for marine-based *n*-3 FAs, EPA, and DHA (Wang et al., 2006).

The association between coronary heart disease (CHD) mortality and fish intake was assessed in a metaanalysis of over 220,000 persons who were followed for an average of 11.8 years (He et al., 2004b). In contrast to non-fish-eating individuals, persons who consumed fish $\geq$5 times per week had a 38 decrease in CHD mortality risk. In addition, for every 20 g/day increase in fish intake there was a 7% decrease in risk of mortality due to coronary events. Also, a moderate intake of one to three servings of fish each month significantly reduced the risk of stroke (9%) and CHD mortality (11%) (He et al., 2004a,b).

LA is primary *n*-6 FA in the diet, accounting for 85%–90% of *n*-6 PUFA intake (Harris et al., 2009). It is metabolically converted to arachidonic acid (AA); however, the amount of AA in cell membranes is tightly regulated so that only about 0.2% of LA is converted (Hussein et al., 2005). AA is provided by meat, poultry, eggs, and some species of fish, while LA is found in vegetable oils such as corn, soybean, and safflower oil, as well as many seeds and nuts. Results from metaanalysis of 60 feeding studies indicated that substituting PUFAs (between 0.6% and 28.8% energy from *n*-6 FAs) for carbohydrate produced favorable effects on the ratio of total to HDL cholesterol in comparison with other fatty acids (Mensink et al., 2003). However, there are suggestions for decreasing LA intake which have been based on the inflammatory nature of CHD and the fact that AA is a major substrate for the production of several proinflammatory molecules (Libby, 2006). In summary, the evidence for the benefits of *n*-6 FA intake and CHD risk is

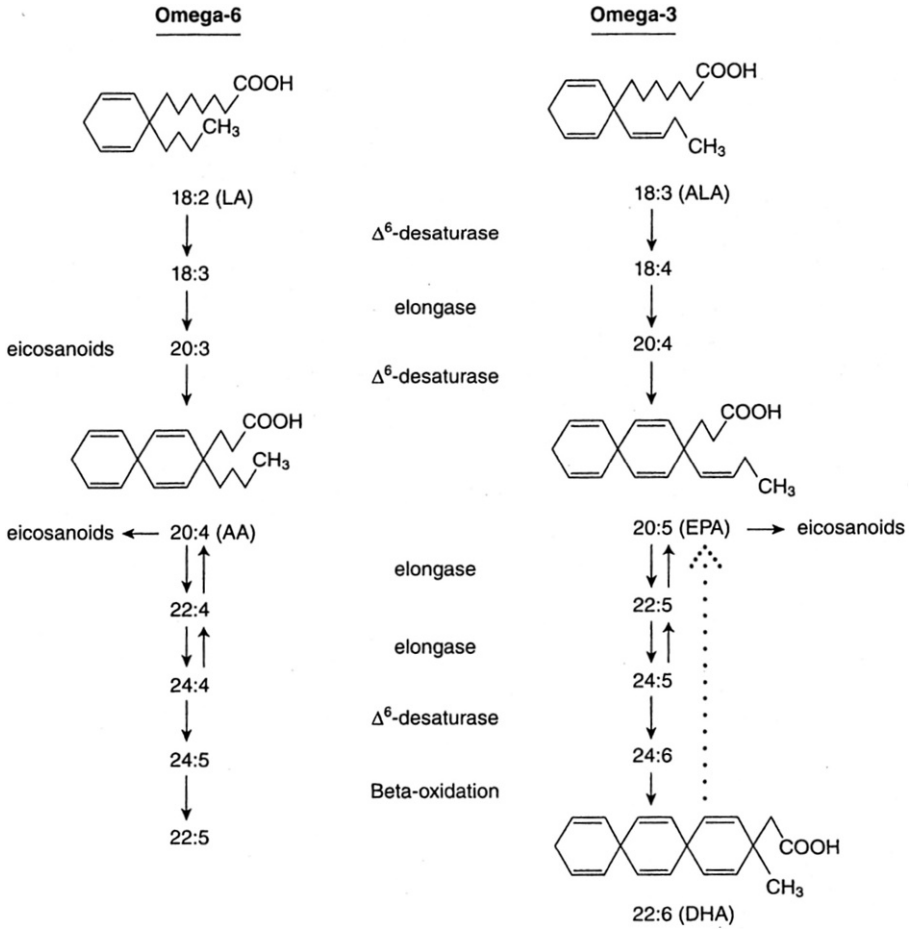

Fig. 3 The metabolism of omega-3 and omega-6 PUFAs. Questions remain about whether DHA is retroconverted to EPA, as shown by the *dotted arrow*.

greater than the evidence to prove otherwise. Estimates show that the ratio of *n*-6 to *n*-3 FAs in the human diet has changed overtime form a ratio of 1:1 to 10:1 or greater, the latter of which is representative of that found in a typical Western diet (Simopoulos et al., 2000).

Intake of long chain *n*-3 FAs is still the most efficient way to increase blood levels of EPA + DHA and thereby decrease risk for CHD, regardless of LA intake. Thus, increasing LC *n*-3 FAs rather than reducing *n*-6 FAs is recommended (Harris, 2006).

Trans Fatty Acid

Trans fatty acids (TFAs) are classified as unsaturated fatty acids containing at least on unsaturated, nonconjugated, double bond in the trans configuration. Industrial TFAs are produced during partial hydrogenation of vegetable oils TFA consumption is of particular concern given the range of its effects on CVD risk. Observational studies and randomised controlled trial (RCTs) have shown adverse effects on lipids such as increased LDL-C levels, reduced HDL-C levels, and increased TC:HDL-C ratio, a proinflammatory response with elevated IL-6 and CRP levels, and TNF-α activity, as well as endothelial dysfunction (Mozaffarian et al., 2009). Epidemiological and clinical studies have shown consistently that TFAs increase cardiovascular risk and adversely affect blood lipids (Ascherio et al., 1999). Studies also have shown that high TFA intakes are associated with increased CHD incidence (Oh et al., 2005), increased levels of inflammatory markers, and impaired endothelial function (Mozaffarian et al., 2004).

CVD and Protein

Protein is a compound in which 20 kinds of amino acids are peptide bonded, nine kinds are essential amino acids. Protein is decomposed and absorbed into amino acids, dipeptides, and tripeptides; reaches the liver via the portal vein; merges with amino acids from body proteins; and is recombined into necessary proteins for the human body. The recommended amount of protein is determined by considering the individual differences in the estimated average requirement calculated by the nitrogen dosing experiment, and it is about 1 g/kg of body weight in adults.

In a cohort study targeting Swedish women, the incidence of CVD was higher as high protein low-carbohydrate diet (Lagiou et al., 2012). It was reported to increase the incidence of CVD in high-protein dietary groups above the recommended amount (Pan et al., 2011), and excessive intake of protein increased the risk of developing diabetes and CVD (Wang et al., 2010).

For obese people, high-protein diet (energy ratio 34%) due to intake energy restrictions improved blood lipids, glucose, insulin and had a positive effect on risk factors for CVD (Clifton et al., 2008). When the effect of high protein (energy ratio 30%) was compared with ordinary protein (15%) under the restriction of energy intake and observation for 4 months, body fat decreased by −22% significantly in high protein diet. Observation without energy limitation for 8 months showed a significant improvement in body composition, TG and HDL-C in high-protein diet (Layman et al., 2009). On the other hand, there was the report that no significant difference was observed in changes in body weight, abdominal circumference, serum LDL cholesterol, triglyceride, systolic blood pressure, diastolic blood pressure, fasting blood glucose, HbA1c, by metaanalyzing the results of intervention studies over 12 months by comparing high-protein food with an energy ratio of 30% or more and normal protein food for CVD and metabolic syndrome (Schwingshackl and Hoffmann, 2013).

However, in studies of Americans, it was reported that the high-protein food group with vegetable protein had significantly lower total mortality and cardiovascular mortality than the animal high-protein food group (de Koning et al., 2011; Fung et al., 2010).These studies suggest that the food source affects the onset of CVD along with protein intake. Vegetarians that are dependent on vegetable proteins tend to have lower CHD mortality than nonvegetarians (Fraser, 2009). Arginine is contained in vegetable protein, and lysine is contained in a large amount in animal protein. The 35-day randomized controlled crossover study was conducted using a high-ratio (lysine/arginine) diet and a low-ratio diet; there was no decrease in serum cholesterol and LDL-C in the low ratio diet (Vega-Lopez et al., 2010). Regarding the relationship between protein and hypertension, it has been reported that the protein has a mild antihypertensive effect in patients who have not yet been treated and whose blood pressure is high (Appel et al., 2005). Soybean protein (Dong et al., 2011), dairy products, and low-fat dairy products (Hodgson et al., 2012; Ralston et al., 2012) also have a hypotensive effect, but its action is minor and should be taken in a balanced manner considering combination with other dietary factors.

CVD and the Macronutrients Balance

Macronutrients, also called energy providing nutrients, produce 4 kcal of protein, 9 kcal of lipid, and 4 kcal of carbohydrate per gram.

As for proper intake of macronutrients, it is necessary to consider the proportion (% energy) of total nutrient intake together with respective quantity and quality from both aspects of avoiding excess and deficiency of each nutrient and prevention of CVD. The intake of macronutrients, for the same ingested energy, if increasing certain nutrients results in a decrease in other nutrients, it is necessary to intake three appropriate proportions of macronutrients.

Among the total energy intake, it is important to secure the recommended amount of protein in the first place. It is effective for improving blood lipids by enabling early weight loss by high-protein diet. But the American Heart Disease Association AHA recommends 15%–20% in terms of energy ratio, to avoid high-protein diet that extremely limits carbohydrates and specific foods (St Jeor et al., 2001).

An extremely low-carbohydrate diet is an excessive intake of protein and lipids, which increases the risk of developing diabetes and CVD (Kurotani et al., 2013; Sluijs et al., 2010).

For example, if the protein energy ratio exceeds 20%, the risk of developing diabetes increases and the CVD increases (Wang et al., 2010). According to a metaanalysis using the interventional study of a high-protein diet with an energy ratio of 30% or more and a normal protein diet, a significant improvement was observed in body weight, body fat, and triglyceride in a short period of 12 weeks on average (Wycherley et al., 2012). In the long term of 12 months or longer, there was not significant difference in changes in body weight, abdominal circumference, serum LDL cholesterol, triglyceride, systolic blood pressure, diastolic blood pressure, fasting blood glucose, HbA1c (Schwingshackl and Hoffmann, 2013). Although high-protein diet has good results in a short period of time, many studies have shown that long-term intervention tests are preferable for prevention of CVD. From the recent systematic review (Pedersen et al., 2013), from the viewpoint of ensuring safety, it is advisable to keep the recommended amount of protein to keep the energy ratio not to exceed 20%.

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Nutrition: Soy and Fish

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Introduction: Longevity-Related Diets

Longevity is the wish of mankind. As was said by William Osler, a man is as old as his arteries, two major cardiovascular diseases (CVD) stroke and coronary heart diseases (CHD) are related to vascular aging, and greatly influence life expectancy and longevity (World Health Organization, 2016). Among them CHD is more closely related to average life expectancy because CHD is more prevalent in younger age compared with stroke (Yamori, 2008).

World Health Statistics indicates Japanese have been maintaining the world longest life expectancy. In particular females have kept the number one position since 1985 (UNDP, 2015) and their CHD mortality is maintained at the lowest in the developed countries in the world. Therefore, the diets good for lowering the prevalence of CHD are related to longevity, and Japanese as well as Mediterranean diets, both regarded as good for CHD reduction and longevity (Willett et al., 1995), are similar in the custom of eating much seafood, vegetables, fruits and nuts, or beans (Pallauf et al., 2013; Aiello et al., 2016).

Nutritional merits of these foods, particularly of fish and soy beans, were first experimentally demonstrated in rat models developing stroke genetically (stroke-prone spontaneously hypertensive rats, SHRSP) (Yamori, 1994), and the benefits of fish and soy intakes were further investigated epidemiologically by estimating the biomarkers of fish and soy in 24-hour urine (24U) in WHO-coordinated Cardiovascular Diseases and Alimentary Comparison (CARDIAC) Study covering over 60 populations in the world (Yamori, 2006a,b, 2008; Yamori et al., 2006, 2010, 2015, 2017).

Therefore, in this article, the nutritional impact of fish and soy on CVD is described from experimental and epidemiological findings and accumulating supportive evidence for beneficial effect of fish and soy from various intervention studies and the mechanisms involved in the effects are briefly reviewed.

Experimental Evidence for CVD Prevention by Soy and Fish Intakes

Genetic stroke models in rats were established by the successive breeding of the offspring of spontaneously hypertension rats (SHRs) which were confirmed by autopsy to have died with cerebrovascular hemorrhage and/or infarction (SHRSP) (Okamoto et al., 1974). Since they developed stroke genetically without exception, they were the appropriate model for experimental prevention of stroke (Yamori, 1989, 1994).

The SHRSP, which were fed on high salt diets, 1% salt in drinking water, quickly developed severe hypertension and died of stroke within 3 months, but when they were fed on high soy or fish protein diets, they could survive longer despite high salt diets (Yamori, 1994; **Fig. 1**). The survival rates of SHRSP fed on soy protein alone or soy protein plus magnesium and/or calcium were shown to be so prolonged that the possible nutritional prevention of stroke were experimentally proven in this genetic model (Yamori, 1989, 1994).

These antihypertensive and antistroke effects of dietary protein intakes first noted in experimental study were later confirmed also in humans (Yamori, 1994; Vasdev and Stuckless, 2010; Zhang et al., 2014).

Epidemiological Studies on the Association of the Biomarkers of Soy and Fish Intakes with CVD in the World

The preventive effects of soy, fish, and other nutrients on stroke experimentally proven in SHRSP led Yamori to propose to WHO the CARDIAC Study for investigating the association of the biomarkers of food intake such as soy, fish, salt, and others with CVD

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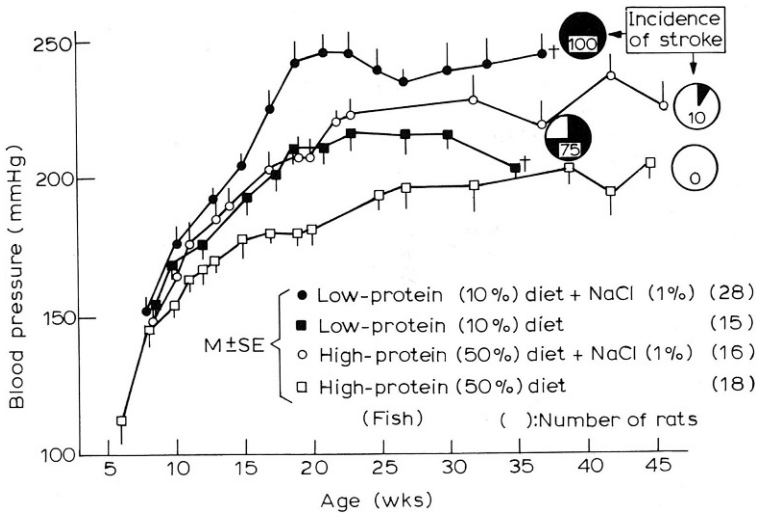
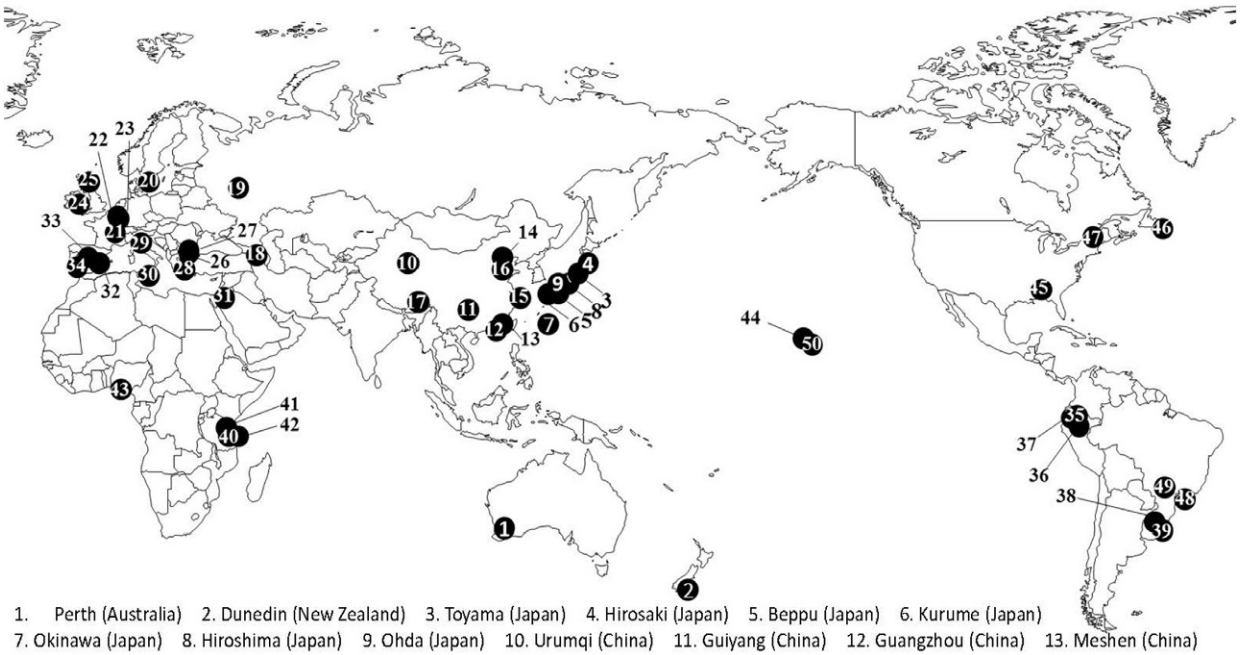


Fig. 1 Preventive effect of fish protein on stroke in stroke-prone spontaneously hypertensive rats (SHRSP) given 1% salt in drinking water or not.



1. Perth (Australia) 2. Dunedin (New Zealand) 3. Toyama (Japan) 4. Hirosaki (Japan) 5. Beppu (Japan) 6. Kurume (Japan) 7. Okinawa (Japan) 8. Hiroshima (Japan) 9. Ohda (Japan) 10. Urumqi (China) 11. Guiyang (China) 12. Guangzhou (China) 13. Meshen (China) 14. Beijing (China) 15. Shanghai (China) 16. Shijiazhuang (China) 17. Lhasa (China) 18. Ossetia (Georgia) 19. Moscow (Russia) 20. Gothenburg (Sweden) 21. Orleans (France) 22. Leuven (Belgium) 23. Ghent (Belgium) 24. Belfast (UK) 25. Stornoway (UK) 26. Sofia (urban) (Bulgaria) 27. Sofia (rural) (Bulgaria) 28. Athens (Greece) 29. Milan (Italy) 30. Palermo (Italy) 31. Tel Aviv (Israel) 32. Navas (Spain) 33. Madrid (Spain) 34. Lisbon (Portugal) 35. Quito (Ecuador) 36. Vilcabamba (Ecuador) 37. Manta (Ecuador) 38. Uruguaiana (Brazil) 39. Bagé (Brazil) 40. Handeni (Tanzania) 41. Shinya (Tanzania) 42. Dar es Salaam (Tanzania) 43. Ibadan (Nigeria) 44. Honolulu (USA) 45. Jackson (USA) 46. Newfoundland (Canada) 47. Montreal (Canada) 48. Sao Paulo (Brazil) 49. Campo Grande (Brazil) 50. Hilo (USA)

Fig. 2 Geographical distribution of population samples of the CARDIAC Study.

(Yamori, 2006a,b; Yamori et al., 2006, 2010,2015, 2017). About 100 males and 100 females aged 46–58 were randomly selected from each population in over 60 study sites in the world (**Fig. 2**). They were invited for health examination to measure height and weight and blood pressure (BP) estimated by automated BP measurement system and to collect fasting blood samples and 24U by using aliquot cups (Yamori et al., 1984; **Fig. 3**), and the age-adjusted standardized mortality rates for CHD and stroke were obtained for the regions encompassing each study sites.

The CHD mortality rates were significantly inversely related with 24U isoflavone excretion, the biomarkers of soy intake (Yamori, 2006a; **Fig. 4**). CHD mortality rates were low in Japan and China where soy bean products were commonly eaten and 24U isoflavone excretion was over 10 μmol/day.

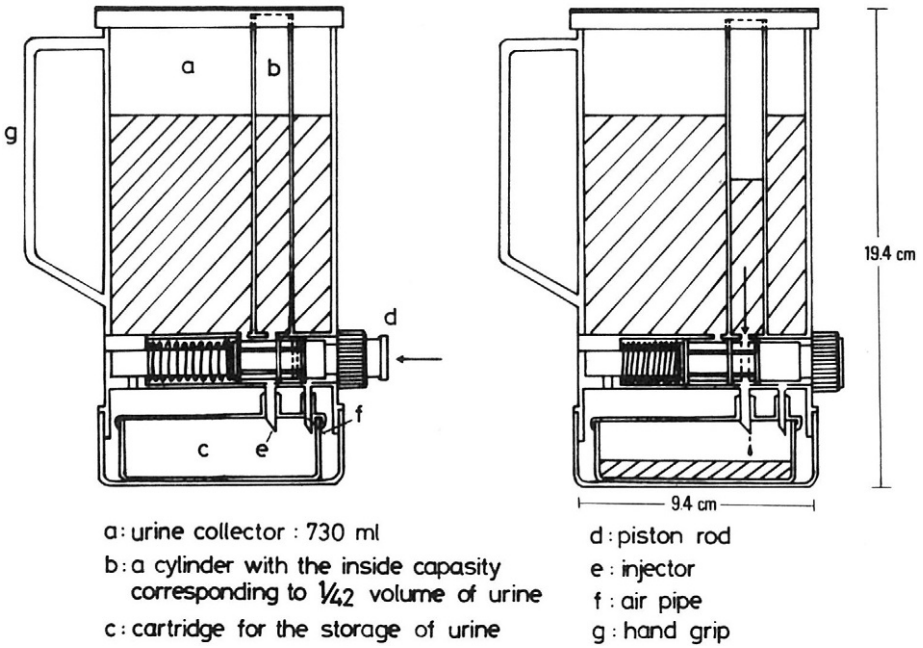
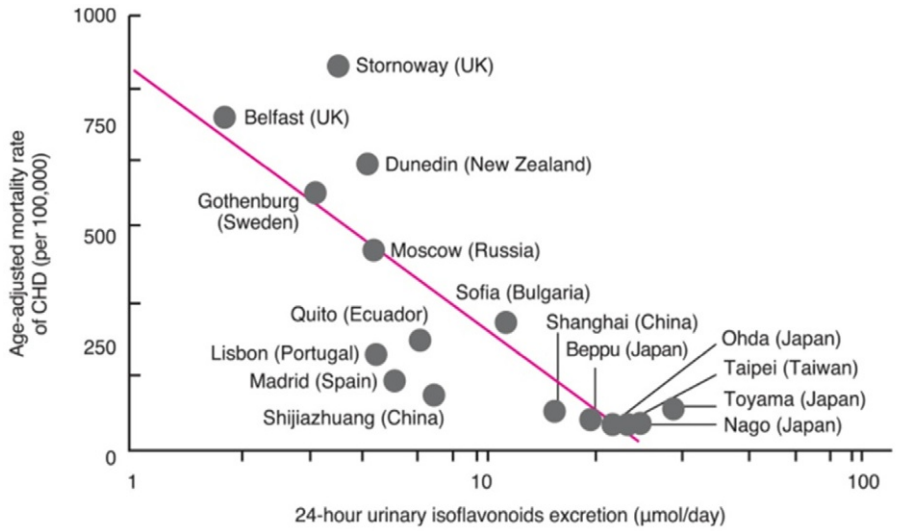


Fig.3 Aliquot cup for collecting 24U samples easily by just pushing a button once to deposit 1/40th of each voided urine.



Line indicates statistically calculated association
between CHD mortality & 24-hour urinary isoflavone excretion.

Graph courtesy Dr. Yamori(2001). Source: WHO ARDIAC Study, 1985-2001

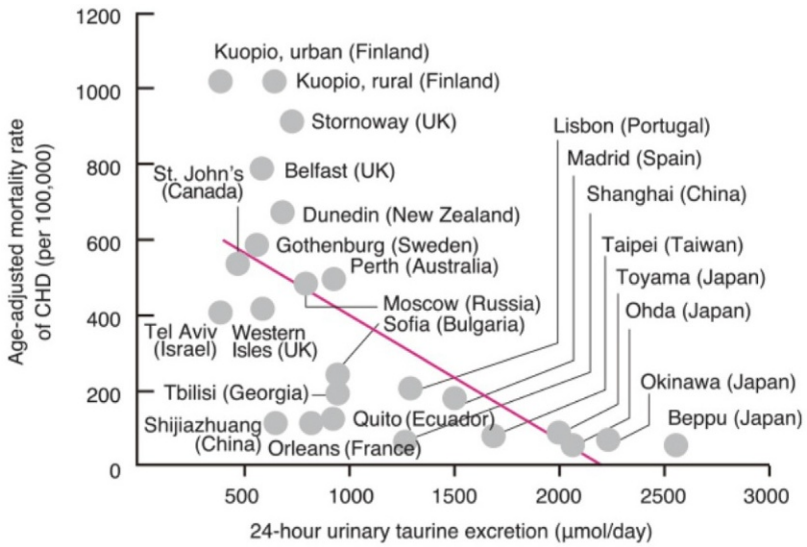
Fig. 4 Association of 24U isoflavone excretion with age-adjusted CHD morality. *Line* indicates statistically calculated association between CHD mortality and 24-h urinary isoflavone excretion. Graph courtesy Dr. Yamori (2001). Source: WHO ARDIAC Study, 1985–2001.

The amount of daily isoflavone intake of 50 mg is expected to be effective for preventing CHD as well as breast and prostate cancers (Yamori, 2006a,b, 2008).

This amount corresponds to 150 g of tofu, 60 g of fermented soy beans, or 30 g of soy powder commonly eaten in Japan.

WHO CARDIAC Study also revealed significant inverse association between CHD mortality and 24U taurine, the biomarker of fish intake (Yamori, 2006a, Yamori et al., 2010) (**Fig. 5**).

CHD mortality rates were low in Japan and Mediterranean countries where 24U taurine excretion exceeded 1000 μmol/day. *N*-3 fatty acids in the blood phospholipids are also the biomarker of fish intake, and their similar significant inverse relationship with



Line indicates statistically calculated association

between CHD mortality & 24-hour urinary taurine excretion.

Graph courtesy Dr. Yamori(2006). Source: WHO ARDIAC Study, 1985-2001

Fig. 5 Association of 24U taurine excretion with age adjusted CHD mortality. *Line* indicates statistically calculated association between CHD mortality and 24-h urinary taurine excretion. Graph courtesy Dr. Yamori (2006). Source: WHO ARDIAC Study, 1985–2001.

CHD mortality was confirmed by CARDIAC Study (Yamori, 2006a, 2008; Yamori et al., 2006). The inverse associations of stroke mortality with 24U isoflavones or taurine were not significant. It was because CARDIAC Study proved a significant positive association of stroke mortality with 24U salt (Yamori et al., 2006) and these participants who excreted significantly higher isoflavones and taurine in 24U were also proven to excrete significantly higher 24U salt (Fig. 6).

The Association of Biomarkers of Soy and Fish Intakes with CHD Risks in the World

Since the biomarkers of soy and fish intakes were inversely associated with CHD mortality, CHD risks such as obesity, hypertension, and hypercholesterolemia were examined in those who were eating soy regularly (soy consumers), excreting 24U isoflavones over 10 μmol/day (Yamori, 2006b), and in those who were eating fish regularly (fish consumers) and excreting 24U taurine over 1000 and 800 μmol/day in males and females, respectively. They were compared with those who were eating soy and fish less frequently (less soy consumers, less fish consumers), excreting 24U isoflavones and taurine less than the abovementioned levels (Fig. 6).

Soy and fish consumers, both in males and females, had significantly lower body mass index and total cholesterol than less soy and fish consumers and significantly lower BP in females. Since soy and fish consumers had lesser risks of obesity and hypercholesterolemia, the major risks of CHD than less soy and fish consumers, these consumers had significantly lower CHD mortality (Fig. 6) As for salt intakes, soy and fish consumers showed significantly higher 24U salt excretion than less consumers, there were no significant difference in BP between the consumers and less consumers in males who were more salt-sensitive than females (Yamori et al., 2001a).

The Association of Soy and Fish Intakes with CHD in Japanese Regularly Taking Soy and Fish

WHO CARDIAC Study compared Japanese 24U excretions of isoflavone and taurine with the 24U excretions of both biomarkers among worldwide populations and demonstrated the ratio of Japanese in the highest quintiles of both biomarkers was nearly 90%, indicating soy and fish eating customs were common in Japanese (Yamori et al., 2017). Further, the Japanese were divided into the tertiles of 24U excretions of isoflavones and taurine and those who were both in the highest tertile were proven to have significantly higher HDL cholesterol and folate levels in the blood than the lowest tertile of both (Yamori et al., 2017). Since HDL cholesterol and folate were related with lower risks of CHD (Abbott et al., 1988), the common intakes of soy and fish could be related with the lowest CHD mortality of the Japanese in developed countries (Yamori, 2008).

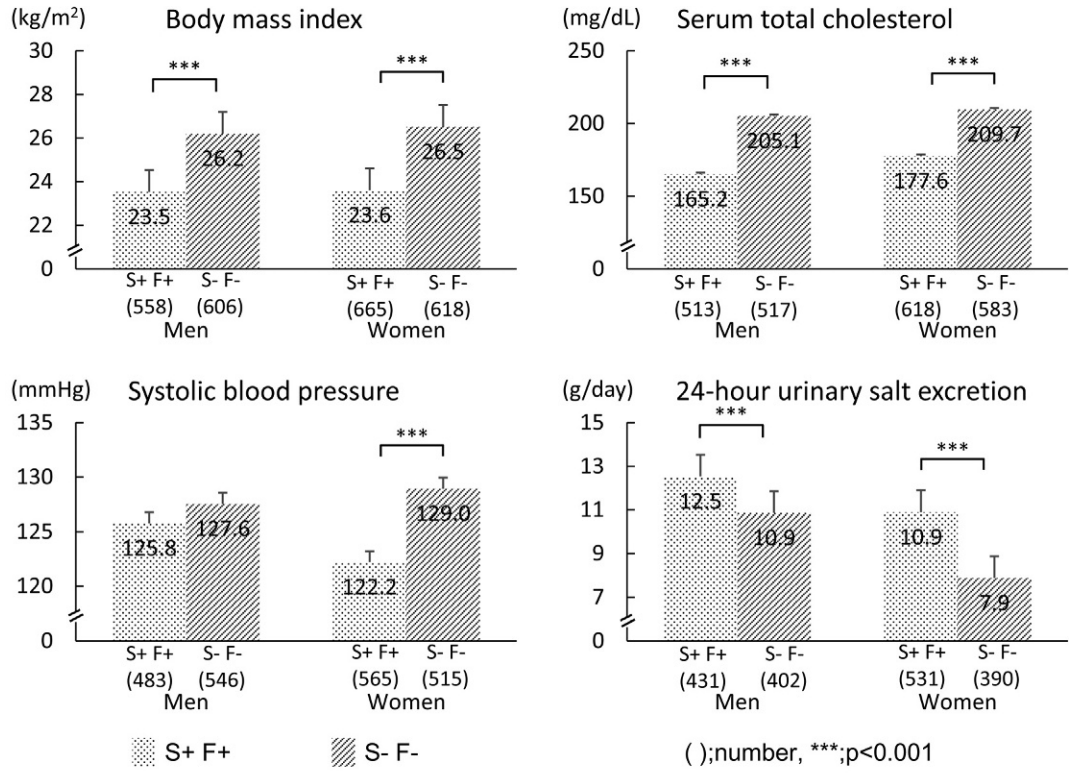


Fig. 6 Cardiovascular risks, body mass index, systolic blood pressure, total cholesterol in soy consumers (S+) and fish consumers (F+) compared with less soy and less fish consumers (S−, F−).

The Japan Public Health Center based (JPHC) study was the most extensive cohort study in Japan, conducted for 40,462 man and women aged 40–59 without CVD or cancer at baseline (1990–1992) and follows up to 2002 (Kokubo et al., 2007). Soy intake was estimated from food frequency questionnaires and was analyzed by the quintiles for the risk of cerebral and myocardial infarction (CI and MI). The multivariable hazard ratios and 95% confidence limits for highest versus the lowest isoflavone quintile were 0.35 (0.21–0.59) for CI, 0.37 (0.14–0.98) for MI, and 0.87 (0.29–2.52) for cardiovascular mortality in women, but no significant association of dietary intake of soy and isoflavones with CI or MI was present in men.

This study indicated the less beneficial effect of soy intakes in man. As Japanese dietary tradition of soy intake was related with high salt intake (Yamori et al., 2017), the adverse effort of high salt intake might reduce the merit of soy intake in men whose BP was significantly associated with salt intake (Yamori et al., 2006). Since salt-associated BP rise was not significant in premenopausal women (Yamori et al., 2001a), the estrogen-agonist effect of isoflavones might reduce the adverse effect of high salt intake accompanied with soy intake in women.

JPHC study also revealed that the higher consumption of fish (eight times per week or 180 g/day) was associated with reduced risk of CHD, more significantly, MI and nonfatal CHD, compared with a modest fish consumption (once a week or 23 g/day) in Japan (Iso et al., 2006). In this study, the *n*-3 fatty acid intakes estimated from questionnaires were also inversely associated with CHD.

The Potential Benefits of Soy Intakes and the Mechanisms

Soy intakes are proven to be beneficial in Japanese taking soy foods regularly, and the intakes have gained attention for the potential role in improving CVD risk factors also in the United States and other Western countries where soy consumption is lower than Asian countries. U.S. Food and Drug Administration (FDA) approved labeling for foods containing soy protein as protective against CHD in 1999 (Erdman, 2000), the effects of soy protein and isoflavones were reviewed from further available articles to report small but significant reduction of total cholesterol or LDL in about half of the literatures (Sacks et al., 2006). Seven human studies on soy protein or isoflavones showed beneficial effects on lipid profiles such as reduced LDL, and ascribed the potential mechanisms to the

reduced intestinal cholesterol absorption by increased fecal bile acid excretion due to soy protein ingestion and/or to the reduction of LDL oxidation by isoflavones (Omoni and Aluko, 2005).

The antihypertensive efforts of soy protein and isoflavones in humane studies were reported (Yang et al., 2005; He et al., 2005) and the BP reduction was ascribed to the higher arginine content of soy protein (Obarzanek et al., 1996) and to enhanced NO production by isoflavones (Si and Liu, 2008; Räthel et al., 2005). Several articles reported no BP effects of soy intervention studies. However, when the compliance of the soy intervention study was confirmed by 24U isoflavone excretion, soy effects on BP and lipid profile were evident (Sagara et al., 2004; Yamori, 2006a,b; Mori and Thorpe, 2005).

The beneficial soy effects may be attributable to other soy nutrients, such as *n*-3 polyunsaturated fatty acids, fibers, vitamins, minerals, saponins, and phytosterols (Omoni and Aluko, 2005).

The other potential benefits of soy on CVD should be the antiinflammatory effect proven by the reduction of C-reactive protein (CRP) (Filiberto et al., 2013) and the antidiabetic effect (Azadbakht et al., 2007) partly through CRP reduction (Xu et al., 2007)and AMP-activated protein kinase activation (Cederroth et al., 2008). The activation of adiponectin (Kim et al., 2014) and the reduction of E-selectin (Rebholz et al., 2013) may also be the benefit of soy and soy products. These pleiotropic effects of soy nutrients may improve arterial compliance (Nestel et al., 1997) and arterial stiffness (Reverri et al., 2015).

The Potential Benefit of Fish Intake and the Mechanisms

Since the pioneering studies on the benefits of fish intakes in 1970 (Bang et al., 1976), fish consumption was proven to be associated with CVD risk reductions by many epidemiological studies (Zhang et al., 1999), including WHO CARDIAC Study in the world as well as in the Chinese (Yuan et al., 2001) and the Japanese consuming the high amount of fish (Iso et al., 2006) as cited in this review.

The merits of fish intakes are mainly considered to be due to *n*-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Hu et al., 2003). These fatty acids have important biological functions such as prevention of arrhythmia, lowering heart rate and BP, decreasing platelet aggregation, and plasma triglycerides (Harris et al., 2008).

Since inflammation has been identified to be involved in the pathogens of atherosclerosis (Libby, 2002) and of CHD, stroke, and diabetes (Libby, 2006; Ridker, 2007), *n*-3 fatty acids reduce CVD risks by the anti inflammatory effects through their stimulation of G protein-coupled receptor 120 in macrophages (Oh et al., 2010).

Flock et al. (2013) reviewed eight meta-analyses of randomized controlled trials evaluating the effects of EPA and DHA on cardiac and/or all cause mortalities and found six out of eight articles reported significant relative risk reduction of cardiac death. The inverse association of blood levels of *n*-3 fatty acids with telomere shortening in leukocytes was observed by over 5-year prospective study on the cohort of 608 ambulatory outpatients with CHD (Erkkilä et al., 2003). Since leukocyte telomere shortening was noted in the patients with premature MI (Brouilette et al., 2003), *n*-3 fatty acid intake may prolong the survival of CHD patients (Farzaneh-Far et al., 2010).

Taurine was used as the biomarker of fish intake by WHO-CARDIAC Study, and its intake was experimentally and epidemiologically proven to lower BP and serum cholesterol (Yamori et al., 2004; Yamori et al., 2010; Murakami et al., 1996). The inverse association of the population means of 24U taurine excretion with the mortality rates of CHD in WHO-CARDIAC Study populations (Yamori et al., 2001b) was further supported by the quintile analysis of 24U taurine/creatinine in all CARDIAC Study participants (Sagara et al., 2015). This study indicated the significant inverse association of 24U taurine with the major CHD risks, such as BMI, BP, and serum cholesterol.

Since 24U taurine excretion in Tibetans with no custom to eat fish was lowest in the world, 1 g of taurine solved in tea, given three times a day for 2 months significantly reduced BP (Yamori et al., 1996).

Taurine is known to work as antioxidant through the formation of taurine chloramine for trapping hypochlorous acid produced by myeloperoxidase in macrophages and neutrophils (Murakami, 2012). Therefore, taurine's effects on endothelial progenitor cells (EPC) were observed in healthy male volunteers and SHR given 1% salt in drinking water which were previously shown to have impaired endothelial function. In humans 3 g taurine administration per day increased EPC colony numbers, and in SHR 3% taurine in drinking water improved the EPC function and significantly decreased free radicals measured as thiobarbituric acid reactive subtract, indicating dietary taurine intake may prevent CVD by improving EPC function (Katakawa et al., 2016).

Combined Intakes of Soy and Fish Nutrients for CVD Prevention

Since CHD morality in the developed countries of the world is lowest in the Japanese consuming soy and fish regularly and HDL cholesterol and folic acids are high in the Japanese taking both foods enough (Yamori et al., 2017), soy and fish intakes are recommended for CHD prevention.

Soy beans are regarded as an eco-friendly source of protein sustainably available widely in the world and able to support 10 times the number of people consuming beef and pork customarily.

Fish consumption, if recommended throughout the world as being eaten in Japan, may lead to an exhaustion of fish from our planet. This environmental concern may be possibly solved in two ways.

One newly developing solution is stearidonic acid, *n*-3 polyunsaturated fatty acid produced by genetically modified soy, (Surette ME, 2013) the safety of which should be still confirmed.

The other practical recommendation in place of fish should be seaweeds. Seaweeds are traditionally eaten in Japan, and contain taurine and *n*-3 fatty acids (MacArtain et al., 2007). Therefore, soy, fish and seaweeds are recommended for the dietary prevention of CVD to achieve eco-friendly global health.

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Obesity and the Obesity Paradox in Heart Failure

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Abbreviations

ADHF	Acute decompensated heart failure
ARIC	Atherosclerosis Risk in Communities (study)
BF	Body fat
BMI	Body mass index
BNP	B-type natriuretic peptide
BP	Blood pressure
CAD	Coronary artery disease
CO	Cardiac output
CRF	Cardiorespiratory fitness
CT	Computed tomography
CV	Cardiovascular
CVD	Cardiovascular disease(s)
DM	Diabetes mellitus
FHS	Framingham Heart Study
HF	Heart failure

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HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
Hs-cTnT	High-sensitivity cardiac troponin T
HTN	Hypertension/hypertensive
LA	Left atrial/atrium
LBM	Lean body mass
LV	Left ventricle/ventricular
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
MESA	Multiethnic study of atherosclerosis
MRI	Magnetic resonance imaging
MUAC	Mid-upper arm circumference
NSTEMI	Non-ST-elevation myocardial infarction
NT-proBNP	N-terminal pro-B-type natriuretic peptide
RAAS	Renin–angiotensin–aldosterone system
RV	Right ventricular
US	United States
VO_2	Oxygen consumption
WC	Waist circumference
% BF	Percent body fat

Overview and Background

Overweight and obesity have become a global epidemic and public health problem (Mozaffarian et al., 2016). Since 1980, the worldwide prevalence of individuals who are either overweight or obese has increased by ~28% among adults and ~58% among children and adolescents (Ng et al., 2014). Recent reports indicated that more than two-thirds of adults in the United States (US) are either overweight or obese and 38% are obese. More concerning is the fact that the prevalence of severe obesity (body mass index [BMI] $\geq$ 40 kg/m^2) has been rising and now totals almost 8% of the US population, with certain demographic groups more commonly affected such as women, non-Hispanic blacks, and middle-aged adults (Ogden et al., 2014; Flegal et al., 2016).

Heart failure (HF) is a heterogenous syndrome with multiple underlying etiologies and pathophysiologies. The lifetime risk of HF for US adults $\geq$40 years of age was reported as 13.8% (Djoussé et al., 2009). HF predominantly affects the elderly population, and it is one of the most common causes of hospitalization in adults $\geq$65 years of age (Yancy et al., 2013). Despite the improvements in the management, HF remains an important cause of death with mortality rates of 20% at 1 year and 53% at 5 years following the diagnosis of HF (Gerber et al., 2015). Patients with HF are commonly classified into two distinct groups based on left ventricular (LV) function: HF with preserved ejection fraction (HFpEF, LV ejection fraction [LVEF] $\geq$50%) and HF with reduced EF (HFrEF, LVEF $\leq$40%). Patients with an LVEF of 41%–49% are referred as borderline or intermediate HFpEF (Yancy et al., 2013). HFpEF is characterized by diastolic dysfunction, and it represents 50% of cases with HF. HFpEF is expected to become the predominant form of HF (Owan et al., 2006; Oktay et al., 2013).

Obesity is a well-established modifiable risk factor for HF and other cardiovascular (CV) diseases (CVD), such as coronary heart disease (CHD), stroke, and atrial fibrillation (Mozaffarian et al., 2016). The strong relationship between obesity and HF is underlain by two main factors. (1) Obesity is strongly associated with other major CVD risk factors such as atherosclerosis, hypertension (HTN), diabetes mellitus (DM), dyslipidemia, and obstructive sleep apnea (OSA). (2) Obesity, independent of the effect of other obesity-associated CVD risk factors, can induce alterations in cardiac structure and function (Lavie et al., 2016a). These alterations are primarily triggered by excessive adipose tissue accumulation and subsequent hemodynamic changes and adverse cardiac remodeling (**Table 1**) (Joyce et al., 2016; Lavie et al., 2016b).

Despite the strong relationship between obesity and development of HF, multiple epidemiological studies have demonstrated an "obesity paradox" which is characterized by a better short- and intermediate-term prognosis in overweight or obese HF patients compared to their leaner or normal-weight counterparts (Lavie et al., 2013a, 2016a).

In this chapter, we discuss the current concepts of the relationship between adiposity, cardiac remodeling, and development of HF. We also review the prognostic implications of the obesity paradox in patients with established HF. Finally, we discuss data on the benefits and risks of weight loss and the prognostic impact of cardiorespiratory fitness (CRF) in patients with HF.

Defining Obesity

In simple terms, obesity is defined as an excess of body adiposity due to increased number and/or size of adipocytes (Caballero, 2007). The increase in body weight and overall adiposity derives from a chronic positive energy balance, meaning energy intake

Table 1 Effects of obesity on the cardiovascular system

Effects of obesity on cardiovascular system	
Hemodynamics	• Increased total and central blood volume • Increased stroke volume/cardiac output • Decreased systemic vascular resistance • Increased arterial pressure • Increased LV wall stress • Pulmonary artery hypertension
Cardiac structure	• LV concentric remodeling • LV hypertrophy (eccentric and concentric) • Left atrial enlargement • RV hypertrophy
Cardiac function	• LV diastolic dysfunction • LV systolic dysfunction • LV myocardial dyssynchrony • RV failure
Inflammation	• Increased C-reactive protein • Overexpression of tumor necrosis factor
Neurohumoral	• Insulin resistance and hyperinsulinemia • Leptin insensitivity and hyperleptinemia • Reduced adiponectin • Sympathetic nervous system activation • Over-activation of renin-angiotensin-aldosterone system • Overexpression of peroxisome proliferator-activator receptor
Cellular	• Hypertrophy • Apoptosis • Fibrosis

LV, left ventricular; *RV*, right ventricular.

Adapted with permission from Lavie, C. J., Sharma, A., Alpert, M. A. et al. (2016). Update on obesity and obesity paradox in heart failure. *Progress in Cardiovascular Diseases* **58** (4), 393–400.

Table 2 Body weight categories

Body weight categories	
Underweight	BMI <18.5 kg/m^2
Normal/acceptable weight	BMI 18.5–24.9 kg/m^2
Overweight	BMI 25.0–29.9 kg/m^2
Obese	BMI ≥ 30 kg/m^2
Grade 1—obese	BMI 30.0–34.9 kg/m^2
Grade 2—obese	BMI 35.0–39.9 kg/m^2
Grade 3 (severe)—obese	BMI ≥ 40 kg/m^2

BMI, body mass index.

more than energy expenditure (or calories consumed >calories burned) (Lavie et al., 2016a). Multiple studies have repeatedly demonstrated that poor dietary choices characterized by excessive calorie intake (particularly with carbohydrates) are largely, if not completely, responsible for the obesity epidemic (DiNicolantonio et al., 2016; Swinburn et al., 2009). Moreover, our recent studies have demonstrated that the obesity epidemic has been contributed by massive declines in occupational physical activity (PA) in both men and women and household management in women during the past 5 decades (Church et al., 2011; Archer et al., 2013).

BMI (the ratio of total weight to height squared [kg/m^2]) is the most commonly used anthropometric measure to assess relative weight and classify obesity (Bastien et al., 2014). Individuals with a BMI between 18.5 and 24.9 kg/m^2 are considered to have normal or acceptable weight, while those with a BMI ≥ 30 kg/m^2 are classified as obese. The classification of body weight based on BMI is given in **table 2** (WHO, 2016). Overall, obesity is associated with increased all-cause mortality in the general population (Engeland et al., 2003). However, recent epidemiologic studies have demonstrated that grading of obesity helps with better prediction of all-cause mortality (Bastien et al., 2014). A systematic review of 97 studies with a combined sample size of 2.88 million individuals revealed that compared to normal weight, grade 2 and grade 3 (but not grade 1) were associated with increased all-cause mortality. Overweight was associated with significantly lower all-cause mortality in relation to normal-weight (Flegal et al., 2013).

Its simplicity and validation in multiple epidemiological studies have made BMI a popular and widely used parameter (Oliveros et al., 2014). We recently demonstrated, in a cohort of more than 60,000 individuals, that very high BMI can accurately predict the risk of CVD mortality in the general population (Ortega et al., 2016). Despite being a very useful measure in population studies,

BMI is flawed in individual-person level. Because it reflects both fat and nonfat mass which mostly consist of muscle and skeletal mass (Lavie et al., 2016b). Generally, metabolic abnormalities occur as a result of excess body fat (BF) rather than a high level of lean body mass (LBM) (Collaboration et al., 2011). A metaanalysis of 25 studies with a combined sample size of approximately 32,000 individuals demonstrated that BMI has high overall specificity (90%) but poor sensitivity (50%) in regard to identifying excess BF (Okorodudu et al., 2010). These results suggested that many individuals who are not obese according to BMI might indeed have excess BF. On the contrary, athletes with a high muscle mass may be misclassified as overweight or obese (even in the absence of excess adiposity) if only BMI is used for assessment (Oliveros et al., 2014).

Several other anthropometric parameters (i.e., waist circumference [WC] and waist-to-hip, waist-to-height, and waist-to-thigh ratios) can be used for assessment of central (or visceral) adiposity and further risk stratification (Oliveros et al., 2014; Lavie et al., 2016b). Increase in WC or related ratios (as above), referred as abdominal obesity, indicates higher risk of CV and metabolic abnormalities independent of BMI (Després, 2012). In a metaanalysis of 15 prospective studies that included >258,000 individuals, every 1 cm increment in WC was associated with a 2% increase in future risk of CVD (de Koning et al., 2007). The World Health Organization expert consensus has proposed the following WC cutoff values based on CVD risk: 94 cm (men) and 80 cm (women) for increased risk, and 102 cm (men) and 88 cm (women) for substantially increased risk (World Health Organization, 2008).

Several other techniques have been developed for BF assessment such as near-infrared interactance, hydrostatic weighing, air displacement plethysmography, dual-energy X-ray absorptiometry, and bioelectric impedance. We are still away from the routine use of these techniques in general clinical practice. In addition, there exist no standardized cutoff values for defining obesity based on percent BF (% BF). The clinical utility and limitations of these techniques were discussed extensively in a scientific statement by the American Heart Association (Cornier et al., 2011).

Computed tomography (CT) and magnetic resonance imaging (MRI) are considered to be the "gold standard" for measurement of adipose tissue and LBM and evaluation of BF distribution (Cornier et al., 2011). CT and MRI are particularly helpful with quantification of visceral adipose tissue. Growing evidence now suggests that visceral adiposity is much more important for CVD risk stratification than subcutaneous adiposity (Neeland et al., 2015; Abraham et al., 2015). Furthermore, visceral adiposity surrounding the heart (referred as pericardial adipose tissue) is now recognized as an independent risk factor for several CVD. Multiple epidemiological studies have revealed that increased pericardial adipose tissue (detected by CT or MRI) is associated with a significantly increased risk of CV morbidity and mortality even after adjustment for other CVD risk factors and obesity parameters (Mahabadi et al., 2013; Thanassoulis et al., 2010; Fox et al., 2009).

Hemodynamic Changes in Obesity

Weight gain and obesity lead to a variety of alterations in central and peripheral hemodynamics. These hemodynamic changes are characterized by increased total and central blood volume, LV stroke volume, and cardiac output (CO) (Lavie et al., 2016b; Alpert et al., 2014, 2016; Alexander et al., 1962). The rise in CO in individuals with grade I and II obesity is mainly driven by lean body mass since skeletal muscle requires a higher amount of blood flow compared to adipose tissue (Alpert et al., 2014; Collis et al., 2001; Carbone et al., 2017). Moreover, obesity is associated with decreased systemic vascular resistance (in normotensive individuals) which facilitates increased CO despite no significant change in heart rate (Lavie et al., 2015a). It is not uncommon to observe CO values as high as 10 L/min in severe obesity (Alpert et al., 2014). Despite the increase in CO, obese individuals usually have reduced blood flow per unit of weight, particularly in severe degrees of obesity. Coexistent HTN in obesity can cause a relatively lower CO due to the impact of increased systemic vascular resistance.

Increased venous return in individuals with grade II and III obesity leads to a rise in preload and LV filling pressure. This results in elevation of the pulmonary artery and right heart pressures (Lavie et al., 2016b; Alpert et al., 2014, 2016). Moreover, chronic hypoxia due to hypoventilation and/or OSA induces vasoconstriction and remodeling of small pulmonary arteries in severely obese individuals. These changes further contribute to the increase in pulmonary vascular resistance and pulmonary artery pressure (Alpert et al., 2014; Alpert, 2016). In individuals with grade III obesity, exercise induces a 20% rise in central blood volume and a 50% rise in LV end-diastolic pressure (Lavie et al., 2016b).

Cardiac Remodeling in Obesity

Obese individuals are more likely to have abnormal LV geometry compared to leaner counterparts (Lavie et al., 2007). Likewise, studies have shown a strong independent correlation between BMI and LV mass, LV wall thickness, and LV internal dimension (Korre et al., 2016; Lauer et al., 1991; Peterson et al., 2004). There exists a significant variation among studies in regard to the reported frequency of LV structural abnormalities in obese individuals. This variation has mainly been attributed to the duration and severity of obesity and presence or absence of comorbidities (Alpert et al., 1995, 2014).

A complex interaction of several mechanisms such as HTN, neurohumoral and metabolic abnormalities, and hemodynamic alterations plays role in obesity-induced LV remodeling (**Fig. 1**) (Lavie et al., 2015a). According to the conventional concept, increased CO and central blood volume in uncomplicated obesity may predispose to LV dilatation and eccentric LV hypertrophy (LVH) (Alpert, 2016). However, we and others have demonstrated that concentric LV geometry is observed as commonly or more

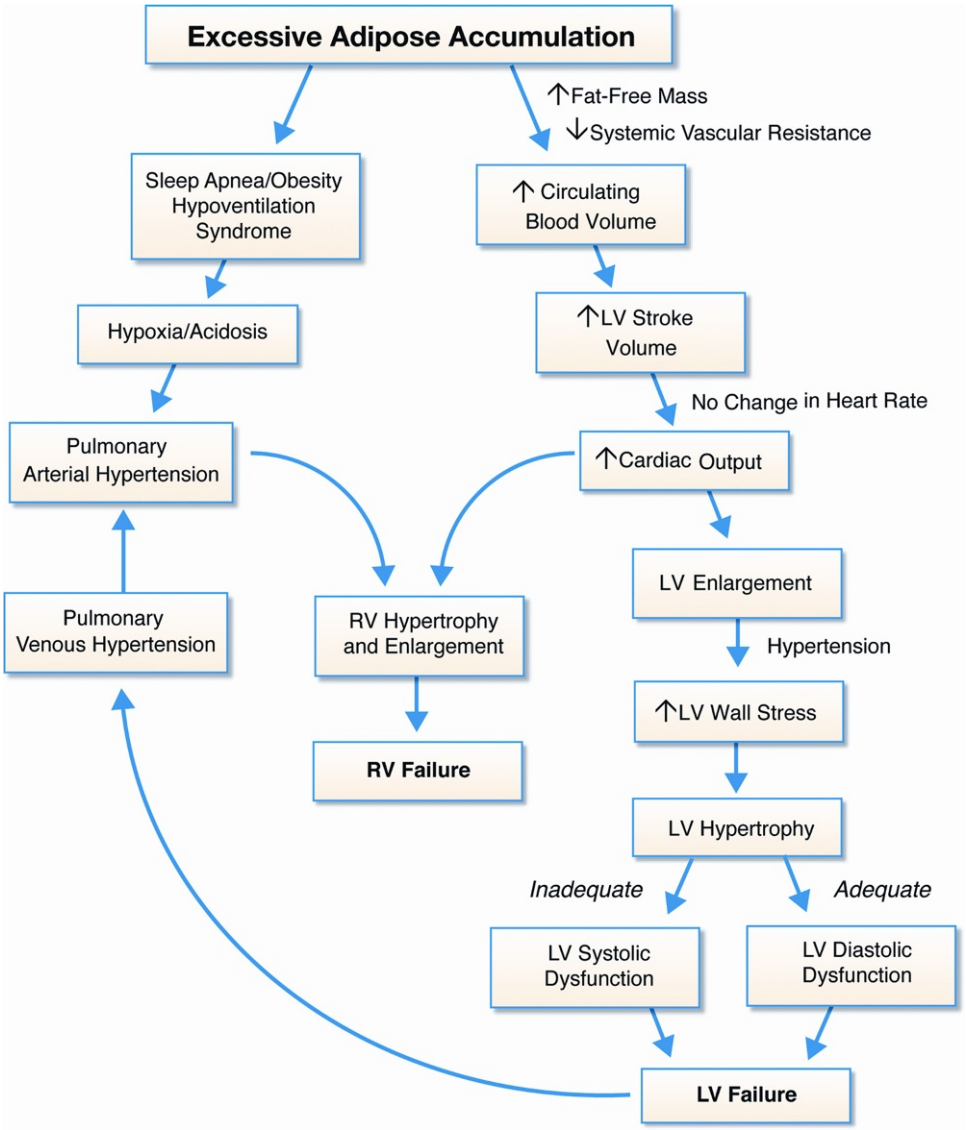

Fig. 1 Pathophysiology of obesity-induced alterations in cardiovascular system. This is a simplified model of mechanisms involved in the development of heart failure due to obesity. *LV*, left ventricular; *RV*, right ventricular. Reproduced with permission from Lavie, C. J., Alpert, M. A., Arena, R., Mehra, M. R., Milani, R. V., Ventura, H. O. (2013). Impact of obesity and the obesity paradox on prevalence and prognosis in heart failure. *JACC: Heart Failure* **1** (2), 93–102.

commonly than eccentric LVH in obese individuals (Lavie et al., 2007; Woodiwiss et al., 2008). And a report from the Multiethnic Study of Atherosclerosis (MESA) ($n = 5098$), which utilized cardiac MRI to assess LV geometry, demonstrated that obesity is more commonly associated with concentric remodeling which is characterized by a greater increase in LV mass relative to that in LV end-diastolic volume (Turkbey et al., 2010). Moreover, distribution of adipose tissue shows a complex relationship with hemodynamics and alterations in cardiac morphology. Neeland et al., in the Dallas Heart Study cohort, found that visceral obesity was independently associated with concentric LV remodeling and a higher systemic vascular resistance and lower CO (Neeland et al., 2013). In contrast, lower body subcutaneous adiposity was associated with an eccentric LV remodeling pattern, and lower systemic vascular resistance and CO.

Left atrial (LA) enlargement is common in obese individuals with obesity even in the absence of other risk factors (Sasson et al., 1996). Multiple epidemiological studies have confirmed that obesity and BMI are among the strongest predictors of LA enlargement (Gottdiener et al., 1997; Stritzke et al., 2009; Shigematsu et al., 2009). In a prospective study from Europe, obesity was found to have almost twice the effect of HTN on LA size (Stritzke et al., 2009).

Right ventricular (RV) structure and function remain understudied in overweight and obese individuals mainly because of the limitations of echocardiography in assessment of the RV (Alpert et al., 2014). Evidence from studies using cardiac MRI showed that compared to leaner counterparts, overweight and obese individuals have greater RV mass, end-diastolic volume, and stroke volume even after adjustment for respective LV parameters (Chahal et al., 2012).

Cardiac Dysfunction in Obesity

LV diastolic dysfunction is common in obese individuals, and its frequency increases in correlation with the severity of obesity (Lavie et al., 2015a). Peterson et al. demonstrated a strong correlation between BMI and diastolic dysfunction in otherwise healthy women (Peterson et al., 2004). A small study from Europe reported the frequency of LV diastolic dysfunction as 12% in individuals with grade I, 35% with grade II, and 45% with grade III obesity (Pascual et al., 2003). Obesity-associated diastolic dysfunction usually occurs in the setting of increased LV mass or LVH. However, LV diastolic dysfunction can be seen in obese individuals even in the absence of LVH (Lavie et al., 2016b; Alpert et al., 2014).

The frequency of LV systolic dysfunction in obese individuals highly varies depending on the definition of systolic dysfunction and the imaging modality utilized (Gupta et al., 2015). Studies using LVEF to evaluate systolic function have reported preserved systolic function in the majority of asymptomatic obese individuals (Turkbey et al., 2010; Kishi et al., 2014; Barbosa et al., 2013). However, studies using more sensitive echocardiographic modalities have revealed a high frequency of subclinical LV systolic dysfunction in obese individuals even with preserved LVEF (Lavie et al., 2016b; Kishi et al., 2014). Wang et al. investigated the relationship of obesity with global longitudinal strain and myocardial dyssynchrony (both were assessed by speckle tracking echocardiography) in a cohort of adults with no known CVD (Wang et al., 2015). The investigators found that compared to nonobese controls, obese individuals had a higher likelihood of impaired systolic mechanics (reflected by lower longitudinal strain) and dyssynchrony, regardless of the presence or the absence of concomitant metabolic syndrome (**Fig. 2**). Barbosa et al. demonstrated that subclinical systolic dysfunction commonly occurs in obese children and adolescents, as well (Barbosa et al., 2013).

Obesity has been associated with RV systolic dysfunction. Chahal et al., in a cohort of 4127 adults from the MESA, demonstrated an association between obesity and a slight decrease in RV ejection fraction determined by cardiac MRI (Chahal et al., 2012). In fact, their study demonstrated 0.5% decrease in RV ejection fraction for every 5 kg/m^2 increase in BMI. A recent report in a large ($n=4343$) biracial cohort of elderly individuals with no cardiac disease showed an inverse correlation between BMI and RV fractional area change on echocardiography (Bello et al., 2016). Tadic et al., in a cohort of adults ($n=127$) with untreated HTN, found a significant correlation between obesity and depressed RV ejection fraction (by three-dimensional echocardiography) and low RV myocardial strain and strain rate (by speckle-tracking echocardiography) (Tadic et al., 2016).

Obesity and Other HF Risk Factors

In addition to its direct effect on cardiac structure and function, obesity contributes to the development of HF through other intermediate risk factors such as HTN, CHD, and type-2 DM.

HTN leads to LV remodeling and LVH due to chronic pressure overload and the related increase in LV filling pressures and wall stress (Oktay et al., 2016). Hypertensives, compared to normotensives, carry 2-fold higher risk in men and 3-fold higher risk in women for development of HF. And at the population level, 39% of HF cases in men and 59% of HF cases in women are attributed to HTN (Levy et al., 1996). The strong relationship between obesity and HTN was demonstrated in the Framingham Heart Study (FHS) 5 decades ago (Kannel et al., 1967). BMI shows a strong correlation with systolic and diastolic blood pressure (BP) and frequency of HTN even after adjusting for other variables (Shihab et al., 2012; Drøyvold et al., 2005). Obese individuals are also at risk for having resistant HTN despite the use of multiple anti-HTN agents (Jordan et al., 2012; Demarco et al., 2014). Intentional weight reduction in overweight or obese individuals can improve BP profiles (Neter et al., 2003).

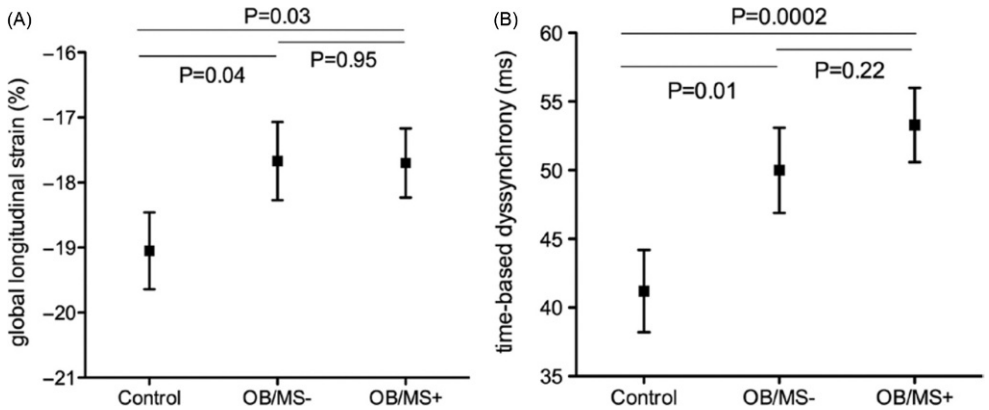

Fig. 2 (A) Global longitudinal strain (%) and (B) time-based dyssynchrony by study groups including nonobese controls, obese individuals without metabolic syndrome (OB/MS-), and obese individuals with metabolic syndrome (OB/MS+). Reproduced with permission from Wang, Y. C., Liang, C. S., Gopal, D. M., et al. (2015). Preclinical systolic and diastolic dysfunctions in metabolically healthy and unhealthy obese individuals. *Circulation. Heart Failure* **8** (5), 897–904.

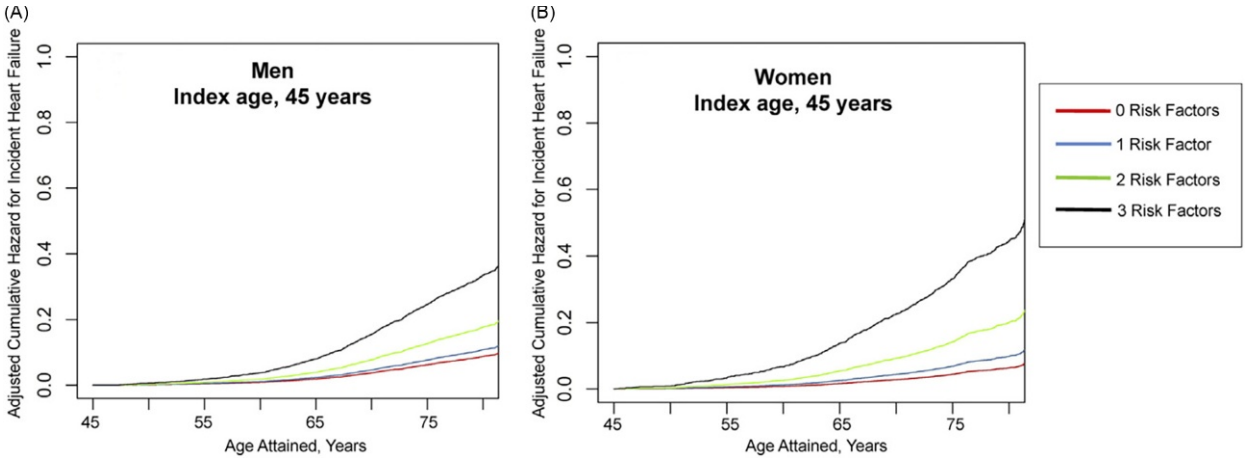

Fig. 3 Adjusted cumulative for incident heart failure by risk factor (hypertension, diabetes mellitus, obesity) burden at an index age of 45 years for men (A) and women (B). The study included 19,249 participants (90% white participants, 10% black participants) from four different cohorts from the United States. Reproduced with permission from Ahmad, F. S, Ning, H., Rich, J. D., Yancy, C. W., Lloyd-Jones, D. M., Wilkins, J. T. (2016). Hypertension, obesity, diabetes, and heart failure-free survival: The cardiovascular disease lifetime risk pooling project. *JACC: Heart Failure* **4** (12), 911–919.

Obesity and related comorbidities play significant role in development of atherosclerosis, the hallmark finding of CHD (Bastien et al., 2014). Consistently, CHD occurs more commonly in overweight or obese individuals compared to their leaner counterparts (Rabkin et al., 1977; Hubert et al., 1983; Wilson et al., 2002). Overweight and obese individuals develop acute coronary syndromes at younger ages than those with a normal BMI. Madala et al. retrospectively examined the relationship between adiposity categories and non-ST-elevation myocardial infarction (NSTEMI) in a large cohort ($n=111,847$) of patients with NSTEMI (Madala et al., 2008). The investigators found that obesity was the strongest predictor of NSTEMI at younger ages followed by tobacco use. In fact, the age at the first NSTEMI was 6.8, 9.4, or 12.0 years earlier in individuals with grade 1, grade 2, or grade 3 obesity, respectively.

Type-2 DM is characterized by insulin resistance which predominantly occurs secondary to increased adiposity. Insulin resistance and DM can independently promote the development of a special form of cardiomyopathy which is also referred as diabetic cardiomyopathy (Jia et al., 2016). Moreover, DM markedly increases HF risk especially in the presence of other HF risk factors such HTN and CHD. A metaanalysis with a combined sample size of 193,424 revealed ~4-fold increased risk of HF in individuals with coexistent DM and HTN compared to those with HTN only (Tocci et al., 2008). A recent study by Ahmad et al., in subjects from 4 different epidemiological cohorts in the United States, investigated the lifetime HF risk associated with concomitant HTN, obesity, and DM in mid-life (Ahmad et al., 2016). The study found that absence of HTN, DM, and obesity at age 45 years was associated with 73% (for men) and 85% (for women) lower lifetime HF risk when compared to the presence of all three risk factors. Moreover, individuals with no HTN, DM, and obesity at age 45 years had 10.6 years (for men) and 14.9 years (for women) longer HF-free survival compared those with all three risk factors (**Fig. 3**).

Obesity and HF

Multiple epidemiological studies have established overweight and obesity as risk factors for the development of HF (Ahmad et al., 2016; Kenchaiah et al., 2002; Huffman et al., 2013; Lee et al., 2007; Ndumele et al., 2014, 2016a). The relationship between obesity and HF appears to be stronger compared to the link of obesity with CAD or stroke (Ndumele et al., 2016b). A seminal report from Kenchaiah et al. examined the association between obesity and HF in the FHS participants ($n=5881$) during a mean follow-up of ~14 years (Kenchaiah et al., 2002). The investigators found that BMI correlates with HF risk in a dose-dependent fashion. For instance, the risk of HF was found to increase by 5% in men and 7% in women for every 1 kg/m^2 (Ng et al., 2014) increment in BMI, even after adjustment for other risk factors. Compared to normal-weight women, overweight and obese women were found to have 50% and 100% higher risk of future HF, respectively. In men, obesity was associated with 90% increase in the risk of HF. However, there was no statistically significant difference between normal weight and overweight men in regard to HF risk. In another prospective cohort with a larger sample size ($n=21,094$ men, mean follow-up 20.5 years), Kenchaiah et al. demonstrated 49% and 180% increased risk of HF in overweight and obese individuals, respectively (compared to leaner counterparts) (Kenchaiah et al., 2009). Moreover, the investigators demonstrated that the HF risk associated with overweightedness and obesity is markedly augmented with the presence of physical inactivity. A large prospective cohort study from Norway ($n=61,299$) demonstrated that obesity is associated with markedly elevated HF risk even in metabolically healthy individuals defined by those without HTN, DM, and dyslipidemia (Mørkedal et al., 2014).

Abdominal obesity assessed by WC or waist-to-hip ratio contributes to the risk of future HF (Mørkedal et al., 2014; Loehr et al., 2009; Hu et al., 2004). A recent metaanalysis of 28 prospective studies with a combined sample size of >600,000 participants found

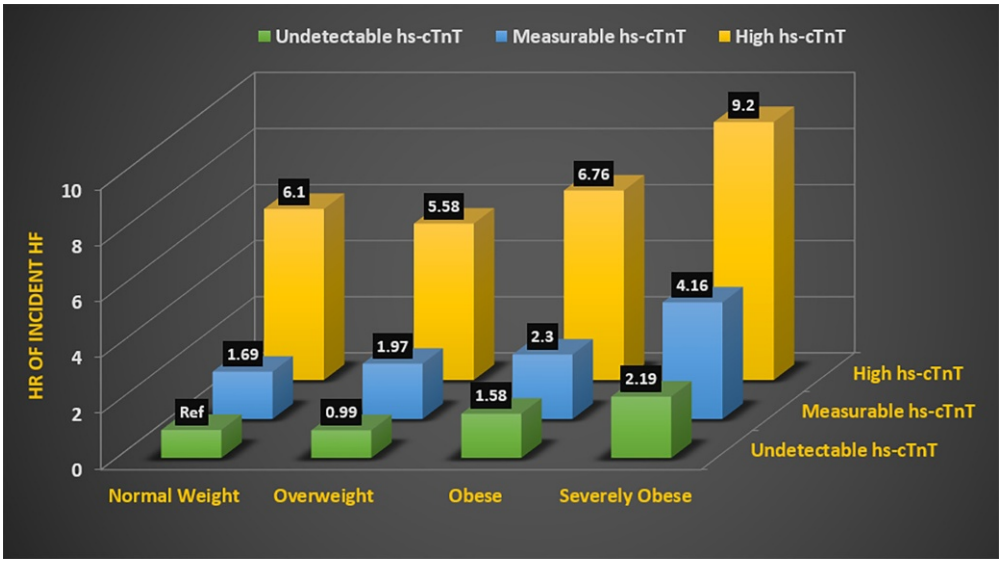

Fig. 4 Combined association of BMI categories and hs-cTnT levels with risk of incident HF. The predicted hazard ratios of incident HF are displayed for both hs-cTnT levels (undetectable [<3 ng/L], measurable [3–13 ng/L], and high [≥14 ng/L]) and BMI categories (normal weight [BMI 18.5 to <25 kg/m^2], overweight [BMI 25 to <30 kg/m^2], obese [BMI 30 to <35 kg/m^2], severely obese [≥35 kg/m^2]) in the Atherosclerosis in The Communities (ARIC) study. The hazard ratio of incident HF was estimated using Cox regression analysis and after adjustment for age, sex, race, smoking status, diabetes mellitus, hypertension, lipid panel, natriuretic peptide level, and estimated glomerular filtration rate. *BMI*, body mass index; *HF*, heart failure; *HR*, hazard ratio; *hs-cTnT*, high sensitivity cardiac troponin T. Reproduced with permission from Ndumele, C. E., Coresh, J., Lazo, M. et al. (2014). Obesity, subclinical myocardial injury and incident heart failure. *JACC: Heart Failure* **2** (6), 600–607.

that every 10 cm increase in WC and 0.1 unit in the waist-to-hip ratio is associated with 29% and 28% higher risk of HF, respectively (Aune et al., 2016). The relationship between BMI and HF risk was found to be nonlinear with a BMI cutoff of 23–24 kg/m^2 for increased HF risk.

Subclinical myocardial injury plays a significant role in the onset of obesity-associated HF. Ndumele et al. evaluated the relationship between adiposity, subclinical myocardial injury, and incident HF in the Atherosclerosis Risk in Communities (ARIC) study participants ($n=9507$) who had no known CVD at baseline (**Fig. 4**) (Ndumele et al., 2014). In the study, the subclinical myocardial injury was assessed by a high-sensitivity cardiac troponin T (hs-cTnT) assay. The study revealed a 2.2 higher rate of subclinical myocardial injury in severely obese. When compared to the reference group (normal weight individuals with undetectable hs-cTnT), individuals with a BMI ≥35 kg/m^2 and undetectable hs-cTnT had 2.2-fold increased risk of incident HF. However, those with a BMI ≥35 kg/m^2 and elevated hs-cTnT had >9-fold higher risk of incident HF than the reference group.

Obesity and Natriuretic Peptides

B-type natriuretic peptide (BNP) and its inactive fragment, N-terminal pro-B-type natriuretic peptide (NT-proBNP), are the biomarkers commonly used in the evaluation and management of patients with HF. In general, natriuretic peptides are secreted from the atria and the ventricles in response to a significant elevation in cardiac filling pressures and related mechanical stretch of the myocardium, with or without the syndrome of HF (Francis et al., 2016). Natriuretic peptides are involved in CV hemostasis and they stimulate natriuresis and diuresis, decrease cardiac preload, and suppress RAAS and sympathetic nervous system (Madamanchi et al., 2014). Data from FHS identified elevated BNP as an independent predictor of future risk of HF, CV events, and death in the general population (without HF at baseline) (Wang et al., 2004a). And BNP-guided prevention strategies were shown to significantly reduce the risk of incident HF in individuals with HF risk factors (Ledwidge et al., 2013; Huelsmann et al., 2013). Moreover, natriuretic peptides are important predictors of adverse outcomes in patients with HFrEF, HFpEF, or acute HF (Anand et al., 2003, 2011; Fonarow et al., 2007a).

BNP and NT-proBNP levels show inverse correlation with BMI in individuals with or without HF (Ndumele et al., 2016a; Wang et al., 2004b; Das et al., 2005; Mehra et al., 2004; Frankenstein et al., 2008). Taylor et al. demonstrated that obese individuals have lower BNP and NT-proBNP despite having higher LV end-diastolic pressure (invasively measured) compared to normal-weight individuals (Taylor et al., 2006). Therefore, a normal BNP does not exclude the diagnosis of HF and volume overload, especially in obese individuals. The cause(s) of lower BNP and NT-proBNP in the setting of obesity and whether this has any role in the increased risk of incident HF in obesity are not well known. The inverse correlation between BMI and natriuretic peptides was initially attributed to the clearance of BNP by adipose tissue (Wang et al., 2004b; Sarzani et al., 1996). However, this assumption was challenged by Das et al. who examined the relationship of BNP and NT-proBNP with body composition (determined by

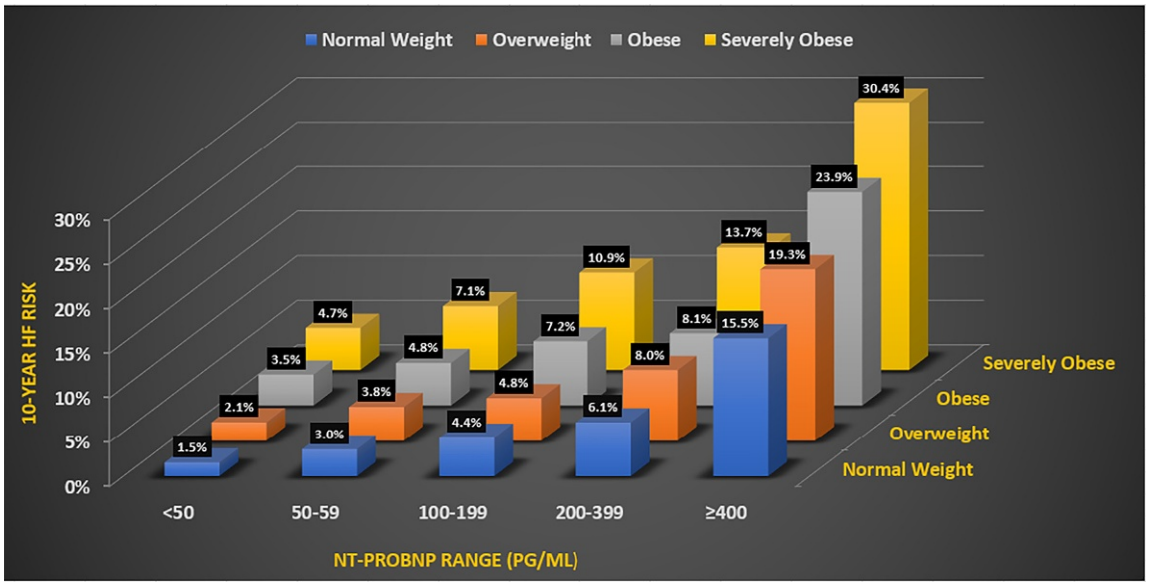

Fig. 5 Combined association of BMI categories and NT-proBNP levels with 10-year Risk of HF. The average predicted 10-year HF risk for the NT-proBNP ranges (<50, 50–99, 100–199, 200–399, ≥400 pg/mL) are displayed for each BMI category (normal weight [BMI 18.5 to <25 kg/m^2], overweight [BMI 25 to <30 kg/m^2], obese [BMI 30 to <35 kg/m^2], severely obese [≥35 kg/m^2]) in the Atherosclerosis in The Communities (ARIC) study. The 10-year HF risk was estimated using Cox regression analysis and after adjustment for age, sex, race, prior coronary artery disease, smoking status, diabetes mellitus, mean systolic blood pressure, use of antihypertensive medications, and heart rate. BMI, body mass index; HF, heart failure; NT-proBNP, N-terminal pro-B-type natriuretic peptide. The figure was drawn based on the data from Ndumele, C. E., Matsushita, K., Sang, Y et al. (2016). N-terminal pro-brain natriuretic peptide and heart failure risk among individuals with and without obesity: The Atherosclerosis Risk in Communities (ARIC) study. *Circulation* **133** (7), 631–638.

dual-energy X-ray absorptiometry) in 2707 subjects from the Dallas Heart Study (Das et al., 2005). The authors found that the association between obesity and BNP/NT-proBNP was mediated by LBM rather than fat mass.

Despite their inverse relationship with BMI, natriuretic peptides still contribute to HF risk prediction in individuals with obesity. The interaction between obesity and NT-proBNP-related HF risk prediction was examined by Ndumele et al. in the ARIC study participants ($n=12230$) who had no HF at baseline (Ndumele et al., 2016a). The investigators found that, even after adjustment for other HF risk factors, elevated NT-proBNP levels were associated with increased relative risk of development of HF within each BMI group and across different demographic subgroups by age, sex, and race. However, relative HF risk attributed to NT-proBNP was blunted among those in the highest BMI groups (Fig. 5). Nadruz et al. examined the interaction between BMI and prognostic impact of natriuretic peptides in patients with HFrEF (Nadruz et al., 2016). The study was conducted in 8217 subjects from the PARADIGM-HF (Prospective Comparison of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure) trial with a median follow-up of 27 months. The investigators found that elevated BNP and NT-proBNP is a predictor of CVD mortality or HF hospitalization across different BMI groups. However, the interaction was weaker in patients with a BMI ≥ 35 kg/m^2 when compared to other BMI groups.

The Obesity Paradox in HF

Despite the significant association between obesity and development of HF, numerous studies have reported an obesity paradox in patients with HF. This phenomenon is usually characterized by a better prognosis in overweight or mildly obese HF patients compared to their leaner counterparts (Lavie et al., 2016b). Moreover, the worst outcomes are usually incurred by underweight HF patients, followed by normal-weight ones (Alpert, 2016). The obesity paradox was first described in 2001 by Horwich et al. who identified higher BMI as an independent predictor of improved survival in patients with advanced HFrEF (Fig. 6) (Horwich et al., 2001). Sharma et al. examined the impact of BMI on HF outcomes in a metaanalysis of six studies with a combined sample size of 22,807 and a mean follow-up duration of 2.9 years (Sharma et al., 2015). The analysis found the highest risk of HF hospitalization and total and CVD mortality in HF patients with the lowest BMI (BMI < 20 kg/m^2). In regard to the same end-points, overweight or obese HF patients had a better prognosis than their counterparts with normal weight (Fig. 7).

Evidence suggests that obesity paradox can be seen with HFrEF, HFpEF and acute decompensated HF (ADHF) (Kenchaiah et al., 2007; Padwal et al., 2014). Fonarow et al., using the ADHF National Registry Data from the United States ($n=108,927$ hospitalizations), demonstrated that a lower in-hospital mortality rate in ADHF patients with a higher BMI (Fonarow et al., 2007b). In fact, every 5-unit increment in BMI resulted in 10% reduction of mortality. A report by Shah et al. in patients with ADHF ($N=6142$) from 12 prospective cohorts followed-up across 4 continents found an association between higher BMI and lower 30-day and 1-year

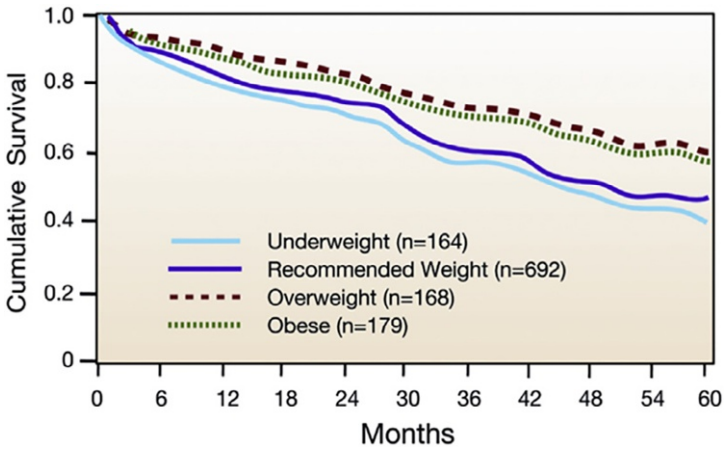

Fig. 6 Risk-adjusted survival curves for four body mass index categories after an average of 5 years of follow-up in a cohort of 1205 patients with advanced heart failure. Survival was significantly better in overweight and obese categories. Reproduced with permission from Horwich, T. B., Fonarow, G. C., Hamilton, M. A., MacLellan, W. R., Woo, M. A., Tillisch, J. H. (2001). The relationship between obesity and mortality in patients with heart failure. *Journal of the American College of Cardiology* **38** (3), 789–795.

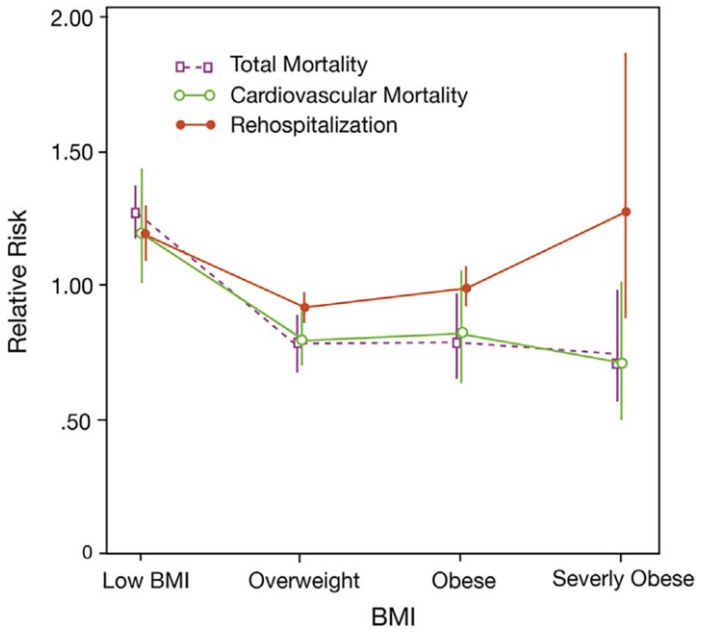

Fig. 7 Metaanalysis of 6 studies ($n = 22{,}807$) demonstrating the impact of BMI on all-cause mortality, cardiovascular mortality, and rehospitalization. *BMI*, body mass index. Reproduced with permission from Sharma, A., Lavie, C. J., Borer, J. S. et al. (2015). Meta-analysis of the relation of body mass index to all-cause and cardiovascular mortality and hospitalization in patients with chronic heart failure. *American Journal of Cardiology* **115** (10), 1428–1434.

mortality following ADHF hospitalizations (Shah et al., 2014). The obesity paradox was marked in older persons (>70 years of age), non-DM, and those with HFrEF and de novo HF diagnosis. Similarly, a study from Europe in chronic HF patients ($n = 2527$, mean follow-up = 4.3 years) showed an obesity paradox for only those without type-2 DM. And the obesity survival benefit was lost in the presence of type-2 DM (Zamora et al., 2016).

Most of the previous studies have reported obesity paradox in both sexes (Lavie and Ventura, 2015). However, a recent study by Vest et al. in patients with HFrEF (mostly advanced HF) suggested a sex-specific obesity paradox in their study population (Vest et al., 2015). The unadjusted data from the study revealed an obesity paradox in both sexes in regard to all-cause mortality. However, BMI-related survival benefit disappeared in males after adjustment for potential confounders. And overweight and obese males had a worse prognosis than normal weight males in the multivariable model. Contrarily, females in the overweight range had the lowest risk of mortality among all females after adjustment for confounders (Fig. 8). The cause of the interaction between sex and the obesity paradox remain poorly understood.

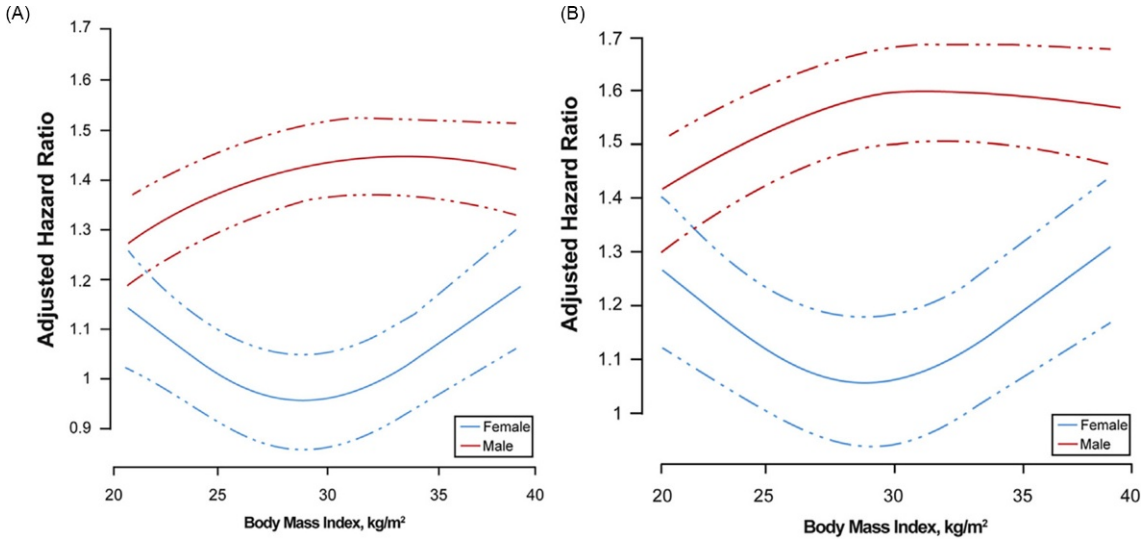

Fig. 8 Interaction plot between BMI and sex for nonischemic (A) and ischemic etiology (B). This retrospective study included 3,811 HFrEF patients who were referred for cardiopulmonary exercise testing. The hazard ratio was adjusted for other variables. Reproduced with permission from Vest, A. R., Wu, Y., Hachamovitch, R., Young, J. B., Cho, L. (2015). The heart failure overweight/obesity survival paradox: The missing sex link. *JACC: Heart Failure* **3** (11), 917–926.

The interaction between BMI and HF prognosis is mostly nonlinear. For instance, in the metaanalysis by Sharma et al., increasing severity of obesity was not associated with further improvement of the outcomes in patients with chronic HF (**Fig. 7**) (Sharma et al., 2015). The obesity paradox observed in the female subjects of the study by Vest et al. had a U-curve-shaped pattern as well (**Fig. 8**) (Vest et al., 2015). In fact, female patients with chronic HF had a nadir in mortality just below 30 kg/m^2 and the mortality risk gradually increased toward the either end of the BMI spectrum. A metaanalysis of 14 HF studies with a total sample size of approximately 24,000 patients found a U-shaped curve for BMI–mortality relationship in both chronic HFrEF and HFpEF (Padwal et al., 2014). Joyce et al. demonstrated a U-shaped curve interaction between BMI and short-term outcomes in patients with ADHF ($n=795$) (Joyce et al., 2016). Different from chronic HF studies, adverse outcomes risk reached a nadir at a higher BMI range (40 kg/m^2).

BMI has been the most commonly tested variable in studies reporting the obesity paradox in HF. Few studies have examined the prognostic role of body composition and other obesity parameters in HF. We previously reported the obesity paradox with both BMI and % BF (estimated with skinfold technique) in 209 patients with chronic HFrEF (Lavie et al., 2003). In fact, every 1% increment in % BF was associated with a 13% reduction in major CVD events. Clark et al. reported improved outcomes with both higher BMI and higher WC in patients with advanced HFrEF (Clark et al., 2011, 2012). The investigators observed the best survival in those with both high BMI and high WC (Clark et al., 2011). A retrospective study by Kamiya et al. examined the prognostic impact of BMI, WC, mid-upper arm circumference (MUAC), a surrogate of muscle mass, in 570 consecutive Japanese patients with ADHF (Kamiya et al., 2016). The study found improvement in the prediction of mortality when BMI was combined with MUAC. Whereas, adding WC to BMI did not change the risk prediction. HF patients with high BMI and high MUAC had the lowest risk, while those with low BMI and low MUAC had the highest risk of mortality. These results suggested that the relationship between adiposity and HF prognosis is altered by muscle mass (**Fig. 9**) (Lavie et al., 2016c). A retrospective study by our group which assessed the effect of body composition on mortality in a large cohort of patients with HFpEF (N=47866) suggested that the protective component of obesity derives from lean body mass rather than fat mass (De Schutter et al., 2014).

Severe Obesity in HF

The prevalence of grade III or severe obesity has been rising dramatically in the general population, and it commonly affects patients with HF (Flegal et al., 2016). Severe obesity is overrepresented in patients with HF partially due to the dose-dependent relationship with BMI and risk of incident HF. Approximately 20% of HF subjects who were enrolled in contemporary ADHF trials were found to have severe obesity (Joyce et al., 2016). Severely obese HF patients tend to have distinctive clinical and biochemical profiles. For example, they are more likely female, younger, hypertensive, and diabetic and have more edema and lower natriuretic peptide levels on presentation (Joyce et al., 2016). HFpEF is more predominantly represented in the severely obese HF population than those in the other BMI groups (Joyce et al., 2016; Fonarow et al., 2007b).

The prognostic impact of severe obesity in patients with HF has not been studied as extensively as that of other weight groups (Alpert, 2016). However, abundant evidence has indicated ominous prognosis associated with severe obesity in HF patients, especially during long-term follow-up (Lavie et al., 2016a; Joyce et al., 2016; Vest et al., 2015; Nagarajan et al., 2013; Nochioka et al., 2010). Joyce et al. demonstrated that the risk of 60-day composite outcome (death, rehospitalization, or unscheduled provider visit)

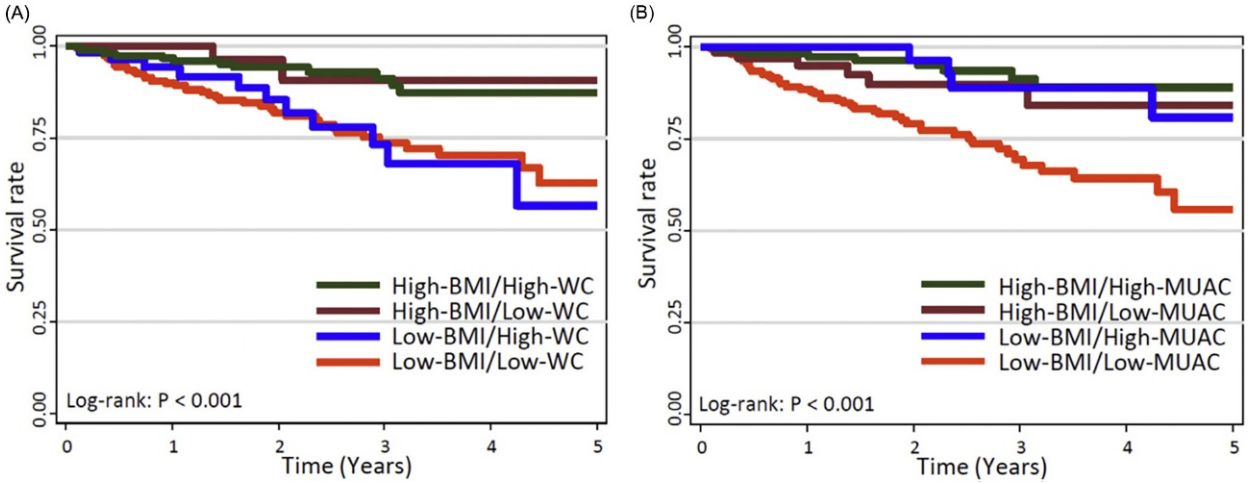

Fig. 9 Kaplan–Meier curves according to combined BMI and WC categories and combined BMI (A) and MUAC categories (B). *BMI*, body mass index; *MUAC*, mid-upper arm circumference; *WC*, waist circumference. Reproduced with permission from Kamiya, K., Masuda, T., Matsue, Y. et al. (2016). Complementary role of arm circumference to body mass index in risk stratification in heart failure. *JACC Heart Failure* **4** (4), 265–273.

sharply rises in ADHF patients with BMI >40 kg/m.^{2} (Joyce et al., 2016) Nagarajan et al. reported that after adjustment for other variables, advanced chronic HF patients with severe obesity (BMI $\geq$40 kg/m^2) had ~2.5-fold higher risk of all-cause mortality/transplantation compared to their counterparts with grade I or II obesity (BMI 30.1–40 kg/m^2) (Nagarajan et al., 2013).

Frailty and Cachexia in HF

Frailty is defined as a state of declining overall and physiologic function that is associated with increased susceptibility to disability (Lavie et al., 2015a; Gustafsson et al., 2009). Frailty is very common in elderly patients with HF, and it is independently associated with risk of readmission, early disability, and long-term mortality (Vidán et al., 2016).

Cardiac cachexia is a serious and poorly understood syndrome which can affect patients with advanced HF. Unintentional weight loss is the seminal feature of cardiac cachexia. Cachexia was identified as a strong independent predictor of mortality in patients with HF even before the obesity paradox in HF was first described (Anker et al., 1997). Moreover, studies describing the obesity paradox usually demonstrated the greatest risk of adverse outcomes in the underweight or lowest-BMI groups (Sharma et al., 2015; Kenchaiah et al., 2007; Nochioka et al., 2010; Curtis et al., 2005). To some extent, better prognosis with higher BMI in HF may represent the reverse of cachexia or in other terms "reverse epidemiology." (Lavie et al., 2014) Melenovsky et al. examined the relative impact of wasting of fat mass and lean mass on adverse outcomes in advanced HF patients with cachexia (Melenovsky et al., 2013). The study found that loss of fat mass rather than LBM was associated with poor outcomes.

Mechanisms of the Obesity Paradox

The mechanisms of the obesity paradox remain poorly understood. **Table 3** lists the potential mechanisms of the obesity paradox. It has been argued that the obesity paradox may partially be explained by the limitations of BMI in reflecting the true level of adiposity (Lavie et al., 2016a). However, we and others have demonstrated that obesity paradox is not specific to BMI and can be seen with other obesity parameters (Lavie et al., 2003; Clark et al., 2011, 2012).

Higher Energy Reserves

HF is a catabolic state and cachexia may be a marker of disease severity in patients with advanced HF. In addition, overweight and obesity indicate higher energy reserves which may help the HF patients effectively deal with the high-energy requirements of the disease (Sharma et al., 2015; Shah et al., 2014). Inability to control for unintentional weight loss is a limitation for many studies examining the relationship between obesity and prognosis in patients with HF. It has been suggested that the worse prognosis observed in leaner HF patients may be due to unintentional weight loss prior to or during the study (Lavie et al., 2016a; Vest et al., 2015). However, a study by Khalid et al. in ARIC study participants (n = 1487 HF patients) showed an obesity paradox even with the BMI values measured 4.3 ± 3.1 years prior to the first diagnosis of HF (Khalid et al., 2014). In fact, premorbid overweight and obesity were associated with increased risk of incident HF. But still, individuals with premorbid overweight and obesity had better survival after the HF diagnosis than their counterparts with premorbid normal weight. This is against the assumption that cardiac cachexia is the main cause of poor prognosis in leaner HF patients.

Table 3 Mechanisms of the obesity paradox

Mechanisms of the obesity paradox
Higher energy reserves
Better nutritional status
Increased muscle mass and strength
Earlier presentation of heart failure
Compression of morbidity in lean individuals
Reverse epidemiology of detrimental effects of cachexia and frailty
Implications related to cardiorespiratory fitness
Better tolerability of heart failure medications
Attenuated response to blockage of the renin-angiotensin-aldosterone system
Genetic factors
Changes in cytokines and neuroendocrine profiles
Immune modulation
Mitochondrial function augmentation

Nutritional Status

The recent line of evidence suggests that nutritional status is an essential prognostic indicator in HF patients, and it plays a role in the obesity paradox. Gastelurrutia et al. examined the prognostic implications of nutritional status in 214 ambulatory HF patients from a single academic center in Spain (Gastelurrutia et al., 2015). The study defined undernourishment based on several variables such as serum albumin, total lymphocyte count, and anthropometric measures such as skinfold thickness and arm muscle circumference. The study found that the frequency of undernourishment was 60%–100% among underweight, 31%–44% among normal-weight, 4%–11% among overweight, and 0%–3% among obese HF patients. Moreover, malnutrition was a more significant predictor of poor survival compared to BMI. A similar interaction between malnutrition and prognosis has been demonstrated in ADHF patients as well (Casas-Vara et al., 2012).

Muscle Mass

Several studies have confirmed the protective effect of muscle mass and strength in patients with CVD, including HF (Kamiya et al., 2016; Casas-Vara et al., 2012; Fülster et al., 2013). The significant association between muscle mass and BMI appears to contribute to the improved outcomes in overweight or obese HF patients. In the study by Kamiya et al. which included 570 consecutive HF patients, 70% of individuals with high BMI (>23.2. kg/m^2) also had high muscle mass (determined by high MUAC) while only 19% of those with low BMI had high muscle mass (Kamiya et al., 2016). And as stated earlier, the study demonstrated the significant contribution of muscle mass in the prediction of improved survival in patients with HF.

It should be noted that not all obese individuals have increased muscle mass and strength. Low muscle mass in the presence of high BMI has been described as sarcopenic obesity (Carbone et al., 2017). This phenotype, in the general population, has been associated with 24% increased risk of all-cause mortality when compared to obese individuals without sarcopenia (Tian and Xu, 2016). Further research is needed to determine the prognostic impact of sarcopenic obesity phenotype in patients with HF.

Tolerability of HF Medications

Obese and overweight patients with HF are more likely to have higher systemic BP than their leaner counterparts because of the strong correlation between BMI and BP levels. This allows overweight and obese HF patients to tolerate more and higher doses of cardioprotective medications, such as beta-blockers and RAAS blockers (Lavie et al., 2013a; Sharma et al., 2015). Also there exists some evidence that individuals with higher BMI, compared to those with normal weight, experience more pronounced regression of LV remodeling in response to blood pressure lowering. A metaanalysis by Zang et al. found that overweight and obesity are independent predictors of marked LV regression in response to anti-HTN treatment (Zhang et al., 2014). In their analysis, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers were found to be the most potent agents for LVH regression in patients with overweight and obesity.

Compression of Morbidity

Obese individuals usually develop HF at an earlier age compared to their leaner counterparts (Ahmad et al., 2016). Some authors have raised the possibility that the BMI-dependent variation in the age of onset of the disease may contribute to the shorter life expectancy in leaner HF patients (Wang, 2016). This is also referred as "compression of morbidity" phenomenon which was first proposed by James Fries in 1980 (Fries, 1980). This phenomenon occurs if the onset of a chronic disease is postponed more rapidly than the improvement in life-expectancy (Fries, 2005).

Genetic Factors and Others

There exist no studies evaluating the genetic background of the obesity paradox. It can be hypothesized that leaner individuals who develop HF may have a more significant genetic predisposition for HF since they develop HF despite not having other HF risk factors, such as HTN, DM, and systemic inflammation. And this genetic predisposition for the onset of the disease may be associated with a poor clinical prognosis as well (Lavie et al., 2016a).

Several other potential mechanisms have been proposed for the obesity paradox. These include but not limited to adiposity-related changes in cytokines and neuroendocrine profiles (i.e., soluble tumor necrosis factor receptors, leptin, adipokines, etc.), immune modulation, and mitochondrial function augmentation (Shah et al., 2014; Lavie et al., 2014; Chess et al., 2009). We still need further research to test the possible interaction of these mechanisms with the obesity paradox in humans.

CRF, the Obesity Paradox, and HF

CRF is a strong predictor of prognosis in healthy individuals and cohorts with CVD (Lee et al., 2012; Kodama et al., 2009; Lavie et al., 2013b). Many studies have established the prognostic significance of CRF and other variables of cardiopulmonary exercise testing in HF (Lavie et al., 2013b; Guazzi et al., 2016; Osman et al., 2000). The classic cut-off point for classifying HF patients into low- and high-risk groups have been the peak oxygen consumption (VO_2) of 14 mL O_2/kg min (Mancini et al., 1991).

Several studies have indicated a strong interaction between CRF and the obesity paradox in patients with CAD and HF (Lavie et al., 2013b; McAuley et al., 2012). We previously demonstrated in a cohort of 2066 patients with chronic HFrEF that patients with a high CRF (peak $VO_2 \geq 14$ mL/kg min) have a better prognosis and an obesity paradox does not affect this patient population (Lavie et al., 2013b). Contrarily, a strong obesity paradox was present in those with a poor CRF. The worse outcomes were observed in leaner individuals with a poor CRF (**Fig. 10**). Similar results were reported in other cohorts of HFrEF from the United States and Europe (Piepoli et al., 2016; Clark et al., 2015).

Purposeful Weight Reduction in HF

Weight loss, achieved via diet, exercise, and/or bariatric surgery, can result in significant improvements of obesity-associated alterations in hemodynamics and cardiac structure and function (Lavie et al., 2015a). Hemodynamic changes associated with weight loss include a reduction in total and central blood volume, stroke volume, and CO. LV end-diastolic pressure and pulmonary capillary pressure usually, but not always, decrease following weight loss (Alpert et al., 2014).

A large body of evidence have demonstrated that weight reduction in overweight and obese individuals is associated with a decrease in LV mass and wall thickness and regression of LVH (Alpert et al., 2014; Ikonomidis et al., 2007; Jhaveri et al., 2009; Haufe et al., 2012). The improvement of LV geometry in response to weight loss is attributed to several mechanisms, such as reduction in cardiac loading and improvement in neurohormonal and metabolic abnormalities such as RAAS activation, sympathetic nervous system, and insulin resistance (Alpert et al., 2014; Ikonomidis et al., 2007).

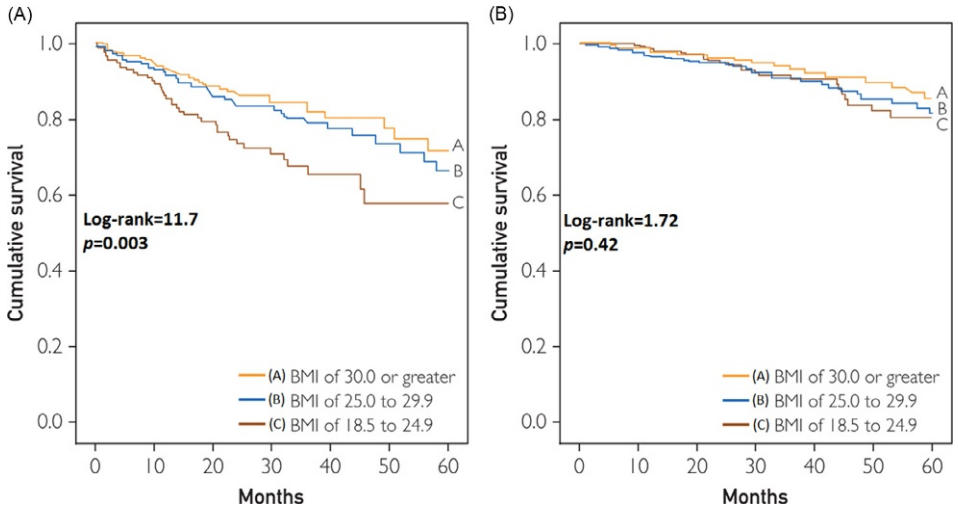

Fig. 10 Kaplan–Meier analysis according to BMI categories in the low cardiorespiratory fitness group (oxygen consumption <14 mL O_2/kg min) (A) and the high cardiorespiratory fitness group (oxygen consumption ≥14 mL O_2/kg min) (B). *BMI*, body mass index. Reproduced with permission from Lavie, C. J., Cahalin, L. P., Chase, P. et al. (2013). Impact of cardiorespiratory fitness on the obesity paradox in patients with heart failure. *Mayo Clinic Proceedings* **88** (3), 251–258.

Weight loss can lead to improvements in diastolic dysfunction (Ikonomidis et al., 2007; Alpert et al., 1997; Ashrafian et al., 2008). Because LVEF is usually within the normal range in obese individuals, most studies have not found any changes in LVEF in response to intentional weight loss (Alpert et al., 2014). A study by Vest et al. demonstrated that weight loss (by bariatric surgery) in severely obese patients with depressed LVEF led to a minimal but statistically significant increase in LVEF (Vest et al., 2016). Recent studies using speckle-tracking echocardiography reported significant improvement in LV and RV strain following bariatric surgeries (Kaier et al., 2014; Koshino et al., 2013; Shin et al., 2016).

Scientific evidence on the safety and efficacy of weight loss with diet, exercise training (ET), or bariatric surgery in obese HF patients remains limited. We still do not know if purposeful weight loss has any impact on mortality in patients with HF. Shimada et al. recently examined HF hospitalization rates in a population-based cohort ($n = 524$) of patients with HF who underwent bariatric surgery (Shimada et al., 2016). The study demonstrated 43% decrease in the rate of ED visits and hospitalizations for ADHF following 2 years after the surgery. Kitzman et al. recently compared the impact of aerobic ET and caloric restriction (via diet) on exercise capacity (measured by peak VO_2 consumption) in obese patients with chronic HFpEF ($n = 100$) (Kitzman et al., 2016). The investigators found that weight loss with diet was associated with significant increase in exercise tolerance. In addition, a combination of diet and exercise training led to a more robust improvement.

The most recent guidelines on the management of patients with HF do not provide firm recommendations regarding purposeful weight loss in obese HF patients (Yancy et al., 2013). Nevertheless, these guidelines recognize the detrimental effect of severe obesity in patients with HF. Based on available data, purposeful weight loss may be reasonable in HF patients with severe obesity. More importantly, interventions in HF patients with obesity should focus on physical activity (PA), ET, and improving CRF, regardless of the effect on BMI (Carbone et al., 2017). Clearly, ET, increased PA, and improved CRF have been demonstrated to reduce the risk of adverse outcomes in patients with HF, regardless of BMI (Lavie et al., 2015b). The ideal type of ET (aerobic-type vs. resistance ET) for HF patients is not well established. Based on the data supporting the protective role of LBM, particularly appendicular muscle mass, resistance ET can be considered to increase muscle mass and strength (Carbone et al., 2017; Lavie et al., 2016c).

Conclusions

Obesity adversely impacts CV structure and function and is associated with increased risk of HF. Not surprisingly, obesity is commonly seen in patients with HF. Despite being a risk factor for the development of HF, overweight and obesity exert protective effects once HF is manifest. The obesity paradox is now a well-established phenomenon which is observed across different ethnicities and regardless of the type (HFpEF vs. HFrEF), acuity (acute vs. chronic) and etiology (ischemic vs. nonischemic) of HF. The relationship between BMI and adverse outcomes in HF usually demonstrates a U-shaped curve pattern with the poorest prognosis in underweight and to a lesser extent in severely obese patients and a nadir of risk with overweight and mild obesity. CRF is the strongest mediator of the interaction between BMI and prognosis in HF. Growing evidence suggests that LBM, compared to fat mass, has more significant prognostic implications in patients with HF. Therefore, instead of just relying on BMI, body composition-based risk stratification in HF may help with identifying patients at higher risk of adverse outcomes. There still exists a knowledge gap in regard to safety, efficacy, and optimal strategy for intentional weight loss in HF patients with obesity. Nevertheless, increased PA/ET with the goal of improved CRF should be considered in all patients with HF to decrease adverse outcomes.

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Relevant Websites

http://aricnews.net/HFCalcs/RiskCalcHFClinFac.html—This risk assessment tool uses the information from the ARIC study and is designed for black or white adults, 55-75 years old. It predicts, using clinical factors, a person's chance of having new onset hospitalized heart failure in the next 10 years.
http://www.heartfailurerisk.org/—This risk assessment tool uses the information from the Meta-Analysis Global Group in Chronic Heart Failure (MAGGIC). The analysis included a cohort of 39372 patients with chronic HF and presents 1- and 3-year mortality estimates in people with chronic HF.

Oral Health and Cardiovascular Disease: Recent Findings and Future View With a Novel Aspect

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Introduction: The Essential Points of Oral Health

The oral cavity is the entrance to the digestive tract, and as such is not only responsible for ingesting the nutrients and fluids essential to life by means of mastication and swallowing, but it is also an important organ for establishing humans as social beings by way of oral communication. The oral cavity is a luminal structure stretching from the lips to the fauces, which form the border with the oropharynx, which is bordered by the surrounding maxillae (palate), mandible, lips, and cheeks and contains the teeth and tongue. The mobile tissues, including the lips, cheeks, mandible, and tongue, are skillfully controlled by the muscles to enable eating and speech to proceed smoothly, and the coordination of their movements with the shape of the teeth, gingiva, palate, and other immobile tissues is critical for this process. The moist environment of the oral cavity is constantly maintained by saliva distributed from the salivary glands to ensure that the mouth remains mobile.

As the entrance to the digestive tract, the oral cavity is responsible for acting to process food into an easily digestible state (by chewing). The mouth is an extremely delicate sensory organ equipped to tactile sense, pressure, temperature, and taste, instantly discriminating between foods to reject dangerous substances and controlling the muscle activity and mandible movement required for mastication in accordance with their physical properties. These oral sensations create the taste of food and are the source of the psychological action of taking pleasure in eating. Saliva is secreted in large quantities during eating to process food into a bolus that is easily swallowed and digest carbohydrates. The antimicrobial and immune substances in saliva fulfill the role of biochemical defense mechanisms in the oral cavity.

The main goals of dental treatment are to develop, maintain, and restore the functions exerted by the normal morphology of the mouth described previously. Caries and periodontal disease (PD), the two major diseases of the oral cavity, are both caused by bacterial infection, but despite the great efforts expended on their suppression and treatment since the establishment of modern dental science, they have yet to be defeated. The association between chronic inflammation of the periodontal tissue due to periodontal pathogen has become a focus of attention in recent years, and since the turn of the century, research on this subject been particularly active. Diseases and abnormalities already found to be associated with PD include arteriosclerosis (Lockhart et al., 2012), cardiovascular disease (Khader et al., 2004), diabetes mellitus (Saito et al., 2004), obesity (Saito et al., 2001), rheumatism (Hashimoto et al., 2015), aspiration pneumonia (Abe et al., 2006), and premature birth (Offeenbacher et al., 1996).

In its initial stage, PD comprises subgingival inflammation of the gingiva due to periodontal bacteria in plaque that has built up in the gingival sulcus. As it progresses, gingival pockets develop, and the bacteria penetrate more deeply, the alveolar bone breaks down, and symptoms such as ulceration, teeth mobility, and occlusal pain appear, ultimately leading to tooth loss. Among individuals aged 35–44 years, the prevalence of periodontal disease is approximately 50% in Europe and North America (Peterson et al., 2005) and approximately 25% in Japan (Ministry of Health, Labour and Welfare, 2011), making it the most important cause of tooth loss in middle age and later, even in developed countries with widely available dental care. Suffering from PD thus not only diminishes oral health in two senses, both through long-term exposure to inflammation and through loss of masticatory function as a result of tooth loss, but it has also been implicated in the development and aggravation of a range of

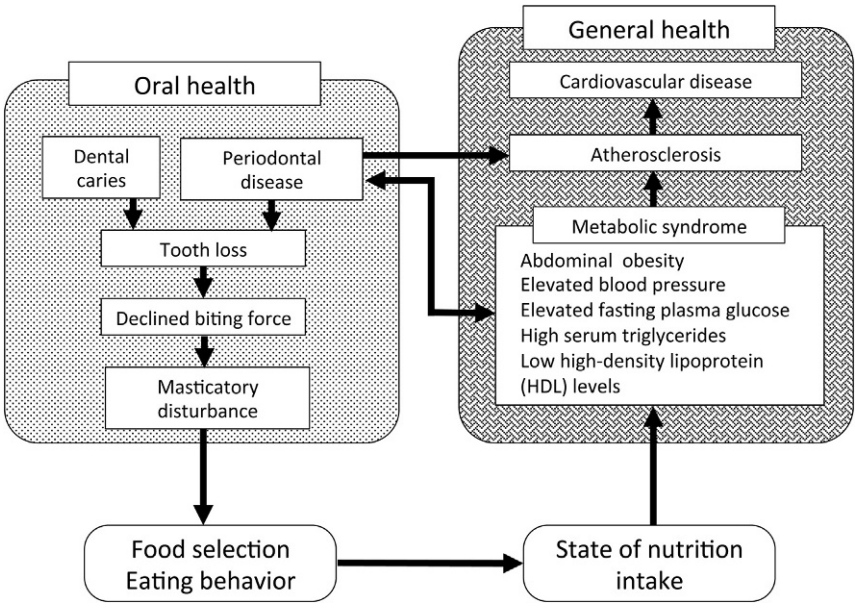

Fig. 1 A simplified image of the impact of oral health on cardiovascular disease with the novel aspect.

systemic conditions. The objective of this review is to outline recent findings on PD as a risk factor for cardiovascular disease and future prospects for its prevention based on the novel aspect of comprehensive oral health (Fig. 1).

Metabolic Syndrome and Oral Health

The association between metabolic syndrome (MetS) and PD has been well studied in the epidemiological way. In this section, we describe findings on the association between MetS and its various risk factors and oral health, particularly PD. Most of studies about the association between MetS and oral health treated PD as a marker of oral health, and few studies treated tooth loss as a surrogate maker of current and past PD. As for the diagnostic criteria for MetS, Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (NCEP-ATP III) criteria was most popularly used in those studies, and the Japanese diagnostic criteria, the American Heart Association and the National Heart, Lung and Blood Institute (AHA/NHLBI) criteria, the International Diabetes Federation (IDF) criteria, the European Group for the Study of Insulin Resistance criteria and integrated versions of these diagnostic criteria were also used.

Individuals with MetS have also been found to be at higher risk of PD than those without (Morita et al., 2009; Timonen et al., 2010; Sora et al., 2013; Minagawa et al., 2015). It was found that patients who are positive for a high number of components of MetS have a higher prevalence of PD (Khader et al., 2008; Kushiyama et al., 2009; Han et al., 2010; Andriankaja et al., 2010; Furuta et al., 2013; Lee et al., 2014). Shimazaki et al. (2007) reported that women who were positive for a higher number of components of MetS were at higher risk of PD, and those who were positive for four or more components were 6.6 times more likely to suffer from PD than were individuals with none. Self-reported number of teeth in Swedish of 70 years old was fewer in those with MetS than those without, and inversely related with inflammatory makers such as leukocyte and C-reactive protein (Holmlund et al., 2007).

Conversely, individuals in a poor periodontal state have been found to be at high risk of MetS (D'Aiuto et al., 2008; Li et al., 2009; Han et al., 2010; Nesbitt et al., 2010; Bensley et al., 2011; Chen et al., 2011; Kwon et al., 2011; Fukui et al., 2012; Tu et al., 2013). In Finish people of 45–74 years old, more than four missing teeth and complete edentulousness were associated with MetS with odds ratio at 1.69 and 1.93 respectively (Hyvärinen et al., 2015). Analysis by the data of The National Health and Nutrition Examination Survey also found the increase of odds ratio for MetS depending on the increasing of missing teeth (Zhu and Hollis, 2015). These conclusions have all been derived from cross-sectional epidemiological studies, and mention of possible causality has been regarded as problematic.

In 2010, however, a cohort study was published that suggested that PD may aggravate MetS (Morita et al., 2010). Moreover, Kaye et al. (2016) found that the risk of tooth loss was 1.39 times greater for individuals who suffered from MetS at baseline compared with those who did not, suggesting that MetS may worsen the condition of the gingiva. At present, few cohort studies of MetS and oral health have been performed, and further evidence is required to elucidate the causal relationship between them.

Studies have addressed not only the relationship between PD and MetS, but also its component factors of obesity (Saito et al., 1998; Chafee and Weston, 2010; Morita et al., 2011), impaired glycometabolism (Taylor, 2001; Mealey, 2006; Demmer et al., 2010; Sora et al., 2013), dyslipidemia (Nibali et al., 2007; Lee et al., 2013), and hypertension (Vidal et al., 2011). With respect to hypertension and oral health, the risk of hypertension increases if tooth loss and other aggravated oral conditions are also present in

addition to PD (Iwashima et al., 2014). Although each causal relationship is still controversial, recent longitudinal and intervention studies suggested pathological pathway between PD and obesity (Gorman et al., 2012), impaired glycometabolism (Sgolastra et al., 2013) and dyslipidemia (Acharya et al., 2010), which could be expected to lead new strategies for preventing MetS.

Ischemic Heart Disease and Oral Health

Since Mattila et al. showed in 1989 that oral health is associated with the occurrence of myocardial infarction, attention has focused on the association between PD and ischemic heart disease, and this has now been well studied. According to a 2004 metaanalysis (Khader et al., 2004) that drew on seven cohort studies and four cross-sectional studies, individuals with PD have a relative risk of coronary artery disease of 1.15, but no association was observed between the number of teeth and coronary artery disease. A later metaanalysis (Bahekar et al., 2007) that drew on five cohort studies, five case-control studies, and five cross-sectional studies found that individuals with PD have a relative risk of ischemic heart disease of 1.14 in cohort studies, with the odds ratio of 2.22 in the case-control studies and 1.59 in the cross-sectional studies. An analysis of the association between the number of teeth and ischemic heart disease also found that, for patients with $\leq$10 teeth, the relative risk of coronary artery disease was 1.24. In 2012, however, the American Heart Association (AHA) published a systematic review in which they compared 500 studies that had reported an association between these conditions, and they found that there was insufficient evidence that PD has any effect on the development or progression of ischemic heart disease (Lockhart et al., 2012). After this statement, it was found by 13 years follow-up survey in 8446 Finish people that having more than five missing teeth was associated with coronary heart disease events and acute myocardial infarction with the relative risk of 1.62–1.99 and 2.10–2.39 respectively (Liljestrand et al., 2015).

The Mechanism of Atherosclerosis due to Periodontal Bacteria

A range of factors related to chronic inflammation has been implicated in the progression of arteriosclerosis, including hyperlipidemia, obesity, smoking, and genetic factors (Okuda et al., 2004). Relationship between periodontal pathogens forming a biofilm and arteriosclerosis was researched since the 1980s. It was suggested that infections due to organisms, bacterium and viruses might contribute to arteriosclerosis by causing inflammation within vascular tissue. Based on a series of studies, the theory that cell damage due to vascular inflammation is implicated in the formation of atheromata was published (Ross, 1999). Studies of the mechanism whereby periodontal bacteria contribute to arteriosclerosis have been carried out on the basis of this theory.

Invasion of the Bloodstream by Periodontal Bacteria

Periodontal pathogens in dental plaque frequently enter the bloodstream. This invasion is particularly common when PD is advanced, with the formation of deep pockets. Various studies have showed that pathogenic periodontal bacteria and their toxic products have been implicated in a range of circulatory problems. Firstly, it was found that the aggregation of human red blood cells is caused by lipopolysaccharides (LPS), an endotoxin of periodontal pathogens (Okuda and Kato, 1986). Later studies have shown that the platelet aggregation is also caused by bacteria in dental plaque (Herzberg and Weyer, 1998; Imamura et al., 2003). Moreover it was showed that the aggregation of human blood cells is caused by the platelet-aggregating action of *Porphyromonas gingivalis* (*P. gingivalis*, Naito et al., 2006). These findings showed that pathogenic periodontal biofilm-forming bacteria such as *P. gingivalis* enter the bloodstream of individuals with PD and cause the blood to coagulate, contributing to the impairment of blood flow.

Detection of Periodontal Bacteria at Sites of Arteriosclerosis

Numerous studies have detected periodontal pathogenic bacteria from atherosclerotic foci. It was firstly reported that *P. gingivalis* was detected in carotid artery samples collected during carotid endarterectomy by carrying out immunostaining with specific antibodies (Chiu, 1999). Later, another study group detected periodontal pathogens in extracted DNA from samples collected during carotid endarterectomy (Haraszthy et al., 2000). Furthermore, the detection rates of periodontal pathogens in the mouths of patients who underwent coronary artery bypass surgery and in samples of the arterial wall obtained during surgery was investigated, it was found that periodontal pathogens were detected in coronary artery wall samples, and that the detection rate was higher in patients with severe PD (Ishihara et al., 2004). These results suggest that periodontal pathogens that have transiently entered the bloodstream reach the vascular wall of the coronary artery, and that the frequency with which this occurs is associated with the severity of PD.

Analysis of the Mechanism from Animal Experiments

As stated previously, there has been a huge clinical data accumulation suggesting the relationship between PD and atherosclerosis. However analysis of etiology by using clinical samples has limitations, so various animal experiments have been conducted in order to detect the mechanism by which periodontal pathogens affect on the formation of atherosclerosis.

The apoE-knockout mice that had been fed by a high-fat diet and infected *P. gingivalis* intravenously had more promotion of arteriosclerosis in the proximal aorta than in uninfected mice (Li et al., 2002). In another study, it was found that there were more sites of aortic atherosclerosis in apoE-knockout mice that had been inoculated with *P. gingivalis* intraorally in the mouth than in uninfected apoE-knockout mice (Lalla et al., 2003). These findings showed that *P. gingivalis* promote progression of arteriosclerosis in apoE-knockout mice. Furthermore, it was found that greater fat deposited in the aortas of rabbits received *P. gingivalis* after ligatures around teeth than in those of unaffected rabbits, suggesting that *P. gingivalis* also acts to promote atherosclerosis formation in normal animals that have not undergone any particular gene knockout (Jain et al., 2003). Later, it was found that *P. gingivalis* developed coronary atherosclerosis by an experiment on pigs after having been inoculated intravenously with *P. gingivalis* (Brodala et al., 2005). Taken together, these early findings from animal experiments suggest that the infiltration of the vascular wall by *P. gingivalis*, or the action on vascular endothelial cells or immunocompetent cells of components of bacterial bodies that have infiltrated the bloodstream, may be implicated in promoting the formation of atherosclerosis. Furthermore it was understood by numerous researches that cytokines produced in the inflated periodontal tissue also entered the circulation and might induce general inflammation (Preshaw and Taylor, 2011).

However, a new pathogenic pathway from PD to general inflammation has been provided in recent years. Firstly, it was found that an elevation of serum inflammatory biomarkers in mice with repeated oral inoculation of *P. gingivalis* was not accompanied with detection of bacteria in the blood and remarkable inflammatory cell infiltrate in the gingival tissue (Maekawa et al., 2011). Based on those results, the same research group tested the hypothesis in mice that disturbances of gut microbiota by swallowed *P. gingivalis* induced a metabolic endotoxemia leading metabolic disorders and demonstrated the alteration of gut microbiota and inflammatory changes in various tissue and organs (Arimatsu et al., 2014). Furthermore, it was found that oral administration of *P. gingivalis* caused the dissemination of enterobacteria to liver by increasing intestinal permeability associating the changes in gut microbiota (Nakajima et al., 2015). These findings suggested that disturbance of the gut microbiota composition by swallowed periodontal pathogen might be causal mechanism between PD and systemic disease.

On the other hand, it was reported that *P. gingivalis* infection from the pulp chamber in obese mice accelerated free fatty acid induced endothelial injury with the in vitro finding that *P. gingivalis* and its LPS both attenuated cytokine production in human umbilical vein endothelial cells, suggesting the pathway by which dental infection of *P. gingivalis* might contribute to pathogenesis of atherosclerosis (Ao et al., 2014). Thus it is expected that further researches using animal model provide novel and comprehensive knowledge about the interrelationship between PD and atherosclerosis as well as relating general diseases.

Periodontal Treatment and Improvements in Risk Markers for Arteriosclerotic Disease

Risk markers for arteriosclerotic disease include serum lipids and serum inflammatory markers. In the Japanese Hisayama Study, HDL cholesterol levels were found to be significantly lower in the population with PD than in those in the population without (Shimazaki et al., 2007). Individuals with PD with a high serum antibody titer for periodontal pathogens have also been found to have significantly higher LDL cholesterol levels (Kudo et al., 2015).

CRP is not only a marker of systemic inflammation, but it has also been found to be a useful risk marker for coronary artery disease (Bisoendial et al., 2005), and serum CRP concentration has been shown to be significantly elevated in individuals with PD compared with healthy individuals (Loos et al., 2000). It has been suggested that the inflammatory cytokines TNF and interleukin (IL-1, IL-6, and IL-8) are also elevated as a result of PD (Loos, 2005), but consensus has yet to be achieved on this point. Further studies are required to ascertain whether the elevation of inflammatory markers as a result of PD is sufficient to cause the development or progression of arteriosclerotic disease. Individuals with PD often also develop obesity, diabetes, hypertension, and other lifestyle-related diseases as a result of their own particular lifestyles, and epidemiological studies must also take this fact into account.

The implementation of treatment for PD has been shown to reduce the levels of factors such as IL-6 and CRP (Nakajima et al., 2010). In terms of serum lipid levels, periodontal treatment has been shown to improve the level of HDL (Pussinen et al., 2004). Some studies have also shown that serum inflammatory markers and lipids were more improved when intensive periodontal treatment was performed (D'Aiuto et al., 2006). Lee et al. compared individuals with PD who underwent treatment with untreated individuals, and they found that the incidence of ischemic heart disease was higher in the untreated group (Lee et al., 2013).

Future Prospects for Research

Gender Difference

Although several studies have shown that the association between MetS and oral health is stronger in women (Shimazaki et al., 2007; Andriankaja et al., 2010; Lee et al., 2014), some studies have found a stronger association in men (Morita et al., 2009). A study of postmenopausal women did not find a significant correlation between MetS and oral health (LaMonte et al., 2014). With respect to the association between obesity and oral health, some studies have found a strong correlation in women (Morita et al., 2011), while others have shown that male obesity increases the risk of progression of PD (Saito et al., 1998). These discrepancies may be due to differences in the diagnostic criteria for MetS used and the assessment of indicators of oral health, as well as the age and race

of subjects. More studies are therefore required to identify the reasons underlying sex differences in the association between oral health and MetS, and which should be considered in the future health promotion.

Diagnostic Criteria for Periodontal Disease

As described in Introduction, PD comprises subgingival inflammation of the gingiva due to periodontal bacteria in its initial stage. As it progresses, periodontal tissue is destroyed, gingival pockets develop and the alveolar bone breaks down. Parameters for diagnosing PD and evaluating its severity are quite various such that bleeding from gingival sulcus, pocket depth, tooth mobility, absorption of alveolar bone measured on the X-ray photo, and so on. Inconsistencies between the results of studies of PD and arteriosclerosis may be due to differences in the indices used to measure PD and the diversity of definitions used, as well as to difficulties in comparing data between studies and from different regions. Larger-scale, long-term epidemiological studies are required that utilize a consistent study design, including disease definitions, and that are based on objective, unified assessment indices. Since studies have also found that many risk factors are common to both PD and ischemic heart disease and, therefore, constitute confounding factors (Peacock and Carson, 1995; Hujoel et al., 2000), these confounding factors must also be taken into consideration.

Masticatory Ability

It was pointed out that tooth loss as a result of PD or dental caries reduced masticatory ability and might change in dietary intake having an adverse effect on nutritional intake (Appollonio et al., 1997; Krall et al., 1998; Sheiham et al., 2001; Kanmori et al., 2003). Recent epidemiological surveys with a large number of participants have provided detailed knowledge how the tooth loss influences on the state of food and nutrition intakes. As for the impact of complete tooth loss (edentulism), questionnaire survey in US women revealed that edentulous US women have higher intakes of saturated fat, trans fat, cholesterol, and vitamin B12, and lower intakes of polyunsaturated fat, fiber, carotene, vitamins C, E and B6, folate, and potassium compared with women with 25–32 teeth (Hung et al., 2005). In Sweden, odds ratio for obesity in edentulous women aged 55–74 years increased from 1.64 in 1980 to 3.17 in 2002 (Österberg et al., 2010). Additionally in Pomerania, Germany, edentulism was associated with the increased odds of high inflammatory makers such as C-reactive protein and fibrinogen comparing with dentate subjects (Meisel et al., 2012). These reports suggested that edentulism might have negative impact on the intake of cardiovascular disease related nutrients and could be the risk for obesity.

As for the impact of number of remaining teeth on food and nutrient intakes, it was found in US adult civilians without prosthesis that people with fewer than 28 teeth had lower intakes of carrots, tossed salads, and dietary fiber than did fully dentate people, and lower serum levels for carotene, folate, and vitamin C (Nowjack-Raymer and Sheiham, 2007), and in Japanese dentists that carotene, vitamins A and C, milk, and vegetables decreased with the decreasing number of remaining teeth and carbohydrate, rice and confectionaries increased among people with fewer teeth (Wakai et al., 2010). These findings suggest that the decline in masticatory function caused by the progression of PD or dental caries and the resulting tooth loss may contribute to MetS by way of eating behavior and nutritional intake.

Against this backdrop, novel objective assessments of masticatory function have been introduced in some cohort studies in Japan. In SONIC Study which investigated the health and longevity of septuagenarians, octogenarians, nonagenarians, and centenarians, the maximal biting force measured by pressure-sensitive sheets was associated with intakes of vitamins and dietary fiber rather than number of remaining teeth (Inomata et al., 2014). In the Suita Study (Kokubo et al., 2008), a cohort study on the risk of cardio- and cerebrovascular diseases in Japanese urban general population including dental examinations with objective masticatory performance assessments using gummy jellies (Kosaka et al., 2014; Kikui et al., 2015; Kosaka et al., 2016), it was found that lower masticatory performance and poorer periodontal state associated with the prevalence of MetS in both cross-sectional (Kikui et al., 2017a,b). It is expected that longitudinal analysis on the association among objectively assessed oral health, food and nutrition intakes, prevalence of MetS components, and incidence cardiovascular disease will provide a lot of knowledge.

Consequences

As shown by the results of the numerous studies described in this article, there appears to be some sort of association between cardiovascular disease, MetS, and oral health, mainly PD. However, at this point, the evidence is insufficient to establish the direction of this association and its causality. More studies are therefore required in the future, and basic research and clinical interventional studies, in addition to longitudinal epidemiological studies, may help to clarify the association among them. It is also expected to establish the objective clear-cut methods for evaluating oral health makers such as the state of periodontal disease, masticatory performance, and so on, which enable more concrete collaboration between physicians and dentists, based on the novel aspect of the prevention of cardiovascular disease.

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Further Reading

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Orthostatic Hypotension and Vasovagal Syncope

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Introduction

Syncope is defined as a sudden transient loss of consciousness secondary to cerebral hypoperfusion with a loss of postural tone followed by a rapid and spontaneous recovery (Moya et al., 2009). It is a very common presenting complaint to many health care providers including physicians working in the emergency department, primary care office, and cardiology or neurology clinics.

There are many underlying etiologies for syncope, however, several broad classifications exist. These include cardiac syncope, neurally mediated (or reflex) syncope, and orthostatic hypotension (OH) (Wieling et al., 2009; Moya et al., 2009). Cardiac syncope refers to syncope occurring secondary to underlying heart disease, most commonly a structural, obstructive, or arrhythmia-based pathology. Vasovagal syncope (VVS), the most common form of neurally mediated syncope, refers to an abrupt change in autonomic control resulting in vasodilation, bradycardia, and subsequent cerebral hypoperfusion (Sheldon et al., 2015). In contrast, OH is most commonly defined as a sustained decline in blood pressure (BP) $\geq$20 mmHg systolic BP or $\geq$10 mmHg diastolic BP within 3 min of assuming an upright position (Freeman et al., 2011).

VVS and OH are two of the most common presentations for syncope. Here, we will review the normal physiology of standing and definitions, etiology, approach to diagnosis, and management strategies for both conditions.

The Physiology of Standing

In a normal individual, approximately 25%–30% of their blood volume is located in the thorax while they are supine (Grubb, 2005). Assuming an upright posture, from either a supine or seated position, triggers the cardiovascular system to react to the increased effects of gravity (**Fig. 1A**). It does so by initiating a downward shift of 500–1000 mL of blood to the capacitance vessels of the lower extremities and splanchnic circulation (Nwazue and Raj, 2013; Chisholm and Anpalahan, 2016). Secondarily, increased hydrostatic pressure in these tissues leads to a substantial loss of plasma volume from the vasculature into the interstitial tissue (Robertson, 2008). These physiological changes result in decreased venous return, decreased cardiac output, and eventually in decreased BP.

The mechanisms involved with the compensatory effects against orthostatic stresses are mainly controlled by the autonomic nervous system, and include both arterial and cardiopulmonary baroreceptors (Tanaka et al., 1996). The high-pressure baroreceptors in the carotid sinus and aortic arch and low-pressure receptors in the heart and lungs are "unloaded," which triggers a reflex sympathetic activation and inhibition of parasympathetic activity (Nwazue and Raj, 2013; Feldstein and Weder, 2012). This causes an increase in heart rate (HR) and systemic vasoconstriction, thereby countering the initial decline in BP (**Fig. 1B**). As a result, in a healthy individual, assumption of an upright posture does not result in major changes in BP. The net effects typically include a transient drop in systolic BP of 10–15 mmHg, a slight increase in diastolic BP of approximately 5–10 mmHg, and a rise in HR of 10–25 bpm (Chisholm and Anpalahan, 2016).

In the instance of a prolonged orthostatic challenge, neurohumoral responses may occur, including the activation of the renin-angiotensin-aldosterone system (Medow et al., 2008). This further contributes to vasoconstriction.

The autonomic nervous system plays a substantial homeostatic role in response to changes in posture. Autonomic dysfunction can result in a lack of counter-regulation and may lead to OH, resulting in falls, presyncope, and syncope (**Fig. 1C**). Alternatively, normal compensatory responses to standing can abruptly turn off leading to a fainting spell, as is the case in VVS (**Fig. 1D**).

Vasovagal Syncope

VVS is defined as a form of syncope that is typically associated with hypotension and relative bradycardia, followed by fatigue (Sheldon et al., 2015). It usually occurs with the assumption of an upright posture for greater than 30 s, or with exposure to emotional stress, pain, or medical settings. There are several common symptoms of the disorder, including diaphoresis, warmth, nausea, and pallor. The 30-s time requirement is used to exclude patients presenting with initial OH (Raj and Sheldon, 2016).

Epidemiology

The incidence of syncope follows a bimodal distribution with an initial peak around 15 years of age with a second rise beginning after 70 years of age (Serletis et al., 2006; Ganzeboom et al., 2006; Colman et al., 2004). The cumulative incidence of an episode of syncope is estimated to be approximately 35% by age 60 (Ganzeboom et al., 2006; Serletis et al., 2006). It is suggested that 30%–50% of patients presenting with syncope have an autonomically mediated form, of which VVS is the most common (Sheldon et al., 2015). VVS is also more common in females (65%F:35%M) (Serletis et al., 2006; Ganzeboom et al., 2006; Sheldon et al., 2006, 2016). VVS is not associated with an increased risk of mortality (Sheldon et al., 2015).

Etiology

The pathophysiological basis for VVS leading to a syncopal episode is secondary to volume depletion leading to decreased venous return and preload; this leads to a baroreceptor response to increase HR, contractility, and increased vascular tone aimed at maintaining BP (Coffin and Raj, 2014a). Such a state can occur following triggers for VVS. The low venous return combined with increased cardiac contractility is thought to lead to an underfilled ventricle, which activates cardiac mechanoreceptors (Coffin and Raj, 2014a). This leads to a sudden reversal of autonomic responses resulting in vasodilation and bradycardia (a form of the Bezold-Jarisch reflex) (Folino, 2007).

The most common precipitants for VVS include pain, fear, orthostatic stress, and emotional distress (Thijs et al., 2009). This form is commonly accompanied by typical prodromal symptoms and is most frequently seen in younger individuals (Freeman et al., 2011).

There are also patients with VVS who present with an atypical presentation, where the precipitating event or trigger is unknown, but patients have a positive response during formal orthostatic stress testing (Alboni, 2015). Older adults are more likely to present clinically in this fashion and will less frequently feature classic prodromal symptoms (Freeman et al., 2011).

Clinical Evaluation

It can be challenging to determine the underlying etiology for a syncopal episode upon initial presentation. An initial assessment of a patient presenting with syncope is estimated to identify the cause of syncope anywhere from 23% to 60% of the time (Crane,

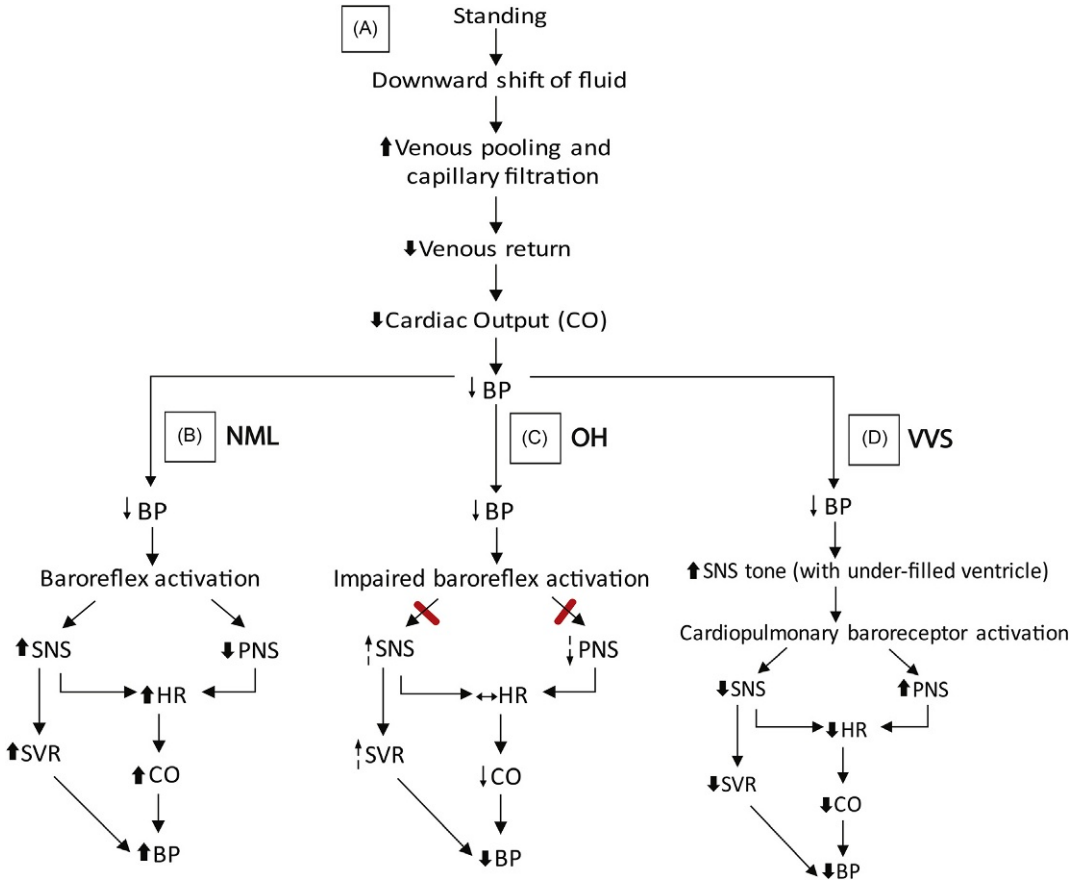

Fig. 1 The physiology of standing (A) with normal compensatory responses (B), as well as the pathophysiology of orthostatic hypotension (C) and vasovagal syncope (D). Normally, the blood pressure decline observed after standing is acutely compensated through a baroreceptor-mediated increase in sympathetic activity and decrease in parasympathetic activity. For patients with orthostatic hypotension, baroreflex responses are impaired resulting in a blunted response that cannot adequately compensate to maintain blood pressure. For patients with vasovagal syncope, standing leads to an underfilled ventricle causing cardiopulmonary baroreceptor activation. This results in a sudden reflex-triggered reversal of normal compensatory responses maintaining blood pressure. *CO*, cardiac output; *BP*, blood pressure; *NML*, normal; *OH*, orthostatic hypotension; *VVS*, vasovagal syncope; *SNS*, sympathetic nervous system; *PNS*, parasympathetic nervous system; *SVR*, systemic vascular resistance.

2002; Croci et al., 2002). This typically will include a careful history, physical examination including orthostatic vital signs, and a resting 12-lead electrocardiogram (ECG) (Moya et al., 2009). Despite the challenges in making the diagnosis on initial evaluation, understanding the key questions to ask on history and signs to look for on examination can be very helpful toward making the correct diagnosis and dictating the need for further investigation.

History and physical exam

The evaluation of a patient presenting with any form of syncope begins with a detailed history. It is important to categorize the nature of the event, including any symptoms or mechanisms leading up to it. Common confounders of a syncopal spell include falls or seizures. It is important to determine if the event was isolated or a recurrent phenomenon and if there were any obvious triggers. A key part of the history is to try and distinguish between an episode of VVS and cardiac syncope; these include inquiring about red flags for an arrhythmia-based precipitant. Concern for an arrhythmia would be heightened if the event happened during (not after) exertion, if palpitations were felt prior to or during the event, if the episode occurred entirely while supine, and if there is any history of sudden cardiac death in the family (Moya et al., 2009). For identifying patients with VVS, there are several diagnostic features to note including prodromal symptoms, physical findings during the event, and during recovery post event (Sheldon et al., 2015).

Distinctive symptoms can occur at different stages of a fainting spell (Wieling et al., 2009). A history of prodromal symptoms can be of value to help discern between different underlying causes. Prodromal symptoms common to VVS include: diaphoresis, abdominal pain, pallor, pupillary dilation, hyperventilation, and nausea (Newman and Graves, 2001; Wieling et al., 2009; Graham and Kenny, 2001). Prodromal symptoms may be noticeably absent in older adults (Wieling et al., 2009). Associated symptoms of cerebral hypoperfusion can also be described including lightheadedness and blurred vision (Alboni, 2015; Graham and Kenny, 2001). Syncope is defined by a transient loss of consciousness that occurs secondary to cerebral hypoperfusion and results in the

patient coming to rest on the ground. "Transient" is a key part of the definition of syncope that is important for distinguishing it from other causes of loss of consciousness including narcolepsy, cataplexy, pseudoseizures, and pseudosyncope (Sheldon, 2015). During a syncopal episode, the patient usually remains unconscious and motionless for only a short period of time (usually less than 1 min) (Sheldon et al., 2015). Syncope patients can have myoclonic shaking with their spell, which can confuse bystanders into thinking that the event is a seizure (Sheldon, 2015; Newman and Graves, 2001). A full recovery will ensue, although patients typically experience profound fatigue for several hours postevent (Graham and Kenny, 2001).

It is important to obtain a detailed past medical history. It is important to note any history of structural heart disease, coronary artery disease, cardiac arrhythmias, or familial sudden cardiac death. A medication history, including any recent medication changes is also valuable, as there are multiple common medications that may predispose an individual to VVS (Hainsworth and Claydon, 2013). Agents that commonly worsen symptoms include diuretics, venodilators, and vasodilators (Coffin and Raj, 2014a).

All patients presenting with a suspected episode of VVS should undergo a physical exam, including a set of orthostatic vitals. These can be helpful in delineating OH from VVS and will be discussed in further detail in the section detailing the clinical evaluation of OH. Patients with VVS should have no significant change in orthostatic HR or BP.

A number of risk stratification tools exist for attempting to identify patients at high risk of adverse events following a syncopal spell (Thiruganasambandamoorthy et al., 2016; Sheldon, 2013; Costantino and Furlan, 2013). These tools are aimed toward emergency department patients presenting with a syncopal episode and can be useful to help identify high-risk features that may necessitate further workup or hospitalization for a patient presenting with a syncopal episode. As of now, there is no gold-standard risk stratification score recommended in current guidelines (Sheldon et al., 2015; Moya et al., 2009).

Overall, a clear history of features consistent with VVS with an absence of any red flags can be sufficient to make the diagnosis (Van Dijk et al., 2008).

Investigations

Overall, the diagnosis of VVS is a clinical diagnosis. Basic laboratory investigations are of limited value in the initial workup of a suspected episode of VVS. Patients with VVS usually have a normal ECG. An abnormal ECG may increase the likelihood of "cardiac syncope." There are no key blood tests that will be of assistance in the diagnosis of VVS, although the presence of laboratory abnormalities such as severe anemia or electrolyte disturbances may increase the likelihood of an alternative diagnosis (Moya et al., 2009). There are a number of tests that should not be performed in a patient presenting with an initial evaluation that is supportive of an episode of VVS. These include computed tomography or magnetic resonance imaging, cardiac perfusion studies, carotid Doppler ultrasound, Holter monitoring, and an electroencephalogram (Moya et al., 2009; Hatoum and Sheldon, 2014).

Autonomic function testing

Patients with a clear history or with a single episode without any red flag features do not need further testing unless special circumstances warrant it (Moya et al., 2009). In selected cases, formal autonomic testing might be helpful. The overall goal of autonomic function testing in the patient presenting with syncope is to assess for the presence and character of autonomic dysfunction in response to different stimuli. Testing will involve assessment of both the sympathetic and the parasympathetic nervous system. Autonomic function testing is not always necessary. This is important given that access to formal autonomic labs is limited given the specialized equipment and trained personal that are required to administer testing. These services are often only available at tertiary care centers.

Head-up tilt table test

A head-up tilt table test involves the patient lying supine for a period of time while having BP and HR assessed optimally by beat-to-beat BP monitoring. After a period of time (anywhere from 5 to 20 min depending on the protocol), the table is tilted upright to an angle usually between 60 and 80 degrees head up, where the patient is maintained in a "standing" position until a presyncopal or syncopal episode is elicited. There are many different protocols for administering a tilt table test. The most basic method is a passive test where the patient is maintained upright for up to 45 min in duration with no added stimulus (Kapoor et al., 1994). Pharmacological provocation in the form of nitroglycerin, isoproterenol, or clomipramine administration can help provoke a patient to faint if an initial period of passive standing (usually 30 min) is not sufficient (Raj and Sheldon, 2011). An argument against this method is the use of medication serves as a nonphysiological stimulus during testing. A final alternative method is through the use of a lower-body negative pressure suction device that can apply graded negative pressure to the lower extremities to promote increased venous pooling and decrease upright tilt duration prior to syncope (El-Bedawi and Hainsworth, 1994; Protheroe et al., 2013).

A positive response to tilt table testing in a patient with VVS results in a characteristic BP and HR response in the moments preceding test termination (**Fig. 2**). In patients with strong pretest probability for VVS, the sensitivity of a head-up tilt is between 78% and 90%, with a specificity of approximately 90% (Sheldon et al., 2015). A tilt table test is not required to make a diagnosis of VVS. The Heart Rhythm Society (HRS) consensus guidelines suggest that head-up tilt table testing can be useful for patients who lack a confident diagnosis of VVS after initial assessment and for differentiating between syncope with convulsions and seizures (Sheldon et al., 2015). A head-up tilt table test may also be of use if it can replicate the symptoms experienced during an event in the community.

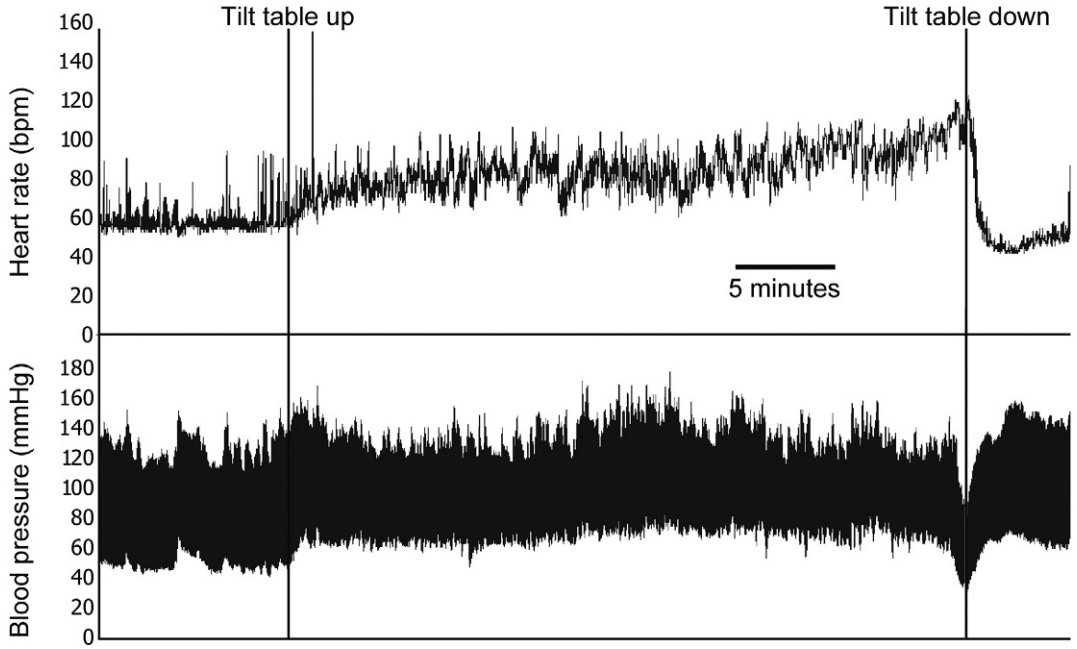

Fig. 2 Sample tracing of vasovagal syncope during head-up tilt testing. Beat-to-beat blood pressure and heart rate responses are shown through the duration of testing. After the patient was tilted upright, blood pressure was maintained for the first 30 min of testing. Heart rate increased during this time. After 35 min of standing, an event consistent with vasovagal syncope occurred, where there was an abrupt decline in blood pressure and heart rate. The test was terminated and the tilt table was brought back to the supine position. *HR*, heart rate; *BP*, blood pressure. From HUTT test chapter 73; Raj and Sheldon.

Management

The management of VVS follows a tiered approach. Most patients respond very well to nonpharmacological management; therefore, these should always be explored and tested prior to pharmacological therapy, as medications will most often not be needed (Raj and Coffin, 2013).

Nonpharmacological approaches

The first step in the management of VVS is to educate the patient on the prognosis and risks associated with this condition. VVS is a common and relatively benign condition (Sheldon et al., 2015). It is important for patients to watch for signs of prodromal symptoms and to avoid precipitating stimuli, such as inadequate hydration and hot environments, or any specific triggers for the individual patient (such as hot showers or needles) (Raj and Coffin, 2013). It is also important to assess for any potential medications that may be contributing to symptoms, such as diuretics, venodilators, and vasodilators and if possible, stopping them (Raj and Coffin, 2013; Coffin and Raj, 2014a).

Increased dietary salt and water intake

The next step in management is to encourage increased intake of dietary salt and water. These recommendations follow a small study that showed that salt supplementation (12 g per day) improved orthostatic tolerance in patients with unexplained syncope (El-Sayed and Hainsworth, 1996). Additionally, acute water ingestion (500 ml) has been shown to improve initial hemodynamics upon standing and overall orthostatic tolerance in healthy controls (Schroeder et al., 2002). In contrast, another study found that increased hydration alone did not improve orthostatic tolerance in patients with neurocardiogenic syncope (Bellard et al., 2007). Overall, there is lack of conclusive evidence for the long-term efficacy of these therapies. In the absence of contraindication against their use (such as preexisting hypertension, heart failure, or chronic kidney disease), salt and/or water supplementation are included with a class I recommendation in the 2015 HRS consensus statement for the management of reflex syncope (Sheldon et al., 2015; Coffin and Raj, 2014a).

Physical counter-measure maneuvers

Physical counter-measure maneuvers are another nonpharmacological therapy that are thought to be beneficial in treating VVS. These maneuvers are performed through isometric contraction of large muscle groups (upper or lower extremities) causing a rise in BP (Raj and Sheldon, 2016; Brignole et al., 2002; Krediet et al., 2002). The thought is to employ these maneuvers when patients experience prodromal symptoms of a syncopal episode. Leg crossing along with clenching of the buttocks is thought to be most effective (Raj and Coffin, 2013). One randomized control trial has shown a 39% risk reduction in patients with syncope employing

physical counter-measure maneuvers (upper and lower extremities) (van Dijk et al., 2006). Overall, the most important maneuver is to lie down flat at the onset of presyncope. If this is not possible, advocate sitting on the ground in a squatting position with knees up against the chest. It is not recommended to sit on a chair as people can faint in this body position. These maneuvers are not felt to be effective in patients who do not experience prodromal symptoms given that patients require time to perform the maneuver to have a beneficial effect (Raj and Coffin, 2013). Given the lack of risk associated with these maneuvers, there is little reason for them to not be employed in the management of VVS.

Compression garments

Compression stockings are often recommended as a potential therapeutic treatment for improving orthostatic intolerance in those with a fainting predisposition based on the physiological rationale that counter-pressure applied to the lower extremities and/or abdomen may reduce venous pooling (Downie et al., 2008; Brown and Hainsworth, 1999) and help improve orthostatic tolerance in patients predisposed to VVS (Privett et al., 2010; Protheroe et al., 2011). The evidence to date is limited primarily to healthy control subjects and astronauts returning from space and does not provide unanimously positive results (Protheroe et al., 2011; Privett et al., 2010; Stenger et al., 2010; Platts et al., 2009). There is also a lack of data in a patient population with VVS. Overall, given that there are very few contraindications or potential harms from their use, compression stockings can be used if a patient does find some symptomatic benefit from using them. The 2015 HRS Expert Statement does not make any recommendations for, or against, their use (Sheldon et al., 2015).

Tilt training

Tilt training is another measure that has been tried in an attempt to do "orthostatic training" as a means to improve orthostatic tolerance through either repeated tilt-tests in the autonomic lab or by standing next to a wall while at home several times a day (Raj and Coffin, 2013). Prolonged inpatient tilt training is impractical and the efficacy and compliance to therapy for outpatient training has been questioned (Coffin and Raj, 2014a). To date, multiple trials have examined outpatient tilt training and have found mixed results, with most studies finding no significant benefit (Foglia-Manzillo et al., 2004; Jang et al., 2013; On et al., 2007; Tan et al., 2010; Zeng et al., 2008). In fact, the 2015 HRS consensus statement does not make any recommendation for its use (Sheldon et al., 2015).

Pharmacological

The majority of patients with VVS do very well with conservative nonpharmacological treatments (estimated at approximately 90%). For the minority of patients that have significant ongoing symptoms despite nonpharmacological management, there are several pharmacological options; these are shown in **Table 1**.

Fludrocortisone

Fludrocortisone is a corticosteroid with primarily mineralocorticoid activity at low doses, resulting in both sodium and water retention. It should be considered as a secondary adjunctive therapy to increased dietary salt and water intake at a dose of 0.2 mg by month (PO) once daily. The Prevention of Syncope Trial (POST)-2 trial examined the effects of fludrocortisone versus placebo in 210 patients with VVS (Sheldon et al., 2016). The study found a nonsignificant trend ($P=0.07$) for a reduction in syncope in the fludrocortisone group by intention to treat analysis. A subanalysis revealed that when dose stabilization was reached (after 2 weeks of treatment), there was a significant reduction in syncope in the group taking fludrocortisone ($P=0.02$). This suggests that there may be benefit for this medication if patients meet the target dose of 0.2 mg once daily. The 2015 HRS consensus statement supports the use of fludrocortisone in appropriate patients given the absence of evidence against its use (Sheldon et al., 2015). Side effects that should be watched for include supine hypertension, fatigue, nausea, and hypokalemia (Lee and Krahn, 2016). The incidence of hypokalemia was very low in the POST2 study (Sheldon et al., 2016).

Table 1 Options for the pharmacological management of vasovagal syncope

Medication	*Dose*	*Comments*	*References*
Fludrocortisone	0.1–0.2 mg PO q daily	The POST2 study suggests that there is a decrease in recurrent syncope if the target dose of 0.2 is reached. Check K^+ for hypokalemia	Sheldon et al. (2016)
Midodrine	2.5–10 mg PO q4h three times daily	Avoid 4 h before bedtime to mitigate risk of supine hypertension. This is being studied in the POST-IV study	Izcovich et al. (2014), Romme et al. (2011), Qingyou et al. (2006), Kaufmann et al. (2002), Ward et al. (1998), Raj et al. (2012)
Metoprolol	25 mg PO BID	Not indicated in younger patients. Maybe helpful in older patients (>40 years) or hypertensive patients. This is being assessed in the POST-V study	Sheldon et al. (2006), Raj et al. (2016)

PO, Per os; *q*, each; *TID*, three times daily.

Midodrine

Midodrine is a prodrug which, when converted to its active form, is an alpha-1 adrenergic receptor agonist. It causes arterial and venous constriction, thereby helping to increase BP and likely venous return by decreasing venous compliance. It has a short-term effect, lasting for about 4 h with a relatively quick onset (20–30 min) (Raj and Coffin, 2013). Typical dosing for the management of VVS is 2.5–10 mg PO every 4 hours three times daily. It is a US Food and Drug administration (FDA) approved medication for the treatment of OH, but is used off-label in the treatment of VVS. Five clinical trials have assessed the efficacy of midodrine, showing an approximate 70% reduction in risk, however none of these trials provide high-level evidence for the drug's use (Izcovich et al., 2014; Sheldon et al., 2015; Raj and Sheldon, 2016). The ongoing POST-IV trial (http://clinicaltrials.gov/show/NCT01456481) is currently comparing the use of midodrine 10 mg three times daily in comparison to placebo (Coffin and Raj, 2014b). There are barriers to the use of midodrine, most notably frequent dosing, supine hypertension, urinary retention in older males, as well as unknown teratogenic effects of the drug (Raj and Sheldon, 2016). The 2015 HRS consensus statement suggests that midodrine is a reasonable option for patient without any contraindications (Sheldon et al., 2015).

Beta-blockers

Beta-blockers have been shown in randomized controlled trials to not be effective for the treatment of VVS (Sheldon et al., 2015). The largest of these trials was the Prevention of Syncope Trial (POST), which tested the effects of metoprolol versus placebo in 208 patients with VVS (Sheldon et al., 2006). There was no benefit for younger patients (<42 years of age). There was, however, a trend suggesting that older patients may benefit. The 2015 HRS consensus statement echoes these findings by suggesting that beta-blockers can be considered in patients older than 40 years of age with recurrent VVS (Sheldon et al., 2015). In the future, the POST-V trial (http://clinicaltrials.gov/show/NCT02123056) may help better clarify the role of beta-blockers in VVS patients >40 years of age. This ongoing study is comparing the use of metoprolol 50 mg twice daily in comparison to placebo (Coffin and Raj, 2014b).

Selective serotonin reuptake inhibitors

Serotonin has an effect that acts to regulate BP and HR. Several studies have examined the use of selective serotonin reuptake inhibitors as a means of increasing serotonin levels to help reduce fainting risk. There have been mixed results in a number of observational studies and small, randomized clinical trials (Takata et al., 2002; Theodorakis et al., 2006; Di Girolamo et al., 1999). To date, the 2015 HRS consensus statement does not provide a recommendation on their use (Sheldon et al., 2015).

Prognosis

The prognosis of VVS is generally quite positive. It is considered a relatively benign condition that is associated with no increased mortality (Colman et al., 2004). Many patients will cease to have episodes of VVS without any medical management needed (Sheldon et al., 2015). For those patients with recurrent VVS requiring management, approximately 90% will do very well on nonpharmacological therapy. Unfortunately, for those individuals with frequent VVS resistant to treatment, quality of life can be greatly impacted (Moya et al., 2009). There are however many ongoing clinical trials to identify better therapies that can help these individuals manage their debilitating symptoms (Coffin and Raj, 2014b).

Orthostatic Hypotension

OH is classically defined as a persistent drop in systolic BP of $\geq$20 mmHg or diastolic BP of $\geq$10 mmHg within 3 min of standing or head-up tilt table testing (Freeman et al., 2011). In patients with baseline hypertension, it may be more appropriate to identify OH as a decline in systolic BP of $\geq$30 mmHg (for hypertension) or in diastolic BP $\geq$15 mmHg (Wieling and Schatz, 2009; Fedorowski et al., 2009) since the magnitude of the BP fall is dependent on the patient's baseline BP (Freeman et al., 2011).

OH is defined by hemodynamic criteria alone. OH can present with symptoms, but also without symptoms. Classic symptoms include lightheadedness, presyncope, and syncope. Others may also report more nonspecific complaints, including weakness, fatigue, classically occipital headaches, "coat-hanger" back and neck pain, orthostatic dyspnea, confusion, and visual blurring.

Epidemiology

The prevalence of OH increases with age (Shibao et al., 2007). OH is associated with overall illness, being much more prevalent in older adults living in long-term care in comparison to an age-matched community cohort (Freeman et al., 2011). OH is independently associated with increased risk of mortality (Masaki et al., 1998; Verwoert et al., 2008) and cardiovascular disease (Luukinen et al., 2004; Rose et al., 2000). A recent meta-analysis suggests that OH is associated with a 36% increased probability of mortality (Angelousi et al., 2014). It is also associated with falling and frailty in older adults (Shaw and Claydon, 2014; Romero-Ortuno et al., 2011). In the United States, OH related hospitalizations occur at a rate of 36 per 100,000 (Shibao et al., 2007). OH is more common in those patients with underlying hypertension and in patients with autonomic dysfunction (Benvenuto and Krakoff, 2011; Fedorowski et al., 2009). It is particularly common in patients with Parkinson's disease and diabetes mellitus (Velseboer et al., 2011; Spallone et al., 2011; Mussi et al., 2009).

Distinguishing VVS From Orthostatic Hypotension

VVS and OH are distinct clinical disorders that can both cause drops in BP and potentially result in syncope; however, they produce distinct hemodynamic patterns during tilt table testing (Nwazue and Raj, 2013). More specifically, the BP falls are experienced at differential rates. VVS involves an intermittent, oversensitive reflex. Patients with VVS usually hold a steady BP for several minutes in an upright posture before developing symptoms and experiencing a rapid drop in their BP and HR. In contrast, patients with classic OH (Fig. 3) undergo immediate and progressive declines in their BPs with upright posture.

Variants of Orthostatic Hypotension

Initial orthostatic hypotension

There are several variants of classic OH. Initial OH is defined as a transient fall in BP (>40 mmHg systolic BP and/or >20 mmHg diastolic BP) within 15 s of standing, with symptoms of cerebral hypoperfusion (Freeman et al., 2011). Patients typically report complaints of lightheadedness immediately on standing, which can occasionally result in syncope.

Initial OH is primarily seen during active standing, and to a significantly lesser extent with passive tilt table testing. It is thought that the initial leg muscle contraction with active standing is a prerequisite for the observed hypotension (Wieling et al., 2007). The fall in BP is proposed to result from a mismatch between cardiac output and vascular resistance as the rapid postural change is experienced. This is a consequence of the normal delay in arterial baroreflex detection and consequent vasoconstriction, as well as the response to gravitational blood volume redistribution (Stewart, 2013). Documentation of Initial OH is very difficult with brachial BP cuffs; it usually requires continuous BP monitoring, as the manual BP equipment (i.e., brachial cuff) is unable to assess the rapid and transient changes in arterial BP (Wieling et al., 2007).

Delayed orthostatic hypotension

Delayed OH is a decrease in BP similar to Classic OH (>20 mmHg systolic BP or >10 mmHg diastolic BP), where this is not observed until after the first 3 min of standing or head-up tilt (Freeman et al., 2011). While the BP drops usually meet the threshold within 10 min, one study also found that 39% of patients experienced BP falls after 10 min of head-up tilt (Gibbons and Freeman, 2006).

Delayed OH may be a consequence of several pathophysiological insults, including increased peripheral venous pooling, increased fluid transudation, and/or gradual impairment of the adaptive neural and humoral mechanisms that counter blood volume redistribution (Ricci et al., 2015; Gibbons and Freeman, 2006). Therefore, it is suspected that these delayed falls in BP may be an early or mild form of sympathetic adrenergic failure.

Confounders for orthostatic hypotension

There are several confounders that may influence the magnitude of the orthostatic BP fall. These include supine hypertension, postprandial hypotension, and diurnal variation in BP.

Orthostatic hypotension with supine hypertension

Supine hypertension can occur in patients with both peripheral and central autonomic failure (Jones et al., 2015). Moreover, supine hypertension can often coexist with neurogenic OH; approximately half of the patients with severe OH also have hypertension when supine (Coffin and Raj, 2014a; Nwazue and Raj, 2013). Upon standing, some of these patients have observed BP drops that fulfill the definition for OH, despite the BP remaining at or above a normal range (Freeman et al., 2011).

Medications used to treat OH may worsen supine hypertension as a side effect; however, a substantial subsection of patients have supine hypertension even prior to pharmacological treatment of OH (Freeman et al., 2011; Goldstein et al., 2003).

Postprandial hypotension

Postprandial hypotension is defined as a decrease in systolic BP of $\geq$20 mmHg, or a decrease in absolute SBP <90 mmHg (from a baseline Systolic BP of $\geq$100 mmHg) within 2 h of meal consumption (Luciano et al., 2010). A significant postprandial BP fall is suggestive of inadequate cardiovascular compensation for meal-induced (or possibly insulin release-induced) splanchnic blood pooling (Trahair et al., 2014; Mathias, 1991). Patients with autonomic failure and the elderly are particularly vulnerable to such meal-associated declines in BP (Freeman et al., 2011; Mathias, 1991). Meal content can also contribute to the degree in which BPs fall; alcohol intake, large meals, and meals high in carbohydrates tend to exacerbate hypotension (Mathias, 1991; Freeman et al., 2011). Specifically, low carbohydrate meals induce significantly smaller BP declines, as well as less frequent and less severe symptoms compared to high carbohydrate meals (Vloet et al., 2001).

Diurnal variation

OH is more common and severe in the mornings compared to other times during the day (Freeman et al., 2011; Ooi et al., 1997). It is suggested that this occurrence is possibly due to higher supine nocturnal BP, which induces an overnight pressure diuresis. This is compounded by a lack of fluid intake overnight. Combined, these cause intravascular volume depletion that is particularly acute in the morning.

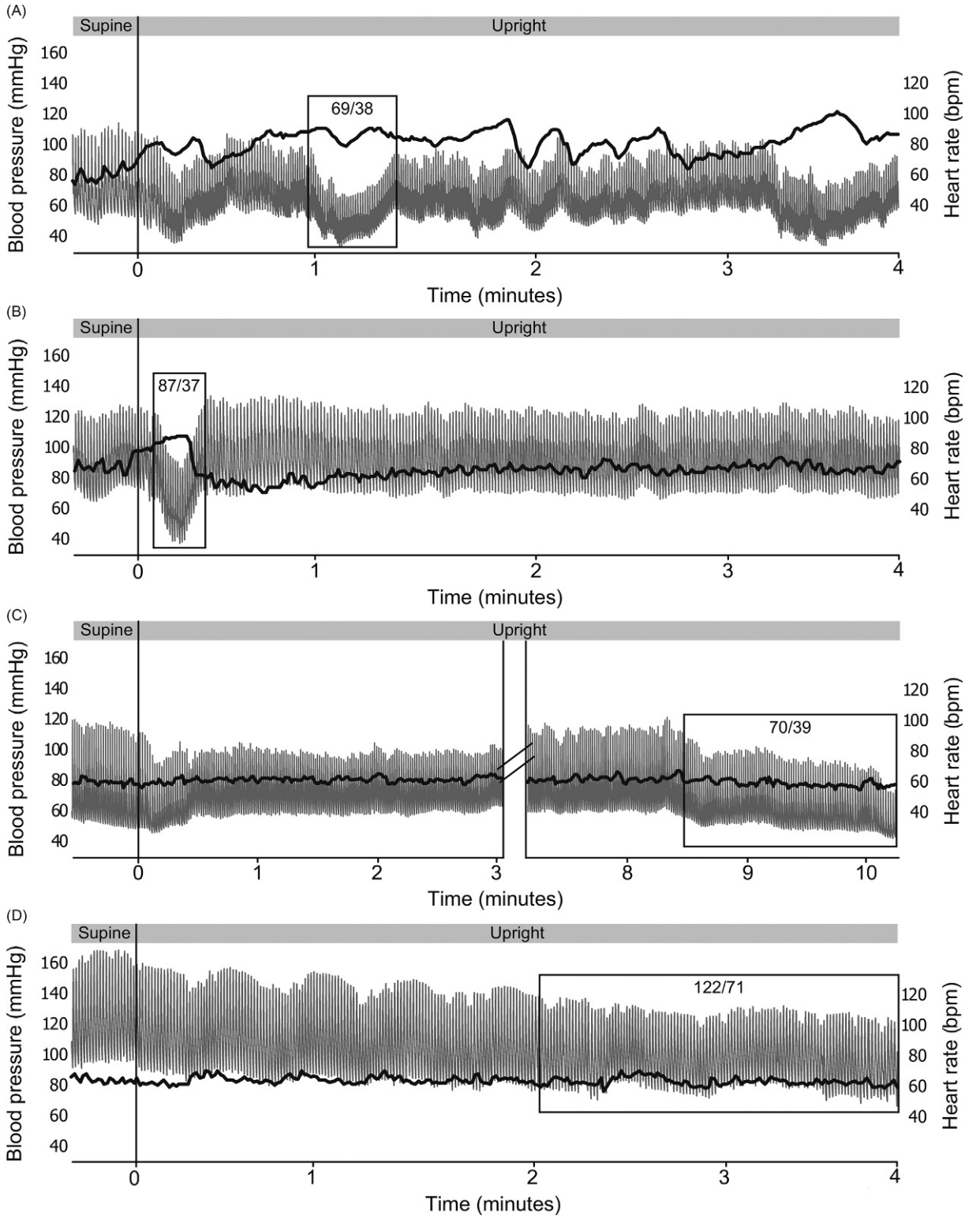

Fig. 3 Examples of orthostatic hypotension variants during head-up tilt testing. Blood pressure and heart rate responses are shown during orthostatic stress testing for patients with (A) classic orthostatic hypotension, in which blood pressure falls during the first 3 min of standing; (B) initial orthostatic hypotension, where blood pressure abruptly falls within the first 30 s of standing; (C) delayed orthostatic hypotension, where blood pressure is maintained during the first 3 min and declines later on during testing; and (D) a patient with classic OH with supine hypertension, who demonstrates a progressive decline in blood pressure with a lack of heart rate response suggestive of a neurogenic cause. From Shaw, B.H.; Claydon, V.E. (2014). The relationship between orthostatic hypotension and falling in older adults. Clinical Autonomic Research 24, 3–13.

Etiology

OH results from inadequate compensatory autonomic nervous system responses to volume redistribution that occurs during standing or position change. The underlying cause for the inadequate compensatory responses during standing can be broken down into nonneurogenic and neurogenic causes of OH.

Nonneurogenic orthostatic hypotension

Causes of nonneurogenic OH are displayed in **Table 2**. There are number of key reversible causes for OH that should be immediately identified including hypovolemia and medications (Gupta and Lipsitz, 2007; Jones et al., 2015). These disturbances lead to diminished cardiac output or decreased effective arterial blood volume resulting in decreased venous return with an appropriate, but inadequate, baroreceptor reflex response leading to a drop in BP. This is frequently accompanied by a compensatory increase in HR.

Neurogenic orthostatic hypotension

Neurogenic OH results from disorders that cause damage to the autonomic nervous system. In contrast to nonneurogenic OH, decreased venous return associated with standing results in an inadequate and impaired baroreceptor reflex response, evidenced by a diminished reflex vasoconstrictive response, resulting in a sustained decrease in BP. Typically, there is also a limited compensatory HR response to orthostatic stress, but the presence of a reflex tachycardia does not rule out neurogenic OH. Other associated manifestations of autonomic failure can include anhidrosis, neurogenic bladder with urinary retention, sexual dysfunction, and gastrointestinal dysmotility (Benarroch, 2014). The etiologies that lead to neurogenic OH can be divided into neurodegenerative, autoimmune, spinal cord injury, and peripheral autonomic causes (**Table 2**).

Neurodegenerative causes

Neurodegenerative disorders that result in the accumulation of alpha-synuclein lead to primary autonomic failure (Low, 2015). Classic causes include Lewy body disorders and multiple system atrophy.

Lewy body disorders Lewy bodies are neurons, which accumulate alpha-synuclein. Lewy body disorders include Parkinson's disease, Lewy body dementia, and pure autonomic failure. Patients with Parkinson's disease typically present with a resting tremor, rigidity, bradykinesia, and postural instability. Constipation is a particularly prominent feature of autonomic dysfunction in Parkinson's disease that becomes more prominent with disease duration (Knudsen et al., 2017). Other autonomic symptoms include bladder dysfunction, erectile dysfunction, and hyperhidrosis (Magerkurth et al., 2005). The prevalence of OH in Parkinson's disease is estimated to be approximately 30% (Velseboer et al., 2011).

Lewy body dementia presents with symptoms of rapidly progressive dementia, autonomic dysfunction, parkinsonian features, and visual hallucinations (Jones et al., 2015). Patients with Lewy body dementia have been found to have lower standing systolic BP in comparison to patients with Alzheimer's disease (Sonnesyn et al., 2009). Among patients with Parkinson's disease, multiple

Table 2 Causes of orthostatic hypotension

Nonneurogenic causes	*Neurogenic causes*
Drugs	Neurodegenerative
Diuretics	Lewy body disorders
Vasodilators	Parkinson's disease
Venodilators	Lewy body dementia
Antidepressants	Pure autonomic failure
Hypovolemia	Multiple system atrophy
Cardiac pump failure	Autoimmune
Cardiac arrhythmias	Autoimmune autonomic ganglionopathy
Valvular heart disease	Paraneoplastic autoimmune neuropathy
Myocardial infarction	Acute autonomic neuropathy
	Sjögren's syndrome
	Spinal cord pathology
	Tumors
	Transverse myelitis
	Tabes dorsalis
	Peripheral Neuropathy
	Diabetes
	Amyloidosis
	Guillain-Barré syndrome

system atrophy and Lewy body dementia, those with Lewy body dementia have been found to be the most affected by autonomic dysfunction (Thaisetthawatkul et al., 2004).

Pure autonomic failure is progressive autonomic failure in the absence of any other neurological deficits (Benarroch, 2014; Thaisetthawatkul, 2016). In contrast to the other Lewy body diseases, pure autonomic failure is an alpha-synucleinopathy isolated to the autonomic nervous system (Thaisetthawatkul, 2016). Patients typically present with typical symptoms of orthostatic hypotension. Other symptoms include urinary symptoms, erectile dysfunction, and chronic constipation (Thaisetthawatkul, 2016).

Multiple system atrophy Multiple system atrophy is a progressive, sporadic, neurodegenerative disorder characterized by combined features of autonomic dysfunction, parkinsonism, cerebellar ataxia, pyramidal symptoms, and urogenital dysfunction (Gilman et al., 2008; Benarroch, 2014). The primary pathological features of multiple system atrophy are glial cytoplasmic alpha-synuclein inclusions, as opposed to neuronal alpha-synuclein in Lewy Bodies (Asi et al., 2014; Papp et al., 1989). The prevalence of multiple system atrophy is estimated to be 4.4 cases per 100,000 (Schrag et al., 1999). Multiple system atrophy can be further delineated by the predominance of parkinsonian features or predominate cerebellar ataxia at initial presentation (Gilman et al., 2008).

Autoimmune causes

Autoimmune causes of autonomic failure include autoimmune autonomic ganglionopathy, paraneoplastic autonomic neuropathy, acute autonomic and sensory neuropathy, and Sjögren's syndrome (Benarroch, 2014). Autoimmune autonomic ganglionopathy is associated with an acetylcholine receptor ganglionic antibody in approximately 50% of cases (Vernino et al., 2009; Sandroni and Low, 2009; Muppidi and Vernino, 2013). These antibodies decrease the synaptic transmission at autonomic ganglia, leading to autonomic failure (Muppidi and Vernino, 2013). Patients often report a subacute onset, often with a preceding event, such as viral illness, recent immunization or surgery (Sandroni and Low, 2009; Muppidi and Vernino, 2013). The majority (70%) of cases will have enteric nervous system involvement, typically in the form of gastroparesis and severe constipation; this can be an important feature to help distinguish this from other autonomic nervous system disorders that may be on the differential (Muppidi and Vernino, 2013).

A paraneoplastic autonomic neuropathy presents in a similar manner to autoimmune autonomic ganglionopathy, but is secondary to a neoplasm, most commonly small-cell lung cancer, adenocarcinoma, or thymoma (Muppidi and Vernino, 2014). Antibodies that may be involved include anti-Hu, voltage-gated potassium channel antibodies or acetylcholine receptor ganglionic antibody (Muppidi and Vernino, 2014).

Acute autonomic and sensory neuropathy is a rare condition that differs from autoimmune autonomic ganglionopathy in that the sensory nervous system is also involved (McKeon and Benarroch, 2016). The most common presenting autonomic symptom is gastrointestinal dysmotility; the sensory deficits tend to be asymmetric, segmental, and affect more proximal areas of the body (McKeon and Benarroch, 2016). To date, no antibody has been identified for this condition (McKeon and Benarroch, 2016).

Sjögren's syndrome classically presents with dry eyes, dry mouth, and a sensory neuropathy, but it can also have autonomic nervous system involvement (Muppidi and Vernino, 2013).

Peripheral causes

Peripheral neuropathies including diabetes, amyloidosis, and Guillain-Barré syndrome can cause autonomic impairment leading to OH. Diabetes is by far the most common cause; the incidence of OH is estimated to be approximately 8%–9% in patients with type 1 diabetes mellitus and slightly lower (7%–8%) in patients with type 2 diabetes mellitus (Low et al., 2004). It is unusual for a patient to develop autonomic failure as a complication of diabetes mellitus in the absence of sensory neuropathy.

Clinical Evaluation

The clinical evaluation of OH is also built on the foundation of a detailed history and physical exam. In contrast to patients with VVS, OH can present with symptoms ranging from being asymptomatic to a complete loss of consciousness (Goldstein and Sharabi, 2009). Common prodromal symptoms include general weakness, lightheadedness, and narrowed vision (Goldstein and Sharabi, 2009).

It is important to ask questions about any precipitating events or recent medication changes. This includes ruling out causes of nonneurogenic cause of OH including dehydration, infection, blood loss, or new onset cardiovascular disease. Common medications that can precipitate OH include diuretics, venodilators, direct vasodilators, and antidepressants (Jones et al., 2015). A detailed past medical history is vital in order to note any medical conditions that may precipitate autonomic dysfunction such as diabetes mellitus or Parkinson's disease.

Similar to the workup of VVS, patients presenting with OH should undergo a physical exam, including a set of orthostatic vital signs. Orthostatic vital signs can be obtained through a variety of testing methods. The most simple, commonly accepted orthostatic stress test is a supine to standing maneuver. A patient is instructed to lie on an examining table for a few minutes. Supine recordings of BP and HR should be recorded with the use of a manual BP cuff. The patient is then instructed to move into a fully upright position. BP and HR measurements are then made at the 1-, 3-, and 5-min intervals while upright. Changes in HR and BP can be used to assess for the presence of orthostatic hypotension at 3-min. The 5-min recording is useful to better define the pattern of the

BP response once OH is detected (Gehrking et al., 2005). In the presence of BP changes suggestive of OH, the severity of OH can be quantified based on duration of standing tolerated, frequency and severity of symptoms, and impact on daily activities (Low, 2015). Changes in HR can be useful to help differentiate between nonneurogenic and neurogenic OH. A lack of HR increase upon standing is consistent with the diagnosis of neurogenic OH; if HR increases, the underlying etiology could still be neurogenic or nonneurogenic in origin.

Investigations

As with the workup of the patient presenting after a syncopal episode, an ECG is a simple, inexpensive test that can help diagnose conduction disease or other abnormalities that could predispose an individual to syncope. Laboratory investigations will generally be guided based on the results of history and physical exam. Common blood work may include a complete blood count and electrolytes to look for severe anemia or electrolyte disturbances. In patients without a clear cause for suspected neurogenic OH further investigations may include a screening test for diabetes mellitus, vitamin B12 levels, and a paraneoplastic or autoimmune workup.

Plasma norepinephrine levels taken while supine and again while standing (after at least the first 5–10 min) can help examine the functioning of adrenergic terminals and the baroreflex (Low et al., 2013; Low, 2015; Goldstein et al., 1989). The supine value provides a baseline measure of postganglionic adrenergic fiber activity; the change between supine and upright can be helpful in describing the degree of baroreflex unloading. A normal change is considered a doubling of norepinephrine levels with standing (Low et al., 2013). Patients with pure autonomic failure will typically have low levels of supine norepinephrine (Low et al., 2013). Patients with OH who have Parkinson's disease, multiple system atrophy, or pure autonomic failure will typically have a blunted increase in plasma norepinephrine levels upon standing (Goldstein, 2014).

Autonomic function testing

Autonomic function testing can be useful in understanding the pattern and degree of autonomic impairment in patients with neurogenic OH. It is of much less utility in patients who have a clear nonneurogenic cause. Similar to the workup for VVS, testing may include head-up tilt testing and Valsalva maneuver. Additional testing for patients with neurogenic OH may include BP changes during hyperventilation or in response to cold pressor, assessment of HR variability, quantitative sudomotor axon reflex testing, thermoregulatory sweat testing, and cardiac iodine-123 metaiodobenzylguanidine (MIBG) uptake (Low, 2015; Nwazue and Raj, 2013; Jones et al., 2015).

Head-up tilt testing

The pattern of BP and HR responses observed during head-up tilt testing can be useful in identifying the type and severity of OH (**Fig. 3**) (Kim et al., 2016; Shaw and Claydon, 2014). It is used primarily as a metric of sympathetic adrenergic function. Patients with neurogenic OH will display a decline in BP that meets criteria for OH as well as a blunted HR increase in response to tilt, in a manner similar to that seen with the supine to stand test.

Valsalva maneuver

The Valsalva maneuver can provide a useful assessment of autonomic function. The test is performed by having the patient perform forced expiration against a closed glottis at a pressure of 40 mmHg for 15 s (Jones and Gibbons, 2014). Continuous recording of BP and HR is vital to interpret the results. The resulting BP and HR waveform can be broken down into four phases (**Fig. 4**). Patients with neurogenic OH can show varying degrees of autonomic impairment during a Valsalva maneuver (Kim et al., 2016). Typically, patients with OH have a poor HR response to the decline in BP experienced during early phase II suggestive of cardiovagal impairment (Low et al., 2013). Patients also display signs of adrenergic dysfunction by the absence of a late phase II systolic BP recovery, absence of phase IV systolic BP overshoot, and a prolonged BP recovery time (Low et al., 2013). These changes become more pronounced with worsening autonomic impairment (Kim et al., 2016).

Other autonomic tests

There are other autonomic function tests that can be used to further delineate changes in cardiovagal and adrenergic function. Cardiovagal function can be assessed by observing variation in HR in response to deep breathing (respiratory sinus arrhythmia) (Bennett et al., 1977); this is performed by inspiring for 5 s (6 breaths per minute), then exhaling for 5 s, and repeating this for 6–8 breathing cycles (Jones et al., 2015). The ratio of HR during expiration divided by the HR during inspiration is the sinus arrhythmia ratio; higher values represent intact cardiovagal function (Low et al., 2013). Patients with impaired cardiovagal function will have ratios closer to 1 (showing a lack of variability).

Cold-pressor testing is a maneuver that uses a cold stimulus to elicit increases in adrenergic activity causing reflex vasoconstriction raising BP as well as increasing HR (Feliciani et al., 2016). The test involves immersing the patient's hand in ice water for 1 min, while recording BP and HR, ideally with beat-to-beat BP technology. A minimal or absent response to cold water immersion can be helpful for indicating adrenergic dysfunction (Feliciani et al., 2016).

Sweat testing is used to test sympathetic sudomotor axon function (Low et al., 2013). Quantitative sudomotor reflex testing (QSART) can test the ability to sweat. It is performed by acetylcholine iontophoresis into the skin, which elicits an axon reflex activating muscarinic receptors at sweat glands (Low, 1993). Sweat responses are typically recorded from four standard sites and compared to a normative data from healthy subjects (Illigens and Gibbons, 2009; Low et al., 1997a). Thermoregulatory sweat

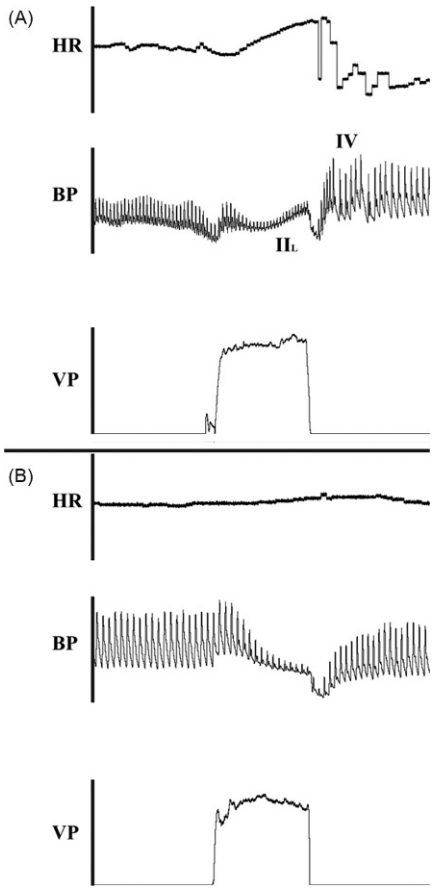

Fig. 4 Valsalva maneuver in a healthy subject (A) and a patient with neurogenic orthostatic hypotension (B). The test is performed by generating approximately 40 mmHg of Valsalva pressure by having the subject expire against a closed glottis. The increased intrathoracic pressure that occurs during Valsalva strain causes decreased venous return resulting in decreased stroke volume and a subsequent baroreflex mediated compensatory increase in heart rate and systemic vascular resistance. In the healthy subject, these compensatory responses create a late phase II (II_L) increase in blood pressure. Upon termination of Valsalva, intrathoracic pressure is suddenly reduced resulting in an increase in venous return and stroke volume. Blood pressure now increases as part of a phase IV (IV) overshoot in systolic blood pressure until the baroreceptor-mediated changes in vascular resistance reverses. In the patient with neurogenic orthostatic hypotension, the compensatory responses to blood pressure change are blunted. This results in a Valsalva pattern with reduced late phase II or phase IV overshoot blood pressure responses. *HR*, heart rate; *BP*, blood pressure; *VP*, Valsalva pressure.

testing can test for an intact sweating response. It elicits a sweating response by increasing body temperature beyond the thermoregulatory set point of the hypothalamus; the test provides an assessment of sympathetic sudomotor pathways traveling from the hypothalamus to the sweat glands (Low et al., 2013). This testing is performed by having the patient lie supine and undressed, so the exposed body can be covered with an indicator powder. The body temperature is warmed to 38°C by increasing ambient room temperature, eliciting a sweating response (Illigens and Gibbons, 2009). As the body sweats, the indicator powder changes color from brown to purple. A photograph can be taken to identify the distribution of sweating to and indicate areas of anhidrosis. The pattern of sweating can be helpful in identifying different types of autonomic disorders, as well as track disease progression over time, and evaluate the effect of treatment (Illigens and Gibbons, 2009; Low et al., 2013).

Planar nuclear imaging can be used to assess the quantitative uptake of iodine-123 metaiodobenzylguanidine (MIBG) (Low et al., 2013). MIBG is a norepinephrine analog that is taken up by presynaptic norepinephrine transporters on postganglionic adrenergic sympathetic terminals in the heart (Nwazue and Raj, 2013). The pattern of uptake can be helpful for differentiating between several autonomic conditions including MSA, pure autonomic failure, and Parkinson's disease (**Fig. 5**) (Nwazue and Raj, 2013).

Management

The initial management of OH is very similar to that of VVS. A detailed review of current medications including recent medication changes is valuable to identify any pharmacological causes for the patient's OH. Overall, the evidence for the treatment of OH (both nonpharmacological and pharmacological) is of poor quality (Logan and Witham, 2012). However, there do exist a number of important recommendations for managing these patients.

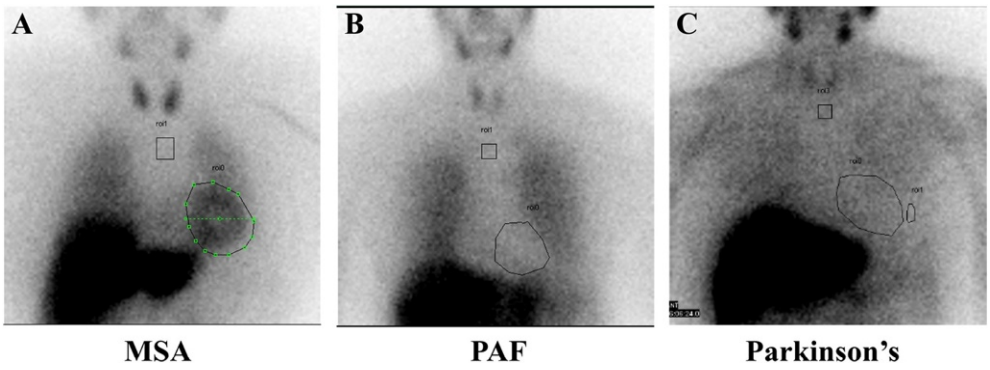

Fig. 5 Planar nuclear imaging assessing uptake of iodine-123 metaiodobenzylguanidine (MIBG) in patients with multiple system atrophy (A), pure autonomic failure (B), and Parkinson's disease (C). MIGB is taken up by postganglionic sympathetic neurons. In a patient with a preganglionic (central) autonomic disorder, such as MSA (A), the uptake of MIGB is normal. In patients with postganglionic disorders, such as primary autonomic failure (B) or Parkinson's disease (C), MIGB uptake is decreased.

Nonpharmacological

It is important to educate the patient of the risks and likely cause of OH in that individual patient. Low and Tomalia (2015) suggest four key educational themes for patients dealing with neurogenic OH: (i) The patient must understand the complications of impaired baroreflex control including OH, supine hypertension, and loss of diurnal BP variation; (ii) the goal of managing their OH will be to alleviate symptoms without causing supine hypertension; (iii) OH severity will vary with different physiological variables including the patient's volume status, meal ingestion, time of day, ambient temperature, and exercise; and (iv) patients have the ability to control plasma volume, degree of venous pooling, and vasomotor tone, which can help improve symptoms (Low and Tomalia, 2015).

Increased dietary salt and water intake

Hydration plays an important role for the patient to help maintain effective plasma volume for managing their symptoms of OH. Patients can be very preload dependent and do not tolerate even mild volume depletion very well. Patients can be advised to consume 2–3 L of water per day. Additionally, dietary salt intake can be increased up to 6–9 g per day.

Rapid ingestion of water can also be beneficial in the management of OH through the osmopressor response. This response can be elicited by rapidly drinking 500 mL of water over 2–3 min. This has been shown to cause an average rise in systolic BP of over 30 mmHg in patients with neurogenic OH (Jordan et al., 1999, 2000). The process begins within about 5 min, peaks between 20 and 40 min postingestion and lasts over 60 min in duration (Jordan et al., 2000). Water has been shown to be most effective in comparison to other solutions, such as salt water, which has been shown to in fact attenuate the response (Raj et al., 2006). This, taken along with work performed in murine models, is suggestive that the osmolality (or hypo-osmolality) of the solution is important (McHugh et al., 2010).

Compression garments

Another nonpharmacological target for patients is to reduce the effects of venous pooling while standing. Both compression stockings and abdominal binders have been shown to reduce the effects of venous pooling in the legs and splanchnic vasculature respectively. The greatest proportion of pooling occurs in the lower abdomen, which suggests that abdominal binding would provide the most effective therapeutic target (Diedrich and Biaggioni, 2004). Several studies have found efficacy with the use of either (or both) of an abdominal binder or waist high compression garments (Smit et al., 2004; Denq et al., 1997; Podoleanu et al., 2006). There are challenges with these treatments. The abdominal binder needs to be tight fitting (applying 20–30 mmHg of pressure) to have much benefit, which can be practically challenging to achieve (Ricci et al., 2015). A recent study used a servo-controlled device that inflated on standing and found it be as effective as midodrine for acutely increasing orthostatic tolerance and decreasing symptom burden (Okamoto et al., 2016). Compression stockings need to be custom fitted and to start, should exert a pressure of 30–40 mmHg (Raj and Coffin, 2013). Proper adherence can be difficult, especially in older adults, given the tightness of the stockings can make them difficult to put on.

Pharmacological

A number of pharmacological therapies exist for the treatment of OH (**Table 3**). There are two key mechanisms to counteract the physiological deficits leading to OH that medication can assist with: volume expansion and/or by increasing the pressor response during standing.

Table 3 Options for the pharmacological management of orthostatic hypotension

Medication	*Dose*	*Comments*	*References*
Volume expansion			
Fludrocortisone	0.1–0.2 mg PO q daily	Significant risk of supine hypertension	Chobanian et al. (1979)
EPO	25–50 units/kg SC	Dosing for Hgb $<$ 120; high risk of supine hypertension; expensive; injections required	Biaggioni et al. (1994), Hoeldtke et al. (1995)
Pressor agents			
Midodrine	2.5–10 mg PO q4 h $\times$3	Should not be taken within 4 h of bedtime to mitigate supine hypertension; agent of choice in the setting of supine hypertension	Wright et al. (1998), Low et al. (1997b), Kaufmann et al. (1988), Dhruva and Redberg (2010), Ramirez et al. (2014), Smith et al. (2016)
Droxidopa	100 mg PO TID up to 600 mg TID	FDA black box warning for supine hypertension	Kaufmann et al. (2014)
Yohimbine	5.4–10.8 mg PO TID	No longer commercially produced; may need to use a compounding pharmacy	Jordan et al. (1998)
Pyridostigmine	60 mg PO TID	A "smarter" drug that works more when patient upright than while supine, but not very potent; increases intestinal motility	Singer et al. (2003), Shibao et al. (2010)
Atomoxetine	18–40 mg PO BID	Low doses only are needed; more potent in patients with MSA than peripheral autonomic failure	Jordan et al. (2015), Ramirez et al. (2014)
Refractory OH			
Pseudoephedrine	40 mg PO TID	Indirect sympathomimetic	Fouad-Tarazi et al. (1995)
Octreotide	12.5–50 μg SC BID	Injectable peptide; often can be effective when other drugs fail	Hoeldtke et al. (1995)
Cafergot	100 mg/1 mg tab PO BID	No longer commercially produced; may need to use a compounding pharmacy	Dewey et al. (1998), Arnold et al. (2014)

PO, Per os; *q*, each; *TID*, three times daily; *BID*, twice daily; *SC*, subcutaneous injection; *Hgb*, hemoglobin; *OH*, orthostatic hypotension.

Volume expansion

Similar to the treatment of VVS, fludrocortisone can be considered as a secondary adjunctive therapy to increased dietary salt and water intake that can help with volume expansion. These expansion effects may provide a short-lived increase in BP. It is believed that long-term exposure to fludrocortisone enhances sensitivity to norepinephrine, thus providing a pressor response in addition to volume expansion (Chobanian et al., 1979). In patients with OH who also have supine hypertension, fludrocortisone could exacerbate the supine hypertension at night. Other side effects include leg swelling, hypokalemia, hypomagnesaemia, and heart failure (Sinn and Gibbons, 2016).

Recombinant erythropoietin (EPO) can be considered in patients with concomitant anemia and OH, which can be a common finding in patients with neurogenic OH (Biaggioni et al., 1994). EPO can be helpful as a volume expander through increased number of red blood cells. The red blood cells and hemoglobin may also cause increased peripheral vasoconstriction by acting as nitric oxide scavengers, providing a second mechanism to boost BP (Rao and Stamler, 2002). The major downsides to EPO administration are the need for injections, high cost, and high incidence of severe supine hypertension (Biaggioni et al., 1994; Hoeldtke et al., 1995).

Pressor agents

Midodrine is an US FDA approved medication for the treatment of OH. Midodrine is useful in that it provides an alpha agonistic effect to raise BP in any position. Midodrine does not however have any impact on the change in BP between positions (Singer et al., 2014). A number of studies have shown that midodrine increases BP and orthostatic tolerance in patients with OH, but the evidence that it provides a major symptomatic improvement has not been clearly demonstrated (Wright et al., 1998; Low et al., 1997b; Kaufmann et al., 1988; Dhruva and Redberg, 2010; Ramirez et al., 2014; Smith et al., 2016).

Droxidopa is a synthetic amino acid precursor to norepinephrine that has been approved by the FDA in 2014 for the treatment of neurogenic OH. Droxidopa is converted to norepinephrine by the enzyme L-amino acid decarboxylase within sympathetic neurons, and it subsequently increases circulating levels of norepinephrine. A recent randomized control trial in 162 patients with symptomatic neurogenic OH found that droxidopa increased standing BP, improved symptoms, decreased symptom impact on daily activities, and was fairly well tolerated (Kaufmann et al., 2014).

Yohimbine is an alpha-2 antagonist. Alpha-2 receptors act both centrally and peripherally in the autonomic nervous system to reduce sympathetic nervous system outflow. Yohimbine is the pharmacological opposite of clonidine. As an alpha-2 antagonist, yohimbine acts to increase sympathetic flow resulting in increased peripheral vasoconstriction and higher BP. The effects of yohimbine on BP have been shown to be similar to that of midodrine (Jordan et al., 1998).

Pyridostigmine is a reversible, peripherally acting acetylcholinesterase inhibitor. It acts by increasing acetylcholine transmission at the level of synaptic junctions that are equipped with nicotinic or muscarinic acetylcholine receptors. Since pyridostigmine amplifies acetylcholine action, but does not directly activate receptors, the effect of pyridostigmine is dependent on synaptic transmission levels. There is less physiological synaptic transmission when supine compared to when upright (Singer et al., 2003). Thus, pyridostigmine works to reduce the decline in BP with standing and does not tend to cause as much supine hypertension (Sinn and Gibbons, 2016). The pressor effects of pyridostigmine on BP are modest compared with agents like midodrine and droxidopa, and may not provide major symptomatic benefit in patients with severe OH (Shibao et al., 2010).

Atomoxetine is a norepinephrine reuptake transporter inhibitor that can help increase BP by raising synaptic norepinephrine levels since it blocks a "clearance transporter" in sympathetic neurons. This medication has been shown to significantly increase BP in patients with OH secondary to MSA (Jordan et al., 2015). One recent study in patients with pure autonomic failure found that atomoxetine induced a greater upright BP and a greater improvement in OH symptoms compared to midodrine (Ramirez et al., 2014). Further studies comparing the effects of atomoxetine in OH to placebo are currently under way (http://clinicaltrials.gov/show/NCT02784535).

There are several other options that can be used in cases of refractory orthostatic hypotension. Octreotide is a peptide that is thought to cause splanchnic vasoconstriction, which helps shunt blood into the peripheral circulation. Octreotide is a peptide that requires refrigeration and parenteral administration. Cafergot is a combination tablet consisting of caffeine and ergotamine that causes vasoconstriction through nonsympathomimetic mechanisms (Dewey et al., 1998). One small study in patients with autonomic failure found that cafergot improved seated BP to a similar degree as midodrine and improved presyncopal symptoms (Arnold et al., 2014). Pseudoephedrine is a mixed alpha-agonist, stimulating alpha-1, beta-1, and beta-2 receptors. This agent has not been found to be superior to midodrine for increasing BP or decreasing symptoms (Fouad-Tarazi et al., 1995).

Conclusions

VVS and OH are two common presentations of syncope. The diagnosis of these conditions begins with a detailed history and physical exam and may require formal autonomic function testing if the diagnosis is in doubt. Management of both conditions starts with a strong foundation of nonpharmacological management with a focus on patient education to understand the risks of both conditions, as well as day-to-day activities and precipitants that can make the conditions worse. To date, the pharmacological options for both conditions are far from perfect and should only be considered in cases that fail conservative management. Overall, VVS and OH represent two relatively common conditions that are far from being completely understood, defined, and effectively treated. There remains a great need for future research and collaboration to help the large number of patients that are affected by these conditions.

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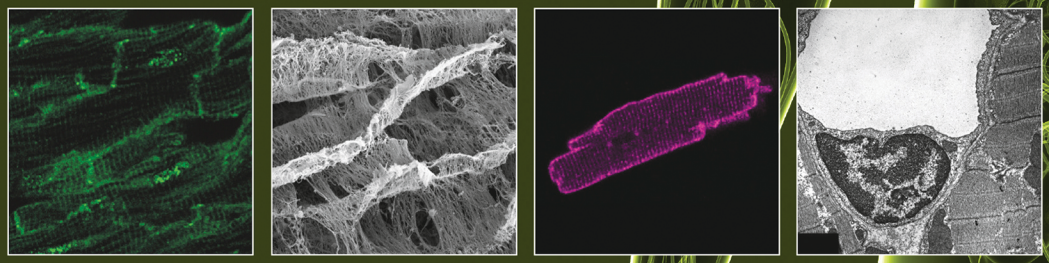

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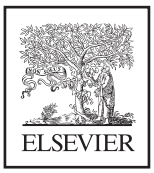

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PREFACE

The burden of cardiovascular disease is growing across the globe as the leading noncommunicable cause of morbidity and mortality. This is despite outstanding research that has improved our understanding of cardiovascular biology, the determinants of disease, and development of new therapies. We are optimistic that the next generation of scientists and clinicians will further these efforts and ultimately bring about changes that will reduce risk and improve outcomes for patients worldwide. We have assembled this encyclopedia with the explicit intent to provide this generation with easy access to past and current wisdom, with the hope that this helps to accelerate innovation in cardiovascular medicine.

This encyclopedia represents both a dream and a labor of love. The dream is to create a scientific compendium that recognizes that the methods and tools used to access and study the scientific literature have changed, with a burgeoning impact of digital technology—the so-called pocket book encyclopedia that is accessible on a smart phone fitting into medical overalls, and that can also be read as a textbook at a desk! These changes in the access of publications have been accompanied by the evolving need for studying different aspects of biology and disease as stand-alone chapters not necessarily read sequentially. Furthermore, as editors we recognize the fundamental importance of conveying knowledge through brilliantly illustrated texts that are profusely embedded with links to key references. The critical importance to use multimedia tools and to provide downloadable slides for educational purposes is reflected in this encyclopedia. Additionally, we want to ensure the feasibility of updating text, figures, and other reading material iteratively even after a publication date, recognizing the rapid advent of information and huge strides made in cardiovascular medicine every day. Thus, this work represents the end of the beginning of a live text that will metamorphose over time, being guided by the rapid strides and advances in both cardiovascular medicine and publication science.

Overall, we conceptualized this work as providing broad coverage of science and also serving the expansive needs of a readership that is quite diverse, that is, encompassing medical students, residents, fellows, and postdoctoral scientists interested in cardiovascular medicine. We hope the labor of love of an outstanding team of editors and a stellar group of authors is readily evident to the broad readership we have targeted.

Assembling this compendium had many challenges. Early on we made the choice to cover the broad range of cardiovascular biology, pathophysiology, epidemiology, and treatment of disease. We sought to assure equal coverage to foundational cardiovascular cellular and molecular biology as well as state-of-the-art treatments. Covering the breadth inevitably led to some overlap between some chapters, and yet we certainly have some residual gaps. We hope to have the opportunity to fill these gaps in future editions. There is also inevitable variation in the style and scope of articles in this multiauthor publication. We and the section editors felt it was more important to allow our expert authors freedom in how they chose to present their topic.

We invite feedback on how this encyclopedia can be improved as a resource for trainees worldwide. Undoubtedly, as noted above, there are gaps in content areas that need to be bridged in future iterations of this encyclopedia. We look forward to hearing from and listening to our readership about how the text can be better organized or better compiled to serve their scholarship better in future years.

This project was made possible through the hard work of many people from across the globe. We were pleased to have section editors and authors from around the world. Drs. Kessler and Schunkert, renowned cardiologists with an outstanding record of accomplishment in genetic research, have compiled a series of chapters on genetics and genomics. Dr. Baliga, a distinguished cardiologist and educator, has conceptualized a tour de force of chapters that capture all aspects of the heart failure syndrome, whereas Dr. DiSalvo, a master clinician and educator, has synthesized a fine tapestry of chapters focusing on diagnostic testing with key sections on cardiac imaging, interventions, and device therapy. Dr. Raj, an outstanding clinical investigator and

electrophysiologist, brought together an exemplary collection of chapters that offer coverage of a broad spectrum of topics in cardiac electrophysiology. Drs. Major and Liaw, both highly accomplished basic scientists and educators, were instrumental in identifying topics of fundamental importance to the field of cardiovascular biology and medicine. Dr. Lenihan, a pioneering clinical scientist and outstanding cardiologist, brought together a special set of articles that represent interactions between cardiovascular and other fields of medicine. Dr. Gale, a celebrated cardiologist with expertise in comparative effectiveness research and clinical cardiology, offers a series of stellar chapters underscoring various aspects of the pathogenesis and management of atherosclerotic cardiovascular disease. Dr. Kokubo, a leading preventive cardiologist with specialization in cardiovascular epidemiology, has orchestrated state-of-the-art chapters on cardiovascular epidemiology.

We would like to thank our colleagues at Elsevier who provided steadfast support of an exemplary nature throughout this long journey. Last but not the least, the editors would like to thank their families, their mentors, and their respective institutions for the constant encouragement, advice, and support over the years.

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P

Palliative Care in Advanced Heart Failure

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Introduction

The incidence of heart failure (HF) is on the rise due to multiple cardiovascular risk factors, including coronary artery disease, diabetes mellitus, obesity, and hypertension. Its prevalence is projected to increase by 46% from 2012 to 2030 to 8 million people in the United States (Writing Group Members et al., 2016). HF patients who are refractory to conventional medical and device therapies are classified under the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) Guideline for the Management of HF as Stage D HF (Yancy et al., 2013). The proportion of advanced or stage D HF patients is uncertain, although it has been estimated to be 5%–10%, which suggests that there are 300,000–600,000 patients in the United States with stage D HF (Mancini and Lietz, 2010). The mortality rate of stage D HF patients remains high with an estimated 1-year mortality rate of 29.6% and 5-year mortality of 50% (Mancini and Lietz, 2010). These patients have limited options, which include cardiac transplantation, mechanical circulatory support, home inotropes, and hospice care (Hunt et al., 2001).

Advanced HF patients have progressive or persistent severe symptoms of HF such as dyspnea, fatigue, and fluid retention which lead to a poor quality of life (Mancini and Lietz, 2010; Metra et al., 2007). Chronic ill-health due to HF can also lead to psychiatric disorders such as depression and anxiety (Mancini and Lietz, 2010). Many patients with advanced HF may not be candidates for advanced therapies or may choose not to avail of these advanced therapies. Palliative care is an important modality in the management of these patients and helps to alleviate their symptoms and improve their quality of life. Palliative care does not exclude conventional life-prolonging treatment, which can be integrated into the overall care of the advanced HF patient (**Fig. 1**) (Ginwalla, 2016a).

Definition of Palliative Care

Various healthcare agencies have defined palliative care in different ways:

- World Health Organization (WHO): An approach that improves the quality of life of patients and their families facing the problem associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other problems, physical, psychosocial, and spiritual (World Health Organization, 2016).
- Institute of Medicine (IOM): The care that provides relief from pain and other symptoms supports quality of life, and is focused on patients with serious advanced illness and their families (IOM (Institute of Medicine), 2015).
- National Hospice and Palliative care organization: A patient and family-centered care that optimizes quality of life by anticipating, preventing, and treating suffering and it addresses physical, intellectual, emotional, social, and spiritual needs of patient and families and facilitate patient autonomy, access to information, and choice (Organization NHaPC, 2016).

The Conundrum of Timing of Referral to Palliative Care

One dilemma that healthcare providers face is the timing of referral to palliative care. Advanced HF patients go through the waxing and waning phases of their disease and it may be challenging to decide when to offer palliative care to these patients (Allen et al., 2012). The common misconception in the general medical community is equating palliative care to end of life and hospice care (Ziehm et al., 2016). In addition to advanced HF, palliative care can also help in the management of patients with chronic symptomatic HF, and consideration for referral should be made during the entire treatment course by their providers (Goodlin, 2009).

Clinical markers and predictive tools may help in identifying patients with advanced HF (**Table 1**). According to the 2013 ACCF/AHA Guideline for the management of HF, "palliative and supportive care is effective for patients with symptomatic advanced HF to improve quality of life" (Class I, Level of Evidence B). "Before hospital discharge, at the first postdischarge visit, and in subsequent follow-up visits, the need for palliative care and hospice should be addressed in all HF patients" (Class I, Level of Evidence B) (Yancy et al., 2013).

Additionally, all patients undergoing advanced therapy options including cardiac transplantation, LVAD implantation, and home inotrope therapy should be evaluated by the palliative care team for advance care planning. This is the process of defining the types of medical and nonmedical care that a patient would and would not like to receive compatible with individual goals and values. It is helpful to involve the patient's family in the advance care planning process to ensure their awareness of the patient's decisions.

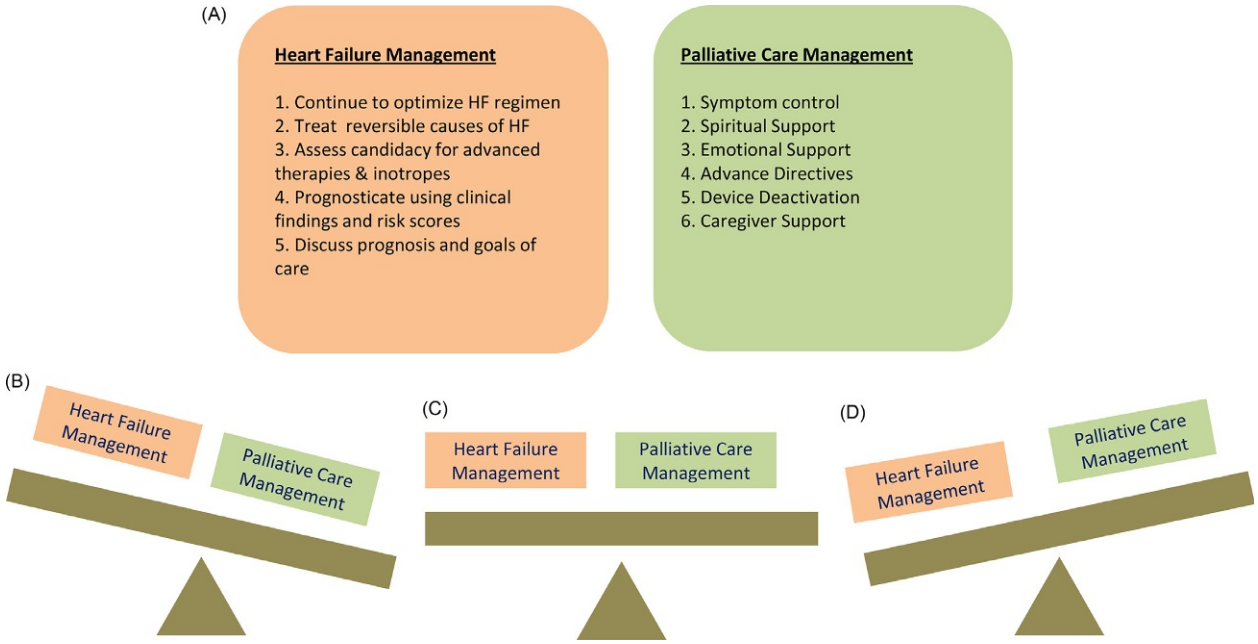

Fig. 1 Balance between the Components of Heart Failure and Palliative Care Management. (A) Shows the components of Heart Failure and Palliative Care Management in patients with heart failure. As the disease progresses, the balance changes at different stages during the course of the patient's illness—during the active medical care (B), advanced stage (C), and end-stage when patients have no other therapeutic options (D).

Table 1 Triggers for referral of heart failure patients to palliative care

1.	Severe heart failure symptoms at rest or with minimal exertion (NYHA class IIIb/IV)
2.	$\geq$1 episode of decompensation leading to hospitalization in the past 6 months despite maximal guideline-directed medical therapy
3.	Need for escalating doses of diuretics or unable to tolerate diuretics due to renal dysfunction or hypotension
4.	Patient intolerant to previously well-tolerated HF medications (renin angiotensin aldosterone blockade or mineralocorticoid receptor antagonist)
5.	Evidence of end-organ failure including renal and hepatic dysfunction
6.	Need for short-term or continuous inotropic support
7.	Reduced functional capacity as evidenced by inability to exercise, 6MWT $<$300 m, peak VO_2 $<$12–14 mL/kg/min
8.	Increased mortality risk ($>$50% within a year) as predicted by various risk scoring models (Seattle Heart Failure Model, Heart Failure Survival Score)
9.	Consideration for Advanced HF therapies such as LVAD or transplant
10.	Signs of cardiac cachexia
11.	Malignant arrhythmias

Modified from Ginwalla (2016a).

Economic Benefits of Palliative Care in Advanced HF

The cost of HF care was nearly $30.7 billion in 2012, 68% of which was attributable to direct medical costs. This cost will increase almost 127% to $69.7 billion in 2030 (Mozaffarian et al., 2015). A major contribution to the cost of HF care is likely related to recurrent hospitalizations, complications, and advanced therapy options for patients with advanced HF.

Palliative care and hospice are documented to be underutilized in advanced HF patients, with some studies reporting less than 10% referral of advanced HF patients (Greener et al., 2014; Bakitas et al., 2013). The goal of palliative care is to alleviate symptoms and improve the quality of life of HF patients and can be integrated into the treatment plan at any stage of HF (Whellan et al., 2014).

In a retrospective analysis of hospital costs of patients with advanced diseases including congestive HF enrolled in Medicaid between 2004 and 2007 at four New York State hospitals, Morrison et al. (Morrison et al., 2011) found that palliative care team consultations reduced the in-hospital costs by $6,900 ($4,098 for patients discharged alive, and $7,563 for patients who died in the hospital) during a given admission for patients who received palliative care than a matched group of patients who received usual care. Patients who received palliative care spent less time in intensive care units and were less likely to die in those units (Morrison et al., 2011). They estimated a potential savings benefit of $84–252 million reduction in annual Medicaid hospital spending in New York State (assuming that 2% and 6% of Medicaid patients discharged from the hospital received palliative care, respectively), if every hospital with $\geq$150 beds had a fully operational palliative care consult team (Morrison et al., 2011). Similarly, post-discharge palliative care program has been found to reduce readmissions and improving symptom control among patients with end-stage HF (Wong et al., 2016). Enrollment of advanced HF patients into a hospice program has been shown to reduce the mean Medicare cost per patient from $53 528 to $46 792 (Pyenson et al., 2004). With increasing healthcare costs, the integration of palliative care in the management of advanced HF patients may reduce costs associated with HF and help patients and their families with overall improved care.

Pharmacologic Therapies in Palliative Care

Patients with advanced HF have multiple symptoms which may be treated by optimizing their HF medications and symptom-directed therapy by the palliative care specialist.

A. *Dyspnea and edema:* Shortness of breath is the cardinal symptom of HF and is usually alleviated with oral diuretics. Torsemide and bumetanide have better bioavailability compared to furosemide and are preferred in advanced HF patients. In patients unable to take oral diuretics and those resistant to orals, intravenous or rarely even subcutaneous (Farless et al., 2013) route can be used for symptom relief. Guideline-directed medical therapy including angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blocker (ARB), digoxin, mineralocorticoid antagonists, and beta-blockers should be continued unless intolerant. There is significant variability among different hospice services about that (Lum et al., 2016). Dietary salt and fluid restriction are appropriate to reduce the progression of volume overload and need for diuretics (Whellan et al., 2014). Palliative intravenous inotropic support with milrinone or dobutamine may be considered for symptom control in patients with stage D HF who are not eligible for either MCS or cardiac transplantation (Class IIb, Level of Evidence B) (Yancy et al., 2013; Gorodeski et al., 2009). However, long-term use of these therapies may increase the overall mortality due to malignant arrhythmias (Stevenson, 2003; Hauptman et al., 2006).

   Opioid analgesics are helpful in relieving air hunger and dyspnea in patients with advanced HF (Whellan et al., 2014). Hydromorphone and oxycodone can be used orally in the short term but in those patients with worsening renal function, methadone or fentanyl are better options due to less accumulation of toxic metabolites. Oxygen supplementation is beneficial in hypoxic patients and anxiolytics such as benzodiazepines can relieve dyspnea caused by concomitant anxiety (Goodlin, 2009).

B. *Pain:* Pain in advanced HF patients may be due to cardiac ischemia and swelling of the extremities. Effective control of pain can help alleviate secondary disease manifestations such as depression and dyspnea. Opioids may be used to help control pain. Nonsteroidal antiinflammatory agents are generally avoided due to increased risk of gastrointestinal bleed and worsening of renal function.

C. *Depression:* Depression is widely prevalent in HF patients with NYHA functional class IV (Rutledge et al., 2006). Selective serotonin reuptake inhibitors (SSRIs) at low doses are generally considered to be first-line agents for the treatment of depression in HF (Goodlin, 2009; Whellan et al., 2014; Adler et al., 2009). Fluid retention and hyponatremia may occur in patients with renal insufficiency and some antidepressants prolong the QTc interval.

D. *Fatigue:* Low cardiac output, loss of muscle mass, deconditioning due to immobility, and comorbid conditions such as anemia, thyroid dysfunction, sleep disorders, and depression may lead to fatigue and weakness in patients with advanced HF. Regular exercise, including aerobic exercise and resistance training with light weights, should be encouraged in patients who are able to exercise (Goodlin, 2009; Whellan et al., 2014; Adler et al., 2009).

E. *Anxiety:* Anxiety can be a prominent symptom as the disease progresses both due to advanced HF symptoms and fear of dying. Norpharmacologic therapy such as involvement of family members, support group, and HF education about controlling their symptoms may decrease the emotional stress (Sullivan et al., 2009). Short acting benzodiazepines such as lorazepam and sometimes even morphine may alleviate the anxiety in these patients (Goodlin, 2009).

Home Inotrope Therapy

Continuous home inotropic infusion with dobutamine, dopamine, and milrinone can be used as a palliative measure to help improve symptoms in patients with advanced HF (Stevenson, 2003; Hauptman et al., 2006; Sindone et al., 1997). Newer inotropic agents including Levosimendan are currently under investigation in the United States (Fruhwald et al., 2016). Inotrope use in the current era has been shown to have a median survival of 9–18 months, compared with 3–6 months in the previous era, which may be due to the increased use of beta-blockers and defibrillators in this population (Ginwalla, 2016b).

The ACC/AHA guidelines support the use of palliative home inotropes in patients with stage D HF as a IIb (Level of evidence B) recommendation (Yancy et al., 2013). Home inotrope therapy is recommended only if there is clear demonstration of both symptomatic and hemodynamic benefit from the inotrope infusion (20% improvement in cardiac index or reduction in pulmonary capillary wedge pressure) (Fang et al., 2015). Due to the cost associated with home inotropes, very few hospice agencies cover its use in hospice patients.

Pleural and Peritoneal Catheters and Dialysis for Symptom Relief in Palliative Care

Patients who are symptomatic due to pleural effusions or ascites can benefit from thoracentesis or paracentesis. Sometimes permanent indwelling catheter placement may be required if the fluid collection is persistent and is not relieved with intermittent drainage (Harris and Chalhoub, 2012; Herlihy et al., 2009). Those patients who have progressive disease with worsening renal function or who are resistant to diuresis can benefit from hemodialysis (HD). However HD is not recommended as the primary treatment method for end-stage HF patients with worsening renal function. Peritoneal dialysis (Lu et al., 2015; Francois et al., 2015) or ultrafiltration (Frohlich et al., 2015) may be a better option for advanced HF patients who do not tolerate HD due to hypotension.

Palliative Medicine in Patients With Left Ventricular Assist Devices as Destination Therapy

Patients with advanced HF may be candidates for a left ventricular assist device (LVAD) implantation. These can be used as a bridge to transplant (BTT) or as destination therapy (DT) (Feldman et al., 2013). These terms are interchangeable as patients who receive a BTT LVAD may develop contraindications to transplant and continue as DT, and conversely DT patients may become eligible for an LVAD.

Palliative care consultation is recommended in all patients with advanced HF undergoing consideration for mechanical support implantation (Feldman et al., 2013). Although patients often experience significant improvement in their symptoms and functional capacity, other symptoms including pain, depression, and adjustment disorders may remain or occur de novo after device implantation. These patients may develop complications including acute stroke, LVAD-related infections, and gastrointestinal bleeding. Early consultation of palliative care provides patient and caregiver support and help with symptom management longitudinally during the continuum of their illness (Brush et al., 2010).

In addition to symptom management, goals and preferences for end of life including establishing advance directives and a living will should be discussed with patients receiving MCS (Feldman et al., 2013). Advance care planning in these patients, also called preparedness planning, is important to document decisions regarding appointment of a patient surrogate decision maker and patient care preferences in the event of device complications including strokes and acute device failure (Swetz et al., 2011). The 2013 ACCF/AHA HF guideline recommends that clinicians should address symptom control, psychological distress, health-related quality of life, patient preferences, and caregiver support, while providing ongoing access to evidence-based optimal medical therapy (Yancy et al., 2013). Addition of a palliative care member to the core interdisciplinary LVAD team is now included as a requirement for Joint Commission MCS certification (The_Joint_Commission, 2014).

The management of LVAD patients near the end of life poses unique challenges to the patient, family, and care providers. Patients may have acute complications or a progressive decline, and early involvement of the palliative care team can help guide the process and ensure the patient's symptoms are controlled when the LVAD is discontinued. They can also help provide adequate caregiver support and ease the decision-making process at the time of withdrawal of LVAD support.

Device Therapy and Deactivation in Advanced HF

Implantable cardioverter defibrillators reduce mortality from sudden cardiac death in HF patients, however they are not beneficial in the advanced HF with less than 1 year survival (Yancy et al., 2013; Tracy et al., 2012). Cardiac resynchronization therapy (CRT) can improve the functional capacity in advanced HF patients and improve survival, however it is not indicated for patients whose comorbidities limit survival to less than 1 year (Yancy et al., 2013). Sometimes, faster pacing in advanced HF patients may generate higher cardiac output and may improve symptoms. Despite these perceived benefits of ICD and CRT, patients with advanced HF are more likely to receive frequent shocks from ICD, which cause significant pain and anxiety (Sears and Conti, 2002). Device deactivation should be considered to alleviate the discomfort from frequent ICD shocks in the terminal stages of HF.

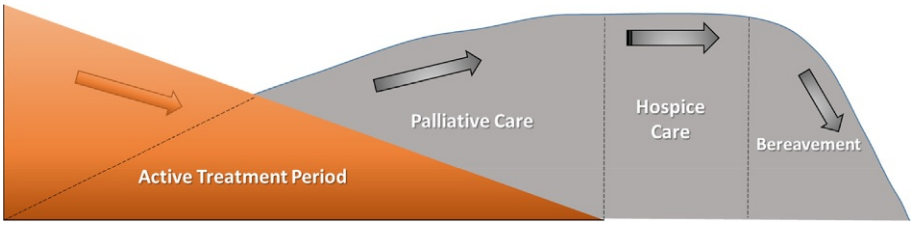

Fig. 2 The integrative model for palliative care with heart failure. Palliative care may be initiated at any point during the care of a heart failure patient. Hospice care ensues when the patient has an expected life expectancy of less than 6 months. Bereavement services help support the patient's family after the patient's demise.

Healthcare providers are recommended to initiate the conversation regarding device deactivation during early stages of the disease, while the patient is still capable of participating in the discussion and able to make decisions (Nohria et al., 2002). Physicians need to help patients determine how the benefits and burdens of device therapy align with their desired outcomes for their health care (Lampert et al., 2010). Multiple options are available with regard to the extent of device deactivation modalities ranging from programming off only certain features such as shock therapy, to discontinuation of all therapy to not replacing a depleting device (Lampert et al., 2010).

Unlike defibrillators, CRT has been shown to improve quality of life. Therefore, it may be appropriate to continue biventricular pacing with CRT for patients even when the decision has been made to turn off the ICDs (Lampert et al., 2010; Cleland et al., 2005). In situations when there is no consensus about discontinuing device support, consideration may be given to consulting with the hospital ethicist or ethics board (Feldman et al., 2013).

Hospice Care

Patients with advanced HF may decide to forego all life-prolonging medications and transition to hospice as the disease progresses (Fig. 2). Hospice provides palliative care for the dying patient who has a prognosis of less than 6 months, and does not want any life-prolonging treatments aimed at curing his or her primary illness. (Adler et al., 2009)

Despite the benefits of hospice care in alleviating discomfort for the patient in the terminal stage and their families, this service is underutilized. Nearly 37% of the patients enrolled in hospice have cancer as a primary diagnosis, and only 14.7% have a primary diagnosis of heart disease (Organization NHaPC, 2015). Reasons for underutilization are proposed to be uncertain prognosis, patient preferences, spiritual beliefs, increasing number of available therapies, lack of awareness of palliative care and hospice services, and inadequate training (Unroe et al., 2012). However, there has been a trend in increased use of hospice service in the past decade.

When a patient is referred to hospice, grieving families can access bereavement counseling and support for up to 1 year following the patient's death (Organization NHaPC, 2015; Goldstein et al., 2011). In 2014, an average of two family members of every deceased patient enrolled in hospice received bereavement support from their hospice which included follow-up phone calls, visits, and mailing (Organization NHaPC, 2015).

Advance directives specifying limitations in end-of-life care have been associated with significantly lower levels of Medicare spending, lower likelihood of in-hospital death, and higher use of hospice care in regions characterized by higher levels of end-of-life spending (Nicholas et al., 2011). Although the primary goal of hospice is symptom alleviation at the time of death, there is data to suggest that HF patients who received hospice had a higher mean survival by 81 days as compared with advanced HF patients who did not receive hospice (Connor et al., 2007).

Future Directions

It is increasingly recognized that in addition to cancer, high-quality end-of-life care should be an integral part of care that is provided for those with other advancing chronic life-limiting conditions such as advanced HF. Despite the recent advances in HF management with newer neurohormonal agents, device therapies, and mechanical circulatory support, there is significant morbidity and mortality associated with advanced HF. Palliative care is an integral part of the advanced HF team to help manage symptoms, complications, and end-of-life issues in these patients.

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Pediatric Catheter Ablation

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Introduction

The past two decades have witnessed astounding advances in the efficacy and safety of catheter ablation techniques as definitive therapy of supraventricular and ventricular arrhythmias, allowing curative therapy for many of the arrhythmias affecting young patients. In many regards, catheter ablation in young patients is similar to ablation in adults; the same tools and techniques are utilized. However, the indications and approaches to ablation among the young often require variances in approach and considerations with respect to size, natural history, and mechanisms. This review will cover both general considerations in pediatric ablation and more specific technical issues as they apply to various arrhythmia scenarios. Additionally, recommendations for appropriate use of ablation will be cited throughout the discussion.

Background

The stage for catheter ablation was first predicated on the recognition of accessory pathways as the mechanism underlying the Wolff-Parkinson-White EKG pattern, subsequently followed by the first described surgical interruption of such pathways (Wolferth and Wood, 1933; Sealy et al., 1976). Since the description by Morady and coworkers of nonsurgical endocardial ablation using DC energy, catheter ablation has emerged as the definitive and often preferred treatment for many forms of SVT and Ventricular tachycardia (Morady and Scheinman, 1984; Scheinman, 1995; Calkins et al., 1999; Scheinman and Huang, 2000; Morady, 2004). No less remarkable has been the application of ablation therapy not only to pediatric patients with structurally normal hearts but

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also to those with a wide spectrum of congenital structural heart disease (Morady, 2004; Van Hare et al., 1991, 2004a; Dick et al., 1991; Kugler et al., 1997; Chetaille et al., 2004; Triedman et al., 2002). In the three decades since, success rates and the ability to approach increasingly complex mechanisms have been enhanced by technical advances in the tools available to the interventional electrophysiologist. Likewise, through large multicenter collaborative efforts, an improved understanding of special considerations in children has allowed improved safety of this technique, as well as an appreciation for situations for which ablation carries an increased risk. This has led to formal guidelines for appropriate use of catheter ablation in this population (Saul et al., 2016). As technical advances continue to emerge, the ability to provide safe and effective cure of the majority of pediatric arrhythmias continues to improve.

General Considerations

Electrogram Recordings

High-fidelity recording and accurate interpretation of intracardiac electrograms remain the keystone of successful mapping and ablation. Recording of biopotentials ranging from 25 μV to 5 mV with optimized signal-to-noise ratio requires rigorous attention to every detail from physical and electrical organization of the electrophysiology laboratory through signal display on the monitor (Venkatachalam et al., 2011a). Amplification and filtering of the recorded signals must be chosen appropriate to the intracardiac recording site, and interference from nearby electrical equipment must be addressed with careful shielding, grounding, and isolation (Venkatachalam et al., 2011a). The influence of electrode size and spacing on bipolar recordings requires thoughtful catheter selection, and the nuances of "unipolar" recordings utilizing a remote indifferent electrode should be understood. Artifact can arise from amplifier saturation, inappropriate clipping, pacing output, radiofrequency energy, or physical electrode contact (Venkatachalam et al., 2011b). Even when "perfect" electrograms are recorded, accurate interpretation remains in part a subjective art. For example, early in the accessory pathway ablation experience a number of electrogram features such as signal stability, timing of local ventricular signal relative to local atrial signal or the surface ECG, and the presence of an accessory pathway potential or continuous electrical activity were recognized as independent predictors of successful ablation, but the prognostic value of any single feature varied substantially between studies (Calkins et al., 1992; Grimm et al., 1994; Bashir et al., 1993; Silka et al., 1992; Ceresnak et al., 2012; Scheinman et al., 1982). More recently, systems have emerged to provide rapid, high-density maps using automatic acquisition and measurement of electrograms from multipolar catheters as they are moved within the cardiac chambers (Bollmann et al., 2016). It remains to be seen how these systems can be best utilized alongside classic diagnostic techniques such as pacing maneuvers and EGM interpretation which are often nuanced and even subjective, but serve as the cornerstone of interventional electrophysiology.

Energy Sources for Ablation

Direct current ablation utilized nondeflectable catheters which were guided under fluoroscopic imaging to target sites based upon the timing and morphology of bipolar local signal recordings (Scheinman et al., 1982; Gallagher et al., 1982). Similarly, RF ablation initially utilized RF generators developed for neurosurgical techniques as the ablation energy source. Since then, an entire industry has emerged, and ablation catheters today allow deflection and torque with multiple degrees of freedom and bidirectional and even omnidirectional bends when used alone or in conjunction with long, shaped or deflectable sheaths. (Peichl et al., 2009; Schwagten et al., 2010). Catheter placement can be further guided by direct visualization by electroanatomic mapping systems, intracardiac echocardiography (Peichl et al., 2009), or magnetic navigation systems (Schwagten et al., 2010; Wu et al., 2010; Van Hare et al., 2002).

The actual process of mapping most frequently consists of point-by-point acquisition of signals recorded from the distal electrode pair of a mapping catheter placed serially in direct contact with multiple sites along the endocardial surface. Alternative techniques can be utilized in certain scenarios. For targets that do not display sustained electrical stability, noncontact mapping systems utilize a multielectrode array placed within the chamber of interest to derive "virtual" local potentials at remote sites by use of a mathematical inverse solution (Kadish et al., 1999). As mentioned above, automated high-density EGM acquisition is now available for increasingly complex tachycardia mechanisms such as scar-related tachycardias (Bollmann et al., 2016). Recently, there has been a resurgence of interest in body surface mapping; when combined with 3D CT or MRI cardiac imaging, it may be increasingly possible to identify target sites before catheters are even placed within the heart.

Mapping

Regardless of the mapping methodology, the fundamental goal is to determine the activation timing of multiple recorded (or derived) electrograms relative to a stable fiducial reference and apply these to their anatomic location. Initially, this required fluoroscopic imaging and a "mentally visualized" impression of the cardiac anatomy, sometimes augmented by cineangiography or echocardiography (transesophageal, intracardiac).

Increasingly, electroanatomic mapping systems (originally developed for complex anatomic and mechanistic situations) are being utilized with regularity in the majority of ablations in young patients. These systems overlay recorded or derived electrical activation on a 3D anatomic virtual shell portraying the cardiac chamber(s) of interest. Such anatomic shells are constructed in real-time during electrogram acquisition as the catheter moves throughout an externally applied magnetic or electrical current field (Gepstein and Evans, 1998; Wittkampf et al., 1999). Anatomic details can be further resolved by fusing such electroanatomic maps

with anatomy derived by other techniques such as CT, MRI, Intracardiac echo, or rotational angiograph (Knackstedt et al., 2008). Although advanced mapping systems may not be essential for effective routine ablation of supraventricular tachycardia, their use has been associated with improved outcomes during ablation of intra-atrial reentrant tachycardia in patients with congenital heart disease as well as decreased fluoroscopy times in routine pediatric ablation cases (Triedman et al., 2002; Kwong et al., 2012; Miyake et al., 2011).

Energy Application and Lesion Formation

Once the arrhythmogenic target has been precisely localized, delivery of ablative energy to that site allows definitive cure. Radiofrequency energy remains the primary means of destroying tissue containing the arrhythmia target. This involves delivery of an unmodulated sine wave of alternating current in unipolar fashion between the tip of the catheter and a ground electrode located on the patient's skin, with resistive heating of the tissue directly adjacent to the tip electrode and conductive heating of a wider rim of tissue (Nath et al., 1994). Many factors influence the extent of thermal injury achieved in the myocardium, including the maximum temperature achieved, steady-state power amplitude, and stability of tip contact. Given that a common reason of ablation failure in patients with congenital heart disease is inadequate lesion formation, it is important to recognize how these variables may be manipulated to increase depth of thermal injury. Because the radius of the lesion size is directly proportional to the radius of the heat source, catheters with 8 mm or even 10 mm tip electrodes may be used in place of the standard 4 mm tip ablation catheter (Haines et al., 1990; Tsai et al., 1999). Active convective cooling of the catheter tip with open irrigated catheter systems can allow for delivery of higher power, with deeper lesion formation (Jaïs et al., 1998; Triedman et al., 2005). Lastly, there is recent report of real-time measurement of tip electrode contact force (Kuck et al., 2012); however, the translation of this data to improved safety and efficacy remains clinically unproven at this time.

The alternative frequently utilized energy source for ablation is cryoablation therapy. Liquid refrigerant is delivered under pressure to the distal catheter tip, where an accelerated liquid-to-gas phase change produces rapid cooling (Khairy and Dubuc, 2008). Heat extraction from the adjacent tissue produces initial cellular changes that are reversible, allowing assessment of sites without permanent tissue destruction. Further cooling produces direct cell injury with first extracellular and subsequently intracellular ice crystal formation at temperatures colder than −40°C (Gage and Baust, 1998). When repeat lesions are delivered in a "freeze-thaw" pattern, there is additional tissue destruction mediated by vascular changes, with initial vasoconstriction and ischemic necrosis followed by hyperemia and edema (Cesario et al., 2009). Post-ablation, within the myocardium there is further expansion of tissue destruction over time to an extent similar to that seen with radiofrequency ablation lesions (Khairy et al., 2011; Saul et al., 1994). The initial clinical experience with cryoablation in the pediatric population was characterized by an excellent safety profile but acute efficacy and recurrence outcomes that trended inferior to that observed with radiofrequency ablation (Kirsh et al., 2005; Collins et al., 2006). The use of larger catheter tip size and variations on lesion delivery have produced acute success and recurrence rates for slow pathway modification and septal accessory pathway ablation in children similar to that reported with radiofrequency ablation (Silver et al., 2010; Czosek et al., 2010). However, to date there has not yet been a randomized comparison of the two energy sources in pediatrics such as has been performed in adults (Deisenhofer et al., 2010).

Special Considerations in Children

Although there are many common ablation tools shared across pediatric and adult populations, the manner in which they are applied at times diverges widely. Natural history, patient size, procedural sedation, radiation effects, and the potentially broad anatomical spectrum of congenital heart disease each influence the clinical decision-making surrounding ablation in children. For instance, patient age during episodes of orthodromic reciprocating tachycardia mediated by an accessory pathway has a well-described bimodal distribution with a "honeymoon" period free of symptoms through much of early childhood (Perry and Garson, 1990). This pattern, combined with the knowledge that 30%–40% of pathways presenting in infancy will spontaneously regress (Benson et al., 1987), makes the need for ablation in infancy and very young children a generally rare event. An initially conservative approach in these very young children has been further supported by the observation of a higher rate of complications when radiofrequency ablation is undertaken (Kugler et al., 1994; Kantoch et al., 2011). Children of all ages are less tolerant of a procedural sedation strategy relying on conscious sedation, but different general anesthetic agents differentially influence cardiac conduction (Niksch et al., 2010; Lavoie et al., 1995). Ionizing radiation has been the traditional imaging modality allowing visualization of intracardiac catheter position; exposure during childhood carries a different risk-benefit balance than in adults (Mah and Triedman, 2011), and there has been rapidly growing interest in primary use of alternative, nonfluoroscopy systems (Miyake et al., 2011; Smith and Clark, 2007).

The location of both the arrhythmogenic substrate and the normal conduction system can vary widely in children. Children with Ebstein's malformation of the tricuspid valve not infrequently have multiple accessory pathways positioned along the atrialized right ventricle; the fractionated signals that arise diffusely from the abnormal endocardium can confound precise localization of the pathway(s) (Cappato et al., 1996; Roten et al., 2011). Anatomic complexities may be more subtle at initial presentation: physical adherence of either atrial appendage to the adjacent ventricular epicardial surface may harbor accessory atrioventricular (AV) connections that can be challenging to effectively access via a transcatheter approach (Mah et al., 2010; Shah et al., 2001). Conversely, the anatomy of the normal conduction system is strikingly unusual in some forms of congenital heart disease: the compact AV node is positioned anteriorly in children with congenitally corrected transposition of the great arteries (Anderson et al., 1974), and posteriorly in patients with primum atrial septal defect/complete atrioventricular septal defect (Feldt et al., 1970). A less common but fascinating congenital variation is the so-called twin atrioventricular nodes in some patients with atrioventricular discordance, with the first pathologic description by Mönckeberg in 1913 followed almost 90 years later by description of such

substrate supporting clinical AV reciprocating tachycardia (Mönckeberg, 1913; Epstein et al., 2001). Surgical interventions performed to repair or palliate congenital defects introduce an additional layer of complexity, with atriotomy scars and baffle suture lines serving as iatrogenic "central obstacles" creating a myriad of unique macro-reentrant circuits (Love et al., 2001; Zrenner et al., 2003). Independent of the arrhythmia circuit, direct catheter access to the target endocardial surface may be obstructed by congenital anomalies such as interrupted inferior vena cava or surgical intra-atrial baffles placed in the process of Fontan palliation or atrial switch procedures, and trans-hepatic, retrograde, or trans-baffle approach may be necessary (Singh et al., 2011; El Yaman et al., 2009; Perry et al., 2003).

Ablation of Supraventricular Tachycardia in Children: Indications and Outcomes

Given these special considerations in children, it comes as no surprise that pediatric-specific guidelines for ablation have been formulated. In 2002 the North American Society for Pacing and Electrophysiology released consensus indications for ablation (Friedman et al., 2002), and revised in 2016. Details are summarized in **Table 1** (Saul et al., 2016).

There are a variety of ways that ablation outcomes may be measured. Pooled efficacy and complication data from the two largest prospective multicenter studies/registries is presented in **Table 2** (Kugler et al., 1997; Van Hare et al., 2004a,b). The differences between the two studies—performed in different era—are illustrative of the well-documented learning curve, with a strong exponential correlation between prior experience and success and complication rates (Danford and Kugler, 1995). Although there has been recent growing interest in the development of quality of life measures specific for the general congenital heart disease population (Marino et al., 2010), to date there is only pilot data reporting positive patient-oriented outcomes following ablation in children (Strieper et al., 2010). Likewise, cost-effectiveness analysis has been limited, with current pediatric-specific analysis limited to a single study performed in the context of a developing country (Vida et al., 2006).

Target-Specific Considerations

Accessory Pathway

Both surgical and transvenous catheter ablations were initially applied to accessory pathways, as they represent a discrete structural target which can be directly mapped (Wallace et al., 1974; Jackman et al., 1991). Strategies for accessory pathway ablation depend upon several factors, including whether there is manifest preexcitation; location of the pathway, particularly proximity to the AV node; and whether the target represents a typical accessory pathway with rapid, nondecremental conduction or an atypical accessory pathway with slow, decremental conduction, as with a Mahaim fiber or with permanent junctional reciprocating tachycardia. In addition to mapping the sites of earliest activation of the proximal or distal insertion, direct recording of an accessory pathway potential may often prove useful (Otomo et al., 2001; McClelland et al., 1994).

In pediatric patients, there are specific considerations related to pathway location. In many pediatric centers, left-sided pathways are approached exclusively via transseptal puncture. The rationale for this approach includes ease of catheter manipulation once the left atrium is entered, and concerns of potential injury to the aortic and mitral valves (Minich et al., 1992; Lau et al., 1994). Other centers prefer a retrograde transaortic approach as the primary mapping strategy; while somewhat more difficult to master, this approach offers the theoretical advantages of catheter stability when the ablation tip is placed beneath the mitral valve leaflet, as well as assuring more secure contact and heat delivery directly to the mitral valve annulus and thus the pathway. However, familiarity with the transseptal approach remains essential, since a retrograde approach may be inadvisable in very small patients, and some sites (e.g., certain locations near the edge between the anterior and posterior mitral leaflets) may prove difficult from a transaortic approach. Neither approach has been demonstrated to be consistently superior across several published single-center studies in children and adults (Law et al., 2001; Vora et al., 1997; Lesh et al., 1993).

For right-sided pathways inserting on the free-wall of the tricuspid valve annulus, catheter stability is an issue. Often, long sheaths can offer additional stability, and these are manufactured in a variety of shapes. Localization of the accessory pathway can sometimes prove difficult, as multiple sites along the annulus appear to show fusion of the atrial and ventricular components of the local electrogram. Some have utilized a small, multi-electrode catheter placed into the right coronary artery to obtain simultaneous activation times along multiple points of the lateral tricuspid valve annulus (Van Hare et al., 2002). An additional strategy which can be utilized is to perform mapping during right atrial pacing rather than during sinus rhythm. The time from the stimulus artifact to earliest ventricular activation may better discriminate between multiple sites displaying "favorable" local electrogram characteristics.

Several concerns may arise during mapping and ablation of septal accessory pathways. Proximity to the AV and His bundle is especially a concern for parahissian/anteroseptal and midseptal pathways. A variety of strategies may be employed to minimize risk of inadvertent injury to the AV node, including use of cryoablation; performing pacing maneuvers to search for a His potential in the pacing catheter when the accessory pathway blocks; and utilizing general anesthesia so that respiration can be held to optimize catheter stability during the lesion (or until adherence as achieved during cryolesions). If cryoablation is performed, pacing maneuvers should be performed during the lesion to ensure intact AV node conduction until preexcitation is eliminated, followed by careful monitoring of the AH interval while the lesion is carried to full duration. If radiofrequency ablation is employed, great care should be taken, and the lesion should be aborted at the first appearance of accelerated junctional rhythm.

Perhaps one of the most challenging situations is the approach to a parahissian accessory pathway with unidirectional retrograde conduction. Since atrial activation over the accessory pathway during ventricular pacing cannot be reliably distinguished from retrograde conduction over the AV node, both mapping and ablation during tachycardia are necessary. In this situation cryoablation is highly advisable, both to avoid injury to the AV node and to avoid dislodgment of the pacing catheter with tachycardia termination.

Table 1 Indications for SVT ablation (>15 kg), indications for ablation of WPW, indications for ablation of inappropriate sinus tachycardia, indications for ablation of atrial fibrillation, indications for ablation of junctional ectopic tachycardia, indications for ablation in patients with congenital heart disease, indications for ablation in infants and small children (<15 kg), indications for ablation of ventricular arrhythmias

Indications for SVT ablation (>15 kg)

- Class I
  - Documented recurrent/persistent SVT and:
    - Assoc w ventricular dysfunction
    - Medical therapy ineffective or not tolerated
    - Family wishes to avoid chronic meds
    - Assoc with hemodynamic compromise/syncope
    - Required emergency medical care or DCCV (>15 kg)
- Class II
  - Clinical symptoms c/w SVT and evidence of AV accessory pathway or inducible SVT
  - Documented SVT and dual AV node physiology but SVT noninducible
    - "cryotherapy should be considered"

Indications for ablation of WPW

- Class I:
  - Following resuscitated cardiac arrest
  - WPW with syncope when there are predictors of high risk for cardiac arrest
  - SPERRI or 1:1 AP ≤250 ms
  - Multiple accessory pathways
- Class IIA
  - Associated ventricular dysfunction
    - Presumed due to dyssynchrony (>15 kg)
    - Medical therapy ineffective/intolerant <15 kg
  - No sxs with high risk for cardiac events > 15 kg
  - With syncope but no predictors for high risk events in larger pts
  - Without symptoms but absence of PE is prerequisite for personal or professional activities
- Class IIB
  - No sxs, no high risk predictors, but pt/family choice to ablate (>15 kg)
- Class III (not indicated)
  - WPW pattern caused by fasciculoventricular pathway
  - WPW without symptoms and <15 kg

Indications for ablation of inappropriate sinus tachycardia

- Class IIb:
  - Autonomic causes (POTS/OI) have been excluded AND medical Rx ineffective or not tolerated
- Class III:
  - Due to POTS or as part of routine care for IST

Indications for ablation of atrial fibrillation

- Class I—none
- Class IIa
  - Any SVT serving as initiating event including single ectopic atrial focus or foci from a single PV
- Class IIb-Wide-area Circumferential PVI
  - Recurrent AF, and
  - Requires repeated DCCV, and
  - Medical Rx ineffective or adverse events
- Class III
  - Rare, not requiring DCCV or well controlled medically
  - Afib incidentally induced at EPS

Indications for ablation of junctional ectopic tachycardia

- Class I: Idiopathic JET with Ventricular dysfxn and
  - ineffective medical Rx
  - Cryoablation 1st choice modality
- Class IIa: Idiopathic JET with No Dysfxn and
  - Med Rx ineffective or poorly tolerated and >15 kg
- Class IIb: Alternative to effective Med Rx and >15 kg
- Class III:
  - Post op
  - Medically controlled and <15 kg

Indications for ablation in patients with congenital heart disease

- Class IIA
  - SVT due to APs or twin AV nodes (heterotaxy)
    - Ineffective medical Rx <15 kg
    - Alternative to medical Rx >15 kg
  - WPW in Ebstein's anomaly (>15 kg)
  - Asymptomatic AT/Flutter >3–6 months post-op at risk for thromboembolic or CHF complications

Table 1 (Continued)

- Adjunct Tx to ICD to reduce shocks
- Frequent VE contributing to ventricular dysfxn >15 kg or meds ineffective and <15 kg
- Sustained monomorphic VT ± ICD
- AVNRT with ineffective or not tolerated meds, >15 kg
- Pre-emptive ablation to prevent post-op arrhythmias
- Prior to Fontan, Ebstein's anomaly (>15 kg)
- Recurrent AT/AF >3–6 months post-op

- Class IIB
  - SVT w/acute hemodynamic compromise, 15 kg
  - AV node ablation/pacing for atrial tachyarrhythmias
    - refractory to all medications
    - Failed substrate-targeted ablation
    - Not candidate for surgical Rx
  - Early post-op arrhythmias that can be managed medically
  - Asymptomatic ectopy with stable ventricular fxn
  - Prophylactic Rx of ventricular arrhythmias deemed to be at risk for SCD in whom ICD is otherwise indicated

Indications for ablation in infants and small children (*<15 kg*)

- Class I
  - WPW and resuscitated cardiac arrest
  - WPW, syncope with predictors of high-risk
  - SVT, JET, VE, or VT w/Ventricular dysfxn and ineffective/intolerable medical Rx
  - CHD with SVT due to AP, Twin AV nodes or post-op AT (>3–6 months) and ineffective/intolerable medical Rx
- Class IIA
  - WPW, dyssynchrony and reduced LVEF with intolerant/ineffective Rx
  - Monomorphic VT causing sxs despite medical Rx
  - Asymptomatic AT >3–6 months and risk of TE Events/CHF and ineffective Med Rx
  - Preemptive pre-op prior to Fontan
  - Frequent ectopy causing dysfunction in CHD, ineffective meds
- Class IIB
  - Sxs of SVT and AP or inducible SVT at EPS
  - Medical Rx should be considered first
  - Cryotherapy should be considered for slow pathway mod and consider for other substrates
  - SVT with acute compromise
  - Idiopathic JET, ineffective/intolerable med Rx
  - PMVT with triggering focus
  - AVN ablation/PM for otherwise refractory atrial tach and not candidate for surgical therapy
- Class III
  - SVT, controlled on medical Rx
  - Asymptomatic WPW
  - Post-op JET
  - Idiopathic JET controlled with meds
  - VT/VE, effectively treated and no vent dysfxn
  - AVR
  - Early post-op AT

Indications for ablation of ventricular arrhythmias

- Class I
  - Frequent ventricular ectopy or VT
    - single focus thought to be causing ventricular dysfxn and >15 kg
    - <15 kg and meds ineffective or not tolerated
  - Idiopathic RVOT/LVOT or fasciular VT
    - Ineffective meds <15 kg
    - Alternative to meds >15 kg
- Class IIA
  - Frequent ectopy correlated with Sx and >15 kg
- Class IIB
  - AVR correlated with Sx and >15 kg
  - Recurrent PMVT with identifiable triggering focus
- Class III
  - VT controlled medically/well-tolerated <15 kg
  - AVR <15 kg
  - Asymptomatic ectopy, VT or AVR not causing or leading to ventricular dysfxn
  - VT due to transient reversible causes (eg, electrolytes, myocarditis, drug toxicity)

Table 2 Pooled efficacy and complication summary data for ablation of SVT in pediatric patients

	Pediatric radiofrequency ablation registry (PRAR) (Dick et al., 1991)	*Prospective assessment after pediatric cardiac ablation (PAPCA) (Kugler et al., 1997; El Yaman et al., 2009)*
Time period (years)	1991–1996	1999–2003
Patients (*n*)	4135	2761
Contributing centers	46	41
Acute success		
● AP—left lateral	95%	98%
● AP—septal	87%	89%
● AP—right free wall	86%	90%
● AVNRT	96%	97%
Freedom from recurrence[a]		
● AP—cumulative	77%	
● AP—left lateral		91%
● AP—right septal		75%
● AP—right free wall		84%
● AVNRT	71%	95%
Procedural complications	3.2%	3.2%
● AV block	0.7%	1.2%
● Emboli	0.2%	<0.1%
● Death	0.1%	0

[a]Denominator in PAPCA study is limited to the 481 patients in prospective (nonregistry) cohort.

Special approaches to septal pathways are sometimes required, including delivery of lesions within the coronary sinus, for which cryoablation probably offers less risk of thrombosis or coronary artery spasm or injury (Collins et al., 2007). Sometimes septal pathways may have insertions to both the rightward and leftward aspects of the septum, necessitating approach from the septal aspect of the mitral valve annulus, the mid-septal left ventricular outflow, or even the noncoronary cusp of the aortic valve. Finally, right anteroseptal or right anterior pathways may be targeted from a superior approach, with similar advantages in stability and contact with the annulus as the retrograde approach offers on the left side.

Wolff-Parkinson-White syndrome

In many regards, catheter ablation of Wolff-Parkinson-White syndrome is the most conceptually straightforward ablation scenario, and when successful provides one of the more dramatic and definitive endpoints. Abrupt loss of preexcitation on the surface ECG during radiofrequency energy application is the hallmark of a successful lesion (**Fig. 1**). Often this can be observed during sinus rhythm, but if preexcitation is subtle on the surface ECG, atrial pacing from the right atrium or coronary sinus to exaggerate the degree of preexcitation may be useful. It is often useful to map both the antegrade and retrograde insertions during right ventricular pacing or tachycardia, since some accessory pathways display a diagonal orientation with respect to the mitral or tricuspid annuli (Otomo et al., 2001).

An important consideration in the evaluation of Wolff-Parkinson-White is whether ablation is warranted. This is increasingly an issue with the widening use of ECG screening for sports clearance or prior to administering medications for attention-deficit/hyperactivity disorder, resulting in inadvertent discovery of Wolff-Parkinson-White in a patient without prior symptoms. The issues and approaches to risk stratification are complex and remain somewhat controversial, thus beyond the scope of this discussion. Certainly the potential risk of sudden cardiac death in previously asymptomatic children is a chief consideration driving the increasingly common recommendation to perform invasive electrophysiology testing for Wolff-Parkinson-White in the absence of symptoms (Santinelli et al., 2009; Campbell et al., 2003). Furthermore, what criteria are used to determine that risk remain a matter of discussion, as is the decision whether to utilize pathway conduction characteristics in the baseline (usually anesthetized state) or whether to assess the degree that accessory pathway conduction is enhanced with adrenergic stimulation, which can sometimes be quite pronounced (German et al., 1983; Moore et al., 2011). Certainly, other considerations enter into the decision as to whether to proceed with catheter ablation in a given patient, including inducibility of AV reciprocating tachycardia (antidromic reciprocating tachycardia likely representing a more ominous risk than orthodromic reciprocating tachycardia). Not every patient with Wolff-Parkinson-White likely warrants ablation—for instance, if rapid antegrade conduction is not demonstrated and tachycardia is not inducible. Conversely, ablation may still be appropriate in certain cases, and the issues should be individualized on a case-to-case basis and discussed carefully with the family.

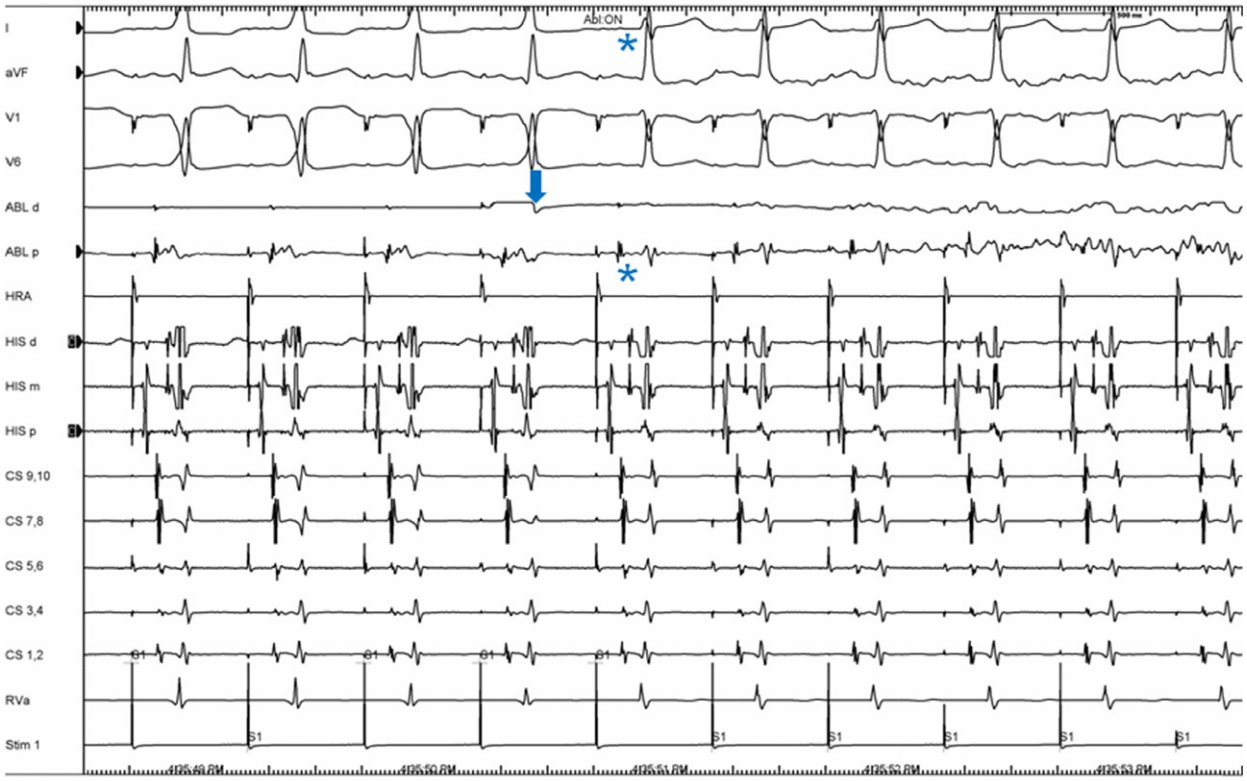

Fig. 1 Acute loss of antegrade accessory pathway conduction with radiofrequency ablation. The solid arrow represents start of lesion; the asterisk marks acute effect as seen on surface ECG and proximal electrode pair on ablation catheter.

Unidirectional retrograde

The approach to a unidirectional retrograde accessory pathway may be rather straightforward for a left-sided accessory pathway. If eccentric VA conduction (early atrial activation in the coronary sinus) is readily displayed during ventricular pacing, this approach can be utilized for both mapping and ablation. The situation becomes somewhat more complex when robust retrograde conduction over the AV node obscures conduction over the accessory pathway during ventricular pacing. These cases require mapping during tachycardia (or at least with orthodromic echo beats), and it may be necessary to ablate during sustained tachycardia. If so, two strategies can minimize the risk of catheter dislodgement with the abrupt rate change accompanying tachycardia termination. Cryoablation offers the clear advantage of adherence, virtually eliminating this risk. Alternatively, ventricular pacing may be performed at a rate slower than the tachycardia rate such that the rate change with tachycardia termination is minimized (**Fig. 2**) (Li et al., 1994). However, it is important to distinguish tachycardia termination due to retrograde block in the accessory pathway due to the lesion itself from VA block due to premature ventricular activation as the result of the asynchronous pacing which results.

For septal pathways with unidirectional retrograde conduction only, the first consideration is verifying the diagnosis, and excluding the alternative possibility of a variant of AV node reentry with VA activation times intermediate between the typical "slow-fast" form of AV node reentry and the atypical "fast-slow" form. A host of pacing maneuvers during tachycardia and sinus rhythm have been described that in sum assist with this distinction (Michaud et al., 2001; Dandamudi et al., 2010; Nakagawa and Jackman, 2005). Since loss of eccentric activation and elimination of inducible tachycardia are the chief markers for successful ablation, careful characterization of any residual VA conduction post-ablation is necessary to identify residual accessory pathway conduction. While adenosine administration during right ventricular pacing is commonly employed in the post-ablation assessment, it should be recognized that transient block with adenosine was demonstrated prior to ablation in up to 20% of accessory pathways in one pediatric study (Fishberger et al., 1995).

Mahaim pathway

The classic description of Mahaim fibers supporting antidromic tachycardia with a left bundle branch block pattern was originally postulated to be nodoventricular or nodofascicular connections. Catheter ablation was instrumental in establishing the current understanding that nearly all accessory pathways displaying decremental antegrade conduction represent atriofascicular connections. The developmental and histologic basis of these structures is uncertain, but they display several characteristics suggesting an accessory AV node-like structure on the right free wall. As such, a His-like potential can usually be recorded over the course of these fibers on the ventricular side of the annulus and extending apically (McClelland et al., 1994). Because the insertion is typically into a fascicle of the right ventricular specialized conduction system, systematic mapping of earliest ventricular activation may not discriminate among widely dispersed recording sites. Therefore, searching for the discrete potential from the atriofascicular fiber

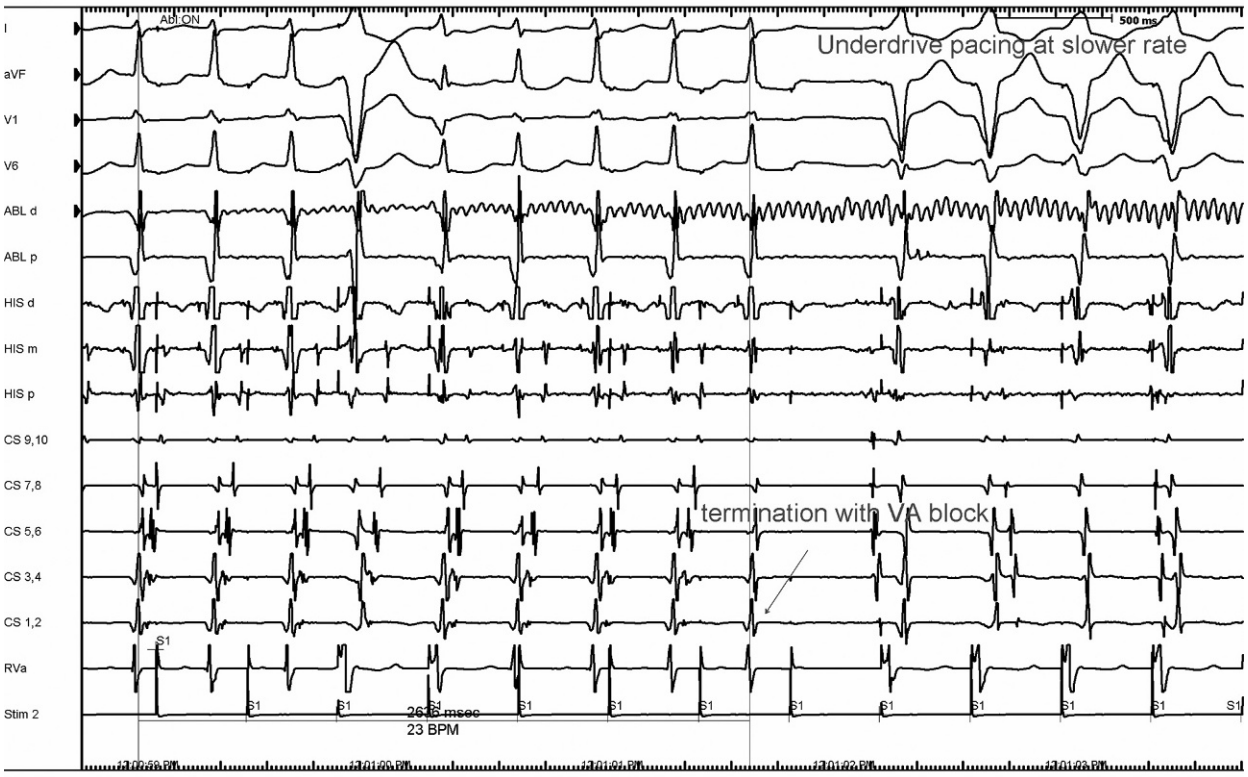

Fig. 2 Ablation of a unidirectional retrograde accessory pathway during orthodromic reciprocating tachycardia. Note that the right ventricular apex is being placed at a cycle length slightly slower than the tachycardia cycle length; when tachycardia terminates during the ablation lesion with acute retrograde block in the pathway, catheter stability is facilitated by the consistent ventricular rate.

is preferable. Ideally, this should be done during ongoing right atrial pacing in order to ensure ongoing conduction through the pathway, since atriofascicular potentials may lie quite superficially and be subject to block with catheter pressure. 3D mapping is helpful in this regard, since the site where such conduction block is encountered can be marked, and if necessary serve as the guide to appropriate ablation sites. Differential pacing from multiple sites along the atrial aspect of the tricuspid valve annulus may also help localize the atrial insertion of the atriofascicular fiber, which should be near the site resulting in the shortest preexcited stimulus to right ventricular interval. However, the pathway's decremental properties may render this a somewhat unreliable maneuver.

Permanent junctional reciprocating tachycardia

Despite the name, the arrhythmia commonly referred to as permanent junctional reciprocating tachycardia is a variant of orthodromic reciprocating tachycardia utilizing a decremental, slowly conducting unidirectional retrograde accessory pathway. In its classic form, the arrhythmia displays several features, including incessant behavior with repeated, self-limited runs of tachycardia with long RP interval which terminate due to VA block; after one or more sinus beats, the arrhythmia spontaneously reinitiates. However, the term permanent junctional reciprocating tachycardia is sometimes used in a broader sense to describe any variant of orthodromic reciprocating tachycardia characterized by a longer than usual VA time such that no sites with VA fusion will be identifiable. While the diagnostic maneuvers to distinguish permanent junctional reciprocating tachycardia from variants of AV node reentry (as well as from atrial tachycardia) are similar to that for characterizing orthodromic reciprocating tachycardia utilizing a septal accessory pathway, the response to ventricular pacing maneuvers may be unique due to the pathway's decremental properties (**Fig. 3**) (Bardy et al., 1985).

Once the diagnosis of permanent junctional reciprocating tachycardia is established, mapping is directed toward the site of earliest atrial activation. Although most commonly the insertion will be septal in location, left-sided and even occasionally right-sided pathways fitting within the spectrum of permanent junctional reciprocating tachycardia will be encountered. Mapping should be performed only during tachycardia to distinguish pathway conduction from retrograde conduction over the AV node. Since the VA time may vary considerably during tachycardia, the site of earliest activation should be measured against a fixed atrial reference site. In some cases, a small electrogram from the pathway itself may be recorded between the ventricular component and the atrial component of the signal at the site of successful ablation. As described above, termination of tachycardia could result in catheter dislodgement, but due to the typically slower rates of this tachycardia, this may be less of a problem than with typical orthodromic reciprocating tachycardia. As discussed above, the factors weighing into the choice of cryoablation versus radiofrequency energy should be considered for each case.

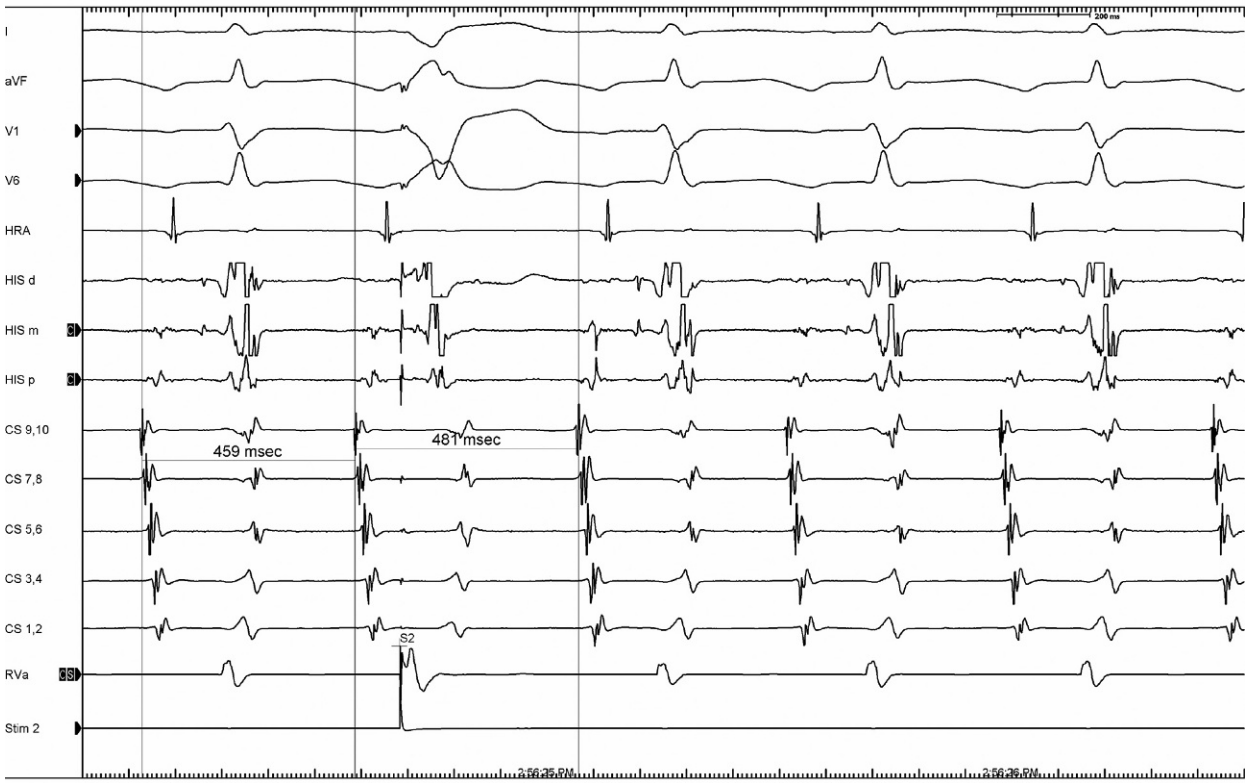

Fig. 3 Delivery of a his-refractory single ventricular premature beat during permanent junctional reciprocating tachycardia produces a paradoxical prolongation of the subsequent retrograde atrial timing, consistent with decremental conduction in the accessory pathway.

AV Nodal Reentrant Tachycardia

In older children and teenagers, AV nodal reentrant tachycardia is encountered with similar frequency as orthodromic reciprocating tachycardia. Thus, the approach to any supraventricular tachycardia in a patient not manifesting preexcitation on the surface ECG should begin with identifying features supportive of either of these two diagnoses. The substrate is usually considered to be two or more regions of inputs to the AV node with differential conduction properties, allowing for reentry into and out of the compact AV node. This concept requires that a portion of atrial tissue in the region of the AV node completes the reentrant circuit. An argument for reentry completely confined to the AV node is still sometimes made, though the distinctions are generally more theoretical than practical in nature. Importantly, a number of variable patterns of AV nodal reentrant tachycardia may be observed, including the potential for antegrade 2:1 conduction due to block above or below the His bundle and atypical locations of the slow pathway inputs.

In general, there is a "fast pathway" and a "slow pathway," which are respectively situated more anteriorly and posteriorly. Usually the slow pathway is in close proximity to the coronary sinus os, and the fast inputs are near the atrial aspect of the apex of Koch's triangle. While interruption of either input should eliminate tachycardia, fast pathway ablation has been all but abandoned as it leaves the patient with first degree AV block and appears to carry a higher risk of inadvertent complete AV block (Jazayeri et al., 1992).

The typical "slow-fast" form of AV nodal reentrant tachycardia is characterized by antegrade conduction over the slow pathway and a very short VA interval. As such, mapping atrial or ventricular activation during this rhythm is of no benefit for determining the site of ablation. In the early experience with this procedure, some investigators stressed the utility of identifying a "slow pathway potential," referring to a low amplitude signal recorded between the local atrial and ventricular components during sinus rhythm (Jackman et al., 1992). However, the identification and interpretation of such signals is at best subjective. It is clear that successful ablation can be achieved at sites not displaying such signals, and conversely, signals which could be construed to be slow pathway potentials may be recorded at sites where effective ablation is not achieved.

For the reasons above, the approach to catheter ablation of the typical form of AV nodal reentrant tachycardia is usually anatomic (Wathen et al., 1992). There can be considerable variation in the site where successful elimination of slow pathway conduction can be achieved. Identification of the AV muscular septum using intracardiac ultrasound has been cited as consistent anatomic marker in adults, but this has not been validated in children (Fisher et al., 1997). It is known that the relative dimensions of this region are decreased in smaller patients, and it has been demonstrated that late fibrosis may emanate from the central scar following radiofrequency ablation with encroachment upon the AV node (Saul et al., 1994; Goldberg et al., 1999). These factors combine to leave younger patients at potentially increased risk for inadvertent AV block (Schaffer et al., 1996). The relative merits of

radiofrequency versus cryoablation are an area of ongoing investigation, as discussed earlier. Because cryotherapy lesions do not elicit junctional acceleration, delivery of cryotherapy during sustained tachycardia may therefore be advisable, with termination of tachycardia due antegrade block serving as the indicator of an effective lesion. If radiofrequency ablation is utilized, careful scrutiny to watch for loss of VA conduction (i.e., loss of conduction over the fast pathway retrograde) during ongoing accelerated junctional rhythm is essential to ensure integrity of the fast pathway.

The most important endpoint of effective ablation is elimination of inducible tachycardia. However, since AV nodal reentrant tachycardia can sometimes be quite difficult to initiate (especially in the sedated or anesthetized patient), surrogate markers of success are useful. Complete elimination of all single AV node echos may be excessively aggressive. Elimination of sustained antegrade slow conduction (manifest as sustained PR > RR interval during atrial pacing) has been proposed as a reasonable proxy endpoint (Kannankeril and Fish, 2006).

In the atypical "fast-slow" form of AV node reentry, retrograde conduction is over the slow pathway. Therefore, direct mapping of the site of earliest atrial activation is a reasonable strategy. Furthermore, this should allow identification of variations in the site of the slow pathway insertions, such as the coronary sinus os, proximal coronary sinus, and less commonly the leftward aspect of the atrial septum. If targeting the slow pathway in this fashion, ablation would ideally be performed during ongoing tachycardia, observing for termination due to retrograde block.

Focal Atrial Tachycardia

Focal atrial tachycardias can vary considerably with respect to clinical behavior and location. Automatic foci represent the most common incessant subtype, but triggered foci may display incessant burst behavior, while other likely reentrant foci have paroxysmal behavior and can be initiated and terminated with pacing. In all cases, the key is to map the site of earliest activation. Electroanatomic mapping is highly useful in this setting since these sites may be recorded in virtually any region of the right or left atrium as well as the pulmonary veins or occasionally the superior vena cava. It is important to ensure that electrograms are recorded during spontaneous tachycardia and not during catheter-induced ectopy. To this end it is often useful to have a multi-electrode reference catheter with 5–10 bipolar signals displayed throughout mapping. In cases where ectopy is unusually quiescent, single-beat mapping with noncontact mapping systems may facilitate mapping in the right atrium.

Ectopic atrial tachycardia

Usually automatic in nature, and thus incessant, ectopic atrial tachycardia commonly originates in the region of the right or left atrial appendages, near the pulmonary vein ostia, or near the coronary sinus os. Mapping sites most likely to produce acute success have local electrical activity 20–60 ms prior to the onset of the surface P wave, and ablation at these sites produces a rapid acute effect on the tachycardia (median 2 s) (Walsh et al., 1992). Several recent series have demonstrated specific benefit from use of 3D electro-anatomic mapping, with acute success rates >90% and recurrence rates of 10%–20% (Cummings et al., 2008; Kang et al., 2011).

Pulmonary vein tachycardia

Occasionally, very rapid, repetitive bursts may emanate from a pulmonary vein. These can sometimes be mistaken for atrial flutter or atrial fibrillation, depending on whether the activation in the right atrium is organized or disorganized. This pattern is similar to focal atrial fibrillation as described by Haissaguerre which served as the springboard for contemporary ablation efforts for atrial fibrillation (Haïssaguerre et al., 1998). In young patients, these sites tend to be isolated and discrete and may be amenable to focal ablation at the venous ostium. However, if not readily extinguished by the first few ablation lesions, pulmonary vein isolation with lesions placed outside the venous ostium should be considered to avoid the creation of pulmonary vein stenosis.

Atrial septal tachycardia

Atrial septal tachycardia, sometimes referred to as "tachycardia from the apex of Koch's triangle," is an unusual tachycardia usually observed in adults which can be mistaken for AV node reentrant tachycardia, and may well be an atypical variant thereof. This arrhythmia may display paroxysmal or incessant behavior, but can be induced and terminated with programmed stimulation. However, no clear link between atrial tachycardia and ventricular activation is evident, and Wenckebach AV conduction during tachycardia is common. Typically, a site of very early, discrete activation is recorded along the mid-superior aspect of the tendon of Todaro (which can often be palpable during catheter manipulation). Care must be undertaken to ensure catheter stability during lesions which must be delivered during tachycardia.

Inappropriate sinus tachycardia

Inappropriate sinus tachycardia (IST) is an ill-defined entity describing unexplained tachycardia during risk or light exertion (Olshansky and Sullivan, 2013). Often the cause is autonomic in nature, overlapping with descriptive characterizations as POTS and Orthostatic Intolerance; in this context ablation is generally inadvisable (Sheldon et al., 2015). Occasionally, what appears to be an ectopic focus with subtle but discernible changes in rate and P wave morphology is mapped to the superior aspect of the crista terminalis. Often the site of activation can be observed near the base of the right atrial appendage with "split" electrograms suggesting this represent the extension of the crista onto the *arcuate ridge*, and may be situated on the septal aspect of the appendage (Killu et al., 2009). Accordingly, ablation of this rhythm may not be readily feasible with a single lesion. Slowing of the rate and a progressively more lateral and inferior origin of the underlying sinus rhythm is observed during effective ablation.

Whether these represent truly ectopic foci or an area of the sinus node which is devoid of normal vagal inputs is a matter of conjecture. Regardless, care must be taken to avoid inadvertent injury to the sinoatrial node; if sinus beats are observed efforts

should be made to map their origin as well as the ectopic focus. Likewise, the right phrenic nerve typically lies nearby and measures to identify the course and ongoing integrity of the phrenic are essential with ablation in this area.

Intra-Atrial Reentry Tachycardia in Congenital Heart Disease

Atrial tachycardias represent one of the most common morbidities late after repair of congenital heart disease. Due to the highly variable and potentially complex interplay between anatomic structures, surgical scars, and subsequent scar and fibrosis within the atrial tissue itself, these macro-reentrant or focal tachycardias can be among the most challenging to address with catheter ablation. Both congenital and acquired vascular anomalies often complicate access to the heart, as described earlier. It is not uncommon for multiple atrial tachycardias to be induced in a single congenital heart disease patient, each of which require individual mapping, often in both the right and left atrial chambers. Accordingly, these cases can be quite lengthy in duration. While acute success rates >70%–90% can be achieved in these cases, recurrences are considerably more frequent than for most other forms of supraventricular tachycardia, likely due to the complex anatomy and the difficulty identifying optimally narrow corridors of conduction to allow block of the reentry circuit (Saul et al., 2016; Kannankeril et al., 2003). Furthermore, the factors leading to the genesis of these arrhythmias are ongoing such that new arrhythmias with different patterns of activation are usually encountered when one returns for repeat ablation following recurrence.

The limitations to intra-atrial reentrant tachycardia ablation must be carefully considered and expectations should be realistic when embarking on this therapy. However, given the limited efficacy of antiarrhythmic therapy in this scenario, ablation is reasonable in a center experienced in this technique and familiar with the complexities and variations in congenital cardiac anatomy which include difficulties in catheter access and challenging catheter manipulation (**Fig. 6A** and **B**).

Isthmus-dependent

In most published series, tachycardias dependent on the cavotricuspid valve isthmus have represented more than 50% of cases (Kannankeril et al., 2003; Lukac et al., 2005; Mah et al., 2011). While conceptually this is the most straightforward situation to address diagnostically and therapeutically, ablation may be complicated by an unusually broad isthmus of atrial tissue (the authors have measured up to 6 cm in a patient with tetralogy of Fallot), hypertrophy of the Eustachian ridge which may be relatively resistant to radiofrequency lesions, and anatomic boundaries found after atrial repair for complete transposition of the great arteries or following Fontan palliation for single ventricle disease.

The hallmark of cavotricuspid isthmus-dependent atrial tachycardia is the demonstration of continuous propagation of activation around the tricuspid valve annulus which comprises the entire cycle length of the tachycardia. This can be readily demonstrated by electroanatomic mapping and/or by multi-electrode catheters recording activation along both septal and free wall surfaces of the right atrium. Activation within the cavotricuspid isthmus should fall in the "diastolic zone" of atrial activation. However, similar activation can sometimes be observed with other tachycardia circuits, including left atrial tachycardias, atrial septal tachycardias, or "scar-dependent" circuits from the right atrial free wall, depending on the location and timing of breakout to the remainder of the right atrium. This can lead to the erroneous presumption of an isthmus-dependent mechanism. Careful employment of entrainment maneuvers to ensure that the isthmus is within the tachycardia circuit is therefore advisable before commencing with ablation in this area (**Fig. 4**). Successful ablation of cavotricuspid isthmus-dependent flutter is usually performed during ongoing tachycardia; as a line of conduction block nears completion, progressive lengthening of the tachycardia cycle length is observed before termination. Differential pacing is then performed from near the medial and lateral aspect of the cavotricuspid isthmus to demonstrate conduction times >130 ms in both directions, and ensure that activation traverses the upper atrium to the contralateral atrial wall before arriving to the opposite side of the cavotricuspid isthmus. While this is usually a useful predictor of successful ablation, it is sometimes useful to demonstrate the conduction time across the isthmus at baseline (prior to atrial flutter induction) to ensure that a longer than expected delay is not present at baseline.

Scar-dependent

So-called scar-dependent atrial flutter represents a challenge for the purposes of mapping and ablation. A thorough understanding of all preceding surgeries is helpful in order to anticipate the expected locations of surgical incisions. While most scar-dependent flutters are macro-reentrant in nature, cycling around the incision, focal tachycardias likely representing micro-reentry may also be observed. Detailed activation mapping is necessary, with careful delineation of scar edges (as defined by low amplitude local electrogram, typically <0.1–0.2 mV). Additional attention to complex electrograms ("split" and "fragmented" potentials) and how these relate to the tachycardia circuit are important. It is not uncommon to observe tachycardia supported by conduction through a "protected corridor" between the crista terminalis and an adjacent atriotomy scar, and great care and high density recordings may be necessary to identify and correctly characterize this conduction. Entrainment maneuvers become essential in evaluating such circuits.

It is important to recognize that scar may exist in atria due to both surgical incisions and late fibrosis with regional conduction delay due to abnormal loading conditions. As such, complex interplay with the potential for diverse and multiple circuits may be encountered. These may exist as individually distinct circuits that are separately able to be induced and mapped, as spontaneous transitions between circuits (with changes in rate and activation), or as concurrent "double-loop" patterns which may be unmasked as ablation lesions result in an abrupt change in rate and activation (**Fig. 5**). As such, simultaneous recordings from sufficiently numerous sites are helpful to recognize and distinguish between such multiple circuits.

Ablation of incisions related to a right atriotomy carries the additional risks of injury to the right phrenic nerve or the sinoatrial node. Usually, the phrenic nerve lies in close proximity to the crista terminalis, and the normal sinoatrial node is likewise situated

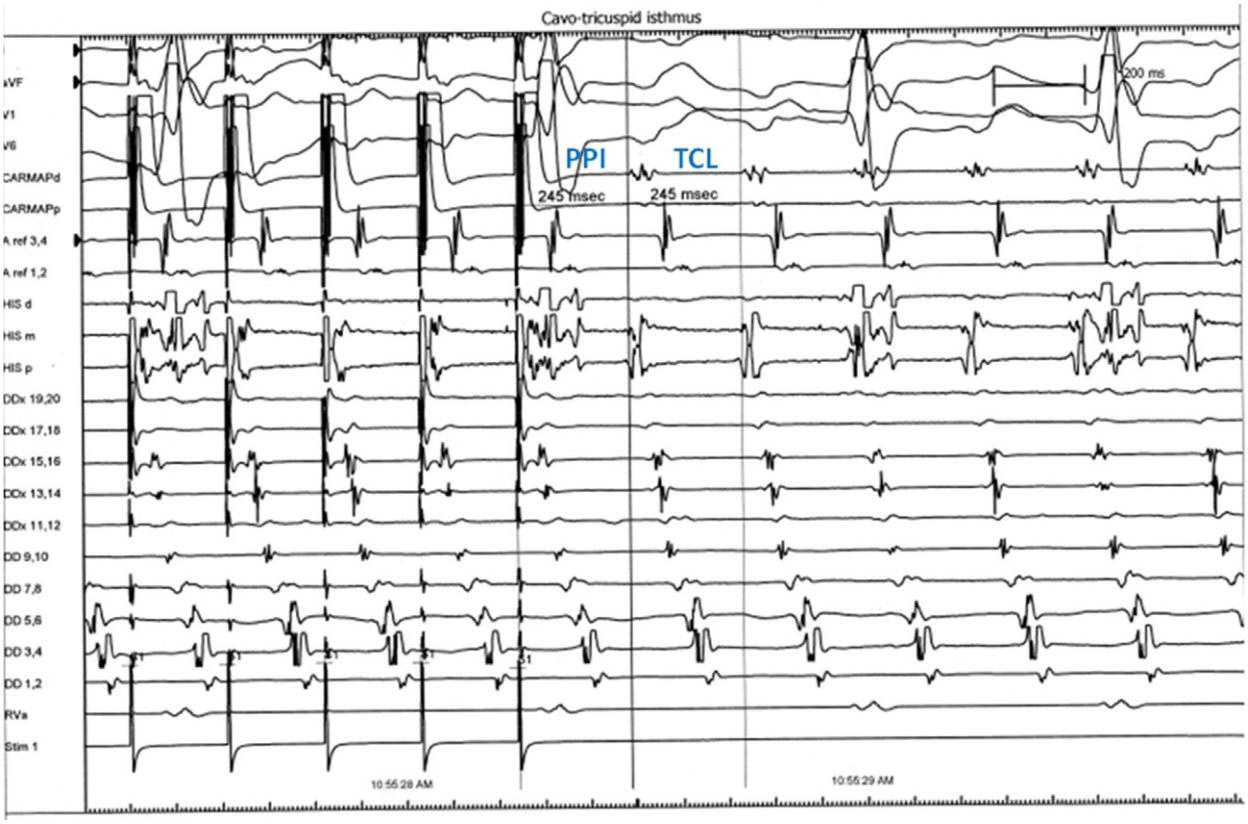

Fig. 4 Entrainment of intra-atrial reentry tachycardia with pacing from the mapping catheter positioned at the cavotricuspid isthmus. Note the concealed fusion, with identical atrial activation pattern during entrainment and tachycardia. The cavotricuspid isthmus is included in the tachycardia circuit, as the post-pacing interval (PPI) and tachycardia cycle length (TCL) are identical. Channels labeled "DD" represent a twenty-pole reference catheter placed from the IVC along the atrial septum and deflected laterally such that poles 1–8 are positioned from low to high along the lateral right atrial wall and poles 11–18 are high to low along the right atrial side of the interatrial septum.

within the crista. To avoid injury to the phrenic nerve, high output pacing while viewing the right diaphragm fluoroscopically should be considered before delivering lesions on the right atrial free wall. When possible, an activation map of the native sinoatrial node at the outset of the study is therefore advisable. Cryoablation may offer less risk of inadvertent injury to these key structures, though may be less likely to be permanently effective in a hypertrophied atrium.

Left-sided circuits

Although most reentrant circuits in adult congenital heart disease patients are right sided, the potential for left-sided circuits may be anticipated in certain lesions, notably patients with mitral valve repair or replacement, repair of partial or total anomalous pulmonary venous connections, and patch repair of an atrial septal defect. Each of these lesions could also predispose to cavotricuspid isthmus-dependent atrial flutter or right-sided scar-related circuits due to the presence of a right atriotomy. The directionality of coronary sinus activation may help in this distinction, but again entrainment maneuvers may be especially important in differentiating between these mechanisms. In the case of reentry around an atrial septal defect patch, it is usually necessary to ablate on the left atrial surface since the medial borders of this circuit are likely to be shielded from right-sided radiofrequency lesions by the patch itself. It may be useful to utilize a double-transseptal technique to deploy both a multi-electrode catheter and a mapping catheter simultaneously in the left atrium.

Junctional Ectopic Tachycardia

Outside of the transient postoperative setting, junctional ectopic tachycardia represents a vexing challenge (Collins et al., 2009). Suppression with antiarrhythmic therapy may be difficult to achieve. The precise mechanism may be unclear, and could include automaticity from within the His bundle (presumed to be the case in the incessant form), intra-Hissian reentry due to partial isolation of parallel tracts within the His bundle, or even a variant of AV node reentrant tachycardia contained within the compact AV node displaying retrograde block. In general, this arrhythmia requires lesions in close proximity to the compact AV node or proximal His bundle, thus carrying an unusually high risk of inadvertent AV block (~20%–30%). When incessant, attenuation of the arrhythmia with catheter pressure may help identify optimal ablation sites. Cryoablation is preferable, both due to the usual advantages of adherence and reversibility in the event of compromised AV node conduction, and to avoid acceleration of the junctional rate which may obscure the endpoint of ablation or make it difficult to ensure the integrity of AV node conduction with radiofrequency lesions. Even so, the increased risk of ablation and potential need for pacing should be clearly outlined with patients and families prior to undertaking ablation for this arrhythmia.

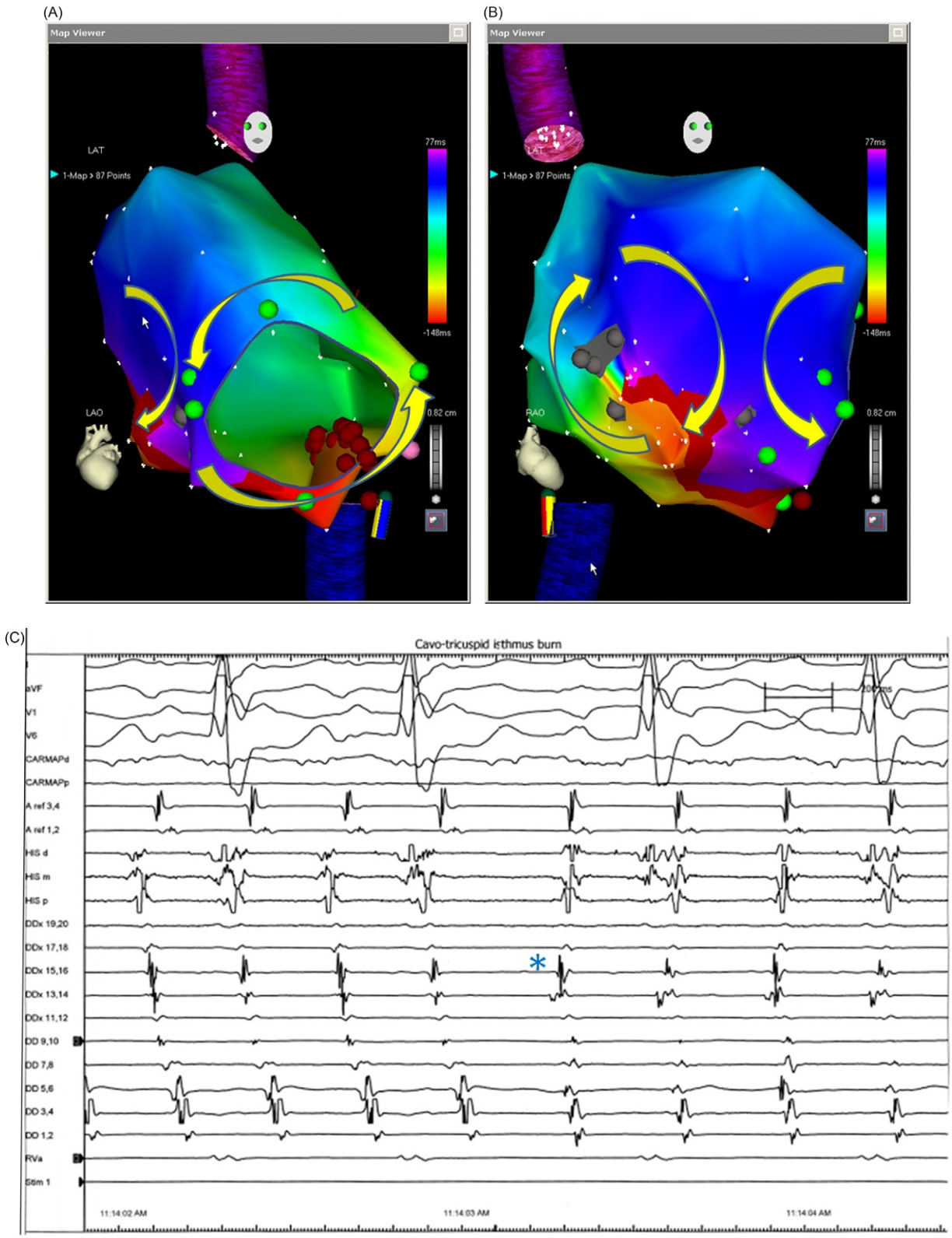

Fig. 5 Example of intra-atrial reentrant tachycardia with a "double-loop" circuit facilitated by both the cavotricuspid isthmus and atriotomy scar. (A) Electroanatomic activation map during tachycardia as viewed from left anterior oblique perspective demonstrating counter-clockwise propagation around the tricuspid annulus. Note proximity of signals in the cavotricuspid isthmus with very early and late timing. Entrainment of this tachycardia from the cavotricuspid isthmus is shown in **Fig. 4**. (B) Electroanatomic activation map of the same patient and tachycardia as seen in Fig. 5A, but from the right anterior oblique perspective. There is clockwise propagation around scar tissue, presumably related to previous lateral atriotomy.

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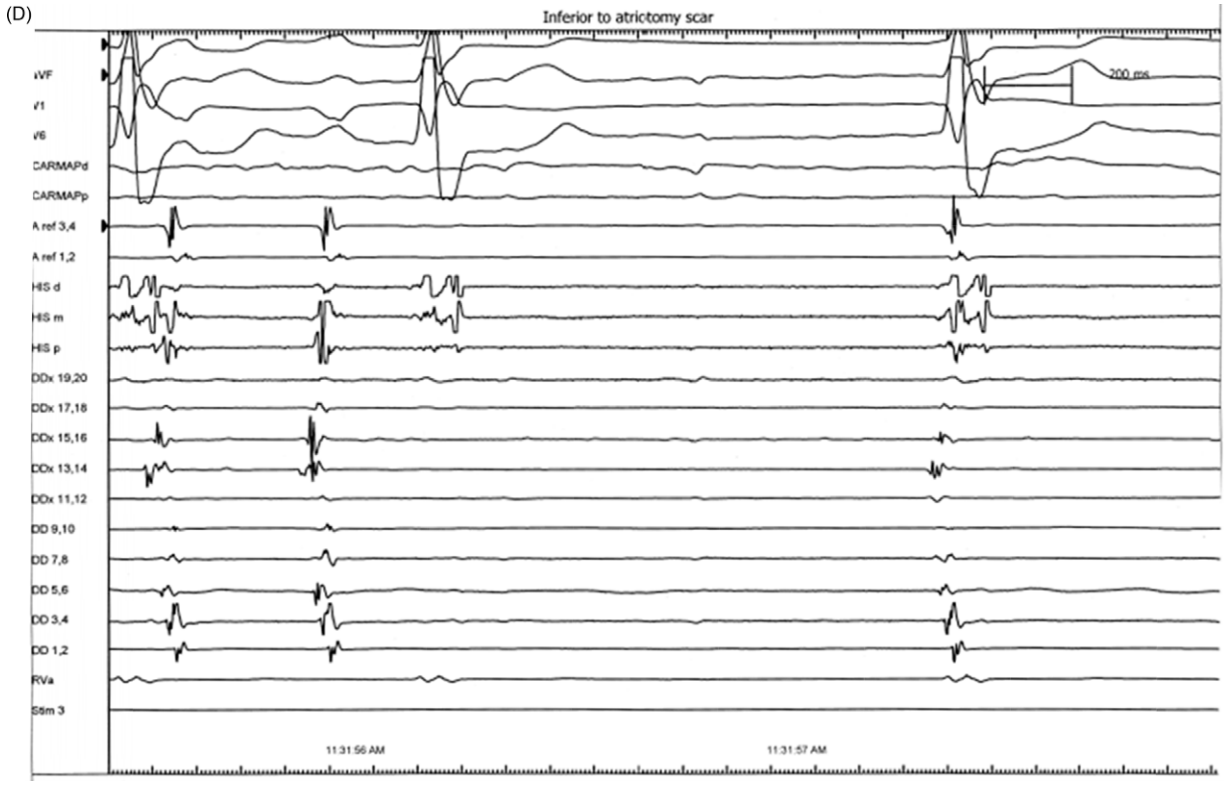

Fig. 5—Cont'd (C) Response of tachycardia shown in **Fig. 4** to completion of line of block across cavotricuspid isthmus (*marks shift from initial tachycardia to new tachycardia). (D) After confirmation of new tachycardia circuit by activation remap and entrainment, acute termination with creation of an additional line of block extended from the lateral atriotomy scar inferiorly to the IVC.

Atrial Fibrillation

While ablation of atrial fibrillation has emerged as the dominant focus of ablation in adults, it remains a very small niche in the pediatric population. Atrial fibrillation is much less common than other supraventricular and atrial arrhythmias among young patients with normal hearts and those with congenital heart disease. When seen, it may be in the context of an intercurrent conduction such as myocarditis or thyrotoxicosis, in certain inherited conditions such as Emery-Dreifuss Muscular Dystrophy, or in congenital defects associated with left-sided valvular disease (Biviano et al., 2010; Boriani et al., 2003; Nazarian et al., 2011). More often, it may represent a secondary rhythm in patients with other arrhythmias such as AVRT, AVNRT, atrial flutter, or chaotic (multifocal) atrial flutter (Ceresnak et al., 2013; Fish et al., 1996). Pacemakers capable of delivering automatic Anti-tachycardia Atrial Pacing are sometimes utilized in CHD patients and may sometimes induce atrial fibrillation, rather than terminate the initial arrhythmia. Care must be made in making the diagnosis of atrial fibrillation electrocardiographically in patients with atypical atrial flutter (IART) in the setting of congenital heart disease; in such patients, typical, saw-toothed "flutter waves" are often lacking, and intermediate atrial rates often result in highly variable atrioventricular conduction patterns with resulting "irregularly-irregular" RR intervals. Indeed, cases are known of patients with IART have undergone ablation inappropriately targeting presumed atrial fibrillation in whom the underlying rhythm was macro-reentrant atrial tachycardia.

In young patients presenting with atrial fibrillation, it is often advisable to perform electrophysiology study in search of other forms of SVT. When identified and targeted, ablation appears to eliminate the likelihood of atrial fibrillation recurrence. In those occasional patients with primary "lone" atrial fibrillation, the relative risk-benefit consideration for ablation is compared with other approaches such as "pill-in-the-pocket" medical therapy or acute DC cardioversion. Most young patients will display a sufficiently rapid ventricular response to allow prompt recognition of arrhythmia recurrence, negating the need for anticoagulation. Likewise, recurrences are often infrequent such that episodic therapy is often adequate. In those with atrial fibrillation in the context of congenital heart disease, it may be preferable to perform surgical ablation at the time of operative interventions addressing the underlying defect.

Limited experience of ablation in pediatric patients with lone atrial fibrillation is beginning to emerge (Balaji et al., 2016).

Ventricular Tachycardias

Ventricular tachycardias in young patients may occur as an idiopathic process, as the result of structural heart disease, or in the context of an inherited arrhythmia syndrome. The latter often predispose to sudden death but are usually polymorphic or pleomorphic in pattern, and thus ablation has little role in their treatment. In contrast, idiopathic ventricular tachycardias from the right and left ventricle are typically non-life-threatening, are amenable to ablation and the relative considerations for ablation vs medical therapy are this similar those for SVT. Monomorphic ventricular tachycardias associated with congenital heart disease are

often amenable to ablation. However, due to a potential risk of sudden death, the role of ablation as primary therapy vs adjunctive therapy in combination with an Internal Cardiac Defibrillator must be an individualized decision based upon lesion, presenting symptoms, and institutional experience and capabilities. Occasionally, recurrent ventricular fibrillation provoked by a focal trigger may offer a target for ablation (Noda et al., 2005; Haissaguerre et al., 2002).

The most common ventricular tachycardias encountered are idiopathic focal tachycardias arising from the right or the left ventricular outflow tract (Badhwar and Scheinman, 2007). These display a characteristic electrocardiographic pattern with a superior QRS axis, tall, typically symmetric QRS complexes in the inferior leads and a left bundle branch block pattern. Often these arrhythmias prevail within an intermediate heart range, and are often partially or completely suppressed during sleep (or while under anesthesia). Likewise, they may increase with physical or emotional stimulation, but suppress during maximal exercise; often sustained tachycardia follows exertion, leading to the notion that this arises from a triggered activity or "triggered automaticity." Sometimes, this rate dependence can be replicated by pacing at a given heart rate to provoke ectopy. However, induction with traditional programmed stimulation suggests a reentrant, rather than focal mechanism and a search for underlying pathology should follow (Stratemann et al., 2008; Hornung et al., 2016).

Catheter ablation of idiopathic outflow tract ventricular tachycardia begins with assessment of the clinical scenario, the electrocardiographic appearance of the VT as well as EKG during sinus rhythm, and imaging to ensure no underlying structural heart disease, particularly Arrhythmogenic Right Ventricular Cardiomyopathy. Note that localized wall motion abnormalities may be seen on MRI in patients with idiopathic RVOT ventricular tachycardia and should not be construed as "diagnostic" of ARVC.

The surface EKG is useful in helping to discern the origin of the tachycardia. As a simple guide, the more rightward the QRS transition from dominant S to dominant R, the more left the origin. More detailed criteria have been described to help distinguish between foci on the anterior RVOT free-wall, ROVT septum, aortic valve cusps, or LV outflow tract (Efimova et al., 2015). This is useful in planning mapping strategy and anticipating the area warranting the most intense mapping focus.

The strategy of activation mapping and ablation for outflow tract ventricular tachycardia is inherently straightforward, targeting the site of earliest ventricular activation which should precede QRS onset by at least 20–30 ms (**Fig. 7A–C**). Pacing at this site should result in a QRS morphology identical to the VT (pacemapping), and algorithms are available to assess the degree of QRS match (Kurosaki et al., 2009). Failure to replicate the VT morphology might indicate that "early activation" was actually recorded during a catheter-induced ectopic beat.

However, a number of pitfalls may be encountered, and it is important that these be anticipated to enable a successful ablation. Perhaps the most important in young patients being the potential for significant, even complete arrhythmia suppression after induction of anesthesia. While it may be feasible to perform mapping and ablation in an awake young patient, his/her ability to remain still and calm for the duration of a case cannot be presumed. It is helpful to monitor a patient for a period of time and obtain a representative 12 lead of the tachycardia to serve as a template for later pacemapping. Indeed, if arrhythmia suppression follows and tachycardia or ectopy cannot be elicited by pacing or pharmacologic maneuvers, pacemapping may be the sole guide for targeting the arrhythmia (Kurosaki et al., 2009).

Electroanatomic mapping is highly advantageous in mapping outflow tract VT. If an otherwise active focus is sudden inhibited by catheter position, the last ectopic complex should be captured and this site should be marked to verify early local activation. If ectopy remains suppressed, and pacemapping at this site is concordant, energy delivery is reasonable (Kühne et al., 2010). Care must be taken to avoid incorporation of ectopy induced mechanically by the catheter into the activation map. This may be more difficult to ensure with automated mapping systems, and use of QRS-matching algorithms can help in this, as can appreciating that the early activation at the ectopic focus is typically low amplitude and fragmented compared to the high amplitude EGM observed during mechanical ectopy. When there is insufficient ectopy for activation mapping, a variety of measures can be attempted, including changing anesthetic agents or allowing the patient to emerge from anesthesia; administering catecholamine infusions (isoproterenol, epinephrine); or administering methylxanthines (aminophylline, caffeine). Sometimes, delivering sustained pacing bursts can provoke transient ectopy (possibly by loading the SR with calcium, inducing calcium transients). When all else fails, pacemapping alone can often lead to successful ablation, but procedural efficacy vs failure becomes evident only during the postprocedural monitoring.

Typically it is our practice to systematically begin with RV mapping then move sequentially to aortic cusps, LVOT, and finally to the cardiac summit. This not only allows transition from lower to higher risk sites but also allows the anatomic geometry to be "built" in a manner that facilitates ablation in more complex areas. When mapping the aortic cusps, it is essential to avoid lesions in or on the coronary arteries by identifying their take-offs; this can be done by angiography, integrated ice images, or directly marking the sites with the mapping system. It is also useful to note the proximity of the left cusp and the high anterior outflow tract at the mitral annulus and the right cusp to the septal RVOT. Note that pacemapping is usually not feasible from the cusps. Occasionally, an outflow focus can only be approached via advancing the catheter via the coronary sinus into the transition from the anterior interventricular vein to the great cardiac vein (**Fig. 8A** and **B**). Typically, RF energy should be delivered at low energy with an irrigated tip catheter to minimize adjacent tissue injury and venous thrombosis. Due to multiple approach to the outflow tracts and relatively thin-walled myocardium in this region, epicardial mapping and ablation is rarely required for this arrhythmia.

The second common idiopathic VT is that arising from the fascicular conduction system, most commonly the left posterior fascicular region, resulting in a QRS activation mimicking Bifascicular conduction block (RBBB with L superior axis) (Collins et al., 2013). This arrhythmia is typically paroxysmal in nature and often provoked during exercise. In the EP laboratory it can sometimes be induced with atrial pacing and sometimes entrained and terminated with atrial pacing as well. Although there is sometimes disagreement as to the underlying mechanism of this arrhythmia, it typically arises in a region on the LV septum roughly half to two-thirds of the distance from base to apex. During activation mapping, pre-QRS potentials are sought, and sometimes these can be simultaneously identified during diastole in pre-systole, confirming a local reentrant circuit (**Fig. 9**) (Nakagawa et al., 1993). Ablation at such sites is typically effective. Of note, when PP potentials can be recorded from adjacent sites during VT and during sinus, the activation pattern should be reversed during VT. Although much less common, VT can also arise from the L anterior

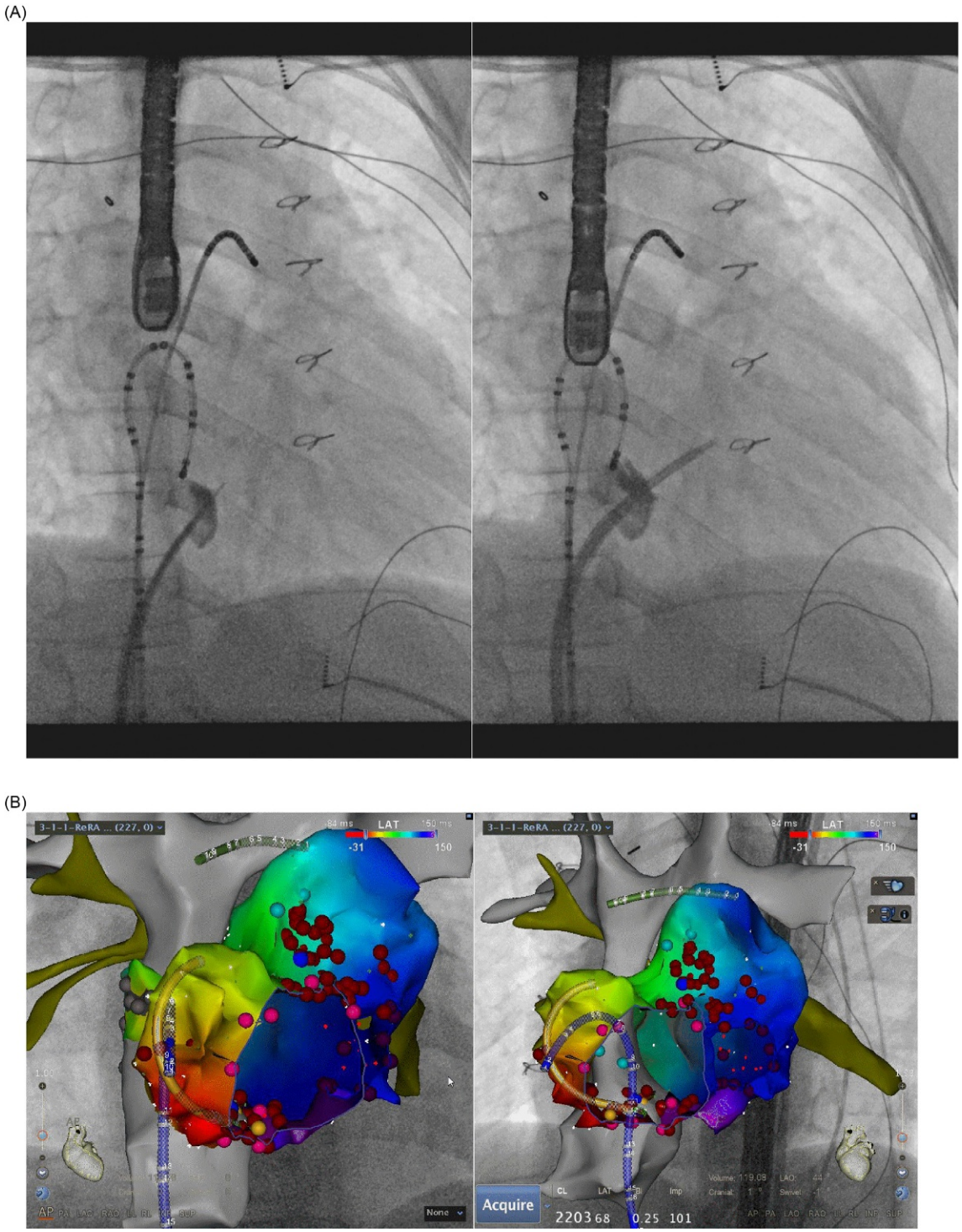

Fig. 6 (A) Puncture from extracardiac conduit fontan into common atrium in patient with double inlet left ventricle and atrial flutter (intra-atrial reentry). (B) Electroanatomic map of atrial flutter (intra-atrial reentry) in 13yo with extra-cardiac conduit fontan for double-inlet left ventricle.

fascicle and is often ablated along the mitral valve annulus. In a large single center series in mostly young adults L anterior fascicular VT accounted for only 2% of fascicular VT cases (Liu et al., 2015).

Pitfalls encountered with this arrhythmia are, as with RVOT, an inability to induce tachycardia despite aggressive stimulation with catecholamine infusions. In such cases, an anatomic approach can be utilized, placing a line of lesions along the mid-apical septum orthogonal to the long axis of the LV (Lin et al., 2005). Of note, activation mapping and pacemapping are of limited value for fascicular VTs. Since the VT engages the rapid conduction system, spread from the focus is rapid, making it difficult to discriminate from sites based on local ventricular activation. Likewise, pacing from multiple sites may replicate the VT if the fascicular system is engaged, whereas capturing local myocardium in addition to the conduction system may actually fail to replicate the VT morphology when pacing from the site of origin (**Fig. 10**).

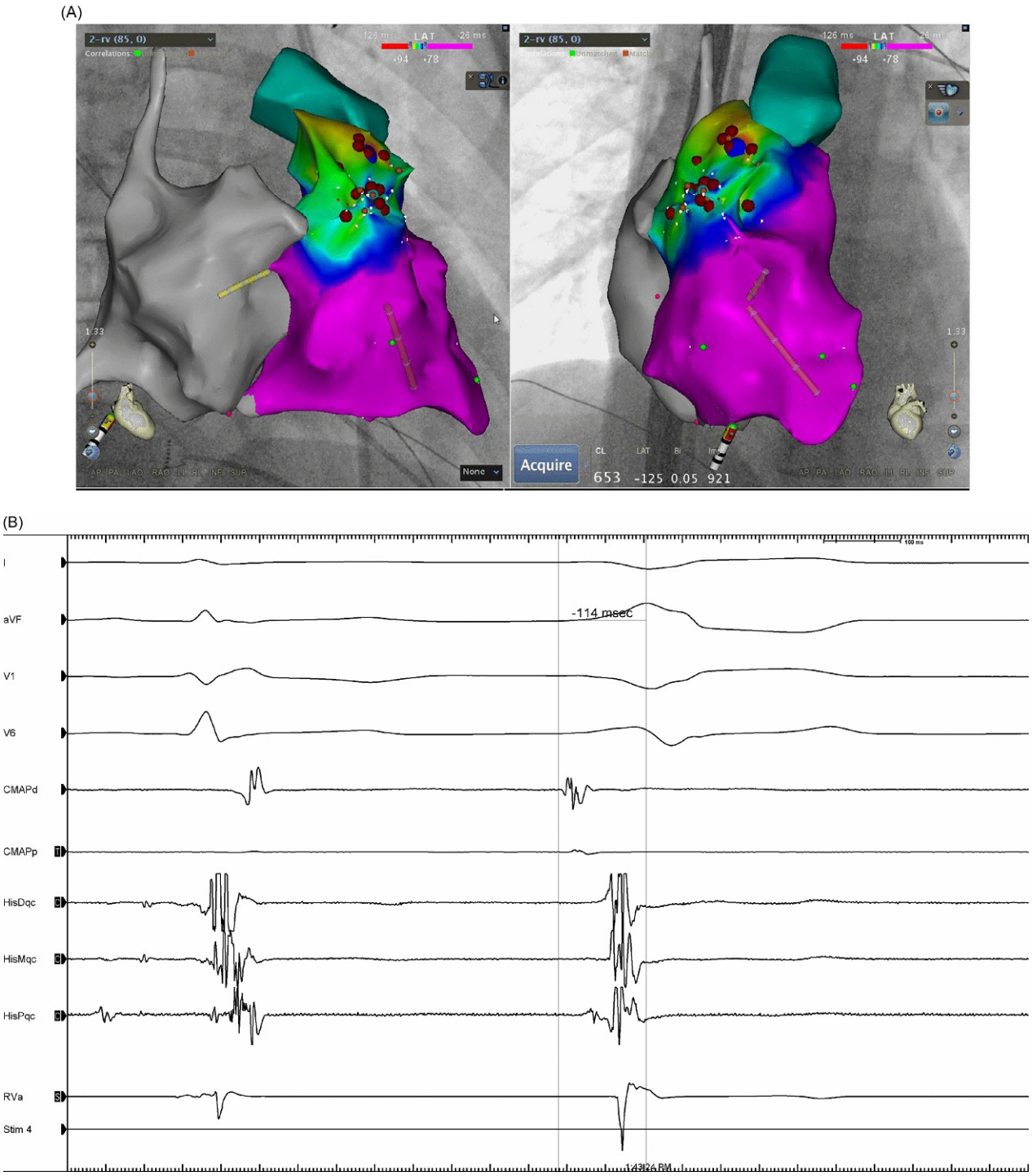

Fig. 7 (A) Electroanatomic map of idiopathic RVOT tachycardia (*red* indicates early activation; *blue* "dot" represents site of effective ablation).

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Ventricular Premature Beats

Ventricular premature beats are a common, often asymptomatic finding in adolescents and teens. Although generally benign in the absence of structural heart disease (Gaita et al., 2001; Guerrier et al., 2015; Niwano et al., 2009; Cha et al., 2012), the potential for the development of ventricular dysfunction has been described, and ablation of VPBs has resulted in recovery of dysfunction (Efremidis et al., 2008; Hasdemir et al., 2013; Satish et al., 2005). While this appears to occur more in older patients, cases have been described of generally mild dysfunction in young patients (Hasdemir et al., 2013; Bertels et al., 2017). Thus, it is deemed appropriate (Class I) to perform VPB ablation in the presence of ventricular dysfunction (Saul et al., 2016). In patients with VPBs describing symptoms, ablation is also deemed appropriate (Class II), though it should be noted that the mere awareness of VPBs



Fig. 7—Cont'd (B) Early (pre-QRS) activation in mapping catheter (CMAPd) at effective ablation site. (C) Acceleration followed by termination of RVOT tachycardia with application of RF energy.

may or may not constitute sufficient rationale for ablation, and the risk vs benefit of ablation should be considered on an individual basis, including the potential need to ablate from an epicardial approach in some cases to achieve success.

Ventricular Tachycardia With Structural Heart Disease

The role of ablation for ventricular tachycardias associated with structural heart disease continues to evolve in young patients, as in adults. This is a consequence of improved tools and strategies for mapping and ablation juxtaposed against an increasing appreciation for the frequency of complications encountered with ICD implantation in young patients (primarily due to lead failure). The approach to VT ablation has been perhaps best studied in patients with postoperative Tetralogy of Fallot in whom 4 potential sites have been described, each of which can serve as the critical isthmus of slow/protected conduction supporting macroreentry (Kapel et al., 2015). The principles of VT mapping and ablation are similar to those described following myocardial dysfunction, though the substrate tends to be more discrete, usually displaying only a single VT morphology. Activation mapping, pacemapping, characterization of the arrhythmia substrate (scar mapping), and careful assessment of putative targets using entrainment maneuvers all play essential roles in achieving an effective result. We have encountered both macroreentrant and focal reentrant mechanisms in this population (**Fig. 11A**). Saline-irrigated and/or large tip catheters are advisable to enhance the likelihood of achieving transmural lesions. In patients with hemodynamically unstable VT, some reports have utilized noncontact mapping to identify critical isthmuses and avoid the need to allow tachycardia to sustain (Schneider et al., 2012). Alternatively, hemodynamic support with ECMO is being increasingly utilized for mapping and ablation of unstable VT in adult patients (Lu et al., 2013; Riskallah et al., 2013), and we have utilized this approach effectively in postoperative VT (**Fig. 11B**). Long-term results have varied so that ICD implantation should be considered not only in those with unsuccessful ablation but also in patients with poor ventricular function. Laredo et al. (2017) recently reported favorable long-term outcomes in a single-center cohort of 34 patients with TOF undergoing VT ablation: freedom from death and arrhythmia recurrence was 94% at 5 years, 81% at 10 years, and 70% at 20 years.

It is especially useful to consider provocative EPS with mapping and ablation in patients with TOF and similar anatomy prior to performing either surgical revision of the RV outflow tract or prior/during placement of a transcatheter pulmonary valve. In the former scenario, the results of mapping and ablation can be utilized to place additional intraoperative cryotherapy at the critical isthmus supporting VT; if immediately postablation, the RF lesions may be grossly visible to the surgeon. In the latter scenario, the concern is that insertion of a stent into the outflow tract may limit access to RVOT tissue which could be critical to the VT circuit. While epicardial ablation could be attempted, access to large regions of the epicardial surface may be limited due to adherence of the

pericardium. Although Nguyen and coworkers reported early reduction in nonsustained ventricular tachycardia (Nguyen et al., 2016), a longer study showed no reduction or prevention of arrhythmias (Prioprintr et al., 2016).

Ablation of ventricular tachycardia has been described in a number of other types of congenital and structural heart disease, including Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) (Philips et al., 2012, 2014) and a recently described entity of focal, reentrant subepicardial VT originating from the RVOT of endurance athletes (Venlet et al., 2017). Although reserved primarily to decrease VT burden and frequency of ICD shocks in patients with ARVC, Pokushalov et al. (2010) reported no VT recurrence during a follow-up of 6 months to 4 years among 16 young patients following epicardial ablation.

With the emergence of the Cone Procedure for addressing severe Ebstein's anomaly, preoperative EPS has identified ventricular tachycardia, often arising from the atrialized region of the RV (Shivapour et al., 2014). TV repair or replacement may completely thwart future access to these regions, whereas preemptive catheter ablation may be curative; as in patients with TOF, catheter mapping and ablation can also guide application of intraoperative cryolesions to the site of VT origin.

Ablation in Infants

As previously discussed, ablation in infants carries an increased risk of complications, including cardiac perforation, AV block, as well as myocardial and coronary injury. This increased risk was observed in patients weighing less than 15 kg (Kugler et al., 1994). Nevertheless, in patients with supraventricular tachycardia refractory aggressive medical therapy, ablation should be considered. Early registry data suggested that experienced centers were capable of performing ablation in infants less than or equal to 18 months old with safety and efficacy comparable to older children (Sheldon et al., 2015). A recent single-center report by Jiang et al. (2016) described ablation of 109 consecutive children under age 3 years with a 94.5% acute success rate, and recurrence rate of 6.8% with no reported complications.

In small patients, vascular complications may be decreased by use of esophageal reference catheters and microcatheters (Van Hare et al., 2002; Blaufox et al., 2001), and there may be avoidance of collateral damage with delivery of test lesions with decreased

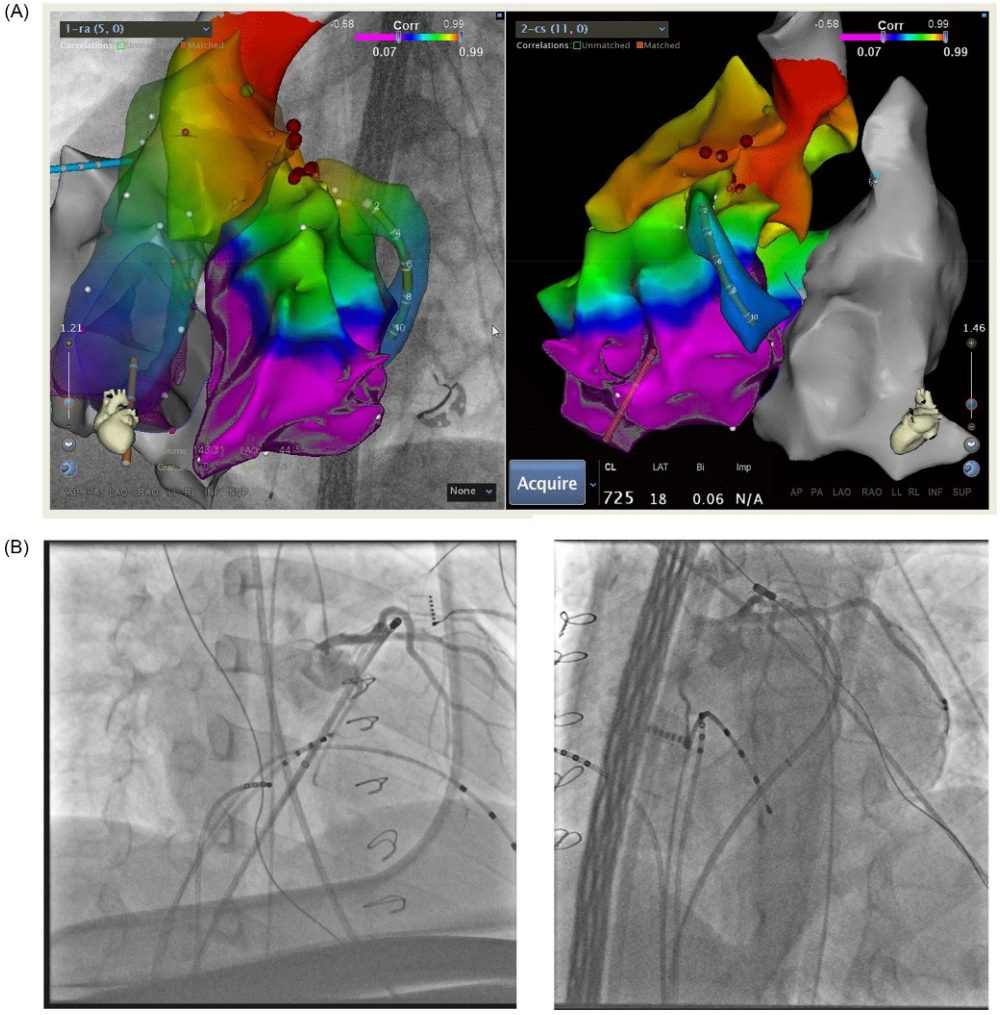

Fig. 8 (A) Idiopathic Ventricular Tachycardia from the Left Ventricular Outflow Tract, accessed via the coronary sinus and great cardiac vein. Note that early activation (depicted by *red*) was recorded in aortic root and septal RVOT, but effective ablation was only achieved via the great cardiac vein. (B) Selective left coronary angiogram to delineate proximity of ablation site to bifurcation of L anterior descending and L circumflex arteries.

Continued



Fig. 8—Cont'd (C) Electrogram (ABLd) at site of earliest local activation of LV outflow tract VT where effective ablation was achieved in the great cardiac vein. (D) Termination of LV outflow tract VT with RF energy applied in the great cardiac vein.

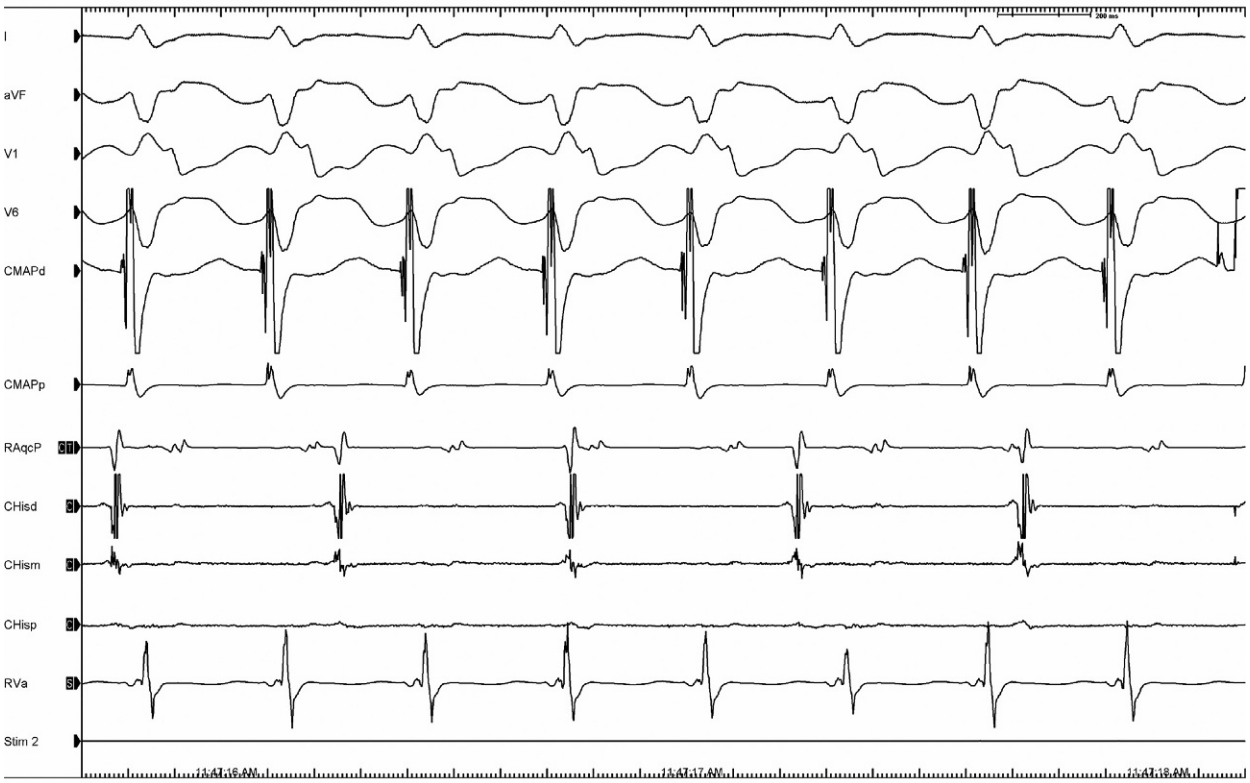

Fig. 9 Presystolic potentials at effective ablation site in idiopathic ventricular tachycardia arising from the L posterior fascicle.

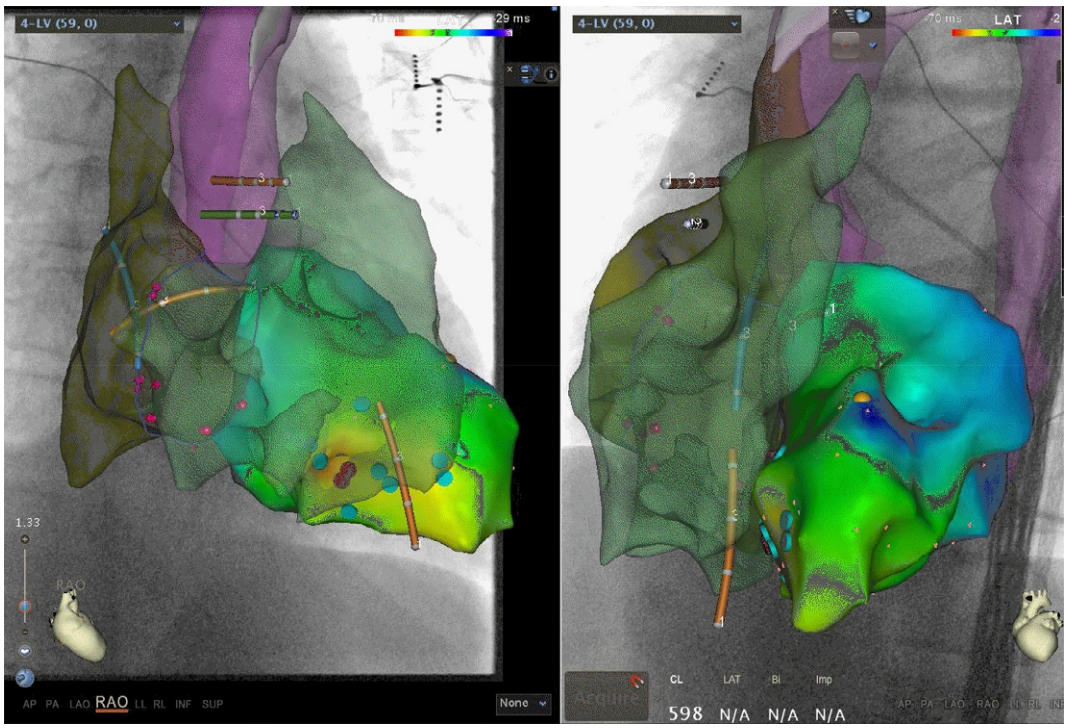

Fig. 10 Ablation site for L posterior fascicular VT (red dots indicate ablation sites; light blue dots indicate sites of Purkinje potentials during sinus rhythm).

temperature and use of ablation catheters with decreased tip size may all improve safety (Cote et al., 1996; Tanel et al., 1998). Other technical modifications advised in the Pediatric Ablation Guidelines included limiting lesions to 20 s and favoring the use of cryoablation when feasible.

Procedural considerations in young patients

It is paramount for young patients, particularly those under the age of 12 years, that ablation be performed in a setting capable of managing the technical, emotional, and recovery needs of small patients, and especially so for patients <15 kg or with moderate or complex Congenital Heart Disease. Even adolescent and teenage patients display a range of ability to undergo procedures under moderate sedation, and ready availability of anesthesia is advisable even in this older age group. Accordingly, a number of recommendations for ablation in young patients were advised in the 2016 guidelines (Saul et al., 2016). These include:

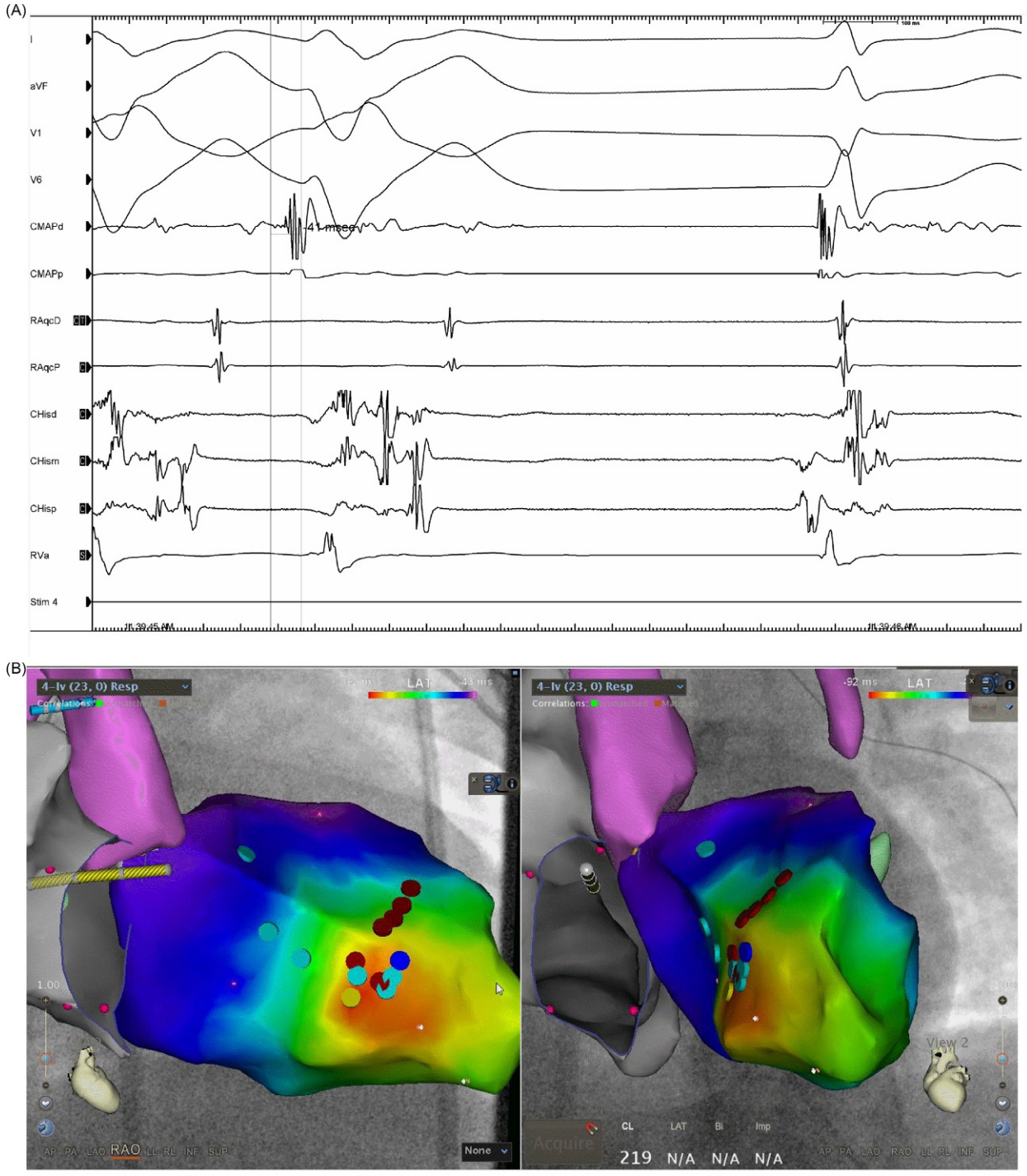

Fig. 11 (A) Left posterior fascicular VT terminating with catheter pressure at site where early diastolic and late diastolic (pre-QRS) potentials were recorded and could not be reinitiated. Linear RF lesion set was delivered. (B) Linear lesion site for left posterior fascicular VT which could not be reinitiated. Red dots indicate RF lesions; dark blue dots indicate site of termination.

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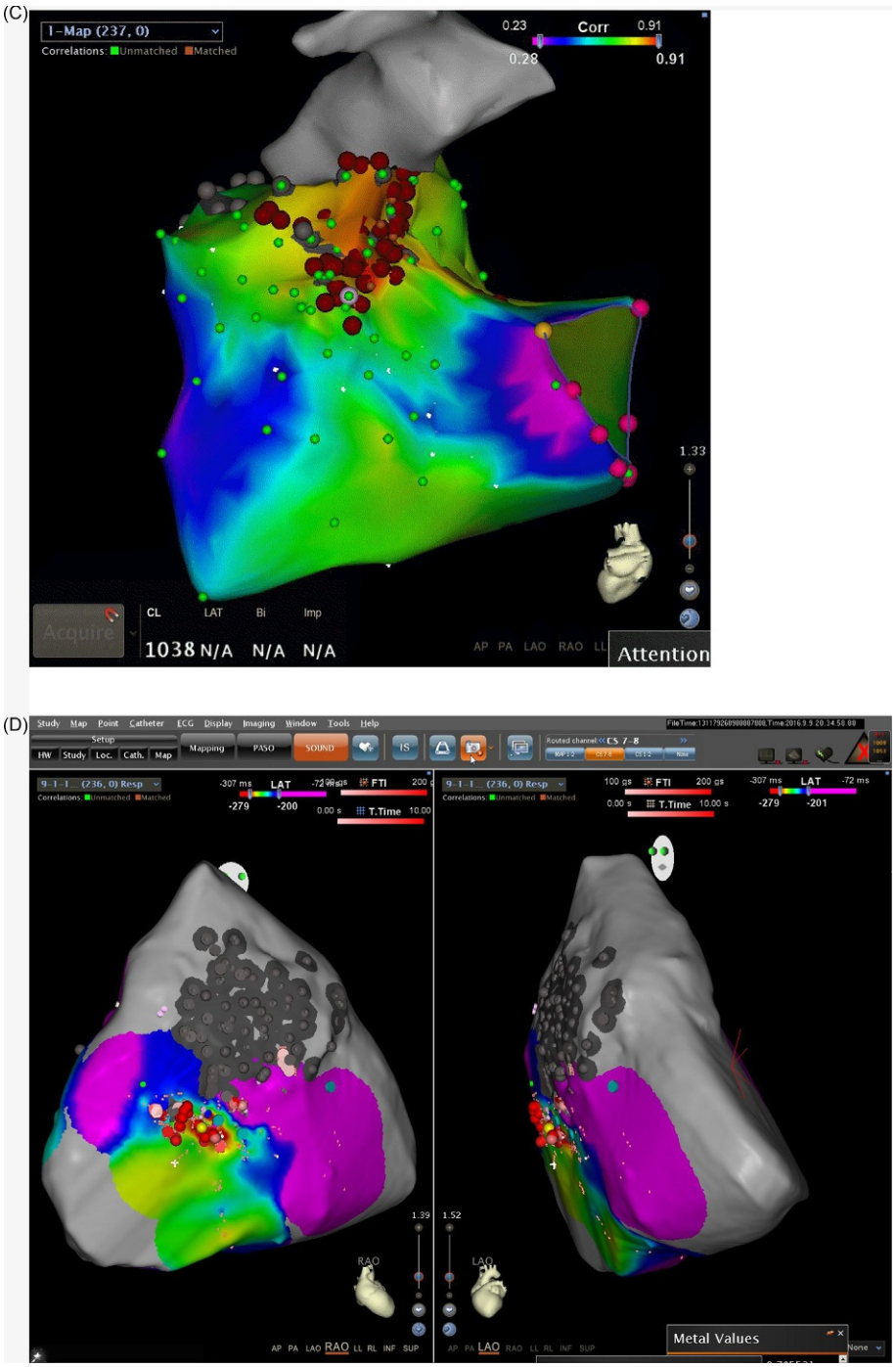

Fig. 11—Cont'd (C) Focal (reentrant) VT in patient with Tetralogy of Fallot, mapped to high RV septum. (D) Electroanatomic map of hemodynamically unstable reentrant VT in patient with Tetralogy of Fallot with early activation (in *red*) along border zone of large RV outflow tract. ECMO support was required to enable mapping and ablation.

1. Available child life support to minimize patient distress and anxiety before the procedure
2. A pediatric (or congenital) cardiovascular surgical program at the same institution where the ablation is performed for patients 12 years of age and younger, and in-house pediatric cardiovascular surgical support for smaller patients (less than 15 kg).
3. 3D nonfluoroscopic imaging as an adjunct for ablation of SVT; and it is recommended that if available, 3D nonfluoroscopic imaging be used for all complex ablation procedures. However, efforts to minimize radiation exposure should not compromise patient safety or procedural success.
4. Routine use of moderate to deep sedation or general anesthesia
5. Removal of sheaths while the patient remains under sedation or anesthesia. Application of local anesthesia at the catheter insertion

6. General anesthesia with endotracheal intubation recommended for (but not limited to): age 12 years and younger; significant CHD or other hemodynamic abnormalities; respiratory comorbidities; other significant systemic comorbidities; anticipation of a prolonged procedure; the need for complete immobility (e.g., a procedure near the coronary orifices or AV conduction system, percutaneous epicardial approach); and patient or parent choice.

Likewise, appropriate follow-up early (within first 2 months of ablation) and later at 6–12 months should be a minimum. Occasional follow-up beyond one year may be useful, both to ensure appropriate evaluation of any late-appearing symptoms suggestive of arrhythmia recurrence or procedural complication, and to ensure that symptom-free patients are not being inappropriately limited or restricted with respect to sports and exercise or access to other appropriate care. It may be necessary to periodically provide ongoing assurance to parents and physicians that it is safe for postablation patients who remain arrhythmia free to receive treatments for conductions such as ADHD or asthma, and may undergo other procedures and options safely without special clearance or perioperative care.

Future Directions

There clearly remain areas for improvement. Although acute efficacy is excellent, recurrences continue to be described following ablation of many substrates. There are opportunities for technical advances facilitating delivery of more lasting lesion formation without extending damage to bystander, normal structures; this is particularly true in those patients with atrial myocardium altered by prior surgeries and longstanding pressure and volume load. There may be further decrease in complication rates by identifying and prioritizing those routine practices yielding safest outcomes, while simultaneously allowing for the elimination of unnecessary precautions. Ongoing minimization of exposure to ionizing radiation by further refinement of nonfluoroscopic techniques remains an area of much current interest. As was previously alluded, to date there has been minimal prospective data regarding patient-oriented outcomes and cost-effectiveness of ablation in children. With all of the above goals in mind, there is currently organization among many pediatric electrophysiologists to establish a prospective quality-improvement database undergirding this next era of pediatric ablation, with anticipated benefits similar to that beginning to be realized in the pediatric cardiothoracic surgical and interventional cardiology disciplines with the STS and IMPACT registries, respectively (Jacobs et al., 2004; Martin et al., 2010).

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Perioperative Management in Heart Failure

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Introduction

Heart failure (HF) is a clinical syndrome that can result from a variety of lesions of the heart (myocardium, pericardium, heart valves, or great vessels) or metabolic abnormalities, which lead to an impairment of ventricular relaxation, contraction, or both (Writing Committee Members et al., 2013). HF is a public health matter: it becomes more common with age; moreover, aging of global population and improvement of HF therapeutics lead to a raising number of patients with HF condition (Braunwald, 2015). Concurrently, the number of surgical procedures performed in the elderly is regularly increasing, with more than a third of the procedures being performed in patients who are 65 years and older (Semel et al., 2012). As a result, patients with HF undertaking surgery are more and more frequently encountered.

Surgery and anesthesia induce hemodynamic stress as well as fluids and metabolic variations that could be compared to a stressing exercise for the myocardium and lead to acute HF, myocardial ischemia, and death (Devereaux and Sessler, 2015).

The latest guidelines on perioperative cardiovascular evaluation and management of patients undergoing noncardiac surgery propose to separate the surgical procedures into two categories (Fleisher et al., 2014), according to their risk of perioperative major cardiovascular events (MACE). MACE are defined by death from a cardiovascular cause or nonfatal myocardial infarction. During a low-risk procedure, the combined surgical and patient characteristics predict a risk of perioperative MACE below 1% whereas elevated risk surgeries are associated with MACE of at least 1%.

In this article, we will review the consequences of HF during surgery and the management of HF patients in the perioperative settings.

HF Increases the Risk of Perioperative Morbidity and Mortality

HF is a well-established perioperative risk factor (Hernandez et al., 2004). Among patients of 65 years and older who undergo major noncardiac surgery, the perioperative mortality rate is at least doubled if they have HF (Hammill et al., 2008). Moreover, among patients who have HF, those who have a more severely impaired ejection fraction have a worse outcome: in patients with HF who had a high-risk noncardiac surgical procedure, Healy and colleagues reported that severely reduced ejection fraction (<30%), but not moderately (30%–40%) or mildly (40%–50%) impaired ejection fraction, was associated with more adverse perioperative events including myocardial infarction, HF exacerbation, and mortality within 30 days after surgery (Healy et al., 2010). In this population, the three independent risk factors of adverse perioperative events were an age above 80 years, an ejection fraction below 30%, and diabetes.

Because HF is such a strong risk factor of perioperative morbidity and mortality, it is a component of indices that predict the risk of perioperative MACE. The revised cardiac risk index (RCRI) is the oldest and easiest validated score that predicts the risk of perioperative MACE. RCRI contains six items, including HF, ischemic heart disease, stroke, diabetes, renal dysfunction, and high-risk surgical procedure. Having at least two items of the RCRI classify a patient as high risk of perioperative MACE (Fleisher et al., 2014; Kristensen et al., 2014).

Patients who have a known HF with symptoms can easily be classified. However patients who do not present symptoms but who are at risk in developing HF should be detected as well. In fact, even in the absence of symptoms of HF, isolated diastolic left ventricular dysfunction and systolic left ventricular dysfunction are associated with increased morbidity (30-day cardiovascular events) and long-term cardiovascular mortality (Flu et al., 2010).

Preoperative Time

Before surgery, the consultation with the anesthesiologist is a major step of the care of a HF patient. During that time, clinical examination and the performance of additional tests will allow to specify the cardiovascular state and risks of a patient. Consequently, management of the perioperative time can be planned, with the adaptation of the medical treatment and the timing of the surgery as well as the discussion of the surgical and the anesthetic strategies.

Clinical Examination

In the presence of a known HF, the anesthesiologist should assess the stability of the signs of chronic HF (peripheral venous congestion including jugular venous distention, lower extremity edema, and abdominal ascites) and look for signs of acute HF (dyspnea, weight gain, orthopnea, paroxysmal nocturnal dyspnea).

In the absence of a known HF, the anesthesiologist should inquire for the presence of the different cardiovascular risks factor, quantitate them, and look for signs of either chronic or acute HF.

Because the functional status is a strong predictor of perioperative and long-term cardiac events, it should be assessed during the preoperative time. The functional capacity can be assessed by the ability to perform daily activities, expressed in terms of metabolic equivalents (METs) (Hlatky et al., 1989). One MET is equivalent to the basal metabolic rate of a resting 40-year-old, 70 kg man. A poor functional capacity is considered when METs <4 and is associated with high risk to develop postoperative cardiac events. A good or very good functional status is considered if METs ≥ 4; in the latest recommendations on perioperative cardiovascular evaluation, before high-risk surgery, it excludes the need to investigate for coronary artery disease and likely also for pulmonary and cardiac disease (Fleisher et al., 2014; Kristensen et al., 2014).

Preoperative Investigations

The requirement for additional investigations depends on the patient history, the clinical examination, and the existence of recent tests.

In the case of a patient with a known HF and symptoms that are unchanged since the last investigation (if under one year), no further test is needed. However, if the anesthesiologist suspects HF, the performance of three additional tests should be considered: an electrocardiogram, measurement of plasmatic natriuretic peptides (NP) levels, and a transthoracic echocardiography (TTE).

It is recommended to obtain a 12-lead electrocardiogram in any patients who have coronary heart disease, arrhythmia, peripheral arterial disease, or cerebrovascular disease, unless they undergo a low-risk procedure, or have had an electrocardiogram within 3 months (Fleisher et al., 2014).

A TTE is recommended for patients who undergo high-risk surgery, if signs and symptoms have changed since the last investigation (Fleisher et al., 2014). TTE gives the quantification of the left ventricle systolic and diastolic functions, the assessment of filling pressures, and the search for a cause of HF such as valvular disease. As importantly, TTE should assess right ventricular function, pulmonary artery systolic pressure (if doable), and inferior vena cava diameter as right ventricular loading conditions may change during some high-risk surgeries. Knowledge of TTE abnormalities does not have prognostic value but is of great interest to adapt the perioperative management of patients.

NPs include brain natriuretic peptide (BNP) or the inactive N-terminal fragment (NT-proBNP), which are primarily synthetized by the cardiomyocytes of the ventricles, in response to an increase of cardiac wall stress. NPs are markers of ventricular dysfunction. Other factors, such as age, renal failure, sepsis, and acute coronary syndrome, can participate in increasing plasmatic NPs levels (Writing Committee Members et al., 2013). In case of acute dyspnea, plasmatic levels of BNP are known diagnostic tools to attribute (if BNP >400 pg/mL) or rule out (if BNP <100 pg/mL) congestive cardiac heart failure (Writing Committee Members et al., 2013). In case of chronic HF, NPs are strong prognostic markers that can be used to direct the therapeutic strategy and improve the prognostic of such patients (Pfisterer et al., 2009; Lainchbury et al., 2009). In addition, plasmatic low levels of BNP (<100 pg/mL) or NT-proBNP (<125 pg/mL) exclude a diagnosis of chronic HF.

In perioperative settings, increased BNP or NT-proBNP levels are powerful independent predictors of short- and long-term MACE, as well as all-cause mortality (Rodseth et al., 2011, 2013; Ryding et al., 2009). In noncardiac surgery, the sole value of preoperative BNP levels has a better ability to predict cardiovascular events than the RCRI (Rodseth et al., 2011), and addition of the BNP levels to the RCRI increases its predictive value. These results suggest that measurement of BNP levels could permit a better prediction of cardiovascular risk and stratification of the patients. However, because multiple factors can influence NPs levels, the threshold of NPs levels that would allow a good distinction between high and low risks patients remains uncertain. NPs-guided therapy has not been studied in perioperative settings. However, preoperative NPs levels could be used as a tool to stratified preoperative investigations. In a prospective study, patients who were about to undergo hip surgery had a systematic measurement of plasmatic BNP and a TTE (Pili-Floury et al., 2012). Major echocardiographic abnormalities (defined by increased left ventricular filling pressure, severe systolic dysfunction, pulmonary hypertension, and severe valvular disease) were mostly found in patients with increased levels of plasmatic BNP. Whether patients had a major echocardiographic abnormality or not was best predicted when plasmatic BNP levels were above 285 pg/mL.

Adaptation of the Medical Treatment

Chronic HF treatment is based on beta-blockers, renin-angiotensin-aldosterone system (RAAS) inhibitors, and diuretics (Writing Committee Members et al., 2013). These therapeutics should be stopped for the shortest time possible. However, some particularities exist depending on the class of the agents.

Treatment by beta-blockers should be continued until the day of surgery (Fleisher et al., 2014; Hopper et al., 2014). When needed, a treatment by beta-blockers should be started but not be initiated within days preceding a major surgery (Study Group et al., 2008).

RAAS inhibitors and diuretics could be stopped the day before surgery (Fleisher et al., 2014; Kristensen et al., 2014).

Discontinuation of antiplatelet agents should be discussed according to the reason of the prescription (primary prevention, drug eluting stent, bare-metal stent...), the time between the beginning of antiplatelet agents and surgery, and the bleeding risk of the procedure (Fleisher et al., 2014).

Timing of the Procedure

Decompensated HF forbids any surgical procedure unless life or limb is threatened if not in the operating room within 24 h (Fleisher et al., 2014).

When possible, in the presence of signs of acute HF, postponing surgery by at least 3 months would give time for stabilization and/or improvement of ventricular function and ventricular remodeling before undertaking the stress of a surgical procedure (Upshaw and Kiernan, 2013).

In patients with valvular heart disease, the need for a valvular replacement or valvuloplasty should be discussed depending on the severity of the valvular disease (assessed by clinical symptoms and TTE) as well as the cardiovascular risk and urgency of the surgery (Fleisher et al., 2014).

Before noncardiac surgery, revascularization should be done according to the general guidelines, but not as a prophylactic treatment (Fleisher et al., 2014). If the indication of such revascularization is retained, it can delay the surgery by several months.

For patients with severe alteration of the left ventricular function and left bundle branch block, cardiac resynchronization therapy or defibrillator should be discussed before major surgery (Kristensen et al., 2014).

Surgical Strategies

Advantages of endovascular surgery are not straightforward in patients with multiple comorbidities (Fleisher et al., 2014; Kristensen et al., 2014). Choice of endovascular surgery, over an open procedure, should be done according to the type of surgery and the patient's risks.

Compared to opened abdominal procedures, endoscopic surgery presents benefits in terms of postoperative rehabilitation (bowel movements, pain, and pulmonary function). However, the pneumoperitoneum created during endoscopic procedures induces a decrease in venous return and an increase in cardiac afterload that can be threatening for HF patients. Hence, the choice of endoscopic surgery should be balanced between advantages and risks.

Anesthetic Strategies

The discussion should be focused on the possibility of performing any locoregional anesthesia and on the modalities of general anesthesia.

Neuraxial anesthesia is associated with a sympathetic blockade leading to high variations of intra vascular volume and arterial pressure. Hence, the beneficial effects of neuraxial anesthesia, compared to general anesthesia, are not evident. However, epidural anesthesia should be considered for postoperative pain relief (Fleisher et al., 2014).

Peripheral nerve blockade does not affect the cardiovascular system as much as neuraxial or general anesthesia. Therefore, peripheral nerve blockade should be proposed to HF patients, whenever it is applicable.

During general anesthesia, the objective is to maintain homeostasis as much as possible. In order to control loading conditions on the left ventricle and maintain a balanced rate of fluids infusion/lost, the monitoring of invasive arterial pressure and cardiac output (by the use of esophageal Doppler or pulse contour analysis) should be considered. Anesthetic drugs should be titrated to use the minimal amount of drug to obtain the intended effects with limited adverse effects, by monitoring the depth of anesthesia (using, for instance, the bispectral index). Finally metabolic constrains should be closely watched in order to avoid hypercapnia, hypoxia, hypothermia, and anemia.

Postoperative Time

Postoperative time remains a very sensitive period. Several factors such as withdrawal of anesthetic drugs, awakening, extubation, pain, hypothermia, and anemia participate to activate the sympathetic system and increase oxygen consumption. Additionally, because of the inability to take oral drugs during the postoperative time, after procedures such as major gastro intestinal tract

surgery, the withdrawal of HF drugs can increase the risk of arrhythmia, hypertension, and acute HF. Hence, careful attention should be placed on preventing/treating any tachycardia, preventing the shivering, giving appropriate analgesia and avoiding hypertension. Moreover, regular treatment by RAAS inhibitors and beta-blockers treatment should be restarted as early as possible (Fleisher et al., 2014).

Postoperative observation of patients with severe HF could be done in intensive care. According to the number of cardiovascular risk factors and the perioperative events, a myocardial ischemia should be monitored by the performance of an ECG and troponin levels measurements.

Postoperative increase of NPs levels is associated with increased mortality or nonfatal myocardial infarction within 30 days or 180 days after noncardiac surgery (Rodseth et al., 2011, 2014). However, the interest of postoperative measurement of NPs remains to be demonstrated.

Postoperative HF

The clinical signs of postoperative acute HF are not specific of that time; patients develop signs of acute HF potentially associated with signs of severity such as hypotension, alteration of consciousness, cyanosis, mottling, and renal or hepatic failure.

Several triggers specific of postoperative time such as ischemia, hypoxia, increased systemic blood pressure, fluid overload, or anemia should be searched for. Cardiac surgery is associated with risks of HF related to the specificity of the surgery such as spasm or occlusion of a coronary graft, prosthetic paravalvular regurgitation, cardiac tamponade, or hemothorax.

TTE is a major tool to diagnose postoperative HF.

The management of postoperative HF consists in noninvasive ventilation together with the treatment of the cause, which can include correcting blood pressure, giving appropriate analgesia and discussing a treatment by antiplatelet and anticoagulant agents together with the anesthesiologist, the cardiologist, and the surgeon. Depending on the severity of acute HF, new inotropic drugs (levosimendan, nesiritide) could be more useful than the more traditional treatment to restore hemodynamic balance (Toller and Metzler, 2005).

Conclusion

As the population is aging and the medicine is improving, the perioperative management of HF patients becomes a frequent situation that is associated with increased risk of morbidity and mortality. Every step before, during, and after surgery is sensitive and should be carefully assessed and treated.

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Peripartum Cardiomyopathy

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Introduction

Cardiovascular disease is an uncommon, yet serious complication of pregnancies worldwide. Heart failure associated with pregnancy was recognized as early as the 1800s (Haghikia et al., 2016; Ritchie, 1849) and was increasingly described in the literature thereafter (Gouley et al., 1937; Hull, 1937; Seftel and Susser, 1961). The term "peripartum cardiomyopathy" was first used and defined by Demakis and others as the development of heart failure in the last month of pregnancy or within 5 months of delivery, in the absence of any preexisting heart disease or other apparent cause (Demakis and Rahimtoola, 1971; Demakis et al., 1971). More recently, an additional criterion was added to incorporate echocardiographic evidence of left ventricular dysfunction: a left ventricular ejection fraction (EF) of <45% (Hibbard et al., 1999; Pearson et al., 2000, Sliwa et al., 2010b). The diagnosis of peripartum cardiomyopathy (PPCM) relies on the exclusion of other etiologies of cardiomyopathy, as well as the normal symptoms of pregnancy.

Epidemiology

The incidence of PPCM varies widely by geographic location, race, and ethnicity and differs between studies. Reported estimates range from as high as 1:100 in Nigeria and 1:300 in Haiti, to as low as 1:4000 in the United States and other Western societies (Brar et al., 2007; Gunderson et al., 2011; Isezuo and Abubakar, 2007; Mielniczuk et al., 2006). Within the United States, the incidence has been shown to be higher in blacks than in whites (Brar et al., 2007; Gentry et al., 2010; Harper et al., 2012). The incidence in the United States also seems to have increased in the last few decades, based on recent data which suggests an incidence closer to 1:1000 (Kolte et al., 2014; Mielniczuk et al., 2006). This may reflect improved understanding and detection of the condition or may mirror demographic trends, such as advancing maternal age, the effects of obesity and insulin resistance, and the increased use of fertility treatments resulting in higher frequencies of multiple gestations.

Risk Factors and Associated Conditions

Several demographic risk factors have been identified in association with PPCM, although the pathophysiology of each is unclear. The odds of developing the condition increase with maternal age; the majority of cases occur after 30 years of age (Arany and Elkayam, 2016). As mentioned earlier, the incidence of PPCM is highest in women of African descent in all geographic locations. However, the incidence in Hispanics is lower than in Caucasians (Kolte et al., 2014).

The relationship of PPCM with multiple gestations and multiparous status is unclear. Many cases of twin and triplet gestations in women who developed PPCM have been reported (Altun et al., 2015). However, in a recent study of 34,000 women with PPCM in

the United States from 2004 to 2011, single gestation pregnancies actually had higher rates of PPCM (Kolte et al., 2014). Sixty-seven percent of women with PPCM in 10 studies reviewed by Bello et al were multiparous (Bello et al., 2013), although recommendations against subsequent pregnancies in these women are expected to decrease this frequency in the coming years.

Hypertension, preeclampsia, and eclampsia, three distinct maternal conditions with considerable continuity and overlap, are all strongly associated with the development of PPCM. A recent metaanalysis found the prevalence of preeclampsia in women with PPCM to be four times higher than in pregnancies not complicated by PPCM (Bello et al., 2013). Another study found nearly half of women with PPCM had hypertension and a third had preeclampsia (Kao et al., 2013). The odds of developing PPCM for a patient with eclampsia has been shown to be 27.9 times higher than in a noneclamptic pregnancy (Kolte et al., 2014). These conditions can all cause pulmonary edema and heart failure on their own, due primarily to prolonged elevations in blood pressure, which often clouds the clinical picture and makes diagnosis challenging. However, preeclampsia and eclampsia also seem to create cardiotoxic conditions predisposing to cardiomyopathy that are independent of elevated blood pressures and are poorly understood (Powe et al., 2011).

Pathogenesis

In the past, PPCM had been attributed to a maladaptive response to the hemodynamic stresses of pregnancy; however, most of the physiologic hemodynamic changes occur in the first two trimesters, whereas PPCM typically arises late in the trimester, or even postpartum, when hemodynamics are returning to their previous (i.e., nonpregnant) state. Myocarditis has been implicated in many documented cases, but there exist many more cases where this has been ruled out. Further confounding this, in comparison studies, evidence of viral infections are often found in control cases as well (Bültmann et al., 2005; Fett, 2006).

In recent years, several new hypotheses have been put forward to explain why some women may be more vulnerable to the physiologic stressors in a normal pregnancy. Focus has surrounded the balance between oxidative stresses and antioxidant capacity. In normal pregnancy, there is an increase in reactive oxygen species (ROS), thought to be part of the physiologic defense system to ward off infection during pregnancy and delivery. However, antioxidant capacity increases in parallel to protect maternal health; when this mechanism is impaired, cellular damage occurs without recourse, and angiogenesis, the generation of new blood vessels, is compromised.

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor involved in the antioxidant pathway, participating in the balance of oxidative stress and angiogenesis. STAT3 expression is reduced in the left ventricles of women with heart failure caused by PPCM. Loss of STAT3 in cardiomyocytes has been shown in mouse models to reduce expression of genes that protect the heart from ROS. In an environment favoring ROS, the hormone prolactin is cleaved into an antiangiogenic 16 kDa fragment, which promotes apoptosis in endothelial cells. In this setting, the mice subsequently developed PPCM (Hilfiker-Kleiner et al., 2007).

The 16 kDa fragment of prolactin does not have a direct effect on cardiomyocytes but activates nuclear factor (NF)-ƙB, which upregulates microRNA-146a (miR-146a). miR-146a enters cardiomyocytes directly and its signaling pathways decrease the metabolic activity of the cells and impair communications between the cardiomyocytes and endothelial cells. Circulating levels of miR-146a are markedly elevated in women with PPCM, and in mouse models, inhibition was able to partially rescue cardiac function (Halkein et al., 2013). Prolactin and its downstream counterpart, miR-146a, seem to play a large role in the imbalances in oxidative stress and angiogenesis, contributing to the development of PPCM.

Another pathway has been proposed involving the relationship between ROS, angiogenesis, and PPCM. Similar to STAT3, proliferator-activated receptor-gamma coactivator-1α (PGC-1α) suppresses ROS and drives the expression of vascular endothelial growth factor (VEGF), an angiogenic factor. In a mouse model with cardiac-specific deletion of PGC-1α, the same 16 kDa prolactin pathway was activated, and VEGF pathways were downregulated. In pregnant mammals, the VEGF pathway is thought to be initiated by secretion of soluble Fms-like tyrosine kinase 1 (sFlt1) by the placenta in late gestation. sFlt1 neutralizes much of the circulating VEGF; this can be circumvented in other organs by local secretion, but in the PGC-1α mouse model, the cardiomyocytes are unable to defend themselves. sFlt1 secretion by the placenta has been shown to be markedly elevated in women with preeclampsia (Powe et al., 2011) and twin gestations (Bdolah et al., 2008). Thus, it appears that ROS and angiogenesis predispose to PPCM through multiple pathways.

Several genes have been linked to the development of PPCM in small studies as well, although familial links are sparsely reported in the literature. *PTHLH*, thought to regulate vascular homeostasis, and *TTN*, which codes for a sarcomeric protein called titin, have both been implicated. Women with gene mutations in these regions may have a higher predilection to develop PPCM, but it is likely that environmental factors, such as the vasculohormonal pathways mentioned above, are required to develop the disease.

Diagnosis

Typical Presentation

Most patients develop symptoms in the early postpartum period, but patients can present in the third trimester or as late as 3–4 months postpartum (Demakis et al., 1971; Elkayam, 2011). Classic symptoms of heart failure are common, such as dyspnea, orthopnea, and paroxysmal nocturnal dyspnea, as well as peripheral edema and palpitations. Many of these symptoms, particularly

exertional dyspnea, are nonspecific and may be experienced as part of a normal pregnancy and delivery, which often delays diagnosis. A high index of suspicion is required for early diagnosis; physical exam and diagnostic studies may be prudent in any uncertain cases.

As expected, physical examination is likely to confirm signs of heart failure, including rales, elevated jugular venous pressure, and peripheral edema. Advanced signs of left ventricular enlargement may be noted, such as a third heart sound (S3) or displaced apical impulse.

Differential Diagnosis

Pulmonary embolism is a risk throughout the peripartum period due to the hypercoagulable state occurring with pregnancy. Infections such as pneumonia and myocarditis should be considered as well. As mentioned earlier, hypertension, preeclampsia, and eclampsia increase women's odds of developing PPCM, but they can also independently cause acute pulmonary edema, most commonly related to hypertensive diastolic dysfunction. Lastly, peripartum myocardial infarction and Takotsubo cardiomyopathy should each be considered.

Laboratory Studies and Electrocardiogram

While no specific biomarkers are currently available, basic laboratory studies may help to differentiate PPCM from symptoms of a normal pregnancy. Brain natriuretic peptide (BNP) and troponin are likely to be elevated in PPCM, whereas they are usually within normal range during a healthy pregnancy or after delivery. BNP and troponin would not, however, separate heart failure related to hypertension, preeclampsia, eclampsia, or other conditions. Novel biomarkers have been proposed; as mentioned above, circulating levels of miR-146a are elevated in women with PPCM, and miR-146a may be useful to differentiate PPCM from other types of heart failure. However, further study is necessary and there is currently no clinical laboratory testing that confirms the diagnosis of PPCM. Given the recent discoveries regarding the proposed vasculohormonal pathways to PPCM, novel biomarker development may be a possibility in the future.

Electrocardiogram (ECG) findings are nonspecific and can range from relatively normal to abnormal, showing sinus tachycardia, T-wave changes, P-wave abnormalities, QRS-axis deviation, or a bundle branch block (Tibazarwa et al., 2012). Since PPCM occurs most often in healthy women, there is rarely a previous ECG available for comparison, making interpretation of acute changes more challenging.

Imaging Studies

Imaging is likely to be most useful in the diagnostic process. A chest X-ray will often show signs of pulmonary congestion and may also reveal cardiomegaly. Echocardiography is useful to evaluate ventricular function in addition to associated abnormalities such as left ventricular dilatation, right ventricular and biatrial enlargement, associated valvular dysfunction, thrombi, pericardial effusion, and pulmonary hypertension (Hibbard et al., 1999). Lastly, an echocardiogram can help to differentiate between other cardiac diagnoses such as myocardial infarction and Takotsubo cardiomyopathy.

The role of cardiac magnetic resonance imaging is yet undefined, but may provide further information regarding myocardial structure, and can also be used when echocardiographic images lead to uncertainty (**Figs. 1** and **2**). Noncontrast MRI can be safely performed in pregnant women; however, gadolinium crosses the placenta and therefore should not be administered (Bouabdallaoui et al., 2015). Contrast agents can be used safely in the postpartum period, with only small amounts being found in breast milk.

Management

Obstetric Considerations

Cases of PPCM occurring during the third trimester require consideration of the fetus as well as the safety of the delivery process. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are contraindicated. Beta-1 selective agents are preferred when using beta blockers to avoid impacting uterine activity. Diuretic therapy may be necessary but must be administered carefully to avoid impacting perfusion of the placenta. There is no evidence to suggest that delivery should be planned early or by caesarian section. Decisions should involve a multidisciplinary team of obstetricians, cardiologists, and neonatologists.

Medical Therapies

Available therapies for PPCM are largely similar to the staples used for other types of systolic heart failure; no studies specifically address these agents in PPCM. Beta-blockers are considered safe, and should be initiated in hemodynamically stable patients and titrated as tolerated for cardioprotection. ACE or ARB agents should be initiated in the postpartum period; enalapril and captopril are preferable during breastfeeding (Bouabdallaoui et al., 2015; Guidelines, 2010). Diuretic therapy is used for volume control; mineralocorticoid receptor antagonists are recommended as part of this regimen, particularly if the patient is not breastfeeding.



Fig. 1 Cardiac magnetic resonance (CMR) delayed enhancement image in the coronal plane, showing diffuse patchy mid-myocardial fibrosis in a patient with PPCM.

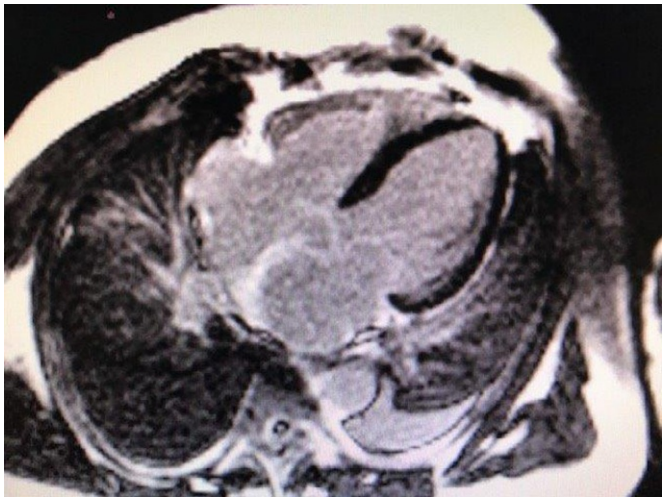

Fig. 2 A CMR delayed enhancement image in the horizontal plane for the same patient pictured in **Fig. 1**

No studies exist to establish the appropriate length of therapy with these agents, but a beta blocker and ACE or ARB (and mineralocorticoid antagonists, if used) should generally be continued for at least 6 months after full recovery of LV structure and function, and weaned off carefully thereafter if desired (Hilfiker-Kleiner et al., 2015). However, many experts recommend continuing therapy for at least 12 months, and in patients with persistently reduced LV function, therapy may be lifelong.

Ivabradine, which blocks f-channels (also called "funny" channels) and has been used successfully in other types of cardiomyopathies, has been used to reduce heart rate in patients with PPCM who cannot tolerate maximal beta blockade due to low blood pressures. A recent retrospective study of 20 patients from the German PPCM Study showed significant improvements in LVEF with ivabradine, used in addition to standard heart failure therapies and bromocriptine (Haghikia et al., 2016).

New and Experimental Therapies

Several targeted therapies are currently being studied for the treatment of PPCM in addition to optimized heart failure therapies. As discussed earlier, prolactin is thought to play a role in the imbalance of oxidative stresses and angiogenesis leading to PPCM. In mouse models, the administration of bromocriptine, which blocks prolactin secretion from the pituitary gland, prevented and even reversed PPCM (Hilfiker-Kleiner et al., 2007). Early studies have shown benefit in human patients as well; one small randomized trial of 20 African women has shown significant benefit of bromocriptine on LV recovery in 6 months (Sliwa et al., 2010a). Larger studies in Europe are ongoing. The benefits of bromocriptine must be weighed against the risks, however; there is a dose-dependent risk of thromboembolic phenomena associated with its use, and consideration of concomitant anticoagulation is strongly encouraged. Adverse events such as myocardial infarction and cerebral ischemia have been reported with its use (Fett, 2008). In addition, bromocriptine use is not compatible with lactation, and the loss of benefits to the infant must be considered, particularly in areas of the world where breast milk is the infant's sole source of nutrition.

Pentoxifylline, a TNFα inhibitor, has also been studied as a possible agent for treatment of PPCM. Thus far, one small study using historical controls has been conducted, which did show improved outcomes in patients treated with pentoxifylline (Sliwa et al., 2002). Further studies are needed with larger sample sizes. Levosimendan, an inotropic agent utilizing calcium sensitization, has been examined for use in PPCM causing cardiogenic shock. Catecholaminergic agents such as dobutamine are less favorable in PPCM than in other causes of acutely decompensated heart failure, which may be related to differences in STAT3 expression (Stapel et al., 2017). Levosimendan does not increase myocardial oxygen demand and may therefore be advantageous. However, studies thus far have showed conflicting results (Biteker et al., 2011; Labbene et al., 2016).

Invasive Therapies

Some women with severe PPCM may require mechanical cardiovascular or circulatory support during the acute phase of illness in cases when inotropic agents are insufficient. Many device options exist. Percutaneously delivered devices, such as intraaortic balloon pumps (IABP) or Impella devices, offer temporary augmentation of cardiac output without requiring open surgery and can be used for patients with adequate oxygenation. Percutaneously delivered devices may also serve as a bridge to more invasive options for support. Extracorporeal membrane oxygenation (ECMO) can be used in the setting of cardiogenic shock with inadequate tissue oxygenation, as a bridge to recovery or to left ventricular assist device (LVAD) placement. LVAD implantation is most appropriately used as a bridge to cardiac transplantation; in a recent study, only 4% of women with PPCM who received LVAD support were able to be weaned and explanted due to recovery, whereas 48% were ultimately transplanted (Loyaga-Rendon et al., 2014). Nearly a quarter of women with PPCM require transplantation, and PPCM accounts for 5% of heart transplants performed in women in the United States (Rasmusson et al., 2012).

Complications and Associated Conditions

Thromboembolism

PPCM is associated with a higher risk of thromboembolic phenomenon than other types of cardiomyopathies, likely owing to the hypercoagulable state of the peripartum period. Presentations of PPCM associated with pulmonary embolism, left ventricular thrombus, and even coronary emboli have been reported (Elkayam, 2011). Thromboembolism was identified as the most common serious complication of PPCM in one database (Kolte et al., 2014). Therefore, anticoagulation should be considered when potential benefits outweigh risks, particularly during pregnancy, and in the first 1–2 months postpartum (Arany and Elkayam, 2016; Bouabdallaoui et al., 2015; Elkayam, 2011). Heparin and its low-molecular-weight varietal are preferred. Vitamin K antagonists, such as warfarin, are known teratogens in the first trimester; although theoretically safe later in pregnancy, difficulties with reversal during delivery make them less desirable agents. Other therapies such as newer oral anticoagulants (NOACs) and synthetic heparins have not been sufficiently studied in pregnant women.

Arrhythmias

PPCM patients are at increased risk of sudden death due to ventricular arrhythmias. However, due to the high rates of recovery of LV function in PPCM, early placement of implantable cardioverter defibrillators (ICDs) is not recommended. Instead, a wearable cardioverter/defibrillator (WCD) should be considered in women with severe LV dysfunction as a bridge to recovery or permanent ICD placement. In a small German study including seven women with WCDs observed over a median follow-up period of 81 days, four events of ventricular fibrillation with successful shocks were recorded (Duncker et al., 2014). Retrospective data from 107 women with PPCM who required a WCD showed that only 20% received a permanent ICD after a 4-month study period (Saltzberg et al., 2012).

Prognosis

In general, the prognosis for patients with PPCM is quite good, particularly when compared to other types of cardiomyopathies. The Investigations of Pregnancy Associated Cardiomyopathy (IPAC) study, recently completed in the United States, included 100 women with 1 year of follow-up. During that period, 71% of women recovered (defined as EF >50%), generally by 6 months. 13%

had persistent cardiomyopathy with EF <35% or experienced major cardiovascular events, and 4% died (McNamara et al., 2015). Older studies both within and outside of the United States have reported recovery rates ranging widely between 20% and 60%, with mortality rates of 0%–28% (Arany and Elkayam, 2016; Bouabdallaoui et al., 2015).

Prognostic indicators include the degree of LV dysfunction and remodeling at the time of presentation, as well as troponin and BNP levels. Higher LVEF at presentation (LVEF >30%) and a lesser degree of LV remodeling (LV end diastolic diameter, LVEDD <55 mm) predicted a greater rate of recovery in several studies (Duran et al., 2008; Goland et al., 2011; Haghikia et al., 2013; Li et al., 2016; Safirstein et al., 2012). In the IPAC study, 90% of women who presented with LVEF >30% recovered (McNamara et al., 2015). Lower levels of troponin and BNP at the time of presentation and throughout the clinical course have also been associated with eventual LVEF recovery (Hu et al., 2007; Li et al., 2016).

African women, both in the United States and within their countries of origin, tend to fare worse than their Caucasian counterparts. LVEF at presentation is often lower in blacks than in whites (Goland et al., 2013; McNamara et al., 2015); however, outcomes have also been shown to be worse in African-American patients even when their LVEF is similar to Caucasians on presentation (Goland et al., 2013). Causes for this discrepancy are unclear, but may reflect genetic differences, which may also explain the increased incidence in this group. Socioeconomic factors likely play a role as well.

Many women with PPCM desire subsequent pregnancies; however, they are at significant risk of adverse results, particularly those who have not achieved complete recovery of LV function. A recent review of 191 pregnancies in women with a history of PPCM revealed a recurrence in 27% of women who had normal LV function prior to the subsequent pregnancy, with no reported deaths. By contrast, 48% of women who had persistent LV dysfunction worsened with the subsequent pregnancy, with 16% mortality (Elkayam, 2014). All women with a history of PPCM should be educated about the risks of future pregnancies, and contraception should be recommended, particularly in the first 6–12 months when LV recovery is ongoing.

Summary

PPCM is an important cause of cardiovascular morbidity and mortality in women, particularly those of African descent. The pathogenesis is thought to be related to an imbalance between oxidative stresses and factors promoting angiogenesis, but studies are ongoing to improve our understanding of this condition and its risk factors. Techniques for diagnosis and management are similar to those utilized for other causes of cardiomyopathy and heart failure, although a higher degree of suspicion is required to recognize PPCM in the healthy young women who are affected. Most women have a positive prognosis, though outcomes can be severe in a minority of patients. Studies providing evidence for new targeted therapies are needed.

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Peripheral Arterial Disease

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Introduction

Herein, we define peripheral arterial disease (PAD) as significant obstructive atherosclerosis in the arterial system of the lower extremities. Representative clinical manifestations of PAD include intermittent claudication and tissue loss of the feet or toes. Both are typically due to the extensive accumulation of atherosclerotic plaques that lead to tissue ischemia and, in the case of tissue loss, necrosis. In some cases, arterial revascularization is performed to restore adequate oxygen supply distal to the obstruction.

The flow-limiting atherosclerosis in the lower extremities of patients with PAD results in a pressure drop distal to the luminal obstruction. Foregoing a diagnosis of subclavian stenosis, this pressure drop results in a lower systolic blood pressure (BP) in the lower extremity relative to the systolic BP in the upper extremity. The ratio of the lower extremity to upper extremity systolic BP is referred to as the ankle-brachial index (ABI). As it is indicative of significant atherosclerotic disease in the lower extremity [or extremities], an abnormal ABI is typically considered diagnostic for PAD. Importantly, an abnormal ABI may be present in the absence of associated symptomatology, while approximately 30% of those with intermittent claudication have an abnormal ABI (Criqui et al., 1985, 1996). In this way, the ABI may be viewed as a measure of subclinical cardiovascular disease (CVD).

Burden of PAD

Globally, and based on 2010 figures, it has been estimated that just over 200 million individuals have PAD (Fowkes et al., 2013). Community-based studies in the United States have estimated the prevalence of PAD to be about 8 million individuals (Allison et al., 2007). PAD is rare in those under age 50, but the prevalence increases rapidly by approximately doubling with every decade increase in age. Moreover, there are significant differences in the prevalence by race or ethnic background. Specifically, African-Americans have been shown to have the highest rates while non-Hispanic Whites and those of Eastern Asian background (i.e., Chinese-Americans) have the lowest (Allison et al., 2007).

Beyond the morbidity associated with lower extremity ischemia, a diagnosis of PAD is important since individuals with this condition have an increased risk for incident total and cardiovascular mortality when compared to those without PAD (see the following text). This, coupled with the relatively high prevalence, makes PAD a significant public health problem. Accordingly, the ABI may be an appropriate candidate for screening of subclinical atherosclerosis to reduce the burden of not only PAD, but also other CVD morbidity and mortality.

The Ankle-Brachial Index

In persons without lower extremity atherosclerosis, and because of increasing impedance associated with taper in the diameter of the arterial system distally, arterial pressures increase with greater distance from the heart. This phenomenon results in higher systolic pressures at the ankle compared to the brachial arteries in persons without PAD.

The ABI is defined as the ratio of Doppler-recorded systolic pressures in the lower to upper extremities that utilizes the increasing distal pressure in the legs to identify significant obstructive disease. The ABI is a simple, noninvasive tool to assess the presence and extent of atherosclerosis in the lower extremities. The methodology for obtaining the systolic BPs necessary to calculate the ABI is standard, but there are variations in how many measurements are obtained in each extremity and which artery is used in the ankles.

ABI Measurement Technique (Fig. 1)

The examination should be conducted in a quiet, warm, and comfortable room. Have the participant lie supine on an examination table. The head and heels must be at the same level. The entire head and both feet must be on the table and not overhanging as having the feet even slightly lower than the rest of the body will produce an invalid ABI measurement.

Have the participant rest quietly for at least 5 min before beginning the measurement. Place an appropriately sized BP cuff around both arms, based on arm circumference at the midpoint. The cuff width must be at least 40% of the arm circumference. The three cuff sizes should be employed as follows:

1. Adult (12 cm width) cuff for an arm circumference of <32 cm
2. Large adult (16 cm width) cuff for an arm circumference of 32–42 cm
3. Thigh (20 cm width) cuff for an arm circumference of ≥ 43 cm

Place an adult (12 cm) cuff size on each ankle. Place the cuff so that the lower portion rests 3 cm above the greatest protuberance of the medial malleolus. All four cuffs should be in place before the first systolic BP is taken.

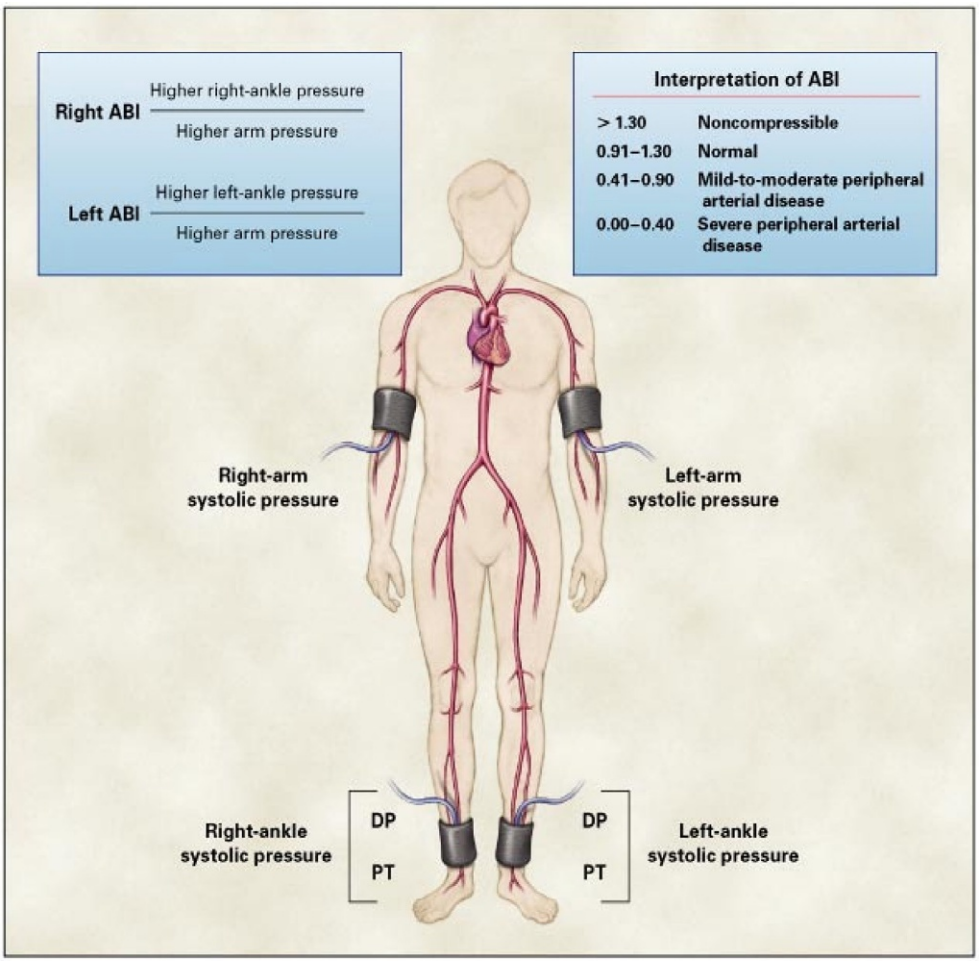

Fig. 1 Method for determining the ankle-brachial index (Hiatt, 2001).

By palpation, locate the brachial artery on both arms as well as the dorsalis pedis and posterior tibial arteries on both legs. Mark the location of each artery. Sometimes, an ankle pulse will not be palpable but can be found with the Doppler probe. Place ultrasound conducting gel over the end of the Doppler. After palpating the location of the pulse, turn on the Doppler and place the probe over the artery. Place the probe in line with the artery, and move it from side to side until the strongest pulse is heard. Inflate the cuff to 20 mmHg above the last audible pulse sound. If the pulse cannot be obliterated, raise the pressure to a maximum of 300 mmHg or to maximum patient tolerance. If not obliterated at this point, the artery is considered "noncompressible." Deflate the cuff slowly allowing the pressure to drop at a rate of 2 mmHg/s. Record the pressure at which the first sustained (more than one beat) pulse reappears. This is the systolic pressure at this location. Deflate the cuff completely. Using the procedure just described, systolic BPs are obtained in the following locations:

1. Right and left brachial arteries
2. Right and left posterior tibial arteries
3. Right and left dorsalis pedis arteries

It is important to record if the pressure in an artery is not detectable or noncompressible as these values will need to be considered when computing the ABI value.

Different Ways of Calculating the ABI

The ABI can be calculated for each lower extremity artery by dividing the systolic pressure measured in the dorsalis pedis and posterior tibial arteries in each limb by the brachial artery pressure. The traditional method of ABI calculation is to use the highest arterial pressure in each limb to calculate the ABI (Fowkes et al., 2013). However, prior studies have shown that the sensitivity of the ABI for diagnosing PAD varies according to how lower extremity arterial pressures are used to calculate the ABI. In one study in which 107 patients (208 limbs) underwent angiographic evaluation using digital subtraction angiography, investigators found that the sensitivity of the ABI for PAD (defined as ABI <0.90) varied from 69% when the highest arterial pressure in the ankle was used to 84% when the lowest arterial pressure in the ankle was used to calculate the ABI (Niazi et al., 2006). Similarly, the specificity of the ABI for a diagnosis of PAD varied from 83% when the highest arterial pressure at the ankle was used to 64% when the lowest arterial pressure at the ankle was used to calculate the ABI. In a separate study of 216 individuals who underwent arterial duplex ultrasonography, the sensitivity and specificity of the ABI for PAD (defined as ABI <0.90) were 89% and 63%, respectively, when the lowest arterial pressure was used to calculate the ABI (Schröder et al., 2006). The sensitivity and specificity of the ABI for PAD were 68% and 99%, respectively, when the highest arterial pressure was used to calculate the ABI (Aboyans et al., 2007).

Interpretation of the ABI

In individuals without manifest CVD, the mean ABI is about 1.13 (Aboyans et al., 2007). Traditionally, an ABI cut point of 0.90 or less has been considered evidence of significant atherosclerosis in the lower extremities and, therefore, PAD. Moreover, an ABI of 0.90 or less has been consistently shown to be associated with prevalent and incident CVD morbidity and mortality (see the following text). There is also evidence that as the ABI value decreases below this cut point, the more severe the atherosclerosis and the more sensitive the ABI is for detecting CVD. However, studies have challenged the use of the 0.90 cut point as the best discriminator of abnormal from normal (Aboyans et al., 2007; Allison et al., 2006; McDermott et al., 2005). These studies suggest that it may be more appropriate to consider the entire range of the ABI values. For example, several studies have now demonstrated that those with an ABI between 0.90 and 1.00 have higher levels of several CVD risk factors and greater subclinical atherosclerosis in other vascular beds compared to those with an ABI above 1.00 (Aboyans et al., 2007; Allison et al., 2006). There is also evidence that those with an ABI between 1.0 and 1.1 have higher levels of carotid intimal medial thickness (McDermott et al., 2005). Finally, when the ABI is analyzed as a continuous variable, those individuals with no CVD risk factors have values closest to 1.13 (Aboyans et al., 2007).

To further substantiate the use of the entire range of ABI values, research has revealed that higher (>1.30 or 1.40) values may be clinically relevant. An ABI value greater than 1.30 is significantly associated with higher levels of coronary artery calcium, diabetes, and higher body mass index (Resnick et al., 2004). Similarly, an ABI >1.40 is associated with a significantly worse quality of life (QOL) on several scales, higher rates of CVD comorbidities, and a higher risk for incident CVD (Resnick et al., 2004; O'Hare et al., 2006). These "high ABI" values represent "stiff" arteries in the lower extremities that may be due to medial calcification that is often present in patients with diabetes mellitus or chronic kidney disease. Importantly, there is a nonperfect overlap between arterial stiffness and atherosclerosis, which begs the question of whether these high ABI values should be classified as PAD or not because they may be masking significant underlying obstructive disease.

PAD and Clinical Outcomes

A wide range of adverse outcomes have been reported in persons with PAD including cognitive decline or bone fracture (Hyde et al., 2013; Laukka et al., 2014). However, in this section, we focus on two types of outcomes that are particularly relevant to patients with PAD and have robust evidence, cardiovascular and limb outcomes.

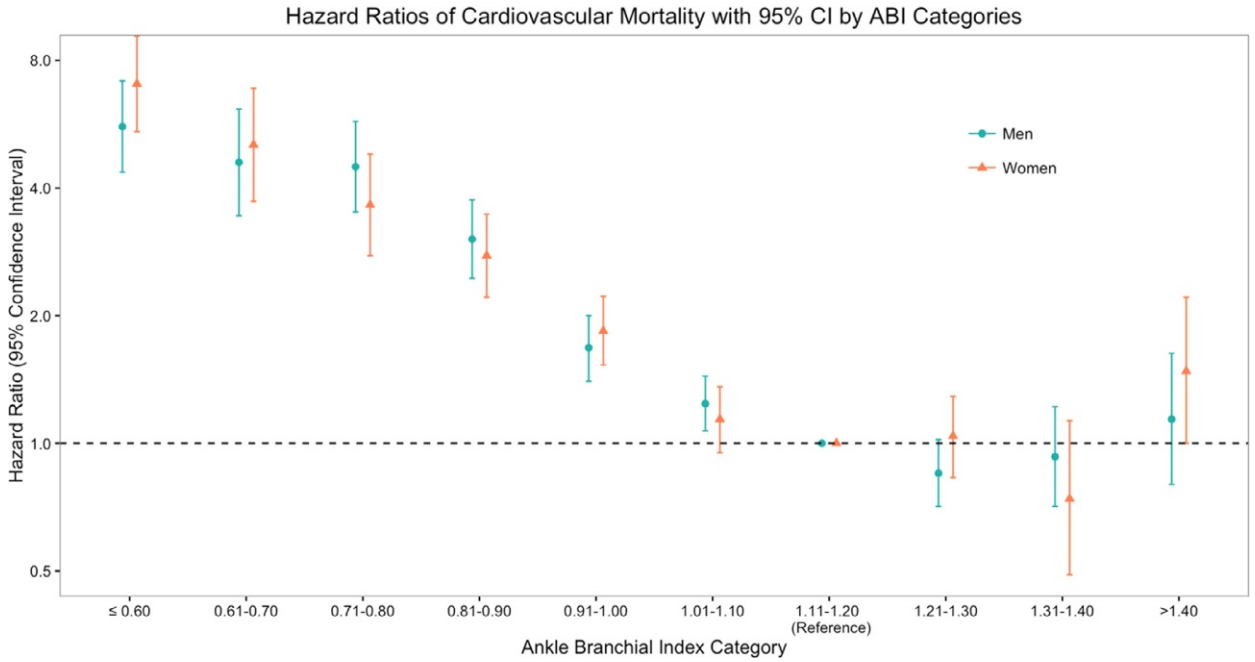

Fig. 2 Crude hazard ratios of cardiovascular mortality according to ABI categories by sex. Modified from Fowkes, F. G. R., Murray, G. D., Butcher, I., et al. (2008). Ankle brachial index combined with Framingham Risk Score to predict cardiovascular events and mortality: A meta-analysis. *JAMA: The Journal of the American Medical Association* **300**(2), 197–208.

PAD and Cardiovascular Outcomes

Persons with PAD have a higher mortality risk compared to those without PAD, mainly due to high short-term risk of cardiac disease and stroke (Criqui et al., 1992). Indeed, the Ankle-Brachial Index Collaboration demonstrated that a low resting ABI is associated with CVD mortality in a graded fashion, with three- to sevenfold increased risk below the ABI $\leq$0.9 compared to the reference ABI of 1.1–1.2 (**Fig. 2**) (Fowkes et al., 2008). Of note, an ABI of 0.9–1.0, the category currently considered borderline (Aboyans et al., 2012), also conferred 70%–80% increased risk of CVD mortality. Importantly, asymptomatic patients with PAD have been reported to have a similar CVD risk as symptomatic PAD patients (Leng et al., 1996).

A low ABI has been associated with myocardial infarction, stroke, heart failure, and atrial fibrillation (Griffin et al., 2016; Gupta et al., 2014; Heald et al., 2006). Therefore, patients with PAD require a comprehensive approach to prevent and manage CVD. Indeed, the American Heart Association (AHA) and American College of Cardiology (ACC) 2016 PAD guideline recommends statin therapy (class I for all PAD patients) and antiplatelet therapy (class I if symptomatic and IIa if asymptomatic) in patients with PAD (more details in the section of **Medical Treatment and Walking Regimen**) (Gerhard-Herman et al., 2017a).

Given its strong association with CVD risk, the AHA/ACC Guideline on the Assessment of Cardiovascular Risk lists ABI along with C-reactive protein and coronary artery calcium as additional biomarkers to be used to refine CVD risk prediction (Goff et al., 2014). Of these nontraditional biomarkers, coronary artery calcium appears to significantly improve the CVD risk prediction beyond traditional risk factors (Blaha et al., 2016; Matsushita et al., 2015). However, the ABI could still serve as a key noninvasive biomarker without radiation exposure (Goff et al., 2014; Kramer et al., 2013). Also, the ABI will uniquely provide information of lower extremity stenosis, which will inform healthcare providers of risk for limb outcomes.

PAD and Limb-Related Outcomes

Critical limb ischemia (CLI) is the most severe manifestation of PAD and is characterized by ischemic nonhealing wounds, tissue loss, or rest pain (Varu et al., 2010). It is estimated that ~1% of PAD patients have CLI as an initial manifestation of PAD, and additional 1%–2% of PAD patients develop CLI in 5 years after the diagnosis of PAD (Hirsch et al., 2006a). Although only a part of PAD patients develop CLI, it is a devastating condition, with ~25% of mortality risk and ~30% of leg amputation risk within 1 year after its diagnosis (**Fig. 3**).

When the limb cannot be salvaged, patients will require limb amputation. Although the rate of leg amputation has decreased by 40% from 1996 and 2011 in the United States (Goodney et al., 2015), approximately 150,000 leg amputations due to PAD are still performed annually in the United States (Hirsch and Duval, 2013). Notably, the dramatic decline of leg amputations is mainly for major amputations such as above-knee amputation, and the rate of forefoot or toe amputations remains similar between 1996 and 2011 (Goodney et al., 2015).

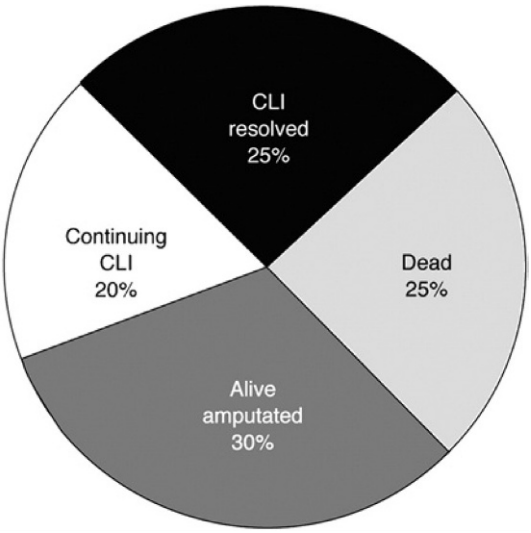

Fig. 3 One-year outcomes of patients presenting with CLI. From *Journal of Vascular Surgery* 2007; **45**(Suppl S), S5–67).

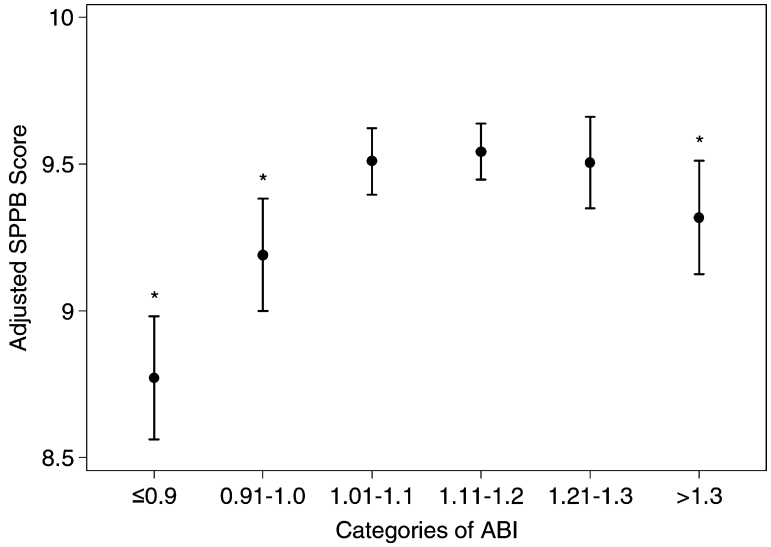

Fig. 4 Adjusted (Adjusted for age, race, sex, education, smoking, alcohol, body mass index, antihypertensive drugs, systolic blood pressure, lipid-lowering drugs, total and hdl cholesterols, diabetes, and history of coronary disease, heart failure, and stroke.) short physical performance battery (SPPB) score according to ABI categories. *Statistical significance with ABI 1.11–1.20 as the reference. From Matsushita, K., Ballew, S. H., Sang, Y., et al. (2017). Ankle-brachial index and physical function in older individuals: The Atherosclerosis Risk in Communities (ARIC) study. *Atherosclerosis* **257**, 208–215.

PAD and Physical Function

Reduced physical function (e.g., slower walking speed) was initially described in patients with PAD referred to vascular laboratory, compared to controls without PAD (Mcdermott et al., 1998, 2001; McDermott et al., 2004). Reduced physical function was identified even in PAD patients without leg symptoms. Recently, two studies have demonstrated a dose–response relationship between lower ABI and reduced physical function among community-dwelling older adults without clinical diagnosis of PAD (Fig. 4). Since this etiology of functional impairment can be improved by several evidence-based therapies (e.g., supervised exercise (Bendermacher et al., 2007) and cilostazol(Regensteiner et al., 2002) [detailed in **Medical Treatment and Walking Regimen**]), identification of PAD as a potential cause of physical dysfunction can have therapeutic implications.

Skeletal muscle atrophy, regenerating muscle fibers, muscle inflammation, and signs of denervation have been reported in the diseased legs of PAD patients (particularly when severe) (Hedberg et al., 1989; Regensteiner et al., 1993), which are considered probable pathophysiologic mechanisms behind reduced physical function. Of note, a recent study has demonstrated the association of low ABI with reduced grip strength (Matsushita et al., 2017). So, it is likely that both leg ischemia due to PAD and systemic atherosclerosis play a role in reduced physical function among patients with PAD.

PAD and QOL

As anticipated from its contribution to physical function and leg pain, several studies have reported reduced health-related QOL in persons with PAD (Inglis et al., 2013; Korhonen et al., 2012; Regensteiner et al., 2008). Most studies consistently showed more evident reduction in physical aspects of QOL (e.g., physical functioning) rather than mental aspects of QOL (e.g., social functioning) in the context of PAD (**Fig. 5**) (Inglis et al., 2013; Korhonen et al., 2012; Regensteiner et al., 2008). The PARTNERS study, a national, community-based PAD detection program, compared PAD with other CVDs (e.g., coronary heart disease and stroke), with respect to their impact on QOL, and reported that PAD may influence QOL similarly as other CVDs (Regensteiner et al., 2008). These findings highlight the importance of multidisciplinary and comprehensive management for patients with PAD.

Medical Treatment and Walking Regimen

Evaluation of the patient with PAD will often start with the primary care physician (PCP). The PCP may start a patient on a walking regimen with medical treatment following sufficient vascular exam and noninvasive studies. The patients suitable for this include those with lifestyle-limiting vascular claudication with abnormal vascular lab testing.

Prior to treatment of the disease itself, PCPs need to also focus on minimizing the risk of concomitant CVD. This is done by thoroughly evaluating the patient's risks factors for coronary artery disease. Antiplatelet therapy, smoking cessation, lipid-lowering therapy, treatment of diabetes and hypertension are central in minimizing CVD risk (Gerhard-Herman et al., 2017b; Alonso-Coello et al., 2012; Society for Vascular Surgery Lower Extremity Guidelines Writing Group et al., 2015). Antiplatelet therapy is particularly helpful in patients with PAD in preventing cardiovascular events (Antithrombotic Trialists' (ATT) Collaboration et al., 2009; Antithrombotic Trialists' Collaboration, 2002; Antiplatelet Trialists' Collaboration, 1994).

Cilostazol, a phosphodiesterase inhibitor that suppresses platelet aggregation and acts as an arterial vasodilator, may also improve the symptoms of claudication (Reilly and Mohler, 2001). This medication should be used carefully in this patient population as many have insufficient cardiopulmonary reserve. Many studies have shown improvement in maximal walking distance (MWD) and pain-free walking distance (PFWD) with cilostazol, as compared to placebo (Bedenis et al., 2014; Thompson et al., 2002; Pande et al., 2010). Other medications under investigation include Naftidrofuryl which is currently available in Europe. Mechanisms of this drug appear to be promoting glucose uptake and increasing adenosine triphosphate levels, and it appears to have equal or greater improvements in PFWD than cilostazol or pentoxifylline (Stevens et al., 2012).

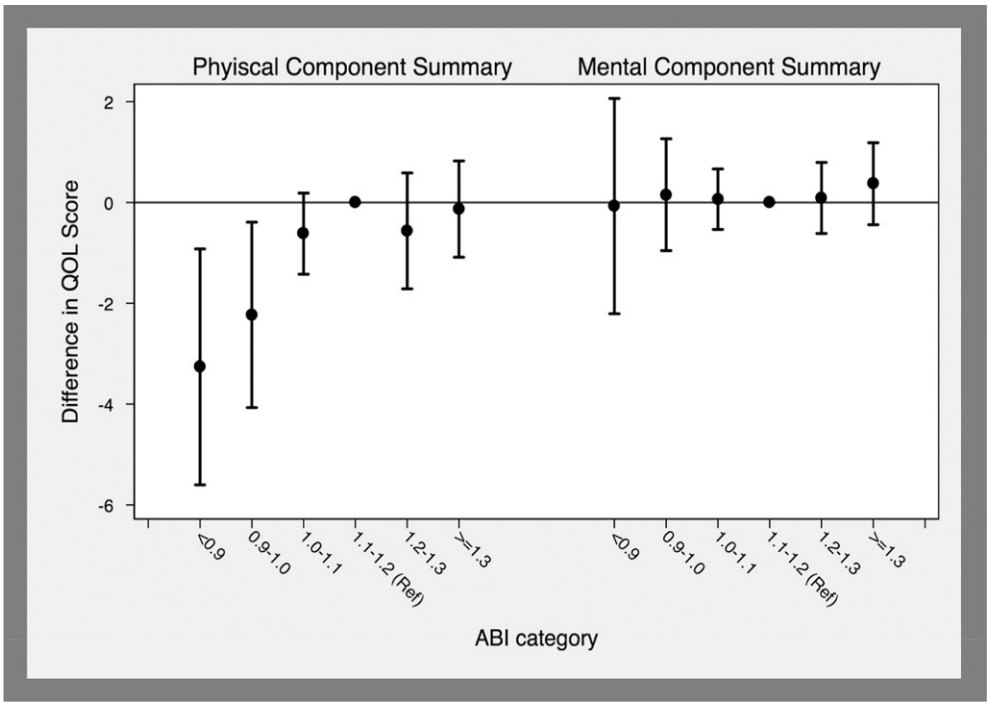

Fig. 5 Adjusted (Adjusted for age, sex, ethnicity, education level, economic status, current smoking/drinking status, body mass index, total cholesterol level, diabetes mellitus, hypertension, history of coronary heart disease, heart failure, stroke, lung disease, and reduced kidney function.) difference in QOL scores (based on SF-12) according to ABI. Modified from *Journal of the American Heart Association* **2017**(6), E004519.

Supervised exercise programs are largely underutilized despite evidence of their benefit. Issues remain regarding the best regimen to help in rehabilitation from claudication symptoms (Fakhry et al., 2012; Gardner et al., 2012). Consistent characteristics among all programs are as follows:

- Supervision by exercise physiologist, physical therapist, or nurse.
- Sessions lasting 45–60 min
- Use of a treadmill
- Three times a week or more
- Total duration of 12 weeks or more

Walking programs appear to double MWD and PFWD when maintained. However, when the program terminates many patients will return to their baseline symptomatology (Hirsch et al., 2006b). Comparing the types of exercise between walking, cycling, and upper extremity devices, there was found to be no difference in the MWD and PFWD improvements (Lauret et al., 2014). Optimal intensity within the regimen is also a matter of debate as many programs have been evaluated with longer duration and less intensity with similar results (Gardner et al., 2005). Other programs, including intermittent compression of the calf, have improved claudication distance (de Haro et al., 2010).

Surgical Intervention

Surgical intervention for PAD, and especially for CLI, has been studied for almost 100 years (Menzoian et al., 2011). Current guidelines are to treat occlusions from proximal to distal, if they coexist. Groups are generally unanimous in recommending intervention for PAD when a patient has rest pain or tissue loss when the ABI is less than 0.8. Another recommendation is to perform inline reconstructions of proximal disease whenever possible (Hirsch et al., 2006b; 2011 Writing Group Members et al., 2011).

Inflow Disease

Open surgery for inflow disease is still the gold standard and, for many, the preferred method of treating symptomatic aortoiliac occlusive disease (AIOD) in low-risk patients. The usual repair is an aorto-bifemoral bypass with prosthetic graft. The perioperative mortality rates are under 5% for severe claudication but 30%–40% for CLI.

Patency rates for open surgical interventions for AIOD are 88% at 5 years and 75% at 10 years. The potential risks of open surgery include graft infection which threaten outcomes including patency and survival (Kieffer et al., 2001). Outcomes for patients undergoing repair for CLI are worse than lifestyle-limiting claudication with 5- and 10-year secondary patencies of 63% and 49%, respectively (Harris et al., 1998; Poulias et al., 1992). Reconstruction methods vary with approaches that are trans-abdominal or retroperitoneal, and reconstructions with ePTFE, Dacron, cryopreserved graft, or varying anastomosis (**Fig. 6**).

Other surgical approaches of reconstruction vary the location of inflow for the femoral vessels. Axillobifemoral bypass offers a reasonable repair to high-risk patients, as there is no clamping of the aorta, entrance into the abdominal cavity, and potential to be done under local anesthesia with cumulative patency of roughly 70% at 5 years in favorable anatomy or similar outcomes in the long term (McPhee et al., 2016; Passman et al., 1996). Other options include femoral to femoral bypass grafting in patients with unilateral iliac occlusive disease or originating the bypass graft from the thoracic aorta.

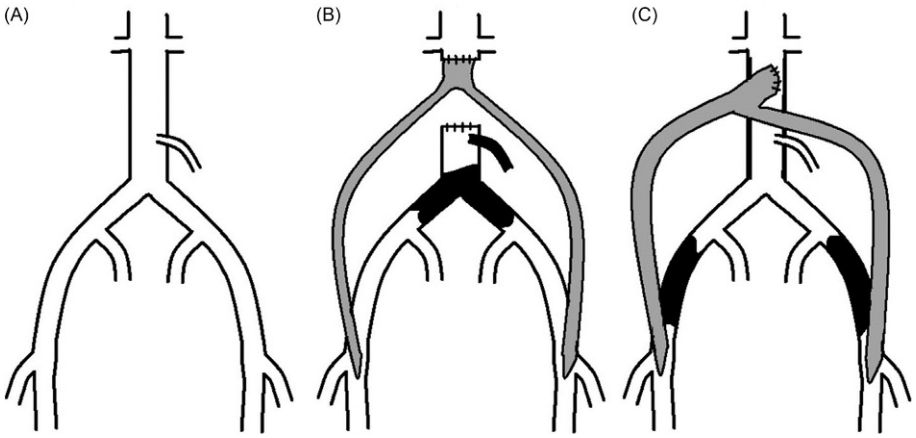

Fig. 6 Reconstruction of AOID with normal vasculature (A), end-to-end proximal reconstruction (B), and end-to-side proximal reconstruction (C) (Gerhard-Herman et al., 2017b).

Graft choice is another opportunity to introduce variability amongst patient outcomes and repair performance. The choice graft for first-time repairs includes PTFE or Dacron with minimal differences in outcomes or complication rates (Davidovic et al., 2004). Formation of a graft from femoral deep or superficial veins or cryopreserved grafts are alternative options, especially in patient with infection of previously placed grafts (Clagett et al., 1993; Fatima et al., 2013).

Infrainguinal Disease

There are several vascular tenants to guide revascularization procedures in the infrainguinal location. For intermittent claudication of the calf, endovascular procedures are usually the intervention of choice. These lesions are often amenable to endovascular intervention, but, when they are not, they require a similar approach, as if it were for CLI. The ideal surgical intervention has normal inflow, normal outflow, and optimal conduit. In most patients, there is some compromise of one of these factors. Many patients with CLI have limited or compromised outflow or other conduits besides autologous vein.

Bypasses often originate from the femoral artery, as it is the closest open proximal vessel to an occlusion with less than 20% stenosis (Hirsch et al., 2006b; 2011 Writing Group Members et al., 2011). Targets vary from above-knee popliteal artery and below-knee popliteal artery to tibial vessels. Bypasses are named by convention with the origin of the bypass, the target, and the conduit used (e.g., fem-peroneal bypass with vein).

The measures of both infrainguinal surgical and endovascular interventions are based on different types of patency. Primary patency is if there is uninterrupted flow without a procedure needed to manage occlusive disease in the conduit or in an adjacent native vessel. Primary assisted patency refers to continuous flow while needing revision either by open surgical revision or endovascular repair prior to occlusion of the conduit. Secondary patency refers to resurrection of a bypass conduit following thrombosis or occlusion from another cause (e.g., neointimal hyperplasia). Other common endpoints for surgical bypass include systemic complications such as myocardial infarction, stroke, respiratory failure, and renal failure. Disease-specific endpoints include the above-mentioned patencies, failure of wound healing, and limb loss (amputation).

In general, shorter bypasses do better than longer bypasses, and bypasses to larger vessels do better than those with smaller caliber target vessels. Superior conduit performance is in the following order: autologous single-segment vein > autologous multiple-segment vein > PTFE > cryopreserved graft. Many studies have also been performed attempting to analyze other aspects of bypass including location of bypass tunnel, orientation of bypass vein, type of PTFE, type of anastomosis with vein or PTFE, and inclusion of surgically created fistula. Level 1 recommendations for surgical intervention are in **Table 1** (2011 Writing Group Members et al., 2011). A summary of randomized trials for surgical intervention is included in **Table 2** (Ziegler et al., 2011).

Endovascular Intervention

Percutaneous in endovascular intervention is a less invasive method at correcting PAD both for AOID and infrainguinal disease. This involves obtaining wire access across an occlusive lesion and treating it with one or multiple devices. There are several devices/treatment modalities approved by the FDA including:

- Subintimal angioplasty
  - Covered stents
  - Coiled stents—e.g., Supera
  - Brachytherapy—e.g., XRT

Table 1 Level 1 recommendations for treatment of critical limb ischemia according to the ACC/AHA guideline summary (Gupta et al., 2014)

Class I—There is evidence and/or general agreement for the following approach to the management of critical limb ischemia
● Rapid evaluation and treatment of factors known to increase the risk of primary amputation. These include significant necrosis of weight-bearing parts of the foot in ambulatory patients, an uncorrectable flexion contracture, paresis of the extremity, ischemic rest pain, sepsis, or limited life expectancy due to comorbid disease.
● The development of acute limb symptoms in patients at risk for CLI (those with diabetes, neuropathy, chronic renal failure, or infection) is a potential vascular emergency that should be assessed immediately and treated by a specialist in vascular disease.
● Prompt initiation of systemic antibiotics in patients with skin ulceration and evidence of limb infection.
● Evaluation for aneurysmal disease (e.g., in the abdominal aorta or popliteal or common femoral artery) in patients with evidence of atheroembolization, such as blue toe syndrome or livedo reticularis.
● Catheter-based thrombolysis for acute limb ischemia (Rutherford category I or IIa) of less than 14-day duration.
● Preoperative estimation of cardiovascular risk if open surgical repair is anticipated.
● When endovascular treatment or surgery is performed for combined inflow and outflow disease, the inflow lesions should be addressed first. If symptoms or infection persist after inflow revascularization, an outflow revascularization should be performed.
● If it is uncertain if there is hemodynamically significant inflow disease, intra-arterial pressure should be measured before and after administration of a vasodilator.
● Patients with skin breakdown should be referred to a healthcare provider with expertise in wound care.
● Patients who have been successfully treated for CLI are at relatively high risk for recurrence. They should be evaluated at least twice yearly by a vascular specialist and be given verbal and written instructions for self-surveillance for recurrence.

Table 2 Aggregate outcomes for surgical infrainguinal revascularization procedures (Goodney et al., 2015)

	Primary patency (%)				*Limb salvage (%)*				*Survival (%)*			
Bypass level	*1 month*	*1 year*	*3 years*	*5 years*	*1 month*	*1 year*	*3 years*	*5 years*	*1 month*	*1 year*	*3 years*	*5 y ears*
Femoropopliteal (above knee)												
Vein	96	86	79	75	94	82	81	80	x	x	78	62
Synthetic	96	80	58	53	94	86	79	72	x	86	81	70
Femoropopliteal (below knee)												
Vein	93	86	83	71	92	85	81	78	x	x	74	68
Synthetic	78	74	54	44	87	74	71	63	x	x	x	42
Femorotibial/femorocrural												
Vein	93	82	72	69	93	82	72	69	x	85	69	60
Synthetic	88	60	40	24	88	60	40	24	x	74	55	40
Pedal/ankle												
Vein	93	74	64	59	93	74	64	59	98	89	65	63

- Drug-eluting stents—e.g., Zilver PTX
- Cryoballoon—e.g., PolarCath
- Laser atherectomy—e.g., Eximer laser
- Directional atherectomy—e.g., SilverHawk catheter
- Rotational atherectomy—e.g., CSI, Rotablator
- Drug-coated balloons
- Absorbable stents

The treatment of AIOD with percutaneous means has been gaining popularity both with patients, as there are smaller incisions and morbidity, and with practitioners as well. One recent summary found that morbidity, mortality, and outcomes rival those of traditional open repair with 4/5-year primary and secondary patency rates ranging from 60% to 86% and 80% to 98%, respectively (Jongkind et al., 2010). There may also be a patency advantage in this patient population when using covered stents versus uncovered stents likely due to the resistance to neointimal hyperplasis (Mwipatayi et al., 2016; Piazza et al., 2015).

The BASIL trial was the first randomized controlled trial evaluating endovascular approaches to CLI to open surgical approaches. In summary, the data found less short-term morbidity in angioplasty with selective stenting to open surgery. Long-term outcomes supported open surgery first approaches to CLI, especially when patient outcomes were evaluated beyond 2 years (Adam et al., 2005). Other trials have analyzed other datasets for which type of approach leads to improved outcomes. Wiseman et al. found when evaluating 5928 patients following intervention for infrainguinal PAD that an endovascular first approach is associated with improved amputation-free survival over the long term with only a modest relative increased risk of subsequent intervention (Wiseman et al., 2017). Further adjuncts to balloon angioplasty and selective stenting in large series have failed to show a clear advantage such as atherectomy (Ambler et al., 2014; Dattilo et al., 2014; Diamantopoulos and Katsanos, 2014), but other technologies such as drug-coated balloon angioplasty and interwoven stents are showing some advantages in the short term, but long-term data has yet to be collected (Kayssi et al., 2016; Katsanos et al., 2016; Steiner et al., 2016).

Summary

PAD is a relatively common condition that ranges clinically from being asymptomatic to significant pain in the lower extremities. PAD is typically associated with an increased risk for incident CVD and some loss of physical function. In more extreme cases, amputation at some level in the leg may be required. There are several medical and surgical therapies that can be employed, which have been demonstrated to be effective in improving PFWD and/or preventing limb loss.

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Pharmacogenomics of Antiarrhythmic Drug Therapy for Atrial Fibrillation

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Introduction

Individuals vary widely in their response to therapy with most cardiovascular drugs. In some patients, drugs are highly effective and cause few or no side effects. In others, the same drug is highly ineffective and produces serious side effects. Antiarrhythmic drugs (AADs) used to treat cardiac arrhythmias are no exception. In fact, variability in membrane-active drug action is so marked that study of the underlying mechanisms has provided important insights into variable responses in general. The sources of inter-individual variability in drug response have traditionally been classified as "pharmacokinetic" and "pharmacodynamic" with the former referring to variability in the conventional measures of drug disposition such as absorption, distribution, metabolism, and elimination, and the latter reflecting the relationship between the drug concentration and its effect. Pharmacokinetic and pharmacodynamic variability as it relates to AAD therapy has extensively been reviewed previously (Darbar and Roden, 2006; Roden et al., 2009). This monograph will outline the challenges and opportunities presented in applying contemporary genetic approaches to better understanding variability in response to AAD therapy for cardiac arrhythmias.

Atrial Fibrillation

Atrial fibrillation (AF), the most common sustained cardiac arrhythmia requiring therapy, is associated with increased risk of stroke, heart failure, myocardial infarction, and death. The global burden of AF was recently estimated at ~33.5 million with progressive increase in incidence, prevalence, and AF-related mortality having major implications for healthcare costs and public health policy (Chugh et al., 2001). The socioeconomic impacts of AF are substantial, as are the consequence of repeated hospital admissions, chronic disease management, and its resultant disabilities. Furthermore, AF remains one of the most challenging cardiac arrhythmias to manage in clinical practice.

Most patients with AF have concomitant conditions such as hypertension that have been associated with the arrhythmia. In a minority, AF occurs in the absence of underlying structural abnormalities and is traditionally termed "lone" but now more correctly categorized as early-onset AF (Kirchhof et al., 2013). A family history of the arrhythmia is common especially in those with early-onset AF (Darbar et al., 2003). Over the last decade, a number of newer risk factors have been identified including race, obstructive sleep apnea, metabolic syndrome, and chemotherapeutic agents (Darbar and Roden, 2013). One setting in which AF is especially common is in patients who have undergone cardiac surgery, where the incidence can be as high 40% in the first week after surgery. Studies suggest that even postoperative AF may also have a genetic basis (Kolek et al., 2015).

Clinical presentations in AF are highly heterogeneous. AF results in loss of atrial activity, with rapid ventricular rates due to conduction of atrial fibrillatory activity down the atrioventricular node (AVN). Therapies are aimed at restoring and maintaining sinus rhythm (rhythm control) or slowing down conduction over the AVN (rate control). Currently, the drug management of AF in

 https://doi.org/10.1016/B978-0-12-809657-4.99763-1

clinical practice varies considerably due to the lack of reliably effective therapies for maintaining sinus rhythm, the toxicities associated with AADs, and the evidence that, on average, rate and rhythm control are approximately equivalent strategies (Wyse et al., 2002). However, a rhythm control strategy is necessary in a large percentage of patients because of intolerable symptoms while in AF and may be appropriate for broader use since patients in the AFFIRM trial had better outcomes if sinus rhythm was achieved and maintained (Corley et al., 2004). Without the ability to match an individual patient with the treatment option most likely to be successful, the current strategy for a trial of AAD therapy focuses primarily not on efficacy, but on the side-effect profile of available medications. In patients who are highly symptomatic with their AF, a rate-control strategy is often ineffective. In such individuals, a trial of AADs is undertaken before consideration of nonpharmacologic, catheter-based ablative therapies. The highly variable clinical presentations of AF including variable and unpredictable responses to drugs and other therapies, unclear etiologies, variable associations with underlying heart disease, and familial aggregation, all support a potential role for genetic variants in the etiology of the arrhythmia as well as in variable response to drugs.

The last decade has witnessed tremendous progress in our understanding the genetic basis of AF. Diverse genetic approaches including linkage analyses and candidate gene approaches as well as next-generation sequencing have identified mutations encoding cardiac ion channels, transcription factors and signaling proteins linked to early-onset familial AF. Conversely, large population-based studies have uncovered common single-nucleotide polymorphisms (SNPs) that modulate AF risk with the strongest association at the chromosome (chr) 4q25 locus (Darbar and Roden, 2013).

Genetic Approaches to AF

One reason for the lack of effective therapies for AF stems from incomplete understanding of the complex pathophysiology of the AF. One approach to unraveling the molecular pathogenesis of AF is through the identification of genes linked with familial early-onset forms of the arrhythmia (Fig. 1).

Evidence for Heritability of AF

Evidence for a genetic contribution to the development of AF was first provided in 1943 by Wolff (Wolff, 1943) who documented transmission of AF in a family with an autosomal dominant pattern of inheritance. Since then, many epidemiologic studies have documented heritability (~62%) in AF especially early-onset AF (Darbar et al., 2003; Fox et al., 2004; Arnar et al., 2006).

Linkage Studies

Several genes and loci have been identified for rare Mendelian forms of early-onset AF (Brugada et al., 1997; Ellinor et al., 2003; Schott et al., 2004; Oberti et al., 2004; Volders et al., 2007). We recently identified a novel AF locus on chromosome 5p15 and established an abnormally prolonged P-wave determined as an "endophenotype" in a family with early-onset AF (Darbar et al., 2002). Linkage analyses have facilitated the identification of individual mutations in several familial AF kindreds. In 2003, the first gene (*KCNQ1*, which encodes a cardiac K^+ channel) responsible for a rare autosomal dominant form of AF was identified (Chen et al., 2003). This discovery was followed by studies that implicated genes encoding additional ion channels and other proteins in the pathogenesis of familial AF. For example, we identified a novel heterozygous frameshift mutation in the natriuretic peptide precursor A (*NPPA*) gene that encodes atrial natriuretic peptide (Hodgson-Zingman et al., 2008). Variations in diverse proteins are

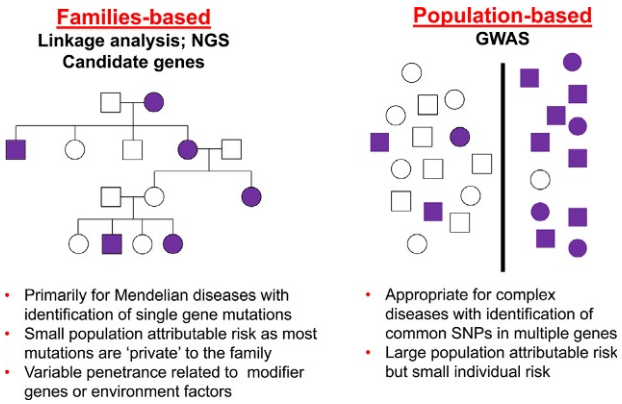

Fig. 1 Genetic approaches to atrial fibrillation (AF). Linkage analysis, next-generation sequencing (NGS) and candidate gene approaches have identified mutations in single genes in large families with early-onset AF. Conversely, genome-wide association studies (GWAS) have uncovered common genetic variants associated with AF in many genes. (Filled in symbols indicate individuals with AF and unfilled symbols represent controls.)

thought to predispose to these uncommon early-onset forms of AF through different mechanisms, a notion that emphasizes the heterogeneity that governs AF initiation and maintenance.

Candidate Gene Studies

Based on the work relating *KCNQ1* to familial AF, investigators considered other potassium and ion channels as potential candidate genes and screened for mutations in AF patients and their families; several rare variants were identified. Otway et al. (2007) examined 50 kindreds with AF and identified a single mutation (R14C) in *KCNQ1* in one family. Although the R14C mutation had no effect on KCNQ1/KCNE1 current amplitudes in cultured cells at baseline, upon exposure to hypotonic solution, mutant channels exhibited a marked increase in currents compared with wild-type channels. Interestingly, only those patients with left atrial dilatation had AF. These findings support the concept of a *double-hit* hypothesis (i.e., the combination of a genetic variant with acquired risk factors, e.g., structural remodeling, is important for AF pathogenesis). A recent study showed that common AF-susceptibility variants on chr4q25 modify the clinical expression of cardiac ion channel and signaling proteins associated with early-onset familial AF—further supporting the double-hit concept (Ritchie et al., 2012).

Association Studies

Most patients with AF have concomitant comorbid conditions such as hypertension or structural heart disease. However, many patients with the same risk factors do not develop the arrhythmia. Thus, a working hypothesis is that genetic determinants increase AF susceptibility in some individuals with identifiable risk factors—a basis for the "double-hit" hypothesis. Initially, association studies comparing AF cases to controls typically tested a small number of variants in candidate genes previously believed to be involved in AF pathogenesis. However, recent advances in next-generation sequencing have made it possible to assay hundreds of thousands of SNPs over the entire human genome and thus made GWAS possible. In 2007, a locus on chromosome 4q25 was identified in a GWAS as having a highly significant association with AF with a relative risk ranging from 1.39 to 1.72 (**Fig. 2**) (Gudbjartsson et al., 2007). Although the mechanism for this observed association remains unknown, the locus is adjacent to the paired-like homeodomain transcription factor 2 (*PITX2*) gene which is critical for left-right cardiac differentiation and development of the pulmonary myocardium. Nine additional AF loci and five novel AF genes were identified when a metaanalysis of AF GWAS was recently conducted (Ellinor et al., 2012; Sinner et al., 2014).

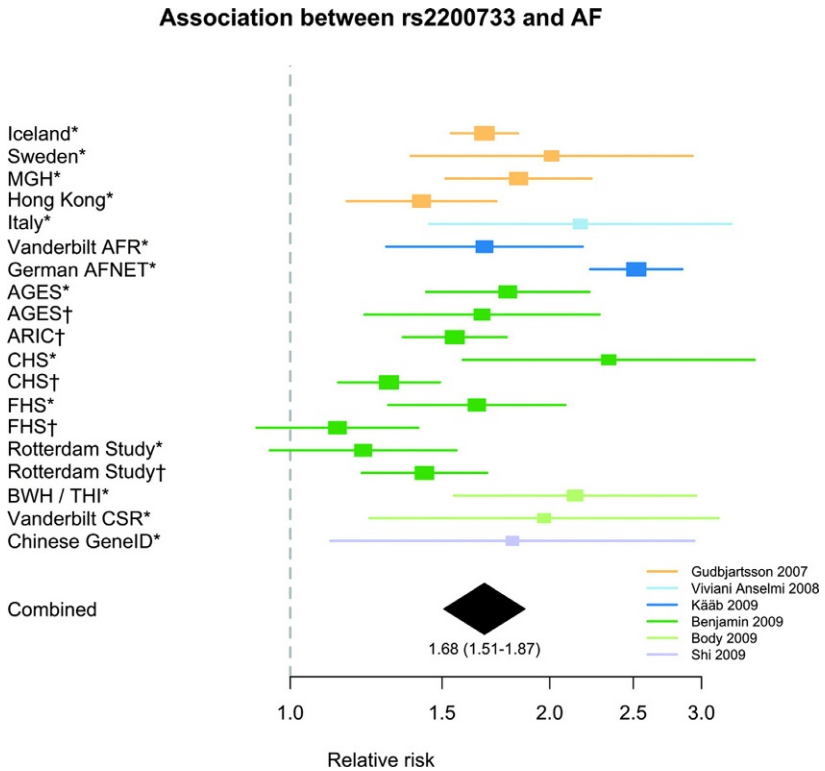

Fig. 2 Replication of a chromosome 4q25 single-nucleotide polymorphism (SNP) (rs22200733) associated with AF in multiple cohorts. Reproduced with permission from Wolters Kluwer Health Inc. Lubitz et al. Circulation: Arrhythmia and Electrophysiology, Genetics of Atrial Fibrillation 3, 291–299 (http://circep.ahajournals.org/).

Impact of Genetic Studies of AF

Insights Into Genetic Mechanisms

Genetic approaches to AF have provided important insights into the underlying mechanisms of the arrhythmia. One mechanism by which gain-of-function mutations in genes encoding cardiac potassium, sodium, and calcium channels may lead to AF is reduction in atrial refractoriness creating a substrate for reentrant arrhythmias (Darbar and Roden, 2013). We identified a novel mutation in the gene (*KCNA5*) that encodes for the ultra-rapid potassium current (I_{Kur}) in a family with early-onset AF (Yang et al., 2010). While *NPPA* mutations may also mediate increased risk for AF by modulating the atrial action potential duration (APD), a transgenic mouse model overexpressing the humanized *NPPA* mutation suggests that other mechanisms may also be involved (Savio-Galimberti et al., 2012). A second mechanism by which mutations in cardiac ion channels may predispose to AF is by lengthening of the atrial APD. A loss-of-function mutation in *KCNA5* lengthened the atrial APD and induced early afterdepolarizations in human atrial myocytes (Olson et al., 2006; Yang et al., 2009; Christophersen et al., 2013). A third mechanism involves modulating expression of gap junction proteins important for cell-to-cell communication. Both somatic and germ-like mutations in *GJA5*, encoding connexin-40, have been associated with early-onset AF (Firouzi et al., 2004; Gollob et al., 2006).

Common Genetic Variants Modulate Pulmonary Vein Development

Although the strongest signal consistently associated with AF in GWAS is located at the chr4q25 locus, the precise mechanisms by which these SNPs modulate AF risk remain unclear (Gudbjartsson et al., 2007). The closest gene to the chr4q25 locus is *PITX2* with the "c" isoform implicated in pulmonary vein development. There is emerging evidence that chr4q25 SNPs likely code for a regulatory element that modulates transcription of *PITX2c* (Lubitz et al., 2010; Nobrega et al., 2003; Pennacchio et al., 2006) and signaling pathways that regulate the electrophysiologic substrate responsible for AF by shortening the atrial APD in heterozygous mice that are more prone to inducible AF (Kirchhof et al., 2011). Atrial fibrosis may also play a critical role in the pathogenesis of AF. $Pitx2c^{-/-}$ knock-out mice not only developed 4-chamber cardiac enlargement but also had histological evidence of atrial fibrosis with upregulation of collagen precursor genes (Mommersteeg et al., 2007). In contrast, $Pitx2c^{+/-}$ heterozygous mice show no evidence of cardiac enlargement, but there is increased expression of genes regulating Wnt signaling, a key fibrosis pathway (Kirchhof et al., 2011; Darbar and Roden, 2013; Disertori et al., 2013). However, both the $Pitx2c^{-/-}$ knock-out and heterozygous mice have easily inducible AF. While these studies support a critical role of the pulmonary veins in triggering AF (Haissaguerre et al., 1998), functional studies of PITX2c suggest that modulation of PV development may be one common pathway by which chr4q25 SNPs increase susceptibility to AF.

The Need for a More Rational Approach to AAD Therapy for AF

Despite recent advances in catheter-based and surgical therapies, AADs remain the mainstay of treatment for most patients with symptomatic AF (January et al., 2014). However, response in an individual is highly variable with over 50% of patients treated with AADs having a recurrence of AF over a 6–12 month period. The limited success of therapy is in part related to poor understanding of the underlying mechanisms of AF, heterogeneity of the electrical substrate, and our inability to target mechanism-based therapies in individual patients. In addition, contemporary membrane-active drugs used to suppress AF carry significant risks of serious adverse drug events including drug-induced long QT syndrome, other forms of proarrhythmia, increased mortality, and extracardiac toxicity. Furthermore, this current "one-size fits all" approach to selecting AAD therapy for a patient with symptomatic AF is based largely on minimizing the risk of adverse events rather than on the likelihood of efficacy. There is emerging evidence that variability in response to antiarrhythmic therapy is modulated by common genetic variants associated with AF (Darbar and Roden, 2013).

Genomic Variation Determines Response to AF Therapies

We have used the Vanderbilt and University of Illinois at Chicago (UIC) AF Registries that now include baseline and follow-up clinical information and biosamples in over 3000 subjects with AF and family members to not only identify new common and rare variants that confer increased susceptibility to AF but have also enabled testing of the hypothesis that common SNPs modulate response to drug and catheter-based therapies to control rate or to maintain sinus rhythm (Darbar and Roden, 2013).

Symptomatic Response to AADs Is Modulated by a Common AF Susceptibility Allele

In a retrospective study, Kolek et al. (Parvez et al., 2012) determined whether symptomatic response to AAD therapy is modulated by the three common AF susceptibility loci on chr4q25 (near *PITX2*), 16q22 (in *ZFHX3*), and 1q21 (in *KCNN3*). The discovery cohort consisted of 478 patients with a validation cohort of 198 that were age and gender matched white patients of European American descent. Response was defined as successful rhythm control if the patient remained on the same AAD for a minimum of 6 months with $\geq$75% reduction in AF symptoms. Multiple clinical variables (including age, hypertension, early-onset AF) failed to

Table 1 Response distribution of antiarrhythmic drugs by class among responders for chromosome 4q25 SNP rs10033464

Discovery cohort			
Drug class	*Wild type (n = 309)*	*Minor allele carriers (n = 90)*	P *value*
Class 1	87 (28%)	37 (41%)	0.02
Class III	222 (72%)	53 (59%)	0.02
Validation cohort			
Drug class	*Wild type (n = 97)*	*Minor allele carriers (n = 46)*	P *value*
Class 1	37 (39%)	30 (67%)	0.01
Class III	60 (61%)	16 (34%)	0.02

predict response to AADs. However, a SNP at the 4q25 locus (rs10033464) was significantly associated with successful symptom control (odds ratio (OR) 2.97, 95% confidence interval (CI) 1.42–6.21, $P = 0.003$). Furthermore, individuals who carried the chr4q25 SNP responded better to Class I versus Class III AADs in both the discovery and validation cohorts (**Table 1**). These findings suggest that a common AF risk allele differentially regulates response to Class I versus Class III AADs in patients with frequent symptomatic AF.

The underlying mechanism(s) by which a chr4q25 AF risk allele modulates response to AADs is not well understood (Parvez et al., 2012). However, measurement of left atrial mRNA levels in 95 patients undergoing thoracoscopic AF ablation and assessment of the effects of flecainide and sotalol in *Pitx2c* mice have provided important insights Syeda et al. (2016) not only showed that variable *PITX2* expression modulates atrial resting membrane potential but confirmed our earlier clinical observation that sodium channel blockers are more effective at suppressing AF than potassium channel blockers (**Table 1**) (Parvez et al., 2012). However, it is important to note that our data and the recent study are based on the assumption that chr4q25 SNPs modify *PITX2* expression. However, this association has not definitively been proven. Nonetheless, even if the presumed link between *PITX2* expression and chr25 SNPs remains elusive, these observations clearly show variability in response to AADs in patients with AF based on chr4q25 genotype.

Common AF Risk Alleles at chr4q25 Predict Rafter Catheter-Based AF Ablation and Electrical Cardioversion

To test the hypothesis that 4q25 AF risk alleles can predict response to AF ablation, we studied 378 patients who underwent catheter-based AF ablations (median age 60 years; 71% men; 89% typical AF) between 2004 and 2011. The primary end point was time to recurrence of any nonsinus atrial tachyarrhythmia (atrial tachycardia, atrial flutter, or AF). There were 200 arrhythmia recurrences (53%) and in multivariable analysis, the rs2200733 risk allele predicted a 24% shorter recurrence-free time (survival time ratio 0.76; 95% confidence interval (CI) 0.6–0.95; $P = 0.016$) compared with the reference allele. The heterozygous haplotype demonstrated a 21% shorter recurrence-free time (survival time ratio 0.79; 95% CI 0.62–0.99) and the homozygous risk allele carriers a 39% shorter recurrence-free time (survival time ratio 0.61; 95% CI 0.37–1.0; $P = 0.037$). The second 4q25 SNP (rs10033464) did not affect recurrence-free survival time. In exploratory analyses, the 1q21 rs13376333 risk allele was found to confer a 22% shorter recurrence free time (median survival ratio = 0.78, 95% CI 0.62–0.97, $P = 0.026$) and the 16q22 rs7193343 risk allele did not. Kolek et al. showed that the chr4q25 AF risk SNP is also an independent predictor of AF recurrence after restoration of sinus rhythm after direct current cardioversion (Parvez et al., 2013).

Translation of Genetic Discoveries to the Bedside

Genetic approaches have not only provided important insights into the underlying mechanisms of AF but also identified genetic subtypes with differential response to therapy (Darbar and Roden, 2013). However, the impact of these discoveries have thus far been limited given the lack of evidence-based data to support mechanism- and genotype-based treatment approaches; incomplete understanding of the underlying mechanisms associated with AF genetic variants; limitations of using noncardiac cell lines; animal models failing to recapitulate human cellular electrophysiology; and our inability to study functional atrial myocytes. While in vitro methods for assessing the role of ion channel variants in AF are well-established and have been used by many (Darbar et al., 2008; Abraham et al., 2010), cardiac myocytes have very distinct electrophysiologic properties and heterologous systems cannot capture the full spectrum of functional changes associated with AF-associated genetic variants. Importantly, this discrepancy between in vitro versus in vivo studies was recently highlighted when Vanderbilt investigators showed that an *SCN5A* mutation (D1275N), linked with cardiac conduction disease, dilated cardiomyopathy and arrhythmias when expressed in multiple heterologous expression systems, showed near-normal sodium channel function (Watanabe et al., 2011). However, expression in a mouse model was associated with conduction slowing, AF, and a dilated cardiomyopathy.

Transgenic murine models have provided important insights into the pathogenesis of AF. However, cardiac ion channels are highly species-specific. Hence, mouse models of AF may not reliably and precisely reproduce the human AF phenotype. Recently, we showed that overexpression of the humanized *NPPA* mutation in mice is associated with increased susceptibility to AF (Savio-Galimberti et al., 2012). However, the absence of atrial fibrosis in the mutant mice suggests that atrial structural remodeling does not play a major role in the pathogenesis of AF in this murine model. The limitations of heterologous expression systems and murine models of AF suggest that an alternative system is urgently needed to elucidate the underlying genetic mechanisms by which chr4q25 AF risk alleles modulate response to AAD therapy.

Challenges and Opportunities

AF Burden as an Endpoint to Evaluate Response to AF Therapies

Due to historical limitations in noninvasive ambulatory monitoring technology and lack of reliable algorithms to identify and provide quantitative summaries for specific arrhythmias, AF burden has in general not been used as a clinical or study end point. One widely used symptom-based metric is "time to first symptomatic AF episode," but this correlates poorly with frequency of symptomatic episodes (Savelieva and Camm, 2000). The unreliability of symptoms to estimate AF burden and to identify patients with and without AF have been pointed out by pacemaker trials and also in patients without implanted devices. However, recent technological advances that allow continuous monitoring of cardiac rhythm have now made it possible to rigorously define AF burden as a surrogate end point for AF therapy (Friedman et al., 2001; Israel et al., 2004). Measurement of AF burden includes symptomatic and asymptomatic episodes and may be a more robust end point for AF therapy because it is less subject to investigator bias and does not have the sampling error associated with episodic monitoring or reliance on patient symptoms (Euler and Friedman, 2003). Furthermore, AF burden is favored to become the metric of choice to assess to response to AADs and monitoring after AF ablation therapy (Calkins et al., 2012a). Insertable cardiac monitors (ICMs) are able to continuously monitor the heart through a single-lead ECG based on a specific algorithm that detects AF by evaluating the irregularity of R–R intervals (Euler and Friedman, 2003; Calkins et al., 2012b). These devices are able to correctly classify AF in 96.1% of patients and correctly exclude AF in 97.4% of subjects. The device is able to monitor the heart for up to 3 years.

Lack of randomized genotype-driven pharmacogenomic trials

One reason for the failure to translate genetic discoveries to the bedside care of patients with AF relates is due to the lack of genotype-directed prospective trials (Darbar and Roden, 2013). We showed that a common chr4q25 SNP associated with AF not only predicted successful symptom control in patients treated with AADs for AF but that individuals who carried the risk variant responded better to Class I (sodium channel blockers) versus Class III (potassium channel blockers) AADs (Parvez et al., 2012).

Based on this data and a recently published study confirming the differential response to sodium channel blockers based on reduced PITX2c expression in AF patients (Syeda et al., 2016) a prospective pharmacogenomic trial is being conducted where a cohort of patients with frequent symptomatic AF are randomized to flecainide (Class I) or sotalol (Class III) to determine if response to therapy is differentially regulated by a chr4q25 SNP using AF burden as a metric of drug efficacy (Fig. 3). This study utilizes a crossover design to minimize interindividual variability and maximize statistical power to detect an interaction between chr4q25 genotype and reduction in AF burden with flecainide versus sotalol. Subjects are monitored with an ICM to assess AF burden and will be asked to complete a comprehensive, validated 20-item AF specific questionnaire (AF Effect on QualiTy-of-life) (Spertus et al., 2011) at baseline, and monthly thereafter for the duration of the study. At the end of the 3-month trial period, the AAD is discontinued and patients are switched to the other AAD and followed for another 3-month period in a crossover trial design.

Summary

Over the last 15 years, tremendous advances have been made in defining the genetic basis of AF, identifying the underlying molecular mechanisms, and subtyping response to AAD and other therapies for AF. However, the translation of these discoveries

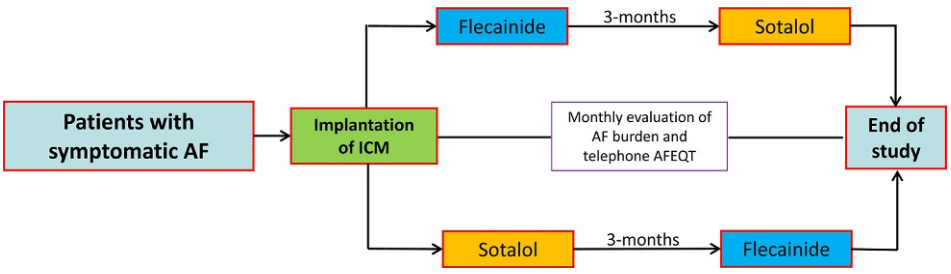

Fig. 3 A randomized sequential crossover study to evaluate if response to sotalol versus flecainide is modulated by chromosome 4q25 associated SNPs. *AFEQT*, AF-specific quality of life questionnaire; *ICM*, insertable cardiac monitor.

when it comes to the bedside management of an individual patient with AF has not yet been fully realized. This may in part relate to the retrospective and observational nature of the studies demonstrating that response to AADs, and other therapies are modulated by common genetic variants at the chr4q25 locus. The time for conducting appropriately-designed pharmacogenomic clinical trials that are genotype driven is upon us.

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Pharmacology of Medications Used in the Treatment of Atherosclerotic Cardiovascular Disease

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Atherosclerosis initially occurs asymptomatically, but can progress to cause symptoms of angina and may culminate in coronary thrombosis and acute myocardial infarction (MI). This article discusses the medications used in atherosclerotic cardiovascular disease, focusing on those used in the acute setting following MI, as well as those used in primary and secondary prevention of cardiovascular disease. For each medicine or class of medicines, their mechanism of action, pharmacokinetics, adverse effects, and interactions will be covered. Practical tips for clinical use will also be provided. Symptomatic management of stable angina, which aims to ease cardiac chest pain by reducing cardiac workload, is beyond the scope of this article.

Medication Used in the Treatment of Acute Coronary Syndromes

Preventing or minimizing irreversible myocardial ischemia and avoiding further thrombosis are important aims in the early treatment of MI. Drugs used to restore normal coronary flow will be discussed here. The immediate symptomatic treatment of suspected acute coronary syndrome (ACS), including opioid analgesia, antiemetics, and oxygen are beyond the scope of this article. Oral antiplatelets, which are used both in the acute and long-term management of cardiovascular disease, will be discussed as part of primary and secondary prevention.

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Pharmacological action	Mechanism of action	Adverse effects
Relief of pain associated with acute angina through: • Coronary artery dilation, reduced afterload and increased oxygen delivery to the myocardium. • Peripheral venodilation, reduced preload and reduced myocardial oxygen demand.	• Multistep conversion to nitric oxide in the vascular endothelium • Nitric oxide-mediated decrease in intracellular calcium causes smooth muscle relaxation	• Headache • Flushing • Hypotension • Dizziness • Tachyphylaxis and tolerance • Methaemoglobinaemia

Fig. 1 Summary of the pharmacological action, mechanism of action, and adverse effects of nitrates.

Nitrates

Nitrates can be administered intravenously as isosorbide dinitrate or glyceryl trinitrate (GTN) in the management of ACS. Sublingual GTN, in spray or tablet form, is also used to relieve acute episodes of angina. Longer acting formulations, such as GTN patch and isosorbide dinitrate and mononitrate tablets, are used to prevent angina. The focus will be on the use of sublingual and intravenous GTN in the acute setting. **Fig. 1** summarizes the pharmacological action, mechanism of action, and adverse effects of nitrates.

Mechanism of action

Nitrates reduce myocardial workload by causing systemic and coronary vasodilation. Coronary artery dilation reduces afterload, increases oxygen supply to the myocardium, and reduces coronary vasospasm. Dilation of collaterals allows blood to bypass narrowed coronary arteries to improve blood flow and oxygen delivery to the ischemic area (Matsunaga et al., 2000). Perhaps more importantly, peripheral venodilation reduces preload, which reduces left ventricular wall stress and subsequently myocardial oxygen demand (Abrams, 1985). In the vascular endothelium, nitrates undergo a multistep conversion to nitric oxide (NO). The activation of guanylate cyclase by NO increases the concentration of cyclic guanosine monophosphate, which mediates a decrease in intracellular calcium leading to smooth muscle relaxation (Jerca et al., 2002). When high doses of nitrates are administered, blood pressure is reduced, which further reduces myocardial wall stress and oxygen demand.

Pharmacokinetics

GTN is widely distributed in the tissues, particularly to red blood cells and vascular smooth muscle cells. The pharmacological effect is observed within 1–2 min of intravenous and 1–3 min of sublingual administration. It undergoes rapid biotransformation, principally by de-nitration in the liver to less active dinitrate and mononitrate metabolites. The duration of action following intravenous and sublingual administration is 3–5 min and 30–60 min, respectively (Bogaert, 1987).

Adverse effects

Flushing, headache, hypotension, and dizziness are common adverse effects directly related to vasodilation (Joint Formulary Committee, 2016). Nitrates should be avoided in patients with systolic blood pressure lower than 90 mmHg, or with marked brady- or tachycardia, due to the risk of hemodynamic compromise in patients with ischemic heart disease (Kushner et al., 2009). Patients with severe aortic stenosis are at high risk of profound hypotension, therefore nitrates should be avoided. Prolonged intravenous infusion of nitrates can rarely cause methemoglobinemia (Joint Formulary Committee, 2016).

An acute desensitization, termed tachyphylaxis, can occur with prolonged (>24 h) intravenous infusion of intermediate to high dose nitrates (Gruppo Italiano per to Studio della Soprawivenza nell'Infarto Miocardica, 1994); however, prolonged treatment is not usually indicated. Tolerance, a separate phenomenon, is associated with chronic use of longer acting nitrates for prophylaxis of angina. The mechanism by which tolerance develops has not been defined. Hypotheses include reduced conversion of nitrates to NO; reduced bioactivity of NO; or activation of renin–angiotensin–aldosterone-system (RAAS) induced vasoconstriction, which counters the effects of NO (Parker and Parker, 1998). Tolerance is prevented by dosing tablets asymmetrically or removing patches, to allow an 8 h nitrate-free period, usually overnight. It is unlikely to occur with sublingual GTN due to the intermittent nature of administration.

Drug interactions

Caution is required when administering nitrates with antihypertensives due to additive risk of hypotension. Phosphodiesterase type 5 inhibitors can exacerbate the vasodilatory action of nitrates and cause severe, life-threatening hypotension which contraindicates concurrent use (Joint Formulary Committee, 2016). Patients wishing to use both drugs must be provided information on appropriate timing of doses (see practical points).

Practical points

- 10 min sublingual GTN rule for patients—At the onset of angina: stop, sit down, and rest. If pain persists, use 1 spray or tablet and wait 5 min. If pain persists, repeat and wait 5 min. If pain persists, call for an ambulance and unlock your door.

Pharmacological action	Mechanism of action	Adverse effects
• Dissolution of clots and restoration of perfusion	• Catalysis of conversion of plasminogen to plasmin through proteolysis • Clot-selective fibrin degradation	• Haemorrhage • Hypotension • Arrhythmias • Embolisation • Hypersensitivity reactions (streptokinase)

Fig. 2 Summary of the pharmacological action, mechanism of action, and adverse effects of fibrinolytics.

- Sublingual GTN tablets are volatile; therefore they must be kept in their original container and have a reduced expiry once opened. Spray formulations avoid this.
- Phosphodiesterase type 5 inhibitors: patients should not use GTN within 24 h of sildenafil or vardenafil, and 48 h of tadalafil. If chest pain occurs following administration of a phosphodiesterase type 5 inhibitor and does not resolve with rest, an ambulance should be called immediately.

Fibrinolytics

Although largely replaced by primary percutaneous coronary intervention, thrombolytics can be used to dissolve clots and restore perfusion in patients with acute MI with ST elevation (Joint Formulary Committee, 2016). **Fig. 2** summarizes the pharmacological action, mechanism of action, and adverse effects of fibrinolytics.

Mechanism of action

Streptokinase is a protein extracted from streptococci cultures. It forms a complex with plasminogen, promoting the conversion of this inactive precursor molecule to the active plasmin, which degrades fibrin clots by proteolysis (Anderson and Willerson, 1993). It has largely been superseded by recombinant tissue plasminogen activators (tPAs), which have a more acceptable side effect profile.

tPA is a naturally occurring protease released from endothelial cells of occluded blood vessels. Pharmacological tPAs (alteplase, reteplase, and tenecteplase) are modified forms of tPA produced by recombinant DNA technology. They catalyze conversion of fibrin-bound plasminogen to plasmin, causing clot-selective degradation of fibrin (Collen and Lijnen, 2009).

Pharmacokinetics

Streptokinase and recombinant tPAs are catabolized to small peptides in the liver, which are cleared renally. Streptokinse and alteplase have short half-lives and must be administered by intravenous infusion. Reteplase and tenecteplase have longer half-lives due to reduced binding to hepatic receptors (Ulrich et al., 1992; Keyt et al., 1994). Reteplase can be administered as 2 intravenous bolus doses, 30 min apart, and tenecteplase as a single bolus dose (Joint Formulary Committee, 2016).

Adverse effects

The most common adverse effect of fibrinolytics is hemorrhage, ranging from minor bleeding to major gastro-intestinal (GI) bleed and hemorrhagic stroke. Fibrinolytics are contraindicated in active bleeding and in patients at a high risk of bleeding, for example, recent hemorrhage, trauma or surgery, recent history of hemorrhagic stroke, uncontrolled hypertension, coagulation defects, endocarditis, aortic dissection, and esophageal varices. Caution is required in severe hepatic disease due to associated coagulopathies.

Streptokinase, due to its biological origin, can cause fever, hypersensitivity reactions, and anaphylaxis. Antibodies blocking the action of streptokinase appear within 4 days of use, rendering repeated administration of streptokinase therapeutically ineffective and high risk for hypersensitivity. Recombinant tPAs do not stimulate an immune response and use can be repeated.

Other adverse effects include hypotension, arrhythmias and rarely, acute embolization due to break up of the targeted clot (Joint Formulary Committee, 2016).

Drug interactions

Although combination of fibrinolytics with antiplatelets or antithrombins has known therapeutic benefits, caution is required due to increased risk of bleeding. Other groups of medicines which increase bleeding risk, such as nonsteroidal antiinflammatory drugs (NSAIDs), should be avoided or used with caution (Joint Formulary Committee, 2016).

Practical points

- Hypotension may resolve on elevating the patient's legs. Alternatively the infusion rate can be reduced or temporarily stopped.
- Hypersensitivity reactions can be treated with antihistamines and corticosteroids. Adrenaline should be available when administering streptokinase, in case of anaphylaxis.
- Recombinant tPAs can be used in patients who are likely to have antibodies to streptokinase.

Antithrombins

Antithrombins reduce thrombus-related events by inhibiting thrombin generation at various points in the clotting cascade (see Fig. 3). They include unfractionated heparin (UFH), low molecular weight heparins (LMWH), fondaparinux, and bivalirudin. Fig. 4 summarizes the pharmacological action, mechanism of action, and adverse effects of antithrombins.

Heparin and fondaparinux

Mechanism of action

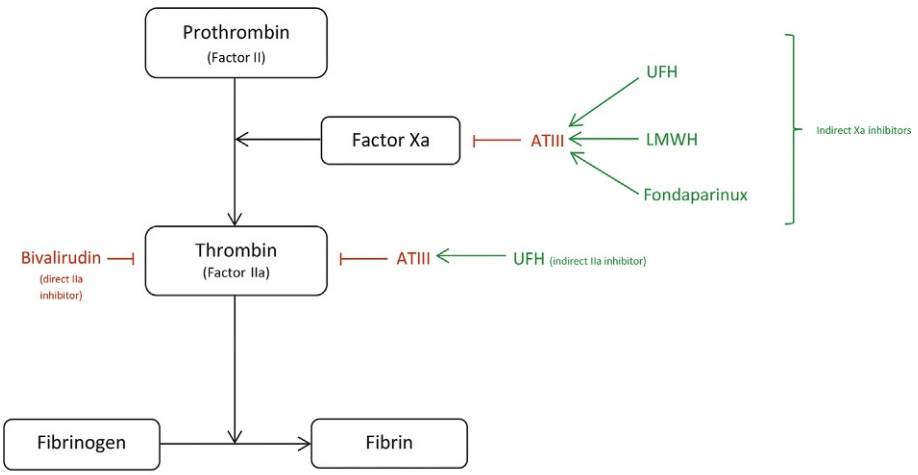
Antithrombin III inhibits clotting factors IIa (thrombin), Xa, and to a lesser extent IXa and XIIa. UFH and LMWH bind to antithrombin III via a pentasaccharide group, inducing a conformational change which enhances antithrombin-mediated inhibition of these clotting factors. Fondaparinux is a synthetic pentasaccharide, with a higher affinity for antithrombin III than UFH or LMWH. To accelerate thrombin inhibition, UFH must bind to thrombin itself, in addition to antithrombin III. LMWH and fondaparinux, which are too small to simultaneously bind antithrombin III and thrombin, selectively inhibit factor Xa, for which the additional binding is less crucial (Hirsh et al., 2001a).

Pharmacokinetics

The effect of UFH occurs immediately following intravenous, or 1–2 h following subcutaneous administration (Hirst et al., 2001b). Binding of heparin to plasma proteins and various tissues contributes to interindividual variability in its effect, necessitating dose adjustment according to regular monitoring of activated partial thromboplastin time (APTT) (Hirsh et al., 2001a). UFH is favorable when a rapid onset and offset of activity is required, for example, patients undergoing angioplasty.

LMWH (dalteparin, enoxaparin, and tinzaparin) and fondaparinux (synthetic pentasaccharide) are administered by subcutaneous injection. They have a greater bioavailability and longer duration of action compared with UFH, allowing once or twice daily dosing (Hirsh et al., 2001b, 2007). Additionally, the therapeutic effect is more predictable than UFH, owing to a smaller degree of binding to plasma proteins and tissues (Hirsh et al., 2001a). Unlike UFH, LMWH and fondaparinux do not require routine monitoring; however, antifactor Xa levels can be monitored.

Clearance of heparins is largely by renal excretion of metabolites and unchanged drug (Hirsh. et al., 2001b). Fondaparinux is excreted unchanged in the urine (Hirsh et al., 2007).



ATIII = antithrombin III; UFH = unfractionated heparin; LMWH = low molecular weight heparin

Fig. 3 Sites of action of antithrombins in the clotting cascade.

Pharmacological action	Mechanism of action	Adverse effects
• Reduction of thrombus related events by inhibiting thrombin or its generation	• Indirect inhibition of thrombin (UFH) or factor Xa (UFH, LMWH, fondaparinux) through potentiating antithrombin III; or direct inhibition of thrombin (bivalirudin)	All: • Haemorrhage UFH/LMWH • Hyperkalaemia • Heparin-induced thrombocytopenia • Osteoporosis (long term use)

Fig. 4 Summary of the pharmacological action, mechanism of action, and adverse effects of antithrombins.

Bivalirudin

Mechanism of action

Bivalirudin is a synthetic 20 amino-acid peptide analog of hirudin. It reversibly inhibits both soluble and clot-bound thrombin by binding both to the active site and at a second, remote binding site (Accord, 2016).

Pharmacokinetics

Bivalirudin, when administered as an intravenous bolus followed by infusion, has an immediate anticoagulant effect and an approximate half-life of 25 min. Its effect can be monitored using activated clotting time or APTT. It is cleared primarily by protease metabolism and renal excretion, such that the infusion rate must be adjusted for patients with severe renal impairment (Accord, 2016).

Adverse effects

Antithrombins increase the risk of hemorrhage and should not be used in patients who are actively bleeding. Extreme caution is required if heparins must be used in patients with a high risk of severe bleeding, including hemophilias, thrombocytopenia, severe hypertension, and recent high risk surgery. Protamine is a specific antidote, which reverses anticoagulation in patients who experience hemorrhage when treated with UFH. It can also be used to treat bleeding due to LMWH; however, it only partially neutralizes the antifactor Xa activity of LMWH. No specific antidotes exist for fondaparinux or bivalirudin.

UFH and LMWH can increase serum potassium; however, this is usually only important for patients with increased risk of hyperkalemia, for example in diabetes and renal impairment. Hypersensitivity can occur and osteoporosis is associated with long-term use (Joint Formulary Committee, 2016).

Heparin-induced thrombocytopenia (HIT) occurs when the immune system produces antibodies to the antigenic complex formed between heparin and platelet factor 4 (PF4). Binding of antibodies to this complex activates platelets, leading to hypercoaguability, characterized by a fall in platelet count and venous or arterial thrombosis (Linkins, 2015). HIT usually presents after 5–15 days of treatment with heparin, or sooner if the patient has recently received heparin. Baseline and regular monitoring of platelets should be performed. The formation of the PF4 complex is dependent upon the size of the heparin molecule; therefore, HIT is less likely to occur with LMWH (Hirsh et al., 2001b). Fondaparinux has a very low risk of causing HIT, due to its much reduced interaction with proteins and cells (Linkins, 2015). Bivalirudin is not implicated in HIT and may be used as a treatment (Linkins et al., 2012).

In severe renal impairment, fondaparinux is contraindicated and LMWH should be used cautiously. The infusion rate of bivalirudin should be reduced; however the standard bolus dose can be used. UFH may be preferred. Caution is required in severe liver disease due to the inherent increased risk of bleeding.

Drug interactions

Although combination of antithrombins with antiplatelets has therapeutic benefits, caution is required due to increased risk of bleeding. Other agents which increase bleeding risk, such as NSAIDs, should be avoided or used with caution (Joint Formulary Committee, 2016).

Practical points

- UFH and LMWH are porcine-derived. For patient groups where porcine-derived products may not be acceptable, for example, practicing Jewish and Muslim patients, fondaparinux or bivalirudin may provide an appropriate alternative
- Pharmacokinetics of LMWH may be less predictable in obesity, renal impairment, and pregnancy. Consideration to monitoring antifactor Xa levels should be given when treating with LMWH in these patient groups.

Intravenous Antiplatelets

Glycoprotein IIb/IIIa inhibitors

Mechanism of action

Activated platelets undergo a structural change in the active site of the glycoprotein IIb/IIIa (GP IIb/IIIa) receptor, creating a binding site for pro-aggregatory molecules, such as fibrinogen, von Willebrand factor, and prothrombin (Stangl and Lewis, 2010). Intravenously administered GP IIb/IIIa inhibitors block platelet activity at the final point of convergence of platelet activation pathways (Quinn et al., 2002). This has a theoretical advantage of inhibiting platelet activity irrespective of the agonist.

Abciximab is a monoclonal antibody fragment which noncompetitively, irreversibly inhibits GP IIb/IIIa receptors. Eptifibatide—a hexapeptide, and tirofiban—a nonpeptide, are selective, reversible GP IIb/IIIa antagonists (Warltier et al., 2002). **Fig. 5** summarizes the pharmacological action, mechanism of action, and adverse effects of GP IIb/IIIa inhibitors.

Pharmacokinetics

Abciximab has a high affinity for the GP IIb/IIIa receptor, resulting in a long duration of receptor blockade and short plasma half-life. Although platelet function is restored over 48 h, abciximab can remain in the circulation in its platelet-bound form for up to 15 days (Lily, 2013).

Pharmacological action	Mechanism of action	Adverse effects
• Prevention of blood clots and myocardial infarction by inhibiting platelet aggregation	• Inhibition of binding of pro-aggregatory molecules to platelets • Block at the final point of convergence of platelet activation pathways	• Haemorrhage • Thrombocytopenia • Pseudothrombocytopenia • Hypotension • Rash and hypersensitivity

Fig. 5 Summary of the pharmacological action, mechanism of action, and adverse effects of glycoprotein IIb/IIIa inhibitors.

Conversely, eptifibatide and tirofiban have lower affinity for the receptor, resulting in a shorter duration of blockade (Stangl and Lewis, 2010).

Adverse effects

Bleeding is the most common adverse effect of GP IIb/IIIa inhibitors and caution is required in patients at increased risk of hemorrhage (Stangl and Lewis, 2010). Thrombocytopenia has also been reported with all GP IIb/IIIa inhibitors, warranting platelet monitoring in the elderly and those with a low platelet count at baseline. Pseudothrombocytopenia can also occur, due to changes in the structure of the platelet membrane components (Warltier et al., 2002). Other side effects include hypotension, nausea, headache, fever, rash, and hypersensitivity (Joint Formulary Committee, 2016).

Eptifibatide and tirofiban are partially renally cleared and require dose adjustment in renal impairment (Stangl and Lewis, 2010). Caution is necessary if using abciximab in patients with severe renal impairment and avoidance in hemodialysis is recommended. Abciximab administration should not be repeated, owing to the development of antibodies in 5.8% of recipients, which could cause reduced efficacy or hypersensitivity (Lily, 2013).

Drug interactions

Combining GP IIb/IIIa inhibitors with other antiplatelets, antithrombins, and fibrinolytics has known therapeutic benefits; however, caution is required due to increased bleeding risk. Use with other drugs known to increase the risk of bleeding, such as NSAIDs, should be avoided or used with caution.

Practical points

- Intended for use with heparin and oral antiplatelet(s)
- Licensed for both ST and non-ST elevation MI
- Can be used for patients who are at high risk of further coronary thrombosis when PCI is not immediately available

Medication Used in the Primary and Secondary Prevention of Cardiovascular Events

Patients with established atherosclerotic cardiovascular disease should receive medication, in addition to adopting lifestyle changes, as secondary prevention to reduce the risk of future cardiovascular events. Primary prevention is indicated for patients with a 10-year risk of cardiovascular disease of 10% or greater. Patients eligible for primary prevention are likely to have a combination of risk factors including diabetes, obesity, elevated cholesterol, hypertension, and smoking history.

Antiplatelets

Platelets play a pivotal role in atherosclerotic cardiovascular disease, such that platelet activation pathways have become valuable therapeutic targets. The sites of action of the antiplatelets agents to be discussed in this article are shown in **Fig. 6**.

Aspirin

Fig. 7 summarizes the pharmacological action, mechanism of action, and adverse effects of aspirin.

Mechanism of action

Thomboxane A2 is a prostaglandin produced by activated platelets in response to stimuli such as collagen and thrombin. It exerts its prothrombotic effects through promotion of platelet aggregation and activation of new platelets. Arachidonic acid undergoes metabolic conversion by cyclooxygenase-1 (COX-1) enzyme to form prostaglandins G2 and H2, which are converted to a number of prostaglandins, including thromboxane A2 (Awtry and Loscalzo, 2000).

Aspirin exerts its antiplatelet action through the irreversible acetylation and inactivation of COX-1 enzyme, inhibiting synthesis of the precursor prostaglandins G2 and H2. The resultant reduction in platelet thromboxane A2 production reduces platelet activation and aggregation (Awtry and Loscalzo, 2001). Since platelets lack a nucleus, they have limited capacity to synthesize new COX-1 enzyme; therefore, thromboxane A2 production is diminished for the 7–10 day lifespan of the platelet.

In addition to its antiplatelet activity, aspirin may reduce the inflammatory response and slow the progression of atherosclerosis in patients with coronary artery disease, through a combination of antioxidant processes (Awtry and Loscalzo, 2000).

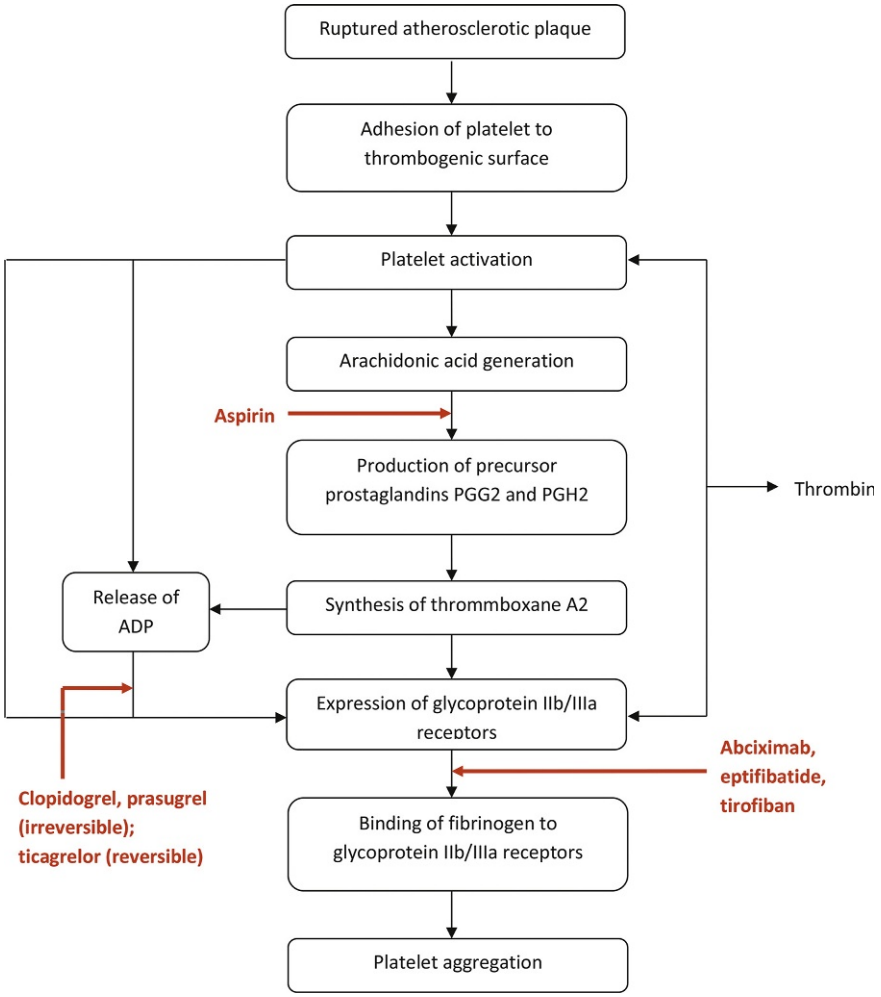


Fig. 6 Sites of action of antiplatelets in the platelet aggregation pathway.

Pharmacological action	Mechanism of action	Adverse effects
• Inhibition of platelet aggregation and activation of new platelets • Possible reduction of inflammatory process and slowing of progression of atherosclerosis	• Irreversible inhibition of cyclooxygenase-1 (COX-1) enzyme, resulting in decreased platelet thromboxane A2	• Haemorrhage • Gastro-intestinal irritation and ulceration • Urticarial rash • Bronchoconstriction

Fig. 7 Summary of the pharmacological action, mechanism of action, and adverse effects of aspirin.

Although there is a multitude of evidence demonstrating the efficacy of aspirin in preventing and treating atherosclerotic cardiovascular disease, it is a relatively weak antiplatelet when used alone. Firstly, aspirin does not completely inhibit thromboxane A2 and secondly, platelet aggregation pathways independent of thromboxane A2 exist and are not affected by treatment with aspirin (Awtry and Loscalzo, 2000). For some indications, better therapeutic outcomes may be achieved by combining aspirin with a newer agent which targets alternative platelet aggregation pathways.

Pharmacokinetics

Aspirin is rapidly absorbed in the acidic conditions of the upper GI tract. It exerts a measurable effect on platelet function within 60 min and has a short plasma half-life of 20 min (Patrono et al., 2001). Cumulative inhibition means that, in chronic administration of low-dose aspirin, the threshold 95% thromboxane A2 inhibition necessary to suppress thromboxane A2-mediated platelet aggregation is achieved after several days (Hoogendijk and ten Cate, 1980). Therapeutic benefits of aspirin have been demonstrated over the dose range 30–1500 mg. In practice, higher doses are associated with an increased risk of adverse effects,

without enhanced therapeutic efficacy (Patrono et al., 2001). Aspirin is 170 times more selective for COX-1 than the COX-2 isoform (Vane et al., 1998), explaining the difference in the dose required for antithrombotic compared with antiinflammatory action.

Enteric coating of aspirin necessitates alkaline conditions for absorption, which is thus delayed until the small intestine (Awtry and Loscalzo, 2000). The link between the resulting reduced bioavailability and therapeutic efficacy of low-dose enteric coated aspirin has not been established; however, it may result in reduced platelet inhibition compared with standard or dispersible aspirin (Hawthorne et al., 1991).

Adverse effects

As described earlier, thromboxane A2 is one of many end products resulting from COX-mediated metabolism of arachidonic acid to prostaglandins (Awtry and Loscalzo, 2000). As aspirin also inhibits COX-1 in gastric mucosa, this suppresses the gastro-protective action of prostaglandin E2, which explains the increased incidence of adverse effects on the GI system in patients receiving aspirin. Dose-related adverse GI effects reported in trials range from minor symptoms (nausea, heartburn, indigestion) to major bleeding (Roderick et al., 1993).

A small proportion of patients suffering with asthma are sensitive to aspirin, which may result in symptoms ranging from urticaria and rhinitis to bronchoconstriction and respiratory compromise.

Drug interactions

Combining aspirin with other antiplatelets, antithrombins, and fibrinolytics has known therapeutic benefits; however, caution is required due to increased bleeding risk. Use with other drugs known to increase the risk of bleeding, such as NSAIDs, should be avoided or used with caution. Antisecretory drugs such as proton pump inhibitors (PPIs) can be used to reduce the risk of GI bleeding.

Adenosine diphosphate inhibitors

Adenosine diphosphate (ADP), which is released from platelet dense granules and damaged cells, binds to P2Y12 receptors on the surface of platelets, causing platelet aggregation and adhesion. ADP binding reduces intracellular cyclic adenosine monophosphate (cAMP), which activates the GP IIb/IIIa receptor and platelet aggregation (Quinn and Fitzgerald, 1999; Kalantzi et al., 2012). ADP inhibitors noncompetitively antagonize the binding of ADP by interacting with the P2Y12 receptor at a site distinct from the ADP binding site. There are the *thienopyridine ADP inhibitors*: clopidogrel, prasugrel, and ticlopidine, and the *cyclopentyltriazolopyrimidine ADP inhibitor*: ticagrelor. **Fig. 8** summarizes the pharmacological action, mechanism of action, and adverse effects of ADP inhibitors.

Mechanism of action

Following the hepatic metabolism of thienopyridine prodrugs to their active forms, covalent disulfide bonding to the P2Y12 receptor facilitates irreversible platelet inhibition (Kalantzi et al., 2012). The inhibitory action of thienopyridines on ADP released from platelet dense granules reduces platelet aggregation induced by other molecules, including collagen, platelet activating factor, and to some extent, thrombin (Quinn and Fitzgerald, 1999). Like aspirin, irreversible inhibition by thienopyridines means platelet function recovers only when new platelets are generated (approximately 7 days) (Patrono et al., 2001).

Ticlopidine is no longer used due to its unacceptable safety profile, namely hematological dyscrasias, and clopidogrel's comparable efficacy (Quinn and Fitzgerald, 1999). The thienopyridines currently in clinical use are clopidogrel and prasugrel.

Tigacrelor exerts its antiplatelets effect by reversible, noncompetitive antagonism of ADP at the P2Y12 receptor (AstraZeneca, 2016a). It inhibits ADP-mediated platelet activation and aggregation more potently than the thienopyridines. The main differences in its mode of action are: firstly, ticagrelor does not require metabolism to an active form, overcoming the delay in therapeutic effect seen with clopidogrel. Secondly, reversible inhibition means that, following cessation of therapy platelet function returns to normal more rapidly, within approximately 5 days (Gurbel et al., 2009).

Pharmacokinetics

Clopidogrel undergoes rapid intestinal absorption and the peak plasma concentration occurs 45 min after oral administration (Sanofi, 2015). The cumulative antiplatelet effect of clopidogrel reaches a maximum of 50%–60% after 4–7 days of repeated administration of 50–100 mg dose (Patrono et al., 2004). A delay in platelet inhibition following administration of clopidogrel is observed, owing to the need for prodrug activation. This two-step conversion, involving cytochrome P450 enzymes, may be the

Pharmacological action	Mechanism of action	Adverse effects
• Reduction in platelet aggregation induced by other molecules, including collagen and platelet activating factor.	• Irreversible (clopidogrel, prasugrel) or reversible (ticagrelor) non-competitive antagonism of ADP at the P2Y12 receptor • Clopidogrel and prasugrel are prodrugs, which undergo hepatic metabolism into their active forms	• Haemorrhage (ticagrelor > prasugrel > clopidogrel) • Hyporesponsiveness to clopidogrel, leading to diminished platelet inhibition • Dyspnoea with ticagrelor

Fig. 8 Summary of the pharmacological action, mechanism of action, and adverse effects of adenosine diphosphate (ADP) inhibitors.

source of interindividual variability in platelet inhibition. Clopidogrel "resistance" or "hyporesponsiveness," seen in up to one-third of patients treated with the drug, can be partially explained by genetic polymorphisms in CYP2C19 alleles coding for loss of function. Additionally, genetic variability in the ABCB1 gene encoding the P-glycoprotein efflux transporter may modulate absorption of clopidogrel (Kalantzi et al., 2012). The active compound undergoes hepatic transformation to inactive metabolites, which are excreted in the urine and feces (Sanofi, 2015).

Prasugrel is rapidly hydrolyzed by intestinal esterases such that the prodrug is not detected in the plasma (Wiviott et al., 2010). The following cytochrome P450 dependent activation results in a peak plasma concentration of active metabolite 30 min after oral administration (Farid et al., 2007). This single cytochrome P450 step facilitates a more rapid onset of antiplatelet activity and renders activation of the parent molecule less susceptible to genetic variation, making prasugrel a more powerful and predictable antiplatelet agent (Kalantzi et al., 2012).

Ticagrelor is rapidly absorbed from the GI tract (Teng, 2012); with a time to maximum therapeutic effect of 2–4 h and a plasma half-life of 7 h (AstraZeneca, 2016a). It undergoes hepatic biotransformation, principally by CYP3A4 and CYP3A5, to a combination of active and inactive metabolites, the majority of which are eliminated by biliary secretion (Teng, 2012).

Adverse effects

Thienopyridines, by their nature, increase the risk of bleeding; however, prasugrel has a greater bleeding risk than clopidogrel (Wiviott et al., 2007). Prasugrel can rarely cause hypersensitivity reactions. Life-threatening blood dyscrasias observed in patients treated with ticlopidine are extremely rare with the newer thienopyridines (Joint Formulary Committee, 2016).

As described earlier, hyporesponsiveness to clopidogrel caused by variant alleles for CYP2C19 can lead to diminished platelet inhibition, and subsequent treatment failure.

Ticagrelor has a higher bleeding risk than the thienopyridine ADP inhibitors owing to its greater potency. The other common side effect is adenosine-mediated dyspnoea, caused by ticagrelor inhibition of cellular uptake and metabolism of adenosine (Cattaneo et al., 2014). Other side effects reported include GI disturbances, headache, dizziness, and raised uric acid levels. Asymptomatic ventricular pauses were reported in early clinical studies (AstraZeneca, 2016a); therefore caution is required when used in patients at risk of bradycardia. Although no dose adjustment in renal function is necessary, increased creatinine levels have been reported during use, warranting monitoring of renal function.

Drug interactions

The heavy reliance on cytochrome P450 enzymes for conversion of clopidogrel to its active metabolite makes it susceptible to metabolic drug–drug interactions. Drugs which inhibit CYP2C19, such as omeprazole, carbamazepine, cimetidine, and erythromycin, may reduce the plasma concentration of clopidogrel's active metabolite, decreasing its antiplatelet action and increasing the risk of therapeutic failure (Bates et al., 2011). The clinical significance of the interaction between clopidogrel and PPIs has come into question due to discrepancy between the randomized clinical trials and observational studies examining this interaction (Melloni et al., 2015).

Prasugrel is not reported to have clinically significant interactions with inhibitors or inducers of cytochrome P450 enzymes.

Strong inducers, such as carbamazepine, and inhibitors, such as ketoconazole, of CYP3A4 can decrease or increase plasma ticagrelor levels, respectively, and concomitant administration should be avoided. Ticagrelor is also a weak CYP3A4 inhibitor; therefore caution is required when administered with CYP3A4 substrates with a narrow therapeutic window. Ticagrelor's interaction with P glycoprotein transporters warrants monitoring of digoxin and ciclosporin levels when administered concurrently (AstraZeneca, 2016a).

Practical points

- Oral antiplatelets require loading on initiation
- Dual antiplatelet therapy increases the risk of hemorrhage. Antisecretory agents, such as PPIs, should be used to reduce the risk of GI ulceration and bleeding
- Combination of one or more antiplatelets with anticoagulants is sometimes indicated, for example, in patients with mechanical valve replacements or atrial fibrillation who present with an ACS. This significantly increases bleeding risk and should be used for the shortest possible time

Lipid-Lowering Therapies

Dyslipidemia is an important risk factor for atherosclerosis and thrombotic MI, which can be modified both by diet and drugs. The lipid-lowering drugs discussed target various lipid transport processes to reduce low-density lipoprotein (LDL)-cholesterol and atheroma.

HMG-CoA reductase inhibitors: statins

Fig. 9 summarizes the pharmacological action, mechanism of action, and adverse effects of statins.

Pharmacological action	Mechanism of action	Adverse effects
• Reduction of LDL cholesterol and atheroma • Mild reduction of triglycerides and increase of HDL cholesterol • 'Pleiotropic' effects, including stabilisation of atherosclerotic plaques against rupture	• Reversible, non-competitive inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase • Upregulation of LDL receptors to increase removal of LDL cholesterol from blood into hepatocyte, where it is enzymatically degraded	• Muscle-related side effects, ranging from myalgia to rhabdomyolysis • Transient elevation of hepatic transaminases • Dose related increased risk of glycaemia and insulin resistance

Fig. 9 Summary of the pharmacological action, mechanism of action, and adverse effects of statins.

Table 1 Grouping of statins and doses according to intensity

	Reduction in low-density lipoprotein cholesterol (%)				
Dose (mg/day)	5	10	20	40	80
Fluvastatin	-	-	21	27	33
Pravastatin	-	20	24	29	-
Simvastatin	-	27	32	37	42
Atorvastatin	-	37	43	49	55
Rosuvastatin		38	43	48	53

Low intensity :20-30%
Medium intensity: 31-40%
High intensity: over 40%

Source: National Institute for Health and Care Excellence (NICE) (2014). *Cardiovascular disease: risk assessment and reduction, including lipid modification [Clinical guideline 181]*. London: National Institute for Health and Care Excellence.

Mechanism of action

3-Hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase catalyzes the metabolism of HMG-CoA to mevalonic acid. Statins are reversible, competitive inhibitors of HMG-CoA (Knopp, 1999) and bind with a higher affinity than the natural substrate (Holdgate et al., 2003). By inhibiting the rate-limiting step in cholesterol synthesis, HMG-CoA reductase inhibitors reduce hepatocyte cholesterol, which leads to upregulation of hepatic LDL cholesterol receptors. Since LDL receptors transport LDL-cholesterol from the plasma into hepatocytes, statins remove LDL-cholesterol from the plasma into hepatocytes, where it subsequently undergoes enzymatic degradation. They also mildly reduce triglycerides and increase HDL-cholesterol. Lowering LDL-cholesterol can reduce the lipid content of atherosclerotic plaques and slow the progression of atherosclerosis (Ferrieres, 2009).

In addition to their cholesterol-lowering action, statins have numerous "pleiotropic" effects. The most important of which, in terms of atherosclerotic disease, is the stabilization of atherosclerotic plaques against rupture. Statins in clinical use are simvastatin, pravastatin, fluvastatin, atorvastatin, and rosuvastatin. Statins vary in potency, with atorvastatin and rosuvastatin producing the greatest reduction in LDL-cholesterol (see **Table 1**). The aim of intensive statin therapy is to achieve a 40% reduction in LDL-cholesterol within 3 months (NICE, 2014).

Pharmacokinetics

Statins are rapidly absorbed following oral administration; however, they are subject to a high degree of first pass metabolism, which limits the amount of drug reaching the systemic circulation (Desager and Horsmans, 1996). Simvastatin is a prodrug, which must undergo hydrolysis to its active form, the beta-hydroxy fatty acid. Fluvastatin, pravastatin, atorvastatin, and rosuvastatin are active in their administered form (Srinivasa et al., 2011).

Short acting statins: simvastatin, fluvastatin, and pravastatin, have a half-life between 0.5 and 3 h. They should be administered at night, to achieve maximal inhibition of cholesterol synthesis in the morning, at its natural peak. Atorvastatin and rosuvastatin are longer acting, with half-lives of 14 and 19 h, respectively (Pfizer, 2016a; AstraZeneca, 2016b); therefore their administration is less time-critical.

Simvastatin, fluvastatin, and atorvastatin are highly hepatically metabolized by cytochrome P450 enzymes. Rosuvastatin undergoes CYP2C9 metabolism to a small degree (AstraZeneca, 2016b), while pravastatin is chemically degraded by cytochrome P450-independent mechanisms (Accord, 2015). This is summarized in **Table 2**. After biotransformation, statins are eliminated primarily in the feces, by biliary excretion, with minimal renal excretion (Srinivasa et al., 2011).

Table 2 Pharmacokinetics of statins (Srinivasa et al., 2011)

	Metabolism			
	Extent	*Mechanism*	*Site*	*Half-life (h)*
Atorvastatin	Extensive	CYP3A4, glucuronidation	Liver	14
Fluvastatin	Extensive	CYP2C9 (CYP2C8, CYP3A4)	Stomach	1.2
Pravastatin	Moderate	Chemical degradation	Liver	1.8
Rosuvastatin	Minimal	CYP2C9 (CYP2C19, CYP2D6, CYP3A4)	Liver	19
Simvastatin	Extensive	CYP3A4	Liver	2.0

Adverse effects

Common unwanted effects of statins include GI disturbance, insomnia, rash, myalgia, and altered liver function tests (Joint Formulary Committee, 2016).

Muscle-related side effects of statins are dose related and range from uncomplicated myalgia to, rarely, serious toxicity including rhabdomyolysis. The most common presentation is myalgia, which is reported at a frequency of 1%–5% in clinical trials and 11%–29% in observational studies (Rosenson et al., 2014). It is important to note that statins are often used in a patient population at an elevated risk of musculoskeletal complaints even in the absence of treatment. Creatine kinase, a marker of muscle breakdown, can be measured if patients present with muscle pain or weakness (Pasternak et al., 2002).

Rises in hepatic transaminases are usually transient and often resolve without cessation or alteration of statin therapy (McKenny et al., 2006). Hepatic failure has been reported rarely with statins in clinical use (Joint Formulary Committee, 2016). Baseline liver function tests should be measured prior to initiation of therapy. Rosuvastatin can cause renal dysfunction, which is more strongly associated with higher doses (AstraZeneca, 2016b).

Statins are associated with an increased risk of type II diabetes. The mechanism is not fully understood; however, glycemia and insulin resistance seen with statins is thought to be associated with HMG-CoA reductase inhibition. The risk is dose-related, with high intensity statins presenting a greater risk than moderate intensity; however, this risk is not thought to outweigh the benefits of improved cardiovascular outcomes (Sattar et al., 2010).

Drug interactions

As many statins are metabolized by cytochrome P450 enzymes, the risk of adverse effects, particularly muscle-related, increases when administered concomitantly with medicines that inhibit this enzyme system. Major examples include azole antifungals, antiretrovirals, macrolides, amiodarone, calcium channel blockers, and grapefruit. It may be necessary to avoid concurrent use or to reduce the dose of the inhibitor to avoid muscle toxicity (Joint Formulary Committee, 2016). Pravastatin and rosuvastatin are not subject to CYP-mediated metabolic interactions.

Cholesterol absorption inhibitor: ezetimibe

Fig. 10 summarizes the pharmacological action, mechanism of action, and adverse effects of ezetimibe.

Mechanism of action

Cholesterol absorption from the small intestinal lumen into endothelial cells is mediated by the transport protein NPC1L1 (Betters and Yu, 2010). Ezetimibe acts at the duodenal brush border to inhibit this protein, preventing the uptake of cholesterol and increasing its excretion in the feces. The resulting decrease in plasma cholesterol causes a reduction in hepatocyte cholesterol stores, which increases expression of LDL receptors to increase clearance of LDL-cholesterol from the plasma into hepatocytes (van Heek and Davis, 2002). Ezetimibe elicits a reduction in LDL-cholesterol of up to 20% (Bruckert et al., 2003).

The additional of ezetimibe to simvastatin has been demonstrated to produce further reductions in cardiovascular risk compared with simvastatin alone in a secondary prevention setting (Cannon et al., 2015a). The synergy observed when ezetimibe is combined with a statin may be explained by the statin off-setting the compensatory increase in cholesterol synthesis caused by ezetimibe (van Heek and Davis, 2002).

Pharmacokinetics

Ezetimibe is rapidly absorbed following oral administration (Merck Sharpe and Dohme, 2016) and is largely glucuronidated to an active metabolite which peaks in the plasma after 1–2 h (Bruckert et al., 2003). Ezetimibe and ezetimibe-glucuronide undergo enterohepatic recycling, which ensure repeated exposure of drug to the site of action in the duodenum (van Heek and Davis, 2002). Both compounds are slowly eliminated in the feces and urine with a half-life of 22 h (Bruckert et al., 2003).

Adverse effects

The most frequent unwanted effects of ezetimibe therapy are abdominal pain, diarrhea, and headache. Rarer side effects include hypersensitivity, altered liver function, hepatitis, pancreatitis, and thrombocytopenia (Joint Formulary Committee, 2016). Muscle

Pharmacological action	Mechanism of action	Adverse effects
• Reduction of plasma LDL cholesterol by up to 20% • No effect on triglycerides • Can be combined with a statin to reduce cardiovascular events in ischaemic heart disease	• Inhibition of the transport protein NPC1L1 at the duodenal brush border to reduce absorption of cholesterol • Subsequent upregulation of LDL receptors and increase in clearance of LDL cholesterol from the plasma into hepatocytes	• Headache • Abdominal pain and diarrhoea • Hepatitis • Pancreatitis • Thrombocytopaenia • Muscle toxicity

Fig. 10 Summary of the pharmacological action, mechanism of action, and adverse effects of ezetimibe.

Pharmacological action	Mechanism of action	Adverse effects
• Significant reduction in plasma cholesterol in patients who are intolerant to statins or have failed to achieve target reductions in cholesterol despite maximal statin therapy	• Fully human monoclonal antibodies • Prevention of PCSK9 mediated degradation of LDL receptors • Increased clearance of LDL cholesterol from the plasma into hepatocytes	• Reaction at the site of subcutaneous injection • Flu-like symptoms • Rash • Nausea

Fig. 11 Summary of the pharmacological action, mechanism of action, and adverse effects of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors.

toxicity, with raises in creatine kinase and very rarely rhabdomyolysis, has been reported in patients taking ezetimibe (Merck Sharpe and Dohme, 2016).

Drug interactions

Caution is required when ezetimibe is combined with a statin, due to the increased risk of muscle toxicity (Cannon et al., 2015a).

Proprotein convertase subtilisin/kexin type 9 inhibitors

Fig. 11 summarizes the pharmacological action, mechanism of action, and adverse effects of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors.

Mechanism of action

In 2003, when research highlighted an association between high levels of PCSK9 and familial hypercholesterolemia (Abifadel et al., 2003), PCSK9 became an interesting new target for lipid-lowering therapies.

PCSK9 is a protein produced in hepatocytes. It binds to internalized LDL receptors and labels them for liposomal degradation. In the presence of PCSK9, the increased degradation leads to lower expression of LDL receptors on the surface of hepatocytes, which reduces clearance of LDL-cholesterol from the plasma (Lagace, 2014). PSCK9 inhibitors prevent the targeted degradation of LDL receptors, leading to increased clearance of cholesterol from the plasma.

There are currently two licensed PCSK9 inhibitors: alirocumab and evolocumab. They are fully human monoclonal antibodies, which have a high affinity for the LDL receptor binding domain of PCSK9 (Cicero et al., 2015). Clinical trials have demonstrated significant reductions in cholesterol in participants who are intolerant to statins or have failed to achieve target cholesterol reductions despite maximal statin therapy (Cannon et al., 2015b; Stroes et al., 2014); however, evidence that this translates to a reduction in cardiovascular risk is still awaited (Everett et al., 2015).

PCSK9 inhibitors are thought to work synergistically with statins, by inhibiting the compensatory increase in PCSK9 induced by the inhibition of cholesterol synthesis (Fazio, 2015).

Pharmacokinetics

Both alirocumab and evolocumab are administered by subcutaneous injection and should be given every 2–4 weeks, depending on the agent and the dose (Joint Formulary Committee, 2016). Peak plasma concentrations occur at 3–7 days (Sanofi, 2016; Amgen, 2016).

Evolocumab has a half-life of 11–17 days (Amgen, 2016) and alirocumab 17–20 days, or 12 days when administered with a statin (Sanofi, 2016). Like other monoclonal antibodies, they are eliminated by degradation to short peptides and amino acids.

Adverse effects

Common unwanted effects include injection site reactions, flu-like symptoms, rash, and nausea (Joint Formulary Committee, 2016); however, postmarketing surveillance may highlight further adverse effects when the drugs are used over a wider population.

Drug interactions

PCSK9 inhibitors are not thought to interact with other medicinal products and do not interact with cytochrome P450 enzymes.

Practical points

- Patients should be educated about the risk of muscle toxicity and asked to report signs and symptoms of myopathy.
- If muscle-related adverse effects occur with statins the following strategies may be used: stop the statin and restart the same treatment when symptoms have resolved; reduce the dose of the statin; change to a different statin.
- "Statin intolerance" is used when patients are found to be intolerant to three different statins that they tried (NICE, 2014).

Beta Blockers

When endogenous catecholamines bind to G-protein coupled beta adrenergic receptors, they stimulate an increase in cAMP. This activates protein kinase A (PKA), which:

(a) Phosphorylates membrane calcium channels, resulting in increased intracellular calcium
(b) Increases release of calcium from the sarcoplasmic reticulum (Ladage et al., 2013)

Of the three main beta receptor subtypes, beta-1 receptors are distributed primarily in the heart (and also in the kidneys), beta-2 in the vascular and bronchial smooth muscle, and beta-3 in the adipocytes and heart (Mansoor and Kaul, 2009). Beta adrenergic stimulation causes increased heart rate, increased force of myocardial contraction, increased blood pressure, and relaxation of bronchial smooth muscle. **Fig. 12** summarizes the pharmacological action, mechanism of action, and adverse effects of beta blockers.

Mechanism of action

Beta blockers competitively antagonize the action of norepinephrine and epinephrine at beta receptors to prevent the accumulation of cAMP, activation of PKA, and the subsequent changes in intracellular calcium which mediate the adrenergic response (Mansoor and Kaul, 2009). The adrenergic effects on the cardiovascular system are mediated primarily through beta-1 receptors. Antagonism at beta-1 receptors in the sino-atrial node reduces heart rate, in the myocardium reduces force of contraction, and in the kidney reduces peripheral resistance through inhibition of renin release. These combined actions reduce myocardial workload and oxygen demand to reduce the symptoms of angina and reduce the risk of ACS. By attenuating the damaging effects of excessive adrenergic activity, beta blockers have a protective effect on left ventricular structure and function (Gheorghiade et al., 2003). Their antiarrhythmic properties may also prevent tachyarrhythmias.

Although beta blockers share similar properties, subtle pharmacological differences may affect the choice of agent for an individual patient. They can be categorized according to cardioselectivity, hemodynamic properties, and the presence/absence of vasodilatory activity (Ladage et al., 2013), summarized in **Table 3**.

Cardioselective beta blockers have a higher affinity for beta-1 receptors and subsequently fewer adverse effects of beta-2 blockade, although this can be lost at high doses (Ladage et al., 2003). Nebivolol demonstrates the highest degree of cardioselectivity (Weber, 2005). Beta blockers with intrinsic sympathomimetic activity, mediated through partial agonism, decrease blood pressure with less effect on heart rate and cardiac output (Prichard, 1987); they should not be used in patients with atherosclerotic cardiovascular disease (Freemantle et al., 1999). Beta blockers with vasodilatory effects also reduce peripheral resistance (Joint Formulary Committee, 2016). This can be mediated either by co-existing alpha blockade or, in the case of nebivolol, direct effects on the endothelial production of NO (Weber, 2005).

Pharmacokinetics

Beta blockers are generally well absorbed following oral administration (Wood, 1984) and their biological half-lives are considerably longer than their plasma half-lives (Weber, 2005). The wide pharmacokinetic variability between beta blockers stems from differences in lipophilicity (Weber, 2005). Lipophilic agents such as propranolol, metroprolol, and labetalol are more readily absorbed from the GI tract and exhibit a greater degree of plasma protein binding. Lipophilic beta blockers are highly hepatically metabolized and have short plasma half-lives; whereas hydrophilic beta blockers, such as atenolol and nadalol, undergo direct renal elimination and have longer plasma half-lives (Weber, 2005).

Pharmacological action	Mechanism of action	Adverse effects
• Reduction of myocardial oxygen demand by decreasing heart rate, strength of myocardial contraction and blood pressure • Reduction in symptoms of angina and risk of myocardial infarction • Protection of left ventricular structure and function	• Competitive antagonism of epinephrine and norepinephrine at beta receptors • Cardiovascular effects medicated by action at the beta-1 subtype of receptors located in the heart • 'Cardioselective' beta blockers have a higher affinity for beta-1 receptors and have fewer adverse effects of beta-2 blockade	• Hypotension, bradycardia • Fatigue, malaise • Sexual dysfunction • Cold extremities • Nightmares, hallucinations • Masking of hypoglycaemia and hyperthyroidism • New-onset diabetes • Bronchospasm (beta-2 blockade) • Rebound hypertension, tachycardia and increase in myocardial contractility

Fig. 12 Summary of the pharmacological action, mechanism of action, and adverse effects of beta blockers.

Table 3 Characteristics of beta blockers

	Cardioselectivity	*Intrinsic sympathomimetic activity*	*Direct vasodilation*	*Combined alpha and beta blockade*
Acebutolol	+	+	−	−
Atenolol	+	−	−	−
Bisoprolol	+	−	−	−
Carvedilol	−	−	−	+
Labetalol	−	−	−	+
Metoprolol	+	−	−	−
Nadalol	−	−	−	−
Nebivolol	+	−	+	−
Pindolol	−	+	−	−
Propranolol	−	−	−	−
Timolol	−	−	−	−

Source: Joint Formulary Committee (2016). *British National Formulary* (72nd edn.). London: BMJ Group and Pharmaceutical Press.

Adverse effects

Common unwanted effects of beta blockers include fatigue, malaise, sedation, low mood, and sexual dysfunction. As a direct extension of the therapeutic effect, beta blockers can cause hypotension, bradycardia, and cardiac conduction disturbances including second- and third-degree heart block. Use should be avoided in significant sinus bradycardia and atrioventricular block, and should not be initiated during acutely decompensated heart failure. Beta blockers may also mask signs of hypoglycemia and hyperthyroidism (Joint Formulary Committee, 2016).

Beta blockers have been associated with deleterious effects on glucose and lipid metabolism, including increased risk of new onset diabetes (Weber, 2015). Various factors may contribute to this increased risk, including inhibition of beta-2 receptor-mediated insulin release from the pancreas (Luna and Feinglos, 2001). Weight gain and reductions in insulin sensitivity and lipolysis through beta-3 blockade may potentiate this (Cruickshank, 2007). These metabolic disturbances are much less likely to occur with the most highly selective beta blockers: bisoprolol and nebivolol (Weber, 2005).

Blockade of beta-2 receptors in the airways can induce dyspnoea and bronchospasm. Although nonselective beta blockers are more likely to cause bronchoconstriction, the risk is not absent from even highly cardioselective agents. The use of all beta blockers requires caution in chronic obstructive pulmonary disease and is relatively contraindicated in asthmatics.

Cold extremities and exacerbation of Reynaud's have been reported with beta blockers due to peripheral vasoconstriction; however, there is no evidence of increased claudication or decreased walking distance in patients with peripheral vascular disease (Paravastu et al., 2013).

Effects on the central nervous system (CNS) include hallucinations, nightmares, confusion, and depression (Joint Formulary Committee, 2016). Theoretically, lipophilic beta blockers would be more likely to cause adverse CNS effects, owing to their greater ability to penetrate the blood brain barrier.

Abrupt cessation of beta blockers can lead to hypertension, tachycardia, and increased myocardial contractility. This "rebound phenomenon" may result from an increase in beta adrenergic receptors induced by treatment with beta blockers and the subsequent increased sensitivity to circulating catecholamines when the drug is withdrawn (Karachalios et al., 2005). It is particularly dangerous in patients with atherosclerotic cardiovascular disease, in whom the inability of myocardial oxygen delivery to meet the rapidly increased demand can lead to myocardial ischemia, ACS, and arrhythmias.

Drug interactions

Pharmacodynamic interactions can occur when beta blockers are given concomitantly with other drugs with similar therapeutic effects, including antihypertensives and drugs with negative inotropic and chronotropic effects, such as digoxin, amiodarone, and rate-limiting calcium channel blockers. While there are known therapeutic benefits to combining such drugs in certain patients groups, caution should be exercised. Administration of beta blockers with verapamil is usually contraindicated. Pharmacokinetic interactions do occur, but are unlikely to be of clinical significance since there is poor correlation between plasma concentration and therapeutic effect.

Practical points

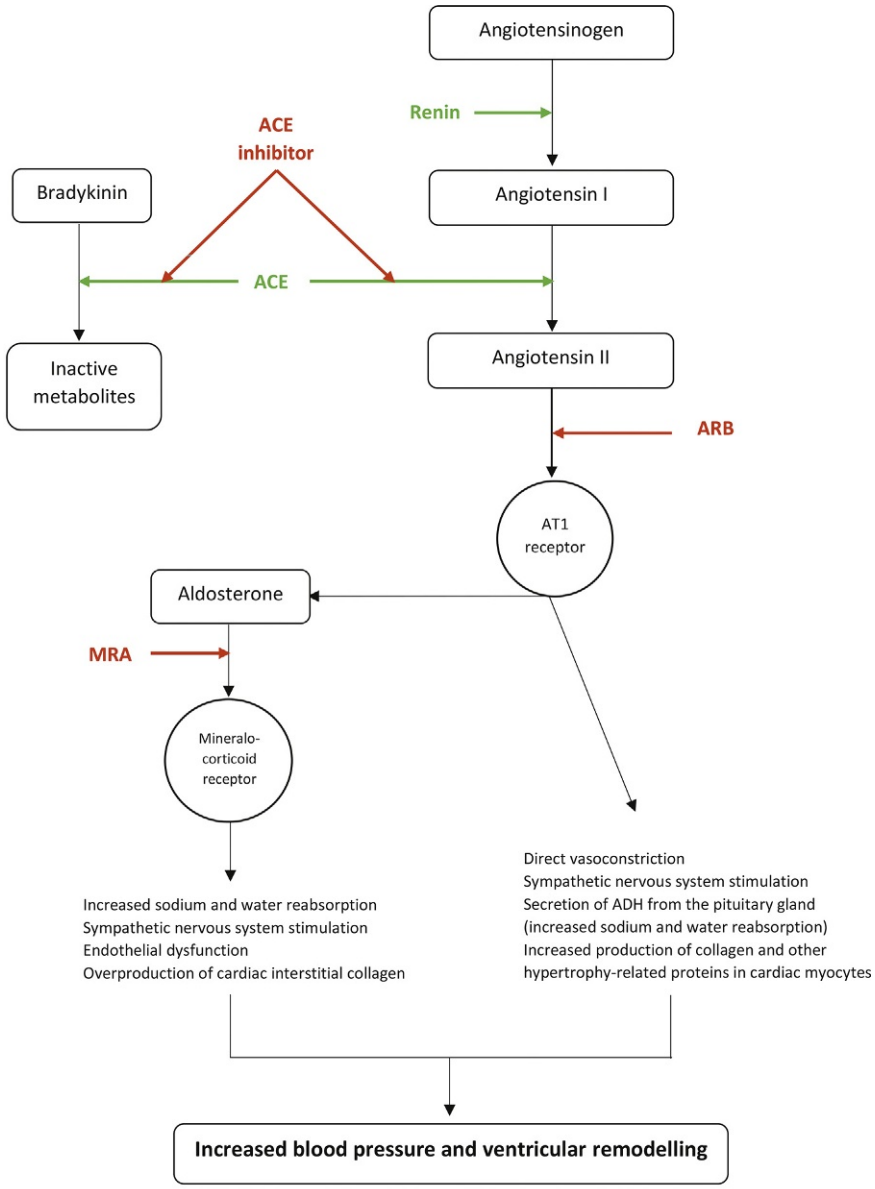
- Systemic absorption can occur following ocular administration of beta blockers and should be borne in mind when considering adverse effects and interactions
- Inform patients about the risks of abrupt cessation of treatment and encourage them to report unacceptable side effects so therapy can be reviewed
- Dose should be started low and uptitrated to a target or maximum tolerated dose. This will be dictated by blood pressure, heart rate, and side effects

Drugs Acting on the RAAS

The RAAS regulates blood pressure and extra cellular volume. Renin is released from the juxtaglomerular apparatus when baroreceptors detect low volume passing through kidneys. This enzyme catalyzes the conversion of circulating angiotensinogen to angiotensin I. When angiotensin I passes through the pulmonary circulation, angiotensin-converting enzyme (ACE) catalyzes proteolysis of angiotensin I to form angiotensin II (Brown and Vaughan, 1998). ACE is a nonspecific enzyme and inactivates a number of other small peptides including bradykinin, a potent vasodilator (Wang et al., 2011). The mechanisms of action of drugs acting on the RAAS are summarized in **Fig. 13** and explained in detail later.

The collective effects of angiotensin II, which are exerted primarily through interaction with angiotensin II type 1 (AT1) receptors distributed in various tissues, lead to increased blood pressure. The effects of angiotensin II, both directly and as a result of aldosterone secretion, are summarized in **Table 4**.

Blocking the effects of angiotensin II has the greatest impact on blood pressure when the RAAS is highly activated; therefore may not be as effective if renin is low, for example, in elderly or Afro-Caribbean patients. Angiotensin II is also implicated in structural changes in the heart through various mechanisms (De Mello and Danser, 2000). Blocking the action of angiotensin II can therefore reduce ventricular remodeling, particularly following acute MI, through the following actions:



ACE = angiotensin converting enzyme; AT1 = angiotensin II type 1; MRA = mineralocorticoid receptor antagonist

Fig. 13 Sites of action of drugs acting on the renin–angiotensin–aldosterone system (RAAS).

Table 4 Summary of the effects of angiotensin II

AT1 receptor location	*Effect of angiotensin II*	*Effect of blocking angiotensin II*
Vascular endothelium	Vasoconstriction (direct effect)	Vasodilation and reduced blood pressure without reflex tachycardia
Sympathetic nervous system	Catecholamine-mediated vasoconstriction (indirect effect)	Reduced norepinephrine, epinephrine, and vasopressin leading to vasodilation
Adrenal gland	Secretion of aldosterone, which acts on mineralocorticoid receptors in the collecting duct of the kidney, causing reabsorption of sodium and water to increase	Increased sodium and water excretion by the kidney, corresponding to decreased blood volume and pressure
Posterior pituitary gland	Release of anti diuretic hormone (ADH), which causes insertion of aquaporins in the collecting duct to further enhance the reabsorption of water by the kidney	Reduced aquaporins in the collecting duct, allowing more sodium and water to be excreted by the kidney, corresponding to decreased blood volume and pressure

Source: Brown, N. J. and Vaughan, D. E. (1998). Angiotensin-converting enzyme inhibitors. *Circulation* **97**, 1411–1420; Wang, X., Wu, S., Xu, D., Xie, D. and Guoc, H. (2011). Inhibitor and substrate binding by angiotensin-converting enzyme: quantum mechanical/molecular mechanical molecular dynamics studies. *Journal of Chemical Information and Modelling* **51**, 1074–1082.

Pharmacological action	Mechanism of action	Adverse effects
• Reduction of myocardial oxygen demand by lowering blood pressure • Reduction of left ventricular remodelling	• Competitive inhibition of ACE at the active site to prevent the conversion of angiotensin I to angiotensin II • Vasodilation • Reduced aldosterone, leading to increased sodium and water retention	• Hypotension and dizziness • Profound first dose hypotension • Renal impairment • Hyperkalaemia • Cough (due to increased bradykinin) • Angioedema

Fig. 14 Summary of the pharmacological action, mechanism of action, and adverse effects of angiotensin-converting enzyme (ACE) inhibitors.

- Decreased sympathetic drive reduces the action of norepinephrine at alpha receptors in cardiac myocytes, preventing the expression of genes involved in cardiac myocyte hypertrophy (St. John Sutton and Sharpe, 2000).
- Decreased preload reduces cardiac myocyte membrane stretch and the associated local release of angiotensin II and expression of genes coding for hypertrophy (Morrone and Marzilli, 2010).
- Blocking of AT1-receptor-mediated synthesis of collagen and hypertrophy related proteins in cardiac myocytes (Morrone and Marzilli, 2010).
- Decreased secretion of aldosterone reduces mineralocorticoid-receptor-mediated myocardial fibrosis and collagen synthesis (St. John Sutton and Sharpe, 2000).

ACE inhibitors

Fig. 14 summarizes the pharmacological action, mechanism of action, and adverse effects of ACE inhibitors.

Mechanism of action

ACE inhibitors competitively inhibit ACE by binding to its active site. ACE is found in the circulation; however inhibition of tissue ACE is thought to be more important. ACE is expressed predominantly the lungs, but also the vascular endothelium, kidneys, brain, heart, and adrenal gland.

In the short term, ACE inhibitors reduce levels of angiotensin II and aldosterone, but do not inhibit the effect of angiotensin II produced by ACE-independent dependent pathways. However, with long-term treatment, angiotensin II and aldosterone return to "pretreatment" levels. This indicates that while acute changes in blood pressure are attributed to inhibition of angiotensin II-mediated vasoconstriction and aldosterone and ADH-mediated sodium and water retention, the long-term therapeutic benefits of ACE inhibitors may be more in keeping with increased production of vasodilatory prostaglandins and NO (Hoyer et al., 1993). ACE inhibitors may also improve endothelial function and slow progression of atherosclerosis by increasing bradykinin-induced NO (Lonn et al., 1994).

Pharmacokinetics

Absorption following oral administration is highly variable between different ACE inhibitors. Maximum plasma concentrations are reached within 1–4 h; however, food can affect the rate, but not extent, of absorption (Wang et al., 2011). Captopril and lisinopril are administered in their active form, while all other ACE inhibitors are formulated as prodrugs to increase their lipophilicity and

oral bioavailability (Brown and Vaughan, 1998). They are converted to active drug in the liver and GI tract (Wang et al., 2011). Half-lives vary between 10 and 24 h, allowing for once or twice daily administration. The primary route of elimination for the majority of ACE inhibitors is renal excretion. Trandolapril and fosinapril undergo a combination of hepatic metabolism and renal excretion (Ujhelyi et al., 1989). Accumulation can therefore occur in renal impairment and in other conditions associated with reduced renal perfusion, such as heart failure. Dose adjustment may be necessary.

Adverse effects

ACE inhibitors generally cause unwanted effects as a class, related to their mechanism of action, and include dizziness, fatigue, headache, and nausea (Joint Formulary Committee, 2016).

Profound first-dose hypotension can occur, particularly in patients who are salt-depleted due to co-administration with diuretics (Wang et al., 2011). Caution is required in patients with aortic stenosis and left ventricular outflow obstruction (Joint Formulary Committee, 2016), as severe hypotension can occur if cardiac output cannot increase to counteract the vasodilatory effects.

A persistent dry cough occurs in up to approximately 20% of patients treated with ACE inhibitors (Ravid et al., 1994). The mechanism is unclear; however it may be due to an increase in pulmonary bradykinin or substance P. It can develop as early as 1 week after initiation or can be delayed by months; however, symptoms resolve on cessation of therapy (Wang et al., 2011).

Hyperkalemia, due to decreased aldosterone-mediated regulation of potassium, can be a problem in patients with renal impairment or heart failure, the elderly and those receiving other medicines which increase potassium. ACE inhibitor therapy should be stopped if potassium rises above 6 mmol/L (Wang et al., 2011).

ACE inhibitors can protect the kidneys; however, in patients with reduced renal perfusion, glomerular filtration is reduced, leading to increased creatinine and acute kidney injury. The risk is highest in patients with heart failure, renal artery stenosis, those treated with diuretics, and the elderly. ACE inhibitors should be avoided in patients with bilateral renal artery stenosis or stenosis of the renal artery supplying a single functioning kidney (Joint Formulary Committee, 2016).

ACE inhibitors can rarely cause potentially life-threatening angioedema, which may be related to accumulation of bradykinin. The risk is highest in black American and African Caribbean patients (Gibbs et al., 1999). It usually occurs within hours to weeks of initiating treatment; however delayed presentation has also been observed. It can affect the GI tract, brain, tongue, and larynx. Immediate cessation of ACE inhibitor is necessary and treatment with adrenaline is warranted if there is potential for airway obstruction. ACE inhibitor should not be restarted (Joint Formulary Committee, 2016).

Side effects not attributed to ACE inhibition include taste disturbances, rash, and neutropenia (Brown and Vaughan, 1998).

Angiotensin II receptor blockers

Fig. 15 summarizes the pharmacological action, mechanism of action, and adverse effects of angiotensin II receptor blockers (ARBs).

Mechanism of action

ARBs, including candesartan, irbesartan, losartan, telmisartan, and valsartan, antagonize the effects of angiotensin II at the AT1 receptor. ARBs bind the AT1 receptor competitively and dissociate slowly (Burnier, 2001). Unlike ACE inhibitors, ARBs inhibit the action of angiotensin II produced by all pathways; however the clinical significance of ACE-independent production of angiotensin II is unclear. Despite differences in pharmacology, the clinical effects of ACE inhibitors and ARBs are similar.

Pharmacokinetics

Pharmacokinetic differences between ARBs are linked to differences in their chemical structure (Israili, 2000). Oral bioavailability is variable according to lipophilicity. Valsartan is the only ARB for which absorption is limited by administration with food. Time to peak plasma concentration varies between 0.5 and 4 h and half-life ranges from 6 to 24 h. All ARBs exhibit a high degree of plasma protein binding (>95%) (Barreras and Gurk-Turner, 2003). ARBs are excreted as unchanged drug and metabolites in the urine and via bile in the feces to varying degrees (Burnier, 2001).

Adverse effects

ARBs have a similar side effect profile to ACE inhibitors. Unlike ACE inhibitors, ARBs do not inhibit the degradation of bradykinin and are therefore much less likely to cause cough (Lacourcière et al., 1994). Angioedema, which may also be linked to elevated

Pharmacological action	Mechanism of action	Adverse effects
• Reduction of myocardial oxygen demand by lowering blood pressure • Reduction of left ventricular remodelling	• Antagonism of angiotensin II at the angiotensin-II-type 1 (AT1) receptor • Vasodilation • Reduced aldosterone, leading to increased sodium and water retention	• Hypotension and dizziness • Profound first dose hypotension • Renal impairment • Hyperkalaemia • Angioedema (less frequently than with ACE inhibitors)

Fig. 15 Summary of the pharmacological action, mechanism of action, and adverse effects of angiotensin II receptor blockers (ARBs).

bradykinin, occurs less frequently with ARBs; however, some cases have been reported (van Rijnsoever et al., 1998). ARBs may be an option for patients with a pressing indication for therapy who are unable to take ACE inhibitors due to angioedema; however, extreme caution is necessary as cross-reactivity may occur (Beavers et al., 2011).

Drug interactions

Many interactions of ACE inhibitors and ARBs relate to additive risk of hypotension, hyperkalemia, or renal impairment when used alongside other medications that share these actions. Combining ACE inhibitors with ARBs increases risk of hypotension, hyperkalemia, renal impairment, and angioedema. Careful monitoring is required when ACE inhibitors or ARBs are combined with mineralocorticoid receptor antagonists, potassium sparing diuretics, antihypertensives and ciclosporin. Plasma concentration of lithium and digoxin can be increased in the presence of ACE inhibitors and ARBs; concurrent use warrants therapeutic close monitoring. NSAIDs may counter vasodilatory effects.

Practical points

- Dose should be started low and uptitrated to a target or maximum tolerated dose. This will be dictated by blood pressure, renal function, and serum potassium.
- Administering ACE inhibitors and ARBs in two divided doses may increase tolerability.
- Symptomatic hypotension, particularly profound first-dose hypotension, may be overcome by administering ACE inhibitors or ARBs at night.

Mineralocorticoid receptor antagonists

This class includes both spironolactone and eplerenone. However, currently it is only eplerenone which is licensed for use post MI in patients with reduced left ventricular ejection fraction and hence the focus on eplerenone. **Fig. 16** summarizes the pharmacological action, mechanism of action, and adverse effects of eplerenone.

Mechanism of action

Eplerenone is a competitive antagonist of aldosterone, which selectively binds to the mineralocorticoid receptor over other steroid receptors (Brown, 2003). This prevents the binding of aldosterone and subsequent gene expression. It is used following acute MI only in those patients who experience heart failure symptoms with a left ventricular ejection fraction of less than 40% (Joint Formulary Committee, 2016). Its effects on plasma volume through reduction of sodium and water reabsorption may contribute to its therapeutic benefit; however, it is likely that other mechanisms are more important for its cardioprotective effects (Pitt et al., 2003). Aldosterone, both in the heart and plasma, is increased following acute MI and contributes to ventricular remodeling by stimulating the sympathetic nervous system, endothelial dysfunction, and overproduction cardiac interstitial collagen. Eplerenone reduces these effects, to attenuate ventricular remodeling and improve left ventricular systolic function (Fraccarollo et al., 2003).

Pharmacokinetics

Eplerenone peaks in the plasma 1.5 h after oral administration. It is converted to inactive metabolites, primarily by CYP3A4 and has a half-life of 4–6 h (Brown, 2003).

Adverse effects

Eplerenone may cause headache, drowsiness, GI disturbances, and diarrhea (Joint Formulary Committee, 2016). Hypercholesterolemia and raised liver transaminases have also been reported (Brown, 2003).

Eplerenone decreases excretion of potassium, which can lead to hyperkalemia. The incidence and severity of hyperkalemia is increased in patients with renal impairment, diabetes, and those taking other medication which increase serum potassium. Eplerenone should be avoided in patients with severe renal impairment. Smaller starting doses should be used and potassium monitored closely for those with moderate renal impairment (Pfizer, 2016b).

Due to its selectivity for mineralocorticoid receptors, eplerenone has a lower incidence of hormone-related side effects, such as gynecomastia and mastodynia in men and vaginal bleeding in women, compared to other aldosterone antagonists such as spironolactone (Brown, 2003).

Pharmacological action	Mechanism of action	Adverse effects
• Lowering of blood pressure by reducing sodium and water retention • Prevention of left ventricular remodelling by reducing collagen overproduction and endothelial dysfunction	• Competitive antagonism of aldosterone • Selective for the mineralocorticoid receptor	• Headache • GI disturbance and diarrhoea • Renal impairment • Hyperkalaemia • Gynaecomastia, mastodynia and vaginal bleeding (less frequently than spironolactone)

Fig. 16 Summary of the pharmacological action, mechanism of action, and adverse effects of eplerenone.

Drug interactions

Administration of eplerenone alongside ACE inhibitors, ARBs, potassium sparing diuretics, and trimethoprim can increase the risk of hyperkalemia. Renal function and potassium should be monitored. Similarly, tacrolimus and ciclosporin can adversely affect renal function which, in the presence of eplerenone, further increases the risk of hyperkalemia. Concomitant use should be avoided. NSAIDs should be used with caution due to their potential to cause renal impairment (Joint Formulary Committee, 2016).

Potent inhibitors of CYP3A4 can greatly increase the plasma concentration of eplerenone, thus increasing the risk of adverse effects. Concomitant administration of potent inhibitors such as clarithromycin, itraconazole, and ritonavir should be avoided. Careful monitoring and dose adjustment may be required with mild to moderate CYP3A4 inhibitors, such as erythromycin, amiodarone, diltiazem, and verapamil. Strong inducers of CYP3A4, including carbamazepine, rifampicin, phenytoin, and St John's wort, may reduce the efficacy of eplerenone (Pfizer, 2016b).

Practical points

- Indicated only post-acute MI with evidence of left ventricular failure
- Increased hypotensive effect when administered with other antihypertensives—monitor blood pressure
- ACE inhibitor, ARB, and eplerenone should not be given together as a triple combination

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Physical Examination: Heritable Cardiovascular Syndromes

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Physician Examination: Heritable Cardiovascular Syndromes

The savvy clinician is a detective in pursuit of keys to unlock the conundrum of a patient's current concerns and future implications. A critical avenue of that pursuit is apprehension of physical exam findings. Physical exam findings are then leveraged by clinical training to induce information on diagnoses, needed treatments, and prognosis. Included in clinical training are education about genetic syndromes, congenital associations, and congenital sequences which exemplify this methodology, allowing one or a collection of particular exam findings to trigger identification of more exam findings, relevant diagnoses, and prognosticate the future. A genetic syndrome or a genetic disorder is defined as a pattern of congenital malformations that are commonly associated together, with a known causal or underlying genetic change. In comparison, a congenital association is a set of physical examination findings occurring together more frequently than would be expected by chance, without a known genetic mutation identified (e.g., VACTERL association). A congenital sequence, finally, is a combination of physical anomalies which occur due to an instigating underlying event or anomaly with other malformations occurring as a consequence (e.g., Pierre–Robin sequence, in which underdevelopment of the mandible leads to a small mouth, posterior displacement of the tongue, cleft palate, and airway difficulties).

While congenital heart disease (CHD) is a common birth defect and is present in about 9 of every 1000 children, the majority of CHD is not associated with genetic syndromes (Blue et al., 2018). With the emergence of widespread, high-throughput gene sequencing and analysis technologies being performed in numbers allowing economics of scale, an increasing number of variations of single-gene mutations, deletions, or duplications with more subtle effect sizes are being associated with CHD, in addition to the more well-known and more penetrant Mendelian disorders. An increasing number of new mutations are being studied in relation to CHD, and broader ranges of presentation are being found for well-known genes. Furthermore, previously well-described, often dismal associations of chromosomal aneuploidy with CHD are also evolving due to clinical courage to treat these children robustly, leading to new, modified natural histories in terms of postoperative care complications, long-term survival, recurrence risk, and care needs (Postma et al., 2015).

What follows is discussion on a selection of genetic syndromes associated with CHD sorted by type of genetic alteration, with specific exposition of dysmorphology, cardiac and noncardiac phenotype, and their genetic testing. In addition, at the end of the chapter, we briefly discuss the arrhythmia-prone long-QT syndromes.

Syndromes With Abnormal Chromosome Number

Down Syndrome

Down syndrome (DS) (Fig. 1) is the most common genetic syndrome associated with CHD, found in about 1 in 1200 live births, and roughly 50% of DS patients will have CHD (Presson et al., 2013; Kazemi et al., 2016). The variety of CHD in these patients is dominated by the atrioventricular septal defects (AVSD), comprising about 60% of CHD in DS patients. The remainder is constituted by secundum atrial septal defects (ASD), ventricular septal defects (VSD), and tetralogy of Fallot (ToF) (Goldmuntz et al., 2016).

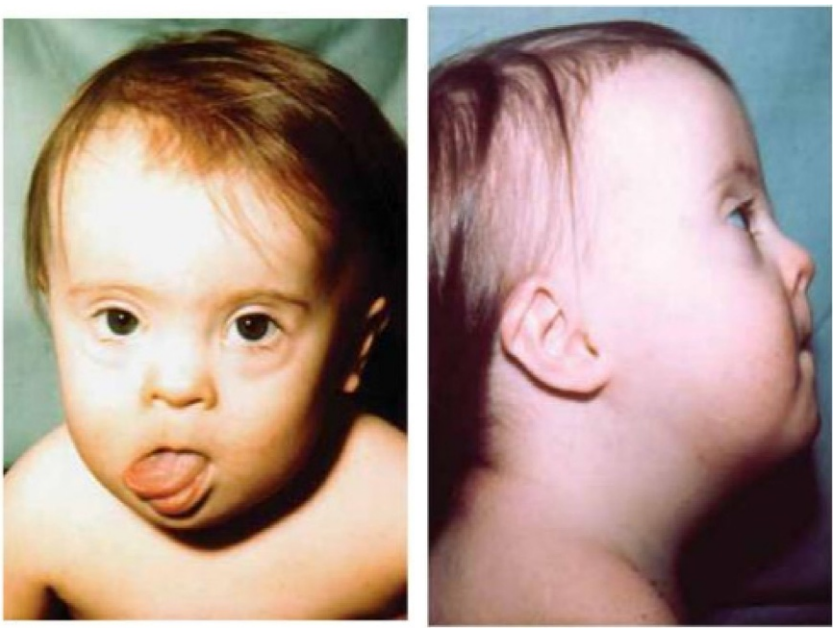

Fig. 1 Down syndrome. Young infant. Flat facies, straight hair, protrusion of tongue. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

Physical exam

In the current era, a majority of DS is diagnosed in the newborn and infant stage, with generalized hypotonia (low muscle tone), protruding tongue, epicanthal fold (skin fold over the eye), transverse palmar crease, shortened and curved fingers due to abnormal development of interphalangeal joints (clinodactyly), and a gap between the first and second toes. As children, they may have difficulty feeding due to poor tone, in addition to possible congestive heart failure (CHF) symptoms. Children with DS with CHD have a 5 times higher risk of dying in the first year of life, but 20-year survival rate is 82% (Presson et al., 2013). Recent reports indicate that the median survival is age 49 years (Presson et al., 2013).

Given the high awareness about the condition, the diagnosis of DS may be made at birth. As per the American Academy of Pediatrics' recommendation for health supervision, all infants diagnosed with DS should undergo an echocardiogram to diagnose CHD (Bull, 2011). The typical CHD lesions (AVSD, VSD) in DS present with features of CHF and pulmonary overcirculation around 2–4 months of age (Goldmuntz et al., 2016). The pulmonary vascular resistance which is high at birth gradually drops which allows the shunting left to right across the lesion to increase, which returns back to the heart and cycle begins again. This progressive cycle overtime leads to much of the heart's work and the baby's energy intake going to the inefficient circular flow from the left heart, across the lesion to the right heart, to the lungs, and back to the left heart. If the CHD goes undiagnosed, these children may continue to overcirculate and then develop pulmonary hypertensive vascular changes. This is accompanied by a seeming improvement in the clinical picture, with lesser overcirculation and better weight gain. However this actually marks a closing window for opportunity for surgical intervention, as the lesions may become inoperable if the pulmonary vascular changes become irreversible (Goldmuntz et al., 2016). After CHD surgery, they have equivalent survival as compared to nontrisomy 21 patients (Goldmuntz et al., 2016; Fudge et al., 2010). While the overall surgical mortality for AVSDs in particular in the current surgical era is less than 3%, the need for reintervention due to atrioventricular valve regurgitation or stenosis remains about 10%–15% at the 5-year postoperative mark (Hoohenkerk et al., 2010; Pontailler et al., 2014).

Children with DS also have a higher risk for development of pulmonary hypertension, with or without preceding pulmonary overcirculation. Autopsy studies and molecular pathology in mouse models has suggested that this may be attributed to the overexpression of antiangiogenic factors from chromosome 21 (Galambos et al., 2016; Bush et al., 2017). This leads to disruption of normal development of alveoli, alveolar simplification, persistence of double capillary networks, and development of intrapulmonary bronchopulmonary anastomoses, leading to pulmonary hypertensive vascular changes (Bush et al., 2017). Several mouse models of trisomy 21 have been developed and studied, with the Ts65Dn being the most popular. It has replicated analogous neurological features of DS, but not the cardiac anatomic lesions (Gupta et al., 2016).

Genetic testing

The most common underlying genetic change is a complete trisomy of the chromosome 21, which are most commonly de novo changes during meiosis during egg or sperm formation (Kazemi et al., 2016). About 90% of these are inherited through the egg, while the remainder are through the sperm. Overall, about 6% of the cases have a partial trisomy due to mosaicism. These may be de

novo, or occur in the parents. The parents are typically unaffected, with a translocation of chromosome 21 material onto another chromosome (Kazemi et al., 2016). During meiosis, this may lead to triple copies of chromosome 21 material, which may also be inherited further. Karyotype can confirm the diagnosis of trisomy 21. While more high-definition testing like chromosomal microarray and sequencing will also make the diagnosis, they are unnecessary unless additional syndromes or defects are suspected.

Turner Syndrome

Turner syndrome (TS) (**Fig. 2**) is present in 1 of 2500 live-born females. About half of the patients with TS have CHD, most commonly left-sided lesions including bicuspid aortic valve (BAV), aortic stenosis (AS), coarctation of the aorta (CoA), or hypoplastic left heart syndrome (HLHS) (Levitsky et al., 2015; Cramer et al., 2014). About 10% of TS patients have CoA. The classic phenotype of a vascular malformation leading to the webbing of the neck and lymphedema is also associated with the findings of left-sided obstructive lesions (Levitsky et al., 2015). This co-occurrence has spurred the hypothesis that the vascular malformations causing the lymphedema may also cause the cardiac lesions. Further, about half of the adults with TS have hypertension (HTN). The risk of aortic dissection in TS is six times that of the general population, and occurs at relatively lesser degrees of aortic dilation (Carlson et al., 2012). These have further prompted the suspicion for an underlying vasculopathic process underpinning these manifestations (Loscalzo et al., 2005).

Physical exam

Fetuses with TS may have lymphedema, hydrops, or lymphatic malformations in the neck. On birth, these newborns may demonstrate neck webbing, low hairline, and puffy extremities (Levitsky et al., 2015). As a child and young adult, they may present with hypertension (about 25% of adolescents), short stature, or infertility, or have a wide carrying angle (cubitus valgus at the elbow) and a broad chest with wide-set nipples on exam. The short stature is thought to be due to decreased sensitivity to growth



Fig. 2 Turner syndrome. Note prominent ears, loose folds of skin in posterior neck with low hairline, broad chest with widely spaced nipples. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

hormone and is responsive to growth hormone administration (Hjerrild et al., 2008). Another common feature is premature ovarian failure, with an accelerated destruction of germ cells starting at 15–18 weeks of gestation. Follicle-stimulating hormone and luteinizing hormone concentrations approach menopausal levels by the end of adolescence. While about 30% of the girls with TS will have some signs of puberty, only about 2%–3% will have spontaneous sustained menstrual cycles (Hjerrild et al., 2008). The deficiency of sex hormones also has a deleterious impact on bone mass, with lower bone mineral density and greater fracture risk. Initiation of sex hormone replacement therapy allows pubertal development at appropriate time, as well as better maintenance of bone health (Hjerrild et al., 2008). TS patients also have neurodevelopmental abnormalities, with average to below-average intelligence and preserved verbal domain skills. However there is impairment of executive functioning and processing, including visual-motor and arithmetic skills (Hong and Reiss, 2014).

Genetic testing

The most common genotype for TS is monosomy of the X chromosome, followed by 46 XY/45 XO mosaicism which is phenotypically less severe (Levitsky et al., 2015; Hjerrild et al., 2008). These aneuploidies are detected by karyotype analysis. Girls with the typical physical exam findings, infertility, primary amenorrhea, or pathologically short stature should undergo karyotype testing for TS (Levitsky et al., 2015; Hjerrild et al., 2008).

Trisomy 18 and 13

Trisomy 18 (Edwards syndrome) (**Figs. 3** and **4**) and Trisomy 13 (Patau syndrome) (**Figs. 5** and **6**) are present in 1 in 6700 and 1 in 12,300 live births, respectively (Bruns, 2011; Macias and Riley, 2016). About 80% of children with trisomy 13% and 90% of the patients with trisomy 18 have associated CHD (Macias and Riley, 2016). The most common lesions in trisomy 13 include double outlet right ventricle (DORV), VSDs and ASDs. The most common lesions in trisomy 18 patients include polyvalvar dysplasia (stenosis or regurgitation), VSDs and ASDs (Bruns, 2011).

There has been a recent shift in the approach to these patients, to enhance quality of life with palliative cardiac surgery in order to obtain stable enough physiology to facilitate the parents taking the baby home (Meyer et al., 2016; Maeda et al., 2011; Janvier et al., 2016). While the greatest mortality for these disorders is in the first week of life, population-based studies have shown an improvement in 1- and 5-year survival in these disorders. Reported 1-year survival from a population cohort from 1999 to 2007 is 11% for trisomy 13% and 13% for trisomy 18, with a 5-year survival of about 10% for trisomy 13 and about 12% for trisomy 18 (Meyer et al., 2016). These numbers are higher than the 1-year survival rate of about 5% reported from a cohort from 1968 to 1999 (Rasmussen et al., 2003). Only in cases of a major CHD like truncus arteriosus, transposition of the great arteries (TGA), ToF, CoA, aortic stenosis, AVSD, or HLHS has CHD been shown to be associated with mortality (Maeda et al., 2011; Janvier et al., 2016).



Fig. 3 Trisomy 18. Clenched hand with index finger overlying third and hypoplasia of fingernails. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.



Fig. 4 Older child with trisomy 18 syndrome. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.



Fig. 5 Newborn child with trisomy 13 syndrome. Note sloping forehead with variable defect in facial development. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

Physical exam

Trisomy 18 patients have increased extensor muscle tone, with scissoring of the legs and overlapping fingers of the hand, in addition to rocker-bottom feet, small mouth (micrognathia), microcephaly, and renal anomalies (Roberts et al., 2016). Patients with trisomy 13 demonstrate polydactyly, cleft lip/palate, cutis aplasia of the scalp, small eyes (microphthalmia), and holoprosencephaly (cerebral hemisphere division failure) (Rasmussen et al., 2003).



Fig. 6 Aplasia cutis congenital over posterior occiput. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

Genetic testing

Diagnosis of trisomy 13 or 18 can be made on karyotype testing. Any child with polyvalvar disease or multiple valves with dysplasia or thickening should be assessed for these underlying genetic trisomies (Maeda et al., 2011; Janvier et al., 2016).

Deletion Syndromes

22q11 Deletion Syndrome

DiGeorge syndrome, velocardiofacial syndrome, conotruncal anomaly (Takao) syndrome, and isolated outflow tract defects of the heart are different names for what we now understand to be a family of syndromes associated with a microdeletion on the long arm of chromosome 22, the 22q11.2 deletion (**Fig. 7**). This occurs in 1 in about 4000–6000 live births and is associated with CHD in about 75%–80% of the patients (Perez and Sullivan, 2002; Hacıhamdioğlu et al., 2015). The CHD spectrum is quite distinct, including interrupted aortic arch, ToF, truncus arteriosus, and perimembranous VSD with aortic arch anomalies. Children diagnosed with these specific CHD lesions are screened for 22q11.2 deletion due to the strength of the association. The deleted region encapsulates >35 genes; however TBX-1 is the most studied gene in this region (Perez and Sullivan, 2002; Hacıhamdioğlu et al., 2015). It is expressed in the pharyngeal arches, is an important transcription factor for development of the second heart field during cardiac embryogenesis, and is needed for the migration of the cardiac neural crest cells in cardiac outflow tract development and other locations. Hence, a mutation in TBX-1 causes defective development in multiple locations, leading to the cardiac, thymic, and parathyroid deficiencies, as well as the facial anomalies and possibly the feeding difficulties found in these patients (Perez and Sullivan, 2002; Hacıhamdioğlu et al., 2015). As repairs for the associated CHD have improved, longer survival has uncovered myriad manifestations of the syndrome. Children with 22q11.2 deletion often have feeding difficulties, and developmental delay, along with behavioral problems like impulsivity and overactivity (Norkett et al., 2017). Nearly one-third are diagnosed with attention deficit hyperactivity disorders, and autism spectrum disorders or general social impairment in about 20%–30% of the cases. They may also go on to be diagnosed with anxiety and mood disorder and may have progressive decline in cognitive abilities (Roberts et al., 2016). About 20%–30% of the adults with 22q11.2 deletion are unfortunately diagnosed with frank schizophrenia or other psychotic illnesses (Norkett et al., 2017).

Physical exam

In the age of prenatal CHD diagnosis, 22q11.2 deletion syndromes are often detected in during infancy. Physical examination features suggestive of this syndrome are—small mouth (micrognathia), flat cheek bones (malar flattening), small nostrils



Fig. 7 Photograph of 12-year-old boy. Facial features emphasizing the nasal configuration. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

(hypoplastic alae nasi), cleft lip/palate, hypocalcemia, immunodeficiency, airway anomalies due to vascular rings, renal anomalies, developmental delay, and behavioral disorders (Perez and Sullivan, 2002). A memory aid for these syndromes is CATCH-22—CHD, abnormal facies, hypoplasia of the thymus (immunodeficiency), cleft palate, and calcium (hypocalcemia—due to hypoparathyroidism). The presence of an aortic arch anomaly increases the chance of 22.q11 deletion, regardless of the underlying CHD.

Genetic testing

Diagnosis of 22q11.2 deletion can be made on fluorescent in situ hybridization (FISH) testing (Perez and Sullivan, 2002; Hacıhamdioğlu et al., 2015). It may also be made by multiplex ligation-dependent probe amplification (MLPA) or chromosomal microarray analysis, the latter of which will also be beneficial in detecting CHARGE (Coloboma, Heart defects, choanal Atresia, Growth retardation, and Genital anomalies and Ear abnormalities) syndrome, since many of the physical exam findings may overlap with 22q11 deletion syndromes.

Williams–Beuren Syndrome (Williams Syndrome)

Williams syndrome (WBS) (**Figs. 8** and 9) is a multisystem genetic cardiovascular disorder occurring in about 1 in 10,000–20,000 live births (Collins, 2013). It is characterized by typical facial features, hypercalcemia, CHD, and a diffuse arteriopathy with stenotic lesions and fibrosis. Most of these children have developmental delay and cognitive weakness, and a characteristic social personality (cocktail party personality). The elastin (ELN) gene has been mapped to the region which experiences a mutation in WBS. This may explain the vascular findings of the condition discussed below (Merla et al., 2012).

Physical exam

WBS facies are characterized as elfin, with a broad forehead, star-shaped irides, flattened nasal bridge with rounded tip of nose, wide mouth and full lips (Collins, 2013). About half the patients have hypercalcemia, which is noted in infancy and thereafter usually is asymptomatic. CHD is present in about 80% of the patients, with about 75% having supravalvar aortic stenosis, 40%–50% having pulmonary artery stenosis, and about 5% having coronary artery stenotic lesions (Merla et al., 2012; Zalzstein et al., 1991). The pulmonary artery lesions typically involve the branch pulmonary arteries or the more distal branches. While the aortic lesions may remain stable or worsen over time, the pulmonary lesions tend to either remain stable or improve as the child grows older (Zalzstein et al., 1991). However in cases where the pulmonic stenosis do not improve, the stenoses tend to be deep into the lung and diffuse, making them difficult to remediate by surgery or cath. A special concern exists with sedation since coronary artery stenoses can be silent until pharmacologically induced changes in arterial pressure may become insufficient to overcome these coronary stenoses and cause sudden death (Bird et al., 2004). A varied range of patients are also being reported to have stenosis of the thoracic aorta, and the elastin deficiency is the putative mechanism of these systemic vasculopathic features (Bird et al., 2004).

Genetic testing

Williams syndrome can be attributed to 7q11.23 deletion in about 90% of the patients (Morris, 1999). This can be detected on FISH or MLPA testing or chromosomal microarray analysis.



Fig. 8 Williams syndrome. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

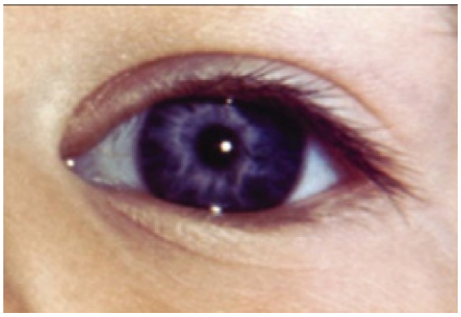

Fig. 9 Note the typical stellate pattern of the iris. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

Jacobsen Syndrome

Jacobsen syndrome is a syndrome caused by a deletion on chromosome 11, found in about 1 in 100,000 live births. It is characterized by developmental and behavioral problems, distinctive facial features, coagulopathy, heart defects, and immunodeficiency (Tassano et al., 2016; Dalm et al., 2015).

Physical exam

The typical facies of Jacobsen syndrome include low-set ears, hypertelorism (wide-spaced eyes), ptosis (drooping eyelids), epicanthal folds, broad nasal bridge, downturned corners of the mouth, and a thin upper lip, in the setting of macrocephaly (Tassano et al., 2016; Dalm et al., 2015). Over 90% of the patients have a bleeding disorder called Paris–Trousseau syndrome which affects the alpha-granules in the platelets. This manifests as easy bruising and abnormal bleeding. CHD is present in just over 50% of the patients (Goldmuntz et al., 2016). The cardiac anomalies are predominantly VSDs, followed by left-sided obstructive lesions like mitral or aortic stenosis, Shone's complex and variants, and hypoplastic left heart syndrome (Goldmuntz et al., 2016; Tassano et al., 2016).

Genetic testing

Jacobsen syndrome is caused by deletion of the terminal material of the long arm of chromosome 11 (Tassano et al., 2016; Dalm et al., 2015). This deletion is large enough to be assessed on karyotyping or readily by FISH testing. The mutation is de novo in about 85% of the cases.

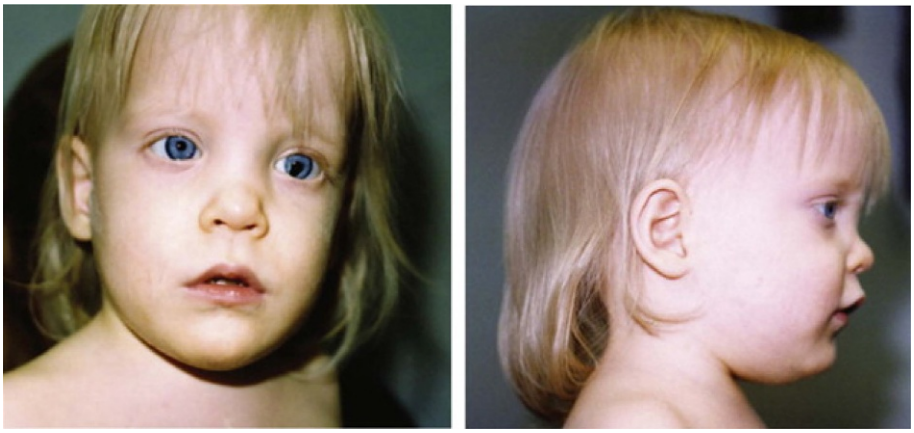

Fig. 10 Cat-eye syndrome. Infant showing coloboma of iris and ocular hypertelorism. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

The cat-eye syndrome (**Fig. 10**) is reported in about 1 in 150,000 live births and is named for its association with vertical colobomas, insensitively called "cat-eye." (Rosias et al., 2001)

Cat Eye Syndrome

Physical exam

The classic triad of this syndrome includes iris colobomas, ear anomalies, and anal malformations (Rosias et al., 2001). Other eye anomalies include hypertelorism and down slanting palpebral fissures, and the facies may also demonstrate ear anomalies and a broad nasal bridge. Cardiac anomalies present in this syndrome include total anomalous pulmonary venous return, ToF, and VSD (Rosias et al., 2001; Timoney and MacNicholas, 2014). Anorectal malformations and genitourinary defects may also be present.

Genetic testing

This syndrome is caused by the partial tetrasomy of chromosome 22, which can be tested for by karyotyping. Confirmatory testing may also be performed by FISH or chromosomal analysis (Rosias et al., 2001; Timoney and MacNicholas, 2014).

Single Gene Deletion Syndromes

Noonan Syndrome

Noonan syndrome (NS) (**Figs. 11** and **12**) is an autosomal dominant condition with characteristics facies and habitus, cardiac disease, and developmental delay. NS occurs in 1 in about 1000–2500 live births (Tafazoli et al., 2017). About 50%–66% of patients with NS have an affected parent. NS is one of a family of disease classified as the RASopathies, conditions with mutations in the RAS-mitogen activated protein kinase (RAS-MAPK) pathway (Tafazoli et al., 2017; Tartaglia et al., 2011). This pathway helps in the communication of growth factor signals into cells, and influences cell division, differentiation and proliferation. These RASopathies include NS, neurofibromatosis type 1, NS with multiple lentigines (discussed below), Costello syndrome, and cardiofaciocutaneous syndrome, among other conditions (Tartaglia et al., 2011). This collection of disorders are seen to have physical craniofacial dysmorphisms, cardiovascular abnormalities, cutaneous findings, musculoskeletal findings, and cognitive impairment. Patients with NS who go on to later develop pigmented spots called lentigines during childhood may be diagnosed with the NS with multiple lentigines. This is a rare multisystem congenital disorder that has findings involving the skin, facial, and cardiac anomalies (Aoki et al., 2016). NS patients commonly suffer from developmental delay as well as growth retardation and short stature. There is also a greater prevalence of attention deficit hyperactivity disorder and learning delays (Tartaglia et al., 2011).

Physical exam

NS has characteristics facies with hypertelorism, micrognathia, low-set fleshy ears, and high-arched palate, a webbed neck, a broad chest with widely spaced nipples, decrease in growth velocity around puberty, and delayed development (Tafazoli et al., 2017; Tartaglia et al., 2011). Their cardiac anomalies include valvar pulmonic stenosis, hypertrophic cardiomyopathy, and electrocardiographic anomalies like right or left axis deviation. Patients with NS with lentigines can also carry the label LEOPARD syndrome: Lentigines, ECG abnormalities, Ocular hypertelorism, Pulmonic stenosis, Abnormal genitalia, Retardation of growth, and Deafness



Fig. 11 Noonan syndrome. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.



Fig. 12 Affected mother and daughter. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

Genetic testing

About half of NS patients carry mutations in the PTPN11 gene (Tafazoli et al., 2017; Tartaglia et al., 2011; Aoki et al., 2016). The remainder are accounted for by six other genes—SOS1, RAF1, KRAS, NRAS, SHOC2, and CBL. PTPN11 may be tested by a targeted gene testing. Currently there is a panel that can be ordered which tests for multiple genes whose mutations can lead to a phenotype of or similar to Noonan syndrome (Tafazoli et al., 2017; Tartaglia et al., 2011; Aoki et al., 2016).

CHARGE Syndrome

CHARGE syndrome (**Fig. 13**) abbreviating Coloboma, Heart defects, choanal Atresia, Growth retardation, and Genital anomalies and Ear abnormalities is a multisystem disorder found in 1 in 8500–10,000 live births. When this constellation of findings is suspected, early involvement of the respective specialist is recommended (Lalani et al., 2012; Bergman et al., 2011).

Physical exam

Patients have a typical facies with prominent forehead, flat midface and prominent nasal bridge. Ear anomalies present in over 90% of the patients include low set anteverted cup-shaped ears, and occasionally microtia or preauricular tags (Lalani et al., 2012;



Fig. 13 CHARGE syndrome. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

Bergman et al., 2011). Absence of semicircular canals detected on head imaging is highly associated with CHARGE syndrome. Colobomas can be present in one or both eyes in about 80%–90% of the patients and are most commonly chorioretinal in location (Lalani et al., 2012). CHD is present in about 80% of the patients and are most commonly conotruncal defects (interrupted aortic arch, ToF, DORV) or AVSD, although almost any type of CHD can be found in these patients (Goldmuntz et al., 2016; Lalani et al., 2012). Choanal atresia is reported in about 65% of the patients, although over half of the patients may also have obstruction distal to the choanae and have multiple levels of obstruction. About 15%–20% of the patients may have cleft lip or palate and a similar frequency may have tracheoesophageal fistula (Lalani et al., 2012; Bergman et al., 2011). Genital anomalies include hypoplastic genitalia. Patients with CHARGE syndrome experience somatic growth restriction in late infancy, global developmental delays, and delayed puberty (Bergman et al., 2011).

Genetic testing

CHD7 mutations, most commonly deletions, account for 90%–95% of the patients with CHARGE syndrome (Lalani et al., 2012; Bergman et al., 2011). A vast majority of the mutations occur de novo and there is no family history, although the inheritance pattern thereafter is autosomal dominant (Lalani et al., 2012).

Marfan Syndrome and Loeys–Dietz Syndrome

Marfan syndrome (MFS) (**Figs. 14** and **15**) is an autosomal dominant negative condition, with manifestations involving the connective tissue of the cardiovascular system, the eyes, and the musculoskeletal system. It occurs in 1 in about 10,000–15,000 people (Groth et al., 2015; Loeys et al., 2010). It is caused by the fibrillin gene (FBN1) mutation. It is also a part of the family of conditions impacting the thoracic aorta, known as the heritable thoracic aortic diseases, and FBN1 is one of the 13 genes known to be causative (Loeys et al., 2010; Romaniello et al., 2014). In MFS, the aorta may dilate over time leading to sudden catastrophic rupture. Loeys–Dietz syndrome (LDS) is another vasculopathy with potentially catastrophic outcomes, associated with mutations in the genes for TGF-β receptors, TGFBR1 and TGFBR2. It is characterized by aneurysmal involvement of the aorta, increased vascular tortuosity, and higher risk of aortic dissection (MacCarrick et al., 2014; Van Laer et al., 2014).

Physical exam

MFS is diagnosed by the revised Ghent criteria, which include aortic root dilation, ectopia lentis, and fibrillin gene mutation as cornerstone criteria, the presence of any two of which clinches the diagnosis (**Table 1**) (Romaniello et al., 2014). The presence of any one of these along with a confirmed family history of MFS based on the revised Ghent criteria is also adequate to confirm the MFS diagnosis. Further, a physical exam to evaluate for the "wrist sign" and the "thumb sign," pectus carinatum, hindfoot deformity, decreased upper segment to lower segment ratio and increased arm to height ratio, reduced elbow extension, scoliosis or thoracolumbar kyphosis, skin striae, and facial features including a long face (dolichocephaly), posteriorly displaced eyeballs within socket (enophthalmos), downslanting palpebral fissures, malar hypoplasia and retrognathia, are a part of the systemic



Fig. 14 Marfan syndrome. Note the long slim limbs, pectus excavatum, narrow face, and reduced elbow extension. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

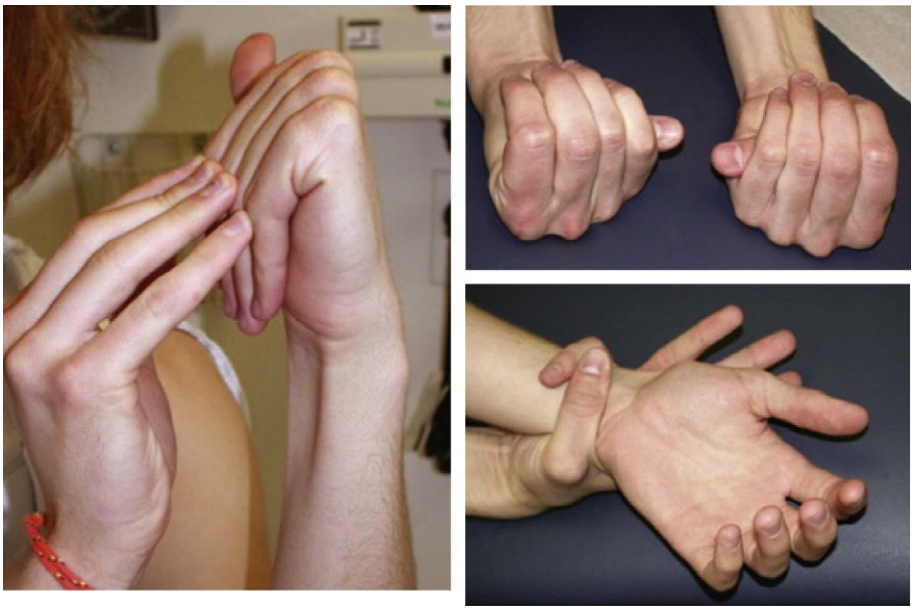

Fig. 15 Joint laxity, Steinberg thumb sign, and ability to join thumb and fifth finger around the wrist (Walker–Murdoch sign). Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

scoring system (Romaniello et al., 2014). A score equal to or greater than 7 along with any one of the 3 major criteria will also confirm the diagnosis of Marfan syndrome (Romaniello et al., 2014).

LDS may be diagnosed in patients with wide-set eyes (hypertelorism), cleft palate, craniosynostosis, and aneurysmal dilation of the aortic root, or other segments of the aorta (MacCarrick et al., 2014; Van Laer et al., 2014). They may also have other skeletal features of kyphosis or joint laxity overlapping with MFS. About 98% of the patients of LDS have aortic root aneurysms, while about 10% have abdominal aortic aneurysms. Patients with LDS are also more likely to have an atrial septal defect or patent ductus arteriosus than the general population, with these lesions found in about 20%–30% of the patients. LDS patients are at greater risk

Table 1 Diagnostic criteria of MFS according to the Ghent-2 nosology

In the absence of family history
1. Aortic root diameter (Z-score ≥2) and ectopia lentis = MFS*
2. Aortic root diameter (Z-score ≥2) and causal *FBN1* mutation = MFS
3. Aortic root diameter (Z-score ≥2) and systemic score ≥7 points = MFS*
4. Ectopia lentis and causal *FBN1* mutation with known aortic root dilatation = MFS

In the presence of family history
5. Ectopia lentis and family history of MFS (as defined above) = MFS.
6. Systemic score ≥7 points and family history of MFS (as defined above) = MFS*
7. Aortic root diameter (Z-score ≥2 above 20 years old, ≥3 below 20 years) and family history of MFS (as defined above) = MFS*

*Caveat: without discriminating features of Shprintzen–Goldberg syndrome, Loeys–Dietz syndrome or vascular form of Ehlers–Danlos syndrome AND after *TGFBR1/2*, collagen biochemistry, *COL3A1* testing if indicated.

Scoring of systemic features of MFS
1. Wrist and thumb sign—3 points (wrist or thumb sign—1 point)
2. Pectus carinatum deformity—2 points (pectus excavatum or chest asymmetry—point)
3. Hindfoot deformity—2 points (plain pes planus—1 point)
4. Protrusio acetabuli—2 points
5. Reduced upper segment/lower body segment ratio and increased arm/height and no severe scoliosis – 1 point
6. Scoliosis or thoracolumbar kyphosis—1 point
7. Reduced elbow extension—1 point
8. Facial features (3/5)—1 point (dolichocephaly, enophthalmos, downslanting palpebral fissures, malar hypoplasia, retrognathia)
9. Pneumothorax—2 points
10. Skin striae—1 point
11. Myopia .3 diopters—1 point
12. Mitral valve prolapse (all types)—1 point
13. Dural ectasia—2 points

The systemic features number 1–13 are used for the systemic score in the Ghent-2 nosology, where a maximum total score points of 20 points can be obtained. We number the systemic features as 1–8 and address these as "skeletal score," and the systemic features as 9–12 and address these as "nonskeletal score".

Copyright © 2010. BMJ Publishing Group Ltd. Reproduced from Loeys, B. L., Dietz, H. C., Braverman, A. C. et al. (2010). The revised Ghent nosology for the Marfan syndrome. *Journal of Medical Genetics* **47** (7), 476–485.

MFS, Marfan syndrome.

for aortic dissection and aneurysm rupture, and hence typically undergo intervention at an earlier stage of dilation (aortic root diameter >4 cm or increasing at rate >0.5 cm per year) (Williams et al., 2007).

Genetic testing

When a complete diagnosis of MFS can be made by clinical signs, genetic testing is not essential to proceed with management. Testing for FBN1 mutation can help diagnose the mutation and allow for testing of future generations that may meet subdiagnostic clinical criteria (Loeys et al., 2010; Romaniello et al., 2014). The first test recommended is the FBN1 gene sequence analysis, followed by the targeted gene deletion/duplication testing if the sequencing is negative. In patients who have distinct physical examination features raising clinical suspicion for LDS, target gene testing can be performed to confirm diagnosis (MacCarrick et al., 2014; Van Laer et al., 2014). If specific gene testing is normal and the concerning clinical finding is thoracic aortic disease, another panel exists to test for the panel of genes associated with other heritable thoracic aortic diseases.

Alagille's Syndrome

Alagille's syndrome (**Fig. 16**) is an autosomal dominant condition found in about 1 in 30,000 live births (Press, 2016). Alagille's syndrome is diagnosed by the presence of classic phenotypic features in a patient with cholestasis. Alagille's syndrome is a multisystem disorder of varying expression, with studies showing that about half of mutation-positive individuals would not meet clinical criteria (Press, 2016). Prognosis is also determined by the number, extent, and severity of organ involvement.

Physical exam

The following criteria help define Alagille's syndrome: cholestasis, ophthalmologic abnormalities (most commonly embryotoxon), characteristic facial features, skeletal abnormalities, cardiac abnormalities, and renal and vascular problems (Press, 2016; Saleh et al., 2016). The facies are characterized by a prominent forehead, hypertelorism, deep set eyes, and a pointed chin. Posterior embryotoxon of the eye, a thickened and centrally displaced anterior border of the ring of Schwalbe visible on external examination without a slit lamp, occurs in about 80%–90% patients. Cardiac involvement occurs in about 90% of the patients including right-sided obstructive lesions (Press, 2016; Saleh et al., 2016). A majority have pulmonary stenosis (about 67%–70%), with ToF being the second most common (~17%). Pulmonic stenosis in these patients can also be distal in the vascular arborization and poorly responsive to intervention (Sugiyama et al., 2004). Skeletal abnormalities seen commonly are butterfly vertebrae and clefting of vertebral bodies. Renal and vascular anomalies seen in these patients include renal artery stenosis, renal cysts, and intracranial



Fig. 16 Alagille syndrome. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

aneurysms, the latter accounting for up to a third of the mortality in one report. The widespread vascular findings, and the finding of hypertension suggest that there may be an underlying vasculopathy at play in this condition (Salem et al., 2012). Other exam findings associated with cholestasis will include pruritus and subsequent excoriations, scleral icterus, acholic stools, and xanthomas. Liver biopsy reveals a paucity of bile ducts although a biopsy is not essential for diagnosis per the current guidelines, and cholestasis with two other classic phenotypic features with suffice. If there is a first degree relative with Alagille's syndrome, then only 2 of the clinical features suffice to make a diagnosis (Press, 2016; Saleh et al., 2016).

Genetic testing

About 95% of patients Alagille's syndrome have a mutation in chromosome 20p12 in the JAG1 gene for the Notch ligand (Press, 2016; Saleh et al., 2016). The remainder of the patients have a NOTCH2 mutations in the chromosome 1p12-p11 region. Looking for the mutations on chromosomal microarray, MLPA as well as targeted gene sequencing testing for the specific deletions aids in diagnosis.

Holt–Oram Syndrome

Holt–Oram syndrome (**Fig. 17**) is an association of birth defects within the family of so-called heart hand syndromes characterized by cardiac septal defects and limb anomalies. The estimated frequency is about 1 in 100,000 live births (McDermott et al., 2004).

Physical exam

The thumb may be triphalangeal, syndactylized to the index finger or resting in the plane of the fingers. The forearm may be involved, with possible deficient or absent radius (McDermott et al., 2004; Da-guang et al., 1986). The upper limb anomalies are often bilateral. Rarely the lower limbs may be involved. ASDs and VSDs are the most common CHD lesions seen in this condition, followed by conduction defects.

Genetic testing

TBX5 is a transcription factor located at 12q24.1 (McDermott et al., 2004). Mutations in this gene resulting in a deletion are associated with Holt–Oram syndrome, although only about a third of the diagnosed cases are associated with known mutations. The condition demonstrates an autosomal dominant transmission with full penetrance.



Fig. 17 Holt–Oram syndrome. Reproduced from Jones, K. L., Jones, M. C., Del Campo, M. (2014). Moderate short stature, facial and genital. In: Jones, K. L., Jones, M. C., Del Campo, M. (eds.) Smith's recognizable patterns of human malformation (7th edn.), Philadelphia, PA: Elsevier. Permission from Elsevier.

Long-QT Syndrome

Long-QT syndrome (LQTS) occurs in about 1 in 2000 people, and is characterized by an increased risk of sudden cardiac death in an otherwise normal individual with a structurally normal heart (Nakano and Shimizu, 2016; Mizusawa et al., 2014). Some forms of LQTS may have also have associated extracardiac features:

(a) **Jervell and Lange–Nielsen syndrome (JLNS)**—JLNS is a rare multisystem disorder with LQTS, in association with profound sensorineural hearing loss. It occurs in 1 in 2–6 million people, with an exceptionally higher prevalence in the Scandinavian nations of Denmark, Sweden, and Norway, at about 1 in 200,000 (Tranebjærg et al., 2002).

Physical exam

Diagnosis of JLNS is based on presence of QTc interval >480 ms and a history of congenital deafness, and difficult to control tachyarrhythmias inspite of beta blocker therapy (Tranebjærg et al., 2002). These patients are at high risk of sudden cardiac death.

Genetic testing

It is caused by an autosomal recessive mutation in the KCNQ1 (90% cases) or the KCNE1 (10% cases) gene, causing defective outflow of potassium ions across membranes in cardiac cells (Tranebjærg et al., 2002). This defect also leads to deficient production of endolymph leading to the congenital deafness seen in this disorder. Testing for this syndrome includes targeted gene analysis (especially if known gene mutation in family), gene sequence analysis (starting with the KCNQ1 mutation), or multigene testing during LQTS evaluation.

(b) **Anderson–Tawil Syndrome (ATS)** (Statland et al., 2004)—This is a rare disorder that has been reported in about a 100 patients across the world. The chief cardiac finding is a prolonged QTc longer than 480 msec and an increased risk of ventricular tachyarrhythmias and sudden death. Bidirectional ventricular tachycardia is a classic finding, which is otherwise found in only two other situations, namely CPVT and digoxin toxicity.

Physical exam

This diagnosis is suspected in patients with two of the following three criteria: (i) dysmorphic facies, (ii) episodic paralysis, and (iii) history of ventricular tachyarhythmias or prolonged QTc. The dysmorphisms are characterized by micrognathia, hypertelorism, low-set ears, clinodactyly of the fifth digit, syndactyly of second and third toes, scoliosis, and short stature (Statland et al., 2004). The periodic paralysis may be in the setting of hypokalemia (absolute <3 mmol/L or relative drop in potassium within the normal serum range) and can be treated with potassium administration. If an individual has one of the above listed three criteria and has a family member who meets at least two of those criteria may also raise concern for this diagnosis.

Genetic testing

It is caused by a mutation in KCJN2 gene, coding for an inward rectifier potassium channel 2 protein, which leads to delayed repolarization (Statland et al., 2004). ATS is inherited in an autosomal dominant pattern, however is de novo mutation in about half the cases. Specific gene testing using sequence analysis or deletion/duplication analysis for KCJN2 will help make the diagnosis.

(c) **Timothy Syndrome**—Timothy syndrome is a rare LQTS disorder with extra cardiac anomalies in the facies, nervous system, and extremities (Napolitano et al., 2006). The chief cardiac finding is again the prolonged QTc greater than 480 msec, and life-threatening ventricular tachyarrhythmias like torsades de pointes and ventricular fibrillation, although some patients may also have structural findings like patent foramen ovale, patent ductus arteriosus, ToF, and VSDs.

Physical exam

The facies of Timothy syndrome are significant for a flattened nasal bridge, mandibular hypoplasia, thin upper lip, small teeth, and low-set ears (Napolitano et al., 2006). Neurologic and behavioral anomalies seen in this syndrome include autism spectrum disorders or pervasive developmental disorders. The hands and feet may exhibit webbed syndactyly with fusion of the skin between the fingers or toes.

Genetic testing

It is caused by a mutation in the CACNA1C gene, causing a prolonged opening of calcium channels with inward calcium flow. Testing for this syndrome includes targeted gene analysis (especially if known gene mutation in family), gene sequence analysis, or multigene testing during LQTS evaluation. It is considered to have autosomal dominant inheritance, however with its high mortality the average age of death is about 2.5years and hence most of the diagnosed patients exhibit a new mutation with no affected family members (Napolitano et al., 2006).

While, the majority of CHD is not associated with genetic syndromes, more frequent genetic testing and investigation has determined several syndromes associated with CHD (Blue et al., 2018). Well-known Mendelian disorders are being redefined and new genetic associations are being described. Many of these persons are making their way into adulthood where familiarity with these diagnoses is emerging. Whereas in times past the combination of genetic or chromosomal disorder with CHD was a contraindication to intervention, modern intervention safety has arrived at a point where these babies can be helped to a more stable physical status and enjoy time and quality of life with family at home. These new practice paradigms are not only changing the survival curves of these unfortunate persons, but uncovering previously unknown phenotypic characteristics. More investigation may lead to better care for these unlucky members of the human family.

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Physical Examination: Normal Examination in Adult Acquired and Congenital Heart Disease

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Introduction

Master clinicians place physical examination at the heart of diagnosis, prognosis, and continuing management for good reason (Hurst et al., 2011; Park, 2014). After obtaining the available historical information, the savvy clinician observes and interrogates the patient's body for additional clues to refine the differential diagnosis, evaluate effects of therapy, and suggest events that may occur in the future. In this chapter, physical exam findings are discussed along with a few key clinical insights based on those exam findings for the interested reader. The chapter is presented within the framework order of how a clinician may usually encounter the physical exam finding in the course of real-world clinical evaluation: vital signs, observation, auscultation, and palpation.

Vital Signs

Heart Rate

Tachycardia

Heart rates above the expected normative value for age warrant additional consideration. In practice, consideration of context such as an emergency room location or presenting complaint such as a broken bone is often sufficient to allay concern. If overall clinical

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status is compromised, tachycardia may belong to the constellation of physical findings signaling the patient is in extremis. In some cases, investigation is needed often in the form of an electrocardiogram, to distinguish between sinus tachycardia and tachyarrhythmia.

Sinus tachycardia: While some causes of sinus tachycardia can be obvious (e.g., pain, fever, anxiety), others may be more difficult to elicit (dehydration or stimulant exposure). Sinus tachycardia can be a sign of cardiac or noncardiac pathology. A key concern from tachycardia is that it is a signal of myocardial dysfunction since inadequate cardiac output induces sympathetic nervous system activation which in turn elicits sinus tachycardia as a compensatory mechanism to support flagging cardiac output. As the list of potential etiologies for myocardial dysfunction is broad (myocardial infarction, pericardial effusion, cardiomyopathy, myocarditis, etc.), the patient evaluation should be expansive. Potential clues to the underlying etiology suggestive of myocardial dysfunction are described in this chapter. Sinus tachycardia can also be seen in the setting of a pulmonary embolus. In that case, it is frequently associated with tachypnea and hypoxemia. In the absence of a primary cardiac or pulmonary etiology, other relatively serious causes of sinus tachycardia include anemia, hyperthyroidism, or less commonly pheochromocytoma.

Tachyarrhythmia: While the different types of tachyarrhythmias and their diagnosis and management are discussed in other chapters, the physical exam can provide helpful clues regarding type and severity. Hypotension or hypoxemia are concerning findings suggestive of cardiovascular compromise. As outlined below, evaluation of the heart rhythm (especially variability and regularity) as well as associated findings (e.g., cannon a waves) can help develop a differential diagnosis prior to any further work-up.

Bradycardia

A resting heart rate below the lower limits of normal is less likely to be achieved by mental state or other common transient features. Therefore, more attention needs to be paid to other physical signs and ECG in order to distinguish sinus bradycardia from intracardiac conduction abnormalities.

Sinus bradycardia: Sinus bradycardia is frequently seen in healthy athletes as a sign of efficient stroke work allowing for lower resting heart rate to deliver the same cardiac output over a given time period. The heart rate usually increases appropriately with activity (e.g., jumping jacks in the office), followed by a rapid return to baseline during recovery. On the opposite end of the spectrum, sinus bradycardia is not uncommon in the elderly population. In that setting, it may represent degenerative changes of the conduction system. This degeneration can be progressive and, if severe, may require pacemaker implantation. Sinus bradycardia can also be a sign of increased vagal tone (such as with increased intracranial pressure), a side effect of many common medications (beta blockers, digoxin, lithium, etc.) or a finding in patients with hypothyroidism. The two most important cardiac causes of sinus bradycardia are myocardial infarction and primary diseases of the sinus node (sick sinus syndrome) (John and Kumar, 2016). While the latter is rare, the former warrants close attention, and the patient should be examined for other findings suggesting myocardial ischemia (e.g., chest pain, diaphoresis, hypotension, new onset left bundle branch block).

Conduction abnormalities: The different types of heart block should be distinguishable based on a surface ECG. Their pathophysiology and management are discussed in other chapters. However, the physical exam can provide helpful clues regarding cause and severity. Cannon a waves appearing as prominent pulsations in the jugular venous pulse, for example, can be a sign of atrioventricular dissociation by virtue of dyssynchronous atrial pulsations occurring against a closed atrioventricular valve. Hypotension, changes in mental status, or crackles on pulmonary auscultation are concerning findings suggestive of cardiovascular compromise. Hepatomegaly and peripheral edema may indicate a more subacute or even chronic duration.

Blood Pressure

While normotension does not rule out heart disease, the deviations from normal deserve particular attention.

Hypertension: Elevated blood pressure is the most common cause of cardiovascular death around the world and is responsible for substantial morbidity and mortality from myocardial infarction, stroke, and congestive heart failure (Lozano et al., 2012). Hypertension is a virtually ubiquitous part of aging with 90% of Americans developing hypertension by age 80 years (Vasan et al., 2002). In addition to primary hypertension, blood pressure can rise in response to other transient or chronic illnesses as well as due to mental state or inappropriate technique. Therefore true positives, false positives, and false negatives all may transpire with varying consequences. Proper interpretation of blood pressure is predicated on proper technique and recognition of clinical scenario, such as severe pain indirectly causing high blood pressure, or other signs, such as coincident bradycardia and bradypnea signaling an intracranial process. But most importantly, recognition is the first step to proper management.

Hypotension: Hypotension can be secondary to dehydration, increased vagal tone, certain medications (e.g., beta blockers), or hypothyroidism. It can more importantly be an alarming finding in patients especially when accompanied by tachycardia, heart block, signs of myocardial dysfunction, or with mental status changes. This should prompt rapid assessment and management.

Blood pressure gradients: In healthy adults, systolic blood pressures in the lower extremities are usually slightly higher than in the upper extremities, historically attributed to peripheral pulse wave amplification, although this may not be true the entire lifecourse (Mitchell et al., 2010). In patients with coarctation, this physiologic gradient can be absent or even reversed (lower systolic blood pressures in the lower extremities, compared to the upper extremities). As a rule, this finding is always abnormal and should prompt further evaluation to rule out coarctation (Dijkema et al., 2017). Recent evidence suggests a blood pressure gradient between the arms may also signal vascular disease requiring further work-up (Clark et al., 2012).

Pulsus paradoxus: In healthy individuals, the systolic blood pressure decreases slightly (by less than 10 mmHg) with inspiration (Hoit, 2017). This respiratory variation is attributed to increased pulmonary vascular capacitance resulting in a mild decrease in

pulmonary venous blood return to the left ventricle and, consequently, a mild decrease in cardiac output. In patients with a pericardial effusion who develop tamponade physiology, the blood pressure decrease with inspiration is more pronounced (greater than 10 mmHg). This phenomenon is referred to as "pulsus paradoxus." In the setting of cardiac tamponade, the increased right ventricular filling normally seen with inspiration compromises left ventricular diastolic filling since the right ventricle is unable to expand into the pericardial space filled with noncompressible fluid. This decrease in left ventricular filling directly causes a decrease in cardiac output, indicated by an inspiratory blood pressure drop. Pulsus paradoxus is best assessed with a manual blood pressure cuff, although suggestions may be gleaned from amplitude differences in a stable, optimal pulse oximetry waveform. Initially, the first Korotkoff sound will only be audible in expiration, but disappear with deep inspiration. With further deflation of the blood pressure cuff, the first Korotkoff sound will become audible during both expiration and inspiration. The difference between these two systolic blood pressures is noted. Differences greater than 10 mmHg are abnormal and may indicate cardiac tamponade.

Respiratory Rate

Tachypnea: An increased respiratory rate is most commonly secondary to primary lung disease, such as infections, asthma, cystic fibrosis, chronic obstructive pulmonary disease, pleural effusions, or malignancy. It can also be seen in certain metabolic disorders, as respiratory compensation of metabolic acidosis. From a cardiovascular perspective, tachypnea can be a sign of a pulmonary embolus, especially in the setting of risk factors, sudden onset, associated hypoxemia, and an otherwise unremarkable lung exam. Cardiac lesions that cause tachypnea due to pulmonary edema commonly fall into three categories: primary myocardial dysfunction, large left-to-right shunts (VSD, PDA), and left-sided obstructive/regurgitant lesions (aortic stenosis or regurgitation, mitral stenosis or regurgitation, coarctation). However, while tachypnea is a key finding in infants with large left-to-right shunts, adult patients with undiagnosed left to right shunt are more likely to present with signs of pulmonary hypertension, such as dyspnea on exertion or hypoxemia. In patients with suspected left-sided obstructive or regurgitant lesions, cardiac auscultation (outlined below) is crucial for formulating a differential diagnosis. In the setting of coarctation, blood pressure gradients between the upper and lower extremities can be a helpful clue, as outlined above. If pulmonary vascular congestion is secondary to an underlying arrhythmia, examination of heart rate and rhythm is obviously the next and most important step. Tachyarrhythmias can cause pulmonary congestion in different ways: via impaired diastolic ventricular filling due to the fast rate, via the loss of synchrony between atria and ventricles, and/or due to arrhythmia-induced myocardial dysfunction. In the latter case, this will often be accompanied by signs of impaired cardiac output, such as poor perfusion, hypotension, and/or mental status changes. Patients with primary myocardial dysfunction and dyspnea may have signs of impaired cardiac output if there is systolic dysfunction. However, in patients with predominantly diastolic heart failure and preserved systolic function these signs may be absent.

Hypopnea/Bradypnea: Abnormally slow, shallow, and/or irregular respirations can be seen in the setting of intoxication, increased intracranial pressure, or neuromuscular disorders. The latter can be associated with cardiomyopathy and myocardial dysfunction. Hypopnea or bradypnea in isolation represents an unusual circumstance that may require timely support.

Oxygen Saturation

Hypoxemia/cyanosis: An abnormally low oxygen saturation, measured by peripheral pulse oximetry, can represent either peripheral cyanosis or central cyanosis. In peripheral cyanosis, the oxygen content of arterial blood leaving the heart is normal, but it is lower than normal in the peripheral vascular bed due to increased peripheral oxygen extraction or low cardiac output. Peripheral cyanosis from depressed cardiac output is distinctly rare. More commonly, it is caused by peripheral vasoconstriction and sluggish blood flow leading to increased oxygen extraction. In central cyanosis, on the other hand, the arterial blood leaving the heart is already desaturated. On exam, this can be distinguished based on the distribution of cyanosis. If oral mucosa and tongue appear pink and well saturated, the cyanosis seen in the extremities is likely peripheral cyanosis. However, if oral mucosa and periphery appear purplish and dusky, this likely represents central cyanosis. Central cyanosis can be due to intracardiac right-to-left shunting (e.g., Tetralogy of Fallot) or pulmonary venous desaturations. The latter, in turn, can be caused by intrapulmonary right-to-left shunting (arteriovenous malformations), ventilation-perfusion mismatch, intrinsic lung disease, or pulmonary edema. Cardiac lesions leading to pulmonary edema were outlined above under "Tachypnea." The pulmonary exam can be particularly helpful in this setting, since patients with right-to-left shunts will likely have a normal lung exam, whereas adult patients with pulmonary edema will likely be tachypneic and have crackles on lung auscultation. As stated above, tachy- and bradyarrhythmias significant enough to cause pulmonary congestion are often also associated with signs of impaired cardiac output, such as poor perfusion, hypotension, and/or mental status changes. This is similar in patients with primary myocardial dysfunction.

Differential cyanosis: A decrease in oxygen saturation from upper to lower extremities (>5%) in adults can be found in the setting of a PDA and pulmonary hypertension, leading to right-to-left shunting across the PDA. A murmur may be absent in that case, and further testing is warranted.

Anthropometrics

Anthropometric data like height and weight can provide helpful clues in patients with suspected heart disease.

Failure to thrive: Failure to thrive is a common presenting symptom in infants and children with congenital heart disease. In adults, cachexia can be seen in patients with heart failure (cardiac cachexia) and is usually associated with a poor prognosis (von Haehling et al., 2017). In addition, cachexia can be an indicator of an underlying malignancy. This is important to note as

chemotherapy-induced cardiomyopathy is a well-described entity. Furthermore, unintended weight loss can be an early symptom in patients with Takayasu's arteritis (Direskeneli, 2017).

Obesity: Excess body weight is a common component of metabolic syndrome with well-known cardiac sequelae described elsewhere. It can also be an indicator of a sedentary lifestyle that is due to underlying heart disease with poor exercise tolerance. Beyond that, obesity may suggest an underlying genetic syndrome having a cardiomyopathy component, such as Bardet–Biedl syndrome (Suspitsin and Imyanitov, 2016).

Short stature: Unusually low height can be an important clue to an underlying genetic syndrome (Ko, 2015). For example, Turner syndrome is associated with short stature, primary gonadal failure, and in about one-third of cases, congenital heart disease, including excessive risk of aortic dissection. Noonan syndrome is also characterized by short stature and congenital heart disease including pulmonary valve dysplasia, pulmonary stenosis, and hypertrophic cardiomyopathy. Both conditions require lifelong cardiac care.

Tall stature: Unusually large height may reflect a person's unique nutritional and hereditary endowment or may signal more sinister processes. For example, tall stature is a hallmark of patients with Marfan syndrome or other connective tissue disorders, especially when associated with excessive chest wall growth like pectus deformities or excessive back curvature like scoliosis (Loeys et al., 2010). Connective tissue disorders are associated with excesses in valve structure, typically mitral or tricuspid valve prolapse and dysfunction, and aortic dilation (particularly of the aortic root) with its dreaded complication, aortic dissection.

Inspection

Even before laying hands on the patient, vital clues can be gathered from careful, informed, visual inspection.

Distress

Pain, often considered the fifth vital sign, should be assessed in every patient. Although more commonly noncardiac in nature, chest pain in particular can be the hallmark of myocardial ischemia, myo-/pericarditis, aortic dissection, or a pulmonary embolism.

Respiratory distress, as outlined above under "tachypnea" and "hypoxemia," is visually evident by vital signs but also by apperception of labored breathing, excessive muscle use with breathing, and visibly apparent fatigue as an indicator of prolonged accessory muscle use. Respiratory distress may be the result of underlying lung disease or various types of heart disease, both congenital and acquired. The presence of respiratory distress mandates rapid assessment and prompt management to prevent respiratory failure.

More subtle may be *distress that is limited to activity*. Patients may appear comfortable at rest but display significant limitations with activities of daily living. Subconsciously, such individuals may self-limit activity and therefore never consciously appreciate the fairly profound limitation in activities of daily living. In addition to a thorough history, asking the patient to walk across the room, down the hall or up a flight of stairs may reveal otherwise missed signs of heart failure.

Inappropriate diaphoresis at rest or with light activity should also catch the examiners attention. While this is a common presenting sign in infants with congestive heart failure and increased sympathetic tone, in the adult population this can be a consequence of diabetic autonomic neuropathy leading to autonomic dysregulation. If diaphoresis is caused by acute *sense of doom*, it may be an alarming sign associated with myocardial infarction, aortic dissection, or pulmonary embolism.

Similarly subtle to detect on exam may be *changes in mental health, affect, or mood*. While these are more commonly noncardiac in nature, lethargy and fatigue may be signs of underlying heart failure and require specific attention. Less common, but no less severe, is a form of cardiomyopathy referred to as Takotsubo cardiomyopathy or "broken heart syndrome." (Chen and Dilsizian, 2017) As a response to emotional stress, significant organic cardiac dysfunction has been described, similar to myocardial ischemia, but with normal coronary perfusion. Thankfully, Takotsubo cardiomyopathy is often characterized by spontaneous recovery. Finally, mental health disorders are now recognized as cardiometabolic risk factors worthy of attention for cardiovascular prevention (Goldstein et al., 2015).

Dysmorphism

As described extensively in other chapters, several genetic syndromes can be associated with congenital (or acquired) heart disease (Ko, 2015). Many of these syndromes are characterized by classic dysmorphic features that should be assessed in every patient. From a cardiac perspective, the most notable of these syndromes are: Down syndrome, Turner syndrome, Noonan syndrome, Marfan syndrome, Ehlers–Danlos syndrome, Holt–Oram syndrome, 22q11.2 deletion syndromes, CHARGE syndrome, and VACTERL association.

Stigmata of Systemic Disease

Many systemic diseases (or their treatments) can be associated with congenital or acquired heart disease. In the absence of a known diagnosis, small visual clues can be of great importance.

- Osler nodes (painful, red, raised lesions on hands or feet), Janeway lesions (small nontender, erythematous macules on palms or soles), or splinter hemorrhages (tiny linear blood clots of the nailbeds) can be signs of infective endocarditis (Baddour et al., 2015).

- A malar rash, a flat red or purplish eruption of the cheeks and nasal bridge, can be a sign of Systemic Lupus Erythematosus, which, in turn, can be associated with pericarditis, myocarditis, conduction defects, valvular disease, and coronary artery disease (Chen et al., 2016). Malar rash may also suggest dermatomyositis. Dermatomyositis has cardiac involvement with frequent subclinical manifestations including conduction abnormalities and arrhythmia and uncommon clinically overt manifestations of congestive heart failure, arrhythmia/conduction abnormalities, and coronary artery disease, possibly due to small vessel involvement (Lundberg, 2006; Schwartz et al., 2016).
- Skin induration and tightening of the face or the fingers (sclerodactyly) is a classic finding in scleroderma, which, in its systemic form, may lead to myocardial disease, conduction system abnormalities, arrhythmias, or pericardial disease (Lambova, 2014).
- The presence of lupus pernio (bluish-red or violaceous nodules and plaques over the nose, cheeks, and ears) or erythema nodosum (reddish, tender lumps most commonly located in the front of the legs below the knees) may be a sign of sarcoidosis which can involve the myocardium and has been associated with sudden cardiac death (Birnie et al., 2016).
- Characteristic joint deformities can point to Rheumatoid Arthritis (RA). RA significantly increases the risk of atherosclerosis, myocardial infarction, and stroke. Recent data suggest disease modulating therapies also prevent CVD (Prasad et al., 2015).
- The presence of an enlarged thyroid (goiter) should prompt evaluation for thyroid disorders. Thyroid disorders are commonly associated with heart rate changes and/or dyslipidemia.
- Acanthosis nigricans (dark, velvety discoloration in body folds and creases) can point at underlying dysglycemia, whereas xanthomata (yellow to orange papules, commonly over joints and tendons) or xanthelasma (when present over the eye lids) can be associated with dyslipidemia.
- Muscle weakness or atrophy as well as calf pseudohypertrophy are classic findings in patients with muscular dystrophy. Many forms of muscular dystrophy are associated with cardiomyopathy, which requires lifelong cardiac care (Palladino et al., 2016).
- Jaundice, as a nonspecific sign of liver disease, can be important to notice since it may point at congestive hepatopathy or cirrhosis in the setting of heart failure. It may also be a complication of alcoholism which, in turn, can lead to cardiomyopathy. Less commonly, jaundice in an adult can lead to a late diagnosis of Alagille syndrome which is often associated with different types and degrees of pulmonary stenosis (Saleh et al., 2016).
- Lastly, a rare but important clue can be the presence of a cochlear implant, since congenital deafness is part of Jervell and Lange–Nielson syndrome, a subtype of long QT syndrome, associated with ventricular tachycardia and sudden cardiac death (Lu and Kass, 2010).

Cyanosis

Visible cyanosis of the skin is a key hallmark of many forms of congenital heart disease. Central, peripheral, and differential cyanosis are discussed above, under "Oxygen saturation."

Clubbing

Nail clubbing of the fingers and/or toes is a nonspecific finding in many chronic diseases. Among those, chronic lung disease and cyanotic heart disease are most prominent, along with gastrointestinal conditions and various malignancies. From a cardiac perspective, any heart disease associated with chronic hypoxemia can lead to the development of progressive clubbing over time. The ubiquity of clubbing is due to its nature as a nonspecific vascular proliferative response to low-oxygen tension. It is important to note both presence and distribution of nail clubbing. Occasionally, clubbing may occur in the lower limbs only, sparing the upper limbs. This is known as differential clubbing and may occur in patients with a PDA associated with pulmonary hypertension and right to left shunting across the PDA. In this setting, there is also differential hypoxia/cyanosis, as outlined above.

Jugular Venous Distention:

Jugular venous distention is assessed with the patient sitting at a 45 degree angle with the head turned slightly sideways. The height of the oscillating top of the internal jugular venous pulse above the sternal angle is noted. Right atrial pressure is then approximated by adding 5 cm to the height of the venous pulse, since it is assumed that the right atrium is located about 5 cm below the sternal angle. When the venous pressure is high, the venous pulsations are best seen when the trunk is elevated to 90 degree. Elevated venous pressure can be a sign of superior vena cava obstruction (prior surgery, malignancy, etc.) or elevated right atrial pressure, such as with right ventricular dysfunction, pulmonary hypertension, or tricuspid regurgitation. In any case, an elevated venous pressure estimate based on jugular venous distention should prompt further evaluation.

Auscultation

Cardiac auscultation should occur in an environment with minimal noise using the stethoscope on the patient's bare chest. The order of conducting an auscultation exam is probably less important than a complete and thorough evaluation. The following order may serve as a suggestion:

Rate

The heart rate can be assessed and counted manually by auscultation to complement the heart rate measured during vital sign assessment with careful attention to any changes between the two time points. For a differential diagnosis of tachycardia and bradycardia, please refer to the section on "Heart rate" above.

Rhythm

- Normal sinus rhythm should be regular with mild, physiologic variation throughout the respiratory cycle (slight rate increase during inspiration, followed by a decrease during expiration).
- A rhythm that is regular but tachycardic and without obvious variability should raise the suspicion for an arrhythmia, such as atrial flutter, supraventricular tachycardia, or an unusually stable ventricular tachycardia.
- If the rhythm is irregular, it can be helpful to determine whether the rhythm is regularly irregular or irregularly irregular. A regularly irregular rhythm follows a particular pattern, such as a dropped beat every few beats. This could be due to second degree heart block with Wenckebach (Mobitz 1) in which the PR interval progressively lengthens, until a beat is no longer conducted. An irregularly irregular rhythm appears erratic without a particular pattern. This can be due to pronounced sinus arrhythmia, atrial fibrillation, or premature atrial or ventricular contractions

Heart Sounds

S1: The first heart sound is caused by closure of the atrioventricular (tricuspid and mitral) valves. It can be soft/muffled in the setting of pericardial effusion. Rarely, S1 can be split. An abnormally wide split can be found in the setting of right bundle branch block or Ebstein's anomaly of the tricuspid valve. A wide split should be distinguished from an ejection click and S4 gallop described below. In patients following mechanical atrioventricular valve replacement, S1 often has a "metallic" quality.

S2: The second heart sound is caused by closure of the semilunar (pulmonary and aortic) valves and consists of a pulmonary component (P2) and an aortic component (A2). It can be soft/muffled in the setting of pericardial effusion. It is often loud (i.e., louder than S1) in the setting of pulmonary hypertension. In a normal heart, A2 and P2 occur simultaneously during expiration, but split slightly (A2 occurring earlier than P2) during inspiration, secondary to increased right heart filling and flow across the pulmonary valve during inspiration. This behavior is considered physiologic splitting. Abnormally wide or fixed splitting of S2 is seen in the setting of right ventricular volume overload (ASD, partial anomalous pulmonary venous return), right bundle branch block, or severe pulmonary stenosis. Furthermore, the second heart sound can be single if there is only one semilunar valve (e.g., aortic atresia or pulmonary atresia), both semilunar valves are connected to the same vessel (following a Damus–Kaye–Stansel procedure), P2 is early (pulmonary hypertension), or A2 is delayed (severe aortic stenosis). In patients following mechanical aortic or pulmonary valve replacement, S2 often has a "metallic" quality.

S3: A third heart sound is a low-frequency sound in early diastole (following S2), caused by rapid filling of the ventricles. It can be normal in children and young adults. In the setting of tachycardia, however, it constitutes an abnormal S3 gallop and could be a sign of congestive heart failure. A variant of S3 is the so-called *pericardial knock*. This is a somewhat higher pitch early diastolic sound that occurs distinctly earlier than the usual S3, due to rapid ventricular filling being abruptly halted by a restricting pericardium, such as with restrictive pericarditis.

S4: A fourth heart sound is a low-frequency sound in late diastole (preceding S1). It is thought to be caused by turbulent blood flow during atrial contraction into a ventricle with reduced compliance. S4 is always pathologic and commonly associated with heart failure, often in the form of a tachycardic S4 gallop.

Murmurs

A murmur is an audible sound caused by nonlaminar blood flow. As outlined in the following, nonlaminar flow need not always indicate pathology, and most murmurs are benign.

Timing

Systolic

Midsystolic (ejection type): This type of murmur begins shortly after S1 and ends before S2 with a crescendo–decrescendo contour. To define this pattern, S1 and S2 should be clearly audible and unobscured by the murmur. It is usually associated with turbulent flow across semilunar valves (aortic or pulmonary) due to mismatch between the size and shape of the orifice and the flow traversing the orifice. For example, a structural defect in the semilunar valve such as in aortic or pulmonary stenosis may cause this murmur by being an inaudible obstruction to flow until the ventricle generates enough pressure to overcome the obstruction. Once that pressure is achieved, the valve structure itself causes turbulent, nonlaminar flow audible as the murmur. Similarly, an atrial septal defect inducing markedly increased flow across a normal pulmonary valve may lead to nonlaminar flow patterns again causing this murmur. The location of the midsystolic murmur (see below) can help distinguish aortic from pulmonary stenosis. A midsystolic (ejection type) murmur also occurs as an innocent flow murmur (Still's murmur) described below.

Holosystolic (regurgitant): This type of murmur begins with S1 and continues through S2. There is no delay between S1 and the murmur, which is an important feature distinguishing it from a midsystolic murmur. The intensity of the murmur is usually constant throughout systole (band-like). Holosystolic murmurs are cause by mitral regurgitation, tricuspid regurgitation or VSDs, circumstances in which the pressure difference between the two chambers involved is different from very beginning to the end of systole. When the murmur is new onset and associated with clinical compromise an acute life-threatening process may be occurring such as either mitral papillary muscle rupture or necrotic VSD following myocardial infarction.

Early systolic (regurgitant): This type of murmur begins with S1, decreases quickly in intensity (decrescendo), and ends well before S2. It is caused by the same conditions that cause holosystolic murmurs, however, with more rapid equalization of pressures and, hence, earlier cessation of flow.

Late systolic: A late systolic murmur begins in late systole and lasts up to S2. It is most commonly caused by mitral regurgitation due to mitral valve prolapse. In that case it is preceded by a midsystolic click.

Diastolic

Early diastolic: This murmur type begins with or even obscures S2 and quickly decreases in intensity (decrescendo). It is associated with semilunar valve (aortic or pulmonary) regurgitation. Although the location of the murmur is similar in both cases (left sternal border), the murmur of aortic regurgitation tends to be higher pitch due to the higher pressure difference between upstream and backflow chambers.

Mid- or late-diastolic: These low-frequency murmurs begin well after S2 (mid diastolic) or just before S1 (late diastolic). They are caused by turbulent flow across the tricuspid or mitral valve. This can happen due to the mismatch between atrioventricular valve structure and flow. Structural mitral stenosis or less commonly tricuspid stenosis may cause such a murmur. Alternatively, increased flow across a normal valve such as a large VSD or PDA leading to excess flow across the mitral valve or a large ASD or partial anomalous pulmonary venous return leading to excessive flow across the tricuspid valve.

Continuous

Hallmark of a continuous murmur is that it begins in systole and lasts without interruption into diastole. It need not last throughout the entire cardiac cycle since all that is required is a connection between two chambers that have a pressure difference and possible flow in systole and diastole. A continuous murmur can be caused by a PDA, aortopulmonary collaterals, coronary artery fistula, or arteriovenous fistula. Of note, in patients with a PDA and pulmonary hypertension, the diastolic component of the murmur may be abolished due to equalized pulmonary and systemic pressure during diastole. Continuous murmurs are distinct from combined midsystolic and early diastolic murmurs ("to-and-fro" murmurs) which occur with mixed semilunar valve disease (stenosis plus regurgitation).

Intensity

Grade I: Barely audible. Audible to roughly half of examiners.
Grade II: Soft, but easily audible
Grade III: Moderately loud, but not accompanied by a thrill
Grade IV: Loud and associated with a thrill
Grade V: Audible with the stethoscope barely on the chest. Thrill present
Grade VI: Audible with the stethoscope off the chest. Thrill present

Location

See Fig. 1.

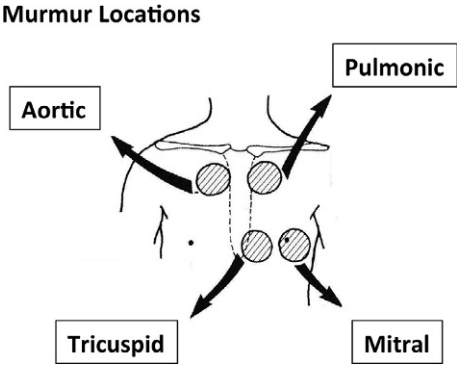

Fig. 1 Murmur locations. Aortic (right upper sternal border); pulmonary (left upper sternal border); tricuspid (left lower sternal border); mitral (apex). Modified from Parks (2014).

Radiation

The pattern of radiation can be useful in determining the etiology of a murmur. Murmurs of aortic origin are more likely to radiate to the right upper sternal border and neck along the course of the downstream chamber, the carotid arteries. Murmurs of pulmonary origin are more likely to radiate into the back and the axillae along the course of the proximal pulmonary arteries. A mitral regurgitation murmur is best heard over the LLSB with radiation into the lower back. A murmur prominently heard in the interscapular area may signal narrowing at classic site for coarctation of the aorta. A continuous murmur in the intraclavicular region with radiation to both left and right chest could indicate a PDA or other aortopulmonary collateral.

Innocent murmurs

Still's murmur: This is the most common innocent murmur. It is a midsystolic ejection type murmur, no more than III/VI in intensity (never with a thrill), and best heard along the left sternal border. The hallmark of this murmur is its vibratory "twangy" quality that is also often described as musical. The murmur is more prominent in the supine position.

Outflow tract murmurs: These murmurs are caused by exaggeration of normal ejection vibrations along the right or left ventricular outflow tract. They are midsystolic ejection type murmurs, no more than III/VI in intensity, and best heard along the left upper sternal border (RVOT) or right upper sternal border (LVOT). Since they occur in the setting of normal anatomy, there are no associated clicks or thrills.

Venous hum: This murmur is caused by turbulence in the jugular venous system. It is a soft, continuous murmur, best heard over the right or left infraclavicular area. It is only heard in the upright position and usually disappears in the supine position. It may be confused with a PDA murmur. However, the diastolic component of a venous hum is louder than the systolic component. The reverse is true for the PDA murmur. In addition, the PDA murmur does not disappear in the supine position. A venous hum can be obliterated in the upright position by turning the neck so as to occlude the jugular vein, or direct application of light pressure, taking care to avoid the carotid bulb.

Clicks

Ejection click: An ejection click is a high-pitch sound that follows S1 closely. It is caused by sudden systolic doming of an abnormal semilunar valve. An aortic click (often in the setting of a bicuspid aortic valve) is best heard at the left lower sternal border. It does not vary with inspiration. A pulmonary click (often in the setting of a dysplastic or bicuspid pulmonary valve) is best heard over the left upper sternal border and becomes softer with inspiration.

Midsystolic click: This sound occurs later is systole in the setting of mitral valve prolapse. If mitral regurgitation is present, it may be accompanied by a late systolic murmur (see above).

Opening snap: This early diastolic sound is caused by the opening of a stenotic atrioventricular valve. It is best heard over the LLSB (tricuspid stenosis) or apex (mitral stenosis).

Other Adventitial Sounds

Rubs: Pericardial friction rubs usually occur in the setting of pericarditis. They are caused by friction between the inflamed pericardial surfaces. It is a rocking to-and-fro sound that resembles walking in fresh snow. It is more pronounced with the patient leaning forward. Of note, once a large pericardial effusion develops, the rub may become softer or disappear. This should not be misinterpreted as a resolution of pericarditis.

Bruits: A bruit is a continuous murmur caused by turbulent arterial flow. This can occur either in the setting of arterial obstruction or due to increased flow through an unobstructed artery.

Carotid and femoral artery bruits should raise concern for peripheral artery disease and warrant further evaluation (Writing Committee M et al., 2017). In patients who have undergone a prior cardiac catheterization with femoral arterial access, a femoral bruit may be a sign of an iatrogenic AV fistula.

Renal artery stenosis can cause a bruit over the renal arteries. This is best auscultated above the level of the umbilicus along the lateral edge of either rectus muscle. This is particularly important in the evaluation of a hypertensive patient.

Bruits heard over the *liver* can be a sign of an underlying liver malignancy, alcoholic hepatitis/cirrhosis, or of an arteriovenous malformation.

Cerebral bruits (auscultated best over the cranium, through a fontanelle, or over the orbit/eyeball) can occur in the setting of high cardiac output or decreased blood viscosity, such as with severe anemia. In the absence of those conditions, cerebral bruits may be a sign of intracranial hemangiomas or arteriovenous malformations. Less commonly, they can be appreciated in the setting of Paget's disease due to increased blood flow through the abnormal bone structure.

Palpation

Palpation, that is, manual investigation, of the cardiovascular system is an ancient practice which is falling out of favor in modern clinical practice. Since personal manual sensation of physical phenomena may not be uniform or easily standardizable among examiners, palpation is in danger of becoming a relic of bygone times. Focusing on aspects still relevant to modern practice,

palpation is classifiable by frequency, duration, timing, and location. Some sources state that while fingertips are the prime tools for palpable investigation, fingertips are ill-suited for long duration events, especially at low frequencies (Hurst et al., 2011). Palms may be better suited to longer duration events.

First palpation of pulses, typically with the examiners fingertips, is a requisite for every physical examination. While much information can be gleaned from pulse qualities since ancient times, in modern practice pulse exam is limited to presence versus absence and timing delay (O'Rourke et al., 2001). Pulses where arteries course over bony prominences like the wrist, dorsum of the foot, medial malleolus of the ankle, neck location of the carotid, and the temporal artery. The absence of pulses must first be investigated as being caused by low cardiac output such as shock. More stable situations where pulses are absent would include very rare Takayasu's arteritis or much more common congenital absence of the dorsalis pedis pulse of the foot (Chavatzas, 1974). The abnormal presence of a brisk or bounding pulse at the palms of a premature infant can be a clue for the presence of an important patent ductus arteriosus, even when the baby might be intubated and covered in monitoring equipment precluding proper auscultative apprehension of a continuous murmur. The timing of pulses can be important such as when a perceptible delay exists between two sites. For example a delay between femoral pulse in the groin versus carotid pulse in the neck may signal collateral vessels supplying the femorals due to coarctation of the aorta. The quality of the palpation may also signal important concerns. For example, "diffusion" of a pulse from a sharp, smooth, brisk phenomenon to a longer sensation described as "worms crawling below the fingertips" signals the presence of a thrill. A thrill suggests the presence of nonlaminar flow, such as a thrill at the carotid site suggesting the occurrence of significant aortic valve stenosis.

Second palpation of the chest and trunk can be informative. Many of the features of palpation in these locations can be enhanced by using the palm rather than the fingertips. The point of maximum impulse is the point usually on the fifth intercostal space at the midclavicular line. This point may show visible pulsation of the heart in small or young people and may have a palpable impulse in larger older persons. However the point of maximal impulse may not always be properly assigned to that location. One example would be the rare circumstance where the cardiac mass is not located in the left chest in so-called dextrocardia when in the right chest or mesocardia when the cardiac mass is more midline. Another example would be changes in LV dimensions such as dilated cardiomyopathy that can lead to the point of maximal impulse to be displaced leftwards. As yet another example, right ventricular pressure overload or right ventricular volume overload can cause a right ventricular heave or palpable pulsation through the chest wall just to the left of the sternum at the fourth or fifth intercostal space, termed an RV heave. Referenced to carotid pulse for timing, sensitive examiners may be able to distinguish an early-in-systole heave consistent with volume overload versus more prominent later-in-systole heave which is more consistent with pressure overload. The difference in timing may be due to the time needed for the ventricle to generate the pressure needed to overcome the pressure overload and initiate outflow. Another key aspect of precordial palpation is the presence of thrill accompanying a murmur defining a more "severe" murmur of at least four out of six on the Levine scale (Freeman and Levine, 1933). Severe chest pain accompanied by a palpable thrill or pulsation in the sternoclavicular area can suggest a prominence of the aortic contour rising to the cranial border of the sternum, possibly due to aneurysm or pseudoaneurysm with impending rupture. Finally, a timing delay between the apical impulse on the chest and the carotid pulse can be a useful signal of important aortic stenosis, especially when echocardiography-based gradient measures can be problematic (Schlingmann et al., 2015).

Conclusion

Physical Examination is still the entry-point for diagnosis and treatment for clinical patients of all ages. Proper attention should be paid to vital signs and general appearance to glean enormous amounts of actionable information. Discovery and confirmation through auscultation and palpation still offer uniquely valuable data points on diagnosis, treatment, and prognosis even in this age of high-resource-intensity solutions to old problems. Retrenchment into time-tested physical exam diagnostic skills would serve modern medicine very well. A lack of focus on the physical exam would be a loss of practical value and an irreparable disruption in the tradition of medical care.

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Physiological Adaptations of the Heart in Elite Athletes

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Introduction

Competitive athletes and highly active individuals are a growing segment of the global population. This growth is driven by increasing recognition of the health benefits of routine physical exercise and greater access to competitive sporting opportunities and recreational exercise programs.

The American College of Cardiology and European Society of Cardiology (ESC) (Mitten et al., 2015) identify a qualitative definition for a competitive athlete, as a person who participates in regular competition, in which emphasis is placed on excellence and achievement, with a usual intense systematic training and a tendency to physical limits during exertion.

From a quantitative point of view, an athlete may be considered "elite" if competing regularly at the regional, national, or international level and exercising for ≥ 6 h/week (Sheikh et al., 2014).

Long-term physical training in elite athletes is characterized by physiological adaptation of cardiovascular structures, with functional, structural, and electrical remodeling, defined as athlete's heart.

The identification of these changes is essential to distinguish athlete's heart from other pathological conditions. Moreover some of the exercise-induced changes may be associated with acute and chronic cardiac damage, and in a small number of athletes this may predispose to atrial and ventricular arrhythmias (D'Andrea et al., 2015).

Cardiac remodeling may be evaluated not only at rest but also during exercise with electrocardiography (ECG), standard echocardiography, advanced ultrasound techniques, and other noninvasive cardiac imaging modalities.

The aim of this chapter is to provide a comprehensive assessment of physiological adaption of the heart in elite athletes.

Physiological Adaptation

Morganroth identified two main models of training adaptation characterized by two distinct patterns of cardiac remodeling (myocardial hypertrophy) (Morganroth et al. 1975). Endurance training is typical of aerobic sports with dynamic–isotonic muscular involvement, such as long-distance swimming and running. These activities cause a gradual decrease in systemic arterial resistance and an increase in venous return, with a predominant volume overload, resulting in higher left ventricular (LV) end-diastolic volume (EDV) and stroke volume (eccentric hypertrophy).

In contrast, strength training is typical of anaerobic sports with predominant static–isometric muscular exercise, such as body-building, short-distance running, and swimming. These sport categories cause mainly an increase in myocardial wall thickness rather than cavity diameters (concentric remodeling and hypertrophy), in response to the predominant pressure overload.

Morganroth's original hypothesis has been criticized because cardiac remodeling is also influenced by other factors like ethnicity, age, sex, genetics, and body size. Moreover it has to be noted that most sports are actually characterized by a variable combination of both endurance and strength exercise, rather than only one of them (**Fig. 1**).

Heart rate reduction and stroke volume increases, associated with systemic vascular resistance reduction are typical hemodynamic changes in athlete's heart: it results in a slight elevation of cardiac output ($=SV \times HR$) during exercise. The long-run hemodynamic changes during exercise cause an increase in both left ventricular (LV) internal size (LV dilation) and LV hypertrophy

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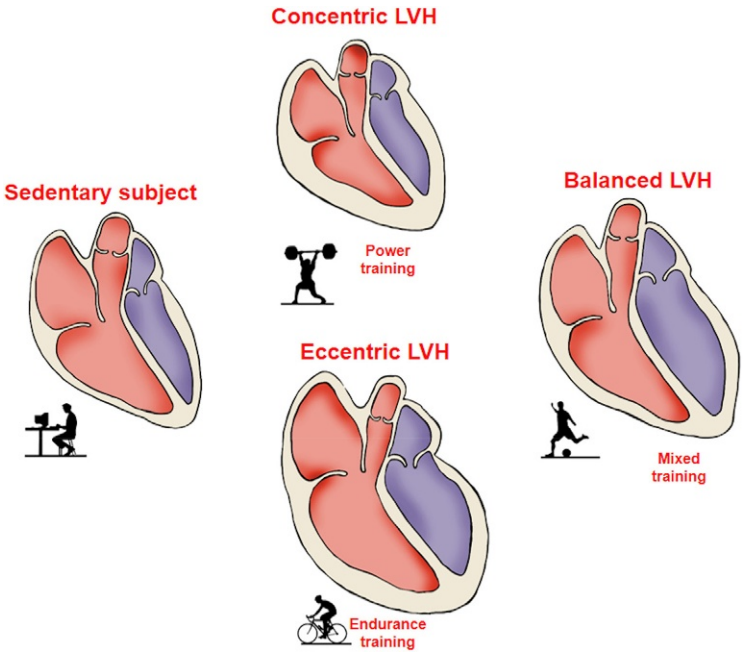

Fig. 1 Models of cardiac remodeling in elite athletes.

(LVH), a response that normalizes LV wall stress. LV dilation and LVH may be pronounced enough to mimic a pathological state. However LV systolic and diastolic function are normal or even supranormal in athlete's heart (Galderisi et al., 2015).

Moreover ejection fraction (EF) calculated as (=100 × SV/LV end-diastolic volume) is normal or can be even marginally reduced at rest in athletes: EF increases markedly during effort when larger LV end-diastolic volume allows to best utilize the Frank–Starling mechanism (the higher preload, the more rapid diastolic filling, and the smaller LV end-systolic volume) and increase SV compared with nonathletes (Abergel et al., 2004).

Concerning the molecular adaptations of the heart, exercise-induced cardiac remodeling is associated with cardiac myocyte hypertrophy and proliferation, sarcomeric and mitochondrial adaptations, electrical remodeling and increased endogenous cardiac stem cell, induced by signaling pathways activated by growth factors, hormones (thyroid and growth), and mechanical stretch. In contrast the adverse effects of endurance training are more similar to the disease-induced pathological remodeling, with beta1-adrenoreceptor desensitization and prolonged tumor necrosis factor alpha-nuclear factor kappa B-p38 signaling (Bernardo and McMullen, 2016).

ECG

The 12-lead ECG in elite athletes has typical characteristics, and it is influenced by the physiologic, electrical, structural, and functional adaptations related to intensive physical activity.

Some ECG findings of athlete's heart may overlap with those described in pathological conditions, thus creating a grey area in ECG interpretation and the need of further investigation (Corrado et al., 2010).

Competitive athletes have increased vagal tone responsible of bradyarrhythmias, such as sinus bradycardia greater than 30 beats/min (bpm), first-degree and second-degree Mobitz type 1 atrioventricular block, sinus arrhythmias, wandering atrial pacemaker, and ectopic atrial rhythm.

Increased vagal tone influences ethnic-specific repolarization changes: white athletes typically show concave ST segment elevation, while Afro-Carribean/black athletes have convex ST segment elevation, often associated with biphasic or deep T-wave inversion in V1–V4 (Yeo and Sharma, 2016).

Manifestation of increased cardiac chamber size and wall thickness includes incomplete right bundle branch block and isolated Sokolow–Lyon voltage criteria (combined amplitude of S wave in V1 + largest R wave in V5 or V6 >3.5 mV, or R wave in aVL >1.1 mV), often regarded as physiologic adaptation in athlete's heart (Yeo and Sharma, 2016).

Standard ECG identifies right atrial abnormalities (RAA) as P-wave amplitude >2.5 mm in DII, DIII, or aVF leads and left atrial abnormalities (LAA) as prolonged P-wave duration greater than 120 ms in leads DI or DII with negative portion of the P-wave greater than or equal to 1 mm in depth and greater than or equal to 40 ms in duration in lead V1. Concerning the prevalence of atrial abnormalities in athletes it may be more common in younger athletes, but there is a low prevalence in adult athletes: Pelliccia et al. (2000) identified the prevalence of LAA to be 4% and RAA to be 0.8% in 1005 trained athletes. As a consequence, in adult athletes, atrial abnormalities should be regarded as abnormal and lead to deeper investigations (Fig. 2).

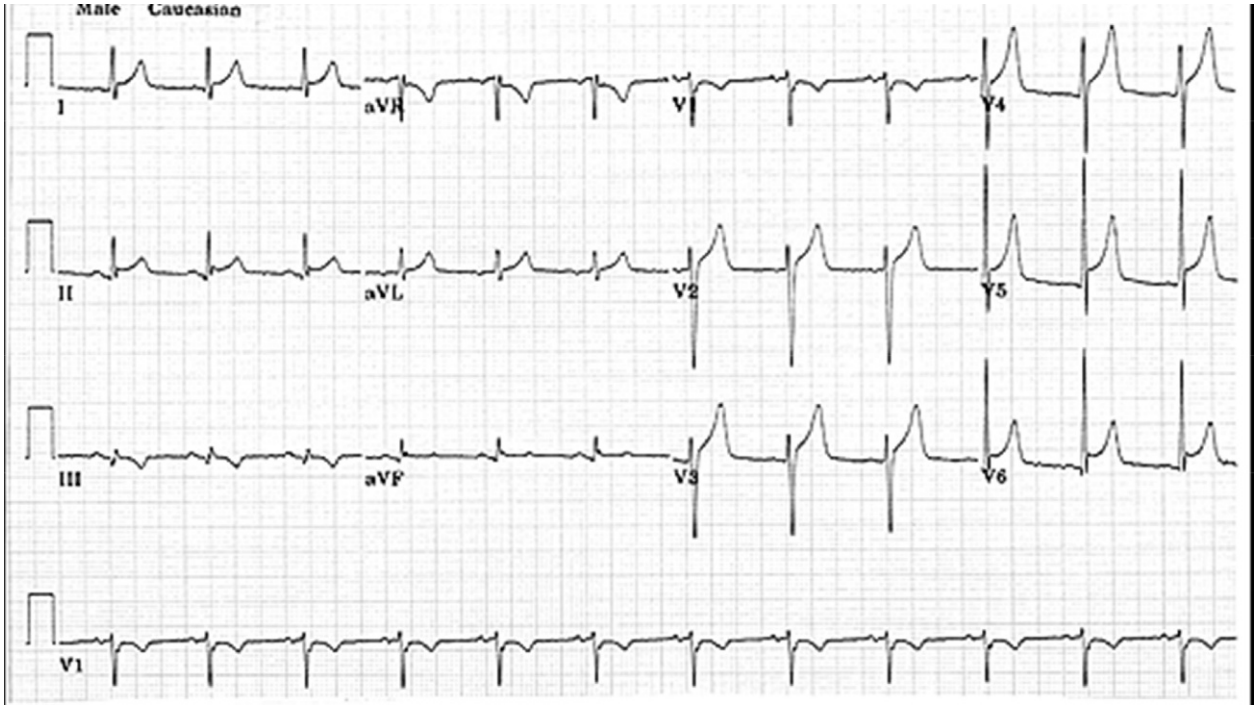

Fig. 2 Common training-related features in an athlete's ECG: isolated QRS voltage criteria for LVH, associated with early repolarization.

ESC 2010 recommendations for interpretation of 12-lead electrocardiogram in the athlete incorporated physiologic adaptations commonly found in athletes, by classifying ECG abnormalities into common and training-related findings versus uncommon and training-unrelated changes, useful to identify those pathological conditions as HCM and arrhythmogenic right ventricle cardiomyopathy (ARVC), with higher risk of sudden cardiac death in competitive athletes (Corrado et al., 2010).

In 2013 the Seattle Criteria (**Table 1**) provided quantitative cut-off, not specified in previous ESC document: these criteria were structured to distinguish normal versus abnormal findings in athletes. The Seattle Criteria include revised contemporary limits for QTc intervals and T-wave inversion and are therefore superior to the ESC criteria in specificity, with further reduction of false-positive findings (Drezner et al., 2013).

The main determinants of ECG patterns in athlete's heart include age, gender, ethnicity, type, and intensity of sport (**Table 2**) (Yeo and Sharma, 2016).

Echocardiographic Analysis of Physiological Adaptations

Echocardiographic analysis is essential in revealing athlete's heart characteristics and in differentiating physiological and pathological LV remodeling.

Left Ventricle

Previous studies on elite athletes reported that 55% had increased LV end-diastolic diameter, and only 15% of them had values >60 mm, with preserved EF; moreover LVH involved all myocardial segments, with a maximal wall thickness <12 mm.(Pelliccia et al., 1991) Conversely an increased wall thickness >15 mm mainly in basal septum is typical of patients with Hypertrophic cardiomyopathy (HCM), with a grey zone between 13 and 15 mm. After a deconditioning period of at least three months a reduction in wall thickness can be observed in athletes, but not in HCM (D'Andrea et al., 2015).

In elite athletes, LV diastolic function is often supranormal (E/A >2 with a low A velocity), in particular in endurance sports, due to a LV remodeling associated with normal or increased myocardial relaxation, as an expression of increased elastic recoil, different from HCM patients, in whom diastolic dysfunction is typically present (E/A < 1) (George et al., 2011).

Pulsed tissue Doppler (TDI)-derived early diastolic myocardial velocity (e') of basal septal and basal lateral wall is increased in athletes, without regional dysfunction ($e'/a' < 1$) and with low E/e' ratio; after ultra long-duration exercise, as marathons, a reduction of e' velocity of both septal and lateral annulus is common (Cardim et al., 2003).

Finally pulsed TDI gives additional information regarding myocardial systolic performance at rest, showing normal or supranormal values in athlete's heart, with normal EF, normal or supranormal stroke volume, and systolic peak velocity (s') > 9 cm/s (Zoncu et al., 2002) (**Fig. 3**).

Table 1 Modified 2013 Seattle criteria (Drezner et al., 2013)

Normal variants in athletes: *do not require further diagnostic evaluation*	
Sinus bradycardia	≥30 bpm
Sinus pauses	<3 s
Ectopic atrial rhythm or junctional escape rhythm	
I AV block	PR interval >200 ms
Mobitz Type 1–II AV block	
Incomplete RBBB	
Isolated QRS voltage criteria for LVH	Except: QRS voltage criteria for LVH occurring with any nonvoltage criteria for LVH (left atrial enlargement, left axis deviation, pathological Q waves, ST segment depression, T-wave inversion)
Early repolarization	J-point elevation, ST elevation, J-waves, or terminal QRS slurring
Convex ST segment elevation associated with T-wave inversion in leads V1–V4 in black/African athletes	
Abnormal variants in athletes: *require further diagnostic evaluation*	
Left atrial enlargement	Prolonged P-wave duration of >120 ms in leads I or II (negative portion of the P wave ≥1 mm in depth and ≥40 ms in duration in lead V1)
Atrial tachyarrhythmias	Supraventricular tachycardia, atrial-fibrillation, atrial-flutter
Extreme sinus bradycardia	<30 bpm
Sinus pauses	≥3 s
Complete LBBB	
Complete RBBB	
Left axis deviation	−30 to −90 degrees
T-wave inversion	>1 mm in depth in two or more leads V2–V6, II and aVF, or I and aVL (excludes III, aVR, and V1)
Right ventricular hypertrophy pattern	R − V1 + S − V5 > 10.5 mm AND right axis deviation >120°
Ventricular preexcitation	PR interval <120 ms with a delta wave and wide QRS (>120 ms)
Long QT interval	QTc ≥ 470 ms (male) QTc ≥ 480 ms (female)
Short QT interval	QTc ≤ 320 ms
Brugada-like ECG pattern	High take-off and downsloping ST segment elevation followed by a negative T wave in ≥2 leads in V1–V3
ST segment depression	≥0.5 mm in depth in two or more leads
Pathologic Q waves	>3 mm in depth or >40 ms in duration in two or more leads (except for III and aVR)
Premature ventricular contractions	≥2 PVCs per 10 s tracing
Ventricular arrhythmias	Couplets, triplets, and nonsustained ventricular tachycardia

AV, atrioventricular; *bpm*, beats per minute; *LVH*, left ventricular hypertrophy; *ms*, milliseconds; *RBBB*, right bundle branch block; *LBBB*, left bundle branch block.

Table 2 Determinants of ECG patterns

Determinants of ECG patterns	*Characteristics*
Age	Adolescent athletes have more frequent juvenile ECG pattern with T-wave inversion in V1–V3 (Migliore et al., 2012)
Gender	Abnormal ECG patterns are four times more frequent in male athletes compared with their female counterparts (Pelliccia et al., 1996)
Ethnicity	Black athletes have more frequent repolarization abnormalities including T-wave inversion, voltage criteria for left and right ventricular hypertrophy, and left and right atrial enlargement (Papadakis et al., 2011)
Type and intensity of sport	Endurance athletes have more common right ventricle hypertrophy and T-wave inversion in V1–V3 Elite athletes show abnormal ECG changes more frequently than amateurs (Pelliccia et al., 1991)

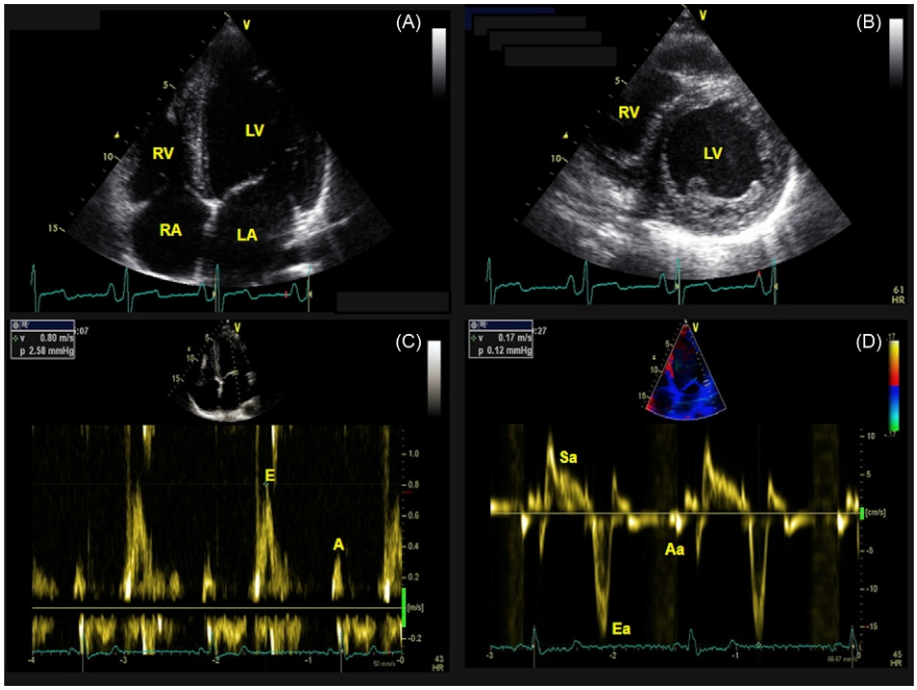

Fig. 3 Standard echocardiography and tissue Doppler in an endurace athlete. Apical 4-chamber view (A) and parasternal short axis view (B) showing eccentric left ventricular (LV) hypertrophy and right ventricular (RV) enlargement. Transmitral flow pattern (C) and Tissue Doppler (D) evidencing "supernormal" early diastolic function. LA: left atrium; RA: right atrium.

Concerning the application of strain variation in competitive athletes at different loading conditions, there is LV adaptation at rest and a load dependency of strain measurements; athletes have normal longitudinal deformation despite left ventricular hypertrophy and higher values for transverse, radial, and circumferential strains when compared with HCM (Richand et al., 2007).

A speckle-tracking echocardiography (STE) analysis shows a different pattern of myocardial deformation between endurance and strength athletes' heart: while global radial strain (GRS) is similar, GLS is lower in runners and global circumferential strain (GCS) is lower in bodybuilders (Szauder et al., 2015).

Finally, STE shows reduction of longitudinal, circumferential, and radial strains and also reduction and delay of peak twisting in triathletes soon after ultra long-duration exercises (Douglas et al., 1990) (**Fig. 4**).

Using three-dimensional (3D) echocardiography Caselli et al. showed LV end-diastolic volumes and mass increases in athletes compared to untrained controls, with preserved LV systolic function; male gender and endurance disciplines had the highest impacts on LV end-diastolic volume and mass (Caselli et al., 2011).

Athlete's heart is characterized by harmonic LV remodeling, differently from patients with hypertrophic or dilated cardiomyopathy. De Castro et al. measured LV remodeling index (LVRI) to describe the pattern of LV remodeling in athletes: athletes' LVRI was similar to that of controls, suggesting that the LV remodeling associated with intensive athletic conditioning does not alter LV geometry (De Castro et al., 2006).

Left Atrium

In a large population of elite athletes, mild left atrial enlargement is relatively common and may be identified as a physiologic adaptation to exercise conditioning. Pelliccia et al. (2005) stated that enlarged left atrial dimension greater than or equal to 40 mm was relatively common in athletes (20%), with the upper limit of 45 mm in women and 50 mm in men, distinguishing physiologic cardiac remodeling from pathologic cardiac conditions. Moreover a mild enlargement of left atrial volume index (LAVi) is relatively common in athlete's heart: D'Andrea et al. (2010) analyzed 615 consecutive elite athletes 370 endurance versus 245 strength-trained and identified a mild enlarged LAVi (29–33 mL/mq) in 150 athletes (24.3%) and a moderate enlarged LAVi (greater or equal to 34 mL/mq) only in 20 (3.2%), all male athletes. Lavi was significantly greater in endurance athletes.

Left atrial myocardial deformation assessed by strain is normal in competitive athletes compared to sedentary controls and hypertensive patients, and it is closely associated with functional capacity.

Especially in middle-aged male athletes with a history of intensive long-term endurance training, left atrial remodeling is associated with a higher risk of atrial fibrillation (D'Andrea et al., 2016).

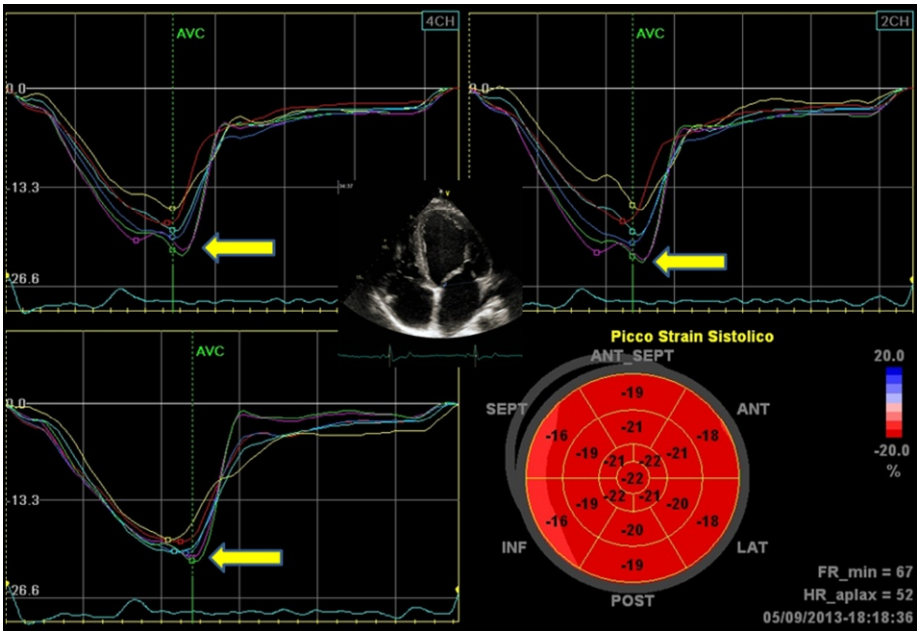

Fig. 4 Automated function imaging (AFI) of left ventricular 2-D strain of endurance athlete, showing normal longitudinal regional myocardial deformation despite left ventricular hypertrophy (arrows) (Douglas et al., 1990).

Right Ventricle

Endurance exercise is associated with cardiac remodeling of the right ventricle (RV), while strength training has minimal impact on RV function. Structural and functional recovery of RV changes in elite athletes might be incomplete after a detraining period (D'Andrea et al., 2009). The right heart (RH) clearly participates in the process of enlargement of the athlete's heart, with an increase in internal diameters and thickness of its free walls.

D'Andrea et al. (2003) documented that RH measures were all significantly greater in highly trained endurance athletes, compared to age and sex-matched, strength-trained athletes.

Resting RV global systolic function, measured by fractional area change (FAC) and Tricuspid Annular Plane Systolic Excursion (TAPSE), may be lower in endurance athletes compared with nonathletic controls. The reduction is more pronounced in the presence of higher RV dilation (D'Andrea et al., 2013).

D'Andrea et al. (2012) identified comparable 2D and 3D RV systolic indexes between endurance athletes and controls. In this setting, a mild reduction in global RV function could be considered a physiological consequence of RV dilation, since an efficient stroke volume will be reached with higher end-diastolic volumes and therefore at lower ejection fraction. On the other hand, a severe reduction in RV global systolic function should be considered an abnormal finding even among athletes (**Fig. 5**).

In athlete's heart RV characteristics can resemble those found in ARVC: in ARVC the enlargement of the RV cavity involves both RV inflow and outflow and may be associated with RV wall segmental morphological and functional abnormalities; in athletes RV enlargement involves only the inflow tract and systolic function is typically normal (Sen-Chowdhry et al., 2004).

The RV is more susceptible than the LV to prolonged exercises, with dysfunction after long-term endurance training (Neilan et al., 2006).

D'Andrea et al. (2010) observed RV dilatation following an ultraendurance triathlon without changes of LV dimension, by using M-mode, 2D echo, and STE (reduction of longitudinal strain about 15% relative to baseline values).

High altitude training induces increased hypoxic pulmonary vasoconstriction and raised hematocrits due to polycythaemia: both changes contribute to raised pulmonary arterial pressure and RV hypertrophy. These RV adaptations are usually reversible in high-altitude sports athletes.

Concerning the pulmonary vascular hemodynamic, whose upper limit of normal was 40 mmHg, endurance-trained athletes show the highest values, compared with strength-trained athletes (D'Andrea et al., 2011).

Finally the inferior vena cava appears to be dilated in a study involving 58 endurance athletes (Goldhammer et al., 1999).

Athlete's Heart and Exercise

Exercise ECG

Dynamic exercise is associated with increased sympathetic drive and a reduction of vagal tone. Rhythm disturbances such as sinus bradycardia, sinus arrest, and wandering pacemaker, as well as the atrioventricular conduction defects, all disappear with exercise.

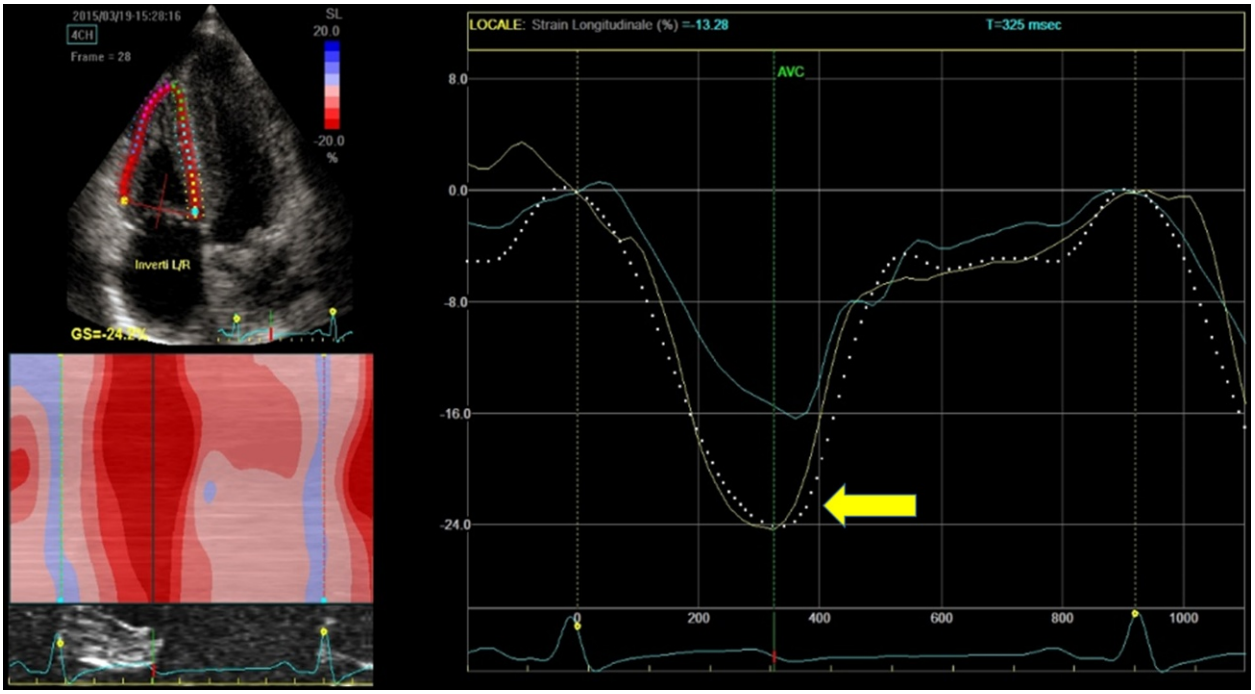

Fig. 5 Right ventricular 2-D strain of endurance athlete, showing normal myocardial longitudinal deformation (arrow) (D'Andrea et al., 2012).

Repolarization abnormalities also normalize with exercise. These observations lead to the conclusion that the described alterations are functional characteristics of athlete's heart and not structural abnormalities (Fagard, 2003).

Exercise Stress Echocardiography

Exercise stress echocardiography (SE) is a feasible, low cost, widely available, and safe technique and is better accepted than pharmacological stress echocardiography by athletes. It gives information about cardiac function, reserve, exercise capacity, and arrhythmias.

SE exercise in athletes may have three different clinically relevant targets besides assessment of ischemia: intraventricular gradients (detected by Continuous Wave (CW) Doppler examination of the Left Ventricular Outflow Tract (LVOT)), pulmonary hemodynamic (Systolic Pulmonary Arterial Pressure (SPAP), LV filling pressure), and evolution of mitral regurgitation (by color Doppler echocardiography), lung sonography for detection of pulmonary congestion (as B-lines). An exercise SE test in athletes can give valuable information about cardiac function, reserve, exercise capacity, and arrhythmias (Lancellotti et al., 2016).

Exercise stress echo is of particular interest in endurance-trained athletes with EF <45%–50% at rest to test if there is contractile reserve (EF increase >5%) during exercise; a considerable EF increase suggests low EF at rest to be related to low preload, and not to LV systolic dysfunction (Galderisi et al., 2015).

SE performed in athletes with LV hypertrophy complaining of shortness of breath or tendency to syncope; a suggestive finding could be an LVOT gradient >50 mmHg during or immediately after exercise in the presence of symptoms. The development of a gradient during exercise in symptomatic athletes is, however, a frequent finding and might help link the reported symptoms (postexercise dizziness or syncope) to a potential cause (the development of an intraventricular gradient) (Cotrim et al., 2010).

Finally stress lung ultrasound (B-lines detection during or immediately postexercise) is useful in two separate settings, HF and extreme physiology.

In healthy elite apnoea, high-altitude trekkers, scuba divers, and extreme athletes involved in sports such as triathlon or marathon, B-lines can be detected in the absence of symptoms of pulmonary oedema (Fagenholz et al., 2007).

Cardiopulmonary Exercise Testing

Cardiopulmonary exercise testing (CET) is a clinically useful technique to assess cardiorespiratory function and exercise capacity in elite athletes.

Previous studies indicated that peak oxygen consumption (pVO2) values in elite athletes usually range between 55 and 70 mL/kg/min and exceed predicted maximum values by as much as 50%; these athletes also have a high anaerobic threshold (AT) and oxygen pulse (O2P) (Sharma et al., 2000).

Anastasakis et al. (2005) compared CET metabolic parameters of 9 strength athletes, 20 endurance athletes, and 27 patients with nonobstructive HCM: endurance athletes had higher pVO2, percentage of predicted Vo2, anaerobic threshold, percentage of

Table 3 Cardiopulmonary exercise testing parameters in elite athletes

	Sharma et al. (2000)	*Anastasakis et al. (2005)*
pVO2 mL/kg/min	66.2 ± 4.1	58 ± 4.5
pVO_2 (% VO_2 max)	156.4 ± 6.8	136.40 ± 11.51
AT l/min	–	2.5 ± 0.4
AT (% VO_2 max)	61.6 ± 1.8	74.55 ± 10.99
O2P mL/beat	27.1 ± 3.2	25.3 ± 2.6
ΔVO2/ΔWR mL/min/W	–	9.2 ± 0.8
VE/VCo2 slope	–	22.3 ± 1.8

AT, anaerobic threshold; *AT%*, percentage of predicted anaerobic threshold; *O2P*, oxygen pulse at peak exercise; *pVo2*, peak oxygen consumption; *pVo2%*, percentage of predicted peak oxygen consumption; *VE*, minute ventilation; *VCo2*, carbon dioxide production; *Vo2*, oxygen consumption; *WR*, work rate.

predicted anaerobic threshold, and oxygen pulse than strength athletes and patients with HCM ($p < 0.05$). None of the CET parameters differed significantly between strength athletes and patients with HCM. The minute ventilation/carbon dioxide production (VE/VCo2) slope was decreased ($p < 0.001$) in the endurance athletes, but similar in patients with HCM and strength athletes. The ΔVo2/Δwork rate (WR) slope was not significantly different between the three groups (**Table 3**).

Sharma et al. (2000) demonstrated a separation in pVO2, AT and O2P between elite athletes with physiological LVH and patients with HCM and mild LVH: a pVO2 >50 mL/kg/min or >20% above the predicted VO2 max, an AT >55% of the predicted VO2 max, and an O2P >20 mL/beat would discriminate physiologic LVH from HCM.

The pVO2 is determined by peak cardiac output and the systemic arterio-venous oxygen difference (A-V): athletes obtain high pVO2 values through large increases in cardiac output and greater widening of the A-V during exercise. The augmentation of stroke volume (SV) is mainly responsible for the increase in cardiac output, while the increase in the A-V is due to the enhanced oxidative capacity within the exercising muscle, resulting from a high cellular mitochondrial concentration (Holloszy, 1967).

Analysis of the oxygen pulse profile (product of SV and A-V) provides evidence for the mechanisms responsible for the higher pVO2 in athletes: the O2P is higher throughout exercise in elite athletes with LVH compared with athletes with HCM, indicating a superior SV and A-V from the beginning to the end of exercise and confirming the physiological differences between the two entities outlined above.

Scharhag et al. analyzed right and left ventricular mass and function in male endurance athletes, demonstrating the correlation of left ventricular mass (LVM) and right ventricular mass (RVM) to maximal oxygen uptake (Vo2max) (Scharhag et al., 2002).

Conclusions

Elite athletes have a physiological remodeling of cardiovascular structures, with structural, functional, and electrical characteristics, caused by intensive and competitive training.

Two main models of sport adaptation of athlete's heart are eccentric hypertrophy, typical of endurance training, and concentric hypertrophy, usual of strength training.

Many instruments, as ECG and echocardiography, are useful to study characteristics of elite athlete's heart and to distinguish the physiological training adaptations of cardiac structures from pathological conditions.

Future improvement in differentiating normal and abnormal cardiac features in athletes is essential to recognize at early-stage cardiac disorders and reduce sudden cardiac death risk.

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Practical Guide to Evidence-Based Management of Heart Failure in the Outpatient Setting

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Introduction

Heart failure remains a highly prevalent and morbid disease. Because of its large public health impact, it has been the focus of avid research for the last several decades. Management has evolved significantly in that time with high-quality evidence demonstrating mortality and quality of life benefits of many pharmacologic and nonpharmacologic interventions (Yancy et al., 2013). In spite of these advances, the three most important patient outcomes in heart failure, namely mortality, hospitalization, and quality of life remain poor. Upon diagnosis, the 5-year life expectancy is 50% (Bui et al., 2011). Heart failure continues to be among the leading causes of hospitalization for patients 65 and older (Hall et al., 2012). There are still many gaps in evidence requiring providers to make clinical decisions based on expert opinion and common sense. However, it is also known that providers are significantly under-prescribing guideline-directed therapies to patients (Mosalpuria et al., 2014).

Given the high prevalence of disease, medical complexity, high hospitalization, and mortality rates of patients with heart failure, in addition to the limited time and resources of providers, outpatient management is challenging. It has been observed that patients cared for by internists are less likely to be on guideline-directed medical therapy (GDMT) than patients cared for by cardiologists (Mosalpuria et al., 2014). This may reflect less familiarity with recommended drugs and concerns about adverse effects. It also suggests that wider dissemination of easily accessible clinical practice guidelines with specific suggestions for safely implementing medication use may improve prescribing habits.

With these considerations in mind, the aim of this review is twofold: (1) help providers improve adherence to guideline-directed care by providing detailed information and practical strategies for implementation and (2) offer practical guidance concerning common management decisions that reflects expert opinion when high-quality evidence is lacking.

Patient Assessment

Heart failure is a clinical syndrome, defined by signs and symptoms that can be caused by dysfunction of virtually any aspect of the heart. However, the majority of heart failure cases are caused by impairment of the left ventricle's ability to fill or eject blood (Yancy et al., 2013). The underlying etiology of heart failure must be identified at diagnosis so that the disease process can be treated and progression can be slowed. Because heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF) are importantly different with regard to demographics, prognosis, and response to therapy, evaluation of ejection fraction is an important branch point in diagnosis and treatment (Yancy et al., 2013). **Table 1** offers the diagnostic cutoffs for HFrEF and HFpEF offered by the AHA/ACC guidelines.

NYHA functional class is another important categorization for standardized clinical assessment of patients with heart failure. It is a simple tool that allows clinicians to communicate the patient's current condition based on the severity of symptoms. Although it relies on subjective information that is based on the patient's report and physician's interpretation of symptoms, it has been shown to correlate with objective measures including the 6 min walk test (Yap et al., 2015). More importantly, it is known from the randomized control trial (RCT) data investigating ACE-inhibitors that NYHA class is a powerful predictor of mortality in HFrEF (Swedberg and Kjekshus, 1988; Cohn et al., 1987) and there is retrospective evidence that it is predictive of mortality in the HFpEF population as well (Ahmed et al., 2006).

The RCTs that underlie the clinical practice guideline recommendations regarding heart failure treatment generally base their inclusion criteria on these definitions. Therefore, selecting the appropriate therapy in patients with heart failure requires evaluation of both a patient's ejection fraction and NYHA class upon diagnosis (**Tables 1** and **2**).

Essentials of the Clinic Visit

Monitoring Clinical Indices

Patient behaviors and understanding of prognosis, medications, and self-care behaviors should be evaluated at every visit. This serves to identify gaps in patient knowledge as well as help patients troubleshoot areas of difficulty with adherence.

Given the prevalence of comorbidities among patients with heart failure, medication review is particularly important not only to ensure appropriate use of heart failure medications, but also address polypharmacy and screen for important medication interactions and contraindications. For example, it is not uncommon for a patient with heart failure to assume taking non-steroidal anti-inflammatory drugs (NSAIDs) for arthritis is safe because it can be purchased without a prescription.

Table 1 Definitions of HFrEF and HFpEF

Ejection fraction (%)	*Classification*	*Description*
≤40	Heart failure with reduced ejection fraction (HFrEF)	Formally referred to as systolic HF
≥50	Heart failure with preserved ejection fraction (HFpEF)	Formally referred to as diastolic heart failure
41–49	HFpEF, borderline	Likely have elements of mild systolic dysfunction and diastolic dysfunction. More research needed to define prognosis and treatment in this group
>40	HFpEF, improved	Include the subset of patients that had once had HFrEF with improvement in EF. Further study is necessary to delineate prognosis and effective treatment in this group

Adapted from AHA/ACC 2013 Guidelines.

Table 2 NYHA Functional Classification

I	No limitations of physical activity
II	Slight limitation of physical activity. Comfortable at rest but symptomatic with activities of daily living
III	Marked limitation of physical activity. Comfortable at rest but symptomatic with less exertion than required for activities of daily living
IV	Symptoms at rest

Adapted from AHA/ACC 2013 Guidelines.

Symptoms

Symptoms of decompensation (e.g., decreased exercise tolerance, orthopnea, worsening dyspnea, increased abdominal girth) and medication side effects and toxicity (e.g., dizziness with standing) should be evaluated at every clinic visit. Patient education regarding symptoms can be performed during this assessment. Patients should be taught to recognize their individual signs of decompensation. Not all patients will develop lower extremity edema or an increase in weight Symptom recognition must be patient-centered.

Signs

Signs of decompensation, dehydration, and medication side effect should be evaluated at every visit.

This includes checking heat rate and rhythm, blood pressure both supine and standing, JVP, pulmonary and cardiac auscultation, abdomen, lower extremities and weight.

Investigations

Laboratory values including creatinine, potassium, magnesium, and sodium should be checked every 3–6 months once the patient is on a stable regime. A complete blood count to check for anemia can be performed routinely once annually. Chest radiograph, cardiac enzymes, and echocardiogram should be performed upon clinical change. The use of basic natriuretic peptide in the monitoring of chronic heart failure is controversial. It is not currently recommended by the AHA/ACC, however RCTs evaluating its utility in chronic heart failure patients are ongoing (Okwuosa et al., 2016) (**Table 3**).

Pharmacological Treatment of Heart Failure

In patients with HFrEF, there are multiple guideline-directed pharmacologic therapies that have a demonstrated mortality benefit. All of these drugs work by addressing the neurohormonal derangements seen in HFrEF. In spite of their demonstrated benefits, it is known that only a fraction of eligible heart failure patients are prescribed these life-prolonging medications (Mosalpuria et al., 2014). Guidelines are much less specific regarding pharmacologic management of HFpEF which represents 50% of patients with heart failure. Although patients with HFpEF have slightly better prognosis than those with HFrEF, (Owan et al., 2006) it is known that their hospitalization rate and quality of life are equally poor (Hoekstra et al., 2011). Therefore, efforts to encourage better adherence to treatment recommendations by providers and patients alike should be undertaken for all patients with heart failure. With this in mind, we offer a review of the medications currently recommended by the AHA/ACC and ESC offering specifics regarding indications, contraindications, and strategies for monitoring in order to address potential concerns about appropriate use by providers who may have limited experience using these medications.

Currently, there are five classes of medications that have demonstrated survival benefit through large high-quality RCTs in the HFrEF population and have therefore been given a Class I recommendation by the ESC and AHA/ACC: angiotensin-converting enzyme inhibitors (ACE-inhibitors), angiotensin receptor blockers (ARBs), aldosterone receptor antagonists (ARAs), beta-blockers (BBs), and angiotensin receptor-neprilysin inhibitors (ARNIs). **Table 4** provides a list of these medications in addition to specific indications, contraindications, common adverse effects, target doses, and strategies for safe titration and monitoring (Yancy et al., 2013; Paul and Page, 2016; McMurray et al., 2014; McIlvennan and Page, 2016; Swedberg et al., 2010).

Table 3 Checklist of outpatient clinic assessment*

Symptoms	*Frequency*
Dyspnea, orthopnea, paroxysmal nocturnal dyspnea, decreased exercise tolerance, chest pain, lower extremity swelling, increasing abdominal girth	Every visit
Physical exam	
HR, BP, weight, JVP, cardiac auscultation, abdomen, lower extremities	Every visit
Investigations	
CBC	Annually
Electrolytes/creatinine	2–3 months
Echocardiogram	Clinical suspicion, worsening symptoms
Cardiac enzymes	Clinical suspicion
Chest X-ray	Clinical suspicion
EKG	Clinical suspicion
Medication review	Every visit
Patient education	
Symptom recognition	Every visit
Medication reconciliation/education	Every visit
Exercise	Every visit
Diet	Every visit
Advanced directive	Progression of disease

*Aspects adapted from Nicholis et al., 2007.

Table 4 Drugs that decrease morbidity and mortality in HFrEF

	Common drugs in the class with initial and target doses	[a]*Class Ia ACC/AHA recommendation*	*Contraindications*	*Common adverse effects*	*How to titrate*	*Monitoring*
Angiotensin-converting enzyme	Lisinopril Initial dose 2.5–5 mg daily Target dose 20–40 mg daily Enalapril Initial dose 2.5 bid Target dose 10–20 mg bid	Recommended in all patients with HFrEF and current or prior HF symptoms to reduce morbidity and mortality	Angioedema' Cr > 2.5 mg/dL, pregnancy, bilateral renal artery stenosis, hyperkalemia (K > 5.0 meq/L)	Cough, Hyperkalemia, neutropenia, renal dysfunction, angioedema, hypotension. Dysgeusia	Increase at intervals no less than 2 weeks to the target dose based on blood pressure and renal function	Serum, renal function, CBC, blood pressure
Angiotensin receptor blocker	Losartan Initial dose: 25–50 mg daily Target dose 50–150 mg daily Valsartan Initial dose 20–40 mg bid Target dose 160 mg bid Candesartan Initial dose 4–8 mg daily Target dose 32 mg daily	Recommended in patients with HFrEF who are ACE-inhibitor intolerant (e.g., cough, angioedema)	Angioedema Cr > 2.5 mg/dl, pregnancy, bilateral renal artery stenosis, hyperkalemia (K > 5.0 meq/L),	Hyperkalemia, neutropenia, renal dysfunction, hypotension	Same as ACE-inhibitors	Same as ACE-inhibitors
Aldosterone receptor antagonists	Spironolactone Initial dose 12.5–25 mg daily Target dose 25 mg daily or bid Eplerenone Initial dose: 25 mg daily Target dose 50 mg daily	Recommended in patients with NYHA class II–IV and who have a LVEF <35% unless contraindicated; recommended in patients with NYHA class II with a history of prior CV hospitalization or elevated natriuretic peptide levels	Serum creatinine <2.5 mg/dL in men and <2.0 mg/dL in women; eGRF <30 mL/min/1.72 m^2)	Gynecomastia (with spironolactone), hyperkalemia, renal dysfunction	Increase dose every 2 weeks based on serum potassium and renal function; potassium supplements may need to be reduced or discontinued	
Close monitoring of potassium and serum creatinine should be checked in 3 days of initiation and at 1 week and at least monthly for the first 3 months; the risk of						

Table 4 (Continued)

	Common drugs in the class with initial and target doses	*Class Ia ACC/AHA recommendation*	*Contraindications*	*Common adverse effects*	*How to titrate*	*Monitoring*
	hyperkalemia is increased with higher doses of ACE-inhibitors and in diabetics					
Beta-blockers	Bisoprolol Initial dose: 1.25 mg daily Target dose: 10 mg daily Carvediolol Initial dose: 3.125 mg BID Target dose 25 mg BID; 50 mg BID for patients >85 kg Metoprolol succinate Initial dose: 12.5–25 mg daily Target dose: 200 mg daily	Recommended for all patients with current or prior symptoms of HFrEF unless contraindicated	Acute decompensated heart failure, 2nd or 3rd degree heart block without a pacer, bronchospastic disease	Fatigue, bradycardia, hypotension	Increase dose every 2 weeks based on heart rate, blood pressure, and weight	Close monitoring of heart rate, daily weights, and blood pressure
Angiotensin-neprilysin inhibitors	Sacubitril/valsartan Initial dose dependent on initial ACE-inhibitor or ARB dose[b] Target dose 97/103 mg BID	Recommended as replacement of an ACE-inhibitor or ARB in patients with chronic HFrEF NYHA class II-III who tolerate an ACE-inhibitor or ARB	Concomitant ACE-inhibitor or aliskiren, history of angioedema with prior ACE-inhibitor or ARB use, pregnancy, severe hepatic dysfunction (Child-Pugh Class C)	Hypotension, hyperkalemia, angioedema, renal dysfunction	Increase dose every 2 weeks based on blood pressure, renal function, and serum potassium	Same as ACE-inhibitors
Vasodilators	Hydralazine/Isosorbide (Bidil) Initiate dose: 37.5 mg/20 mg (1 pill) TID Target dose: 2 pills TID	Recommended for self-described African Americans with NYHA class III-IV HFrEF receiving optimal therapy with an ACE-inhibitor or ARB and a BB, unless contraindicated	Concomitant use of phosphodiesterase type-5 inhibitors (i.e., avanafil, sildenafil, tadalafil, vardenafil)	Headache, dizziness, flushing, tachycardia, drug-induced lupus (hydralazine)	Increase dose every 1–2 weeks based on blood pressure and tolerability	Blood pressure, heart rate, CBC

ACC: American College of Cardiology, *ACE*: angiotensin-converting enzyme, *AHA*: American Heart Association, *ARA*: aldosterone receptor antagoinists, *ARB*: angiotensin receptor blocker, *BID*: twice daily, *eGFR*: estimated glomerular filtration rate, *CBC*: complete blood count, *HFrEF*: heart failure with reduced ejection fraction, *NYHA*: New York Heart Association, *TID*: three times daily.

Enalapril $\leq$10 mg/day or equivalent or valsartan $\leq$160 mg/day or equivalent, or no ACE-inhibitor or ARB: Washout ACE-inhibitor or ARB for 36 h, then begin sacubitril/valsartan 24/26 mg daily.

Severe renal dysfunction (eGFR $<$ 30 mL/min/1.73 m^2) or moderate hepatic dysfunction.

(Child-Pugh Class B): Washout ACE-inhibitor or ARB for 36 h, then begin sacubitril/valsartan 24/26 mg daily.

[a]Strongest recommendation, benefit greatly outweighs risks.

[b]Enalapril $>$10 mg/day or equivalent or valsartan $>$160 mg/day or equivalent: Washout ACE-inhibitor or ARB for 36 h, then begin sacubitril/valsartan 49/51 mg daily.

Drugs With Demonstrated Mortality Benefit in HFrEF

Angiotensin-converting enzyme inhibitors, Angiotensin Receptor Blockers and Beta-Blockers

Angiotensin-converting enzyme inhibitors and ARBs work by counteracting the renin angiotensin system thereby reducing cardiac remodeling and fluid retention leading to improved symptoms. In placebo-controlled trials, ACE-inhibitors have been shown to significantly reduce morbidity and mortality (see **Appendix 1**). Additionally, when compared with captopril, losartan was just as effective in reducing all-cause mortality, sudden death, and hospitalization. Due to a lack of effect on bradykinin, ARBs are indicated in patients who cannot tolerate ACE-inhibitors due to cough or angioedema. Use of ACE-inhibitors and ARBs together is not routinely recommended due to lack of mortality benefit and risk of hyperkalemia and hypotension (Yancy et al., 2013).

It is important to note that only certain beta-blockers have been recommended for use in patients with HFrEF consisting of bisoprolol, carvedilol, and metoprolol succinate (**Appendix 2**). These beta-blockers have been shown to reduce mortality and are thought to offer cardio protection by down regulating the sympathetic nervous system, have been shown to reduce remodeling, as well as improve ejection fraction (Yancy et al., 2013) (see **Appendix 2**) (Yancy et al., 2013).

All patients with HFrEF should be treated with an ACE-inhibitor or an ARB and a guideline-based beta-blocker unless there is a documented contraindication. Documentation of both classes of medication is a *JACHO core measure* (JACHO, 2017). This indicates that current standard of care requires providers to ensure patients who can tolerate these medications receive them and that they are titrated to as close to the target dose as the patient is able to tolerate.

Aldosterone receptor antagonists, Hydralazine/Isosorbide and Angiotensin Receptor-Neprilysin Inhibitors

ARAs, hydralazine/isosorbide, and ARNIs are the three remaining drug interventions that have a demonstrated mortality benefit in selected HFrEF populations and currently have Class 1 indications (Yancy et al., 2013; Yancy et al., 2016). These medications are considered "add on" therapy to the patient already on ACE/ARB and BB (see **Appendices 1** and **2**). There are circumstances in which these medications are given to patients without ACE/ARB and/or BB if ACE/ARB and/or BB are not tolerated.

The combination of hydralazine and isosorbide dinitrate has demonstrated mortality benefit in African Americans with heart failure receives a class 1 recommendation for persistently symptomatic Class III–IV African American patients already on an optimal dose of an ACE-inhibitor and BB (Yancy et al., 2013) (see **Appendix 1**). In patients who cannot tolerate an ACE-inhibitor or ARB (e.g., pregnancy, renal dysfunction), the combination of hydralazine and isosorbide dinitrate can be substituted for the ACE-inhibitor or ARB (Yancy et al., 2013).

In HFrEF, the hormone aldosterone has been associated with several negative pleotropic effects within the heart, kidney, and vasculature. In the heart, aldosterone stimulates growth-promoting and profibrotic effects on myocytes, which could lead to ventricular remodeling, myocardial fibrosis, and premature myocyte cell death. Outside of its effects on the reabsorption of sodium and water in the kidney, stimulation of mineralocorticoid receptors in the renal cortex can lead to ischemic injury, mesangial cell proliferation, and nephrosclerosis. Two ARAs are currently available: spironolactone, a nonselective and eplerenone, a selective ARA. Both are recommended in patients with NYHA class II to IV who have an LVEF of 35% or less, an estimated glomerular filtration rate exceeding 30 mL/min/1.73 m^2, and a serum potassium less than 5 mEq/L to reduce morbidity and mortality. In addition, ARAs are recommended to reduce morbidity and mortality after an acute myocardial infarction in patients who have an LVEF of 40% or less who develop symptoms of heart failure or who have a history of diabetes (Yancy et al., 2013). ARAs can be very effective in removing fluid from the periphery especially in combination with loop diuretics.

ARNIs are a new class of medications for heart failure. They work by up regulating several vasoactive peptides including naturetic peptide and bradykinin, thereby decreasing vasoconstriction, sodium retention, and cardiac remodeling. Sacubitril/valsartan demonstrated a mortality benefit over enalapril (see **Appendix 2**). The AHA/ACC were updated its 2016 Heart Failure Management Guidelines in order to incorporate recommendations with regard to its use. The guidelines now recommend ARNIs replace an ACE-inhibitor or ARB in patients with symptomatic HFrEF that are able to tolerate ACE-inhibitors and ARBs. Importantly ARNIs should not be used within 36 h of the last dose of an ACE-inhibitor due to the risk of angioedema. This requires extra attention when switching a patient from an ACE-I to an ARNI in that the patient will need to be completely off ACE at least 36 h before switching to the ARNI. A clinician's oversite to assure this is done correctly is required. ARNIs should not be administered to patients with a history of angioedema. There is a lower incidence of renal dysfunction and hyperkalemia compared to enalapril with ARNI. However, hypotension is a major concern when initiating sacubitril/valsartan, which can be particularly concerning in the older adult (McMurray et al., 2014; Desai et al., 2017). Since central neprilysin is responsible for degradation of Aβ plaques which are associated with dementia and Alzheimer disease, controversy exists regarding the effect of ARNIs on increased neurocognitive disorders (Campbell, 2017).

Other Recommended Agents

Loop diuretics are a class 1 recommendation in patients with overload for relief of symptoms. Although they have not been shown to improve mortality they have been shown to improve exercise capacity (Ponikowski et al., 2016a). Furosemide can be started once or twice daily and titrated to maintain a patient's dry weight. Patients and care givers can be taught to use a flexible diuretic regime that they can titrate to weight or symptoms (Ponikowski et al., 2016a). Patients with HFrEF do not need to take a loop diuretic every day, or perhaps not at all. The use of loop diuretics should be tailored for the individual patient. Reassessment of the use of loop diuretics should take place at every visit and doses/frequency adjusted if needed. Close monitoring of volume status and electrolytes is recommended for all patients on diuretics as fluctuations in fluid and salt intake can disrupt euvolemia leading to hospitalization for decompensated heart failure as well as acute kidney injury and electrolyte disturbance from over diuresis.

Diuretic resistance is a commonly occurring problem often caused by gut edema or worsening renal function. Switching to bumetanide or torsemide is sometimes helpful as these two agents have better oral bioavailablity than furosemide allowing for a more predictable diuretic response (Yancy et al., 2013). Another strategy for improving diuresis in patients already on a loop diuretic is to add a thiazide like metolazone, although this approach requires close monitoring as overdiureses and electrolyte abnormalities is a common problem (Redfield, 2016). Metolazone can be used as an "emergency" diuretic in addition to the loop diuretic but daily dosing is not recommended.

Ivabridine

Another new class of medications are those that reduce heart rate. In patients with HFrEF, resting heart rate is a strong predictor of adverse outcomes. All-cause mortality, cardiovascular mortality, and HF hospitalizations are directly associated with heart rate alteration, and all increase progressively as heart rate rises above 70 beats per minute (bpm) (Ahmadi-Kashani et al., 2009). Ivabridine, an I_f channel blocker, is a sinus node inhibitor which primarily effects chronotropy and not inotropy. Ivabridine has been shown to reduce hospitalizations thereby earning IIa B-R rating for symptomatic HFrEF patients in normal sinus rhythm that are already on a max tolerated beta-blocker or have contraindication to beta blockade (Yancy et al., 2016). Ivabradine should be avoided in patients with acute decompensated heart failure, systolic blood pressures <90 mm Hg, second or third degree AV block without a pacemaker, and a history of atrial fibrillation (Bohm et al., 2010).

Digoxin

Although digoxin has no known mortality benefit, it has been shown to decrease the frequency of hospitalization in patients with HFrEF, thereby earning it a IIA level B recommendation from the AHA which deems it reasonable for patients who are on guideline recommended therapy but still symptomatic (Digitalis, 1997; Batterman and De Graff, 1947). Its inotropic and chronotropic effects make digoxin most useful in HFrEF patients with relative hypotension and atrial fibrillation. Given its narrow therapeutic range and renal clearance providers should watch for toxicity. Serum digoxin levels should be checked within 7 days of initiating therapy with a goal range between 0.5 and 0.9 ng/mL. As digoxin is cleared renally, dose adjustments should be made in patients with renal dysfunction (Yancy et al., 2013). Patients over the age of 75 rarely need more than every-other-day dosing. Withdrawal of digoxin in these patients can result in decompensation and should be done only when absolutely necessary and with close follow-up.

In their 2016 Management Guidelines, the ESC provided a rational approach to the pharmacotherapy of patients with HFrEF (Ponikowski et al., 2016a) (**Fig. 1**).

Pharmacological Treatment of HFpEF

In spite of many RCTs to identify medications that significantly improve mortality in HFpEF patients, none have been identified. Data suggest that HFpEF patients are hospitalized with the same frequency as HFrEF patients but that they are hospitalized and die more often from comorbid conditions than HFrEF patients (Owan et al., 2006). The classic demographic for the patient with HFpEF is an older woman with longstanding hypertension. The pharmacologic management of HFpEF focuses on optimal management of comorbidities. In addition to hypertension, rate control in atrial fibrillation is particularly important in HFpEF so that diastolic filling of the left ventricle is maximized.

Loop diuretics, for symptom and volume control, remain the only Class 1 recommended drug in HFpEF. Careful titration is important as symptoms can easily occur from over diuresis due to preload dependence. Although the recent TOPCAT trial which investigated the utility of spironolactone in HFpEF management did not demonstrate a mortality benefit, it did show a reduction in hospitalization (Pitt et al., 2014). Clinically, spironolactone can be very helpful to reduce volume overload in these patients.

Management of Important Co-morbidities in Heart Failure

Blood pressure management

Optimal blood pressure management is essential in the management of both HFrEF and HFpEF. From a cardiovascular perspective, blood pressure should be kept as low as is tolerated in HFrEF patients, in order to unload a weakened heart. However, in elderly patients, this goal must be tempered with fall risk and a propensity for orthostasis. Because there are several agents indicated in HFrEF that lower blood pressure, it is often difficult to attain target doses due to hypotension leading to both symptoms and renal dysfunction. Many HFrEF patients are not able to tolerate an ACE-inhibitor or ARB in addition to their BB due to renal dysfunction. Providers must consider all aspects of the patient when titrating GDMT in order to avoid iatrogenic complications.

Given the lack of life extending drugs in the management of HFpEF, optimal management of comorbidities, hypertension being among the most important, is the cornerstone of management. The Eighth Joint National Committee guidelines do not offer blood pressure targets for HFpEF but do recommend a target of less than 150/90 mm Hg for patients 60 or older and less than 140/90 mm Hg in patients with chronic renal insufficiency and proteinuria (James et al., 2014). The most recent landmark trial investigating blood pressure targets, SPRINT, demonstrated a mortality benefit at a target of less than 120 systolic however, patients with heart failure were excluded from this trial (Wright et al., 2015).

Anemia

Initial assessment of patients with heart failure should include screening for anemia given the high incidence in this population (Hindricks et al., 2014). Iron-deficiency anemia is associated with decreased exercise capacity in heart failure

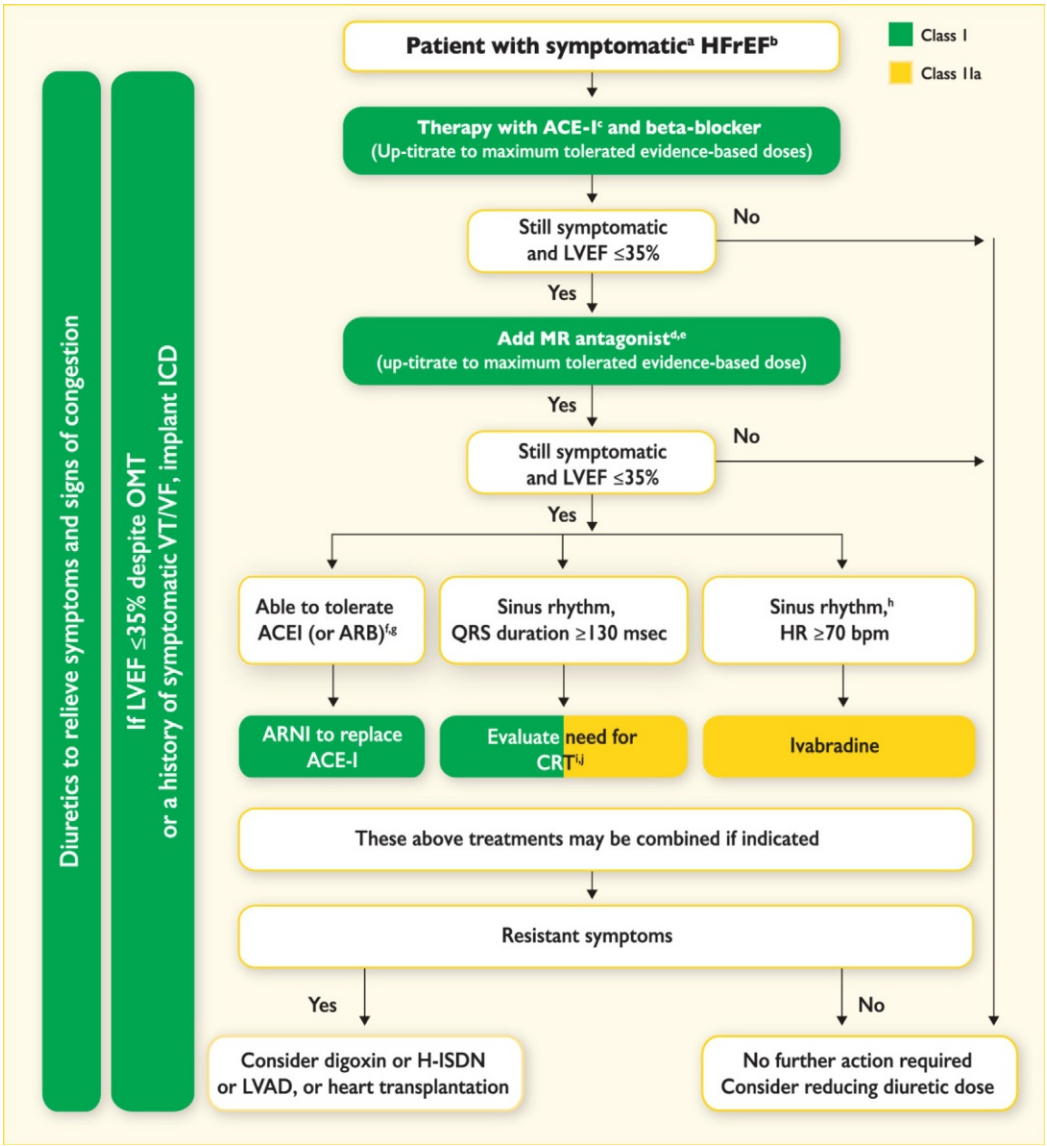

Fig. 1 Therapeutic algorithm for the patient with symptomatic HFrEF. Used with permission for Reference (ESC 2016 Guidelines). *Green* indicates a class I recommendation from the ESC; *yellow* indicates a class IIa recommendation from the ESC. *ACEI* = angiotensin-converting enzyme inhibitor; *ARB* = angiotensin receptor blocker; *ARNI* = angiotensin receptor-neprilysin inhibitor; *BNP* = B-type natriuretic peptide; *CRT* = cardiac resynchronization therapy; *HF* = heart failure; *HFrEF* = heart failure with reduced ejection fraction; *H-ISDN* = hydralazine and isosorbide dinitrate; *HR* = heart rate; *ICD* = implantable cardioverter defibrillator; *LBBB* = left bundle branch block; *LVAD* = left ventricular assist device; *LVEF* = left ventricular ejection fraction; *MR* = mineralocorticoid receptor; *NT-proBNP* = N-terminal pro-B type natriuretic peptide; *NYHA* = New York Heart Association; *OMT* = optimal medical therapy; *VF* = ventricular fibrillation; *VT* = ventricular tachycardia. a = Symptomatic = NYHA Class II-IV. b = HFrEF = LVEF <40%. c = If ACE-inhibitor not tolerated/contraindicated, use ARB. d = If MR antagonist not tolerated/contraindicated, use ARB. e = With a hospital admission for HF within the last 6 months or with elevated natriuretic peptides (BNP > 250 pg/mL or NTproBNP > 500 pg/mL in men and 750 pg/mL in women). f = With an elevated plasma natriuretic peptide level (BNP ≥ 150 pg/mL or plasma NT-proBNP ≥ 600 pg/mL, or if HF hospitalization within recent 12 months plasma BNP ≥ 100 pg/mL or plasma NT-proBNP ≥ 400 pg/mL). g = In doses equivalent to enalapril 10 mg b.i.d. h = With a hospital admission for HF within the previous year. iCRT is recommended if QRS ≥ 130 ms and LBBB (in sinus rhythm). j = CRT should/may be considered if QRS ≥ 130 ms with non-LBBB (in a sinus rhythm) or for patients in AF provided a strategy to ensure biventricular capture in place (individualized decision).

patients (Wright et al., 2015) and the 2017 AHA/ACC/HFDA Management Guidelines now advise intravenous iron repletion as a Class IIb recommendation for patients with NYHA II and III HF with iron deficiency (ferritin <100 ng/mL or 100 to 300 ng/mL if transferrin saturation is <20%). Data from RCTs has suggested that IV iron replacement can improve both functional status and quality of life (Oyanguren et al., 2016; Feltner et al., 2014). Of note, uncertain evidence exists for oral iron repletion in the setting of anemia with heart failure. Additionally, the 2017 Guidelines recommend against erythropoietin-stimulating agents as the body of evidence suggests no benefit and possible harm due to increased risk of venous thromboembolism and stoke (Wright et al., 2015).

Sleep Disordered Breathing

Sleep disorders are a common comorbidity in the heart failure population with 60% having either central or obstructive sleep apnea (Rocha and Menezes Falcao, 2016). The AHA/ACC/HFSA 2017 Management Guidelines recently updated the recommendations to reflect new evidence related to the diagnosis and management of disordered breathing in patients with cardiovascular disease (Wright et al., 2015). The guidelines now deem a formal sleep assessment in patients with NYHA Class II–IV heart failure with suspicion of sleep-disordered breathing or excessive daytime sleepiness as reasonable (Class IIa) because of the importance of distinguishing obstructive from central sleep apnea (Wright et al., 2015). Continuous positive airway pressure (CPAP) is deemed reasonable (Class IIb) in patients with cardiovascular disease based on a recent high-quality RCT demonstrating an improvement in sleep quality, though no difference in the rate of cardiovascular events (Ponikowski et al., 2016c). An increase in mortality has been demonstrated in symptomatic HFrEF patients with central sleep apnea placed on nightly adaptive servo-ventilation and thus it now receives a Class III rating for harm (Wright et al., 2015).

Implantable Cardiac Devices

In addition to medications, certain implantable cardiac devices have been shown to offer mortality benefit in patients with HFrEF. Providers should be aware of guideline indications so that timely referral to an electrophysiologist can be made for placement.

Implantable Cardiac Defibrillators

Implantable cardioverter defibrillators (ICDs) are implantable devices that, depending on the configuration, are able to perform cardioversion, defibrillation, and pacing of the heart in the event of an arrhythmia. The devices are composed of a generator that is implanted in the subcutaneous tissues below the left clavicle and leads that travel from the generator to lodge in the right ventricular septum which is able to cardiovert or defibrillate if an arrhythmia occurs. There is high-quality evidence that ICDs prevent sudden cardiac death (SCD) in patients with both ischemic and nonischemic cardiomyopathy (Okwuosa et al., 2016). Current AHA/ACC guidelines (Yancy et al., 2013) give ICD placement a class IA recommendation for primary prevention of SCD in symptomatic HFrEF patients on optimal GDMT with an EF 35% or less at least 40 days post-MI or 90 days post-PCI or CABG. ICDs receive a Class IB recommendation for primary prevention in NYHA Class I patients on GDMT with an EF of 30% or less at least 40 days post MI. ICDs are not recommended for patients with a prognosis of less than 1 year.

Cardiac Resynchronization Therapy

In patients with significant conduction disease, disynchronous contraction of the left and right ventricles can lead to reduced forward flow and worsening of symptoms. Cardiac resynchronization therapy (CRT) in which both the left and right ventricles are paced has been shown to significantly improve exercise tolerance, ejection fraction, and quality of life in patients with significant systolic dysfunction and intraventricular conduction abnormalities (Okwuosa et al., 2016). CRT is achieved by placement of a biventricular pacing device similar to an ICD but instead of a single lead traveling to the right septum there is a second ventricular lead that travels through the coronary sinus and is lodged in the outer wall of the left ventricle. CRT currently holds an AHA/ACC Class 1A/B recommendation (Yancy et al., 2013) for symptomatic HFrEF patients on GDMT with EF of 35% or less in sinus rhythm with LBBB and QRS of 150 ms or greater.

Implantable Sensors

In an effort to improve outcomes, there have been recent trials investigating the use of implantable monitors that allow earlier detection of volume overload. The information from these implanted devices can be transmitted to providers electronically who can then titrate medications more quickly and frequently, thereby theoretically averting frank decompensation and hospitalization. The two trials that have demonstrated the utility of implantable sensing devices are the CHAMIPION and IN-TIME trials which investigated the use of intrapulmonary pressure monitoring and multiparameter monitoring in patients with ICDs, respectively (Abraham et al., 2016; Hindricks et al., 2014)

In the CHAMPION trial (Abraham et al., 2016), the Cardiomems device was implanted in the pulmonary arteries of study subjects and used to transmit pulmonary artery pressures to study investigators. The transmitted data was reviewed at least weekly and medications were adjusted based on the transmitted information. The device was approved by the FDA for use in chronic heart failure patients after demonstrating a significant reduction in heart failure-associated hospitalizations.

The IN-TIME study (Hindricks et al., 2014) population included HFrEF patients with ICDs. In the intervention group, abnormal telemetry data was transmitted to study investigators at which point weight, medication compliance, and heart failure symptoms were recorded. At 12 months, the mortality rate in the monitored group was lower than that of the control group.

Based on the results of the CHAMPION and IN-TIME trials, these two approaches have received a Class IIB recommendation by the ESC. Whether a provider uses this tool in caring for patients will depend on the resource environment. One of the challenges in using this technology in a real world setting is having the ability to acquire and follow-up on the available data quickly enough to improve outcomes.

Nonpharmacologic, Nondevice Interventions

Multidisciplinary programs

A multidisciplinary approach to the care of patients with heart failure, both HFpEF and HFrEF, has been shown to reduce mortality and readmission rates (Oyanguren et al., 2016; Feltner et al., 2014) and receives a Class IA recommendation by the ESC (Ponikowski et al., 2016a). Although there is heterogeneity among multidisciplinary interventions shown to be effective, key aspects consist of optimization of medical therapy, patient education that focuses on self-care including patient involvement in symptom monitoring, medication adherence, an exercise training (ET) regime, close follow-up and health-care access that allows for prompt assessment and invention when clinical changes occur.

Patient and caregiver education in self-care behaviors

Promoting self-care includes providing patients with information regarding self-monitoring of symptoms, medication adherence, exercise, diet, weight, alcohol consumption, and smoking cessation. There is growing evidence that patient education of self-care practices is an effective way to decrease hospitalization and mortality rates (Rocha and Menezes Falcao, 2016). Thus, a comprehensive patient education program is now considered an essential part of care in patients with heart failure and part of the major society guidelines (Yancy et al., 2013; Ponikowski et al., 2016b).

Time to discuss and clarify aspects of the disease process and management should be part of every clinic appointment. Providers should be prepared to operationalize their recommendations for patients. For example, providers should ensure that patients are weighing themselves in the same way at the same time every morning. Given the time constraints inherent in many physician clinic schedules, mid-level providers as well as pharmacists, physical therapists, and dieticians have become an essential part of many patient education and self-care programs (Mosalpuria et al., 2014). With each clinic visit, patients should receive clarification, coaching, and encouragement on these self-care behaviors. Providers should help patients identify and troubleshoot barriers to self-care.

In addition, a significant proportion of the heart failure population are older adults and cognitive impairment is common (Bressler et al., 2017). Strategies to help improve these patients' ability to adhere to self-care and medication regimes include frequent visits, written directions offered in large print, and importantly, engagement and education of a caregiver (**Tables 5** and **6**).

Behavior change

Even after receiving education regarding self-care, it is often difficult for patients to make the required changes in lifestyle. Given the impact of self-care on patient outcomes, research investigating how providers can effectively facilitate behavioral change is ongoing. Already there is promising data demonstrating that motivational interviewing is associated with improved adherence to self-care behaviors (Masterson Creber et al., 2016) and a decrease in hospitalizations among patients with heart failure (Riegel et al., 2016). Motivate-HF, a RCT designed to confirm the benefit and obtain a better estimate of the magnitude of effect is underway (Vellone et al., 2017). Given the growing evidence for its effectiveness in improving outcomes in patients with heart failure and other chronic diseases, coupled with the relative ease in attaining basic skill in motivational interviewing (Masterson Creber et al., 2015), we encourage providers to seek training and begin engaging heart failure patients in this technique.

Diet

Given one of the common causes of heart failure decompensation is dietary indiscretion, patient education regarding appropriate diet is important. While experts and major society guidelines agree that a low sodium diet is important for the majority of patients

Table 5 Essential goals of patient and caregiver education

Recognize signs and symptoms of early decompensation (which is often individualized) as well as symptoms of drug toxicity
Check and record BP and pulse
Check accurate daily weights (e.g., taken at the same time while wearing the same thing every day on an accurate scale)
Demonstrate an understanding of how to take all prescribed medications and the importance of not missing doses and obtaining refills on time. The provider should assess for rationing of medications and help the patient troubleshoot difficulty with adherence to a medication routine
Participate safely in regular physical activity or structured exercise training program
Impart skills that will allow patients to eat a low sodium diet (e.g., ensure they are able to interpret food labels and have a plan for avoiding convenient but high sodium foods like fast food)

Table 6 Strategies and tools to facilitate self-care

Screen for new barriers at every visit so that the provider can help troubleshoot
Create a learning environment that encourages questions and a feeling of empowerment
Ensure teaching is structured and systematic to help ensure all essential topics are covered
Ensure teaching is at appropriate literacy level and is culturally sensitive
Involve family members and caregivers in teaching sessions so that they can facilitate healthy behaviors
Help patients set realistic short-term goals for themselves
Involve multiple modalities (e.g., provide and encourage symptom, diet, and exercise journaling) and multidisciplinary individuals (e.g., provide referral to nutritionist, pharmacists, physical therapists, and nurses to provide additional education).

with heart failure, there is much controversy regarding exactly how much sodium constitutes a "low sodium" diet. This discrepancy reflects both a paucity of data and conflicting results. Although the current evidence is limited, AHA/ACC recommends a low sodium diet, less than 3 g per day for all symptomatic patients, and those with frequent heart failure exacerbations (Yancy et al., 2013). A careful diet history will help a provider determine if dietary indiscretion is an issue for an individual patient. Further complicating this issue is that patients with heart failure often have additional dietary restrictions secondary to comorbid diseases, like diabetes and chronic renal insufficiency making adherence to all recommendations impractical.

Fluid restriction while not routinely recommended is important in certain patients. Many patients experience excessive thirst due to the upregulation of the renin angiotensin system and antidiuretic hormone. A careful history will help a provider determine if intensification of hormonal blockade will benefit an individual patient in addition recommending limiting fluid intake to 1.5 L per day.

It is important for providers to discuss the details of how patients can adhere to these recommendations effectively. Asking the patient to keep a log of food and fluid consumption to discuss at clinic visits is helpful. Providers should also be mindful of the appropriate diet for a particular patient's comorbidities. Referral to a nutritionist is recommended in order to reinforce and further operationalize recommendations. It should be mentioned that in advanced disease, when goals of care become focused on palliation, dietary recommendations should be changed accordingly.

Exercise

The latest US (Yancy et al., 2013) and European (Ponikowski et al., 2016a) practice guidelines designate ET a Class IA recommendation for patients with stable HFrEF. Evidence underlying this recommendation includes results of HF-ACTION trial, the largest RCT to date evaluating the effects of exercise in HFrEF patients. When its results were adjusted for important prognostic factors, HF-ACTION showed that patients with HFrEF, who were receiving guideline-directed pharmacologic and device therapies plus a supervised home-based aerobic program, had lower rates of hospitalization, all-cause mortality, and better quality of life than patients receiving guideline-directed pharmacologic and device therapies alone (O'Connor et al., 2009). A meta-analysis of earlier smaller RCTs also demonstrated mortality benefit in patients with HFrEF randomized to ET (Piepoli et al., 2004).

Despite the established benefits of regular exercise in patients with HFrEF, only a small percentage of eligible patients are referred to cardiac rehabilitation (Haykowsky et al., 2016). In an effort to improve provider prescription habits, European Association for Cardiovascular Prevention and Rehabilitation have published a position paper (Piepoli et al., 2011) offering practical recommendations regarding how to implement safe and effective ET programs for heart failure patients. The first step in this approach is appropriate patient selection. Major Societal guidelines recommend ET for all clinically stable HFrEF patients, with clinical stability defined as "no recent ($\leq$6 weeks) or planned ($\leq$6 months) major cardiovascular hospitalizations or procedures." by the Centers for Medicare and Medicaid Services (CMS) (CMS, 2017).

Once clinical stability is established, patients must then be evaluated for contraindications to exercise in order to ensure safety. We offer a list of contraindications here (**Table 7**), as uncertainty among providers regarding which patients can safely engage in ET likely contributes to its under prescription. It should be emphasized that several studies, including HF-ACTION, have demonstrated the safety of ET in stable patients (O'Connor et al., 2009; Piepoli et al., 2004).

Exercise training prescriptions should include a clear description of intensity, duration, frequency, type of activity, and location. A rated perceived exertion scale and/or heart rate are recommended ways to quantify intensity. Experts endorse a goal of 30 min daily $\times$ 5 days a week of moderate activity, with a slow uptitration of intensity and duration guided by patients' symptoms and vitals as desirable to obtain and safe to pursue (Piepoli et al., 2011). Whether an individual patient should be monitored while engaging in ET will depend on a patient's risk of adverse events and comorbidities. For instance, patients with known exercise induced ventricular ectopy, supraventricular tachycardia, or baseline hypoxia may benefit from monitoring.

Cardiac rehabilitation receives an AHA/ACC Class IIA recommendation as there is evidence suggesting it improves functional capacity, exercise tolerance, quality of life, and mortality in stable heart failure patients (Yancy et al., 2013). Providers should be aware of the current trends in insurance coverage for cardiac rehabilitation in order to maximize the available resources for their patients. The Centers for Medicare & Medicaid Services (CMS) currently covers at least 6 weeks of cardiac rehabilitation for stable patients with left ventricular ejection fraction of less than 35% who have persistent symptoms (NYAH Class II–IV) despite 6 weeks of optimal heart failure therapy (CMS, 2017).

Presently, third party payers do not cover cardiac rehabilitation for patients with HFpEF. However, although less robust, there is evidence that ET training is beneficial in the HFpEF population as well. It has been shown to improve diastolic dysfunction as measured by echocardiogram (Pearson et al., 2017). In addition, a small pilot RCT, the EX-DHF trial, demonstrated that ET improves quality of life and exercise capacity in patients with HFpEF (Edelmann et al., 2011).

Adherence is known to be low in patients referred to ET (Piepoli et al., 2011). Thus, it is essential that ET programs are adapted to patients' preferences and lifestyles, in order to encourage participation.

While the benefits of a formal ET program are clear, it is important for providers and patients to realize that simply increasing the amount of daily physical activity and having a less sedentary lifestyle will likely provide clinical benefit. This is particularly important for the older adult whose mobility may be limited by comorbidities (e.g., arthritis, frailty) as well as patients struggling with behavioral change for whom a structured exercise program is too daunting a goal. Studies are ongoing to confirm the benefit of this common sense, expert endorsed approach.

Table 7 Summary of contraindications to (A) exercise testing and training exercise, (B) training, and (C) increase risk for exercise training

A. Contraindications to exercise testing and training exercise
Within 2 days of ACS
Untreated malignant cardiac arrhythmias
Decompensated heart failure
Uncontrolled hypertension
Advanced atrioventricular block
Acute myocarditis and pericarditis
Symptomatic aortic stenosis
Severe hypertrophic obstructive cardiomyopathy
Acute systemic illness
Intracardiac thrombus
B. Contraindications to exercise training
Progressive worsening of exercise tolerance or dyspnea at rest over previous 3–5 days
Significant ischemia during low-intensity exercise
Uncontrolled diabetes
Recent embolism
Thrombophlebitis
New onset atrial fibrillation or atrial flutter
C. Increased risk for exercise training
>1.8 kg increase in body mass over the previous 1–3 days
Continuous or intermittent dobutamine therapy
Decrease in systolic blood pressure with exercise
NYHA functional class IV
Complex ventricular arrhythmia at rest or with exertion
Supine resting heart rate of >100 b.p.m
Comorbidities limiting exercise tolerance

Adapted from Haykowsky, M. J., Daniel, K. M., Bhella, P. S., Sarma, S., Kitzman, D. W. (2016). Heart failure: Exercise-based cardiac rehabilitation: Who, when, and how intense? *The Canadian journal of cardiology*.**32** (10s2), S382–s387.

When to Refer to a HF and Advanced HF Program or Cardiologist

Although there is evidence that a general internist can safely manage patients with heart failure that are clinically stable on optimal medical therapy in countries that offer universal access to health care (Ponikowski et al., 2016a), it is unclear if the same is true in the United States. In either case, it is imperative that generalist providers know when referral to a heart failure specialist is needed as life extending advanced therapies such as left ventricular assist devices (LVADs) and heart transplantation may no longer be options if irreversible end organ damage is already present. The ACC/AHA (Yancy et al., 2013) guidelines offer disease milestones that signal end-stage disease and need for evaluation by a heart failure specialist for advanced therapies or referral to hospice:

- NYHA III and IV symptoms in spite of optimal medical therapy
- Worsening renal or liver function
- Bb or ace-inhibitor intolerance due to hypotension
- Diuretic requirements greater than 160 mg/day or use of metolazone
- 2 or more heart failure hospitalization within 6 months
- Cardiac cachexia
- Frequent shocks from implanted cardiac defibrillator
- Worsening hyponatremia, generally less than 133 meq/L

When to Refer Patients to Palliative and Hospice Care

Given the disease trajectory of heart failure and that only a small proportion of NYHA IV patients will be eligible for advanced therapies, major society guidelines now recommend palliative care services be incorporated early into the multidisciplinary team approach to management of patients with heart failure (Yancy et al., 2013; Ponikowski et al., 2016c).

At its inception, the specialty of palliative care was created for end-stage cancer patients in whom effective disease specific treatment was no longer available. Over the last few decades, palliative care has come to encompass a broader scope, becoming more synonymous with "supportive care" rather than simply hospice or end of life care. Palliative care takes a holistic approach to improving the quality of life of patients with highly morbid diseases by addressing the physical, physiological, and spiritual needs of these patients (Chen-Scarabelli et al., 2015). With this change, it can be seamlessly incorporated into the care of patients still receiving disease-targeted therapy. There are multiple advantages to integrating palliative care services to the multidisciplinary care team prior to a patient progressing to end-stage disease. In fact, late referrals to palliative care have been associated with the perception by family of unmet needs, poor care coordination, and poor awareness of prognosis (Fendler et al., 2015). Current evidence indicates that patients' goals for their care change over time and that advanced care planning conversations are an iterative

process that evolve over multiple conversations, typically increasing in frequency as disease becomes more advanced. Palliative care specialists have been shown to facilitate advanced care planning and informed decision making in heart failure patients (Lewin and Schaefer, 2017). In addition, improved provider patient communication, which palliative care specialists can augment, has been shown to enhance patient satisfaction and emotional well-being (Tulsky, 2004).

In contrast to palliative care, which should be introduced early in the disease process, hospice care focuses on aggressive symptom management for patients whose life expectancy is less than 6 months. Providers should recognize when it is appropriate to offer hospice care to patients. Indications include patients with NYHA IV symptoms in spite of optimal medical and device therapies, deemed ineligible for transplant or LVAD, and have recurrent hospitalizations despite good compliance or multisystem organ failure secondary to heart failure or other comorbidities. Given the high symptom burden and poor quality of life associated with end-stage heart failure, all management decisions should be focused on quality of life and alleviation of suffering both physical and psychological. The most common symptoms are dyspnea and fatigue. Opioids, nitrates, and O_2 are effective tools to treat dyspnea. Caffeine and other stimulants may be used to address fatigue.

It should be noted that, hospice care can be quite variable when it comes to the management of end-stage heart failure. Traditionally, hospice care providers have focused on end-stage care of cancer. Many hospice providers will try to decrease or stop heart failure medications (such as ACE-inhibitor or BB). This is the exactly wrong approach in that the patient will decline more rapidly. Discussion with the hospice provider regarding their approach to heart failure and available protocols is important to review.

Discussions and decisions surrounding ICD deactivation arise for many patients with advanced heart failure. The Resuscitation Council of the United Kingdom, British Cardiovascular Society, and National Council for Palliative Care have published a statement to offer guidance to providers for appropriate care surrounding decisions around deactivation of implantable cardiac devices (Pitcher et al., 2016). In it, the authors emphasize that end of life planning should be highly individualized and decisions about Code status and ICD deactivation should be considered separately.

Such discussions must be approached in a sensitive fashion. For patients with an ICD, the decision to be DNR should trigger a conversation about ICD deactivation. Given the discomfort associated with ICD shocks and that tachy-arrhythmias are common in end-stage HF patients, the deactivation of defibrillators is consistent with a focus on comfort and quality of life. By the same reasoning, it is reasonable to continue the CRT capability of an implantable device as it can contribute significantly to symptom management.

Conclusion

While it is true that there continue to be important gaps in the medical literature regarding the outpatient management of heart failure, there are currently many pharmacologic and nonpharmacologic interventions that have been shown to be effective but that are grossly underutilized. While there are many factors that likely contribute to provider variability in prescribing habits of disease modifying interventions, both suboptimal dissemination and difficulties with practical application of clinical practice guidelines likely contribute. In this review, we have attempted to facilitate adherence to clinical practice guidelines by not only reviewing current standard of care but also offering specific practices in an attempt to better operationalize major society recommendations.

It is important to acknowledge that heart failure patients are often elderly, socially isolated individuals with multiple comorbidities that require substantial time and expertise to be managed optimally. Providers caring for heart failure patients are often busy clinicians limited in the amount of time they have available to engage in patient education of self-care activities. We would encourage providers to enlist their pharmacy, dietician, and physical therapist colleagues in the effort to provide essential patient education they may not have the time to offer themselves.

Given the prevalence, poor outcomes, and pace of advances in heart failure treatment, we recommend all providers, particularly generalists, begin or continue referring to the AHA/ACC clinical practice guidelines in order to stay current with standard of care.

Appendix 1

Summary of major clinical trials with vasodilators, ACE-inhibitors, angiotensin receptor blockers, and angiotensin receptor-neprilysin inhibitors in patients with HFrEF

Trial	*Date*	*Study design*	*Treatment arms*	*Population*	*Follow-up time period*	*Primary Outcome*
Vasodilators						
V-HeFT I	1986	RCT	H-ISDN ($n=273$) Prazosin ($n=183$) Placebo ($n=273$)	LVEF < 30%, Mean age 58 years	2.3 years (mean)	H-ISDN was associated with trend in all-cause mortality compared to placebo (38.7% vs. 44.9%, $P=0.09$) by a 34% reduction in morality at 2 years reduced ($P<0.028$)

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Trial	*Date*	*Study design*	*Treatment arms*	*Population*	*Follow-up time period*	*Primary Outcome*
V-HeFT II	1991	RCT	H-ISDN ($n=403$) Enalapril ($n=401$)	LVEF $<0.45\%$, NYHA II-IV, Mean age: 60.5 years	2.5 years (mean)	Enalapril compared to H-ISDN demonstrated a reduction in all-cause mortality (38.2% vs. 32.8%, $P=0.008$)
A-HeFT	2004	RCT	BiDil ($n=518$) Placebo ($n=532$)	LVEF $\leq 30\%$, NYHA III-IV, Mean age: 57 years	12.8 months (mean)	Bidil demonstrated a better composite score than in the placebo group (-0.1 ± 1.9 vs. -0.5 ± 2.0, $P=0.01$; range of possible values, -6 to $+2$)
ACE-inhibitors						
CONSENSUS (The CONSENSUS Trial Study Group, 1987)	1987	RCT	Enalapril ($n=126$) Placebo ($n=127$)	NYHA IV, mean age:70 years	188 days (mean)	Enalapril was associated with a 40% reduction in all-cause mortality at 6 months compared to placebo (26% vs. 44%, $P=0.002$, respectively) and 31% at 1 year (36% vs. 50%, $P=0.001$, respectively)
SOLVD-Treatment	1991	RCT	Enalapril ($n=1285$) Placebo ($n=1284$)	LVEF $\leq 35\%$, NYHA II-III, Mean age: 61 years	41.4 months (mean)	Enalapril was associated with a 16% RR reduction in all-cause mortality (39.7% vs. 35.2%, $P=0.0036$, respectively) and a 26% RR reduction in the composite of death or hospitalization due to heart failure compared to placebo (47.7% vs. 57.3%, $P<0.0001$, respectively)
SOLVD-Prevention	1992	RCT	Enalapril ($n=2117$) Placebo ($n=2111$)	LVEF $\leq 35\%$, NYHA I-II, Mean age: 59 years	37.4 months (mean)	Enalapril was associated with a 8% reduction in all-cause mortality (95% CI: -8% to 21%, $P=0.30$); 12% reduction in cardiovascular mortality (95% CI: -3% to 26%, $P=0.12$); 37% reduction in the development of heart failure (95% CI: 28%–44%, $P<0.001$); 20 reduction in mortality or heart failure hospitalization (95% CI: 9%–30%, $P<0.001$); 36% reduction in first heart failure hospitalization (22%–46%, $P<0.001$); and a 29% reduction in all-cause mortality or development of heart failure (21%–36%, $P<0.0001$)
SAVE	1992	RCT	Captopril ($n=1116$) Placebo ($n=1115$)	LVEF $\leq 40\%$ post MI, Mean age: 59 years	42 months (mean)	Captopril was associated with a 19% RR reduction in all-cause mortality (95% CI: 3%–32%, $P=0.019$), 21% RR reduction in CV deaths (95% CI: 5%–35%, $P<0.001$), 37% RR reduction in development of severe heart failure (95% CI: 20%–50%, $P<0.001$), and a 22% RR reduction in heart failure hospitalization (95% CI: 4%–37%, $P=0.019$)
ATLAS	1999	RCT	Low-dose lisinopril ($n=1596$) High-dose lisinopril ($n=1568$)	LVEF $\leq 30\%$, NYHA II-IV, Mean age: 64 years	47.5 months (median)	High dose was associated with an 8% reduction in all-cause mortality (HR: 0.92, 95% CI: 0.82–1.03, $P=0.128$) and a 10% reduction in cardiovascular mortality (HR: 0.90, 95% CI: 0.81–1.01, $P=0.073$)
Angiotensin receptor blockers						
ELITE I	1997	RCT	Losartan ($n=352$) Captopril ($n=370$)	LVEF $\leq 40\%$, ACE-inhibitor naïve, NYHA II-IV, Mean age: 74 years	48 weeks	No difference between losartan and captopril in the frequency persisting (confirmed) increases in serum creatinine (10.5% in each group, RR: 2% (95% CI -51% to $+36\%$), $P=0.63$)
ELITE II	2000	RCT	Losartan ($n=1578$) Captopril ($n=1574$)	LVEF $\leq 40\%$, ACE-inhibitor naïve, NYHA II-IV, Mean age: 72 years	555 days (mean)	No difference between losartan and captopril in all-cause mortality (17.7% vs. 15.9%, $P=0.16$, respectively) or the composite or all-cause mortality or all-cause hospital admission (47.7% vs. 44.9%, $P=0.18$, respectively)
Val-HeFT	2001	RCT	Valsartan ($n=2511$)	LVEF $\leq 30\%$, NYHA II-IV,	23 months (mean)	No difference between valsartan and placebo in all-cause mortality (RR: 1.02, 97.5% CI:

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Trial	Date	Study design	Treatment arms	Population	Follow-up time period	Primary Outcome
			Placebo ($n=2499$)	93% receiving ACE-inhibitors, Mean age: 63 years		0.88–1.18, $P=0.80$); valsartan had a 13.2% lower risk of the composite of mortality and incidence of cardiac arrest with resuscitation, heart failure hospitalization, or use of intravenous inotropic or vasodilator therapy for at least 4 h (RR: 0.87, 97.5% CI: 0.77–0.97, $P=0.009$)
CHARM-Added	2003	RCT	Candesartan ($n=1276$) Placebo ($n=1272$)	LVEF $\leq 40\%$, NYHA II-IV 100% receiving ACE-inhibitors, Mean age: 64 years	41 months (median)	Candesartan had a 15% reduction in cardiovascular death or unplanned heart failure hospitalization compared to placebo (HR: 0.85, 95% CI: 0.75–0.96, $P=0.011$) and a 9% reduction in all-cause mortality (HR: 0.89, 95% CI: 0.77–1.02, $P=0.86$)
CHARM-ALTERNATIVE	2003	RCT	Candesartan ($n=1013$) Placebo ($n=1015$)	LVEF $\leq 40\%$, NYHA II-IV, Intolerant to ACE-inhibitors, Mean age: 67 years	33.7 months (median)	Compared to placebo, candesartan demonstrated a 30% reduction in the primary endpoint (adjusted HR: 0.70, 95% CI: 0.60–0.81, $P<0.0001$)
HEAAL	2009	RCT	Low-dose losartan ($n=1919$) High-dose losartan ($n=1927$)	LVEF $\leq 40\%$, NYHA II-IV, Intolerant to ACE-inhibitors, Mean age: 66 years	4.7 years (median)	Losartan had a 10% reduction in the composite of all-cause mortality or heart failure hospitalization (HR: 0.90, 95% CI: 0.82–0.99, $P=0.27$)
Angiotensin receptor-neprilysin inhibitor						
PARADIGM-HF[a]	2014	RCT	Sacubitril/valsartan ($n=4187$) Enalapril ($n=4212$)	LVEF $\leq 40\%$, NYHA II-IV, Stable dose of Stabilized on a beta-blocker and an ACE-inhibitor (or ARB) equivalent to at least 10 mg of enalapril daily Mean age: 64 years	27 months (mean)	Compared to enalapril, sacubitril/valsartan demonstrated a 20% reduction in cardiovascular death or heart failure hospitalization (HR: 0.80, 95% CI: 0.71–0.89, $P<0.001$); a 20% reduction in cardiovascular death (HR: 0.80, 95% CI: 0.71–0.89, $P<0.001$); and a 19% reduction in first heart failure hospitalization (HR: 0.79, 95% CI: 0.71–0.89, $P<0.001$)

ACE: Angiotensin-converting enzyme, A-HeFT: African American heart failure trial , ARBs: angiotensin receptor blockers, ATLAS: assessment of the treatment with lisinopril and survival, ARB: angiotensin receptor blocker, CHARM: Candesartan in heart failure assessment of reduction in mortality and morbidity, CONSENSUS: cooperative north scandinavian enalapril survival study , CV: cardiovascular, ELITE: evaluation of losartan in the elderly, LVEF: left ventricular ejection fraction, HEAAL: heart failure endpoint evaluation of angiotensin II antagonist losartan, H-ISDN: hydralazine combined with isosorbide dinitrate, NYHA: New York aeart association functional class, PARADIGM-HF: prospective comparison of ARNI with ACEI to determine impact on global mortality and morbidity in heart failure, RCT: Randomized control trial RR: risk reduction, SAVE: survival and ventricular enlargement, SOLVD: studies of left ventricular dysfunction, Val-HeFT: valsartan heart failure trial, V-HeFT: vasodilator heart failure trial.

[a]Study stopped early.

Modified with permission from Paul, S., Page, R.L. 2nd. (2016). Foundations of pharmacotherapy for heart failure with reduced ejection fraction: Evidence meets practice, Part I. *The Journal of cardiovascular nursing* **31**(2), 101–113; McMurray, J.J., Packer, M., Desai, A.S. et al. (2014). Angiotensin-neprilysin inhibition versus enalapril in heart failure. *New England Journal of Medicine* **371**(11):993–1004.

Appendix 2

Summary of major clinical trials with β blockers, digoxin, aldosterone antagonists, and ivabridine in patients with heart failure with reduced ejection fraction

Trial	Date	Study design	Treatment arms	Population	Follow-up period	Primary outcome
Beta-blockers						
MDC	1993	RCT	Metoprolol tartrate ($n=194$) Placebo ($n=189$)	LVEF $<40\%$, NYHA II-IV, Mean age: 49 years	12 months	Metoprolol tartrate had a 34% reduction in death or need for transplantation compared to placebo (95% CI: 0.06–0.62; $P=0.058$)

(*Continued*)

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Trial	*Date*	*Study design*	*Treatment arms*	*Population*	*Follow-up period*	*Primary outcome*
CIBIS I	1994	RCT	Bisoprolol ($n=320$) Placebo ($n=321$)	LVEF <40%, NYHA III–IV, Mean age: 60 years	1.9 years (mean)	No difference in mortality between bisoprolol and placebo (RR: 0.80, 95% CI: 0.56-1.15; p = 0.22)
CIBIS II[a]	1999	RCT	Bisoprolol (n = 1327) Placebo (n = 1320)	LVEF ≤35%, NYHA III-IV, Mean age: 61 years	1.3 years (mean)	All-cause mortality was significantly lower with bisoprolol than placebo (11.8% vs. 17.3%) (HR: 0.66, 95% CI: 0.54–0.81; $P<0.0001$)
MERIT-HF[a]	1999	RCT	Metoprolol CR/XL ($n=1990$) Placebo ($n=2001$)	LVEF ≤40%, NYHA II-IV, Mean age: 64 years	1 years (mean)	All-cause mortality was significantly lower with metoprolol CR/XL than placebo (7.2%, per patient-year of follow-up vs. 11.0%) (RR: 0.66, 95% CI: 0.53–0.81, $P=0.00009$)
COPERNICUS[a]	2001	RCT	Carvedilol ($n=1156$) Placebo ($n=1133$)	LVEF <25%, Mean age: 63 years	10.4 months (mean)	Carvedilol had a 35% reduction in risk of death compared to placebo (95% CI: 0.19–0.48; $P=0.00013$ [unadjusted])
COMET	2003	RCT	Carvedilol ($n=1511$) Metoprolol tartrate (n = 1518)	LVEF ≤35%, NYHA II-IV, Mean age: 62 years	58 months (mean)	All-cause mortality was 34% for carvedilol vs. 40% for metoprolol (HR: 0.83, 95% CI: 0.74–0.93; $P=0.0017$); the composite endpoint of mortality or all-cause admission occurred in 74% of carvedilol group and 76% of metoprolol group (HR: 0.94, 0.86–1.02; $P=0.122$)
Digoxin						
DIG	1997	RCT	Digoxin ($n=3397$) Placebo ($n=3403$)	LVEF ≤45%, NYHA II-IV, Mean age: 62 years	37 months (mean)	No significant differences in mortality ($P=0.80$); in digoxin group, trend toward a decrease in risk of death due to heart failure (RR: 0.88, 95% CI: 0.77–1.01; $P=0.06$); in digoxin group, 6% fewer hospitalizations and fewer patients hospitalized for heart failure (RR: 0.72, 95% CI: 0.66–0.79; $P<0.001$)
DIG (High Risk Sub-Study)	2013	Posthoc analysis	NYHA III–IV Digoxin ($n=1118$) Placebo ($n=1105$) LVEF ≤ 25% Digoxin ($n=1127$) Placebo ($n=1129$) CTR ≥ 55% Digoxin ($n=1175$) Placebo ($n=1170$)	LVEF ≤45%, NYHA II-IV, Mean age, 62 years	37 months (mean)	Compared to placebo, those receiving digoxin had a significant reduction in the 2-year composite endpoint of HF mortality or HF hospitalization: NYHA III–IV (HR: 0.65, 95% CI 0.57–0.75; $P < 0.001$); LVEF <25% (HR: 0.61, 95% CI 0.53–0.71; $P < 0.001$); and CTR .55% (HR 0.65, 95% CI 0.57–0.75, $P<0.001$)
Aldosterone antagonists						
RALES	1999	RCT	Spironolactone ($n=822$) Placebo (841)	LVEF ≤35%, NYHA III–IV, Mean age: 62 years	24 months (mean)	Spironolactone had a 30% reduction in risk of death compared to placebo (RR: 0.70, 95% CI: 0.60–0.82; $P<0.001$)
EPHESUS	2003	RCT	Eplerenone ($n=3319$) Placebo ($n=3313$)	LVEF ≤40%, Mean age: 64 years	16 months (mean)	Eplerenone had a 15% reduction in risk of death compared to placebo (RR: 0.85, 95% CI: 0.75–0.96; $P=0.008$)
EMPHASIS-HF[a]	2011	RCT	Eplerenone ($n=1364$) Placebo ($n=1373$)	LVEF ≤30%, NYHA II, Mean age: 69 years	21 months (mean)	Eplerenone had a 34% reduction in risk of death from cardiovascular causes or hospitalization for HF compared to placebo (HR: 0.66, 95% CI: 0.56–0.78; $P<0.001$)
Ivabradine						
SHIFT	2010	RCT				

(Continued)

Trial	Date	Study design	Treatment arms	Population	Follow-up period	Primary outcome
			Ivabradine ($n=3241$) Placebo ($n=3264$)	LVEF < 35%, NYHA II-IV, Heart rate > 70 bpm, Stabilized on background treatment including a β blocker, Mean age: 60 years	23 months (median)	Compared to placebo, ivabradine demonstrated an 18% reduction in cardiovascular death or heart failure hospitalization (HR: 0.82, 95% CI: 0.75–0.90, $P<0.0001$)

BPM: Beats per minute, CI: confidence interval, CIBIS: cardiac insufficiency bisoprolol study, COMET: carvedilol or metoprolol European trial, COPERNICUS: carvedilol prospective randomized cumulative survival study, CTR: cardiothoracic ratio, DIG: digitalis investigation group, EMPHASIS-HF: eplerenone in mild patients hospitalization and survival study in heart failure, EPHESUS: eplerenone post-acute myocardial infarction heart failure efficacy and survival study , HF: heart failure, HR: hazard ratio, LVEF: left ventricular ejection fraction, MDC: metoprolol in dilated cardiomyopathy, MERIT-HF: metoprolol CR/XL randomized intervention trial in congestive heart failure, mo: months, NYHA: New York heart association, RALES: randomized aldactone evaluation study, RCT: randomized controlled trial, RR: relative risk, SHIFT: systolic heart failure treatment with the if inhibitor ivabradine trial.

[a]Study stopped early.

Modified with permission from McIlvennan, C.K., Page, R.L., 2nd. (2016). Foundations of pharmacotherapy for heart failure with reduced ejection fraction: Evidence meets practice, Part II. *The Journal of cardiovascular nursing* **31**(6), 545–554; Swedberg, K., Komajda, M., Bohm, M. et al. (2010). Ivabradine and outcomes in chronic heart failure (SHIFT): A randomised placebo-controlled study. *Lancet.* 376(9744), 875–885.

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Preclinical Cardiovascular Imaging

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Introduction to Preclinical Imaging

In the past three decades, there have been significant advances in the field of various imaging modalities that enhance visualization of smaller objects. These advances have propelled the field of cardiovascular imaging forward and allowed the use of small rodent animal models for human cardiovascular disease. The goal of this article is to provide an overview of different imaging modalities that are available for cardiovascular research in the preclinical setting. The most commonly used imaging applications are magnetic resonance imaging (MRI), ultrasound imaging, microcomputed tomography (μCT), positron emission tomography (PET), single photon emission computed tomography (SPECT), and optical imaging. **Table 1** provides a quick comparison of the imaging modalities addressed in this article.

Magnetic Resonance Imaging

A Brief History

In 1930, the Austria-born physicist Isidor Isaac Rabi, who was influenced by the work of Bohr, Pauli, and Heisenberg, determined the nuclear spin and magnetic moment of sodium using a modified Stern–Gerlach apparatus. He continued to determine the nuclear spins of hydrogen and deuterium. His work established the nuclear magnetic resonance method, which is the basis for MRI. Rabi correctly predicted the behavior of nuclei in magnetic fields and his laboratory was the first to observe magnetic resonance in 1938 in lithium chloride molecules. He was awarded the Nobel Prize in 1944 for "his resonance method of recording the magnetic properties of atomic nuclei" (Rigden, 1998).

In 1946, Edward Purcell and Felix Bloch independently developed the method to study the resonance properties of nuclei in solids and liquids. Both received the Nobel Prize in 1952. For two decades, magnetic resonance was only used to determine the chemical and physical properties of substances, but in 1971, Raymond Damadian (Kauffman, 2014) showed that tumor tissue and healthy tissue had different nuclear relaxation times, opening the door for clinical applications for MRI. The development of

Table 1 Overview of imaging modalities, with their corresponding resolution, advantages and disadvantages, and contrast agents used

	Resolution	*Radiation*	*Longitudinal studies*	*Endpoint studies*	*Advantages*	*Disadvantages*	*Contrast agents*
Magnetic resonance imaging (MRI)	In vivo: 80–100 μm In vitro: ~27 μm	Radio waves	Yes, unlimited	Specimen imaging possible	Noninvasive, very good soft tissue contrast	Long scan times, high maintenance costs	Gadolinium, SPIO, ferritin
Positron emission tomography (PET)	1–2 mm	γ Rays (high energy)	Limited by radiation exposure	No	Metabolic activity measurements, high sensitivity	Very expensive, radiation exposure, low resolution, cyclotron needed	Radioactive isotopes: ^{82}Rb, ^{18}F, ^{11}C, ^{13}N, ^{15}O Fluorodeoxyglucose (FDG)
Single photon emission computed tomography (SPECT)	1–2 mm	γ Rays (low energy)	Limited by radiation exposure	No	Longer half-lives of radioisotopes, metabolic activity, dual radioisotope use	Radiation exposure, low resolution	Radioactive isotopes: ^{99m}Tc, ^{123}I, ^{111}In
Microcomputed tomography (μCT)	In vivo: ~67 μm In vitro: 10.5 μm	X-rays	Yes, however, limited by radiation exposure	Yes, for vascular imaging	Fast imaging times, excellent resolution, whole body images possible	Poor soft tissue contrast, vascular imaging requires endpoint study	Microfil, barium, iodine
Ultrasound	50 μm	High-frequency sound waves	Yes, unlimited	No	Fast imaging times, good resolution, least expensive imaging modality	Highly trained personnel to interpret images, low tissue penetration	Microbubbles
Optical imaging	1–3 mm	Visible or near infrared light	Yes	No	Fast imaging times, good resolution, inexpensive	Low tissue depth penetration	Fluorescent dyes and labeled molecules

computers and thus the ability to generate images from magnetic resonances led to the first live human MRI on July 3, 1977. The first commercial MRI systems became available in the 1980s and the development of high-strength magnetic field systems continues until today.

Technique Overview

MRI is based on the fact that charged nuclei that spin generate a magnetic field and thus behave like tiny bar magnets. Several elements such as ^{1}H, ^{19}F, ^{23}Na, ^{31}P, and ^{13}C have odd proton numbers in their nuclei and thus have a magnetic dipole moment. When these nuclei are exposed to a constant magnetic field, they will align with (lower energetic state) or against (higher energetic state) the constant magnetic field. The opposite directionality will cancel most of the nuclei out, however, there is a tiny fraction of nuclei that aligns with the magnetic field and these are the nuclei that contribute to the generation of the signal that is used in MRI. These nuclei can be excited with resonant radio frequencies, which increase their energetic state. Once the radio signal is turned off, these nuclei release energy in the form of a radio signal and relax back to their original energetic state. The released energy from every nucleus is slightly different based on the temporal and spatial encoding used in the MRI sequence and the magnetic gradient applied over the field of view. The integration of all frequency information from every nucleus is used to generate the actual image. The time it takes for the nuclei to release energy and return to their prior energetic state is called relaxation time, which can be distinguished further into spin–lattice (T1) or spin–spin (T2) relaxation times. Dependent on the chemical environment of the nuclei, relaxation times vary and can thus be used to generate contrast between tissues (Hashemi et al., 2012; Mitchell, 2004).

Imaging Applications for Cardiovascular Animal Models

MRI imaging in cardiovascular research can be divided into three major categories:

Real-time imaging of the beating heart using dark blood or white blood imaging applications,
Magnetic resonance angiograms with and without contrast enhancement, and
Tissue perfusion imaging with and without contrast enhancement.

Real-time imaging of the beating heart in small mammals

The development of very fast imaging techniques combined with stronger gradients enables MRI of fast beating small mammalian hearts (anesthetized mouse: 350–450 BPM, anesthetized rat: 120–160 BPM). Two methods stand out for their usefulness in

determining heart muscle morphology and ventricular function: black blood spin echo imaging and bright blood gradient echo imaging. Black blood imaging with spin echo techniques requires slightly longer acquisition times, however, it allows for higher resolution images. Black blood images are very useful for detailed morphological analysis of the heart as well as large blood vessels within the thorax. Gradient echo bright blood images are very fast in the acquisition, have a somewhat lower resolution, but allow ventricular function assessment as well as blood flow measurements, myocardial perfusion, and cardiac valve functional determination (Fig. 1).

Magnetic resonance angiograms

Imaging methods for magnetic resonance angiograms can be distinguished into noncontrast and contrast-enhanced imaging. Contrast-enhanced imaging relies on the fast injection of gadolinium into the venous system of the mouse. Gadolinium has a vascular time of approximately 30 s; thus, any contrast enhanced imaging has to be acquired very quickly. The advantage of contrast-enhanced imaging is that smaller blood vessels can be visualized because the method is not dependent on blood flow. In noncontrast enhanced imaging, blood flow itself is important and contrast is gained from fast moving protons. Time of flight imaging relies heavily on spin labeling of blood outside the area of interest that then flows into the area of interest within a tissue that is saturated (= not visible) by rapidly applied radiofrequency pulses (Fig. 2).

Tissue perfusion imaging with contrast enhancement

MRI is a well-accepted method to image the infarcted heart and determine the area at risk, the infarcted area, as well as determine areas with lower perfusion levels. These imaging methods use gadolinium contrast agents that can be divided into two major classes: extravascular and intravascular gadolinium conjugates. For both classes, gadolinium is complexed because free gadolinium is highly toxic. Gadolinium is paramagnetic with seven free electrons and accelerates the relaxation of water protons in its immediate proximity. On a T1 weighted image, the contrast of water will thus be enhanced (=brighter) in the presence of gadolinium. In murine models of cardiac infarction, late gadolinium contrast enhancement is used to determine the infarcted area. This is based on the increase in extracellular space in dead or compromised tissue and the increased accumulation of gadolinium in this

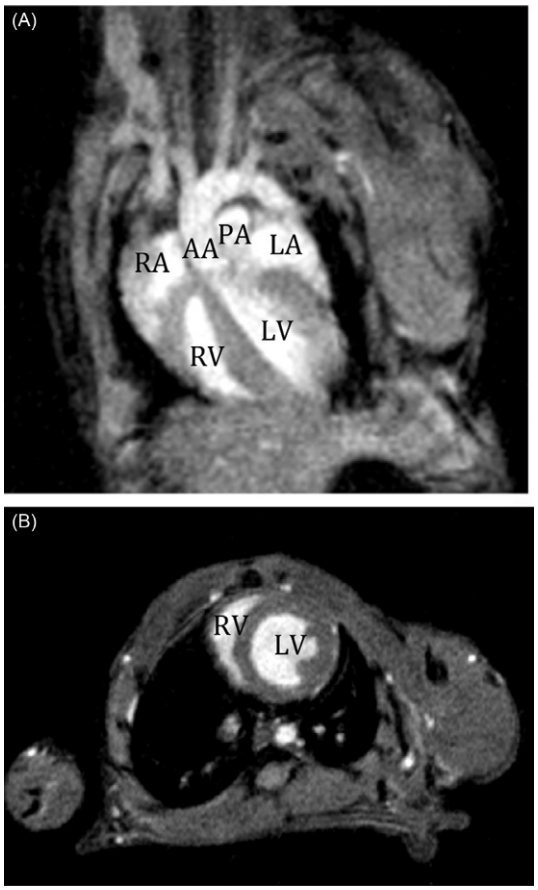

Fig. 1 MRI gradient echo bright blood image of a mouse heart. (A) Four chambers long axis view: ventricular chambers (left ventricle (LV) and right ventricle (RV)) and atrial chambers (right atrium (RA) and left atrium (LA)) can be clearly identified. The ascending aorta (AA) with its three major artery branches can be easily seen as well as the pulmonary artery (PA). The interventricular septum is clearly visible in the long axis and short axis views. (B) Short axis view of the mouse heart during diastole. RV and LV are filled with blood and the papillary muscles can be seen in the LV.

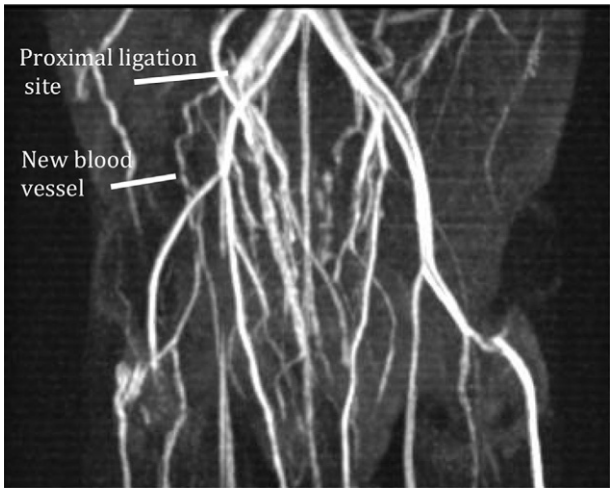

Fig. 2 Magnetic resonance angiogram (MRA) of the lower abdominal and hindlimb area of the mouse. This mouse underwent surgery to resect the left femoral artery and vein. MRA imaging 7 days later showed that blood flow to the ischemic hindlimb was reestablished. In this study, arteries and veins were not distinguished.

extracellular space. A precontrast image is taken and then gadolinium injected. After 10 min (up to 30 min later), a second image is acquired to show enhancement in areas where gadolinium accumulated (Protti et al., 2010; Price et al., 2011; Chapon et al., 2008). Another use of contrast enhancement is in first pass contrast imaging to delineate the perfusion of the myocardium. Initially, this was perceived as too complicated in rodent models of cardiovascular disease; however, several groups have demonstrated that despite high heart rates sufficient contrast can be achieved. Here the image is acquired within seconds after the gadolinium injection to measure "the first pass" through the vasculature before gadolinium diffuses into the extravascular space (Makowski et al., 2010; Coolen et al., 2010).

Ultrasound Imaging

A Brief History

In the 18th century, Carl Linnaeus and Franz Aepinus worked with crystals and found that some crystals generate an electric potential in response to a temperature change. They called this the pyroelectric effect. Pyro is the Greek word for to squeeze or press. René Just Haüy, a French mineralogist, and Antoine César Becquerel (grandfather to Henri Becquerel) continued this work; however, they could never provide proof of the piezoelectric effect. Finally, in 1880, the brothers Pierre and Jacques Curie were successful in demonstrating the direct piezoelectric effect using crystals of tourmaline, quartz, topaz, cane sugar, and Rochelle salt (sodium potassium tartrate tetrahydrate) (Curie and Curie, 1881). They showed that quartz and Rochelle salt exhibited the highest piezoelectricity. In 1881 Gabriel Lippmann (Lippmann, 1881) predicted the reverse piezoelectric effect, which was then confirmed by the Curie brothers.

It took until World War I for the piezoelectrical effect to find an application, which was initially nonmedical with the development of sonar to detect submarines (Katzir, 2012). It took a couple more decades for the technique to be used for human tissue imaging and a landmark paper was published in 1958 (Donald et al., 1958). Just a couple of years later in the early 1960s, the first scanners for clinical imaging were developed and commercially available (see review (Manbachi and Cobbold, 2011)).

Technique Overview

Ultrasound imaging uses sound waves that are emitted into tissue and then reflected back (echoed) from any boundaries these sound waves encounter. The image is constructed from the time differences between returning echoes and the strength of the echoes. The transducer that transmits the sound waves uses piezoelectric crystals to emit the sound and these crystals also receive the echoes back and convert it to an electric current. The development of solid-state transducers for high-resolution ultrasound has improved the user friendliness of these transducers and further improved the resolution for high-resolution ultrasound imaging. This is particularly important for the application of ultrasound imaging in small animal models.

Modes of Imaging

B-mode or 2D mode: In B-mode (brightness mode) ultrasound, a linear array of transducers simultaneously scans a plane through the body that can be viewed as a 2D image on the screen.

M-mode: In M-mode (motion or movie mode) ultrasound, pulses are emitted in quick succession with a stationary transducer while the tissue (for example the heart) moves under the transducer. Over time, this is analogous to recording a video in ultrasound. As the organ boundaries that produce reflections move relative to the probe, this can be used to determine the velocity of heart structures.

Doppler mode: This mode makes use of the Doppler effect in measuring and visualizing blood flow. An analogy to explain Doppler mode is the changing sound of an approaching and then passing ambulance or police car.

Cardiovascular Imaging Applications in Animal Models

- Echocardiography (ECG) is the most utilized method for relatively inexpensive and fast imaging of the heart in mouse and rat models of human heart diseases. The advantages are that images can be obtained fairly quickly with under 15 min of imaging time per animal. The preparation time of the animal uses up most of that time because any fur in the imaging area has to be completely removed, including the hair follicles, using chemical depilators. The disadvantages are that the resolution of the images is low and an experienced and well-trained person is required to interpret the images. **Fig. 3** shows an example of a short axis view of the mouse heart (**Fig. 3A**) with the corresponding M-mode image (**Fig. 3B**) and the ECG tracing (**Fig. 3C**).
- Morphological imaging of blood vessels and Doppler measurements of blood flow:

  Blood vessels can be easily visualized using ultrasound. **Fig. 4** shows an example of the outflow tract of the heart with the ascending aorta and its major branching off blood vessels.
- Contrast enhanced imaging using microbubbles:

  Untargeted: Ultrasound imaging offers a unique approach to measure tissue perfusion or specifically target molecules on the vascular side of blood vessels using microbubbles. Microbubbles are small (~1 μm) lipid micelles that burst when hit by a high-energy ultrasound wave. They can be used untargeted to provide information of tissue perfusion (Qin et al., 2009; Kiessling et al., 2012). In that case, animals receive an intravenous catheter (in the tail vein) and an image of the organ of interest is acquired prior to the microbubble injection. Then microbubbles are injected and the increased return of echo from the bubbles within the blood vessels of the organ reflects the degree of perfusion (**Fig. 5**).

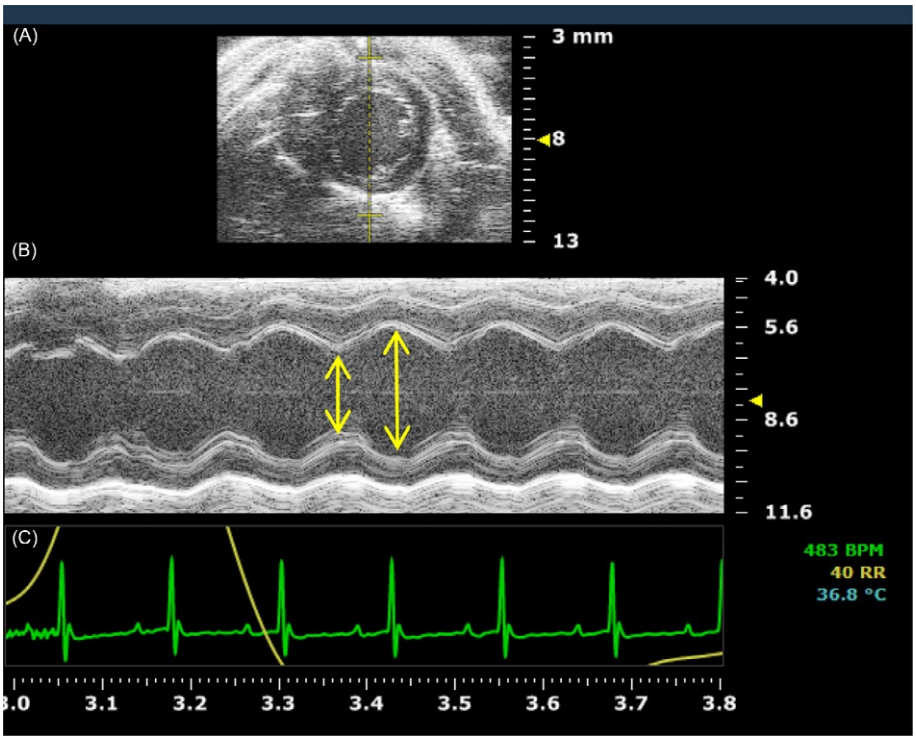

Fig. 3 M-mode echocardiography of a mouse heart. (A) Short axis view through the heart. On the left side and toward the bottom of the left ventricular cavity, the papillary muscle can be seen, which is used as a landmark to position the scan. The *yellow line* marks the position for the M-mode acquisition. (B) M-mode image of the short axis view in (A). The movement of the ventricular walls in diastole ("valleys," longer *yellow double arrow*) and systole ("hills," *shorter yellow arrow*) toward and apart from each other creates the visual appearance of waves. The length of the *arrows* represents the left ventricular chamber size during diastole and systole. The slopes of the waves represent the speed of contraction ("contractility") and the speed of relaxation. The broken *white line* on *top* of the bottom wave reflects the echo shadow of the papillary muscle. (C) Electrocardiogram (*green line*) and respiration (*yellow line*) during the time the M-mode image was taken. Note the disturbance respiration causes at the beginning of the M-mode image.

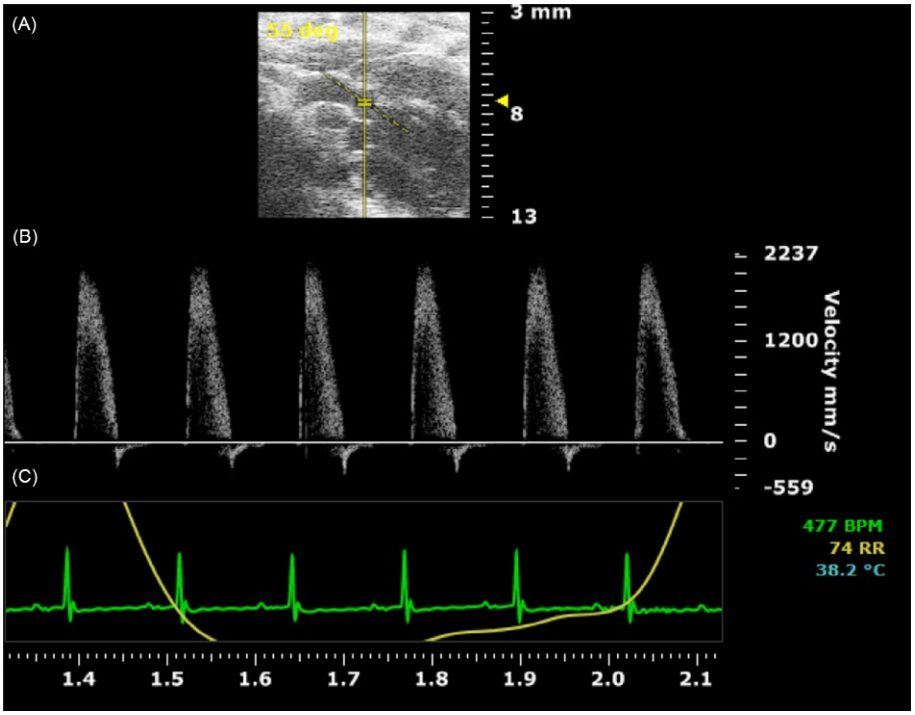

Fig. 4 Blood flow measurement by ultrasound Doppler through the aortic valve of the mouse heart. (A) 2D ultrasound view of the aortic valve. The *solid yellow line* and the equal sign indicate where the measurement is taken. The *broken yellow line* indicates where the maximal velocity of blood flow could be measured. (B) Doppler measurement of the blood flow through the aortic valve in mm/s. The opening of the valve causes the outflow of blood and the fast upstroke of the Doppler signal. The downstroke is blurred due to the changing velocity of blood and the scattering of echoes. There is a very small negative signal that is caused by the closing of the valve and is physiological. (C) Electrocardiogram (*green line*) and respiration (*yellow line*) during the Doppler measurement.

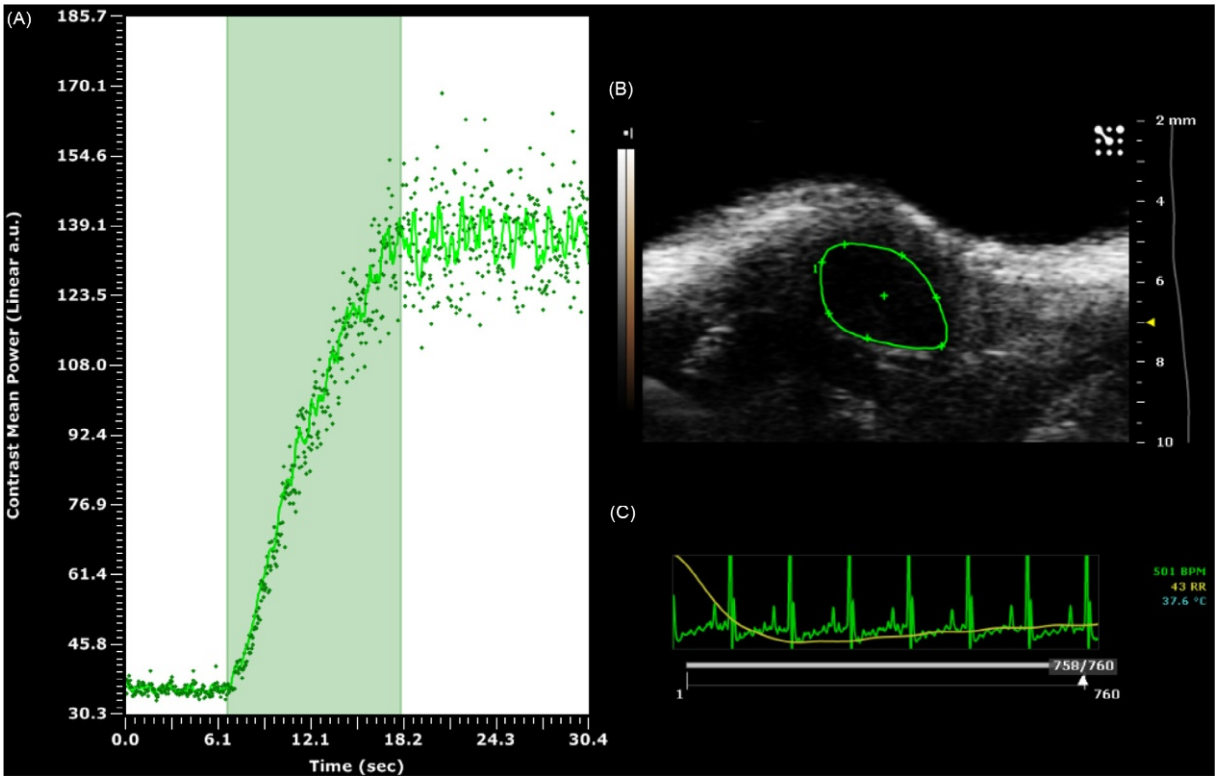

Fig. 5 Cine ultrasound imaging to measure tumor perfusion using untargeted microbubbles. (A) Graphic representation of the contrast change in the area of interest (tumor outline in B) over time. At time 0, untargeted microbubbles were injected in a bolus via a tail vein catheter. The increase in contrast in the *green highlighted box* represents the inflowing microbubbles into the tumor vasculature. The slope of the contrast change, as well as the difference between the minimum and the maximal value of contrast achieved, represents a measure of tumor vascularity. (B) 2D ultrasound image of a tumor in the flank area of a mouse. The tumor is outlined in *green* and delineates where the flow measurement is performed. (C) Corresponding ECG (*green*) and respiration (*yellow*) during image acquisition.

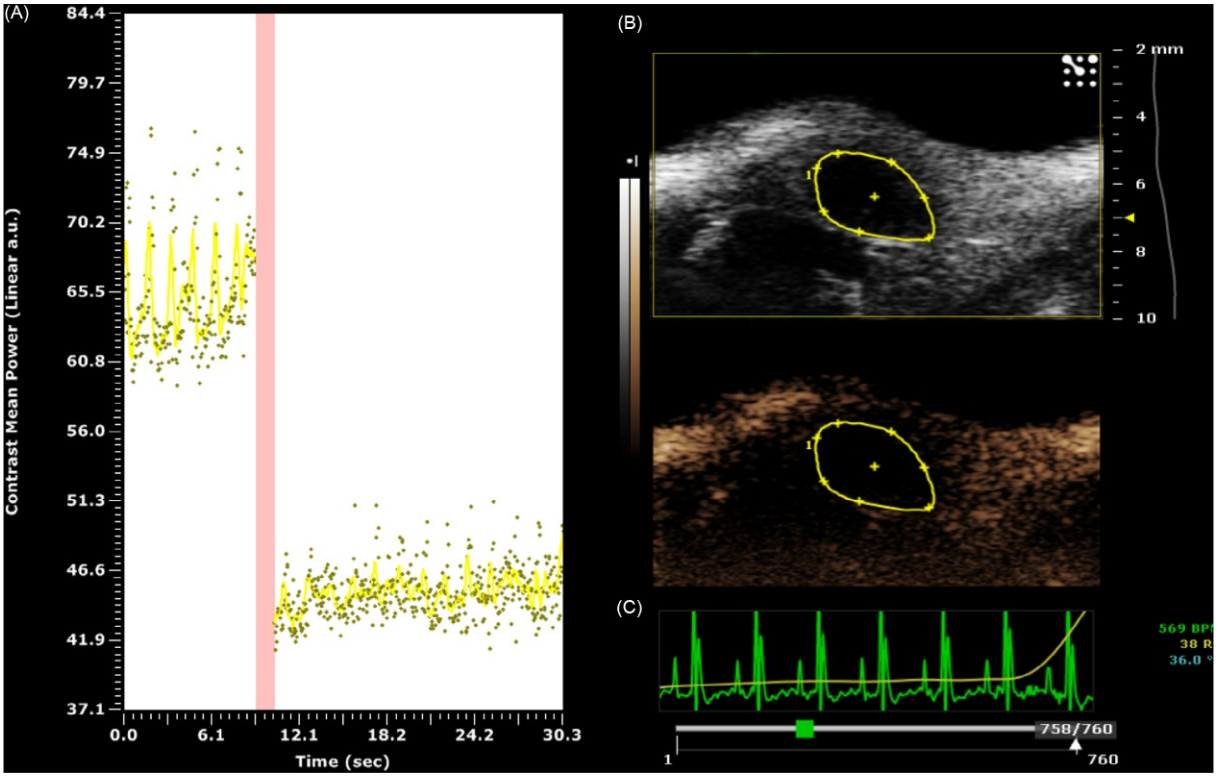

Fig. 6 Targeted microbubbles binding to the vasculature in a mouse tumor. (A) Graphical representation of the change in contrast within the tumor vasculature pre and postburst of microbubbles. Mice were injected with microbubbles targeted to extracellular matrix molecules within the vasculature of tumor tissue. The *yellow line* between 0 and 9 s represents the bound microbubbles. The *pink box* indicates the burst sequence that was applied to burst all bubbles within the area of the tumor. The lower contrast after the burst represents still circulating microbubbles. The difference between pre and postburst contrast is used to calculate the number of microbubbles bound to target molecules and is a measure of the number of target molecules present in the tumor vasculature. (B) 2D images of the tumor pre (*top*) and post (*bottom*) burst. The tumor is outlined in *yellow*. The *mottled gray areas* within the tumor show the echo signal of the bound microbubbles. (C) Corresponding ECG (*green*) and respiration (*yellow*) during image acquisition.

Targeted: Microbubbles can also be specifically targeted to molecules on the intravascular wall by conjugating antibodies to different spacer molecules such as for example biotin or many other chemical conjugation methods (Chopra, 2004; Willmann et al., 2010; Yeh et al., 2015). An isotype control antibody is used as a comparison. For this technique, the microbubbles are injected in a bolus 4–10 min prior to starting a cine loop acquisition. This leaves enough time for the microbubbles to bind their target molecule. The cine loop contains a burst frequence after roughly half of the acquisition time, which bursts all bubbles in the field of view. The difference in contrast between pre and postburst is used to calculate the number of specifically bound microbubbles and can thus be used to quantify target molecules. Fig. 6 shows a representation of pre and postburst signal in a mouse tumor.

Microcomputed Tomography

A Brief History

The development of computer tomography has its beginnings with the findings of Wilhelm Konrad Roentgen, who on November 8, 1895 discovered X-rays. He was awarded the Nobel Prize in Physics in 1901 for his findings that marked the beginning of medical imaging and the term Radiology was born. The Italian scientist Alessandro Vallebona was the first to propose tomographic imaging in the very early 1900s. Tomography meant to obtain an image only from a thin section of the sample. The development of computers in the 1960s accelerated this idea dramatically and also new mathematical methods for image reconstruction were needed to open the field for the wider use of computed tomography. The first computed tomography on a commercial CT scanner was obtained in October 1971 in Great Britain, and the British engineer Sir Godfrey Newbold Hounsfield and the South-African physicist Allan MacLeod Cormack were awarded the Nobel Prize in Medicine in 1979. The continued development of mathematical image reconstruction methods and the increase in computer computational speed, combined with ways to focus the X-ray beam on enabling higher resolution, have led to the development of high-resolution μCT scanners.

Technique Overview

The difference between a preclinical μCT scanner and a clinical CT scanner is that the sample rotates in the preclinical setup and not the X-ray source as in a clinical scanner. In μCT imaging, X-rays are used to obtain a series of 2D planar images that are assembled into 3D images using image analysis software. In high-resolution μCT scanners, the X-ray source sends out highly focused X-ray beams that scan a single slice per view. The sample rotates in small angular increments and an X-ray image is obtained for every angle. Images are then assembled by stacking all 2D images into a 3D image, which in most cases is based on the Feldkamp Cone Beam Reconstruction algorithm (Feldkamp et al., 1984; Johnson et al., 1998). The resolution of μCT is as low as 10.5 μm enabling the visualization of small capillary blood vessels.

Cardiovascular Imaging Applications in Animal Models

The visualization of blood vessel networks using μCT is based on the perfusion of the tissue of interest with Microfil® (FlowTech Inc., Carver, MA), a radiopaque polymer-based solution that solidifies within 2 h after perfusion, leaving a cast of the blood vessel network. This can be imaged using μCT and the number and size of blood vessels can be quantified. Fig. 7 shows an example of a mouse heart, which was perfused in a retrograde fashion with Microfil ex vivo, thus that the ventricular chambers would not fill with the Microfil solution.

Positron Emission Computed Tomography (PET)

A Brief History

The development of PET has its beginning in the early research in isotopes and how to generate them. In 1929, Ernest O. Lawrence invented the cyclotron, which enabled the generation of the isotopes iodine-131, thallium-201, technetium-99m, carbon-14, and gallium-67. In 1939, Lawrence received the Nobel Prize in Physics for his work on isotope generation. To fully use these newly found radiotracers, detectors and scanners were required, thus delaying progress for several decades until 1951, when Benedict Cassen developed the first scanner at UCLA. This was followed just 1 year later by the development of the first gamma camera by Hal Anger at Berkeley, initiating the field of nuclear medicine. In 1953, Gordon Brownell at MIT constructed the first detector for positron–electron annihilation events as an imaging tool, creating, in essence, the first PET scanner. In 1973, scientists developed a faster method for generating thallium-201, enabling routine stress testing in cardiac PET imaging. In 1974, the first PET scanner was built that allowed 3D imaging. In the same year, ^{18}F-flourinedeoxyglucose was synthesized and used for measuring the glucose uptake into various organs, establishing the modern use of PET imaging. In the following decades, the development of more advanced mathematic algorithms and faster computers continued to improve PET imaging (Nutt, 2002; Rich, 1997).

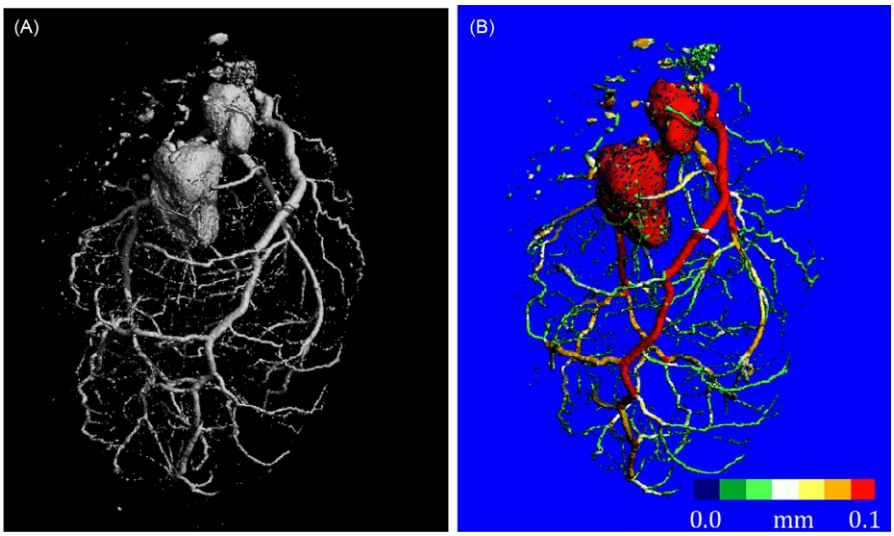

Fig. 7 μCT image of a mouse heart perfused with Microfil. (A) 3D rendering of the coronary vasculature in a mouse heart. The heart was perfused ex vivo in the Langendorff mode to clear blood and then was perfused slowly with Microfil to prevent the ventricular chambers from filling with the contrast agent as well. The specimen was then imaged on a Viva40 (Scanco) high-resolution μCT with a resolution of 10.5 μm. (B) Analysis of coronary artery thickness of the image in (A). This analysis provides the number of the blood vessel at a specific thickness as well as volume calculations for each of the colors.

Technique Overview

PET imaging is the most expensive of all of the imaging techniques covered in this chapter, requiring the generation of very short-lived radiotracers by a cyclotron. Access to a cyclotron is thus a limiting factor and only a few laboratories can use this technique for preclinical imaging. Thus, here we only touch briefly on the advantages of using PET imaging in preclinical cardiovascular imaging and the reader is referred to an excellent review by Kuntner and Stout (Kuntner, 2014) for a detailed overview of preclinical PET imaging.

The biggest advantage of PET imaging is that the metabolic activity of the heart can be imaged in real-time using glucose and lipid analogs that are labeled with radioactive isotopes. As the radioisotope undergoes beta decay, it causes the emission of a positron, an antiparticle of the electron with opposite charge. The emitted positron travels a short distance (less than approximately 1 mm) through tissue (Konopinski and Rose, 1965). Once it has decelerated sufficiently, it can interact with an electron (Ziegler, 2005). The annihilation collision with an electron produces two gamma photons that move in opposite directions from the collision site. These photons are the basis for the image that is generated during PET imaging. The photons are detected by a scintillator within the scanner, which enhances the signal by generating a flash of light that can be detected by photomultiplier tubes. It is important to note that only the simultaneous detection of two photons in opposing directions contributes to image information. Photons that do not arrive in pairs or are not opposite to each other are ignored.

The most commonly used isotope for metabolic imaging in the heart is ^{18}F-fluorodeoxyglucose (^{18}F-FDG) (Higuchi et al., 2007; Rosenbaum et al., 2012). It is a glucose analog that the heart can take up but cannot metabolize past the first phosphorylation step in glycolysis by hexokinase. Thus it accumulates in heart cells and is a direct measure of glucose uptake. FDG can be used to detect hibernating myocardium, in which blood flow and thus glucose uptake is reduced. Similarly, lipids can be labeled with ^{18}F and the uptake measured by PET to complete the picture of substrate utilization by the heart.

In addition, FDG-PET imaging can be used for the detection of atherosclerosis and inflammation. This approach is limited in small animals somewhat by the resolution of PET imaging, however novel multimodal imaging, the combination of two imaging modalities such as PET/CT (Ziegler, 2005) and PET/MRI (Nappi and El Fakhri, 2013), increase the confidence in the low-resolution PET images by overlaying with a high-resolution μCT or MRI scan.

Single Photon Emission Computed Tomography

A Brief History

The early history of the development of SPECT is identical to PET. The development of the gamma camera by Hal Anger in 1952 started the use of SPECT and it soon was used in clinical settings for cardiac stress tests. The availability of isotopes with longer half-lives makes SPECT a more easily accessible method and it is also cheaper than PET imaging.

Technique Overview

SPECT uses radioisotopes to directly measure the perfusion of the heart muscle in an application that is called perfusion imaging. It is cheaper compared to PET imaging because it uses isotopes; for example, ^{99m}Tc-Tetrofosmin (Myoview, GE Healthcare), ^{99m}Tc-Sestamibi (Cardiolite, Bristol-Myers Squibb) that are stable for longer periods of time and less difficult to generate (Kelly et al., 1993). It also relies on the direct detection of emitted gamma rays and is, therefore, more comparable to scintigraphy. However, SPECT provides true 3D images compared to 2D images in conventional scintigraphy. This is achieved by up to three gamma detection cameras that rotate around the imaging object and acquire images at incremental angles of the object, equal to other tomography imaging methods.

Imaging Applications for Cardiovascular Animal Models

In animal models of ischemic heart disease, SPECT imaging allows the detection of underperfused myocardium. Small rodents are anesthetized and injected with a radiotracer. An image is acquired at rest and the heart stressed by applying an adrenergic stimulus (e.g., dobutamine). A second image is acquired shortly after the maximal activity has been achieved, and areas that are ischemic will have lower signal intensity on the SPECT image. SPECT systems specifically for small rodents have been designed, which allow a resolution of structures with about 1 μL volumetric sizes (Beekman et al., 2005). In addition, multimodal imaging in combination with μCT or MRI increases the resolution and interpretation of SPECT images (Hendrikx et al., 2016).

Optical Imaging

Optical imaging of the heart has played an important role in defining physiological processes at the molecular, cellular, and whole organ level. For example, optical imaging has been used to determine calcium transients in isolated cardiomyocytes and hearts, as well as investigate the propagation of the action potential through the heart's conduction system. With the development of new fluorophores and new imaging technologies, optical imaging is emerging as a complementary approach to nuclear imaging modalities.

Fluorescence and bioluminescence are the basis of most optical imaging approaches. Fluorescence imaging is based on the transgenic expression of fluorescent proteins (e.g., green fluorescent protein, GFP or yellow fluorescent protein, YFP) or quantum dots that are used as a source of exogenous contrast agents for in vivo imaging. In fluorescence, the energy of an external photon is absorbed and reemitted at a longer wavelength and lower energy. Biological tissues have a high photon absorbance in both the visible wavelength range (350–700 nm) and in the infrared range (>900 nm; due to lipids and water). However, in the near-infrared region (700–900 nm), the absorbance for all biomolecules is minimal. Thus, near-infrared fluorescence light offers a unique advantage for the imaging of physiological states. The advantage of using near-infrared wavelengths is the relatively deep photon penetration into tissues ($\leq$1 cm), minimal autofluorescence, less scatter, and high optical contrast when exogenous near-infrared fluorophores are used. Near-infrared imaging is now routinely used in animal models of cancer to monitor tumor growth and metastasis.

In bioluminescence, the emitted photons are produced in a chemical reaction for example by luciferin and luciferase. The luciferase enzyme catalyzes the oxidation of luciferin, resulting in light emission.

While there are several advantages in using optical imaging, there are also significant difficulties associated with this technique. First, the shallow penetration depth of most optical imaging probes limits its use for in vivo imaging of the heart. Even with near-infrared imaging, the mice have to undergo major surgery to make the heart directly accessible for the imaging camera. Second, movement artifacts caused by the high heart rate and the high breathing frequency in small rodents can make it difficult to gain useful information from optical in vivo imaging (Efimov, 2004). Image gating on ECG and breathing can prevent some but not all artifacts.

The high heart rate required the development of very high-speed image acquisition capabilities. This was achieved in developing charge-coupled device cameras that allow temporal resolutions to as high as 10,000 frames/s and thus enable imaging of isolated cardiomyocytes and isolated hearts at physiological heart rates of mice.

Optical imaging has been intensely used to describe the calcium transient in isolated cardiomyocytes of different species (Cordeiro et al., 2001; Bing et al., 1991; Pinz et al., 2011), and it has been applied to the study of different cardiac ion channels and gap junctions (Ianoul et al., 2004; Ding et al., 2017; Smyth and Shaw, 2008), as well as investigations in ischemia–reperfusion injury in the mouse heart (Lee et al., 2012).

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Preeclampsia and Hypertension in Pregnancy

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Introduction

Hypertension in pregnancy, including gestational hypertension, preeclampsia, and eclampsia, complicates 10% of pregnancies and are a leading cause of maternal mortality and morbidity worldwide. Women with chronic hypertension are at risk for developing gestational hypertension and preeclampsia/eclampsia. Those with de novo onset of hypertension in pregnancy are in turn at risk for developing later essential hypertension and cardiovascular disease. In this module we review and summarize the epidemiology, pathophysiology, and clinical management of preeclampsia and hypertension in pregnancy.

Hypertensive Disorders of Pregnancy: Definitions

Hypertensive disorders are among the most common medical problems in pregnancy and consist of chronic hypertension, gestational hypertension, preeclampsia, and preeclampsia superimposed upon chronic hypertension.

Chronic hypertension is defined as a systolic blood pressure $\geq$140 mmHg and/or diastolic blood pressure $\geq$90 mmHg that predates conception, is present before the 20th week of pregnancy, or persists longer than 12 weeks postpartum. Gestational hypertension refers to elevated blood pressure first detected after 20 weeks of gestation in the absence of proteinuria or other diagnostic features of preeclampsia. Gestational hypertension generally resolves before 12 weeks postpartum.

Preeclampsia (and eclampsia) are multisystem disorders that are characterized by new onset hypertension (systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg) and proteinuria ($\geq$0.3 g in a 24-h urine specimen) and/or end organ dysfunction after 20 weeks of gestation in a baseline normotensive woman (Cunningham and Lindheimer, 1992). Systemic complications of preeclampsia can affect the renal, hematologic, hepatic, neurologic, cardiac and/or pulmonary systems. Specific manifestations include kidney injury, thrombocytopenia, hepatic insufficiency, stroke, seizure, and pulmonary edema. Preeclampsia can also cause fetal growth restriction and result in placental abruption. In most women, these findings are seen later in pregnancy, after 34 weeks of gestation. In only 10% of cases, preeclampsia develops before 34 weeks of gestation (Cunningham and Lindheimer, 1992). Eclampsia is defined as preeclampsia complicated by neurologic defects including seizures and stroke.

When a woman with chronic hypertension develops worsening hypertension with new onset proteinuria or other cardinal features of preeclampsia, this is referred to as preeclampsia superimposed upon chronic hypertension. Distinguishing between worsening chronic hypertension in pregnancy versus new superimposition of preeclampsia has been highlighted as a particularly challenging diagnostic dilemma by the American College of Obstetrics and Gynecology (2013).

Hypertensive Disorders of Pregnancy: Epidemiology

Preeclampsia and other hypertensive disorders are the leading cause of maternal morbidity worldwide. The incidence of hypertensive disorders is estimated to be between 3% and 10% of all pregnancies. Within the past 20 years the rate of maternal chronic hypertension has nearly doubled in the United States, which is in part due to the obesity epidemic and increasing maternal age (Martin et al., 2011). The rates of chronic hypertension have increased for all racial and Hispanic ethnicity groups, with the largest

increase seen among non-Hispanic Black women (Martin et al., 2011). There are increasing trends in the United States for all types of hypertensive pregnancy disorders with the exception of mild preeclampsia (Kuklina et al., 2009; Hutcheon et al., 2011).

Preeclampsia occurs in 5% of pregnancies worldwide. Eclampsia, which is the occurrence of seizures in addition to the preeclampsia syndrome, occurs in one in 100–1700 pregnancies in the developing world and in approximately 2000 pregnancies in Europe. The range of prevalence reflects the differences in maternal age. In the United States the prevalence of preeclampsia is 2.4%, but 1.5- to 2-fold higher in first pregnancy. The reported incidence of preeclampsia is increasing in the United States due to several factors, including the high prevalence of disorders such as hypertension, diabetes, obesity, delay in child bearing, and the use of artificial reproductive techniques. Rates of eclampsia, however, have declined due to antenatal care and magnesium sulfate (Lo et al., 2013).

Physiologic Blood Pressure Changes During Pregnancy

Understanding the physiologic changes of pregnancy is key to understanding the maladaptation that occurs in preeclampsia. Pregnancy is characterized by significant hemodynamic and structural changes in the cardiovascular system that allow for adequate uteroplacental circulation and fetal development. These changes are dynamic and include increased left ventricular size, mass, heart rate, cardiac output, vascular compliance, and capacitance. Systemic vascular resistance (SVR) and blood pressure decrease during pregnancy (Clapp and Capeless, 1997).

Hormones including estrogen, progesterone, and chorionic gonadotropin as well as prostaglandins and nitric oxide act on the vascular endothelium and smooth muscle to alter vessel structure and tone. As early as 5 weeks' gestation, the vasodilatory effects of these hormones and the low-resistance of the uteroplacental flow lead to a significant decrease in SVR (Ouzounian and Elkayam, 2012). In the first trimester, SVR decreases by around 10% and nadirs in the middle of the second trimester. It then plateaus until approximately 32 weeks, when it minimally increases (Clapp and Capeless, 1997). The change in SVR is associated with greater vascular compliance as well as a decrease in blood pressure and an increase in pulse pressure.

Due to the effects on blood vessel tone and the low resistance of the uteroplacental vascular system, a decrease in arterial pressures is seen in pregnancy. Diastolic blood pressure decreases more significantly than systolic blood pressure. Blood pressure nadirs during the second trimester, dropping by 5–10 mmHg below baseline. These changes in blood pressure have been demonstrated in numerous studies. A prospective cohort study by Grindheim et al. confirmed a statistically significant drop in blood pressure in mid-pregnancy followed by a progressive increase until term (Grindheim et al., 2012; Mahendru et al., 2014).

Pregnancy is also associated with increased plasma volume secondary to activation of the renin–angiotensin–aldosterone system. This results in retention of salt and water, which is augmented by increased vasopressin secretion (Sanghavi and Rutherford, 2014). The degree of volume expansion is directly linked to normal fetal growth and birth weight (Ouzounian and Elkayam, 2012). Cardiac output is also seen to increase throughout gestation. A rise in stroke volume accounts for the increase in cardiac output early in pregnancy, while heart rate is the main contributor later in pregnancy. These changes can augment the cardiac output by up to 45% by 24 weeks (Sanghavi and Rutherford, 2014).

The varied and dynamic effects on the cardiovascular system, including the effects on blood pressure, during pregnancy tend to return to baseline 2 weeks postpartum. However, failure to make the above adaptations is associated with uteroplacental insufficiency and pregnancy complications, such as preeclampsia and other hypertensive disorders of pregnancy.

Placentation in Preeclampsia Versus Normal Pregnancy

Normal placentation involves trophoblast invasion into the inner third of the maternal myometrium including the uterine spiral arteries. These trophoblasts replace the endothelial lining of the spiral arteries. The sum of these processes serve to take a high-resistance, low-flow vascular system and convert it to a low-resistance, high-flow system, thus allowing for adequate fetal perfusion. In preeclampsia however, there is often a relatively shallow invasion of the myometrium and an incomplete replacement of the endothelial lining of the uterine spiral arteries as occurs in normal pregnancy. These abnormalities result in an effective decrease to the vascular perfusion to the fetus and placenta resulting in fetal growth restriction in the more severe cases of preeclampsia and in early-onset preeclampsia. The abnormal placentation is accompanied by a maternal inflammatory response.

Chronic hypertension or renal disease is a risk factor for preeclampsia, along with a family history of preeclampsia. It is uncertain which specific factors in hypertensive women directly contribute to the underlying placental abnormalities that characterize preeclampsia.

Preeclampsia Screening

Given that preeclampsia is a leading cause of maternal death and morbidity, as well as numerous fetal complications, multiple studies have attempted to guide screening. The National Institute for Health and Clinical Excellence (NICE) antenatal care

guideline was published in 2003 and updated in 2008 (NICE, 2008). The preeclampsia community guideline (PRECOG) was published in 2005 in the United Kingdom (Milne et al., 2005). Both of these publications attempt to identify women at risk for preeclampsia who require either subspecialty referral or more frequent monitoring. However, the clinical utility of these guidelines remains controversial (Verghese et al., 2012). Furthermore, no single test has been identified to predict preeclampsia adequately enough to be instituted in clinical practice (Magee et al., 2015a). The American College of Obstetricians and Gynecologists (ACOG) recognizes this fact and recommends obtaining a detailed medical history as the only screening for preeclampsia. ACOG further notes that supplemental predictive tests may do more harm than good secondary to their low positive predictive value (Taghiof et al., 2015).

When taking a clinical history, focusing on risk factors for the development of preeclampsia is key. Strong risk factors include previous preeclampsia, preexisting hypertension, chronic kidney disease, type 1 or 2 diabetes, and autoimmune disorders include systemic lupus erythematosus and antiphospholipid antibody syndrome. Other identified risk factors include maternal age $\geq$40 years old, family history of preeclampsia, BMI of 35 or more, first pregnancy, multiple pregnancy, and polycystic ovarian syndrome (Magee et al., 2015a; Mol et al., 2016; North et al., 2011). Based on the medical history, it is up to the provider to refer to subspecialty treatment, more frequent monitoring, and assess for preventative therapy. Magee et al. recommend that these measures be offered if a woman has one or more strong risk factors. Women with two or more "other" factors should also be offered these interventions (Magee et al., 2015a).

Along with a clinical history, a standard physical exam and blood/urine testing are commonly performed. Knowing that a pregnant woman's blood pressure varies greatly on her positioning, blood pressure should be measured in a seated and upright position or in a left lateral recumbent position. Subsequent blood pressure measurements should be taken in the same position for consistency (Mol et al., 2016; Ouzounian and Elkayam, 2012). Recommended testing includes urinalysis, CBC and film, serum creatinine, and liver enzymes as well as function. Proteinuria should be assessed for early in pregnancy to identify patients with preexisting kidney disease and at $\geq$20 weeks to screen for preeclampsia in those identified as at risk (Magee et al., 2015a). This screening is usually performed by dipstick testing, which has a low mean sensitivity of 55% but an adequate mean specificity of 84% (Magee et al., 2015a).

Although current guidelines recommend against further screening, studies are currently evaluating the utility of maternal blood biomarkers, such as placental growth factor (Mol et al., 2016). Other combination screening procedures are also being studied, including the use of late first-trimester uterine artery Doppler ultrasound, placental growth factor, and pregnancy-associated plasma protein A in maternal blood. These tests combined have been shown to predict early-onset preeclampsia with a sensitivity of 93% (95% CI 76%–98%) and specificity of 95% (95% CI 94%–96%) (Mol et al., 2016; Cuckle and Maymon, 2016). However, they have not been externally validated.

Preeclampsia Prevention

Preeclampsia is associated with an imbalance of thromboxane and prostacyclin resulting in platelet activation and endothelial dysfunction (Leslie et al., 2011; Correa et al., 2016). The efficacy of antiplatelet agents has thus been studied in the prevention of preeclampsia.

In 2010, Bujold et al. published a systematic review suggesting that initiation of low-dose aspirin at $\leq$16 weeks in women at high or moderate risk for preeclampsia significantly reduced the occurrence of preeclampsia (RR: 0.47, 95% CI: 0.34–0.65), severe preeclampsia (RR: 0.09, 95% CI: 0.02–0.37), gestational hypertension (RR: 0.62, 95% CI: 0.45–0.84), intrauterine growth restriction (RR: 0.44, 95% CI: 0.30–0.65), and preterm birth (RR: 0.22, 95% CI: 0.10–0.49) (Bujold et al., 2010). A recent meta-analysis further supported the beneficial effects of low-dose aspirin initiated $\leq$16 weeks. However, this study could find little to no reduction on the relative risk of preeclampsia, severe preeclampsia, and fetal growth restriction when aspirin was initiated after 16 weeks (Roberge et al., 2017). Although multiple studies have reaffirmed a benefit of aspirin in reducing the risk of preeclampsia, identifying the appropriate population to treat, when to initiate therapy, and the optimal dose of aspirin remain unclear (Correa et al., 2016). For the time being, ACOG recommends the initiation of low-dose (60–80 mg) aspirin beginning in the late first trimester in women with a medical history of early-onset preeclampsia and preterm delivery at less than 34 weeks, or preeclampsia in more than one previous pregnancy (American College of Obstetricians and Gynecologists, 2013). Prior clinical trials that have additionally focused on the anticoagulant effects of heparin and dalteparin in preventing preeclampsia demonstrate positive effects. These studies, however, were too small to make definitive conclusions (Mol et al., 2016).

Along with antiplatelet and anticoagulant agents, researchers have focused on the connection between low dietary calcium and preeclampsia. Prior studies have shown a link between low dietary calcium and low serum calcium concentrations with higher rates of preeclampsia (Mol et al., 2016). A Cochrane review published in 2014 showed that high-dose calcium supplementation reduced the risk of preeclampsia in women with low dietary calcium intake (RR 0. 36, 95% CI 0.20–0.65) (Hofmeyr et al., 2014). The World Health Organization (WHO) thus recommends calcium supplementation (1.5–2 g daily) in women with low dietary calcium intake in the second half of pregnancy (Correa et al., 2016).

Further studies on diet and lifestyle interventions, antioxidants, fish oils, magnesium, and garlic in preventing preeclampsia were unable to show a clear benefit (Leslie et al., 2011; Correa et al., 2016). L-Arginine, a precursor to nitric oxide, is also being evaluated and more evidence is needed to confirm any protective effect (Mol et al., 2016; Correa et al., 2016). Pravastatin has shown promising effects in preclinical and pilot clinical studies to prevent preeclampsia, but further randomized controlled trials are needed to validate these findings (Marrs and Costantine, 2016).

Preeclampsia Treatment

Once a woman is diagnosed with preeclampsia, the only cure is delivery (Mol et al., 2016). Subspecialty consultation with high-risk perinatology physicians is recommended and inpatient treatment may be necessary if severe hypertension or end-organ damage develop.

Intravenous fluids should be minimized and hypertension with systolic blood pressure ≥ 160 or diastolic blood pressure ≥ 110 should be treated. The recent CHIPS trial found that treatment of maternal hypertension benefits the mother without increasing pregnancy loss or requirement for high-level neonatal care for more than 48 h (Magee et al., 2015b). Appropriate antihypertensives include methyldopa, labetalol, hydralazine, and nifedipine. ACE inhibitors, angiotensin receptor blockers, prazosin, and atenolol are avoided due to known or suspected teratogenicity. In an emergency setting, IV hydralazine, labetalol, and oral nifedipine are commonly used (Berzan et al., 2014). Repeated doses of these medications every 15–30 min control blood pressure in at least 80% of women (Mol et al., 2016).

The WHO and ACOG recommend magnesium sulfate for prevention of eclampsia in women with severe preeclampsia (Organization, 2011; American College of Obstetricians and Gynecologists, 2013). This therapy has been found to more than halve the risk of eclampsia (Berzan et al., 2014). However, around one hundred women with preeclampsia have to be treated with magnesium sulfate to prevent one seizure. Due to cost and a high number needed to treat, guidelines recommend avoiding magnesium sulfate in women without severe preeclampsia (Magee et al., 2015a).

The timing of delivery in women with preeclampsia is key to treatment. ACOG stipulates that expectant management is appropriate for women with preeclampsia without severe features and no indication for delivery at less than 37 weeks. This should only be done if there are adequate resources to ensure close maternal and fetal monitoring. Such monitoring includes daily assessment of symptoms and fetal movement, twice-weekly measurements of blood pressure, and obtaining platelet counts and liver function weekly (American College of Obstetricians and Gynecologists, 2013). Only 40% of women, however, are eligible for expectant care. For women with preeclampsia without severe features at or past 37 weeks, delivery is recommended. Delivery is also appropriate in women with severe preeclampsia at or beyond 34 weeks, and in women who are unstable or have fetal instability no matter what the gestational age (American College of Obstetricians and Gynecologists, 2013). It is up to the clinician to weigh the risks and benefits as well as patient preferences in pursuing delivery.

After delivery, it is imperative to monitor the mother for complications of preeclampsia, especially in the first 48 h postpartum. Blood pressure control and patient education on symptoms and signs are key (Mol et al., 2016).

Pathophysiology of Preeclampsia and CV Risk

Placental abnormalities in preeclampsia mirror many of the vascular changes seen in cardiovascular disease including acute placental arterial atherosis, fibrin deposition, and accumulation of lipid-laden macrophages (Fisher, 2015). Pathologic placental specimens derived from preeclamptic pregnancies demonstrate placental arterial thrombosis and infarction (Gibbins et al., 2016). Several studies now demonstrate that preeclampsia is an independent risk factor for cardiovascular disease including fatal and nonfatal myocardial infarction (Brown et al., 2013). The underlying mechanisms accounting for the increased risk include (1) shared genetic risk factors for hypertension in pregnancy and CVD, (2) other shared risk factors including hypertension, insulin resistance, obesity, thrombophilia, and endothelial dysfunction. Subclinical atherosclerosis as measured by carotid intima media thickness was elevated prior to preeclampsia (Milic et al., 2017). Whether the preeclamptic pregnancy per se increases a woman's risk of CVD is uncertain.

Preeclampsia and Later CVD in Women

In a recent meta-analysis of 43 studies, preeclampsia was associated with increased pooled odds of CVD: OR 2.28 (95% CI 1.87–2.78), of stroke: OR = 1.76, 95% CI 1.43, 2.21) and of hypertension [relative risk (RR) = 3.13, 95%CI 2.51, 3.89] (Brown et al., 2013). As a result of this growing body of evidence, expert panels now recommend taking a history of preeclampsia as part of cardiovascular disease risk stratification in women including the American Heart Association (Mosca et al., 2011) as well as European expert committees (Heida et al., 2016). However, the question of whether preeclampsia has utility as a novel marker for CVD risk prediction in women over and beyond traditional risk factors has not been systematically evaluated. However,

regardless, preeclampsia likely serves as an early window that may reveal which women are at higher risk for hypertension and cardiovascular diseases.

Postpartum Screening and Management of CV Risk Factors in Women With Gestational Hypertension and Preeclampsia

Women with a history of preeclampsia following pregnancy and delivery should be referred to the primary care providers early for close and aggressive monitoring of CVD risk factors including blood pressure, weight, lipids, and lifestyle factors such as smoking and physical activity. Studies have evaluated the feasibility of referral clinics for women with pregnancy complications such as preeclampsia, which focus on cardiovascular risk factor modification (Cusimano et al., 2014).

Conclusions

Preeclampsia and hypertension in pregnancy are disorders with a growing prevalence in the United States and can lead to substantial maternal morbidity and mortality without treatment and intervention. Similar to essential hypertension, the exact etiology of preeclampsia and gestational hypertension is uncertain; however, specific features of the downstream pathophysiology of preeclampsia substantially overlap with cardiovascular disease. Preeclampsia is a risk factor for heart disease, stroke, and hypertension and therefore can identify the woman at risk for developing these conditions, who may benefit from early and intensive lifestyle modification.

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Pregnancy and Cardiovascular Disease

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Introduction

In western industrialized countries, the prevalence of pregnancies carried by women with cardiovascular disease is 0.2%–4% (Regitz-Zagrosek et al., 2011). This situation poses an increased risk to both mother and fetus. With the declining incidence of rheumatic heart disease in Western countries, most maternal heart disease is now congenital. Other heart diseases include dilated cardiomyopathies, hypertrophic cardiomyopathies, valvular disease, pulmonary arterial hypertension (PAH), and Marfan syndrome. Maternal heart disease remains an important cause of maternal mortality and morbidity during pregnancy. In a report of maternal deaths from 2006 to 2008 in the United Kingdom, heart disease remains the most common cause of indirect maternal death (Cantwell et al., 2011).

Cardiac events may be caused by hemodynamic changes including increased volume load (preload) and hypercoagulable status during pregnancy. It is quite important to understand these physiological changes during pregnancy and the risk factors for cardiac events during pregnancy in each type of heart disease. High-risk pregnancies are shown in **Table 1** (Bonow et al., 2012). Women with pulmonary hypertension, Eisenmenger syndrome, dilated cardiomyopathy (ejection fraction <40%), symptomatic obstructive lesion, Marfan syndrome with aortic root >40 mm, cyanotic lesions, and mechanical prosthetic valves are considered to have high-risk pregnancies. In some cases, patients should avoid pregnancy or in some cases consider termination.

All patients with known cardiac disease, especially these high-risk groups, should be counseled before conception. Risk assessment of cardiac events during pregnancy is important. If possible, needed surgical operations including valvotomy, valve repair, or replacement should be performed before pregnancy. Treatment of arrhythmias and treatment of underlying medical conditions such as hypertension or diabetes should also be performed before pregnancy. To avoid teratogens, medication should be changed before pregnancy; for instance, warfarin or angiotensin-converting enzyme (ACE) inhibitors should be avoided. It is important to discuss anticoagulation factors. Women of child-bearing age using warfarin need to understand its teratogenic potential and the risk of fetal intracranial hemorrhage, and warfarin should be changed, in most cases to heparin, immediately after pregnancy is confirmed, and certainly before sixth weeks of gestation. Women with mechanical valves, in particular, should be counseled on the risk and benefit of warfarin versus low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH), and given the opportunity for an informed choice about their anticoagulant regime during pregnancy. Their pregnancies should be managed by a multidisciplinary team. Especially high-risk patients should be treated in specialized tertiary centers (Regitz-Zagrosek et al., 2011).

Epidemiology

With the decreasing incidence of rheumatic heart disease in Western countries, the most common maternal heart disease is now congenital heart disease. Other maternal cardiovascular diseases include dilated or hypertrophic cardiomyopathies, and peripartum cardiomyopathy, valvular disease, pulmonary hypertension, and Marfan syndrome with aortic root dilatation. In western countries

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Table 1 High-risk pregnancies of cardiovascular disease

Pulmonary hypertension/Eisenmenger syndrome
Dilated cardiomyopathy, ejection fraction <40%
Symptomatic obstructive lesions
Aortic stenosis
Mitral stenosis
Pulmonary stenosis
Coarctation of the aorta
Marfan syndrome with aortic root >40 mm
Cyanotic lesions (oxygen saturation:<85%)
Mechanical prosthetic valves

Modified from Bonow, R. O., Mann, D. L., Zipes, D. P. and Libby, P. (2012). *Braunwald's heart disease: a textbook of cardiovascular medicine* (9th edn.). Philadelphia: Elsevier Saunders.

maternal heart disease is now the predominant cause of maternal death during pregnancy (Cantwell et al., 2011). Hypertensive disorders are the most frequent cardiovascular events during pregnancy, occurring in 6%–8% of all pregnancies (Peters and Flack, 2004).

Physiological Changes in Normal Pregnancy

Hemodynamic Changes in Pregnancy

Hemodynamic changes begin early in the first trimester. The plasma volume begins to increase in the sixth week of pregnancy and at 24 weeks' gestation reaches 30%–50% above baseline. Cardiac output (CO) is increased around 50% (Hunter and Robson, 1992). The heart rate begins to rise at 20 weeks and continues to increase until 32 weeks when it plateaus. It remains high through the immediate postpartum period, 2–5 days after delivery. Total peripheral vascular resistance is reduced. As a result, systemic blood pressure falls early in gestation and diastolic blood pressure is reduced in the second trimester. Increased CO is primarily related to the rise in stroke volume in early pregnancy; on the other hand, heart rate is the major factor in late pregnancy (Robson et al., 1989; Mabie et al., 1994).

These hemodynamic changes may cause problems for pregnant women with cardiac disease. Clearly, the added volume load may compromise a patient who has impaired ventricular function and limited cardiac reserve. Stenotic valvular (e.g., aortic stenosis) lesions are less well tolerated than regurgitant lesions because the decrease in peripheral resistance exaggerates the gradient across the aortic valve. On the other hand, with a lesion such as mitral regurgitation, the afterload reduction helps offset the volume load on the left ventricle that gestation imposes (Bonow et al., 2012).

Hematologic Changes in Pregnancy

During pregnancy, plasma concentrations of coagulation factors are greatly changed (Stirling et al., 1984; Szecsi et al., 2010). The levels of coagulation factors II, V, X, XI, and XII, antithrombin and protein C, as well as the activated partial thromboplastin time (APTT) and prothrombin time remain largely unchanged during pregnancy, delivery, and postpartum, remaining within the nonpregnant reference ranges. However, levels of fibrinogen, D-dimer, and coagulation factors VII, VIII, and IX increase markedly during pregnancy. Protein S activity decreases substantially, while free protein S decreases slightly and total protein S is stable (Szecsi et al., 2010). These procoagulant changes have the physiological function of minimizing intrapartum blood loss, but they do increase the risk of thromboembolism during pregnancy and the postpartum period.

It is also problematic to monitor the anticoagulant effect of a therapeutic dose of heparin during pregnancy. In pregnant women, APTT does not precisely reflect the anticoagulant effect of a therapeutic dose of heparin. However, the measurement of anti-Xa activity can be used to monitor the anticoagulant effect of heparin, since the plasma concentrations of coagulation factors increase in pregnant women. In the presence of UFH, APTT was shortened by increased concentrations of fibrinogen, FVII, or FVIII; the effect was much stronger when the FVIII concentration was increased (Mitsuguro et al., 2015).

Risk Assessment for Pregnancy

Preconception Counseling

Preconception counseling should be offered to all women of reproductive age with known cardiac disease. Risk assessment should focus on both mother and fetus. Risks for the mother include cardiovascular events, maternal death during pregnancy and

postpartum, and persistent deterioration of heart function. Risks for the fetus include the teratogenicity of medication, fetal growth restriction, fetal death, preterm birth, and the inheritance of congenital heart disease, Marfan syndrome, and hypertrophic cardiomyopathy. If necessary, drug schedules should be changed before pregnancy.

Drug Treatment During Pregnancy

There are no standard or uniform recommendations for the medical treatment of pregnant women. Drug treatment during pregnancy should consider both the mother and the fetus. A medication should be given only if the benefits overweigh the potential risk to the fetus. We should consider the use of the lowest dose and shortest duration, if possible. All these issues should be reviewed carefully before pregnancy. Especially we need special caution with the administration of amiodarone, ACE inhibitors, angiotensin II receptor blocker, and warfarin. A list of cardiovascular drugs that might be considered safer options during pregnancy is given in **Table 2**.

Maternal Cardiovascular Risk Assessment

There are some risk stratifications for estimating maternal cardiovascular complications. CARPREG (CARdiac disease in PREGnancy) is the most widely used risk score (Siu et al., 2001). This prospective multicenter study included 599 pregnancies in women with congenital or acquired cardiac lesions or those with cardiac arrhythmias. This study incorporates the four predictors of primary cardiac events as shown in **Table 3**. Predictors of primary cardiac events were incorporated into a revised risk index, in which each pregnancy was assigned 1 point for each predictor when present. The estimated risk of a cardiac event in pregnancies with 0, 1, and >1 points was 5%, 27%, and 75%, respectively. This study showed that pulmonary edema, arrhythmia, stroke, or cardiac death complicated 13% of pregnancies (Siu et al., 2001).

Table 2 Cardiovascular drugs in pregnancy

Drug	*Potential fetal side effects*
Amiodarone (INN)	Thyroid insufficiency, hyperthyroidism, goitre, bradycardia, FGR, premature birth
Angiotensin-converting enzyme inhibitors	Oligohydramnios due to renal failure or renal dysplasia, lung hypoplasia, FGR, IUFD, abnormal bone ossification
Angiotensin II receptor blocker	Oligohydramnios due to renal failure or renal dysplasia, lung hypoplasia, FGR, IUFD, abnormal bone ossification
Acetylsalicylic acid (INN)	Low-dose usage is safe
Beta blockers	Relatively safe; bradycardia and hypoglycemia in fetus; FGR
Calcium channel blockers	Relatively safe; potential synergism with magnesium sulfate may induce hypotension
Digoxin (INN)	Safe; no adverse effects
Flecainide (INN)	Limited data; used to treat fetal arrhythmias
Furosemide (INN)	Safe; caution regarding maternal hypovolemia and reduced placental blood flow; oligohydramnion
Hydralazine (INN)	Safe; no major adverse effects, lupus-like symptoms for mother
Lidocaine (INN)	Safe; high doses may cause neonatal central nervous system depression
Methyldopa (INN)	Safe; one of the choices for hypertension during pregnancy
Procainamide (INN)	Relatively safe; limited data
Warfarin (INN)	Fetal embryopathy (nasal hypoplasia and/or stippled epiphyses; limb hypoplasia) for first-trimester exposure, adverse fetal CNS events and CNS abnormalities with exposure in any trimester, placental and fetal hemorrhage

FGR, fetal growth restriction; *IUFD*, intrauterine fetal death; *INN*, International Nonproprietary Name; CNS, central nervous system.

Modified from Bonow, R. O., Mann, D. L., Zipes, D. P. and Libby, P. (2012). *Braunwald's heart disease: a textbook of cardiovascular medicine* (9th edn.). Philadelphia: Elsevier Saunders.

Table 3 CARPREG maternal cardiovascular risk score

- Prior cardiac event (heart failure, transient ischemic attack, or stroke before pregnancy) or arrhythmia
- Baseline NYHA functional class >II or cyanosis
- Left heart obstruction (mitral valve area <2 cm^2 or aortic valve area <1.5 cm^2 or peak left ventricular outflow tract gradient >30 mmHg by echocardiography)
- Reduced systemic ventricular systolic function (ejection fraction <40%)

NYHA, New York Heart Association.

Modified from Siu, S. C., Sermer, M., Colman, J. M., Alvarez, A. N., Mercier, L. A., Morton, B. C., Kells, C. M., Bergin, M. L., Kiess, M. C., Marcotte, F., Taylor, D. A., Gordon, E. P., Spears, J. C., Tam, J. W., Amankwah, K. S., Smallhorn, J. F., Farine, D., Sorensen, S. and Cardiac Disease in Pregnancy, I. (2001). Prospective multicenter study of pregnancy outcomes in women with heart disease. *Circulation* **104**(5), 515–521.

Table 4 ZAHARA maternal cardiovascular risk scores identified in congenital heart diseases

Zahara
● Prior arrhythmias
● NYHA functional class >II
● Left heart obstruction (peak LVOT gradient >50 mmHg or aortic valve area <1.0 cm^2)
● Mechanical valve prosthesis
● Systemic atrioventricular valve regurgitation (moderate/severe)
● Pulmonary atrioventricular valve regurgitation (moderate/severe)
● Cardiac medication before pregnancy
● Cyanotic heart disease (corrected and uncorrected)

NYHA, New York Heart Association; *LVOT*, LV outflow tract.
Adapted from Balci, A., Sollie-Szarynska, K. M., van der Bijl, A. G., Ruys, T. P., Mulder, B. J., Roos-Hesselink, J. W., van Dijk, A. P., Wajon, E. M., Vliegen, H. W., Drenthen, W., Hillege, H. L., Aarnoudse, J. G., van Veldhuisen, D. J., Pieper, P. G. and Investigators, Z.-I. (2014). Prospective validation and assessment of cardiovascular and offspring risk models for pregnant women with congenital heart disease. *Heart* **100**(17), 1373–1381.

Table 5 Modified WHO classification of maternal cardiovascular risk

WHO class	*Risk for pregnancy by medical condition*
I	No detectable increased risk of maternal mortality or morbidity
II	Small increased risk of maternal mortality or morbidity
III	Significantly increased risk of maternal mortality or severe morbidity. Expert counseling required. If pregnancy is decided upon, intensive specialist cardiac and obstetric monitoring needed throughout pregnancy, childbirth, and puerperium
IV	Extremely high risk of maternal mortality or severe morbidity; pregnancy contraindicated. If pregnancy occurs termination should be discussed. If pregnancy continues, care as for class III

WHO, World Health Organization.
Adapted from Regitz-Zagrosek et al., 2011, which was modified from Thorne, S., MacGregor, A. and Nelson-Piercy, C. (2006). Risks of contraception and pregnancy in heart disease. *Heart* **92**(10), 1520–1525.

The ZAHARA (Zwangerschap bij Aangeboren HARtAfwijkingen) study provides additional risk calculators in pregnant women with congenital heart disease (Drenthen et al., 2010) (**Table 4**). It should be noticed that these CARPREG and ZAHARA studies did not identify important high-risk factors including PAH and dilated aorta, simply because they were underrepresented in these studies.

The Task Force of the European Society of Cardiology (ESC) Guidelines (Regitz-Zagrosek et al., 2011) recommends that maternal risk be assessed according to the modified World Health Organization (WHO) risk classification (Thorne et al., 2006). This risk classification integrates all known maternal cardiovascular risk factors including underlying heart disease and any other comorbidity. It includes contraindications for pregnancy that are not incorporated in the CARPREG and ZAHARA risk scores. The modified WHO classification is shown in **Table 5**. WHO class I indicates low risk, WHO class II indicates low or moderate risk, and WHO class III indicates high risk, for which frequent follow-up by a cardiologist and obstetrician during pregnancy is recommended. WHO class IV constitutes a contraindication for pregnancy; however, if these patients become pregnant and will not consider termination, close and careful follow-up is needed.

Additionally, Balci et al. compared these three maternal cardiovascular risk scores for pregnant women with congenital heart disease: namely, the ZAHARA and CARPREG risk scores and the modified WHO classification. Their study concluded that the WHO classification is the best available risk assessment model for estimating cardiovascular risk in pregnant women with CHD given in **Table 6** (Balci et al., 2014).

Cardiac Events During Pregnancy

Heart failure and arrhythmias are the most common cardiac events during pregnancy.

Heart Failure

Preeclampsia is a common condition that precipitates afterload failure, and chronic hypertension with superimposed preeclampsia is the most frequent cause of heart failure in pregnant women. All patients with heart failure during pregnancy should be admitted to a nursing or medical facility for bed rest. Medical therapy includes diuretics to reduce preload and antihypertensive therapy including hydralazine or another vasodilator to reduce afterload.

Table 6 Modified WHO classification of maternal cardiovascular risk

Conditions in which maternal risk is WHO class I
Uncomplicated, small or mild pulmonary stenosis
Successfully repaired simple lesions (atrial or ventricular septal defect, patent ductus arteriosus, anomalous pulmonary venous drainage)
Conditions in which maternal risk is WHO class II or III
WHO class II (if otherwise well and uncomplicated)
Unoperated atrial or ventricular septal defect, repaired tetralogy of Fallot
WHO class II–III (depending on individual)
Native or tissue valvular heart disease not considered WHO I or IV; repaired coarctation; Marfan syndrome without aortic dilatation, bicuspid valve with aorta <45 mm; mild ventricular impairment
WHO class III
Mechanical valve; systemic right ventricle; Fontan circulation; unrepaired cyanotic heart disease; other complex congenital heart disease; Marfan syndrome with aortic dilation 40–45 mm; bicuspid aortic valve with aortic dilation 45–50 mm
Conditions in which maternal risk is WHO class IV (contra-indicated)
Pulmonary hypertension/Eisenmenger syndrome; systemic ventricular EF <30% or systemic ventricular dysfunction with NYHA class III–IV; severe mitral stenosis, severe symptomatic aortic stenosis, Marfan syndrome with aortic dilation >45 mm; bicuspid aortic valve with aortic dilation >50 mm; native severe coarctation

WHO, World Health Organization; *EF*, ejection fraction.

Adapted from Balci, A., Sollie-Szarynska, K. M., van der Bijl, A. G., Ruys, T. P., Mulder, B. J., Roos-Hesselink, J. W., van Dijk, A. P., Wajon, E. M., Vliegen, H. W., Drenthen, W., Hillege, H. L., Aarnoudse, J. G., van Veldhuisen, D. J., Pieper, P. G. and Investigators, Z.-I. (2014). Prospective validation and assessment of cardiovascular and offspring risk models for pregnant women with congenital heart disease. *Heart* **100**(17), 1373–1381.

During pregnancy, ACE inhibitors, ARBs, and renin inhibitors are contraindicated due to their known fetotoxicity. Hydralazine and nitrates can be used instead of ACE inhibitors and ARBs for afterload reduction. Administration of a β-blocker is indicated for all patients with heart failure, with a preference for β1-selective drugs. Diuretics should be used for only pulmonary edema because they may decrease placental blood flow.

Arrhythmias

Due to the physiological changes of pregnancy, the incidence of arrhythmias may be increased during pregnancy in women with heart disease. If the patient has no underlying heart disease, pharmacologic treatment should be administered when the patient is symptomatic or when arrhythmia is a risk for the mother or fetus. If the patient has underlying heart disease, the precipitating cause should be treated. The most common arrhythmia is atrial reentry tachycardia. The major concern for use of antiarrhythmic drugs during pregnancy is their potential adverse effects on the fetus (**Table 2**). It is important to treat symptomatic tachyarrhythmia by catheter ablation before pregnancy.

High-Risk Maternal Heart Disease: WHO Class III or IV

Pulmonary Hypertension

Pulmonary hypertension is a category of high-risk pregnancy in WHO class IV. PAH is defined as a nonpregnant elevation of the mean pulmonary artery pressure ≥25 mmHg at rest or 30 mmHg at exercise in the absence of a left-to-right shunt (Bedard et al., 2009). Pulmonary artery systolic pressure is usually estimated using Doppler ultrasound to measure the regurgitant jet velocity across the tricuspid valve. A Doppler study is a good diagnostic modality during pregnancy. Pulmonary hypertension includes different pathophysiologies: that is, PAH, pulmonary hypertension due to left heart disease, pulmonary hypertension due to lung disease and/or hypoxia, chronic thromboembolic pulmonary hypertension, and pulmonary hypertension with unclear multifactorial mechanisms (Simonneau et al., 2013). It is important that the pulmonary artery pressure falls in the presence of moderate to severe right ventricular impairment, thus modulating the severity of pulmonary vascular disease. The maternal risk with pulmonary vascular disease is high; previously, the mortality risk for patients with severe PAH and Eisenmenger syndrome was reported as 30%–50% (Weiss et al., 1998), but in recent data the estimate fell to 17%–33% (Bedard et al., 2009), which is still quite significant.

If such patients are pregnant, termination should be considered. Given the risk associated with anesthesia, delivery should be performed in a tertiary center experienced in the management of PAH patients. If patients want to continue pregnancy despite the risk, they should be managed in a center with expertise in PAH with all therapeutic options available (Regitz-Zagrosek et al., 2011).

Marfan Syndrome

Aortic dilatation and dissection are severe complications of pregnancy that may cause maternal death. The overall risk of maternal death is approximately 1%. Women at particularly high risk include those with a poor family history, cardiac involvement and aortic root >4 cm diameter, or a rapidly dilating aorta (Lind and Wallenburg, 2001; Elkayam et al., 1995). Care should be taken during both pregnancy and the postpartum period (Katsuragi et al., 2011). The prevalence of sinus of Valsalva ≥40 mm, aortic size

index (size of sinus of Valsalva/body surface area) ≥ 25 mm/m^2, and faster growth of the sinus of the Valsalva was significantly higher in the group with aortic dilatation or dissection than in groups without aortic dilatation or dissection (Katsuragi et al., 2011).

Mechanical Valve Prosthesis and Anticoagulant Therapy

The management of pregnancy with maternal mechanical valve prosthesis is quite controversial. There is no universal consensus or standard regimen. During pregnancy, maternal blood is highly thrombotic due to increased concentrations of clotting factors and decreased fibrinolysis. These hematological changes increase the risk of valve thrombosis and thromboembolism during pregnancy. Anticoagulant therapy during pregnancy raises specific concerns, because of the increased risk of valve thrombosis, hemorrhagic complications, and offspring complications. At least during the first trimester, that is between the sixth and twelfth weeks of gestation, vitamin K antagonists such as Warfarin should be avoided because of fetal embryopathy (**Table 2**).

Nonetheless, use of such agents still remains despite adverse fetal effects for exposure in each trimester. During the second and third trimesters, in choosing between vitamin K antagonists, UFH, and LMWH, each maternal and fetal risk should be weighed carefully. If vitamin K antagonists are used, their use is limited until 36 weeks of gestation, and then they should be replaced by heparin before delivery (Regitz-Zagrosek et al., 2011).

Conclusion

Heart disease is the major cause of maternal mortality in the United Kingdom. Pulmonary hypertension, Marfan syndrome with aortic dilation more than 40 mm, or mechanical valves are at highest risk of developing cardiac events during pregnancy. These groups are classified as modified WHO class III or IV. All patients with known cardiac disease should be counseled before pregnancy. If at all possible, needed surgical operations should be performed before pregnancy. To avoid teratogens, medication such as warfarin or ACE inhibitors should be swapped out before pregnancy. Such patients should be managed by a multidisciplinary team. Particularly high-risk patients should be treated in specialized tertiary centers. Careful and intensive management is required for these high-risk pregnant women.

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Proteomics

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Abbreviations

1DE	One-dimensional gel electrophoresis
2DE	Two-dimensional gel electrophoresis
CID	Collision-induced dissociation
DDA	Data-dependent acquisition
DIA	Data-independent acquisition
DIGE	Difference gel electrophoresis
ESI	Electrospray ionization
FDR	False discovery rate
HPLC	High-performance liquid chromatography
iTRAQ	Isobaric tagging for relative and absolute quantification
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography tandem-mass spectrometry
MALDI	Matrix-assisted laser desorption ionization
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem-mass spectrometry
PTM	Posttranslational modification
SILAC	Stable isotope labeling by amino acids in cell culture
SRM	Selected reaction monitoring
SWATH	Sequential windowed acquisition of all theoretical mass spectra
TMT	Tandem mass tags

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Introduction to Proteomics

Proteomics as Part of Systems Biology

Biological processes are not linear systems but represent dynamic networks with cross-talk of their molecular components at multiple levels. This concept is embraced by systems biology and supported by the analytical capabilities of -omics technologies. Systems biology uses a holistic, rather than the traditional reductionist approach of studying only a single gene or protein at a time. Thus, systems biology explores mechanisms and interactions in biological systems in a more unbiased way. Omics experiments have become feasible with the advent of high-throughput technologies to obtain and analyze larger amounts of data. Particularly the *Human Genome Project* paved the way to high-throughput sciences (Lander et al., 2001). Proteomics aims to deliver a "protein complement of the genome" (Lottspeich, 2009). It has evolved as an essential part of systems biology, as reflected in the increasing number of publications (Lindsey et al., 2015).

Proteomics: Definition and Challenges

The term "proteome" was first introduced in the mid-1990s in analogy to the term "genome" and refers to the entire set of proteins produced by an organism, a tissue, or a cell at any given time (Wilkins et al., 1996a,b). Proteomics is the large-scale study of the entire proteome including protein isoforms and modifications, their abundance, and structure as well as the interactions between them (Tyers and Mann, 2003). Thus, the overall aim of proteomics is to contribute to a comprehensive description of cellular function. Since proteins are the major effector molecules in biological processes, proteomics is well suited for assessing phenotypes and has the potential for the identification of novel prognostic and diagnostic targets. By systematically analyzing proteins, proteomics holds promise for better patient stratification based on protein biomarker measurements at an unprecedented scale (Langley et al., 2017; Pechlaner et al., 2017).

Previous achievements of genomics were essential for progress in proteomics, providing the "genetic blueprint" of potential protein products. In contrast to genomics, however, proteomics faces the challenge of a higher degree of complexity and dynamics of the proteome (Lottspeich, 2009). The relationship between the genome and proteome is not linear, that is, the concept that one gene encodes one protein does not apply. Posttranscriptional control such as RNA editing and alternative splicing results in a much greater diversity at the protein than the gene level. Moreover, only a certain set of genes are transcribed in any one cell at a given time point. The dynamic nature of the proteome is also dependent on environmental cues. Further complexity is added by posttranslational modifications (PTM), such as glycosylation or phosphorylation, as well as protein degradation. In addition, a particular challenge is the detection of low-abundant proteins in the presence of very high abundant proteins. Especially the plasma proteome represents a major challenge, with protein concentrations spanning 10–12 orders of magnitude (Anderson and Anderson, 2002). This dynamic range of protein concentrations still exceeds the capabilities of current proteomics technologies, which can currently resolve 5–6 orders of magnitude in protein abundance (Zubarev, 2013).

Unlike genetics, proteomics lacks the ability to amplify their targets. Thus, proteomics relies on enrichment and subfractionation of complex samples. Therefore, appropriate sample preparation is essential for reproducible proteomics experiments. An example is the depletion of highly abundant plasma proteins such as albumin or the immunoglobulins (Fu et al., 2007). Another approach to tackle the dynamic range of the plasma proteome is to shift biomarker discovery approaches from plasma to tissue-based proteomics. In tissues, potential biomarkers are less dilute and their cellular origin is less uncertain (Cuello et al., 2014; Langley et al., 2017). Similarly, extracellular matrix (ECM) proteins tend to be less abundant than cellular proteins and are thus less likely to be identified in tissue homogenates. Methods enriching for ECM proteins can address this issue (Didangelos et al., 2010, 2011). Finally, technological advances in proteomics instrumentation, mainly better mass spectrometry (MS) instruments and analysis techniques, will help to overcome these difficulties and will be discussed in the subsequent sections.

Proteomics Versus Traditional Molecular Research

Traditional molecular approaches using Western blotting or immunostaining rely on antibodies to study proteins. This approach will only shed light on part of the protein network (Walther and Mann, 2010). Notably, these techniques are based on a priori assumptions on the proteins of interest and rely on the availability of good antibodies. Though antibodies usually exhibit great specificity, their use is associated with notorious limitations such as epitope masking, nonspecific binding, or cross-reactivity between species. In contrast, proteomics research is not constrained by a priori assumptions but provides a comprehensive analysis of the protein composition in a sample. Thus, proteomics is less a hypothesis-driven but rather a hypothesis-generating approach (Cravatt et al., 2007), that is complementary to traditional molecular biology techniques, and provides an opportunity for new frontiers in biomedical research.

Proteomics Applications

Types of Proteomics Experiments

Three types of proteomics analysis exist. First, proteomics is used for compositional analysis, which aims to identify all proteins present in a sample. Second, proteomics enables characterization of protein–protein, protein–RNA, and protein–DNA interactions.

This is mainly accomplished by affinity purification before analysis by MS. For protein–protein interactions, the protein of interest (bait protein) together with its interaction partners is captured with a ligand and subsequently analyzed by proteomics (Dunham et al., 2012). A limitation of this application is the potential copurification of nonspecific interactors, so-called background contaminants. A database containing common background contaminants has been published (Mellacheruvu et al., 2013) to facilitate the identification of genuine protein–protein interactions. Finally, the main application of proteomics is differential protein expression profiling. In this approach quantitative protein changes between two or more different, well-defined conditions, such as control and diseased samples, are compared in order to identify relevant differences. Differential regulation is not only reflected in protein abundance but might also include differential regulation of PTMs.

Typical Proteomics Workflow

A typical proteomics study involves the following steps (Fig. 1):

(A) Proteomics must start with a well-conceived *experimental design* (sample size, estimated biological variation, costs, etc.). Proteomics analyses are laborious and expensive and most notably generate vast amounts of data. Thus, without a precisely formulated question and a good experimental design, the data interpretation can become very cumbersome.

(B) *Sample preparation* is paramount for accurate and reproducible proteomics analysis. This includes sample collection, handling, and storage conditions. For example, rapid freezing upon sample collection and treatment with protease inhibitors upon thawing is crucial to prevent protein degradation. Protein extraction from a biological sample can be done in a single lysis step or subfractionation methods can be applied. This is performed to reduce sample complexity or to study a specific subproteome (Barallobre-Barreiro et al., 2013; Didangelos et al., 2012; Matt et al., 2008; Yates et al., 2005). Further sample preparation requirements such as protein labeling, denaturing, reduction, or enzymatic digestion are dependent on the method chosen for protein analysis and will be discussed throughout the respective sections.

(C) Due to sample complexity further *separation* steps are required prior to MS analysis. This is necessary to reduce undersampling of low-abundant proteins. Otherwise, the MS data are dominated by high-abundant proteins. This can be achieved at the protein level by methods such as one- or two-dimensional gel electrophoresis (1-DE, 2-DE) or at the peptide level by high-performance liquid chromatography (HPLC). Due to rapid advances in MS technology, laborious gel electrophoresis methods are receding. On the other hand, HPLC separation is a standard procedure in proteomics. The most commonly applied chromatography is reversed-phase LC using C18 columns. The separation is based on hydrophobic interactions of the peptides with the C18 residues on the stationary phase, the HPLC column. The gradient of the mobile phase has an increasing organic content. Depending on the hydrophobicity, peptides elute at different time points from the column, resulting in different retention times for each peptide.

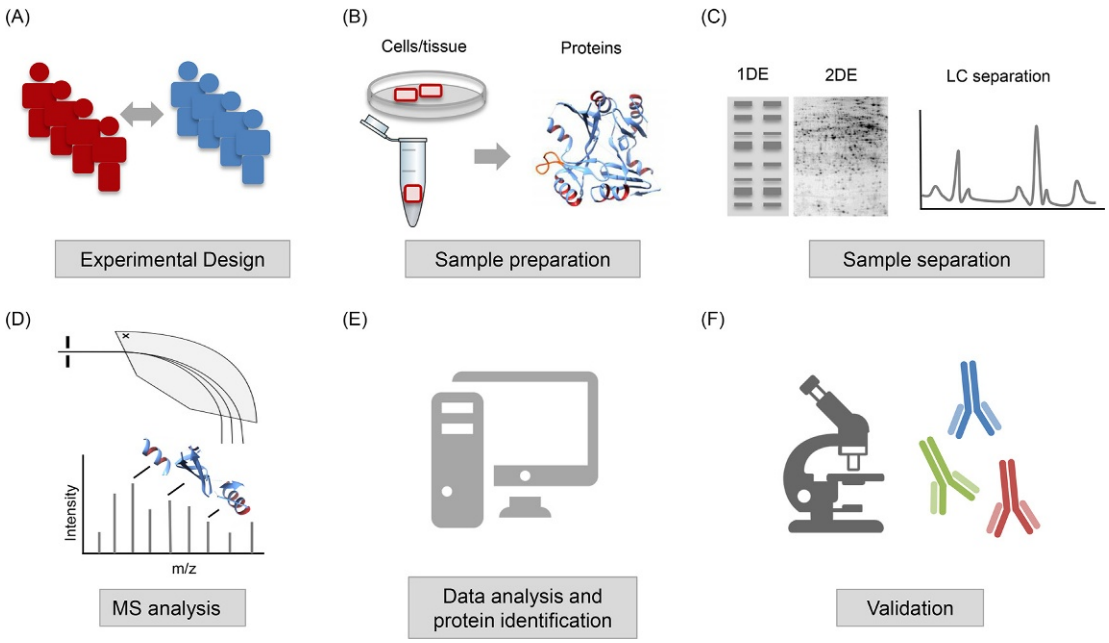

Fig. 1 Proteomics workflow. (A) A proteomics experiment will result in a large number of protein comparisons. Thus, a well-conceived experimental design with sufficient biological and technical replicates is crucial for reliable data interpretation. (B) Protein extraction is critical to obtain a representative sampling of proteins. Certain proteins, that is, membrane proteins or extracellular matrix proteins, are not readily extracted with conventional lysis buffers and may therefore be underrepresented in tissue or cell extracts. (C) Sample complexity is reduced by gel separation of proteins or LC of peptides prior to MS analysis (D). The observed MS spectra are computationally matched to a spectral library to obtain a protein identification (E). Finally, proteins of interest are validated by independent techniques, usually involving antibody-based techniques (F).

(D) Separation is followed by *MS analysis*. MS is the workhorse of modern proteomics and brought the most significant breakthrough in proteomics research. Current MS have exceptional mass accuracy, resolution, and sensitivity, reaching detection limits of attomole concentrations (Thakur et al., 2011). However, the sensitivity of the MS analysis is determined by the complexity of the sample (Nilsson et al., 2010). MS can analyze multiple proteins at the same time and offers high throughput (Arrell et al., 2001). The mass spectrometer determines the mass-to-charge ratio (m/z) of an ionized molecule generating thousands of MS spectra in a single run. A detailed description on MS principles and instrumentation will be given in "MS Principles and Instruments" section.

(E) The next step in the workflow is *data analysis* for *protein identification and quantification*. MS measures the m/z of a peptide. The unambiguous identification of the actual protein occurs by matching the observed MS spectra to a database with the assistance of software tools and scoring algorithms (Martens and Apweiler, 2009). Considering the amount of generated data, this is only possible with automated software solutions. Nevertheless, data analysis can still be a time-consuming part of proteomics analysis. The exact sequence determination process as well as quantification will be discussed in "Protein identification algorithms" section. Another major challenge is to discern the proteins with functional relevance among many differentially expressed proteins. Bioinformatics has become invaluable in harnessing the information obtained from proteomics data. The advent of molecular profiling technologies that focus on a global characterization of whole systems allows exploration of the interconnectivity of biological pathways but brought about the issue of statistical analysis and data integration.

(F) *Validation* is the final step in the workflow after selecting the target proteins for follow-up studies. It is important to realize that proteomics data often require further validation. Usually independent techniques, such as Western blotting or immunohistochemistry, are applied to validate the proteins of interest. Targeted proteomics is also gaining importance as a way to validate findings from discovery-based proteomics, as will be explained in "Targeted Proteomics" section. Follow-up experiments should range from in vitro to in vivo studies to link protein changes to function.

Two-Dimensional Gel Electrophoresis

Even though advances in MS are widely replacing gel-based proteomics techniques, 2-DE is still in use, owing to its unique separation power. It allows the resolution of more than 10,000 proteins on a single gel (Wittmann-Liebold et al., 2006). Since its introduction in 1975 (Klose, 1975; O'Farrell, 1975), the technique has been widely refined, the general principle however is the same: proteins are separated in two dimensions, first based on their isoelectric point and second by their molecular mass using SDS-PAGE. Due to the use of two separation dimensions, 2-DE gels have a higher resolving capacity than 1-DE (SDS-PAGE). In the first dimension, proteins are separated by the concentrating technique of isoelectric focusing (IEF). The sample is loaded onto a strip with an immobilized pH gradient (IPG). These strips consist of acidic and basic buffering groups covalently linked to the gel matrix generating a pH gradient. Wide pH gradients of 3–10 are used for an overview of protein composition, while narrow-range IPG strips of three pH steps provide a better resolution for unambiguous protein identification (Gorg et al., 2004). Upon application of an electric field, proteins migrate according to their intrinsic charge until they reach the pH at which their overall charge is neutral—their isoelectric point. This migration results in focused discrete protein bands on the strip. This strip is then placed on a large SDS polyacrylamide gel for separation in the second dimension. Proteins now migrate orthogonally to their previous movement according to their molecular mass. Thus, 2-DE gels produce a map of intact proteins in form of multiple "spots." The spots are visualized by Coomassie, zinc, silver, or fluorescent staining, with silver being the most commonly applied. Immunodetection or radiolabeling is also used. Following staining, gel images are digitalized and the spots are detected and directly quantified using densitometry. Spot patterns between multiple gels are compared for differential expression with the help of image analysis software, providing automated spot-detection (Kriegel et al., 2000). However, visual inspection is still essential. Spots of interest are excised and the proteins are identified by MS. The same protein can be detected in different spots, since posttranslational modifications, isoforms, or degradation products cause a shift in isoelectric point and molecular weight. Unlike other proteomics techniques, quantitation with 2-DE is performed directly on the gel at the protein level and not by MS. Technical irreproducibility due to gel-to-gel variation remains the main limitation of 2-DE gels.

A significant improvement was achieved with the introduction of difference gel electrophoresis (DIGE) (Unlu et al., 1997). This technique allows the comparison of two or three protein samples simultaneously on the same gel, thus limiting experimental gel-to-gel variation. This is achieved by labeling the proteins of each sample with a different fluorescent Cy-dye (Cy2, 3 and 5), which are designed not to interfere with the migration properties of the labeled proteins. The differently labeled samples are then mixed together in equal amounts and run on the same gel in two dimensions. A differently labeled pool of all experimental samples is included serving as an internal standard for normalization. The gel is analyzed using a fluorescent gel imager detecting the emission wavelength of each Cy-dye. Again, software packages are deployed to determine the differential spots to be picked for MS analysis. DIGE is capable of reliably quantifying protein differences as low as 15% over a protein concentration range of over four orders of magnitude (Viswanathan et al., 2006). However, high-abundant proteins mask less abundant proteins on the gel. Also, DIGE is a tedious process and a bottleneck for large-scale analysis.

Mass Spectrometry-Based Proteomics

MS Principles and Instruments

MS determines the *m/z* of an ionized molecule. Thus, peptides/proteins first need to be transferred from the liquid into the gas phase (volatized) and electrically charged (ionized). This is accomplished using matrix-assisted laser desorption ionization (MALDI) or electrospray ionization (ESI) (Fenn et al., 1989; Karas and Hillenkamp, 1988). It was the advent of these techniques that made MS became applicable for large-scale proteomics analysis, for which John B. Fenn and Koichi Tanaka were awarded the Nobel Prize in Chemistry in 2002. MALDI sublimates and ionizes the samples out of a dry, crystalline matrix via laser pulses (Aebersold and Mann, 2003). In contrast, ESI ionizes proteins/peptides directly out of a solution and is therefore readily interfaced with LC. Thus, ESI-MS coupled to LC is the method of choice for the analysis of complex biological samples.

Briefly, mass spectrometers consist of three parts: (1) an ion source, (2) the mass analyzer(s), and (3) a detector. The mass analyzer records the mass and charge state of the peptide, which is represented in the *m/z* ratio. The detector registers the intensity of ions, thus their relative abundance is displayed as peaks in MS spectra. There are different types of mass analyzers using either static or dynamic and magnetic or electric fields. In proteomics, the main types of mass analyzers are time-of-flight (TOF), ion trap, quadrupole, and Orbitrap analyzers. For a detailed technical review on MS instrumentation see a review by Aebersold and Mann (Aebersold and Mann, 2003). Mass analyzers are characterized by key parameters such as sensitivity, scan speed, resolution, and mass accuracy. A high resolving power allows the MS to distinguish peptides of slightly different *m/z* from each other and display them as separate peaks. Similarly, a high mass accuracy is required for accurate identification. The mass accuracy is measured in parts per million (ppm).

Overview of MS Methods

Due to the rapid development of MS technology, proteomics has shifted from using gel-based (2-DE or 1-DE gel-LC-MS/MS) to gel-free (shotgun proteomics) methods. In terms of quantification proteomics methods use label-free quantitation or isotopic labeling (SILAC, iTRAQ, TMT) techniques. These quantification techniques allow relative but not absolute protein quantification. Further, proteomics is subdivided into "top-down" and "bottom-up" proteomics. "Top-down" proteomics involves the analysis of intact proteins. The "bottom-up" strategy identifies proteins after proteolytic digestion to peptides and is by far the most commonly applied technique. Currently there are three main "bottom-up" strategies (Aebersold and Mann, 2016): The first and most widely used one is "shotgun" or untargeted discovery proteomics with the MS being operated in data-dependent acquisition (DDA) mode. The second strategy is targeted proteomics, carried out by multiple reaction monitoring (MRM). The latest approach is the emerging data-independent acquisition (DIA) method. Both, DDA and DIA are discovery-based methods to profile the proteome of a given sample, while MRM is a targeted method only quantifying preselected proteins of interest with the use of stable-isotope labeled standards. These three "bottom-up" approaches differ with regard to their analytical performance in terms of sensitivity, reproducibility, limit of detection, and resolving power (Domon and Aebersold, 2010). Limit of detection refers to the minimal quantity of an analyte that can be confidently detected. In the following sections, these introduced methods and MS acquisition modes will be discussed in detail. A schematic overview of proteomics approaches is given in **Fig. 2**.

Top-Down Versus Bottom-Up Proteomics

Top-down and bottom-up proteomics are two different approaches for protein and peptide analysis by MS, respectively. In *top-down* proteomics, intact proteins are analyzed by MS without prior digestion of the proteins to peptides. Using MS instruments with high mass accuracy, it initially measures the intact protein before further fragmentation to obtain the amino acid sequence (Kellie et al., 2010). The top-down approach has the advantage that it allows the analysis of protein isoforms and the analysis of the stoichiometry of PTM on a particular protein. The amino acid sequence coverage may vary depending on the size of the protein. Yet, top-down proteomics remains technically challenging, in particular for large proteins and is only feasible in very simple biological matrices, when the protein of interest is present in high abundance. In the *bottom-up* approach, proteins are first digested to peptides. Compared to proteins, peptides have similar chemical properties and are more amenable to separation by liquid chromatography and analysis by MS. Thus, the bottom-up approach has become the method of choice for proteomics analysis. The identified peptides are assigned back to a protein using a method called protein inference (Nesvizhskii and Aebersold, 2005). This computational challenge was solved with the establishment and curation of comprehensive protein databases, the development of algorithms to score MS spectra, and the availability of commercial and open source software (Perkins et al., 1999). A unique peptide can be sufficient to unambiguously identify a protein. However, information on protein isoforms and the stoichiometry of PTMs on a particular protein is lost by peptide analysis as the different protein isoforms generate a set of identical peptides.

Liquid-Chromatography Tandem Mass Spectrometry

Sample preparation

Liquid-chromatography tandem mass spectrometry (LC-MS/MS) is the current gold standard technique for proteomics analysis. It provides a robust method for large-scale, high-throughput protein analysis. Upon protein denaturation (e.g., with urea),

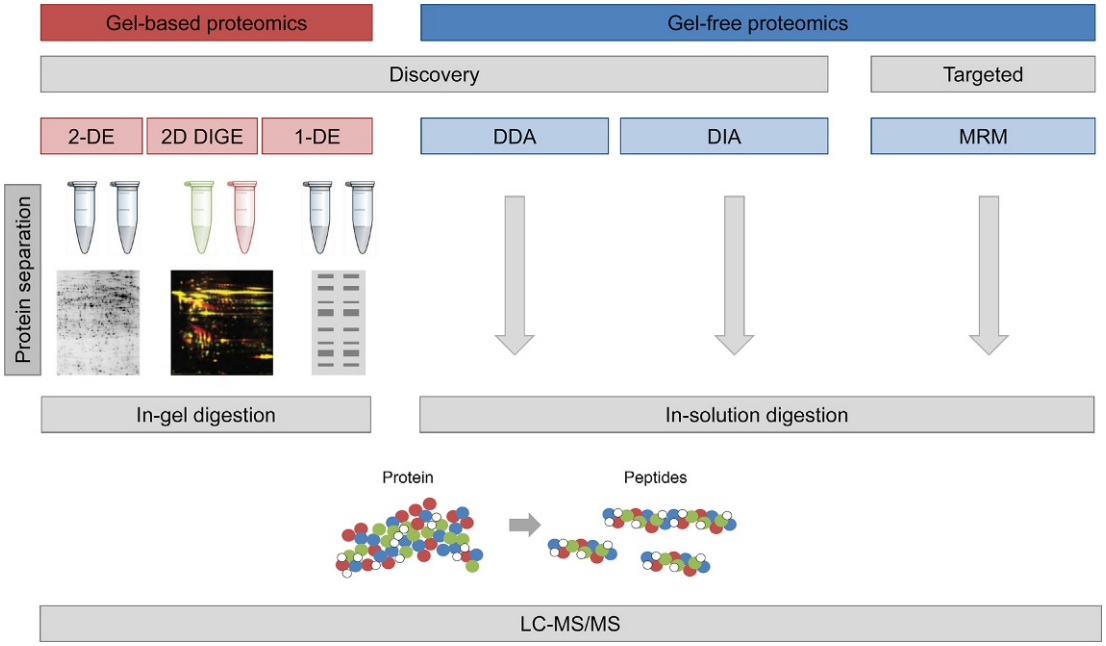

Fig. 2 Overview of proteomics approaches. Using gel-based methods, separation of protein extracts is accomplished at the protein level. Separated proteins are excised and subjected to in-gel digestion before LC-MS/MS analysis. Using gel-free proteomics approaches, protein extracts are directly processed to peptides by in-solution digestion and subject to LC-MS/MS analysis. There are three data acquisition modes in bottom-up proteomics: DDA, DIA, and MRM. MRM is a targeted proteomics approach with a predefined set of proteins of interest, while DDA and DIA are discovery-based approaches without a priori selections of proteins of interest.

reduction (e.g., with dithiothreitol), and alkylation (e.g., with iodoacetamide), proteins are digested into peptides. Trypsin usually generates peptides between 0.5 and 3 kDa, an optimal mass range for chromatographic separation and ionization (Gillet et al., 2016). Enzymatic cleavage with trypsin occurs at the carboxyl side of the amino acids lysine or arginine, except when either is directly followed by proline. Missed cleavages with trypsin need to be considered for the subsequent database search (Siepen et al., 2007). Trypsin has a high cleavage specificity and is efficient and cheap. However, other enzymes are available and can increase the sequence coverage for proteins in LC-MS/MS analyses.

Mass spectrometry analysis

Following enzymatic digestion, peptides are purified mainly using C18 reversed-phase resins and separated by reversed-phase LC according to their hydrophobicity. LC is directly coupled to the MS. Despite high-resolving capabilities of LC, simultaneous coelution and coionization of peptides have an impact on the subsequent MS analysis. The peptides are identified in two steps, hence the term tandem MS or MS/MS: in the first step, MS^1 or survey scan, the m/z and intensity of the intact ionized peptide (precursor ion) is determined. To identify its sequence, in addition to its m/z ratio, the peptide is isolated and fragmented, usually by energetic collision with an inert gas such as helium, nitrogen, or argon, in a process called collision-induced dissociation (CID) (Wells and McLuckey, 2005). The m/z ratios of the generated fragment ions are recorded, resulting in the MS^2 or MS/MS spectrum. Fig. 3A illustrates the LC-MS/MS approach from digestion to protein identification. In a typical LC-MS/MS experiment, an MS^1 spectrum is obtained roughly every one to two seconds. Within this time window, 10–40 peptides are selected for fragmentation and their MS/MS spectra are recorded. Thus, in a usual 2–3 h LC-MS/MS run thousands of spectra are collected (Walther and Mann, 2010).

Protein identification algorithms

Upon obtaining the MS^2 spectra there are two ways of identifying the protein sequence: database search or de novo sequencing (Martens and Apweiler, 2009; Steen and Mann, 2004).

In *de novo sequencing* the sequence is directly retrieved from the spectra, in particular for peptides which did not have a match in the protein databases (Medzihradszky and Chalkley, 2015). Fragmentation at the peptide bond generates peptide fragments of increasing size: b-ions containing the N-terminus or y-ions with the C-terminus. Fragments are numbered depending on the amino acids they contain. For example, a fragment containing the N-terminus with three amino acids is called a b3 ion, while a fragment with the C-terminus and two amino acids is termed a y2 ion. Thus, the sequence information can be deduced from the mass difference between two consecutive fragment ion spectra, each corresponding to an amino acid, for each of which the mass is known (Fig. 3B). However, de novo sequencing is quite an intricate process, and high-throughput experiments are ill-suited for this

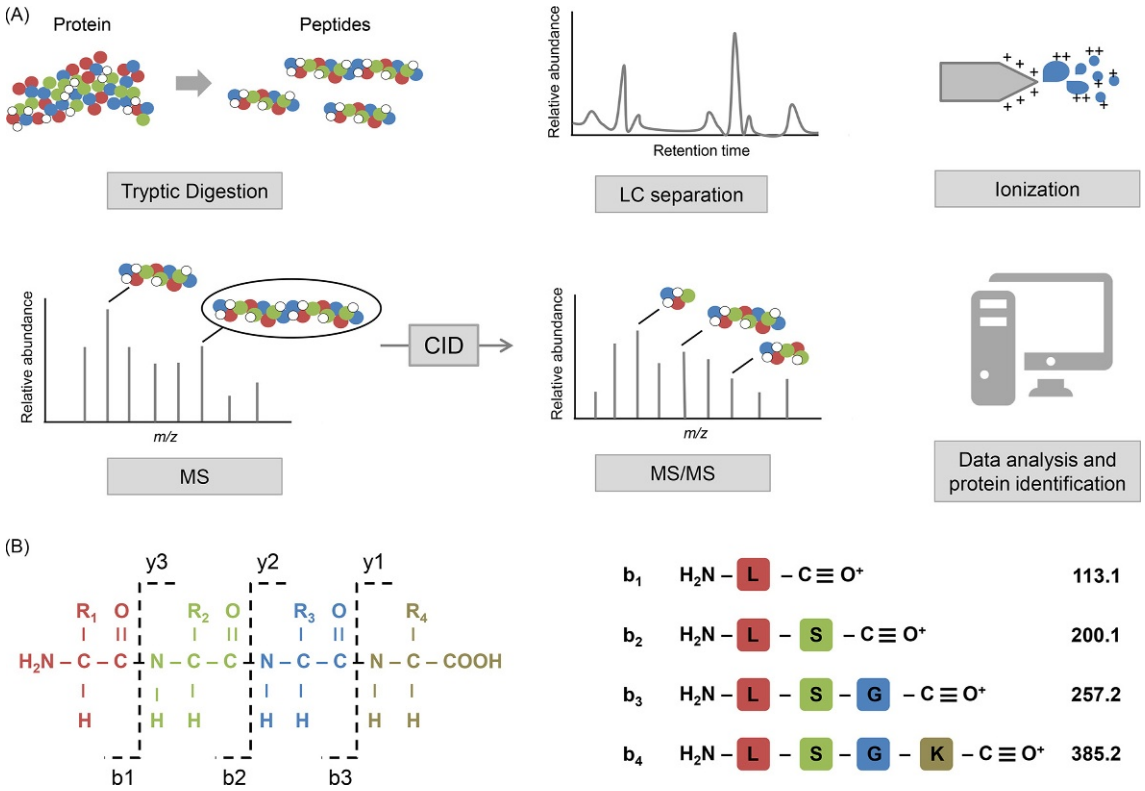

Fig. 3 Protein identification by LC-MS/MS. (A) Tryptic digestion of protein mixtures is followed by LC separation of the resulting peptides to reduce sample complexity. Separated peptides are ionized and injected into the MS. In a survey scan the *m/z* and relative abundance of ionized peptides are determined. Peptides are selected for fragmentation by CID, usually on the basis of their abundance. The *m/z* of the resulting fragment ions is measured by tandem MS (MS/MS). Protein identification is accomplished by database searching, which contains all theoretical MS/MS spectra resulting from in silico fragmentation of all known protein sequences. (B) The protein sequence can also be directly retrieved from the observed MS spectra, independent of protein database matching (de novo sequencing). Fragmentation at the peptide bond generates b-ions containing the N-terminus of the protein and y-ions with the C-terminus. The sequence information is deduced from the mass difference of two consecutive fragment ion spectra, as the mass for each amino acid is known. By analyzing the corresponding b- and y-ion series, the amino acid sequence of the peptide is determined. Here, only b-ions are shown.

approach. Software packages such as PEAKS combine features of de novo sequencing with database search to alleviate these disadvantages (Ma et al., 2003).

A *database search* is more commonly applied for protein identification (Nesvizhskii, 2007; Sadygov et al., 2004). In this approach, the measured fragment spectra are matched against a protein database with a search engine, such as Mascot (Perkins et al., 1999), SEQUEST (Eng et al., 1994), or X!Tandem (Duncan et al., 2005). The databases contain theoretical fragment spectra of in silico digested peptides according to the known cleavage patterns of the used proteases, most commonly trypsin. The match between an observed fragmentation spectrum and a theoretical database spectrum is given a score. Thus, the peptides with the highest matching scores lead to the protein identification. Since mass shifts can result from PTMs or artificial modifications during sample preparation such as a cysteine modification due to alkylation with iodoacetamide, these alterations need to be considered. Spectral matching occurs within a preset mass tolerance. Ultimately, accurate identification relies on the availability of complete and accurately annotated sequence databases (Nesvizhskii and Aebersold, 2005). While for human and mice near complete sequence databases exist, this is more challenging for other species.

Finally, to give confidence in the correct peptide identification or estimate false positive identifications, respectively, a false discovery rate (FDR) can be calculated (Hather et al., 2010). The most common method of estimating FDR is by searching the MS data against a decoy database. A decoy database contains randomized nonnatural peptide sequences, for example, by reversing the genuine amino acid sequences of all database entries. Thus, a match against a decoy database is random and per definition false. FDR can be estimated by comparing distributions of randomized and nonrandomized matches.

Shotgun Proteomics

The term "shotgun proteomics" refers to the application of LC-MS/MS to globally profile proteins in a complex sample in a high-throughput manner. In this method peptide identification is usually accomplished by the DDA method. "Data dependent" in this

context refers to the selection criteria of peptides for fragmentation in MS^2. In complex samples, where multiple peptides are simultaneously ionized, it is not possible to select all peptides in the survey scan for fragmentation, as this would overwhelm the scan speed of most instruments. Instead, a deliberate peptide selection for fragmentation is required, which is based on predefined criteria, hence data dependent. These are abundance, signal intensity, and charge state. Selection is made in real time by the MS software during the survey scan and newer MS are able to select the 15–40 most abundant—"top 15 or top 40"—peptides. Thus, a fixed number of precursor ions is selected in every MS^1 survey scan and analyzed by MS^2. Charge state preselection is used to exclude singly charged ions, which are mostly contaminants (Gillet et al., 2016). In addition, the acquisition parameters can be set to prevent repeated selection of the same peptide, thus reducing interference from high-abundant peptides and also allowing low-abundant peptides to be selected. Nonetheless, undersampling of low-abundant peptides is not eliminated and remains a bias, even in newer instruments with increased scan speed. Also, precursor selection is a stochastic process, which will never be exactly the same between two LC-MS/MS runs due to variability of the chromatographic separation. Thus, reproducible quantification, especially of low-abundant peptides, remains a challenge. However, the run-to-run variability remains within an accepted range for most discovery-based proteomics studies, especially with extensive prefractionation to reduce sample complexity, making shotgun proteomics a robust platform for protein analysis.

Quantitative Proteomics

MS itself is not quantitative due to sequence-specific differences in ionization efficiency of peptides and phenomena such as ion suppression in complex peptides mixtures (high-abundant ions consume most of the charges and reduce the ionization of coeluting low-abundant ions). However, MS-based quantification strategies allow relative quantification between samples. Generally, quantification with MS can be divided into label-free and stable isotope-labeling methods (Mirza, 2012). More precise quantification can be achieved by using internal standard peptides with targeted proteomics as will be discussed in "Quantification using MRM" section.

Label-free quantification

There are two different approaches for label-free quantification using either mass spectrometric signal intensity or spectral counting (Old et al., 2005). The latter is based on the rationale that more abundant peptides are more likely to generate more MS^2 spectra. Therefore, relative quantification can be achieved by counting the number of MS^2 spectra assigned to the same protein across different samples. However, this approach only works in samples with limited complexity and does not take the physical peptide properties into account assuming a linear response for every peptide (Bantscheff et al., 2007). The second approach uses signal intensities (spectral ion currents) for relative quantification of peptides. Advanced pattern comparison software is applied to align ion intensities of the same peptide on MS^1 and MS^2 level in different samples. The main advantages of label-free quantification are that it is cheap, achieves high-proteome coverage, and does not require laborious labeling workflows.

Stable isotope labeling

This approach is based on the incorporation of stable isotope tags into peptides or proteins by a chemical reaction, a metabolic, or enzymatic process. Stable heavy isotopes have the same physicochemical properties as their light counterparts, meaning they behave identically on chromatography. However, due to their slight mass difference, they generate distinguishable signals in the MS, making them ideal for quantification purposes. Compared to label-free methods, the labeling approach provides a higher quantitative accuracy and consistency. This is due to near equal ionization efficiencies of the isotope tags. The most commonly used labeling methods are stable isotope labeling by amino acids in cell culture (SILAC), tandem mass tags (TMT), and isobaric tagging for relative and absolute quantification (iTRAQ) (**Fig. 4**).

Stable isotope labeling by amino acids in cell culture

SILAC is an in vivo metabolic labeling approach on protein level (Ong et al., 2002). Thus, proteins are already labeled during their synthesis. This is achieved by growing cells in a medium containing isotopically labeled amino acids, which are incorporated into the proteins. Mostly arginine and/or lysine are labeled with ^{13}C isotope (instead of ^{12}C) or ^{15}N isotope (instead of ^{14}N) to ensure that all tryptic peptides contain a labeled isotope (**Fig. 4A**). Lysine has the additional advantage of being an essential amino acid, which cannot be produced de novo. At the same time cells of the other experimental condition are grown in normal medium, containing the normal or "light" amino acids. Eventually both samples are multiplexed and analyzed in the same MS run, which reduces experimental variability. The heavy and light labeled protein pairs coelute on chromatography. Due to the characteristic mass shift in their MS^1 spectra (e.g., 6 Da for ^{13}C lysine) the relative quantity for each protein can be calculated. An equal ratio between both samples indicates no difference in abundance. If the heavy labeled peptide exhibits a higher peak intensity, the protein was more abundant in the labeled sample and vice versa.

Application of SILAC goes beyond cells in culture but is also used for mice without affecting their development, growth, or behavior (Kruger et al., 2008) as the proteins are chemically identical except for their mass difference. However, SILAC diet is expensive, thus limiting its use to small animal models. SILAC is also a very useful tool to assess protein turnover (Doherty et al., 2005; Mann, 2006). Upon feeding a SILAC diet to animals the amino acids are gradually incorporated into the newly synthesized proteins and are clearly distinguishable from preexisting proteins. Thus, the ratio between heavy and light peptides indicates the synthesis rate of a protein and can be compared across different proteins.

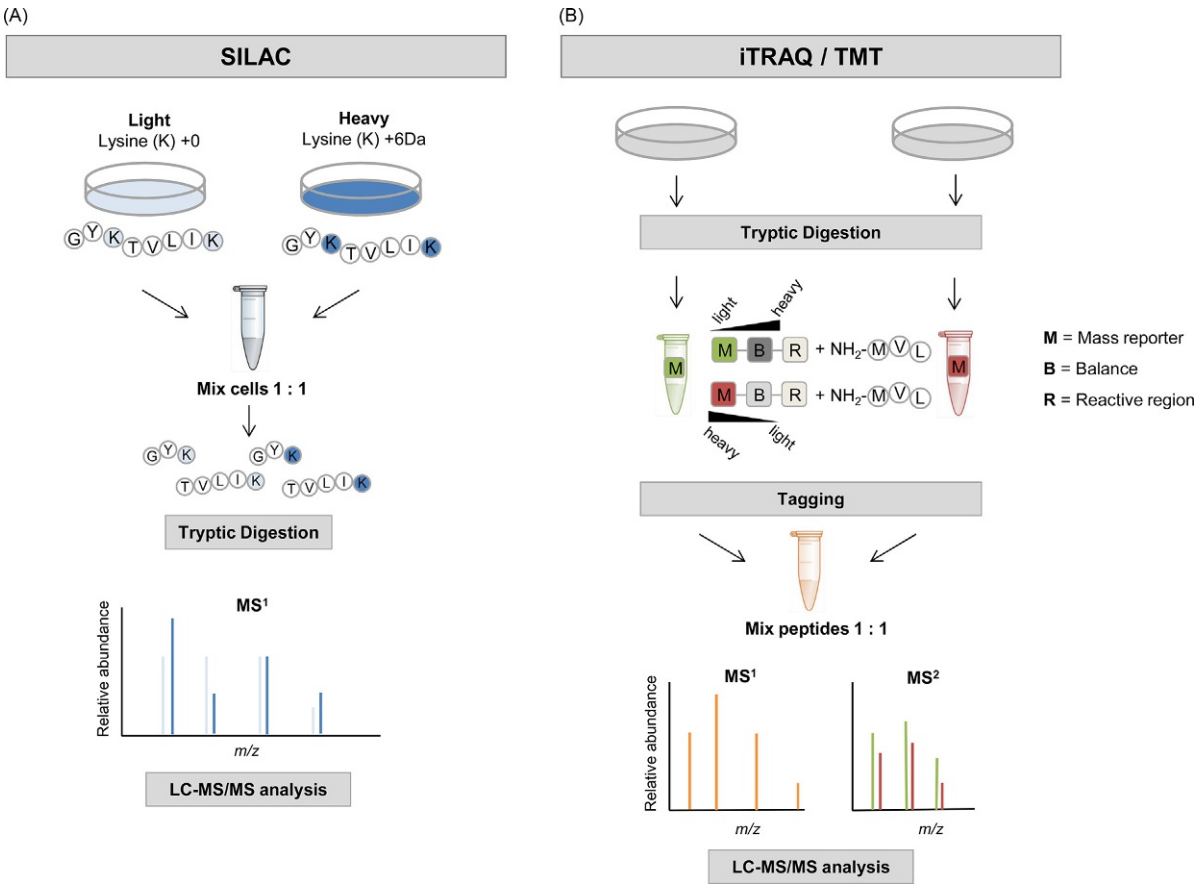

Fig. 4 Stable isotope labeling. (A) The SILAC approach allows metabolic labeling at the protein level during protein synthesis by incorporation of heavy or light amino acids. Protein samples are mixed, digested, and analyzed by LC-MS/MS. Due to their characteristic mass shift in MS^1 the relative quantity of the peptides with light and heavy amino acids can be determined. (B) In contrast, using iTRAQ or TMT labeling is performed at the peptide level. Different tags allow multiplexing and reduce experimental error. Upon individual labeling of each sample, peptides from the different samples are mixed. Both iTRAQ and TMT are "isobaric" tags: the peptides coelute in chromatography and cannot be distinguished in MS^1 due to their balance region. Only in MS^2 peptides can be relatively quantified due to the mass shift in the reporter regions.

Isobaric Tagging

In the mass-tagging approach proteins or peptides are chemically tagged with a stable isotope-containing molecule in vitro. Thus, this approach is also applicable to human tissue samples. The most commonly used tags are TMT (Thompson et al., 2003) and iTRAQ (Ross et al., 2004), both belonging to the category of isobaric mass tags. The tags are available with different isotopic substitutions (reporter regions) creating distinct mass differences to allow multiplexing of currently up to 10 samples with TMT (10-plex) and 8 with iTRAQ (8-plex). Again, multiplexing offers the advantage of reducing experimental error. Each tag consists of three functional regions (Fig. 4B). The amine-reactive region is equal in all tags and allows the binding to the N-terminus and side-chain amines of peptides. In addition, they have a mass reporter and a balance region, which are different and unique to each tag, due to isotopes substituted at various regions. As the name indicates the function of the balance region is to compensate the variable mass of the reporter region so that the intact tag has the same mass in all samples, hence the term "isobaric." Thus, after mixing of all differently labeled samples the peptides remain undistinguishable during chromatographic separation and MS^1. Only upon fragmentation in MS^2, the different reporter regions are released enabling the distinction and quantification of the peptide in different samples.

Cysteine-reactive mass tags are available (iodoTMT) to specifically label cysteine-containing peptides, commonly applied in the field of redox-proteomics. In comparison to the previous isotope-coded affinity tags (ICAT), also with specificity toward sulfhydryl groups, multiplexed quantification (up to 6-plex) is possible. ICAT contains an additional biotin affinity tag (Gygi et al., 1999). This tag enables purification by avidin affinity chromatography. Thus, it allows further reduction of sample complexity. Similarly, TMT-labeled peptides can also be enriched.

The downside of isobaric mass tagging is that labeling occurs at the peptide level, thus later in the experimental workflow. In contrast to the SILAC workflow, potential experimental variations before labeling cannot be excluded. A further caveat is that the complexity of a sample can increase the likelihood of inaccurate quantification. Coisolation of precursors can give rise to mixed MS/

MS spectra from the simultaneous fragmentation of more than one peptide leading to erroneous combinations of reporter ions from different proteins (Langley et al., 2013). This caveat has been addressed in newer instruments by MS^3 fragmentation (Ting et al., 2011).

^{18}O labeling

Another labeling method is the enzymatic incorporation of stable isotopes, such as ^{18}O instead of ^{16}O (Stewart et al., 2001). In this approach, isotopic labeling occurs during the proteolytic digestion of proteins. The cleavage of peptide bonds requires the addition of H_2O. If the reaction is performed in the presence of ^{18}O labeled water ($H_2^{18}O$), the generated peptides have either a 2 or 4 Da mass shift compared to their natural counterparts, depending on cleavage occurring at either one or both ends. Though it is highly specific and universally applicable, a major limitation of this method is the enzyme-mediated back-exchange of ^{18}O with ^{16}O, resulting in complicated data analysis (Mirza et al., 2008). However, by using immobilized trypsin, which is removed upon digestion/labeling, the back-exchange can be prevented (Mirza, 2012; Storms et al., 2006). Also, software tools for efficient quantitative analysis of complex samples have been developed (Hicks et al., 2005).

Further, this principle is also applied for PTM studies, namely for the identification of N-linked glycosylation sites on proteins (Kuster and Mann, 1999). During enzymatic deglycosylation of N-linked glycans under incorporation of H_2O, the enzyme PNGase-F converts asparagine to aspartic acid via a deamidation reaction causing a mass shift of 0.984 Da. Thus, if $H_2^{18}O$ is used, it leads to a mass shift of 2.99 Da, which leads to increased search specificity and indicates the former presence of glycosylation at that particular amino acid.

Targeted Proteomics

In contrast to discovery or shotgun proteomics, targeted proteomics allows the precise quantitation of specific proteins of interest. Thus, it is a hypothesis-driven proteomics strategy (Picotti et al., 2013), which is often employed to validate and complement the findings obtained from discovery approaches. The focus on predetermined target proteins offers higher sensitivity, quantitative accuracy, and reproducibility than the DDA approach. Indeed, due to these characteristics together with its advantages over conventional antibody detection that rely on the specificity of the binder, targeted proteomics has been selected as the method of the year by the journal *Nature Methods (2013)*. The typical MS method for targeted proteomics is selected reaction monitoring (SRM) mainly carried out on triple-quadrupole instruments (QqQ). SRM enables the analysis of multiple peptides in a single run, hence the term MRM is used synonymously with SRM (Picotti and Aebersold, 2012). In SRM, the first mass analyzer (Q1) selects precursor ions within a mass range around the mass of the predetermined target peptides, which are then fragmented by CID in the collision cell (Q2). The specific fragment ions of the targeted peptide are then selected and measured by the second mass analyzer (Q3) (**Fig. 5A**). A specific precursor–fragment ion pair is termed a transition. The high selectivity and specificity of this method is given by the two levels of mass selection in Q1 and Q3 acting as a two-stage mass filter. In principle, the instrument is preprogrammed to consistently monitor the same set of predefined transitions (Q1/Q3 pairs) and record their intensities over the entire chromatography run. This is considered as the basis for its high reproducibility in repeated samples. Thus, analysis time is optimally used on the peptides of interest, largely eliminating interference from high-abundant/contaminant peptides. On the other

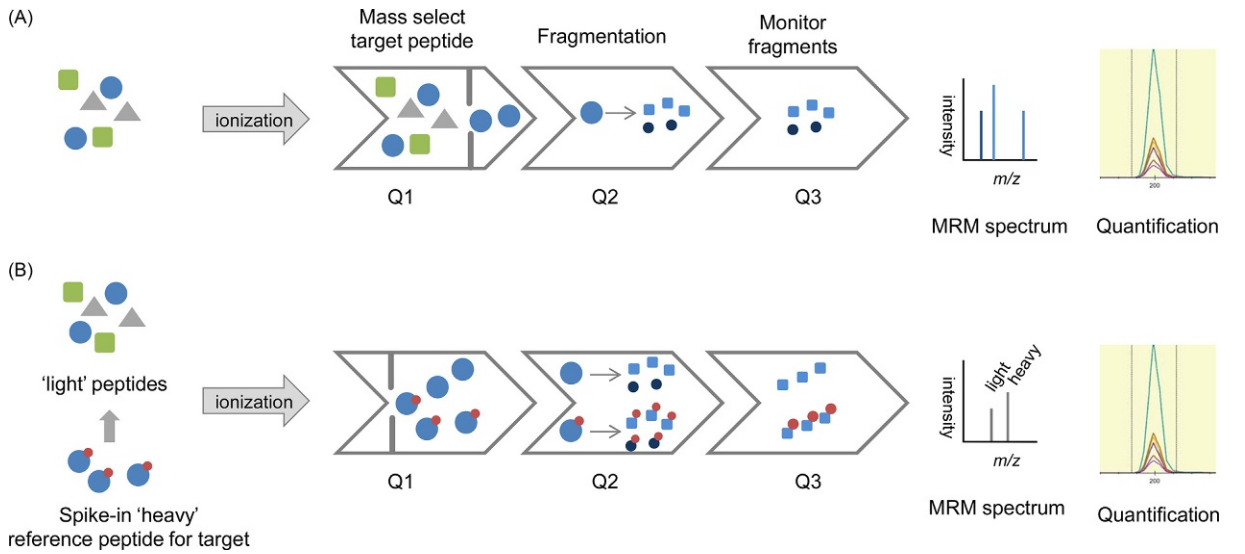

Fig. 5 SRM/MRM analysis on a triple-quadrupole (QqQ) MS. (A) Coeluting peptides are filtered by the first quadrupole (Q1) based on a predetermined list of targeted precursor ions. The selected peptides are subjected to fragmentation by CID in Q2, and their fragment ions are selected and measured by Q3 (transitions). (B) Heavy labeled reference peptides of known concentration can be spiked-in as standards for reliable quantification of the targeted peptides.

hand, it is limited to preselected transitions (Q1/Q3 pairs). Currently the number of peptides that can be monitored in a single run without compromising performance is around 100 peptides. Multiplexing up to 1000 proteins is possible, however, at the cost of reduced quantification accuracy (Picotti et al., 2010).

Multiple reaction monitoring (MRM) assays

A selective transition/inclusion list needs to be generated for MRM experiments. This depends on the knowledge of the MS characteristics of a peptide and its fragmentation products. The required MRM transition data for a given protein is called an MRM assay. This information is mostly derived from previous untargeted MS experiments, or alternatively the MRM assays can be retrieved from an online compendium (see later).

First, a number of rules need to be considered when selecting the optimal peptides (Lange et al., 2008). In essence, the selected peptides need to be unique for the protein of interest (proteotypic). Preferably, the peptide should be of medium length, fully tryptic and should not contain abundant PTMs or amino acids prone to variable chemical modifications such as asparagine (N) deamidation or methionine (M) oxidation. In terms of their MS parameters peptides with the most intense transition peaks are selected. In addition to their m/z values, the optimal collision energy and the chromatographic retention times are retrieved. The latter is used to monitor the expected transitions close to their corresponding elution time (Escher et al., 2012). Due to these timed ion selection windows in the so-called scheduled MRM approach more transitions can be monitored/multiplexed in one run. All these considerations together contribute to the sensitivity of this method. Although initially laborious, once the assays are established, they can be used with great reproducibility across different sample sets. Indeed, the extensive effort required to develop MRM assays has been considered as the main reason for the limited application of MRM in the field of proteomics (Picotti et al., 2010), even though it has been widely applied to the measurement of small molecules such as drugs or metabolites. To facilitate the wider implementation of MRM assays, online transition lists have been made freely accessible, that is, in the SRMAtlas (http://www.srmatlas.org/). This is a compendium of highly specific MRM assays that have enabled the quantification of 99.7% of the >20 000 annotated human proteins (Kusebauch et al., 2016). The assays are developed and validated by the wider proteomics community (Kennedy et al., 2014).

Parallel reaction monitoring (PRM) is a newer targeted proteomics method, where Q3 of a triple-quadrupole MS is replaced with a high-resolution accurate mass analyzer such as TOF or Orbitrap (Peterson et al., 2012). This allows the accurate detection of all fragment ions due to higher resolution, while in MRM the transitions are usually restricted to a maximum of 5. Thus, PRM requires less time for assay development, since it monitors all transitions and alleviates the need for preselecting target transitions.

Quantification using MRM

For analysis of targeted proteomics data, an open-source software *Skyline* is now available and widely used. Even though the software offers an automatic selection of the best transition matches for each peptide, the correct attribution/identification needs to be visually matched and the peak areas manually integrated, making the data analysis a cumbersome process. Most importantly, targeted proteomics allows precise quantification, by spiking-in heavy labeled reference peptides of known concentrations. Briefly, the fragment ion intensities of peptides of interest are related to corresponding signals of isotopically labeled reference peptides with the same sequence (**Fig. 5B**). Without the constraints of antibody-based protein detection and quantification, MRM is emerging as a promising alternative to conventional methods such as enzyme-linked immunosorbent assays (ELISA), at least for medium- to high-abundant proteins. For low-abundant proteins, that is, in the pg/ml range, targeted proteomics may not match the sensitivity of ELISAs. Commercially available kits now allow the quantification of up to 100 proteins in human serum or plasma (Kiseleva et al., 2015). Using MRM, plasma levels of 13 apolipoproteins were compared in a prospective, community-based study and VLDL-associated apolipoproteins were found to predict cardiovascular events (Pechlaner et al., 2017). The increasing importance of targeted MS technologies for clinical application is reviewed by Carr et al. (Gillette and Carr, 2013).

Data-Independent Analysis (DIA)

DIA in comparison with DDA and MRM

Neither the untargeted discovery nor the targeted proteomics approach is able to quantify every detectable component of a proteome. Each method is a compromise that improves analytical performance at some level, while reducing it at another. DDA is biased due to its random selection of peptides for fragmentation. Thus, reproducible quantification, especially of low-abundant peptides, remains its major limitation. On the other hand, MRM is limited to the detection of preselected peptides requiring a priori knowledge. Therefore, it is not suitable for a discovery-based approach. Additionally, it has a limited multiplexing capacity. To overcome the limitations of both methods and combine their advantages the data-independent acquisition (DIA) mode was adopted for proteome analysis. This method aims to obtain the MS^2 spectra of every detectable precursor ion regardless of its abundance or its prior knowledge, hence the term "data independent." Therefore, DIA provides a less biased discovery approach for reproducible and accurate quantification of the detectable proteome in a sample without being restricted to predefined peptides. A comparative summary on the data acquisition mode in all three bottom-up proteomics approaches is given in **Fig. 6**.

Data acquisition in DIA

The key to achieve a comprehensive collection of MS^2 spectra is the sequential isolation window acquisition principle (Sajic et al., 2015). More precisely, the MS triggers fragmentation of *all* precursor ions within a predefined m/z isolation window. Due to

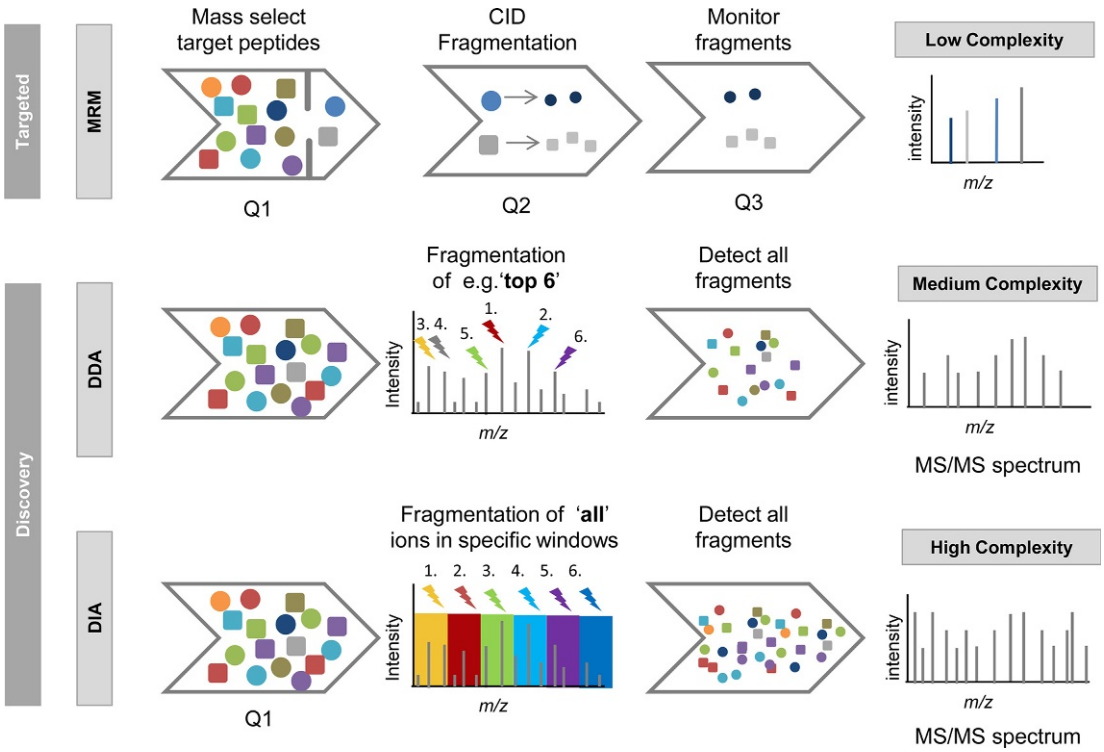

Fig. 6 Comparison of data acquisition using the MRM, DDA, or DIA method. While MRM allows targeted identification of only preselected peptides, both DDA and DIA are discovery proteomics approaches. In the DDA mode, peptides for fragmentation are selected on the basis of their abundance in regular survey scans during the chromatographic separation. The top 6–40 most abundant ions of each survey scan (depending on the MS instruments' scan speed) are fragmented, and their fragment ions result in the full MS/MS spectrum. Due to the stochastic nature of sampling the most abundant ions on a chromatographic time scale, poor run-to-run reproducibility can be a limitation of the DDA mode, especially of low-abundant peptides. In contrast, in the DIA approach all peptides within defined isolation windows are subjected to fragmentation. This results in highly multiplexed MS/MS data, which are analyzed by targeted data extraction based on spectral libraries from these samples.

seamlessly adjacent isolation windows the entire precursor *m/z* range expected for tryptic peptides can be covered in a single duty cycle. The width of a cycle is adjusted to be shorter than the chromatographic elution time of a peptide. Eventually, while the instrument repetitively cycles through the consecutive isolation windows fragmenting each peptide, MS^2 spectra of all possible precursors are recorded in real time. This results in the generation of comprehensive, but very complex fragment ion maps. However, due to this multiplexed recording of MS^2 spectra originating from multiple precursors, the relationship between the fragment ion and its precursor is lost. In comparison, in the DDA method the MS^2 spectrum is generally well associated with the MS^1 spectrum. Thus, the uncoordinated record of precursors and their fragment ions complicates the data analysis. Therefore, the main challenges of the DIA mode are data processing and correct interpretation of multiplexed MS^2 spectra.

Data analysis in DIA

The DIA strategy of unbiased MS^2 data acquisition is not entirely new, several DIA methods with various isolation window widths have been proposed previously (Bern et al., 2010; Panchaud et al., 2009, 2011; Purvine et al., 2003; Venable et al., 2004). However, recent improvements in MS instruments regarding their resolution power, mass accuracy, and scan speed have contributed to the maturation of the DIA strategy. Currently the most advanced DIA strategy is SWATH-MS (sequential windowed acquisition of all theoretical mass spectra) (Gillet et al., 2012). SWATH-MS experiments were first performed on high-resolution quadrupole TOF instruments with fast scan speed. In this setup, 32 fixed precursor isolation windows (swaths) with a width of 25 *m/z* each are used to cover a mass range of 400–1200 *m/z* (allowing an overlap of 1 Da between two adjacent windows). With a dwell time of 100 msec for each window, the total cycle time adds up to 3.2 s. The data from all windows is compiled together in a single map, termed the SWATH map. The reason for the success of SWATH-MS lies in the novel data analysis strategy, which is fundamentally different from the standard database-searching tools applied in previous DIA methods. Though a discovery-based approach, the data analysis is accomplished using a targeted data extraction strategy for confident scoring. The data in generated fragment ion maps are mined using fragment ion spectral libraries supported by software such as OpenSWATH or Skyline. The spectral or assay libraries contain the exact MS coordinates for each targeted peptide including the precursor *m/z*, the fragment ion *m/z*, their intensities, and their chromatographic retention time. Thus, peptides in the map can be uniquely identified with MRM-like reproducibility and quantification accuracy. However, currently DIA cannot compete with MRM in terms of sensitivity, especially

for low-abundant peptides (Sajic et al., 2015; Schmidlin et al., 2016). Moreover, for successful data analysis, availability of high-quality spectral libraries is crucial (Schubert et al., 2015) so that any possible peptide of interest (including isoforms, degradation products, or PTM carrying forms) can be queried in DIA maps. Since the SWATH map is a permanent digital record of fragment ion spectra for all detectable peptides in a sample, it is possible to reanalyze the data as new targets of interest arise. The comprehensive nature of SWATH maps has also compelled researchers to convert tissue biopsy samples or plasma samples into "digital maps" or "digital records" for archiving (Guo et al., 2015). Notably, DIA is quite a recent strategy compared to DDA. Facilitation of DIA analysis by spectral library generation, automated data extraction, or scoring tools is currently work in progress and expected to provide better solutions in the future (Gillet et al., 2016).

Conclusions

A major limitation of cardiovascular research is that a simplistic and reductionist approach is applied to explain different pathologies, often resulting in a focus on few proteins. Proteomics technologies have made tremendous progress over recent years although cardiovascular disease is receiving relatively little attention by the proteomics community (Mayr, 2013). Initiatives, such as the Human Protein Atlas project (www.proteinatlas.org), provide a publically available resource capturing the location of proteins in different tissues. The only cardiovascular tissue in this database is the human heart. No large blood vessels are included. No tissues with different cardiovascular diseases are assessed. It is essential that high-quality cardiovascular proteomics data become publically accessible to advance the field. Proteomics is essential as part of a systems' biology approach to science and offers powerful technologies to place scientific discoveries in a more global unbiased framework by integrating biological information into disease-specific networks that drive pathophysiological changes.

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Further Reading

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Pulmonary Arterial Hypertension

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Introduction

The field of pulmonary arterial hypertension (PAH) has progressed significantly in the past 25 years through greater understanding of pathogenic pulmonary vascular biochemical pathways and the development of numerous PAH-targeted medical therapies (Farber and Loscalzo, 2004). With expert management, PAH is now viewed as a chronic condition with a life expectancy from years to decades as opposed to an invariably fatal disease (Simonneau et al., 2013). Periodic international pulmonary hypertension (PH) symposia, which have developed consensus recommendations regarding PH, have been instrumental in furthering our understanding of the syndrome. The fifth World Symposium on Pulmonary Hypertension (fifth WSPH) held in Nice, France, in 2013 included 12 task forces to address multiple aspects of PH. Task forces were convened in the fields of pathology and pathobiology; genetics and genomics; pathophysiology; classification, definitions, and diagnosis; epidemiology and registries; standard of care; treatment goals for PH; new trial designs and potential therapies for PAH; chronic thromboembolic pulmonary hypertension; PH due left heart disease and lung diseases; and pediatric PH. The proceedings of the fifth WSPH have updated PH clinical classification system, PH diagnostic recommendations, consensus treatment advice concerning rehabilitation, combination therapy, new medications, and lung transplantation and discussed new approaches to PAH (Galie et al., 2013; Simonneau et al., 2013; Soubrier et al., 2013; Hoeper et al., 2013b; McGoon et al., 2013; McLaughlin et al., 2013; Gomberg-Maitland et al., 2013).

Clinical Classification of PH

Prior to the second World Symposium on Pulmonary Hypertension (WSPH) in 1998, PH was classified into just two categories: primary PH or secondary PH based on identifiable causes and/or risk factors (Hatano and Strasser, 1975; Simonneau et al., 2004). The PH classification system has since evolved with the development of categories of PH demonstrating similar clinical, pathological, and hemodynamic characteristics. Five PH diagnostic groups are currently delineated: PAH (group 1), PH owing to left heart disease (group 2), PH owing to chronic hypoxia and/or chronic lung disease (group 3), chronic thromboembolic pulmonary hypertension (CTEPH, group 4), and PH due to unclear multifactorial mechanisms (group 5) (Simonneau et al., 2004; **Table 1**). This review will focus exclusively on PH diagnostic group 1 (PAH).

Table 1 Clinical classification of pulmonary hypertension

Group 1: Pulmonary arterial hypertension
Idiopathic PAH
Heritable PAH
Drug- and toxin-induced
Schistosomiasis
Associated with the following:
● Connective tissue disease
● HIV infection
● Portal hypertension
● Congenital heart diseases
Group 2: Pulmonary hypertension owing to left heart disease
Left ventricular systolic/diastolic dysfunction
Mitral or aortic valve disease
Congenital/acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies
Group 3: Pulmonary hypertension owing to chronic hypoxia and/or chronic lung disease
Obstructive sleep apnea
Interstitial lung disease
Other pulmonary diseases with mixed restrictive and obstructive pattern
Sleep-disordered breathing
Alveolar hypoventilation disorders
Chronic exposure to high altitude
Developmental lung diseases
Group 4: Chronic thromboembolic pulmonary hypertension (CTEPH) and other pulmonary artery obstructions
CTEPH
Other pulmonary artery obstructions are as follows:
● Angiosarcoma
● Other intravascular tumors
● Arteritis
● Congenital pulmonary artery stenosis
● Parasites (hydatidosis)
Group 5: Pulmonary hypertension with unclear multifactorial mechanisms
Hematologic disorders: chronic hemolytic anemia, myeloproliferative disorders, splenectomy
Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis, neurofibromatosis, vasculitis
Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
Others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure, segmental PH

Source: Simonneau, G., Gatzoulis, M. A., Adatia, I., Celermajer, D., Denton, C., Ghofrani, A., Gomez Sanchez, M. A., Krishna Kumar, R., Landzberg, M., Machado, R. F., Olschewski, H., Robbins, I. M. and Souza, R. (2013). Updated clinical classification of pulmonary hypertension. *Journal of the American College of Cardiology***62**, D34–D41.

PAH Definition

Currently, PAH is defined hemodynamically as having a mean pulmonary arterial pressure (mPAP) greater than 25 mmHg, along with a pulmonary capillary wedge pressure (PCWP) or left ventricular end-diastolic pressure (LVEDP) less than 15 mmHg and a pulmonary vascular resistance (PVR) greater than 3 Wood units. There should be no significant (i.e., hypoxemic) chronic lung disease or CTEPH after thorough investigation. It is a rare condition with a prevalence of about 1 per 100,000 in the general population (McLaughlin et al., 2009; Hoeper et al., 2013b; Authors/Task Force et al., 2015).

Clinical Subgroups of PAH (Group 1 PH)

Over the past decade or so, the nomenclature of the different subtypes of group 1 has markedly evolved (**Table 1**). Idiopathic PAH (IPAH, formerly referred to as "primary pulmonary hypertension" or PPH) is the largest subgroup within group 1 PH and accounts for 46% of PAH patients. IPAH has an incidence of about 0.9 cases per million adults in the United States. Approximately 83% of patients affected by IPAH are women, and patients have a mean age of 50 years at diagnosis (McGoon et al., 2013; McGoon and Miller, 2012). Hereditary PAH (HPAH) cases are identified as patients with PAH with at least one other documented PAH case in their family or who possesses a gene mutation known to strongly associate with PAH (Simonneau et al., 2004). Several genetic mutations have been found to be associated to HPAH and IPAH, including bone morphogenic protein receptor type 2 (BMPR2), activin-like receptor kinase-1 (ALK1), endoglin (ENG), suppressor of mad9 (Smad9), caveolin-1 (CAV1), and KCNK3 (Simonneau et al., 2013; Soubrier et al., 2013; Machado et al., 2009; Austin et al., 2012; Ma et al., 2013). A number of drugs and toxins have also been implicated as risk factors for PAH and categorized according to strength of evidence as definite, likely, possible, or unlikely

Table 2 Drugs and toxins associated with PAH

Strength of association	*Drug/toxin*
Definite	Aminorex, fenfluramine, dexfenfluramine, toxic rapeseed oil, benfluorex, SSRI antidepressants
Likely	Cocaine, phenylpropanolamine, chemotherapy, St. John's wort, interferon alpha and beta, amphetamine-like drugs
Possible	Amphetamines, tryptophan, methamphetamines, dasatinib
Unlikely	Oral contraceptive medications, estrogen, cigarette smoke

Source: Simonneau, G., Gatzoulis, M. A., Adatia, I., Celermajer, D., Denton, C., Ghofrani, A., Gomez Sanchez, M. A., Krishna Kumar, R., Landzberg, M., Machado, R. F., Olschewski, H., Robbins, I. M. and Souza, R. (2013). Updated clinical classification of pulmonary hypertension. *Journal of the American College of Cardiology***62**, D34–D41.

(**Table 2**; Simonneau et al., 2013). A definite association is defined as an epidemic or large multicenter epidemiological studies demonstrating an association between a drug and PAH. A likely association is defined as a single case-control study demonstrating an association or a multiple-case series. A possible association is defined as drugs with similar mechanisms of action as those in the definite or likely category but that have not yet been studied. Lastly, an unlikely association is defined as one in which a drug has been studied in epidemiological studies, and an association with PAH has not been demonstrated (Simonneau et al., 2013). There is a known association between PAH and connective tissue diseases. The prevalence is best established in scleroderma, estimated to be between 7% and 12% (Hachulla et al., 2005; Mukerjee et al., 2003). The prognosis of PAH associated with scleroderma is poor with a 1-year mortality of 30% that is considerably worse than seen in IPAH (1-year mortality about 15%) (Mukerjee et al., 2003; Humbert et al., 2010). PAH is found in about 1%–5% of cases of patients with end-stage liver disease and is termed portopulmonary hypertension (POPH). This condition also has a poor prognosis relative to IPAH. In the US REVEAL registry, patients with POPH had a 3-year survival rate of 40% versus 64% in patients with IPAH (Krowka et al., 2012). Pulmonary arterial hypertension is also associated with HIV infection with a stable prevalence of 0.5% over the past decade (Degano et al., 2010). The survival rate at 5 years in a recent French cohort was more than 70% (Sitbon et al., 2008). Schistosomiasis-associated PAH is potentially the most prevalent cause of PAH worldwide. Pulmonary arterial hypertension occurs in patients with primarily hepatic schistosomiasis with an associated mortality rate of about 15% at 3 years (Lapa et al., 2009; dos Santos Fernandes et al., 2010; **Fig. 1**).

Genetics of PAH

Over 300 independent mutations in the BMPR2 have been identified in approximately 75% of families with HPAH with more than one affected member and up to 25% of cases of isolated IPAH (Machado et al., 2009). The protein BMPR2 is a member of the tumor growth factor (TGF)-beta superfamily, which appears to have a prominent role in the development of PAH. Compared with PAH patients without BMPR mutations, those with a BMPR2 mutation develop PAH about 10 years earlier have worse clinical courses and hemodynamic profiles (lower baseline cardiac index, higher PVR, and less likely to be responsive to acute vasodilators). These patients also have an increased risk of death and transplantation (Pfarr et al., 2011; Sztrymf et al., 2008; Rosenzweig et al., 2008; Humbert et al., 2006; Evans et al., 2016). Pathogenic mutations in activin receptor-like kinase-1 (ACVRL1 or ALK1) have been implicated in PAH associated with hereditary hemorrhagic telangiectasia (HHT) (Harrison et al., 2003). Girerd and colleagues demonstrated that ACVRL1 mutation carriers were diagnosed and died at a significantly younger age compared with BMPR2/ACVRL1 noncarriers (Girerd et al., 2010). Despite less severe baseline hemodynamic characteristics compared with those of BMPR2 mutation carriers, age at death among ACLVRL1 mutation carriers was broadly similar to that of BMPR2 mutation carriers, suggesting a more rapid disease progression (Girerd et al., 2010). ACVRL1 haploinsufficiency may lead to a pulmonary vascular status that predisposes to the development of PAH. Interestingly, ACVRL1 mutation carriers with PAH have a predominance of mutations in exon 10 and particularly in the NANDOR box, which is necessary for regulation of TGF-beta signaling. This further supports the hypothesis that disruption of the TGF-beta pathway is central to the development of PAH (Machado et al., 2009; Girerd et al., 2010). Recently, several novel gene mutations have been discovered. CAV1, which encodes a membrane protein of caveolae, is critical to initiation of a cellular signaling cascade involving the TGF-beta superfamily, nitric oxide pathway, and G-protein-coupled receptors (Soubrier et al., 2013; Austin et al., 2012). Exome sequencing of family members with HPAH but without identifiable known mutations found a novel heterozygous missense variant in the potassium channel KCNK3 (Ma et al., 2013). Analysis for additional HPAH cases and IPAH cases identified five additional heterozygous novel missense variants. KCNK3 encodes a pH-sensitive potassium channel in the two-pore domain superfamily (Patel et al., 1999). This potassium channel has been shown to be sensitive to hypoxia and regulates resting membrane potential and pulmonary vascular tone (Hartness et al., 2001; Olschewski et al., 2006; Osipenko et al., 1997). It has been postulated that KCNK3 is also involved in the regulation of pulmonary vascular remodeling and proliferation in patients with PAH through inhibiting apoptosis (Yu and Choi, 2000). Ma et al. showed that some, but not all, mutations were rescued by the phospholipase inhibitor, ONO RS-082 (Ma et al., 2013). In human pulmonary artery smooth muscle cells, KCNK3 can be activated by treprostinil (a stable prostacyclin analog) through cyclic AMP-dependent phosphorylation of the channel induced by protein kinase A (Higenbottam et al., 1984; Moncada et al., 1976). Identification of KNCK3 and other genes as contributing to the development of PAH potentially opens new paths to PAH therapy. Penetrance of PAH disease in those with the BMPR2 mutation is only about 20%. Members of families with HPAH who have

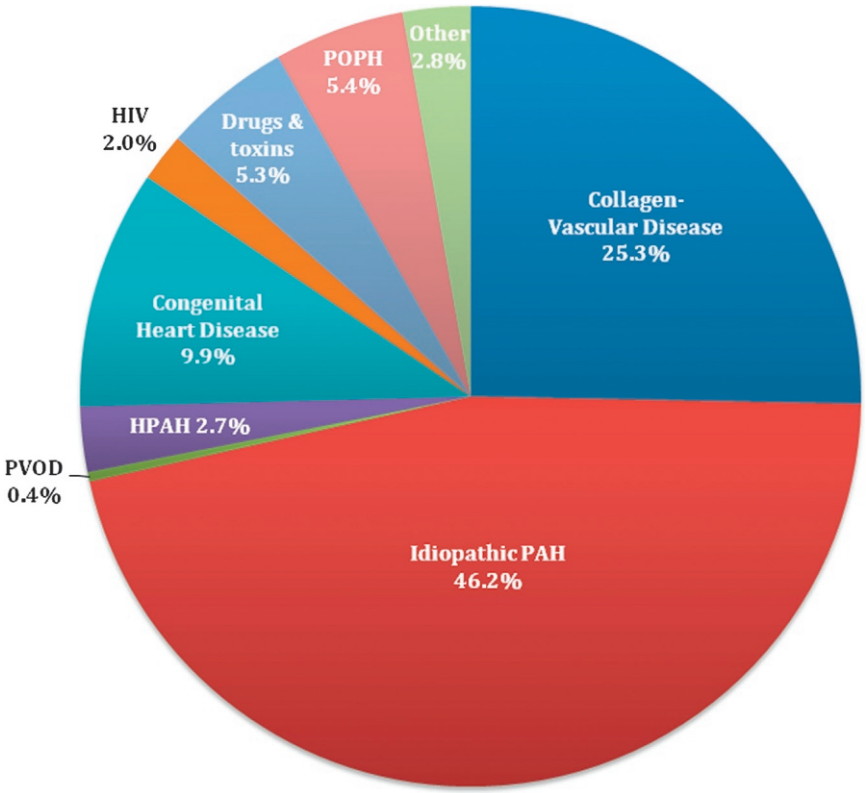

Fig. 1 Subgroups of pulmonary arterial hypertension. *HPAH*, hereditary PAH; *HIV*, human immunodeficiency virus; *POPH*, portopulmonary hypertension; *PVOD*, pulmonary veno-occlusive disease. Adapted from McGoon, M. D. and Miller, D. P. (2012). REVEAL: a contemporary US pulmonary arterial hypertension registry. *European Respiratory Review* **21**, 8–18.

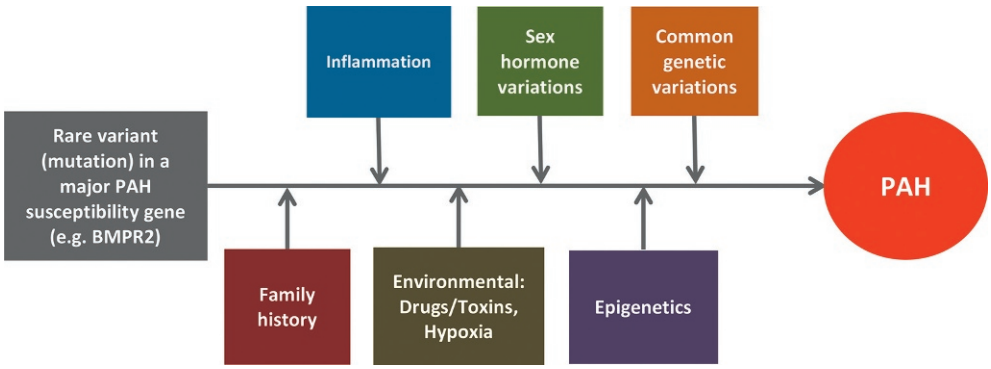

Fig. 2 Factors in the development of PAH.

identified PAH-associated mutations but have no evidence of disease are not uncommonly identified. This may be due to incomplete penetrance of the mutation or due to PAH that has not yet manifested. There is growing evidence to suggest that additional genetic, epigenetic, environmental, and developmental factors exist and modify the development of PAH among susceptible individuals (Austin and Loyd, 2014; **Fig. 2**). Identifying asymptomatic persons who are genetically at risk provides a potential opportunity for early intervention and treatment (Sztrymf et al., 2007; Machado et al., 2005).

Diagnosis of PH

The diagnostic approach to PH is initially based on clinical history and physical exam findings (McGoon et al., 2004). The cardinal symptoms of PH are exertional dyspnea, fatigue, palpitations, and chest discomfort. Syncope is an ominous finding and portends a worse outcome. The most common physical examination finding is an accentuated pulmonic component of the second heart sound

("loud P2") followed by a tricuspid regurgitation murmur (systolic ejection murmur along the left upper sternal border with respiratory variation). A palpable P2, right ventricular heave, and edema are seen in more advanced cases. There may also be symptoms and signs of associated conditions such as Raynaud's phenomenon, liver disease, HHT, left heart disease (systolic or diastolic heart failure and aortic or mitral valve disease), chronic lung disease (chronic obstructive lung disease, pulmonary fibrosis, sleep apnea or kyphoscoliosis), or thromboembolic disease (Badesch et al., 2009). Chest radiograph findings in PAH include prominent central pulmonary arteries, peripheral hypovascularity, and right ventricular enlargement. Signs of associated conditions may also be present. If there is suspicion for PH, transthoracic echocardiography is advised as the preferred initial diagnostic test. Common echocardiographic features of PH include right atrial enlargement, right ventricular enlargement, flattening or reverse curvature of the interventricular septum, and underfilled left heart chambers. The right ventricular systolic pressure (RVSP) can be estimated by measuring the maximal velocity of tricuspid regurgitation and central venous pressure. The RVSP will approximate the peak pulmonary arterial pressure in the absence of pulmonic stenosis. A RVSP of greater than 38–40 mmHg at rest is abnormal. Other findings of PH such as right chamber dilation or reduced right ventricular function (measured by the tricuspid annular plane excursion by "m-mode" or right ventricular fractional area change on a four-chamber view) increase the specificity of echocardiography for the presence of PH. Current guidelines recommend screening high-risk populations for the development of PAH, particularly in cases of limited scleroderma (Hoeper et al., 2013b; Hachulla et al., 2005; Fisher et al., 2009; Brown et al., 2011; Authors/Task Force et al., 2015). Once PH has been identified, additional workup may be indicated to determine the etiology (**Table 3**). Symptomatic patients with an RVSP > 40 mmHg or asymptomatic patients with an RVSP > 50 mmHg should generally undergo further investigation into the etiology of their PH. All therapy for PH is based on the etiology as outlined in the PH classification system. It is therefore important to identify the cause(s) of PH in order to determine the appropriate therapy. The initial diagnostic work up for common etiologies of PH (i.e., groups 2 and 3) includes pulmonary function tests, assessments of oxygenation, EKG, and echocardiogram. Chest computed tomography and polysomnography may also be needed as contingent tests. To exclude group 4 PH (CTEPH), patients should undergo ventilation–perfusion scanning, which has superior diagnostic characteristics to CT pulmonary angiography. A normal or low-probability scan effectively excludes CTEPH. Formal pulmonary angiography by an experienced center is generally needed to confirm the diagnosis (Hoeper et al., 2006; Coulden, 2006). Group 1 PH patients should have further testing performed to exclude collagen vascular disease (ANA and RF) and liver disease (liver function tests and right-upper-quadrant Doppler ultrasound) and thyroid function studies (Hoeper et al., 2013b).

Right heart catheterization (RHC) is essential in all patients with suspected PAH after completion of the noninvasive evaluation. The RHC is the gold standard used for hemodynamic evaluation to confirm the presence of PAH, determine initial prognosis and initial management. There are several essential components to a complete RHC evaluation; this includes oxygen saturations to exclude a left-to-right shunt, cardiac output by the Fick method, right atrial pressure, right ventricular pressure, pulmonary artery pressure (PAP; systolic, diastolic, and mean), PCWP to estimate left atrial pressure, cardiac output and index (traditionally performed by thermodilution method), PVR, systemic blood pressure, and heart rate. If the PCWP is uncertain or elevated, a direct measurement of LVEDP via left heart catheterization is often necessary to definitively exclude left heart disease.

The hemodynamic definition of PAH is a mean PAP greater than 25 mmHg, a PCWP or LVEDP less than 15 mmHg, with a PVR greater than 3 Wood units in the absence of significant (i.e., hypoxemic) chronic lung disease or CTEPH (McLaughlin et al., 2009; Hoeper et al., 2013b; Authors/Task Force et al., 2015). Acute vasodilator/vasoreactivity testing (AVT) should be performed on all IPAH patients. IPAH patients who demonstrate a significant acute vasodilator response have a better prognosis and are more likely to benefit from oral calcium-channel blockers (CCB) (Sitbon et al., 2005; Morales-Blanhir et al., 2004). Once the diagnosis and classification of PAH have been established, exercise testing by 6 min walking distance testing (6MWD) or cardiopulmonary exercise testing should be performed to establish a baseline functional status (Deboeck et al., 2005, 2012; Wensel, 2002; Hansen et al., 2004; ATS/Committee, 2002).

Table 3 Diagnostic evaluation of pulmonary hypertension

PAH subgroup	*1 (PAH)*	*2 (Left heart disease)*	*3 (Lung disease, hypoxemia)*	*4 (CTEPH)*	*5 (Unclear/multifactorial)*

Core testing	• ANA, RF, LFTs, TFTs, HIV
	• RHC
	• 6MWD testing or CPET
		• EKG
		• Echocardiogram
			• PFTs
			• Oximetry
			  • Overnight
			  • Ambulatory
			• Chest radiograph
				• V/Q(Ventilation-Perfusion Scan)
				• Pulmonary angiogram
					• CBC (Complete Blood Count)
					• CMP (Complete Metabolic Panel)

Additional testing	• Acute vasodilator test (for IPAH) • RUQ Doppler ultrasound (for POPH)	• RHC with exercise or volume challenge • LHC (for left heart failure)	• Polysomnogram (for SBD (Sleep Breathing Disorders)) • High-resolution CT (for ILD (Interstitial Lung Disease)) • High altitude simulation test (for flight)	• CTPA (CT Pulmonary Angiogram)	• Other testing as warranted by clinical picture (see **Table 1**)

Clinical Management of PAH

General Measures in the Management of the PAH Patient

General medical care of the PAH patient includes vaccination (influenza and pneumococcal), psychosocial support, and avoidance of pregnancy (Table 4). Supervised exercise training (while avoiding overly strenuous physical activity) is strongly endorsed due to several small but concordant studies demonstrating that PAH patients undergoing supervised cardiopulmonary rehabilitation achieved enhanced physical activity, improved 6MWD testing and patient-reported quality of life, and decreased fatigue when compared with untrained controls (Weinstein et al., 2013; Chan et al., 2013). The optimal timing, methods, intensity, and duration for rehabilitation have yet to be determined. Supplemental long-term domiciliary oxygen should be provided to maintain the partial pressure of blood oxygen above 60 mmHg or oxyhemoglobin saturation above 90% during all conditions encountered (rest, exercise, sleep, and flight/altitude). General medication measures include the use of diuretics to reduce fluid retention (edema or ascites) when necessary. Loop diuretics are generally preferred, and aldosterone antagonists should be added when appropriate. Patients should be monitored for electrolyte derangements, and careful attention should be given to avoiding hypovolemia that can quickly negatively impact renal function. Warfarin anticoagulation should be considered for patients with PAH due to IPAH, heritable PAH, and PAH due to anorexigens. Patients with PAH on long-term IV prostaglandin therapy are generally anticoagulated (absent contraindications) to reduce risk of catheter-associated thrombosis. The targeted international normalized ratio range varies from 1.5–2.5 to 2.0–3.0 depending upon local experience and preferences. Anticoagulation of other group 1 patients is not generally considered beneficial but may be considered for certain individuals where the benefit is thought to outweigh the risks of bleeding. Digoxin may be considered for select PAH patients, however, as with most of the ancillary medications. There are limited data to support this recommendation (Galie et al., 2009).

Acute Vasodilator (Vasoreactivity) Testing and Therapy

Acute vasoreactivity testing (AVT) is mandatory in patients with IPAH to identify patients who are likely to have an excellent long-term outcome to treatment with CCB. AVT should not be performed if the patient is hemodynamically unstable. In cases of associated PAH, AVT is generally not performed due to a very low rate of reactivity found. Inhaled nitric oxide is the preferred agent for acute vasoreactivity testing due to safety, efficacy, and expediency. Epoprostenol or adenosine may also be used; however, these agents carry the risk of systemic hypotension (Galie et al., 2009). Only about 13%–15% of patients with IPAH demonstrate a positive AVT response during the hemodynamic study. This response is defined as a decrease in mPAP by greater than 10 mmHg, a final mPAP under 40 mmHg, and stable or increase in cardiac output (Rich et al., 1992; Sitbon et al., 2005). CCB should only be prescribed to those patients with a positive AVT. Satisfactory results are reported using nifedipine (120–240 mg daily), diltiazem (240–720 mg daily), and amlodipine (up to 20 mg daily). The exact CCB is chosen based upon the baseline heart rate (bradycardia supporting nifedipine or amlodipine and tachycardia supporting diltiazem). The recommended method of beginning CCB is to start with a low dose and increase gradually to the maximum tolerated dose. Patients who meet the criteria for a positive AVT and are treated with CCB therapy must be monitored regularly for safety and efficacy. About half of these patients will continue to do well with CCB in the long run (Rich et al., 1992; Sitbon et al., 2005). If a patient does not show marked hemodynamic improvement to CCB and WHO-FC I or II status within 3–4 months or alternatively loses their responsiveness over time, PAH-specific therapy should be instituted (Galie et al., 2009).

Table 4 General management of the PAH patient

Intervention	*Recommendation(class)*	*Level of evidence*[a]
Supervised exercise training	Recommended/indicated	A
Psychosocial support	Recommended/indicated	C
Avoid strenuous physical activity	Recommended/indicated	C
Avoid pregnancy	Recommended/indicated	C
Influenza and pneumococcal immunization	Recommended/indicated	C
Oral anticoagulants:		
IPAH, heritable PAH, and PAH due to anorexigens	Should be considered	C
APAH	May be considered	C
Diuretics	Recommended/indicated	C
Oxygen	Recommended/indicated	C
Digoxin	May be considered	C

A, Data derived from multiple randomized clinical trials or meta-analyses; *B*, data derived from a single randomized clinical trial or large nonrandomized studies; C, consensus of opinion of the experts and/or small studies, retrospective studies, registries.

[a]Level of evidence definition.

Source: Galie, N., Corris, P. A., Frost, A., Girgis, R. E., Granton, J., Jing, Z. C., Klepetko, W., McGoon, M. D., McLaughlin, V. V., Preston, I. R., Rubin, L. J., Sandoval, J., Seeger, W. and Keogh, A. (2013). Updated treatment algorithm of pulmonary arterial hypertension. *Journal of the American College of Cardiology* **62**, D60–D72.

PAH-Specific Medication Therapy

Presently, in the United States, there are 12 PAH-specific medications in four different classes—endothelin receptor antagonists (ERA), phosphodiesterase-5 inhibitors, soluble guanylate cyclase stimulators, and prostacyclin analogs or agonists. These medications have a variety of administration routes (oral, inhaled, and continuous subcutaneous or intravenous) leading to significant administration complexity. Direct comparisons among different compounds are not available, and there are no evidence-based initial treatment recommendations favoring any specific agent except for the most ill patients. The choice of initial therapy is dependent on a variety of factors including the PAH disease severity (e.g., PAH functional classification), route of administration, side effect profile, and patient preferences. The current standard of care is to use combination therapy with drugs of different classes employed to optimize patient outcomes (Pulido et al., 2013; Galie et al., 2013, 2015; McLaughlin et al., 2010; Ghofrani et al., 2013). A treatment algorithm (**Table 5**) recommends a risk-based management strategy to optimally tailor initial and ongoing therapy based on each individual patient's PAH severity and goals of care (Authors/Task Force et al., 2015; Galie et al., 2013; Taichman et al., 2014). Once initiated on PAH-specific therapy, patients should be regularly monitored with defined treatment goals (McLaughlin et al., 2009, 2013). Treating providers need to be vigilant regarding inadequate clinical responses to initial therapy or clinical disease progression, both of which occur commonly (**Table 6**). Although oral agents are simpler to administer and more commonly used, parenteral therapies remain the preferred treatment as an initial therapy or subsequent intervention for patients with advanced PAH in the appropriate clinical setting (Galie et al., 2013; Authors/Task Force et al., 2015). Continuous IV epoprostenol is recommended as initial therapy for WHO-FC IV PAH patients because of the proved survival benefit in this population. Oral agents are considered as second-line treatments in severely ill patients. Earlier, medical intervention is associated with reduced unwanted clinical outcomes such as hospitalization and urgent initiation of advanced therapies that generally precede death (Burger et al., 2014; Farber et al., 2015a,b; Galie et al., 2015).

Combination PAH-specific medication therapy should be given to patients with a high-risk profile at presentation or who have a continued inadequate clinical response (WHO-FC III or IV, low 6MWD or exercise performance, and elevated biomarkers). The multicenter, prospective, blinded, placebo-controlled AMBITION trial (a randomized, multicenter study of first-line ambrisentan and tadalafil combination therapy in subjects with pulmonary arterial hypertension) compared first-line monotherapy with tadalafil or ambrisentan to combination therapy with tadalafil and ambrisentan as initial treatment strategies for WHO-FC II and III PAH patients. This study showed initial combination therapy resulted in a significantly lower risk of clinical failure events (principally hospitalization for worsening PAH, disease progression, or unsatisfactory long-term clinical response) than either drug as initial monotherapy (Galie et al., 2015). Strategies of "up front" versus sequential combination therapy should both be considered, as the optimal approach and medication combinations are still being determined. For patients with PAH who have a persistently inadequate clinical response and fail to meet treatment goals despite maximal medical therapy, atrial septostomy and/or lung transplantation may be indicated.

Table 5 Recommended initial PAH-specific therapy based on WHO status of most subjects in the pivotal medication clinical trials

	Level[a]	*WHO FC II*	*WHO FC III*	*WHO FC IV*
Medication recommendation	A	Ambrisentan Bosentan Macitentan Sildenafil Tadalafil Riociguat Treprostinil PO Selexipag	Ambrisentan Bosentan Macitentan Sildenafil Tadalafil Riociguat Treprostinil SC, inhaled, or PO Selexipag Iloprost inhaled Epoprostenol IV	Epoprostenol IV
	B		Iloprost IV Treprostinil IV Beraprost Initial combination therapy	Ambrisentan Bosentan Macitentan Sildenafil Tadalafil Riociguat Iloprost inhaled, IV Treprostinil SC, IV, or Inhaled Initial combination therapy

PO, oral route; *SC*, subcutaneous route; *IV*, intravenous route.

[a]Level of recommendation defined as the following: (A) is recommended or indicated and (B) should be considered.

Source: Galie, N., Corris, P. A., Frost, A., Girgis, R. E., Granton, J., Jing, Z. C., Klepetko, W., McGoon, M. D., McLaughlin, V. V., Preston, I. R., Rubin, L. J., Sandoval, J., Seeger, W. and Keogh, A. (2013). Updated treatment algorithm of pulmonary arterial hypertension. *Journal of the American College of Cardiology* **62**, D60–D72.

Table 6 Variables with prognostic value at follow-up and goals of PAH therapy used in clinical practice

Measure type	*Variable*	*Therapeutic goal*
Symptoms	WHO Functional Class	WHO FC I or II
Exercise tolerance	6MWD	>380–440 m
	CPET testing	VO_2 maximum >10.4mL/kg/min
Hemodynamics	Right atrial pressure	RAP <8 mmHg
	Cardiac index	CI >2.5 L/min-m^2
	Sv02	Sv02 >62%
Echocardiogram	RA and RV size and function	Normal or near normal RA and RV size and function
	TAPSE	TAPSE >1.8 cm
	Exercise RVSP	Ability to raise RVSP >30 mmHg during supine exercise
Biomarkers	BNP/NT-proBNP	BNP <180
		NT-proBNP <1250

Source: McLaughlin, V. V., Archer, S. L., Badesch, D. B., Barst, R. J., Farber, H. W., Lindner, J. R., Mathier, M. A., McGoon, M. D., Park, M. H., Rosenson, R. S., Rubin, L. J., Tapson, V. F., Varga, J., Harrington, R. A., Anderson, J. L., Bates, E. R., Bridges, C. R., Eisenberg, M. J., Ferrari, V. A., Grines, C. L., Hlatky, M. A., Jacobs, A. K., Kaul, S., Lichtenberg, R. C., Lindner, J. R., Moliterno, D. J., Mukherjee, D., Pohost, G. M., Rosenson, R. S., Schofield, R. S., Shubrooks, S. J., Stein, J. H., Tracy, C. M., Weitz, H. H., Wesley, D. J. and ACCF/AHA (2009). ACCF/AHA 2009 expert consensus document on pulmonary hypertension: a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association: developed in collaboration with the American College of Chest Physicians, American Thoracic Society, Inc., and the Pulmonary Hypertension Association. *Circulation* **119,** 2250–2294; McLaughlin, V. V., Gaine, S. P., Howard, L. S., Leuchte, H. H., Mathier, M. A., Mehta, S., Palazzini, M., Park, M. H., Tapson, V. F. and Sitbon, O. (2013). Treatment goals of pulmonary hypertension. *Journal of the American College of Cardiology* **62**, D73–D81; Authors/Task Force, Galie, N., Humbert, M., Vachiery, J. L., Gibbs, S., Lang, I., Torbicki, A., Simonneau, G., Peacock, A., Vonk Noordegraaf, A., Beghetti, M., Ghofrani, A., Gomez Sanchez, M. A., Hansmann, G., Klepetko, W., Lancellotti, P., Matucci, M., McDonagh, T., Pierard, L. A., Trindade, P. T., Zompatori, M. and Hoeper, M. (2015). 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS)Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *European Heart Journal*.

Surgical Management of PAH

Atrial Septostomy

Atrial septostomy is a procedure to intentionally create an interatrial right-to-left shunt with aim of depressurizing the right ventricle and improving left ventricular preload to reduce right heart failure and increase cardiac output (Sandoval et al., 1998; Kurzyna et al., 2007). However, these hemodynamic improvements are at the expense of systemic oxygenation due to the inherent right-to-left shunting of venous blood. A majority of patients require supplemental oxygen therapy following atrial septostomy (Olsson et al., 2005). Atrial septostomy was first reported in 1983 as a palliative therapy for patients with refractory PH using a blade balloon technique (Rich and Lam, 1983). The blade-balloon technique has been replaced by graded balloon dilation atrial septostomy (BAS) as it carries reduced risk and equivalent improvement in patient hemodynamics (Rich and Lam, 1983; Althoff et al., 2008). BAS is performed by puncture of the atrial septum with a Brockenbrough needle, followed by serial balloon dilations of the septum (Sandoval et al., 1998). Some patients will have spontaneous closure of the BAS and will need to have the procedure repeated. Smaller defect size is associated with increased incidence of spontaneous closure of the BAS (Olsson et al., 2005).

Patient selection is important to reduce procedural mortality in BAS. The procedure should generally be avoided in patients with right atrial pressure >20 mmHg or oxyhemoglobin saturation <85% at rest on room air. Profound hypoxemia and inability to control the newly created right-to-left shunt is a potential cause of mortality. In the United States, BAS is considered a bridge to transplantation or palliative procedure and is reserved for patients who have refractory right heart failure or syncope and while on maximal medical therapy. In other countries, BAS is performed more commonly related to lack of access to PAH medical therapies (Sandoval et al., 1998; Kurzyna et al., 2007; Chiu et al., 2015; Kuhn et al., 2015). Studies have shown improvement in cardiac index, New York Heart Association functional class and 6 min walk distance, with decreases in right atrial pressure and oxygen saturation post procedure. The BAS procedure should only be performed at centers with experience with the technique (Kurzyna et al., 2007; Chiu et al., 2015; Sandoval et al., 1998).

Transplantation

Transplantation remains a treatment option for patients with PAH despite advances in medical therapy. Referral for transplantation evaluation should occur prior to right ventricular failure. The International Society for Heart and Lung Transplantation found that between 1995 and 2011, about 3% of all lung transplantation procedures in the United States were performed for PAH. The majority of procedures performed for PAH are double-lung transplantations. When compared with the other indicated conditions for lung transplantation, patients with PAH were found to have the highest unadjusted 3-month posttransplantation mortality. PAH is the second most common indication for heart–lung transplantation (Christie et al., 2012).

The survival following lung transplantation for PAH ranges from 52% to 75% at 5 years and between 45% and 66% at 10 years (Toyoda et al., 2008; de Perrot et al., 2012; Fadel et al., 2010). There is debate among transplantation centers whether heart–lung or

double-lung transplantation is superior for patients with PAH, with some centers favoring heart–lung transplantation for those patients with severe right heart failure. Recent studies have shown no significant mortality difference between PAH patients who underwent double-lung transplant and those who underwent heart lung transplantation (Toyoda et al., 2008; de Perrot et al., 2012; Fadel et al., 2010).

Since 2005, the allocation of lungs for transplant has been determined by the lung allocation score (LAS) (Egan et al., 2006). The LAS is calculated from estimates of waiting list survival and posttransplantation survival of each listed patient. Determinants in the LAS calculation include age, body mass index, current mechanical ventilation, diabetes mellitus, diagnosis, forced vital capacity, functional status, oxygen requirement at rest, pulmonary artery systolic pressure, PCWP $\geq$20 mmHg, $PaCO_2$, 6 min walk distance, and serum creatinine (Egan et al., 2006; Colvin-Adams et al., 2012). In patients that are listed for heart–lung transplantation, priority is based on LAS for lung transplantation and medical urgency status code for heart transplant (Colvin-Adams et al., 2012). Since introduction of the LAS, there has been a significant increase in the number of lung transplantations performed (unrelated to an increase in donors), a reduced number of deaths of patients on the waitlist, and an observed increase in 1-year posttransplantation survival (Egan and Edwards, 2016).

Newer Therapies and Approaches to PAH

The majority of the current medical therapies for PAH are focused on well-established biochemical pathways related to pulmonary vasoconstriction and thrombosis. Although a marked improvement in overall survival is associated with appropriate use of the currently available PAH therapies, these agents generally produce only a partial amelioration of abnormal hemodynamics and temporary clinical improvement in for the majority of patients. Current PAH clinical drug development is focused on several areas: use of currently approved agents in combination regimens, use of currently approved agents in specific PAH subpopulations, new delivery methods of currently approved agents, and developing new agents targeted at inflammation and neoplastic pathways implicated in PAH pathogenesis. A novel nonmedical approach involves pulmonary artery denervation (**Table 7**).

Table 7 Current clinical trials in PAH: 2017

Multiagent trials in group 1 PAH	
FREEDOM-Ev	Trial of the early combination of oral treprostinil with background oral monotherapy in subjects with PAH
TAO	Double-blind, randomized, placebo-controlled study of oral treprostinil in combination with tadalafil and ambrisentan compared with tadalafil and ambrisentan alone in WHO group I PAH
TRITON	To compare triple with dual initial oral PAH therapy in newly diagnosed, treatment-naive PAH
"Switch" or treatment strategy trials in group I PAH	
TRANSIT 1	To assess the tolerability and safety of UPTRAVI® (selexipag) in subjects with PAH who transition from inhaled treprostinil to UPTRAVI
REPLACE	Riociguat replacing PDE-5i therapy evaluated against continued PDE-5i therapy
Evaluating efficacy of approved PAH drugs on specific subgroups within group 1	
MAESTRO	Macitentan in Eisenmenger's syndrome to restore exercise capacity
PORTICO	Portopulmonary hypertension treatment with macitentan—a randomized clinical trial
RUBATO	Prospective, multicenter, double-blind, randomized, placebo-controlled, parallel-group study assessing the efficacy and safety of macitentan in Fontan-palliated adult and adolescent subjects
Evaluating efficacy of PAH drugs in groups 2–5 PH	
INCREASE	Safety and efficacy of inhaled treprostinil in adult PH with ILD including CPFE
MELODY 1	Macitentan in combined pre- and postcapillary pulmonary hypertension due to left ventricular dysfunction
MERIT 1	Macitentan in the treatment of inoperable chronic thromboembolic pulmonary hypertension
SOUTHPAW	Double-blind, randomized, placebo-controlled study of oral treprostinil in WHO group 2 PH in HFpEF
IRONS	Phase 3 study of oral treprostinil in patients with sickle cell disease-associated pulmonary hypertension
SOPRANO	Macitentan in pulmonary hypertension post left ventricular assist device implantation
Novel therapeutic agents	
Bardoxolone	LARIAT, CATALYST: investigation of bardoxolone in PAH and CTD-PAH
Dichloroacetate (DCA)	Phase I study of the a proapoptotic molecule in patients with WHO III-IV group 1 PAH
Ubenimex	LIBERTY: phase 2, randomized, placebo-controlled study in patients with FC II/III group 1 PAH
Ralinepag	Phase 2, randomized, double-blind, placebo-controlled trial in patients with group I PAH

Oral Prostacyclin Therapy

Recently, several oral prostacyclin agents and receptor agonists have become commercially available or are in active development.

Oral treprostinil was investigated in the FREEDOM studies, both as monotherapy and in combination with other PAH specific medications. This medication is gradually increased over time, following the paradigm for parenteral dosing. In the FREEDOM-M monotherapy study, significant improvements were seen in 6MWD testing, particularly with dose titrations above 2 mg twice daily (Jing et al., 2013). Higher doses were not well tolerated by many patients due to significant side effects, principally gastrointestinal upset and headaches. The FREEDOM-C studies with background ERA and PDE-5 inhibitors demonstrated no overall improvement in 6MWD test results, time to clinical worsening, mortality, or functional class. Data suggest that significant improvements were possible at higher doses, when these doses were tolerated by patients. Oral treprostinil is currently dosed three times daily, which appears to mitigate the intensity of adverse effects in many patients with the hope of improving tolerability and efficacy (Tapson et al., 2013).

Selexipag, an oral prostacyclin receptor agonist that has both vasodilatory properties and antiproliferation effects, was recently approved at twice daily dosing. The GRIPHON study demonstrated a decrease in the risk of the primary composite end point of death from all causes and complications from PAH, including PAH patients on background therapy (Sitbon et al., 2015). It also requires an up-titration schedule.

A phase 2 study of another oral prostacyclin receptor antagonist, ralinepag, has recently completed enrollment. Pharmacokinetic studies of this agent demonstrate an adequate half-life to potentially allow for once daily dosing (clinicaltrials.gov/ct2/show/NCT02279160).

Upfront Combination PAH-Specific Therapy

The 2015 AMBITION study looked at oral monotherapy with either a PDE5 inhibitor or ERA or the combination of both in patients with both IPAH or CTD-PAH. The event-driven protocol showed that the use of up-front combination therapy decreased death, hospitalization, or clinical worsening by approximately 50% (Galie et al., 2015).

Sitbon and colleagues performed a retrospective analysis pilot study examining 19 patients with FC III/IV PAH treated with up-front triple combination therapy (bosentan, sildenafil, and IV epoprostenol). All patients had improved FC to I/II, and all but one patient showed improvement in 6MWD and right-heart catheterization hemodynamics (Sitbon et al., 2014). The TRITON study, currently recruiting, compares triple with dual initial oral PAH therapy in newly diagnosed, treatment-naive group 1 PAH using the combination of macitentan and tadalafil with either oral treprostinil or placebo. Variations of these up-front studies are abundant (clinicaltrials.gov/ct2/show/NCT02999906).

Inflammation, Immunity and Apoptosis

Plexiform lesions in PAH demonstrate infiltration by variety of immune cells, and the degree of inflammation has been found to correlate with measured mPAPs (Tuder et al., 2013). Bardoxolone methyl is a treprostenoid compound with antioxidative and anti-inflammatory properties, which acts via Nrf2 and NF-κB, suppressing inflammatory mediators and inhibiting the downstream effects of vascular proliferation and remodeling. Since 2013, bardoxolone has been studied in the LARIAT trial, a phase 2, randomized, placebo-controlled study in patients with group 1 PAH (clinicaltrials.gov/ct2/show/NCT02036970). It is also being investigated for use in connective tissue disease associated PAH (CTD-PAH) in the CATALYST trial (clinicaltrials.gov/ct2/show/NCT02657356).

Immune dysregulation in PAH is currently being targeted with both approved medications and novel agents drugs in specific PAH populations (Rabinovitch et al., 2014). Studies are being done within the subgroups of patients with group 1 PAH, with attention to associated diseases. Patients with systemic-sclerosis-associated PAH (SSc-PAH) have higher mortality rates despite use of currently available PAH-specific therapies (Chaisson and Hassoun, 2013). Preclinical data and case reports have suggested a benefit in patients with SSc-PAH who were treated with immunomodulatory therapies. Rituximab, an anti-CD20 monoclonal antibody used in collagen vascular diseases and hematologic malignancies, has been tested in systemic-sclerosis-associated interstitial lung disease and is currently under investigation in a phase 2 studies in patients with SSc-PAH (clinicaltrials.gov/ct2/show/NCT01086540).

Macrophages, regulatory T cells, and leukotrienes have been associated with vascular proliferation in PAH and modification of the leukotriene pathway is currently being investigated as a target in PAH clinical trials (Qian et al., 2015). Inhibition of leukotriene B_4 has been shown to decrease vascular proliferation and occlusion (Tian et al., 2013). Ubenimex, a leukotriene B_4 antagonist, has been in use in Asia as adjunctive therapy in leukemia therapy for more than 2 decades. This agent is being studied in the LIBERTY phase II clinical trial for management of PAH (clinicaltrials.gov/ct2/show/NCT02664558).

Imatinib mesylate, a PDGF inhibitor currently used in hematologic and GI malignancies, which mitigates vascular and smooth muscle cell proliferation, was investigated in the IMPRES study. PAH patients on at least two background therapies were studied for 2 years. Functional class, time to clinical worsening, and mortality did not differ between treatments, and 6MWD was only improved by 32 m at 2 years. Serious adverse events and discontinuations were more frequent with imatinib than placebo, including subdural hematomas in anticoagulated patients (Hoeper et al., 2013a).

The Endothelium in PAH

Pulmonary endothelium is thought to have a pivotal role in the development of PAH, and the role of pathological apoptosis of pulmonary endothelial cells is an area of current investigation. Diminished programmed cell death of pathological endothelial cells may lead to vascular proliferation and remodeling in PAH. Dichloroacetate, a proapoptotic agent that has been shown to cause regression of vascular proliferation, has recently completed early phase testing in PAH patients (clinicaltrials.gov/ct2/show/NCT01083524).

Nonpharmacologic Therapies

Research has shown that obliteration of the sympathetic nerve supply to the renal arteries in severe systemic hypertension can have the effect of lowering systemic blood pressure, albeit with unacceptable risks in most studies. Sympathetic activation has also been shown to be contributing factor to pulmonary arterial pressures. Chen and colleagues safely performed pulmonary artery denervation in 66 PAH subjects resulting in improvement in 6MWD and mean PAP with reduction in PAH medication use (Chen et al., 2015). Studies of the effect of stellate nerve block and/or reserpine in group 2 pulmonary hypertension are in the preclinical stage (NCT02684786).

Current Challenges in the Diagnosis and Management of PAH

With the development of multiple oral agents to treat PAH and expert management, PAH is becoming a chronic condition with an enhanced life expectancy (Simonneau et al., 2013). However, the availability of oral medications for PAH treatment has also seen a migration of care delivery from tertiary care centers to the wider community. Overreliance on oral medication use may lead to a higher rate of emergent interventions for decompensated PAH and reduced survival (Badagliacca et al., 2012). Despite the availability of multiple drugs and evidence-based treatment guidelines for several years, there are ongoing reports of errors of misdiagnosis, delayed diagnosis, and undertreatment of PAH patients within both the community and PAH centers (Deano et al., 2013). Multidisciplinary team-based patient care is felt to be an important strategy to improve health-care quality in chronic diseases (Yarnall et al., 2009). It has been shown that multidisciplinary healthcare teams are clinically effective and can improve the quality of clinical care delivered in a spectrum of acute and chronic care settings (Wagner et al., 2001). Improvements have been demonstrated in medication safety and in clinical and patient-centered outcomes (patient knowledge, medication adherence, and quality of life health scores) (Chisholm-Burns et al., 2010). The fifth World Symposium on Pulmonary Hypertension Task Force on Standard of Care recommends referral of PAH patients to expert centers after diagnosis (Galie et al., 2013). While many areas have well-established centers for the multidisciplinary management of PAH for some time, further organization and coordination between PH centers regionally, nationally, and internationally is progressing and will hopefully positively impact this uncommon but potentially lethal condition (Sahay et al., 2016; Chakinala et al., 2016).

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Pulmonary Embolism

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Introduction

Pulmonary embolism (PE) was first alluded to in Indian texts dating back to 1000 BC. Although others previously described PE, the German pathologist Rudolph Virchow is credited with describing PE in 1846 and hypothesizing the relationship between venous thrombosis and obstruction of the pulmonary arteries. His description of factors leading to clot formation is still used today (Wood, 2009).

Definitions

PE is broadly defined as obstruction in one or more of the pulmonary artery branches. PEs are most often due to thrombus embolization from peripheral veins but can also be composed of bacteria, protozoa, tumor, fat, amniotic fluid, and air. Herein, we will focus on thrombus. PE is generally separated into acute and chronic presentations. Acute PE is by far the more common presentation. Rarely, patients present with the subacute onset of signs and symptoms consistent pulmonary hypertension due to chronic thromboembolic disease.

Epidemiology

The incidence of acute PE is reported between 38 and 80 cases per 100,000 persons per year. In the US, there are approximately 237,000 PE cases diagnosed per year. Some databases demonstrate increasing incidence over the last two decades. However, with widespread use of ventilation perfusion scanning and computed tomography with pulmonary angiography, the increased incidence of PE is likely due to increased detection (Alotaibi et al., 2016; Heit et al., 2005; Huang et al., 2014; Martinez et al., 2014; Silverstein et al., 1998; Wiener et al., 2011). Both the incidence and the risk of death are higher in certain subpopulations including those with malignancy, hospitalization, joint replacement, inherited thrombophilias, and autoimmune disease (Alotaibi et al., 2016; Dalen and Alpert, 1975; Horlander et al., 2003; Lilienfeld, 2000; Wiener et al., 2011).

Death

PE is fatal in up to 10% of patients. In the United States, fatalities due to PE are 50% higher in African-Americans than whites, while deaths in whites are approximately 50% higher than Latinos and Asians Within racial strata, mortality rates are 20%–30% higher for males than females. Most commonly, this is due to failure to adequately diagnose PE as well as failure to prevent it via prophylactic interventions in high risk patients. Patients that receive appropriate prophylaxis and treatment typically do not succumb to PE. Mortality from PE appears to have declined over the past several decades coincident with advances in diagnosis and treatment (Horlander et al., 2003; Lilienfeld, 2000). The true incidence of death from PE may be higher than reported. Various autopsy series have noted up to three times higher incidence of death from PE than what is clinically diagnosed (Karwinski and Svendsen, 1989; Morgenthaler and Ryu, 1995; Rubinstein et al., 1988).

Pathophysiology

Inherited Risk Factors

Thrombus formation results from risk factors defined by Virchow's Triad; hypercoagulability, stasis, and vascular endothelial injury. Hypercoagulable states can be hereditary or acquired. Factor V Leiden is the most common inherited thrombophilia, accounting for up to 50% of cases of identified thrombophilia leading to PE. Five percent of Caucasians are heterozygous, which carries a 5- to 10-fold increased lifetime risk for venous thromboembolism (VTE). Homozygosity for Factor V Leiden is much rarer but carries an 80-fold increase risk of thrombosis. The risk increases even more during pregnancy and with the use of estrogen containing contraceptive pills. It is important to note that only 5% of patients with Factor V Leiden deficiency will develop a VTE in their lifetime (Koster et al., 1993; Rosendaal et al., 1995; Simone et al., 2013). Other common inherited hypercoagulable states include Prothrombin Gene Mutation, Antithrombin III Deficiency, Protein C Deficiency, and Protein S Deficiency (Leiden and Society, 2008; System, 2014).

Acquired Risk Factors

Many patients that develop VTE have underlying inherited risk factors that, in combination with acquired risk factors such as endothelial injury, acute illness, and venous stasis, result in clot formation (Anderson and Spencer, 2003).

Major acquired risk factors include pelvic and lower extremity fractures, orthopedic and major surgery, malignancy, prolonged immobility, and autoimmune diseases. **Table 1** summarizes common risk factors.

Various studies have shown that most patients with VTE have multiple risk factors at the time of diagnosis (Anderson and Spencer, 2003; Ocak et al., 2013; Spencer et al., 2006).

Natural History

Thrombi arise at areas of low and turbulent flow such as valvular cusps, areas of endothelial injury, and on foreign bodies such as venous catheters (Nicolaides et al., 1971). After initial clot formation, the size, extent, and morbidity of the clot depend on many

Table 1 Risk factors for venous thromboembolism (Anderson and Spencer, 2003)

Inherited thrombophilias
Factor V Leiden mutation
Protein C deficiency
Prothrombin gene mutation
Hyperhomocysteinemia
Familial plasminogen deficiency
Acquired surgical risk factors
Lower extremity orthopedic surgery
Neurosurgery
Major thoracoabdominal surgery requiring >30 min of general anesthesia
Fractures of the pelvis, hip, or long bones
Major trauma
Acquired medical risk factors
Malignancy
Pregnancy
Estrogen therapy
Autoimmune diseases
Lupus anticoagulant
Prolonged immobilization
Congestive heart failure
Critical illness

patient factors listed previously. The majority of pulmonary emboli result from clot that originates in the deep veins of the proximal lower extremities (Kakkar et al., 1969; van Langevelde et al., 2013; Nicolaides et al., 1971).

Physiologic Response to PE

PE causes pulmonary and cardiovascular derangements well beyond the simple mechanical obstruction of the clot itself. The mechanical obstruction of the pulmonary vascular bed can cause alterations in ventilation perfusion relationships as well as increased pulmonary arterial pressure. Furthermore, release of inflammatory cytokines and serotonin from platelets dysregulates hypoxemic vasoconstriction, increases pulmonary vascular resistance and worsens V/Q mismatch and hypoxia.

Despite increased dead space ventilation, stimulation of irritant receptors causes alveolar hyperventilation and resultant hypocapnia (Huet et al., 1985). If PE are large enough to cause increased right atrial pressure, the event can induce right to left shunting through a patent foramen ovale and further worsen hypoxia. There is also reflex bronchoconstriction which increases airways resistance, and noncardiogenic pulmonary edema that contributes to decreased lung compliance (D'Angelo, 1997; Elliott, 1992).

With small emboli (<20% of pulmonary vascular bed), the cardiovascular system is able to compensate for the increases in pulmonary pressure by augmenting RV pressure and stroke volume in order to preserve cardiac output. However, the native right ventricle (RV), unlike the left ventricle (LV) is a thin walled, crescent shaped pump unable of generating high pressures. As pulmonary vascular resistance and pulmonary arterial pressure rises, the RV wall tension increases, followed by RV dilation and dysfunction. As the RV dilates, LV filling is impaired and leads to decreased cardiac output and decreases in systemic blood pressure. RV wall tension coupled with decreases in systemic blood pressure impairs right coronary artery perfusion, leading to myocardial ischemia, and worsened cardiac function (Gerges et al., 2014). In up to 10% of patients, pulmonary infarction occurs due to small thrombi occluding distal subsegmental vessels. Infarctions are less common than in other organs due to both bronchial and pulmonary artery circulation. However, in patients with underlying cardiac or pulmonary disease, bronchial circulation and venous return may be impaired and lead to higher rates of pulmonary infarction (Dalen et al., 1977; Stein et al., 1991).

Clinical Presentation and Diagnosis

Symptoms of PE are nonspecific and overlap with other disease states, so a high index of clinical suspicion is a key factor in subsequently establishing a diagnosis. The three most common symptoms are dyspnea (77%), pleuritic chest pain (66%), and lower extremity pain and/or swelling (44%). Cough (37%), wheezing (21%), and hemoptysis (13%) can also be present. Syncope is uncommon in patients with PE. However, in patients admitted with a first episode of syncope, PE is identified in almost 20% of patients (Prandoni et al., 2016). Some patients also describe angina and a sense of doom, which is often associated with massive PE. Dyspnea often occurs within minutes of PE. Patients with delayed presentations tend to have larger, more central clot burden than those with more peripheral clot (dyspnea, pleuritic chest pain) (Stein et al., 2007). The most common physical examination findings include tachypnea (54%), leg swelling (44%), and tachycardia (24%). Other less common findings include inspiratory crackles and fever. With massive PE, patients may have evidence of right ventricular failure—hypotension, jugular venous distension, splitting of S2 and an accentuated pulmonic component of the second heart sound (Stein et al., 2007; Stein and Henry, 1997).

Imaging

Computed tomography

Since the 1990s, due to advances in imaging techniques, computed tomography with pulmonary angiography (CTPA) has become the preferred test in the diagnosis of acute PE because of its high diagnostic accuracy and ability to provide alternate diagnosis for diseases of the lung parenchyma, pleura, pericardium, aorta, heart, thoracic lymph nodes, and mediastinum (van Rossum et al., 1998; Sodhi et al., 2010). Contrast media is rapidly injected into a vein, travels to the pulmonary circulation and the scan takes place while the pulmonary arteries have been opacified. The diagnosis of PE is established on CTPA by direct visualization of one or more low attenuation filling defects that partially or completely occlude a contrast filled artery (Kuriakose and Patel, 2010). Other indirect CTPA findings include areas of lung parenchyma with lower attenuation due to decreased pulmonary blood flow or oligemia, evidence of focal pulmonary hemorrhage, atelectasis, small effusions, and RV dilation (Coche et al., 1998). CTPA has traditionally been less sensitive at detecting smaller, more peripheral emboli. However, newer multidetector CTs have improved the detection of smaller segmental and subsegmental PE (Perrier et al., 2005).

Ventilation perfusion scans

Prior to the widespread use of CTPA, radionuclide ventilation perfusion (V/Q) scans were the imaging modality of choice for diagnosing acute PE. Ventilation perfusion scans are still used in patients with contraindications to CTPA and are the preferred imaging technique in the initial evaluation of chronic thromboembolic disease. A V/Q scan with normal findings effectively excludes PE with a negative predictive value close to 100%. A high-probability scan is highly specific for the diagnosis of PE, allowing definitive treatment. Unfortunately, the majority of patients originally studied had an intermediate probability of having a

PE and require more testing to determine the diagnosis (PIOPED Investigators, 1990). However, V/Q technology and radiology reporting has improved significantly over the past two decades making it a much more useful tool in diagnosing thromboembolic disease (Freeman and Haramati, 2009).

Catheter pulmonary angiography

Catheter pulmonary angiography is an invasive means of diagnosing PE. A catheter is inserted into a central vein and advanced through the RA and RV into the pulmonary arteries under fluoroscopy and contrast is injected to obtain radiographic images to visualize a thrombus. With newer, more rapid technologies of diagnosing PE, catheter pulmonary angiography does not play a major role in diagnosing acute PE (Kuriakose and Patel, 2010). However, it is still routinely used as part of the preoperative evaluation for pulmonary thromboendarterectomy.

Plain film radiographs

Plain film radiographs are not adequate to establish the diagnosis of PE but can demonstrate indirect evidence of PE—atelectasis, small effusions, wedge shaped opacities suggestive of infarction, and hypovascularity.

Echocardiography

Similar to radiographs, echocardiography cannot definitively diagnosis PE. However, in a patient with unexplained shock and a high clinical suspicion for PE, echocardiography can help point toward the diagnosis in those with RV dilation, and may sometimes result in clot visualization in the IVC, RA, or RV. Echocardiography can also suggest alternative diagnoses—congestive heart failure, pericardial effusion, myocardial infarction. In the right clinical context in an unstable patient, echocardiography can provide enough information to allow for immediate therapies to be undertaken at the bedside (Goldhaber, 2002; Miniati et al., 2001).

Electrocardiography

The most common ECG finding in PE is tachycardia. Other abnormalities can include a new right bundle-branch block, evidence of RV strain—anterior ST-segment changes, T wave inversions, and Q waves in the inferior leads. With larger and more hemodynamically significant occlusion, the evidence of right strain can be evidenced by "S1Q3T3" pattern, a pseudoinfarction pattern (QR in V1), a complete or incomplete right bundle-branch block, or right axis deviation (Kucher et al., 2003; Stein et al., 1975). Neither the sensitivity nor the specificity of ECG for PE is high.

Lower extremity ultrasound

Lower extremity ultrasound (LE U/S) is very useful in detecting lower extremity thrombus with sensitivity and specificity (Lensing et al., 1989). However, only 10%–20% of patients with clinical suspicion are found to have DVT. Further, only half of patient with proven PE are found to have DVT (Perrier, 2000). Therefore, LE U/S does not reliably diagnose PE and should not be used as the initial imaging modality unless contraindications to CTPE or V/Q exist (Wells et al., 2001).

Laboratory data

Arterial blood gas analysis—arterial hypoxia is often present and correlates to the degree of the obstruction. A widened alveolar-arterial oxygen gradient and hypocapnia occur but is not specific for PE. Hypercapnia is rare and only occurs in patients with a fixed minute ventilation, such as those sedated and paralyzed on mechanical ventilation (Rodger et al., 2000). Brain natriuretic peptide (BNP) is of limited value in diagnosing PE but is useful in risk stratifying those with submassive PE (Sohne et al., 2006). Similarly cardiac markers such as troponin and CK-MB are not sensitive or specific but may also help to risk stratify and define prognosis (Pruszczyk et al., 2003). D-dimer, a fibrin degradation product produced by fibrinolysis, has low specificity but high sensitivity. It is useful in excluding embolism in patients with low clinical probability of disease and is used in some predictions models mentioned in the following.

Clinical Prediction Models

Clinical prediction models have been created and studied to determine who needs more diagnostic testing and in who PE can be ruled out. The two most validated models are the Wells Score and pulmonary embolism rule out criteria (PERC) score. The Wells Score is most validated and has most clinical use. There are seven criteria and each is assigned points. Based on the score, patients are stratified into low, intermediate, and high pretest probability. This risk stratification is then used to determine which diagnostic test is most appropriate (**Table 2**). In patients with low pretest probability of PE, it is appropriate to order a d-dimer test for screening. If this test is negative, it excludes the diagnosis of PE in almost all cases (Van Belle et al., 2006; Lucassen et al., 2011). Rarely, a false negative can occur in older patients with smaller clot burden. Unfortunately, d-dimer often produces false positive results, especially in patient with acute illness, recent surgery, pregnancy, or decreased renal function (Crowther et al., 2005; Karami-Djurabi et al., 2009; Kline et al., 2008). Those who are at intermediate or higher risk need more advanced diagnostic testing such as CTPE or V/Q scan.

An alternative prediction model for patients with a low pretest probability of PE who present to an emergency department is also used. The PERC, is used when clinicians have a low suspicion of PE (<15%) and who meet specific criteria. If all criteria are met, no d-dimer is needed and clinicians can effectively rule of PE (**Table 3**) (Kline et al., 2008).

Table 2 Wells score to predict pretest probability of pulmonary embolism

Clinical signs and symptoms of DVT	3
PE is the leading diagnosis	3
Heart rate greater than 100	1.5
History of VTE	1.5
Hemoptysis	1
Malignancy	1
Score	*Probability*
>6	High
$2 \leq \text{score} \leq 6$	Moderate
<2	Low

Table 3 Pulmonary embolism rule out criteria (PERC)

Age <50 years old
Heart rate <100
Oxygen saturation $\geq 95\%$
No hemoptysis
No estrogen use
No history of VTE
No surgery or trauma requiring hospitalizations in the previous 4 weeks
No unilateral leg swelling

All must be negative in order to forgo further testing.

Treatment

Anticoagulation

Anticoagulation is the cornerstone of treatment for PE. Heparin was first discovered in 1918 but it was not until 1930s that it was used for venous thrombus (Howell and Holt, 1918; Murray GaB, 1938). Unfractionated heparin and low molecular weight heparin (LMWH) have traditionally been the most common initial forms of anticoagulation in patients with PE (Kearon et al., 2008; Wood, 2009). Unfractionated heparin is preferred in patients with anticipated need for discontinuation or reversal, hemodynamic instability, renal failure, those with extensive clot burden who are at risk for deterioration, need an invasive procedure, and those at high bleeding risk. In patients who are pregnant, have cancer and those who will transition to warfarin, LMWH is generally preferred. Compared to unfractionated heparin, data suggest that LMWH has slightly less thrombotic complications, improved clot resolution, reduced rates of major hemorrhage, improved mortality, and lower rates of heparin induced thrombocytopenia (Erkens and Prins, 2010; Weitz, 1997). Long-term anticoagulation in most patients can be accomplished with vitamin K antagonists or newer, direct factor Xa and thrombin inhibitors (direct oral anticoagulants, or DOACs). Due to increasing evidence demonstrating safety, efficacy, and ease of doing, DOACs are now recommended over warfarin therapy in the absence of renal failure (Kearon et al., 2016). Pregnant patients as well as those with cancer should still be managed with LMWH. If renal failure is present, subcutaneous unfractionated heparin is also an option prior to starting warfarin. In patients with a provoked PE, most clinicians recommend at least 3–6 months of treatment with anticoagulation. In those with unprovoked PE or unresolved risk factors as well as a low bleeding risk, many suggest lifelong anticoagulation due to high rates of recurrence after stopping anticoagulation (Kearon et al., 2016; Prandoni et al., 2007).

Systemic Thrombolysis

The administration of thrombolytic therapy in a patient with PE was first described in 1964 in a case series of four patients. All patients survived and were noted to have improvements in hemodynamic parameters. Since this initial report, multiple trials and reports have evaluated the efficacy and safety of thrombolytic therapy in acute PE. Trials in massive PE have been limited by small size, severity of illness and the ethical issue of withholding a potentially lifesaving therapy. However, all trials demonstrated a trend toward increased survival (Jerjes-Sanchez et al., 1995; Wan et al., 2004). Trials in submassive embolism generally show faster improvements in hemodynamics, less escalation of therapy, but no mortality difference with use of thrombolytic therapy. These benefits come at the expense of increased major and fatal bleeding events (Marti et al., 2014). Absolute contraindications to thrombolytic therapy include a history of intracranial neoplasm, recent (<2 months) intracranial or spinal surgery or trauma, a history of hemorrhagic stroke, active bleeding, and recent (<3 months) nonhemorrhagic stroke. Relative contraindications include severely uncontrolled hypertension, any history of ischemic stroke, CPR or surgery within the prior three weeks, recent invasive procedure, pregnancy, noncompressible vascular punctures, pericarditis, current anticoagulant use, age $>$ 75 years.

Hemodynamically Unstable Patients

Less than 10% of patients presenting with PE have signs of hemodynamic instability (Stein et al., 2007). Massive PE is not identified by clot burden, but is defined as hemodynamic instability due to a PE and is a medical emergency. A more detailed definition of massive PE is a systolic blood pressure less than 90 mmHg for greater than 15 min or a reduction of systolic pressure by more than 40 mgHg from baseline. Clinically, patients are in cardiogenic shock. Initial support should focus on restoration of blood pressure with judicious intravenous fluids and vasopressor support to prevent worsening RV ischemia and function as well as supplemental oxygen. Empiric anticoagulation with intravenous unfractionated heparin should be started immediately without waiting for diagnostic confirmation. Ideally, definitive testing with CTPE or V/Q should be performed. However, if a patient is too unstable for transport, bedside echocardiography can help confirm clinical suspicion or provide an alternative diagnosis, as mentioned previously. For patients with persistent cardiogenic shock either a high clinical suspicions or confirmed diagnosis, systemic thrombolytic therapy is indicated in the absence of contraindications (Kearon et al., 2016).

Hemodynamically Stable Patients

In general, patients without hemodynamic compromise should not be administered thrombolytic therapy. However, in some situations certain patients may benefit from thrombolytics. Those include younger patients with radiographically extensive PE, signs of decompensation, cardiopulmonary arrest due to PE, and free floating clot (Kearon et al., 2016) (**Fig. 1**).

Catheter Directed Therapy

Catheter directed therapy has emerged as an adjunctive treatment for PE. Catheter directed thrombolysis typically uses much lower doses of TPA (~10–25 mg), so the bleeding risk may be decreased. Catheter directed thrombolysis is suggested for patients with hemodynamic instability after systemic administration of thrombolytic therapy, those with high risk of bleeding and other contraindications to systemic thrombolysis (Kearon et al., 2016). In patients with submassive PE with significant RV dysfunction and radiographically extensive embolism, catheter directed therapy is preferable to systemic therapy. Catheter directed thrombolysis can be combined with other mechanical therapies such as ultrasound and thrombectomy to further decrease clot burden. Interestingly, the half-life of alteplase is 4 minutes. A normal cardiac output is 5–6 L per minute. Therefore, the effect of recirculating alteplase is approximately five times greater than the first pass effect from directed catheter administration.

Embolectomy

Traditionally, surgical embolectomy has been used in patients in whom thrombolytic therapy has failed or is contraindicated. Other indications may include atrial and ventricular thrombus (Aklog et al., 2002; Keeling et al., 2015). Catheter directed embolectomy has emerged as an alternative to surgical embolectomy. The procedure consists of mechanical fragmentation and aspiration. Percutaneous embolectomy combined with intracatheter thrombolysis appears to be a relatively safe and effective treatment (Kuo et al., 2009). However, large randomized trials demonstrating efficacy are lacking.

Follow Up and Chronic Thromboembolic Pulmonary Hypertension

Most patients fully recover after an acute PE. The thrombus dissolves by native fibrinolysis, typically assisted by the use of anticoagulation management. However, approximately 1%–5% of patients go on to remodel the thrombus into vascular scar, resulting in chronic thromboembolic pulmonary hypertension (CTEPH). CTEPH is defined by mean pulmonary arterial pressure



Fig. 1 Radiographically extensive submassive saddle pulmonary embolism. CT pulmonary angiogram demonstrating large saddle thrombus at the bifurcation of the main pulmonary arteries as well as thrombus in the left upper lobe segmental arteries.

≥25 mmHg and pulmonary capillary wedge pressure ≤15 mmHg in the presence of multiple chronic, organized occlusive thrombi after at least three months of effective anticoagulation (Fedullo et al., 2011; Guérin et al., 2014; Pengo et al., 2004). Risk factors include radiographically extensive thrombus, prior splenectomy, presence of a lupus anticoagulant, and elevated systolic pulmonary artery pressure greater than 50 mmHg at time of diagnosis (Fedullo et al., 2011; Lang and Madani, 2014).

Routine screening for CTEPH is not recommended in all patients who have had a PE. However, high risk populations may benefit from screening with a follow up 6 months after initial diagnosis echocardiogram and ventilation perfusion scan. Diagnosis is confirmed with right heart catheterization to measure pulmonary artery pressures, pulmonary vascular resistance, and cardiac output. The definitive treatment of CTEPH is surgical pulmonary thromboendarterectomy (Fedullo et al., 2011). However, for patients who are not surgical candidates, medical therapy and pulmonary artery balloon angioplasty may be viable treatment options (Fukui et al., 2014; Ghofrani et al., 2013; Lang and Madani, 2014; Mizoguchi et al., 2012) (**Figs. 2** and **3**).



Fig. 2 Pulmonary thromboendarterectomy surgery. Surgical specimen of a patient who underwent pulmonary thromboendarterectomy for chronic thromboembolic pulmonary hypertension.

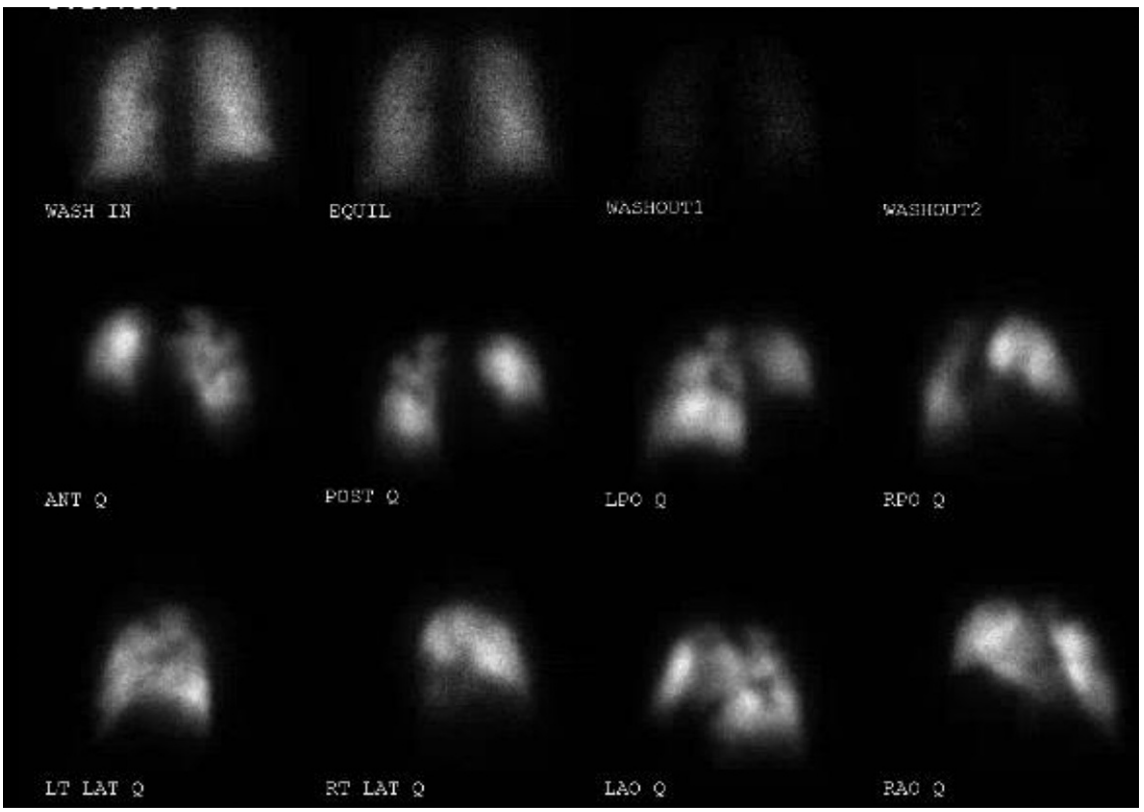

Fig. 3 Abnormal ventilation perfusion scan. The ventilation imaging in the top row demonstrates normal, homogenous uptake, and washout of xenon gas. The perfusion imaging should mirror the ventilation. In this patient, however, there are large unmatched defects in the right middle and lower lobe as well as smaller segmental defects throughout the left lung.

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Pulmonary Hypertension

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A complex disease of the pulmonary vascular system, pulmonary hypertension (PH) is found to exist as both an isolated disease of small pulmonary arteries and as a complication of numerous chronic diseases. The first description of common morphologic findings of PH is attributed to the German physician Ernst Von Romberg in the late 19th century (Romberg, 1891). The "pulmonary vascular sclerosis" described remains an accurate representation of plexiform lesions and muscularization of small pulmonary arteries and arterioles that identifies the histopathology of pulmonary arterial hypertension today. A disease characterized on autopsy, the accurate identification of PH in living patients would have to wait until the 1940s when Drs. Cournand, Ranges, and Richards developed the techniques for right heart catheterization to directly measure pressures within the pulmonary vascular system that remain in use today (Cournand et al., 1944; Bloomfield et al., 1946). Proper identification of PH led to the development of the first national registry of patients with primary pulmonary hypertension, now known as idiopathic pulmonary arterial hypertension (IPAH). Numerous registries now exist throughout the world identifying patients with various forms of pulmonary hypertension. Registry data has led to a better understanding of the pathophysiology and natural history of the disease, leading to advances in basic science, translational, and clinical research which has been paramount to discovery of therapies. The approval of intravenous epoprostenol as the first treatment of IPAH in 1995 came on the heels of a groundbreaking clinical trial showing survival benefit with the use of this agent. This heralded the development of four classes of drug therapy used for PAH today. The future of pulmonary hypertension focuses on continual growth of our knowledge in this disease state, identifying best practices and novel therapeutics to combat both PAH and other forms of pulmonary hypertension.

Definition and Clinical Classification of Pulmonary Hypertension

Elevations in pulmonary artery pressure result from defects at any site of the pulmonary vascular system including the pulmonary artery and arterioles, capillaries, or pulmonary veins and venules. The system is also intricately coupled with the heart, lung parenchyma, and airways such that any number of disease processes affecting the cardiovascular or pulmonary systems can invariably lead to changes in pulmonary hemodynamic measurements. The diversity of pulmonary hypertension has thus necessitated the use of a clinical classification of pulmonary hypertension (**Table 1**). This classification has evolved and currently separates pulmonary hypertension into five groups based on similarities in both pathophysiologic mechanisms and response to therapy: (i) pulmonary arterial hypertension, (ii) pulmonary hypertension owing to left heart disease, (iii) pulmonary hypertension owing to lung diseases and/or hypoxia, (iv) chronic thromboembolic pulmonary hypertension, and (v) pulmonary hypertension with unclear multifactorial mechanisms (Simonneau et al., 2013).

While the hemodynamic definition of pulmonary hypertension requires a mean pulmonary artery pressure (PAP) greater than 25 mmHg, a more robust description of elevated pulmonary artery pressures also distinguish the region of the pulmonary vasculature accounting for this elevation, pre- versus postcapillary PH, and the pulmonary vascular resistance (PVR) which takes into account the cardiac output within the system (**Table 2**). Precapillary PH specifies that the elevated mean PAP is independent of pressures transmitted from the pulmonary venous system and left heart with pulmonary artery wedge pressure (PAWP) and/or left ventricular end-diastolic pressure (LVEDP) being within normal ranges. Postcapillary PH or pulmonary venous hypertension (PVH) describes PH with elevated PAWP or LVEDP, indicating that elevated pressure is being transmitted back to the pulmonary artery

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Table 1 Clinical classification of pulmonary hypertension

1. Pulmonary arterial hypertension (PAH)
   - 1.1 Idiopathic
   - 1.2 Heritable
     - 1.2.1 BMPR2
     - 1.2.2 ALK1, endoglin, SMAD9, CAV1, KCNK3
     - 1.2.3 Unknown
   - 1.3 Drugs and toxins induced
   - 1.4 Associated pulmonary arterial hypertension
     - 1.4.1 Connective tissue diseases
     - 1.4.2 HIV infection
     - 1.4.3 Portal hypertension
     - 1.4.4 Congenital heart disease
     - 1.4.5 Schistosomiasis
     - 1.4.6 Chronic hemolytic anemia

1′ Pulmonary venoocclusive disease and/or pulmonary capillary hemangiomatosis

1″ Persistent Pulmonary Hypertension of the Newborn

2. Pulmonary hypertension owing to left heart disease
   - 2.1 Systolic dysfunction
   - 2.2 Diastolic dysfunction
   - 2.3 Valvular disease
   - 2.4 Congenital/acquired left heart inflow/outflow obstruction
3. Pulmonary hypertension owing to lung diseases and/or hypoxia
   - 3.1 Chronic obstructive pulmonary disease
   - 3.2 Interstitial lung disease
   - 3.3 Mixed restrictive and obstructive pattern
   - 3.4 Sleep-disordered breathing
   - 3.5 Alveolar hypoventilation disorders
   - 3.6 High-altitude pulmonary hypertension
   - 3.7 Developmental lung diseases
4. Chronic thromboembolic pulmonary hypertension
5. Pulmonary hypertension with unclear and/or multifactorial mechanisms
   - 5.1 Hematologic disorders: chronic hemolytic anemia, myeloproliferative disorders, splenectomy
   - 5.2 Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis, lymphangioleiomyomatosis, neurofibromatosis, vasculitis
   - 5.3 Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
   - 5.4 Others: tumor obstruction, fibrosing mediastinitis, chronic renal failure on dialysis, segmental PH

Table 2 Hemodynamic definition of pulmonary hypertension

Definition	*Hemodynamic measurements*	*Clinical classification*
Pulmonary hypertension	Mean PAP $\geq$25 mmHg	All
Precapillary PH	Mean PAP $\geq$25 mmHg PAWP $\leq$15 mmHg	Group 1—Pulmonary arterial hypertension Group 3—PH owing to lung diseases Group 4—chronic thromboembolic PH Group 5—PH with unclear and/or multifactorial mechanisms
Postcapillary PH	Mean PAP $\geq$25 mmHg PAWP $\leq$15 mmHg Cardiac output normal or reduced	Group 2—PH owing to left heart disease
Passive	DPG $\leq$7 mmHg	
Reactive (out of proportion)	DPG $>$7 mmHg	

PH indicates pulmonary hypertension; *PAP*, pulmonary arterial pressure; *PAOP*, pulmonary artery occlusion pressure; *TPG*, transpulmonary gradient.

from the pulmonary venous system and left heart. This is further subdivided into passive and reactive pulmonary venous hypertension indicative of the amount of vascular remodeling that is present. The diastolic pressure gradient (DPG), the difference between the diastolic pulmonary artery pressure and the PAWP, is normally 7 mmHg or less, and any elevation would suggest remodeling of the pulmonary arteries and arterioles. While the treatment of passive and reactive PVH are similar, there may be important clinical significance to this dichotomy (Grigioni et al., 2006; Tatebe et al., 2012). Furthermore, group 1 PAH requires elevated PVR in which increased mean PAP is not induced merely by a high cardiac output.

Pulmonary arterial hypertension consists of precapillary pulmonary hypertension and includes idiopathic pulmonary arterial hypertension, heritable pulmonary arterial hypertension, drug-induced PAH, PAH associated with connective tissue diseases,

HIV-associated PAH, portopulmonary hypertension, congenital heart disease with systemic-to-pulmonary shunt, and shistosomiasis. Idiopathic pulmonary arterial hypertension most clearly represents the entity previously described as primary pulmonary hypertension. It is defined as pulmonary arterial hypertension that occurs sporadically and has no familial inheritance. Hereditary PAH includes all cases with known germline mutations as well as familial cases without an identified mutation. Germline mutation in the bone morphogenetic protein receptor type 2 (BMPR2) gene has been identified in up to 70% of cases of familial pulmonary hypertension with other identified mutations being much less common (Soubrier et al., 2013).

Pulmonary hypertension owing to left heart disease represents PVH in which left heart disease causes increased left atrial pressure which is transmitted to the pulmonary circulation resulting in pulmonary hypertension. This classification is divided between left heart disease resulting from diastolic or systolic dysfunction and left heart disease caused by mitral or aortic valve dysfunction. This group is thought to be the most common form of PH due to the prevalence of heart disease though therapeutic strategies to date have mostly focused on medical or surgical treatment of the underlying heart dysfunction rather than strategies targeting the pulmonary vasculature (Vachiery et al., 2013). Pulmonary hypertension owing to lung diseases and/or hypoxia includes both obstructive and restrictive lung disease with and without parenchymal lung findings as well as high-altitude-induced pulmonary hypertension and intermittent hypoxia causing PH as a result of sleep-disordered breathing. A majority of these patients exhibit mild to moderate pulmonary hypertension with PAP $\leq$40 mmHg. In the largest subgroup, patients with COPD, studies suggest that only 1% of patients have PAP $\geq$40 mmHg (Chaouat et al., 2005). Thromboembolic disease resulting in PH is due to chronic occlusion of the pulmonary vasculature. Estimates suggest that the rate of CTEPH after acute pulmonary embolism is around 3% and disease can develop in both the proximal and distal pulmonary arteries (Pengo et al., 2004). Pulmonary hypertension with unclear multifactorial mechanisms is a group of miscellaneous disorders that do not clearly fit into the above categories or have a multifactorial etiology that has not been defined earlier. This group is split into subgroups based on hematologic disorders, systemic disorders, metabolic disorders, and miscellaneous conditions. This classification system aims to distinguish patients based on common characteristics, but often patients have multiple comorbid conditions which could contribute to PH. Clinical judgment plays an important role when determining the likely etiology and classification of PH.

Clinical Manifestation and Diagnosis

Symptoms and Signs

Pulmonary arterial hypertension is associated with numerous, non-specific symptoms including dyspnea, weakness, fatigue, chest pain, and syncope (**Fig. 1**) (Jing et al., 2009). Symptoms are often more pronounced with exercise or exertion, but as severity advances, the level of symptoms at rest intensifies. It can be difficult to distinguish symptoms directly related to pulmonary hypertension versus those of common comorbid conditions found in these patients. The lack of specific symptoms, along with delays at both the patient and practioner level, may lead to delay in diagnosis (Strange et al., 2013).

The signs of pulmonary arterial hypertension can be broken down into two groups, those that represent increased pulmonary vascular resistance versus signs of right heart dysfunction and failure. In advanced stages of disease both types of clinical signs are often present. In the absence of coexisting heart or lung disease, the lung examination is most often noted to be normal. Signs of increased pulmonary vascular resistance include a parasternal lift, accentuated pulmonary component of the second heart sound, a

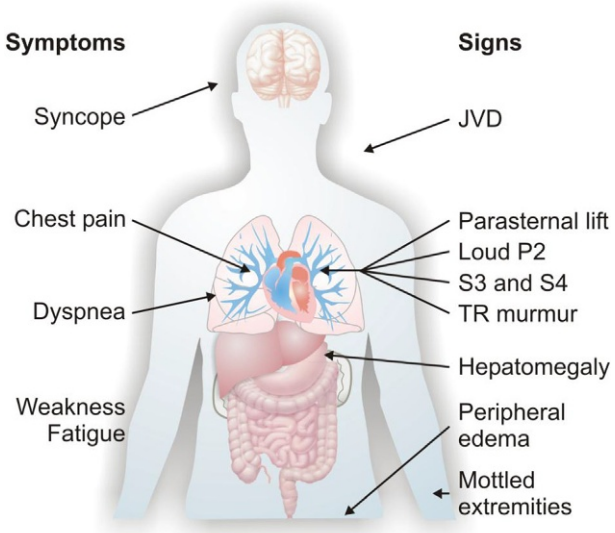

Fig. 1 Symptoms and signs of pulmonary hypertension. JVD, jugular venous distension; P2, second pulmonic heart sounds; S3, third heart sound; S4, fourth heart sound.

soft, pansystolic murmur of tricuspid regurgitation, audible S4, and a diastolic murmur of pulmonary insufficiency. Signs of right heart dysfunction and failure include a third heart sound arising from the right ventricle, best heard at the lower left sternal border and increases with inspiration. Jugular venous distension, peripheral edema, hepatomegaly, hepatojugular reflux and cool, mottled extremities in the setting of severe pulmonary arterial hypertension also indicate right heart failure.

The physical exam is a useful tool in helping to define the etiology of PAH. Signs of scleroderma including sclerodactyly, telangiectasias, Raynaud's phenomenon, and digital ulcers may be present. Connective tissue disease associated with PAH can present with a skin rash and oligoarthritis with synovitis. Advanced liver disease is associated with angiomata, caput madusea, palmar erythema, and testicular atrophy. Fine inspiratory crackles may distinguish a diagnosis of interstitial lung disease.

Noninvasive Testing

There are numerous noninvasive tests that can raise clinical suspicion for PH as well as potentially identify the underlying pathology leading to PH (**Fig. 2**). Electrocardiogram is shown to identify signs of RV hypertrophy in up to 87% of IPAH patients and right axis deviation in 79% though absence of these do not preclude PH (**Fig. 3**) (Rich et al., 1987). Pulmonary function testing is often performed during the routine evaluation of symptoms commonly seen in PH. The most common finding is an impaired lung diffusion capacity for carbon monoxide. Pulmonary function testing can also be used to identify patients with pulmonary hypertension associated with respiratory disorders including airways disease or diffuse parenchymal lung disease. The chest radiograph is also useful to identifying respiratory disorders and can also have findings suggestive of PH (**Fig. 4**). Findings consistent with pulmonary hypertension include central pulmonary arterial dilation; right atrial and ventricular dilation can also be seen. Parenchymal lung disease is often absent or minimal in PAH compared to pulmonary hypertension associated with respiratory diseases. Pulmonary edema and cardiomegaly can be seen with pulmonary hypertension associated with left heart disease. Computed tomography images can identify lung parenchymal abnormalities and thromboembolic disease, both of which can put patients at risk of PH (**Fig. 5**). The ratio of pulmonary artery diameter to aortic diameter has been shown to correlate with directly measured PAP and is predictive of PH when the ratio exceeds 1 (Ng et al., 1999).

A simple, reproducible technique, six-minute walk testing (6MWT) incorporates symptoms, distance, and oxygen saturation in order to define exercise capacity. Two studies have shown worse prognosis in PAH patients when walking distances are limited, $<$332 m or $<$250 m respectively, and desaturation greater than 10% during exercise also indicates worse prognosis (Miyamoto et al., 2000; Paciocco et al., 2001; Sitbon et al., 2002). Test performance is influenced by several factors including height, weight, age, gender, and motivation, which must be considered when interpreting results, yet this remains a vital tool in defining functional capacity to determine prognosis and response to therapy (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002). Cardiopulmonary exercise testing has been suggested as a more accurate method to assess exercise capacity. Ventilation and gas exchange are continuously measured during graded exercise. Oxygen uptake at the anaerobic threshold and peak exercise is reduced as disease severity increases in PAH. In IPAH patients, peak O_2 uptake and peak systolic arterial pressure during exercise is an independent predictor of poor prognosis (Oudiz et al., 2006; Wensel et al., 2002).

Serum markers of disease prognosis and/or progression are constantly expanding, yet in clinical practice the markers used most frequently are brain natriuretic protein (BNP) and troponin. BNP levels have been shown to predict the severity of RV dysfunction in PAH, a major determinant of prognosis and mortality (Nagaya et al., 2000; Andreassen et al., 2006; Fijalkowska et al., 2006; Williams et al., 2006). Troponin, a marker of myocardial damage, is shown to be an independent predictor of 2-year mortality in PAH and possibly CTEPH patients (Torbicki et al., 2003). This likely reflects RV failure and ischemia due to increased chamber pressure overcoming coronary perfusion pressure in the RV. Serum lab testing for connective tissue disease serology, HIV disease, liver and renal function, chronic hemolysis, and genetic mutations are commonly used during initial clinical evaluation to correctly classify the etiology of PH.

Echocardiography has been used for both structural and functional evaluation of the heart for decades (**Fig. 6**). Right heart dynamics and function are distinct from the left side, with its function mainly as a capacitance system with low systolic pressures. Increases in pressure in the pulmonary circulation lead to significant changes in the dynamics of the right heart and blood flow. First described in 1984, echocardiogram-derived estimates of right ventricular systolic pressure using Bernoulli's equation ($\bigtriangledown$Pressure = 4 [Velocity], Cournand et al., 1944) have changed the screening and diagnosis of PAH. Using the mean right atrial pressure and tricuspid regurgitant velocity an estimate of right ventricular systolic pressure can be made (Yock and Popp, 1984). Improvements in estimation of right atrial pressure with IVC size and collapsibility along with better measurements of tricuspid regurtitant velocity by improved Doppler resolution and contrast-enhanced echocardiography have made estimates of RVSP a common screening tool for pulmonary hypertension. There still remains some controversy as both over and underestimation with a high degree of error has been reported. In a recent study, examining directly measured pulmonary artery pressures as compared to echocardiographic estimates, less than half of the echocardiographic estimates were within 10 mmHg of the direct measurements (Fisher et al., 2009). Underestimates were attributed to poor quality of the Doppler measurements, and overestimates were most closely correlated with overestimates of RAP by echocardiogram. Using stringent criteria, Kim et al. established that estimated RVSP $\geq$ 50 mmHg correlated with mean PAP $\geq$ 35 mmHg with a sensitivity of 97% (Kim et al., 2000). This was an accurate way of ruling out moderate to severe pulmonary hypertension, but early detection of mild disease would be missed with this criteria. The most clear guidelines regarding screening come from Barst et al., who recommend using echocardiography as a screening tool in patients with a known familial gene mutation or family member with IPAH, systemic sclerosis, congenital heart disease with systemic-to-pulmonary shunt, or portal hypertension during evaluation for liver transplant (Barst et al., 2004). PASP estimates from 36–50 indicate mild or possible PAH,

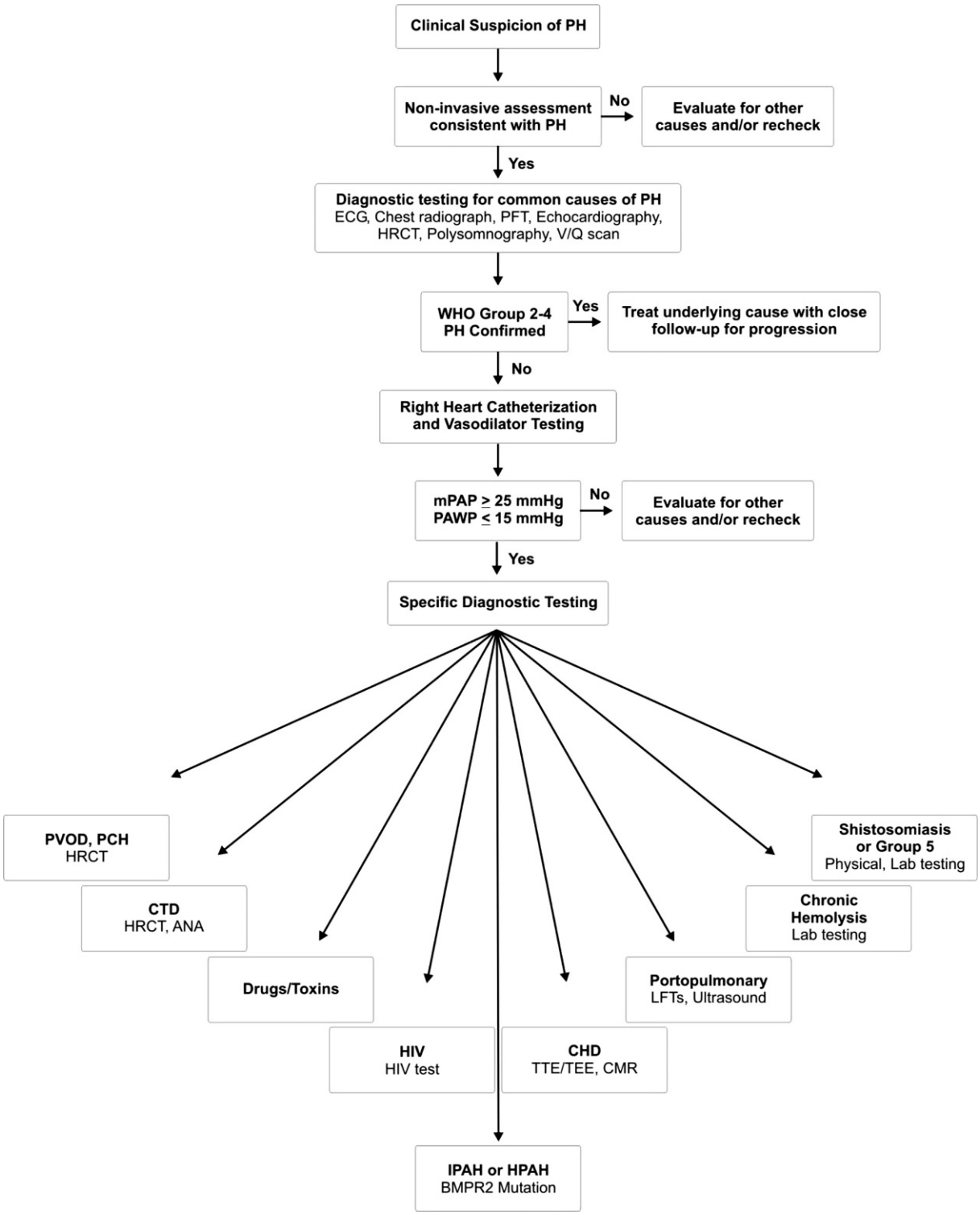

Fig. 2 Diagnostic algorithm for pulmonary hypertension. *PH*, pulmonary hypertension; *ECG*, electrocardiogram; *PFTs*, pulmonary function testing; *HRCT*, high-resolution computed tomography; *V/Q*, ventilation/perfusion; *WHO*, World Health Organization; *mPAP*, mean pulmonary arterial pressure; *PAOP*, pulmonary artery occlusion pressure; *PVOD*, pulmonary veno-occlusive disease; *PCH*, pulmonary capillary hemangiomatosis; *CTD*, connective tissue disease; *HIV*, human immunodeficiency virus; *CHD*, congenital heart disease; *TTE*, transthoracic echocardiography; *TEE*, transesophageal echocardiography; *CMR*, cardiac magnetic resonance imaging; *LFTs*, liver function testing. Adapted with permission from Fraidenburg, D. R., Yuan, J. X. J. and Gaine S.P. (2015). In: Lanzer, P. (ed.) *PanVascular medicine*. Springer, Berlin.

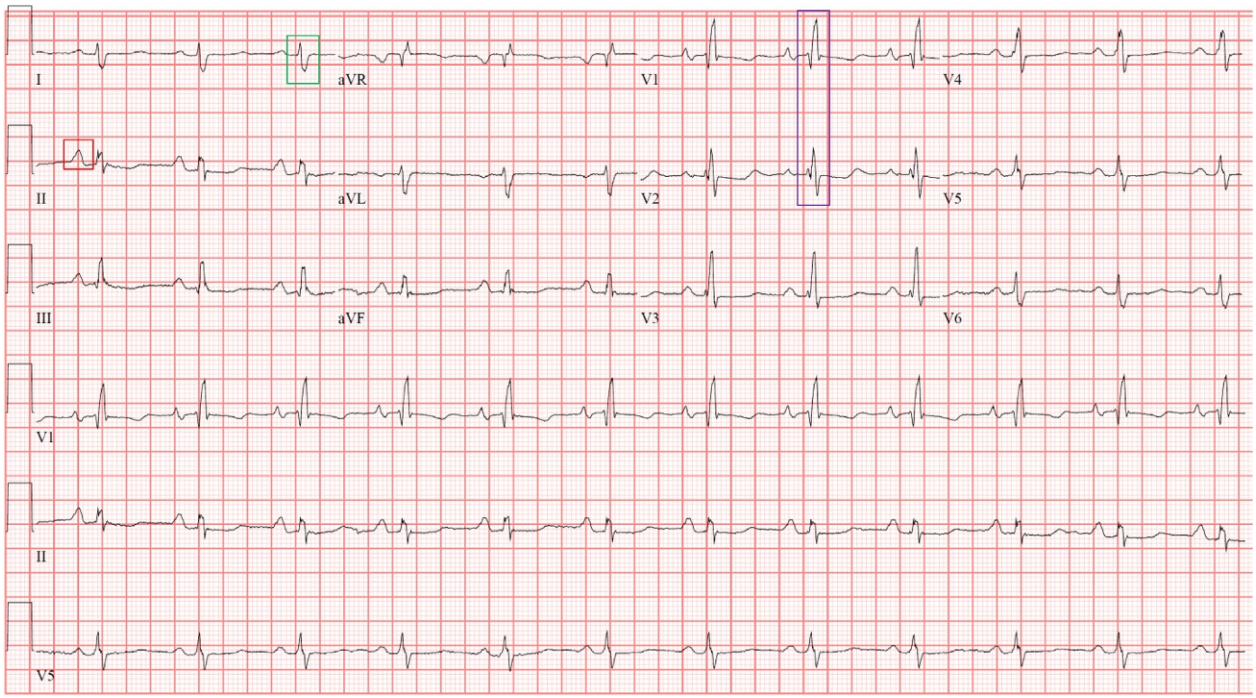

Fig. 3 Electrocardiogram of patient with pulmonary hypertension. Boxes indicate common abnormalities seen in PH including right atrial enlargement (*red box*), right axis deviation (*green box*), and right ventricular hypertrophy (*purple box*).

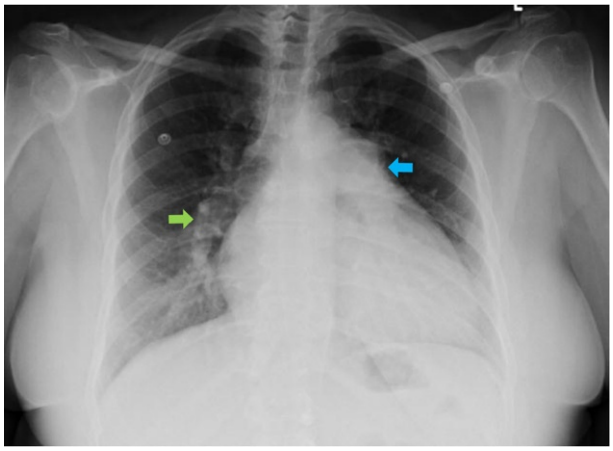

Fig. 4 Chest radiograph of patient with pulmonary hypertension. Frontal chest radiograph from a patient with PAH. *Green arrow* identifies dilated right main pulmonary artery, and *blue arrow* identifies enlarged pulmonary artery trunk and left main pulmonary artery.

with PASP estimates over 50 indicate likely moderate to severe PAH (**Table 2**) (Fisher et al., 2009; Launay et al., 2007). For all patients with suspected PAH, right heart catheterization is still indicated to confirm the diagnosis and perform vasoreactivity testing.

Cardiac magnetic resonance imaging (MRI) is a safe, noninvasive test that can assess multiple parameters in the right heart and pulmonary vasculature (**Fig. 7**). Along with the structural definition seen with MRI techniques, ECG triggering has allowed the measurement of right ventricular mass, volume, and contractility. Velocity encoding with phase contrast MR has also allowed flow measurements within the pulmonary circulation with similar predictive power to that of echocardiography (Sanz et al., 2007; Nogami et al., 2009). Limitations of MRI are that this is an expensive modality with limited availability; the testing is done in the supine position and requires breath holding which can be challenging in pulmonary hypertension patients; and claustrophobia has continued to be a limiting factor in many patients.

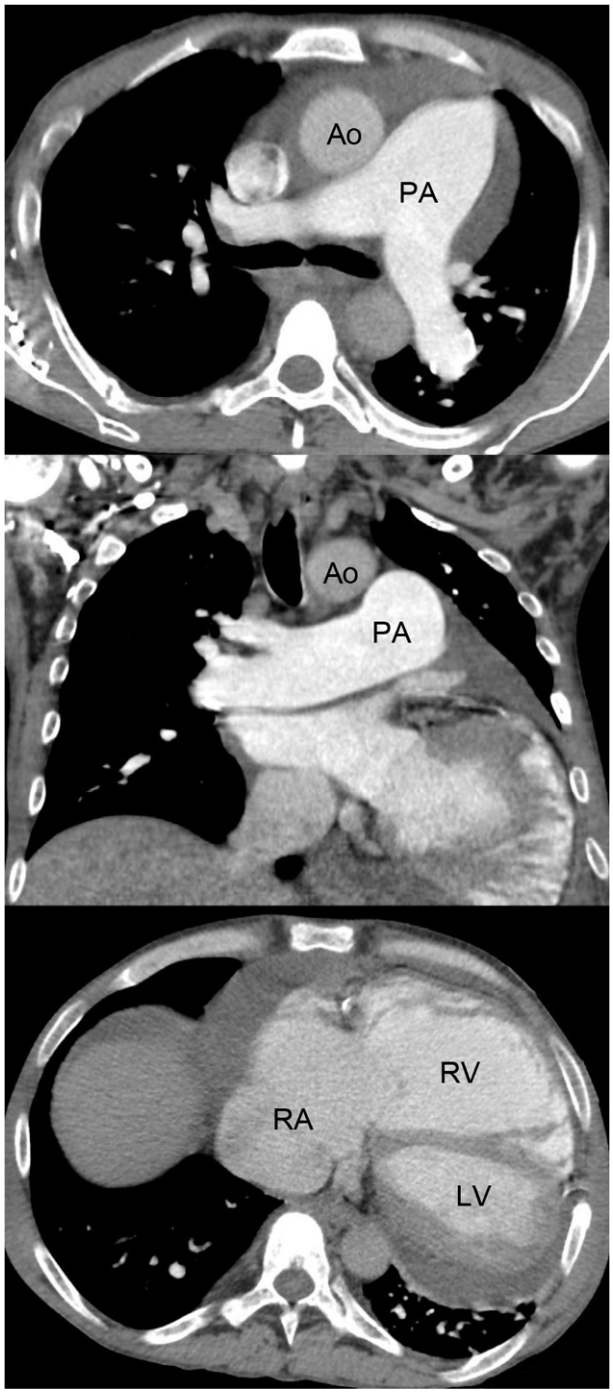

Fig. 5 Computed tomography images of patient with pulmonary hypertension. CT images show enlarged pulmonary artery trunk (*upper and middle panel*) as well as enlarged right atrium and right ventricle (*lower panel*). *Ao*, aorta; *PA*, pulmonary artery; *RA*, right atrium; *RV*, right ventricle; *LV*, left ventricle.

Right Heart Catheterization and Vasoreactivity Testing

Right heart catheterization (RHC) is an essential study to confirm the diagnosis of pulmonary hypertension as well as to direct therapy following vasoreactivity testing. Measurements of diastolic, systolic and mean PAP are recorded, as well as pulmonary artery wedge pressure (PAWP), right atrial and ventricular pressures (Fig. 8). Cardiac output is measured by thermodilution and/or the Fick method. Oxygen saturation must be measured in the superior vena cava, pulmonary artery, and systemic arteries in order to calculate pulmonary vascular resistance (PVR). Elevated PAWP is seen in pulmonary venous hypertension and precludes the diagnosis of precapillary pulmonary hypertension. Measurement of left ventricular end-diastolic pressure may also be useful to

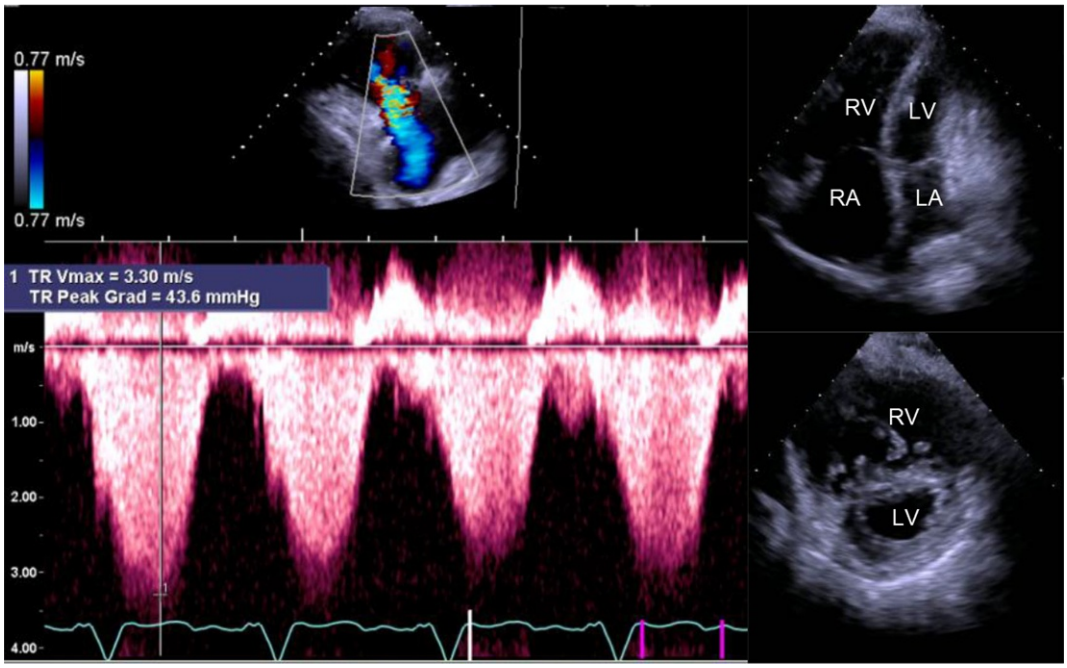

Fig. 6 Echocardiogram of patient with pulmonary hypertension. Echocardiogram with Doppler images showing tricuspid valve regurgitation and elevated TR jet velocity (*left panels*). Right atrium and right ventricular enlargement is seen on a four chamber view (*right-upper panel*) and an enlarged right ventricle with a "D-shaped" bowing of the intraventricular septum is seen on a parasternal short axis view (*right-lower panel*). *TR*, tricuspid regurgitation; *RA*, right atrium; *RV*, right ventricle; *LA*, left atrium; *LV*, left ventricle.

assess left ventricular dysfunction as a source of pulmonary hypertension. RHC is a relatively safe procedure with an estimated morbidity and mortality of 1.1% and 0.055%, respectively and remains the gold standard for diagnosis (Hoeper et al., 2006).

Vasoreactivity testing during RHC is used to identify those patients that may benefit from the use of calcium channel blockers (CCB) (Rich et al., 1992; Sitbon et al., 1998). At the time of diagnosis of PAH, an acute vasodilator challenge should be administered as inhaled nitric oxide (NO), intravenous epoprostenol, or intravenous adenosine (Rich et al., 1992; Galie et al., 1995; McLaughlin et al., 1998; Sitbon et al., 2005). A reduction in mean PAP more $\geq$10 mmHg to an absolute value $\leq$40 mmHg with no reduction in cardiac output represents an acute responder (Sitbon et al., 2005). Acute responders account for about 10% of IPAH patients and represent a population that is more likely to have a long-term response to treatment with high dose CCBs. More importantly, nonresponders have been shown to have increased mortality when treated with high dose CCBs. About 50% of IPAH patients with acute vasodilator response will have persistent, long-term response to CCBs, while the other half will show disease progression despite CCB treatment and will require additional pharmacologic agents. Vasoreactivity testing has been less well studied in other forms of PAH such as those associated with connective tissue disease, HIV, hemolytic anemia, and chronic liver disease, and therefore the rate of acute response to vasodilators and the long-term efficacy of calcium channel blockers remains unknown. There remains no data regarding the use of CCB in other forms of pulmonary hypertension; therefore acute vasoreactivity testing is not recommended.

Pathophysiology

Pathogenic Mechanisms

Pulmonary hypertension develops through pathologic changes at any point along the pulmonary vascular circuit. There are several established mechanisms by which these changes occur, each of which may have unique clinical consequences, with variable progression and reversibility, yet all have been associated with pulmonary vascular remodeling. Obstruction of pulmonary venous outflow, such as left heart disease or mitral stenosis, leads to passive transmission of elevated pressure to the pulmonary artery. Abnormally high blood flow, as seen in congenital heart disease with left-to-right shunt, increases pulmonary artery pressure due to hyperkinetic alterations. Obstruction of the pulmonary artery, seen in pulmonary thromboembolic disease, leads to elevations in pulmonary artery pressure, as does destruction of the pulmonary vascular bed in an obliterative fashion in diseases such as diffuse parenchymal lung disease. Hypoxic vasoconstriction leads to elevated pulmonary vascular resistance in severe lung disease or those residing at high altitudes. Often these mechanisms overlap in complex and end-stage cardiovascular and pulmonary disease. Still, little is known about the inciting mechanism for the persistent pulmonary vascular remodeling encountered in PAH, the idiopathic and hereditary forms in particular.

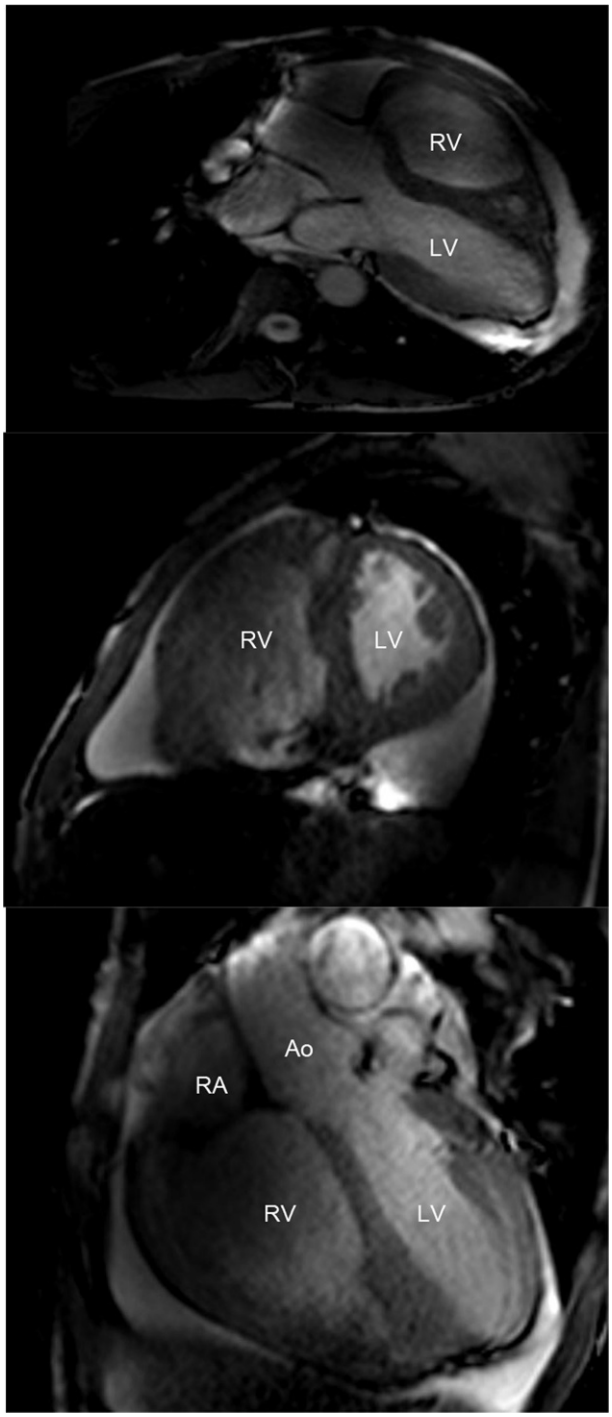

Fig. 7 Cardiac magnetic resonance images of patient with pulmonary hypertension. Cardiac MR images show enlargement of the right ventricle on axial (*top panel*), sagittal (*middle panel*), and coronal (*lower panel*) planes. *RA*, right atrium; *RV*, right ventricle; *LA*, left atrium; *LV*, left ventricle.

Structural and Functional Changes in the Pulmonary Vasculature

Despite this gap in our understanding, much work has been done to describe pathophysiologic mechanisms that lead to increased PVR and thus PAP in PAH. Pulmonary vascular remodeling, sustained pulmonary vasoconstriction, in situ thrombosis, plexiform vasculopathy, and vascular wall stiffening all contribute both individually and collectively in the development and progression of PAH (Fig. 9). Sustained vasoconstriction in small arteries and arterioles powerfully impacts PVR and is the principal functional alteration encountered in PAH. Associated with sustained vasoconstriction are structural changes in which medial hypertrophy is a key feature of changes with the pulmonary vasculature. Pulmonary artery smooth muscle cell hypertrophy and hyperplasia leads to



Fig. 8 Right heart catheter tracings in a patient with pulmonary arterial hypertension. Right heart catheterization with serial pressure measurements in the right atrium (A), right ventricle (B), pulmonary artery (C), and pulmonary artery wedge (D) positions. Elevated mean pulmonary artery pressure is associated with normal pulmonary artery wedge pressure indicating precapillary pulmonary hypertension.

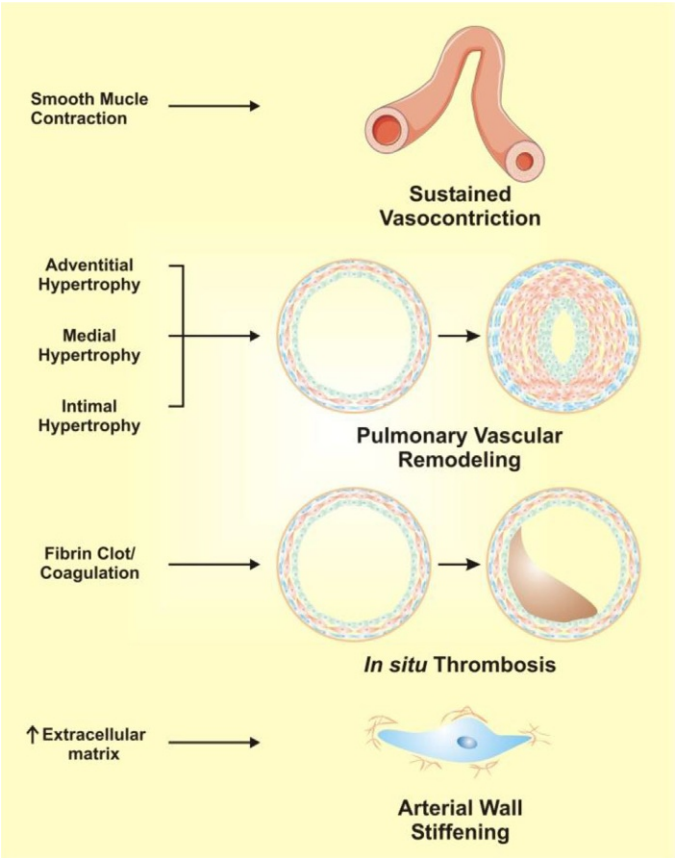

Fig. 9 Major pathogenic components in the development of pulmonary arterial hypertension. Pathogenic factors contributing to the development and progression of PAH include sustained vasoconstriction, pulmonary vascular remodeling, in situ thrombosis, and vascular wall stiffening. *PAP*, pulmonary arterial pressure; *CO*, cardiac output; *PVR*, pulmonary vascular resistance; *r*, radius.

muscularization of small arteries and arterioles which are normally nonmuscularized or only partially muscularized. The elevated PAP and sustained vasoconstriction in PAH can, in turn, enhance PASMC hypertrophy and hyperplasia (Hishikawa et al., 1994; Heath et al., 1987). Pulmonary vascular remodeling also affects the other pulmonary artery cell types, including endothelial cell hyperplasia associated with intimal thickening, extracellular matrix stiffening as fibroblasts differentiate into myofibroblasts (Stenmark et al., 1995). One of the classic structural alterations of small arterioles in PAH is plexiform lesions. These complex lesions result in aneurysmal dilation of arterioles associated with partial or fully occlusive intraluminal collections of various cells and matrix proteins (Mandegar et al., 2004; Pietra, 1997). These functional and structural changes to the pulmonary artery system are explained through numerous cellular and molecular mechanisms in PAH.

Cellular and Molecular Mechanisms of Pulmonary Arterial Hypertension

There are innumerable cellular and molecular mechanisms working in concert to lead to the pathologic changes encountered in PAH. In its simplest design, these entail an imbalance of vasoactive mediators, altered expression, and function of both mitogenic and angiogenic factors, as well as changes to cell survival proteins in each of the various cell types of the pulmonary vasculature. Nitric oxide and prostacyclin, powerful vasodilators in the pulmonary vasculature, are undersupplied when compared to excess of vasoconstrictors such as endothelin-1 and thromboxane A2 (**Fig. 10**). Nitric oxide (NO), released by the vascular endothelial cells (ECs), stimulates the production of cGMP in the PASMC resulting in relaxation and pulmonary vasodilation. A product of the metabolism of arachidonic acid, prostacyclin (PGI_2) in synthesized in the endothelium and secreted, binding to the prostacyclin receptor (IPR) on PASMC which results in increased cyclic adenosine monophosphate (cAMP) to promote vasodilation and exhibit antiproliferative effects (Olschewski et al., 2004). Endothelin (ET-1) plays a central role in the pathogenesis of PAH and exerts its effects as a potent vasoconstrictor which is also shown to increase smooth muscle cell proliferation and decrease apoptosis (Levin, 1995; Yanagisawa et al., 1988). Increased levels of circulating ET-1 have been shown in PAH patients which lead to activation of both ET-A and ET-B receptors (Giaid et al., 1993). ET-A receptors, located on PASMC, are thought to be the primary driver of the vasoconstrictive effect (Seo et al., 1994), while ET-B receptors are primarily located on endothelial cells and have vasodilatory effects (Hirata et al., 1993), though evidence also exists for vasoconstrictive downstream targets of ET-B, including thromboxane A2

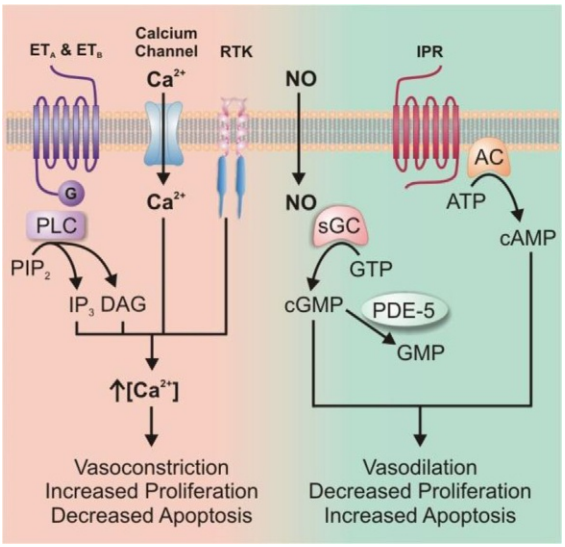

Fig. 10 Cellular and molecular mechanisms of PAH. Pathogenesis of PAH is characterized by an imbalance in vasoactive mediators. Left panel showing major pathways which lead to increased $[Ca^{2+}]_{cyt}$ and resultant vasoconstriction, increased cellular proliferation and decreased apoptosis. The right panel shows pathways by which cGMP and cAMP lead to vasodilation, decreased cellular proliferation, and increased apoptosis. Stimulation of endothelin receptor ET_A, a G-protein coupled receptor, by endothelin-1 leads to activation of phospholipase C and production of IP_3 and DAG, both of which lead to increased $[Ca^{2+}]_{cyt}$. Similarly, distinct receptor tyrosine kinases (RTK), such as PDGF, VEGF, and EGF, can lead to increased IP_3 and DAG and subsequent increases in $[Ca^{2+}]_{cyt}$ through a similar yet distinct pathway. Nitric oxide stimulates soluble guanylate cyclase to produce cGMP which has vasodilatory effects through decreased $[Ca^{2+}]_{cyt}$ as well as anticoagulant and antiproliferative effects that are both dependent on and independent of $[Ca^{2+}]_{cyt}$. cGMP is degraded primarily by phosphodiesterase type 5 in PASMC, which is targeted therapeutically by the PDE-5 inhibitors. The vasodilating properties of cAMP are produced through decreased $[Ca^{2+}]_{cyt}$ as well as anticoagulant and antiproliferative effects that are both dependent on and independent of $[Ca^{2+}]_{cyt}$.

(Dupuis et al., 1985). Other vasoactive mediators such as thromboxane, serotonin, and vasoactive intestinal peptide have various roles in the development and progression of PAH.

Numerous ion channels and membrane receptors have been implicated in the pathogenesis of PAH. Increased cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) is an important common pathway by which ion channel activity and receptor activation exert there effects in the pulmonary vasculature. Rising $[Ca^{2+}]_{cyt}$ in PASMC is a major trigger for PASMC contraction and vasoconstriction as well as an important mediator for PASMC proliferation/migration that results in vascular remodeling. Ca^{2+}-permeable cation channels and K^+-permeable channels (e.g., KCNA5 and KCNK3) in the plasma membrane of PASMC can influence $[Ca^{2+}]_{cyt}$ both directly and indirectly, through membrane depolarization (Mandegar et al., 2004). G protein-coupled receptors (GPCR) and tyrosine kinases receptors (TKR) have been implicated in the development and progression of PAH. These membrane receptors are shown to have various effects on important second messengers such as cGMP, cAMP, diacylglycerol, and inositol triphosphate that lead to pulmonary vascular remodeling. Numerous other cellular and molecular mechanisms have also been identified, including: (i) altered signaling pathways; (ii) changes in expression and function of growth factors; (iii) microparticle release; (iv) epigenetic modifications; and (v) genetic alterations (Mandegar et al., 2004; Kuhr et al., 2012; Morrell et al., 2009).

Genetics and Genomics

While it has long been clear that familial forms of PAH exist, it was the identification of genetic mutations in bone morphogenetic protein receptor II (BMPR2) that really advanced the knowledge on genetic susceptibility in pulmonary hypertension. Found primarily on endothelium and to a lesser extent smooth muscle cells, both reduced expression and functional impairment of BMPR2 are common in the various types of PAH, most notably those with mutations of the *BMP-RII* gene (Atkinson et al., 2002). Germline mutations in *BMP-RII* have been identified in up to 70% of patients with familial PAH, and in 20% of patients with IPAH (Cogan et al., 2006; Deng et al., 2000; Lane et al., 2000; Machado et al., 2006; Newman et al., 2001, 2004; Thomson et al., 2000), as well as disease associated with anorexigens (Humbert et al., 2002), congenital heart disease (Roberts et al., 2004), and pulmonary venoocclusive disease (Runo et al., 2003). Nearly 300 different mutations of BMPR2 have been identified (Machado et al., 2006). Subsequently, mutations of other important constituents of the TGF-β pathway have been discovered, including activin receptor kinase-like 1 (*ALK1*), endoglin (*Eng*), and Smad 8 (*Smad9*) (Shintani et al., 2009). ALK1 is a receptor for TGF-β and endoglin is its coreceptor, while Smad 8 is a downstream second messenger of BMPR2. Mutations in *ALK1* and *Eng* have been shown to confer susceptibility to the development of PAH in patients with hereditary hemorrhagic telangiectasia (Trembath et al., 2001; Chaouat et al., 2004). Patients have also been identified with concomitant Thrombospondin-1 gene mutation (*THBS1*) which is proposed to promote pulmonary hypertension development and increase genetic penetrance (Maloney et al., 2012). Outside of the TGF-β superfamily there have been discovery of rare mutations in *KCNK3* and *CAV1* what are attributed to the development of HPAH

(Austin et al., 2012; Ma et al., 2013). Mutation of *KCNK3* results in a channelopathy that is thought to facilitate calcium-mediated vasoconstriction leading to PAH, while *CAV1* encodes the protein caveolin-1 which is an important membrane protein that has several functions which include cell signaling and membrane trafficking (Chidlow and Sessa, 2010; Parton and Simons, 2007). Further mutations are likely to be revealed as whole exome and whole gene sequencing can identify gene mutations from previously unidentified HPAH patients. Understanding genetic susceptibility in PH and specific gene mutations responsible for much of HPAH, we now have a better understanding of PAH pathogenesis and these discoveries also raise the possibility of gene therapy in the future.

Treatment

The pulmonary vasculature represents a unique environment with mechanisms that are distinctive from the systemic circulation. Therefore, cellular and molecular pathways common throughout the human body have distinct roles in the pulmonary circulation. By understanding and exploiting these pathways in the pulmonary vasculature, therapeutic approaches have been created for the treatment of PAH (**Table 3**).

Supportive Therapies

Anticoagulants, diuretics, and ionotropes represent treatment used for many decades that target specific consequences of pulmonary arterial hypertension including thrombosis and right heart failure, yet have limited effect with regard to any improvement or reversal of the underlying pathophysiology. Diuretics can improve the fluid balance leading to symptomatic relief and quality of life improvements yet there have been no randomized clinical trials with the use of diuretics in PAH. There is also no clear evidence to suggest that long-term oxygen therapy has an effect on PAH; therefore oxygen therapy is not routinely recommended in the absence of severe hypoxemia ($SpO_2 \leq 88\%$ or $PaO_2 \leq 60$ mmHg at rest), which could intensify vasoconstriction. Anticoagulation has been a mainstay of treatment due to an acquired prothrombotic state and is recommended for all PAH patients unless contraindications exist. Warfarin, a vitamin K antagonist, is the most commonly used anticoagulant for PAH with evidence to support its use coming from multiple nonrandomized trials (Frank et al., 1997; Fuster et al., 1984). Digoxin is used as an adjunctive agent and has been shown to improve cardiac output in severe PAH with right ventricular failure (Rich et al., 1998). Dobutamine increases myocardial contractility and reduces vascular resistance in both the pulmonary and systemic circulation. It is often employed as a first line therapy for patients with acute right heart failure, though the necessity of this therapy portends a poor prognosis with survival found to be independently associated with lower requirements of dobutamine (Sztrymf et al., 2010).

Pulmonary Vasodilator Therapies

Vasodilator therapy has been used for nearly three decades to treat PAH. Calcium-channel blockers (CCBs) represent the first, well-accepted vasodilators for the treatment of PAH, with nondihydropyridines being the most common class, particularly Nifedipine and Diltiazem. In order to establish safety and efficacy with this class of medications, pulmonary vasodilatory testing, as described previously, identifies patients that may benefit from this therapy while excluding patients in which CCB could be detrimental. Only

Table 3 Medication summary for pulmonary arterial hypertension

Medication	*Route*
Prostacyclin and prostacyclin analogs	
Epoprostenol	IV
Treprostinil	IV/SC/Oral/inhaled
Iloprost	Inhaled
Selexipag	Oral
Endothelin receptor antagonists	
Bosentan	Oral
Ambrisentan	Oral
Macitentan	Oral
Phosphodiesterase type 5 inhibitors	
Sildenafil	Oral
Tadalafil	Oral
Vardenafil	Oral
Soluble guanylate cyclase stimulator	
Riociguat	Oral
Calcium channel blockers	
Amlodipine	Oral
Nifedipine	Oral
Diltiazem	Oral

a small proportion of IPAH patients, <10%, will have acute vasoreactivity, and of these patients, only about half will have long-term response to calcium channel blockers (Sitbon et al., 2005).

Prostacyclin (PGI_2) plays an important role in vasodilation and has also been shown to inhibit vascular smooth muscle cell proliferation and migration. The first synthetic prostacyclin, epoprostenol, decreases both SVR and PVR with increased cardiac output in a dose-dependent manner in PAH patients (Rubin et al., 1982). The first successful trial of chronic pulmonary vasodilator therapy compared epoprostenol to traditional therapy in PAH patients over a 12-week period and showed a significant mortality benefit (Barst et al., 1996). It remains a mainstay of treatment, particularly in advanced stages of disease as a potent pulmonary vasodilator, though adverse effects of both the drug and the intravenous delivery system may be encountered. Side effects common to this class of medications include jaw pain, headache, and diarrhea.

A prostacyclin analog, treprostinil, has an increased half-life compared to epoprostenol and is available in multiple formulations including intravenous infusion, continuous subcutaneous injection, inhaled, and oral. The risk of bacteremia or other catheter related complications can be reduced by delivering it subcutaneously, and the extended half-life allows increased time for troubleshooting in the cases of pump malfunction. Intravenous treprostinil shows similar efficacy to epoprostenol with a similar side effects between the two groups (McLaughlin et al., 2003). Subcutaneous treprostinil is comparable to both intravenous treprostinil and epoprostenol while minor adverse events are common, with 94% of patients having erythema at subcutaneous pump site and 88% reporting pain at that site (Simonneau et al., 2002). Inhaled formulation of treprostinil has comparable hemodynamic effects to inhaled iloprost, another prostacyclin analog, and also has favorable effects when added to oral therapy with either a PDE5 or ERA (Voswinckel et al., 2006; Channick et al., 2006; McLaughlin et al., 2010; Benza et al., 2011). Oral formulation of treprostinil is efficacious when used alone in PAH patients with moderate functional impairments, yet initial studies did not show a significant benefit when added to existing background therapy (Jing et al., 2013; Tapson et al., 2013). The first prostacyclin analog to be available in an inhaled formulation, iloprost, was designed to target the pulmonary vasculature with minimal systemic side effects. Long-term treatment was able to show improvements in exercise capacity and hemodynamic measurements which were sustained for 1 year (Hoeper et al., 2000). Side effects specific to the inhaled formulation of both iloprost and treprostinil are cough, though this appears to wane over time.

Selexipag is an orally bioactive, highly selective IP receptor agonist that is chemically distinct with unique pharmacological kinetics compared to other prostacyclin analogs. Selexipag is shown to have an acceptable safety profile and to reduce the risk of morbidity and mortality in PAH patients as either initial or add-on therapy (Simonneau et al., 2012; Sitbon et al., 2015). Adverse effects of selexipag are similar to those of prostacyclin analogs.

Endothelin (ET-1) is believed to play a central role in the pathogenesis of PAH as a potent vasoconstrictor which also increases smooth muscle cell proliferation and decrease apoptosis (Levin, 1995; Yanagisawa et al., 1988). Targeting the endothelin receptor has been a powerful tool in the treatment of PAH. Bosentan targets both endothelin receptor A and B as a dual receptor antagonist and is shown to significantly improvement in 6-min walk testing and pulmonary hemodynamic measurements after 12 weeks of use (Channick et al., 2001). Ambrisentan is a selective endothelin A antagonist that is shown to have comparable efficacy to bosentan in PAH patients (Galie et al., 2005a, 2008). Liver toxicity is a feared adverse effect of this class and ambrisentan may have less elevation in liver function testing during treatment. The most recently developed ERA, macitentan, is an orally active, dual endothelin receptor antagonist that is highly lipophilic with an active metabolite to optimize pharmacokinetics. This agent is shown to have similar efficacy for PAH patients as a single agent or in addition to another class of oral or inhaled therapy (Pulido et al., 2013). Minor adverse effects of the class include headache, anemia, and nasopharyngitis. Liver function testing must be followed during active therapy, and these agents are teratogenic, requiring appropriate birth control when used in women of child-bearing age.

The cyclic guanosine monophosphate (cGMP) pathway is an important target of endothelium-derived nitric oxide (NO), an essential vasodilatory agent (Sausbier et al., 2000). Phosphodiesterase type 5 (PDE5) rapidly hydrolyzes cGMP, inactivating its vasodilatory effects. Sildenafil is a specific phosphodiesterase type 5 inhibitor and the first of the class used for PAH, showing significant improvements in walk distance and pulmonary hemodynamics at both short- and long-term follow-up (Galie et al., 2005b; Rubin et al., 2011). Tadalafil is another agent in the class with a longer half-life than sildenafil and shows similar efficacy in the treatment of PAH patients (Galie et al., 2009; Oudiz et al., 2012). Vardenafil has similar effects, though this agent has not been studied as robustly (Jing et al., 2011). Adverse effects of the class are minor; with headache, edema and esophageal reflux being the most common. Soluable guanylate cyclase converts guanosine triphosphate (GTP) to cGMP, leading to vasorelaxation. Riociguat stimulates soluble guanylate cyclase to increase cGMP and promote vasodilation. This agent is used in both PAH and CTEPH patients, showing significant improvement in symptoms, exercise capacity, and pulmonary hemodynamic measurements. Adverse events include headache, dizziness, and gastrointestinal complaints as well as hypotension.

Surgical Treatment

Surgical management for PAH includes atrial septostomy, pulmonary thromboendarterectomy (PTE), and lung transplantation. Puncture of the atrial septum creates a physiological shunt through the atrial septal defect giving reprieve to the high pressures in the right heart. Atrial septostomy has been shown to improve cardiac output without significant change in mean PAP and at the expense of worsening hypoxemia due to the right-to-left shunt (Law et al., 2007). The procedure is associated with high mortality, particularly in patients with higher presurgical right atrial pressures. PTE is the preferred management of CTEPH and is a surgical procedure to remove thromboemboli from the obstructed pulmonary arterial circulation (**Fig. 11**) (Madani et al., 2012).

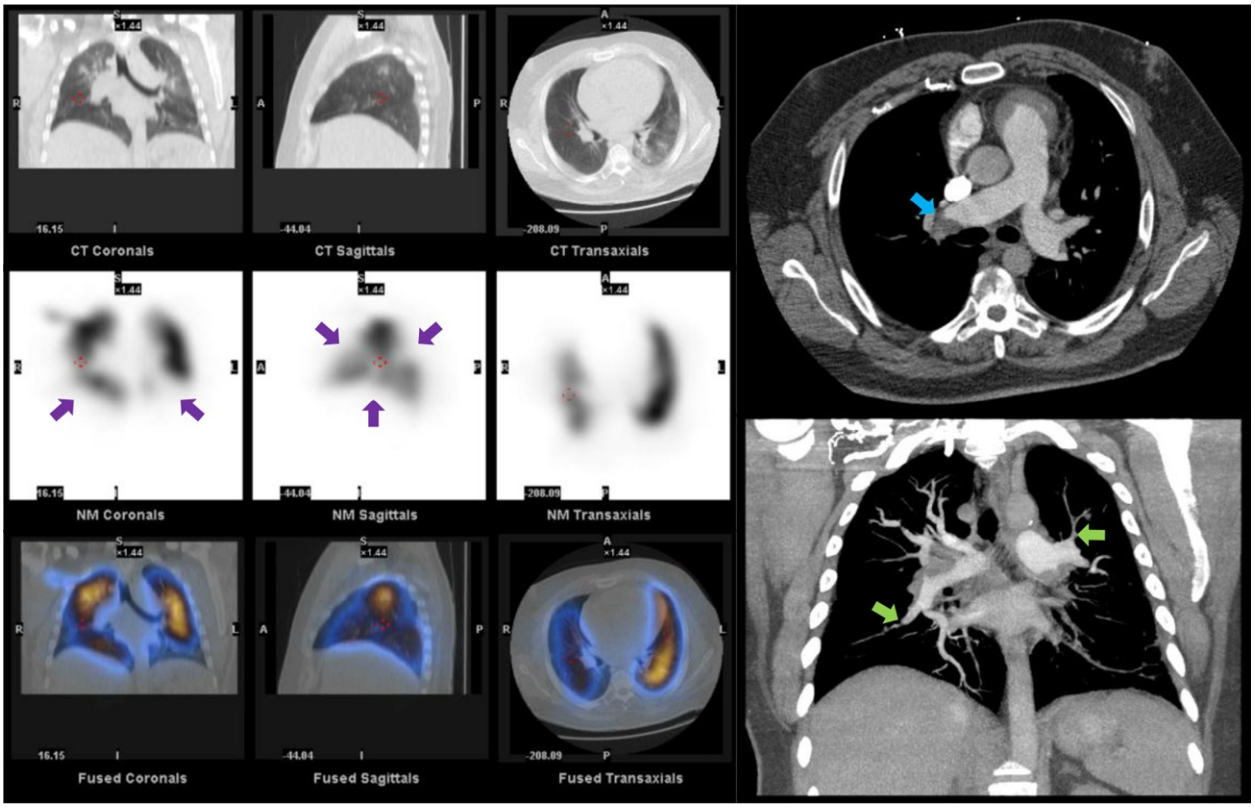

Fig. 11 Imaging findings of a patient with chronic thromboembolic pulmonary hypertension. Perfusion SPECT images (*left panels*) showing unmatched perfusion defects (middle and lower panels). CT angiography (*right panels*) confirms thromboembolic disease through the lung fields, including the main (*blue arrow*) and segmental (*green arrows*) pulmonary arteries.

In operable candidates, this surgery reduces mean PAP and increases cardiac output, though residual pulmonary hypertension can occur in 5%–35% of surgical patients (Condliffe et al., 2008; Corsico et al., 2008; Freed et al., 2011; Thistlethwaite et al., 2006). In these patients, pulmonary vasodilator therapy is used to manage the residual PH. Lung transplant remains the preferred surgical procedure for patients with severe PAH, though significant morbidity and mortality exist, particularly in patients with poor preoperative status (Keogh et al., 2009). The United Network of Organ Sharing showed IPAH lung transplant survival of 83%, 47.5%, and 34.7% at 1, 5, and 10 years, respectively. Surgical management, though associated with high levels of morbidity and mortality, remains essential with insufficient medical therapies available for the most severe patients.

Clinical Management Strategies

Disease severity and prognosis remain a critical step in assessing a patient with PAH. Hemodynamic measurements at the time of RHC have been shown to predict prognosis. Right atrial pressure, CO, and PVR have prognostic relevance and while PAP measurements do show some prognostic correlation, it has repeatedly been shown to be less accurate and declining PAP can result from RV failure in the most severe patients. On echocardiogram, the presence of pericardial effusion, increased right atrium area, decreased tricuspid annular plane systolic excursion (TAPSE), increased LV eccentricity, and RV Doppler index have all been shown to correlate with worse prognosis (Eysmann et al., 1989; Forfia et al., 2006; Raymond et al., 2002; Tei et al., 1996; Yeo et al., 1998). The simplest and most widely used predictor of survival remains WHO functional classification, in which severity of functional limitation increases from class I to class IV. Historical data have shown a median survival of 6 years for WHO functional class I–II drops off precipitously to 2.5 years for WHO class III and 6 months for WHO class IV (D'Alonzo et al., 1991).

Multiple classes of medical therapy with differing potency, adverse effects, and route of delivery require careful consideration and individualization when starting treatment (**Fig. 12**). Patients who fall under WHO functional class II or III, those limited by strenuous or mild to moderate exercise respectively, are shown to benefit from pulmonary vasodilator therapy. Oral formulations, ERAs, PDE5 inhibitors, or sGC stimulators, are usually employed as first line agents due to ease of use, while drug interactions or individual risk factors such as liver disease may favor one agent over another. Patients with symptoms at rest, WHO functional class IV, and those with signs of right heart failure have a much worse prognosis. Treatment in these patients generally involves parenteral therapy with epoprostenol or treprostinil, the most potent pulmonary vasodilators. The goals of treatment include improvement in

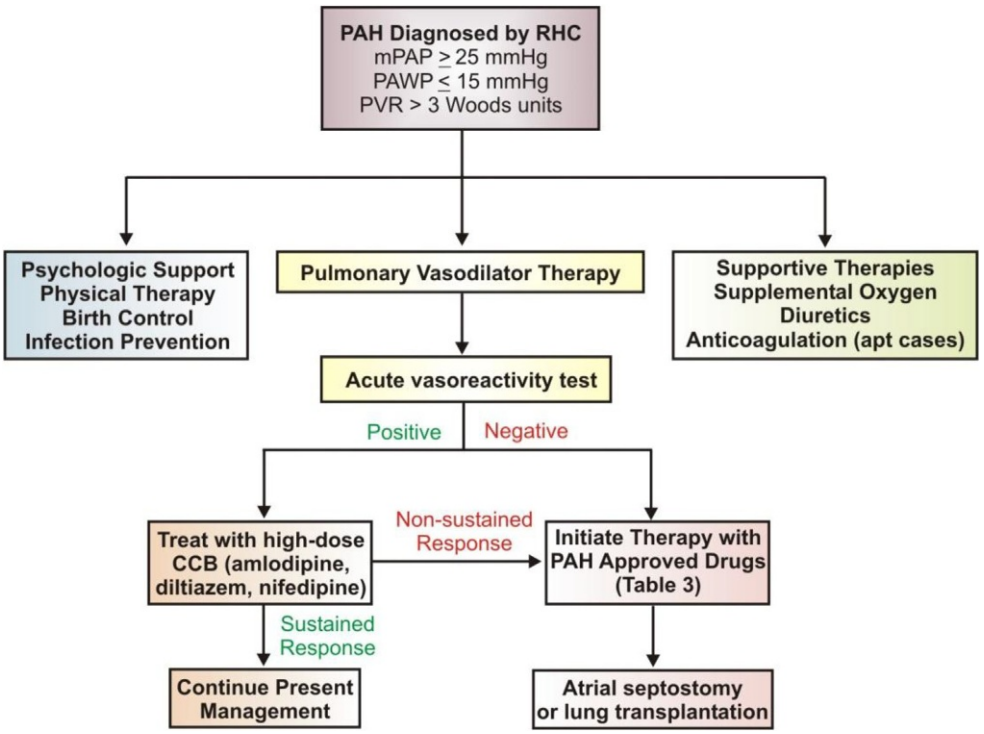

Fig. 12 Treatment algorithm for PAH. Standard care for all individuals diagnosed with PAH includes avoidance of excessive exertion or physical stressors, rehabilitation, and social support services as well as referral to an expert on the treatment of PAH. The patient should undergo vasoreactivity testing to identify the minority of patients who may have a substantial benefit from high-dose calcium channel blocker (CCB) therapy, and these individuals must be monitored closely to ensure a sustained response. Patients without a positive vasodilator response should potentially be started on therapies approved for PAH based on symptoms at presentation. The most severe patient who fails to respond to therapy may need referral for surgical intervention to treat their disease. Adapted with permission from Fraidenburg, D. R., Yuan, J. X. J. and Gaine S.P. (2015). In: Lanzer, P. (ed.) *PanVascular medicine*. Springer, Berlin.

symptoms, such as dyspnea, fatigue, chest pain, and/or syncope; improved functional capacity, including six-minute walk distance; and improvement in pulmonary and right ventricular hemodynamics, as measured by echocardiography and right heart catheterization. Sequential combination therapy with the addition of separate classes of medications is often utilized for severe or progressive disease, and many of the classes of agents have been used concurrently with varying levels of efficacy (Ghofrani and Humbert, 2014). Modest effects with single agents may be enhanced with the use of upfront combinations and could lead to more dramatic improvements in both symptoms and hemodynamic measurements. The most widely used combination for initial treatment of PAH includes an ERA in combination with a PDE5 inhibitor which has shown efficacy beyond the use of either agent alone (Galie et al., 2015). Careful monitoring of side effects and surveillance of disease progression, as either functional limitations or signs of right heart dysfunction, remains critical in adjusting therapy or adding additional agents to existing therapy.

Conclusion

A disorder that carries substantial morbidity and mortality, pulmonary hypertension can often be found as a complication of many serious cardiovascular and pulmonary disorders. In each of these disorders, the presence of pulmonary hypertension is an ominous sign which portends poor outcomes. Early recognition of PH involves connecting non-specific signs and symptoms with findings on both laboratory testing and imaging modalities that may suggest the diagnosis. This will lead to early referral for invasive hemodynamic measurements by right heart catheterization and appropriate diagnosis. The identification of pulmonary arterial hypertension, as idiopathic, hereditary, or linked to one of the many diseases in this category, is important for the initiation of specific pulmonary vasodilators. Prostacyclin analogs, phosphodiesterase type 5 inhibitors, soluble guanylate cyclase stimulators, and endothelin receptor antagonists are all shown to improve symptoms, functional capacity, and pulmonary hemodynamics; representing the armamentarium we have against this progressive disease. The understanding of the precise pathophysiology and genetics of this disorder has consistently advanced; and discovery of novel mechanisms for sustained vasoconstriction, pulmonary vascular remodeling, in-situ thrombosis, plexiform vasculopathy, and extracellular matrix stiffening will broaden our therapeutic possibilities.

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Pulmonary Hypertension Associated With Left-Sided Heart Disease

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Introduction

Pulmonary hypertension (PH) affects a significant number of patients with chronic heart and lung diseases contributing to worsening of their dyspnea and fatigue. Early diagnosis, assessment of severity, prognosis, and aggressive treatment strategies play a vital role in management of the condition and limiting associated morbidity.

PH is defined hemodynamically by a right heart catheterization (RHC), by a mean pulmonary arterial pressure $\geq$25 mmHg at rest (Galie et al., 2016). Pulmonary artery wedge pressure (PAWP) distinguishes pre-capillary ($\leq$15 mmHg) and post-capillary (>15 mmHg) PH in setting of normal or reduced left ventricular ejection fraction (LVEF) (Galie et al., 2016). The prevalence of PH in left-sided heart disease (LHD) occurs in 25%–75% of patients (Vachiery et al., 2013). Severity of PH is directly proportional to severity of underlying heart failure or valvular disease. Patients with PH secondary to LHD are more likely to be elderly females with severe hypertension and features of metabolic syndrome. This article focuses on PH secondary LHD, also known as pulmonary venous hypertension

Pathophysiology of Pulmonary Hypertension in Left-Sided Heart Disease

Elevated left-sided filling pressures secondary to diastolic or systolic dysfunction or valvular lesions eventually result in post-capillary pulmonary hypertension. Venous hypertension is back transmitted via capillaries to the pulmonary arterial system initially in a passive fashion resulting in passive pulmonary arterial hypertension (Harvey et al., 1971). During the passive phase, diuresing the patient is likely to result in complete normalization of pulmonary pressures. Over time, long-standing passive PH results in pulmonary arterial endothelial dysfunction leading to elevated levels of endothelin 1 (ET_1), decreased nitric oxide (NO), and decrease in BNP mediated vasodilatation (Moraes et al., 2000; Guazzi and Galie, 2012). These biochemical changes result in active pulmonary arterial vasospasm leading to further increase in pulmonary arterial pressures. Eventually, pulmonary vascular remodeling occurs with fibrosis and decreased vascular compliance resulting in fixed elevation in pulmonary arterial pressures independent of the pressure changes in the left heart, no longer amenable to reversal with resolution of the fluid overload due to underlying LHD. Eventually, PH results in right heart strain and failure, which is associated with worse prognosis. In view of the natural history of the disease, early diagnosis and management, particularly achievement of euvolemia, is the key to favorable outcomes. The goal is to identify and treat the disease prior to onset of fixed pulmonary arterial hypertension and right heart failure.

Due to the wide spectrum of pathology involved, PH in LHD can have different presentations. Patients can present with pure passive pulmonary venous hypertension with elevated PAWP with no pulmonary vascular disease. They may present with elevated PAWP with significant pulmonary vascular disease. Patients who have undergone aggressive management of their underlying LHD may present with normal (previously elevated) PAWP with significant pulmonary vascular remodeling. The myriad presentations pose a formidable diagnostic challenge to distinguish between pulmonary *arterial (fixed primary PH)* versus *venous (secondary to LHD)*. There is often a delay from onset of symptoms to an appropriate diagnosis of up to 2–3 years (Vachiery et al., 2013). The

diagnosis of PAH is a diagnosis of exclusion. PH in LHD is insidious in onset but often rapidly progressive, and it is important to have a high index of suspicion for individuals at risk due to presence of underlying LHD. Presence of associated scleroderma-related disorders and lupus, sleep apnea, cirrhosis with portal hypertension, human immunodeficiency virus (HIV) infection, family history of PAH, chronic cocaine use, and use of anorexigens (Moraes et al., 2000) further increases the risk of developing PH.

Classification of PH and Associated Triggers

Pulmonary hypertension is better understood if considered as a disease of triggers (Fig. 1). A clinical classification was established in order to individualize different categories of PH sharing similar pathological findings, similar hemodynamic characteristics and, similar therapeutic algorithms (Simonneau et al., 2013). The term *secondary* PH has been abandoned and not longer utilized. Five groups of disorders that cause PH were identified: pulmonary arterial hypertension (WHO Group 1); PH due to LHD (WHO Group 2); PH due to chronic lung disease and/or hypoxia (WHO Group 3); chronic thromboembolic PH (WHO Group 4); and PH due to unclear multifactorial mechanisms (WHO Group 5).

Common Signs and Symptoms in PH

Symptoms attributable to PH are similar to those in left-sided heart failure, valvular disease, and lung disease. PH should be suspected as the cause in the setting of risk factors, triggers, and associated diseases. The symptoms depend on the functional limitation and the degree of hemodynamic impairment.

Each of the symptoms has one or more hemodynamic correlates:

1. *Dyspnea and fatigue* are correlated with decrease in stroke volume (SV) (only with exercise initially but in severe disease also present at rest), cardiac output (CO), and oxygen transport.
2. *Angina-like chest pain* on exertion correlates with increased right ventricular (RV) myocardial oxygen demand and pulmonary artery pressures in excess of aortic pressure.
3. *Presyncope and syncope*: postural-, tussive-, and exercise-induced systemic hypotension are correlated with decreased RV filling, decreased left ventricular (LV) filling, and LV compression by the RV.
4. *Signs of RV failure*: Abdominal pain, anorexia, edema, and ascites are correlated with gastric distention, hepatic congestion, increasing jugular venous pressure, and tricuspid insufficiency.

Physical findings depend on the cause and severity of PH. Patients with lung disease severe enough to cause more than moderate PH can be identified by decreased breath sounds, chest deformities, rales consistent with interstitial lung disease, abnormal jugular venous wave or pressure, a murmur of tricuspid insufficiency, and often clubbing of the fingers and toes and cyanosis.

Long-standing Eisenmenger's syndrome (severe PH associated with congenital intracardiac shunt at the atrial, ventricular, or pulmonary artery level) with predominant right-to-left shunting is characterized by cyanosis at rest or with exercise, clubbing of the

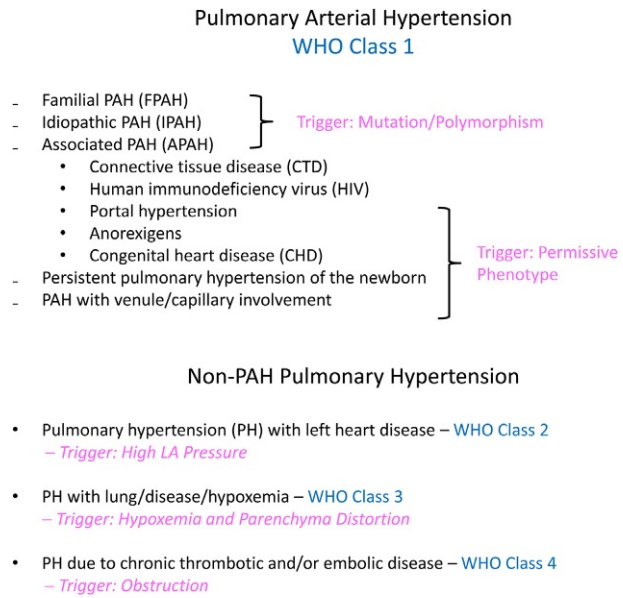

Fig. 1 Pulmonary hypertension a disease of triggers and classification.

fingers and toes, and often murmurs of tricuspid insufficiency and pulmonic insufficiency. Because of the RV hypertrophies over a period of years, patients are often only mildly symptomatic until very late in the course. Patients with atrial septal defects and anomalous pulmonary venous drainage (about 2%) can rapidly develop severe PH that resembles PAH and manifests without clubbing of the fingers and toes or without cyanosis. Cyanosis can be due to intracardiac right-to-left shunting or due to low CO and decreased alveolar-capillary diffusion.

Algorithm for Assessment and Diagnosis of PH

The 5th World Symposium for Pulmonary HTN has proposed a new algorithm for diagnosis of PH to facilitate early identification (Fig. 1) (Hoeper et al., 2013). The algorithm provides a logical sequence to exclude conditions that are mostly associated with PH. The algorithm is aimed at facilitating early and appropriate identification of PH class. PH management requires a multidisciplinary approach and liberal referral to physicians experienced in evaluating and treating PH is emphasized to limit morbidity.

Testing for Evaluation of PH

Electrocardiogram

The electrocardiogram (EKG) has a high degree of sensitivity (more than 75%–80%) (Galie et al., 2016) for detecting RV hypertrophy in symptomatic patients with severe PH. The sensitivity is less than 40% when the EKG is read without clinical information or when the ECG is unedited and computerized. Frequent misdiagnoses include inferior, anterior, and septal infarction; inferior and anterior ischemia; and left posterior fascicular block. EKG is not an effective screening tool in asymptomatic or mildly symptomatic patients.

The most common EKG patterns in PH include right axis deviation, qR in V_1, and increased voltage (>2.5 mm) of the *p* wave in lead II (sometimes called *P pulmonale*). The increased voltage is associated with a fourfold increase in mortality rate (Moraes et al., 2000). Atrial premature beats occur frequently, but atrial fibrillation, atrial flutter, and serious ventricular arrhythmias are not common.

Chest Radiograph

The magnitude of lung disease (emphysema, fibrosis, masses, and skeletal deformity) needed to induce significant PH is usually detectable on the chest radiograph. Chest radiographic indicators of PH include enlargement of the RV, right atrium, superior vena cava, main pulmonary artery, and right/left pulmonary arteries. CXR criteria for PH is found in 95% of patients with primary PH (Class I) and only 4% of controls matched for age, gender, and body surface area.

Echocardiography

Doppler echocardiography is the most effective noninvasive screening tool for detecting PH and for monitoring progression of disease, particularly, the development of RV enlargement and failure (Galie et al., 2016; Simonneau et al., 2013; Franco, 2012). It is an essential test as it is the most useful noninvasive tool to evaluate RV function and estimate pulmonary pressure. An estimated systolic RV pressure (RVSP, equivalent to systolic pulmonary arterial pressure) >40 mm Hg generally warrants further evaluation in the patient with unexplained dyspnea. Although echocardiogram is a very effective screening tool and is useful for monitoring progress of RV size and function, it is less useful in monitoring pulmonary pressures in the setting of severe RV dysfunction.

The tricuspid regurgitant velocity (TRV) is proportional to the gradient between the RV and RA and is used to calculate the RVSP with modified Bernoulli's equation:

$$\text{RVSP} = 4 \times \text{TRV}^2 + \text{estimated RA pressure (mmHg)}$$

RA pressure is estimated from the size of the inferior vena cava and its response to respiration (collapse with inspiration). The TRV (in meters per second) should be provided to allow the clinician to add an estimate of the RA. TRV should be carefully reviewed to assure the tricuspid regurgitation envelop is complete for an adequate estimation of RVSP; otherwise, echo-Doppler is less accurate and it can under- or over-estimate the true pressure compared to cardiac catheterization (Hoeper et al., 2013).

Echocardiography can also identify abnormal systolic and diastolic dysfunction of the left ventricle. An enlarged left atrium in the absence of severe mitral disease suggests elevated left-ventricular pressures. Agitated saline contrast should be used to identify intracardiac (i.e., atrial septal defect) or intrapulmonary shunts. Patent foramen ovale or atrial septal defect can be the explanation for exercise and rest arterial desaturation. The common anatomic findings in severe PH include RA and RV dilatation, flattened or D-shaped interventricular septum, and often a small, compressed left ventricle. Pericardial effusions are not common but may be predictive of poor outcomes regardless of size (Franco, 2012; Benza et al., 2012). Tricuspid annular plane systolic excursion (TAPSE) is a marker of RV function as related to longitudinal axis shortening (thus drawing the tricuspid annular plane toward the apex). A TAPSE ≤18 mm was associated with lower cardiac index, more right heart remodeling, and poorer prognosis in PH patients.

Right Heart Catheterization

RHC is a key diagnostic test in establishing and classifying the diagnosis of PH (Simonneau et al., 2013; Hoeper et al., 2013). Every patient suspected of PH should undergo RHC despite evidence from other diagnostic modalities discussed above. RHC not only confirms and classifies the diagnosis of PH, but also provides information about RV function. An elevated pulmonary capillary wedge pressure (PCWP) (PCWP $\geq$15 mmHg) is indicative of left-sided HF contributing to PH and, if present, a left heart catheterization may also be considered for direct measurement of left ventricular end-diastolic pressure, gradient across mitral valve as well as to perform angiography if coronary artery disease is suspected especially in the setting of systolic dysfunction.

RHC measurements not only aid in diagnosis of PH but also assist in differentiating passive venous pulmonary hypertension from pulmonary arterial hypertension. Up until recently, trans-pulmonary gradient (TPG), defined as the difference of mean pulmonary arterial pressure (PAP) and PCWP (TPG = mPAP − PCWP), was used to identify component of pulmonary vascular disease (mixed disease as a consequence of chronically elevated PCWP that resulted in pulmonary arterial remodeling). If TPG was >12, the terminology "out of proportion" pulmonary hypertension was used to define pulmonary pressure over that can be explained by passive back transmission of elevated left heart pressures, indicative of pulmonary vascular disease. However, "PCWP" is acquired by inflating the right heart catheter balloon in the distal pulmonary arterial system rather than the pulmonary capillaries. Hence, the pressure measured is actually pulmonary arterial wedge pressure (PAWP) and will reflect vascular disease distal to site of RHC balloon inflation in addition to true PCWP/left atrial pressure. Also, TPG is dependent on flow rate and varies with SV/CO. The dependence of TPG on pulmonary vascular flow, resistance, and left heart filling pressures (PAWP) makes it a poor measure of pulmonary vascular disease in setting of LHD (Naeije et al., 2013; Provencher et al., 2008).

In order to accurately assess the development of vascular disease and fixed pulmonary hypertension in patients with LHD, the 5th World Symposium For Management of PH updated guidelines propose the use of diastolic pressure difference (DPD), which is defined as the difference between pulmonary arterial diastolic pressure (dPAP) and PAWP.

$$\mathrm{DPD} = \mathrm{dPAP} - \mathrm{PAWP}$$

dPAP is less sensitive to changes in pulmonary vascular flow rate (SV/CO), resistance, and PAWP (Vachiery et al., 2013; Gerges et al., 2013). It has been shown to be less sensitive to pulmonary vascular distensibility. A study correlating PH with histology has demonstrated that DPD has a higher specificity to identify significant PVD (Gerges et al., 2013). DPD in normal subjects ranges from 1 to 3 mmHg and remains <5 mmHg in most patients with cardiac disease (excluding shunts) (Simonneau et al., 2013; Benza et al., 2012). Elevated DPD is a marker of pulmonary vascular remodeling. Studies correlating DPD with PVR have shown that individuals with DPD <5 mmHg are unlikely to have severe PVD (Rapp et al., 2001). A PAWP >15 mmHg with a DPD <7 mmHg is more likely associated with isolated postcapillary pulmonary hypertension (passive pulmonary venous hypertension). Individuals with PAWP >15 mmHg with a DPD $\geq$7 mmHg have combined pre- and postcapillary pulmonary hypertension ("out-of-proportion" PH) (Gerges et al., 2013). Elevated DPD has been shown to have a prognostic value (Franco, 2012). Patients with TPG >12 mmHg and DPD >7 mmHg have a median survival of ~6.5 years similar to survival in primary pulmonary arterial hypertension. Patients with DPD <7 mmHg have slightly better prognosis with median survival of ~8.4 years.

Fluid challenge and occasional vasoreactive testing are recommended during RHC in cases with borderline PAWP numbers to establish diagnosis of PH secondary to LHD (Hoeper et al., 2013; Robbins et al., 2014). Patients with PAWP in the range of 12–15 mmHg and suspicion of underlying LHD (enlarged left atrium, possible diastolic dysfunction) should undergo fluid challenge with 500 cc normal saline infused over 5 min. If PAWP is >15 mmHg with fluid challenge, patient's PH is most likely secondary to LHD rather than primary pulmonary arterial hypertension. Exercise testing can be used to accomplish a similar outcome; however, as the exercise testing protocols are yet to be standardized, results are subject to speculation. As of today, the role of exercise testing in diagnosing PH in LHD has not been validated but can be used if fluid challenge is not feasible/contraindicated.

The role of vasoreactive testing in diagnosis of PH suspected to be secondary to LHD is not well established. It has been shown to be beneficial in evaluating patients for advanced heart failure therapies and determining transplant candidacy. It is important to choose the appropriate medication to perform testing. Nitroprusside is utilized to determine if pulmonary hypertension is reversible before listing for heart transplantation. It is a strong vasodilator that usually reduced wedge pressure and subsequently PAP. Other pulmonary vasculature vasodilators, like NO or epoprostenol, could potentially precipitate pulmonary edema and hemodynamic decompensations; therefore, it should be reserved for testing in patients with pulmonary arterial hypertension but should be avoided if there is evidence of high wedge pressure. It is best to avoid vasoreactive testing in PH secondary to fixed valvular disease for similar reasons.

Management PH Secondary to LHD

Management Goals

Management of PH secondary to LHD should be geared toward appropriate interventions for the underlying heart disease. Aggressive control of cardiovascular disease risk factors and features of metabolic syndrome is recommended. Systolic and diastolic heart failure should be treated with optimal medical management. Experts recommend a trial of nonspecific vasodilators, nitric oxide, and hydralazine in patients with heart failure with preserved or reduced EF. These have shown improvements in pulmonary pressure especially in patients with diastolic dysfunction and HFpEF. In patients with severe systolic heart failure with secondary PH

not responding to medication should be evaluated for advanced therapy with left ventricular assist device (LVAD). Mechanical LV support has shown to dramatically improve pulmonary pressures and reduce RV failure by unloading the left ventricle. Patients with fixed mitral or aortic valvular disease should be appropriately evaluated for valve repair or replacement. Adequate treatment of the underlying LHD with improvement in left-sided pressures results in significant improvement in secondary PH especially if PVD has not set in. Finally, it is imperative that comorbidities that contribute to PH independently (e.g., lung disease, obstructive sleep apnea, pulmonary thromboembolic disease, obesity) have to be screened for early treatment initiation and treated aggressively in course of the disease.

Pulmonary Vasodilators in Management of Precapillary Pulmonary Hypertension/PVD

Pulmonary vascular remodeling/PVD in PH primarily occurs secondary to endothelial dysfunction with upregulation of endothelin 1 (ET_1) and downregulation of NO with impaired NO-dependent vasodilatation (Moraes et al., 2000; Porter et al., 1993; Spieker et al., 2000). Agents that inhibit ET_1 and upregulate NO have shown to improve pulmonary pressures and exercise capacity in patients with PVD. Inhibition of phosphodiesterase type 5 (PDE5) attenuates LV remodeling and improves vascular, renal, and neuroendocrine function (Tsai and Kass, 2009; Pokreisz et al., 2009; Katz et al., 2000). These are the first-line medication for management of primary pulmonary hypertension. However, in secondary PH, these medications are reserved as second line options for patients not responding to management of underlying LHD or those with persistent PVD despite correction of underlying LHD (e.g., postvalve repair). Utilization of these agents as primary modality of management for PH in LAD carries the risk of pulmonary edema due to increased pulmonary vascular flow in the presence of elevated left heart pressures and further worsening of PH with associated hemodynamic collapse for resultant drop in CO.

There are four major classes of medication available for treatment of PH with PVD: (1) prostanoids (e.g., epoprostenol), (2) endothelin receptor antagonists (e.g., bosentan), (3) phosphodiesterase-5 inhibitors (PDE5I; e.g., sildenafil, tadalafil) and (4) guanyl cyclase stimulators (e.g., riociguat). In large studies, these medications have proven to be valuable in decreasing morbidity in primary pulmonary hypertension. However, there is limited data from small studies that has evaluated the benefit of these medications in PH secondary to LHD with controversial results. Small studies have consistently demonstrated some improvements in hemodynamic status, exercise capacity, and symptoms; sustained and survival benefits remain unclear (Spieker et al., 2000). Also, patients with LHD are on multiple medications with survival benefit that lowers blood pressure making hypotension a limiting factor in the adequate use of pulmonary vasodilators in this group of patients.

Among the pulmonary vasodilators, acute usage of sildenafil 25–50 mg, in prelung transplant patients, has been shown to decrease pulmonary vascular resistance (PVR) with improvement in hemodynamics and oxygen consumption (Barnett and De Marco, 2012; Guazzi et al., 2004; Lewis et al., 2007a; Angel Gomez-Sanchez et al., 2004). Phosphodiesterase-5 inhibitors seem to have a more potent pulmonary vasodilator effect compared to prostanoids. Chronic administration of sildenafil has also been shown to improve pulmonary hemodynamic status before transplantation (Jabbour et al., 2007). In small placebo controlled trials involving patients with PH and reduced EF, sildenafil in dose of 25–75 mg three times a day has shown to improve exercise tolerance (sustained up to 6 months) and decrease PVR even though there was no significant impact on mean PAP (Guazzi et al., 2007; Lewis et al., 2007b). Large trials to evaluate the benefit of PDE5I in PH with LHD are currently underway. PDE5I are not currently FDA approved for PH in LHD but off-label use may be considered in carefully selected cases with evidence of PVD (without fixed valvular disease) when all other modalities of treatment have not resulted in optimal benefits.

Trials that evaluated the benefit of prostanoids and ERA in PH with LHD were negative for benefit with increased adverse reactions from the drugs and a trend to increase in adverse outcomes or mortality (Packer et al., 2005; Kalra et al., 2002; Califf et al., 1997). A small trial looking at the benefit of guanyl cyclase stimulators did not show any benefit in PH with LHD despite the benefit noted in patients with PAH (Bonderman et al., 2013). However, as these act by increasing guanylyl cyclase and hence production of NO, larger trials are currently ongoing to evaluate their benefit in HFpEF (diastolic heart failure). None of these medications have been evaluated in patients with underlying uncorrected fixed valvular disease.

Management of PH in HFpEF

HFpEF is a common cause of PH and is associated with significant morbidity and poor outcomes (Lam et al., 2009; Fang et al., 2012). PH in underlying diastolic dysfunction/HFpEF presents a diagnostic and treatment challenge. In individuals with mean PAP >25 mmHg and borderline normal PAWP, testing with fluid challenge or exercise is recommended to evaluate the presence of diastolic dysfunction especially if there is evidence of diastolic dysfunction on history or imaging. Unfortunately, to date, there are no strong evidence-based recommendations for the management of HFpEF. Despite lack of evidence, optimal medical management as used for HFrEF continues to remain the main stay of therapy. Nonspecific vasodilators (nitrates and hydralazine) have shown to provide only a modest benefit of decrease in PVR and increase in CO at the cost of significant hypotension (Schwartzenberg et al., 2012). Sildenafil has had mixed outcomes in small trials in patients with HFpEF; however, these trials had not appropriately stratified PH to identify the presence of PVD. In one trial, 50 mg of sildenafil three times a day has shown to improve exercise tolerance and hemodynamics at 6 months lasting up to a year in PH with HFpEF (Guazzi et al., 2011). The RELAX study involving a large population of PH with HFpEF did not reveal the benefits of sildenafil. This study however did not evaluate hemodynamics or RV function and nature of PH was not prespecified (Lam et al., 2009). A recently published small randomized controlled trial, use of sildenafil 60 mg three times a day for 12 weeks did not have a favorable effect on PAWP, CO, and peak VO_2 compared with placebo

(Fang et al., 2012). These trials however have validated the safety profile of sildenafil in this subpopulation of patients. Results from larger well-designed trials are awaited. In a recent study, inhaled iloprost resulted in acute reduction of PAP and PVR (Schwartzenberg et al., 2012). Large trials are underway to evaluate the benefit of ERAs, ambrisentan and bosentan, in management of PH in HFpEF. In another randomized controlled trail, riociguat was shown to improve exploratory hemodynamic and echocardiographic parameters with a favorable safety profile but without a significant effect on mPAP (Guazzi et al., 2011). Large well-designed studies are needed to better define the phenotype of patients who may benefit from more selective pulmonary vascular agents.

Summary

Management of PH in LHD is a widely prevalent poorly understood condition lacking evidence-based management approach. A better understanding of the nature of PH in patients with LHD will aid management decisions in this subpopulation of patients. Adequate management of underlying LHD continues to remain the main stay of treatment. There are no approved vasodilator therapies for PH in LHD. Results of large trials evaluating the role of pulmonary vasodilators in PH in LHD are awaited.

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Quality Indicators for the Management of Acute Myocardial Infarction

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Introduction

Cardiovascular disease remains the leading cause of death across the world. According to the World Health Organisation, it was estimated that of the 17.5 million deaths in 2012, 7 million (40%) were due to coronary heart disease (World Health Organization, 2016). However, despite the high death toll, there have been many advancements in coronary heart disease care which have led to improvements in survival over the last 20 years.

The measurement and recording of information that can be used to assess performance within medicine is not a new concept. Dr Codman advocated that surgeons should both track and publish their patient's outcomes to as far back as 1910 to aid their development as surgeons and improve outcomes for patients (O'Leary, 1995). Outcome measurement has been the mainstay of measuring quality of care. One example of this is reporting of operator-level standardized mortality rates for both percutaneous coronary intervention and cardiothoracic surgery by the National Health Service covering all publicly funded procedures performed by all operators within England and Wales.

However, patients' journey from their initial presentation until their discharge and subsequent follow up consists of many more steps than simply the outcome. Therefore, there has been a move to focus on the processes that lead to the outcomes as a method to measure quality (Bebb et al., 2016). The rationale behind this is that if these processes are performed well then this should lead to improved outcomes.

There are several terms used for these new measures including performance measures. Throughout this chapter, however, they will be referred to as quality indicators.

A Brief History of Quality Indicators

The emergence of quality indicators for the measurement of performance first started in 2003 with the publication of the Canadian quality indicators for acute myocardial infarction. The Canadian publication comprised 37 indicators of which 15 were inpatient measures, 13 outpatient measures, and 9 outcome measures (**Table 1**) (Panel et al., 2003). The American Heart Association and American College of Cardiology (AHA/ACC) published their first report on quality indicators for adults with ST-elevation

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Table 1 Temporal evolution of quality indicators

	Quality indicator	Dimension	Type	Canadian 2003	ACC/AHA 2006	ACC/AHA 2008	Canadian 2008	ESC 2016
1	Aspirin on arrival	Treatment (antithrombotic)	Process (pharmacological)					
2	Aspirin on discharge	Treatment (antithrombotic)	Process (pharmacological)					
3	Beta-blocker on arrival	Treatment	Process (pharmacological)					
4	Beta-blocker on discharge	Secondary prevention	Process (pharmacological)					for LVSD
5	LDL cholesterol assessment	Diagnostics	Process (non-pharmacological)			Test measure (T1)		
6	Lipid lowering therapy at discharge	Treatment	Process (pharmacological)					High intensity
7	ACEi/ARB for LVSD on discharge	Secondary prevention	Process (pharmacological)	For all patients			For all patients	
8	Time to fibrinolytic therapy (within 30 minutes of arrival to hospital)	Treatment	Process (pharmacological)		Reported as median			Timely reperfusion
9	Time to PCI (arrival in hospital to time of PCI)	Treatment	Process (non-pharmacological)		FMC to balloon inflation <90min			Timely reperfusion
10	Reperfusion therapy	Treatment	Process (non-pharmacological)		Proportion of those eligible receiving reperfusion			Timely reperfusion
11	Adult smoking cessation advice counselling	Patient Education	Process (non-pharmacological)					
12	Evaluation of LVSF	Diagnostics	Process (non-pharmacological)					
13	Cardiac rehabilitation referral	Patient satisfaction/education	Process (non-pharmacological)					
T2	Excessive initial heparin dose	Treatment	Process (pharmacological)					
T3	Excessive initial enoxaparin dose	Treatment	Process (pharmacological)					
T4	Excessive initial abiciximab dose	Treatment	Process (pharmacological)					
T5	Excessive initial eptifibatide dose	Treatment	Process (pharmacological)					
T6	Excessive initial tirofiban dose	Treatment	Process (pharmacological)					
T7	Anticoagulant dosing protocol	Treatment	Structural					
T8	Anticoagulation error tracking system	Treatment	Structural					
T9	Clopidogrel at discharge	Treatment (antithrombotic)	Process (pharmacological)			Medically managed patients		P2Y12
14	ECG within 10mins of hospital arrival	Treatment	Process (non-pharmacological)					
15	Prehospital ECG	Treatment	System					
16	In hospital mortality	Outcome	Outcome					
17	PPCI within 90mins of hospital arrival	Treatment	Process (non-pharmacological)					Timely reperfusion
18	PPCI within 120mins of call for emergency services	Treatment	System		See QI 9	See QI 9		Timely reperfusion

(*Continued*)

Table 1 (Continued)

	Quality indicator	Dimension	Type	Canadian 2003	ACC/AHA 2006	ACC/AHA 2008	Canadian 2008	ESC 2016
19	Fibrinolysis within 60 mins of call for emergency services	Treatment	System		See QI 8Time	See QI 8		Timely reperfusion
20	Risk stratification (angiogram/ exercise stress testing / perfusion imaging/stress echocardiography)	Treatment	Process (non-pharmacological))					
21	Median length of stay in emergency department	Outcome	Outcome					
22	Coronary angiography in hospital or referral for angiography (test)	Treatment	Process (non-pharmacological)					
23	Coronary angiography within 72hrs (NSTEMI patients only)							
24	Median length of in hospital stay	Outcome	Outcome					
25	P2Y12 inhibition at discharge	Treatment (antithrombotic)	Process (pharmacological)					
26	DAPT at discharge	Treatment (antithrombotic)	Process (pharmacological)					
27	GRACE score documented in notes	Prognosis	Process (non-pharmacological)					
28	CRUSADE score documented in notes	Prognosis	Process (non-pharmacological)					
29	30-day mortality adjusted mortality	Outcome	Outcome					
30	Composite all or none (HF and non HF)	Composite	Outcome					
31	Composite opportunity	Composite	Outcome					

Colour	Present in guideline
	Yes
	Included, but in a different format
	Not included

ACC, American Collage Cardiology; *AHA*, American Heart Association; *ESC*, European Society of Cardiology; *LVSD*, left ventricular systolic dysfunction; *ACEi*, angiotensin converting enzyme inhibitor; *ARB*, angiotensin receptor blocker; *PCI*, percutaneous coronary intervention; *FMC*, first medical contact; *LVSF*, left ventricular systolic function; *PPCI*, primary percutaneous coronary intervention; *DAPT*, dual antiplatelet therapy; *GRACE*, global registry acute coronary events; *CRUSADE*, can rapid risk stratification of unstable angina patients suppress adverse outcomes with early implementation of the ACC/AHA Guidelines; *HF*, heart failure.

myocardial infarction (STEMI) and non ST-elevation myocardial infarction (NSTEMI) in 2006, comprising of 11 measures (**Table 1**) focusing on the inpatient management of STEMI and NSTEMI encompassing medications at arrival and discharge to reperfusion therapy during admission and referral for lifestyle advice at discharge (Krumholz et al., 2006). In 2008, an update of these indicators were published proposing changes to four of the existing measures as well as introducing a further four indicators to include an evaluation of left ventricular function, recording time from arrival in the emergency department to discharge for those patients transferred for percutaneous coronary intervention, and referral to cardiac rehabilitation services (**Table 1**). In the same year, the Canadian society also issued updated quality indicators with 17 inpatient measures and 21 outpatient measures (Tu et al., 2008; Krumholz et al., 2008).

In addition to the quality indicators themselves, the AHA/ACC have published complementary reports on both the classification (Bonow et al., 2008) and methodology (Bonow et al., 2011; Spertus et al., 2010) for defining these quality indicators.

Nearly a decade later, the European Society of Cardiology (ESC) published their own set of 20 quality indicators (Schiele et al., 2016) focussing on the inpatient management of acute myocardial infarction. The ESC indicators offer a more comprehensive set than earlier versions of the AHA/ACC indicators but do not focus on outpatient indicators which feature heavily in the Canadian sets. Moreover, the ESC is the first to include composite measures to assess the overall quality of healthcare providers as part of their quality indicators. Whilst the AHA/ACC have published a position paper on the use of composite measures for healthcare performance assessment, they have not, as yet, recommended their widespread use (Peterson et al., 2010).

Rationale Behind Quality Indicators

Quality indicators are predominantly based upon Class 1A recommendations from the respective national cardiology societies. Class one recommendations are provided for those interventions for which there is agreement that they are beneficial, useful, and

effective. They are usually supported by a level of evidence A (data is based upon multiple randomized clinical trials or metaanalyses).

Quality indicators need to cover all aspects of care that a patient receives on their journey from presentation to diagnosis to discharge and then follow up. These aspects are referred to as either domains or dimensions of care. Initial quality indicator guidelines focussed predominantly on the treatment, in comparison to the ESC guidelines which being the most recent cover seven differing domains of care.

Table 1 compares the different sets of quality indicators that have been recommended by the respective national cardiology societies. It shows that although there is a large degree of overlap between the suggested measures of quality, some key differences remain. Certainly the earlier versions of quality indicators primarily focussed on medication prescription for which there is a plethora of evidence to show their association with improved outcomes both in the form of randomized clinical trials and registry-based analyses (Yusuf et al., 2001; Wallentin et al., 2009; Wiviott et al., 2007; Devita et al., 1994; Pfeffer et al., 1992, 2003; Cannon et al., 2004). However, for some indicators the associations with outcomes are not well evidenced. For example, there is no Class 1A recommendation to show that receipt of echocardiography is associated with outcomes. However, there is an indirect association due to echocardiography being a vital step in identifying patients with an ejection fraction ≤ 0.4 which determines eligibility for angiotensin converting enzyme inhibitors and β-blockers which are both well recognized to improve outcomes and carry Class 1A recommendations.

Several studies have examined the association of quality indicators with mortality (Peterson et al., 2006; Bradley et al., 2006; Bebb et al., 2017). The first considered the association between nine process of care measures (of which six were quality indicators from the 2006 AHA/ACC guidelines) as well as an opportunity based composite score and in-hospital mortality (Peterson et al., 2006). All nine measures were found to have a negative correlation with in-hospital mortality, which was significant for all but β-blocker prescription both acutely and at discharge. Furthermore, improved attainment of the composite score was associated with a significant reduction in in-hospital mortality. The second study examined the relationship between seven measures which were all listed in the AHA/ACC report with 30 day risk-standardized mortality (Bradley et al., 2006). Again an opportunity based composite score (although only five of the seven indicators were included) was calculated and its association with 30 day risk-standardized mortality investigated. The authors demonstrated a negative correlation between all indicators and 30 day risk-standardized mortality, which was greatest for the composite measure. The degree of correlation was not as marked as that shown by Peterson et al. (2006). The most recent analysis by the chapter authors and colleagues used the ESC quality indicators to assess performance and showed that 11 of 12 indicators assessed were associated with a reduction in crude mortality (Bebb et al., 2017).

All three studies demonstrated that in addition to associations with outcomes, there was correlation between the individual quality indicators. The correlations between ESC measures, although evident, were weaker which is likely due to the much wider coverage across the spectrum of care than that covered in the AHA/ACC indicators. It should be noted that the Canadian indicators cover the widest spectrum but as far as the authors are aware there exists no work demonstrating an association between these indicators and outcomes.

In conclusion, although quality indicators are not solely based on Class 1A recommendations, they have been demonstrated to be associated with outcomes such as mortality. Therefore improving adherence to quality indicators should result in a decrease in mortality. One study in Canada examined the effects of publication of "report cards" for acute myocardial infarction patients, they surveyed physicians responses to the "report cards" and over 50% reported launching quality improvement projects as a consequence of the "report cards" (TU and CAMERON, 2003). The Get With The Guidelines program has shown that programs aimed at improved adherence to quality indicators leads to improved outcomes (Lewis et al., 2008).

Design and Attributes of Quality Indicators

Quality Indicators

Quality indicators differ from recommendations as they must not only have a strong evidence base behind them they must also meet further criteria concerning validity and feasibility.

The process of developing a new quality indicator is complex. In this section, the steps in defining quality indicators are explored (**Fig. 1**). Firstly a review of existing evidence is undertaken to identify where guideline recommendations exist and importantly, whether there is variation within provision of these guidelines between providers. These guidelines ideally should cover all domains of patient care. However, there may be some areas where guidelines are lacking and further work is needed, the initial set of indicators proposed by the AHA/ACC covered diagnostics, patient education and treatment, but had no indicators concerning self-management or monitoring of disease states (Krumholz et al., 2006). The European indicators include "the centre should participate in a regular registry or program for quality assessment" which is aimed at developing both regional and national programs to measure quality indicator performance then provide feedback to the individual hospitals (Schiele et al., 2016). This is not based upon any one specific recommendation, but builds upon participation in a regular registry and is a logical next step for the implementation of quality indicators.

Following a review of the evidence, one must determine whether the proposed indicator leads to improved patient outcomes, this is why they are based in the main Class 1A recommendations. The measure also needs to be interpretable by the practitioners who treat patients and finally, the measure must be something that is modifiable.

When indicators are designed they need to have clear precise definitions of the populations for both the denominator (all those eligible for a treatment for example) and numerator (all those receiving a treatment for example) to ensure consistency of

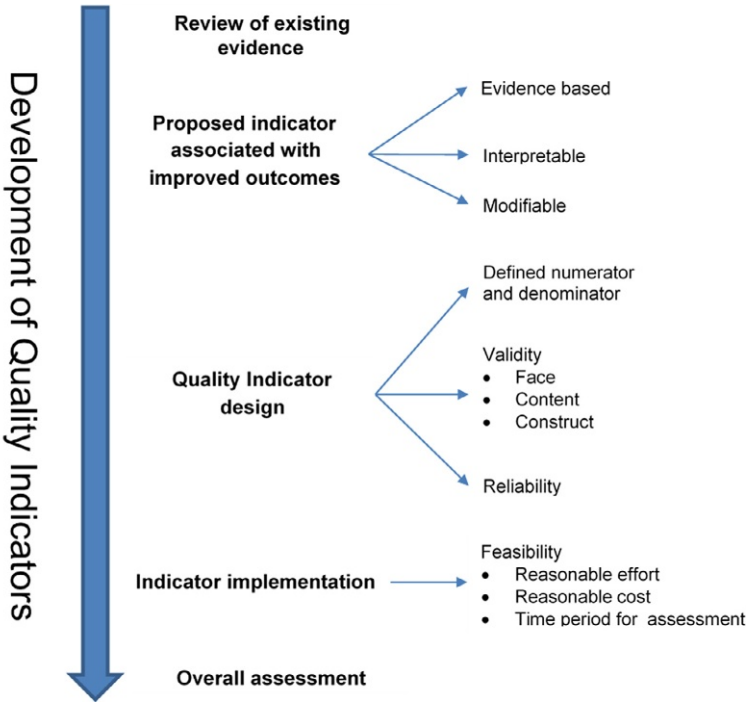

Fig. 1 Flow diagram outlining the process for development of quality indicators.

Box 1 Measuring Time of Reperfusion

There have been several quality indicators for assessing this, the first were time to fibrinolytic therapy and time to PCI, where the numerators are the time from first medical contact to the time that fibrinolysis is initiated or a balloon is inflated.

The reason for choosing these values as opposed to time reperfusion achieved, is that in a small minority of patients despite following all the correct processes, it is not possible to achieve reperfusion. Therefore, these quality indicators provide a more balanced assessment of the care provided, than the outcome of achieving reperfusion. The European guidelines take this a step further and use the passage of the wire as the indicator for the clock to stop, rather than the time of balloon inflation.

calculation and comparability across populations. In addition, indicators must be valid, which can be measured in several different ways including face validity, content validity, and construct validity. Face validity considers whether the indicator assesses what it truly set out to measure. Content validity ensures the indicator captures the most meaningful aspects of care for example you could measure prescription of angiotensin converting enzymes at discharge for all patients, however, the evidence suggests the most benefit is seen in those with heart failure of left ventricular systolic dysfunction. Hence the measure should be prescription for those with heart failure or a left ventricular ejection fraction ≤ 0.4, rather than prescription for all patients (Box 1). Finally, indicators should have construct validity—which means that the measure should correlate well with other measures in the same aspect of care. Lastly when designing new indicators their reliability needs to be considered, will they correctly identify true variation within care or just chance variation, and can they be reproduced across care providers both at a national and international level.

Following the design of an indicator which meets the above criteria, the practical aspects of implementing the indicators need to be considered. First the measure must be feasible, in that it can be measured for a reasonable effort at affordable cost within a predefined time frame. The time frame is an important consideration, as it takes 5 years to measure and report the performance of an indicator, the care that aforementioned indicator covers may have progressed in that time period.

When the proposed indicator has gone through these steps it is put forward to a panel of experts who will then reach a consensus to recommend or reject the indicator.

Quality Metrics

Although the focus of this article is on quality indicators, note should be made of quality metrics which are similar as they both focus on improving the quality of care for patients. The processes for development of quality indicators and metrics are also comparable though quality metrics need not go through the same rigorous process that quality indicators do (Bonow et al., 2008). One fundamental difference between the two is that although quality metrics are developed to support both self-assessment and

quality improvement at all levels (hospital, regional and national), they are not intended for public reporting rather than quality indicators which are primarily intended for public reporting, they do however represent a valuable tool for enhancing patient outcomes. Quality metrics can be often be viewed as tests or preliminary indicators for potential future quality indicators. The AHA/ACC 2008 guideline included nine "test" measures, the majority focusing on anticoagulant management; of these it can be argued that only one of the prescription of clopidogrel at discharge has made the transition to "full" quality indicator (Krumholz et al., 2006).

Types of Quality Indicators

There are several broad classes of quality indicators including process, outcome, structural or system, and composite indicators. Each are discussed later.

Process Indicators

Process indicators focus on measuring aspects of care that if performed well should lead to an improvement in outcomes such as the prescription of medications at discharge, rather than measuring a specified outcome. They can be both pharmacological and nonpharmacological. All three sets of guidelines contain multiple examples of process measures, although there are some such as aspirin on discharge which are found in every set (**Table 1**).

Outcome Indicators

Outcome measures are hard endpoints such as 30-day mortality or readmission to hospital. There remains much debate as to whether outcome measures are still relevant and applicable. A study from 2003 showed that physicians found information regarding prescription rates and waiting times for procedures more useful than outcomes (30-day and 1-year mortality) for driving quality improvement (TU and CAMERON, 2003). They are rather crude measures in that they focus on one outcome that is the consequence of multiple components of care that patients will experience in their journey of treatment (Lilford et al., 2004). The Canadian guidelines (Tu et al., 2008; Panel et al., 2003) have multiple measures focusing on the differing types of acute coronary syndrome, assessing both mortality and readmission at 30 days and 1 year, although these were not cardiac specific or risk adjusted. The American guidelines have no outcome measures within them (Krumholz et al., 2006, 2008) and the European guidelines only contain GRACE adjusted 30-day mortality (Schiele et al., 2016). There is also concern as to whether the methods used for risk adjustment are appropriate, and whether they should be used when considering the quality of care. Finally there is the question of how one differentiates between preventable deaths and deaths from other causes, the Harvard Medical Practice Study showed that only 0.25% of admissions resulted in an avoidable death and hence one can argue that reporting mortality does not reflect the quality of care provided (Brennan et al., 1991).

Structural/System Indicators

Structural measures focus on the facilities and organization of both hospitals and health services, for example the first quality indicator domain from the ESC is that of center organization, the theory behind this being that an organized system designed to facilitate primary PCI will lead to reduced times to reperfusion, without these structural aspects in place it may be harder to deliver an improved service (Schiele et al., 2016). They range from the presence of a system to monitor errors in anticoagulation to the time from call to emergency services to receiving treatment.

Composite Indicators

A composite quality indicator is the combination of a minimum of two or more indicators to summarize performance across multiple dimensions of care. This allows an immediate representation of the global standard of care provided as opposed to one individual clinical parameter. Composite indicators are only officially recommended by the ESC at present, yet there are multiple examples of their use within the cardiovascular literature (Couralet et al., 2011; Eapen et al., 2011; Chen et al., 2013; Simms et al., 2013a,b; Aliprandi-Costa et al., 2016; Bebb et al., 2017).

There are several ways in which a composite score can be achieved and these can be found in **Table 2**. The ESC guidelines advocate the use of both an "opportunity based" and "all or none" approach as each individual method has its own strengths and weaknesses.

The advantages of an opportunity based method is it's reflection of the number of opportunities that a care provider had to offer for a particular patient given their eligibility, which is a fairer method than a simple total or average composite score (total number of times care intervention received over the total number of patients) which could be biased by measures for which some patient groups were not eligible. Although the disadvantage of such measures is that it will be influenced by the most common care indicators, as each indicator is implicitly weighted by the number of eligible patients which can vary between providers. In addition, it may not be apparent whether it is one particular area underperforming or multiple areas.

Table 2 Types of composite measures

Type of composite measure	*Method*
Linear combinations	
Simple average	Take an average of the number of times care indicator provided over the population
Budget allocation	Allows weighting of the indicators
Equal weighted	Every indicator carries the same weight
Unequal weighted	Indicators are weighted differently according to their importance
Opportunity based scores	Number of times care indicator provided over the number of opportunities to provide the care indicator
Logical combinations	
All or none (process measures)	Success is receipt of all care indicators that a patient is eligible for
Any or none (outcome measures)	"Success" is the patient experiencing any adverse outcome from a list of two or more
Incommensurable measurement scales	Where indicators are translated into a common scale prior to being combined together

In contrast, "all or none" measures promote the highest standards of care as only providers giving patients all indicators for which they are eligible can score. Although this is the standard of care which providers should strive towards, the counter argument is that there is a loss of information by reducing the measure to a simple dichotomy and does not account for the relative importance of individual care opportunities. For example, one provider could have achieved optimal care in 60% of patients based on medication prescription and reperfusion therapy, but perhaps was more likely to not provide smoking cessation advice and would therefore be considered as providing suboptimal care. The "lost" information could be important for identifying areas that need quality improvement, hence the supporting information should focus on individual measures alongside composite scores.

Regardless of the method used to calculate the composite score, composite scores have their own strengths and weaknesses. As previously mentioned their strength is in providing an overall assessment of the care provider at a predefined level (patient/care providers), they allow a wider scope of measurement than individual indicators as they can cover all the differing types of indicator. Given that they are a summation of care provided, they can be used for comparing the quality of care provided by care providers, both within and between countries. However, as they are a summation they do not allow easy identification of underperforming areas.

The challenges of using composite measures is in the interpretation, usually this involves benchmarking the composite score against set levels; above national average, within national average, below national average for example. There should be a clear explanation of both, how missing data is dealt with and how the composite is calculated so providers can identify which areas of the composite require improvement.

Finally, just as individual indicators, composite measures have to be valid and they should correlate with an outcome that is regarded as the gold standard of measuring the focus of the composite. For example, for acute myocardial infarction this outcome is usually measured as 30-day mortality. Previous work has demonstrated that composite measures are associated with in hospital mortality (Peterson et al., 2006), and 30-day mortality (Bebb et al., 2017; Eapen et al., 2011; Simms et al., 2013b; Chen et al., 2013; Bradley et al., 2006), whilst some studies have demonstrated the association for 1 year mortality (Simms et al., 2013a). It should be noted that for each of these studies a different composite was used, hence it is important that when a composite indicator is recommended that it undergoes validation promptly, ensuring it is an accurate measure of performance.

Implementation of Quality Indicators

Challenges

Quality indicators cannot always be measured due to the quality and the availability of data. This is one of the obstacles to the widespread implementation of quality indicators. Determining which measures can accurately be assessed from existing data and therefore whether new data needs to be recorded to ensure that all quality indicators to be assessed, is necessary. The Myocardial Ischaemia National Audit Project (Herrett et al., 2010) has been used to validate the recent ESC quality indicator guidelines, the authors showed they could only directly assess 12 of the 20 measures, a further four could be assessed indirectly whilst for the remaining four indicators further data need to be gathered (Bebb et al., 2017).

Secondly, each indicator has very precise numerator and denominator with set exclusion criteria, however it may be that not all the data relating to exclusion criteria is readily available from the hospital records and therefore ineligible patients may be incorrectly included in the denominator which in turn leads to an underestimation of the level of attainment a hospital or provider has achieved.

For those indicators which have a time component, ensuring accurate timing is of paramount importance, ideally all systems used to measure time (ECG machines, emergency service call records, and catheterization laboratory clocks) should all be synchronized, however, this is impractical and unlikely in a busy acute care setting. There is also the issue of transferred patients who in earlier guidelines were excluded from indicators involving timing, in the more recent sets there is differentiation between those admitted to hospitals able to perform primary percutaneous coronary intervention and those who are not. Ensuring these patients are included within quality analysis is important to ensure they are not disadvantaged by requiring transfer between hospitals.

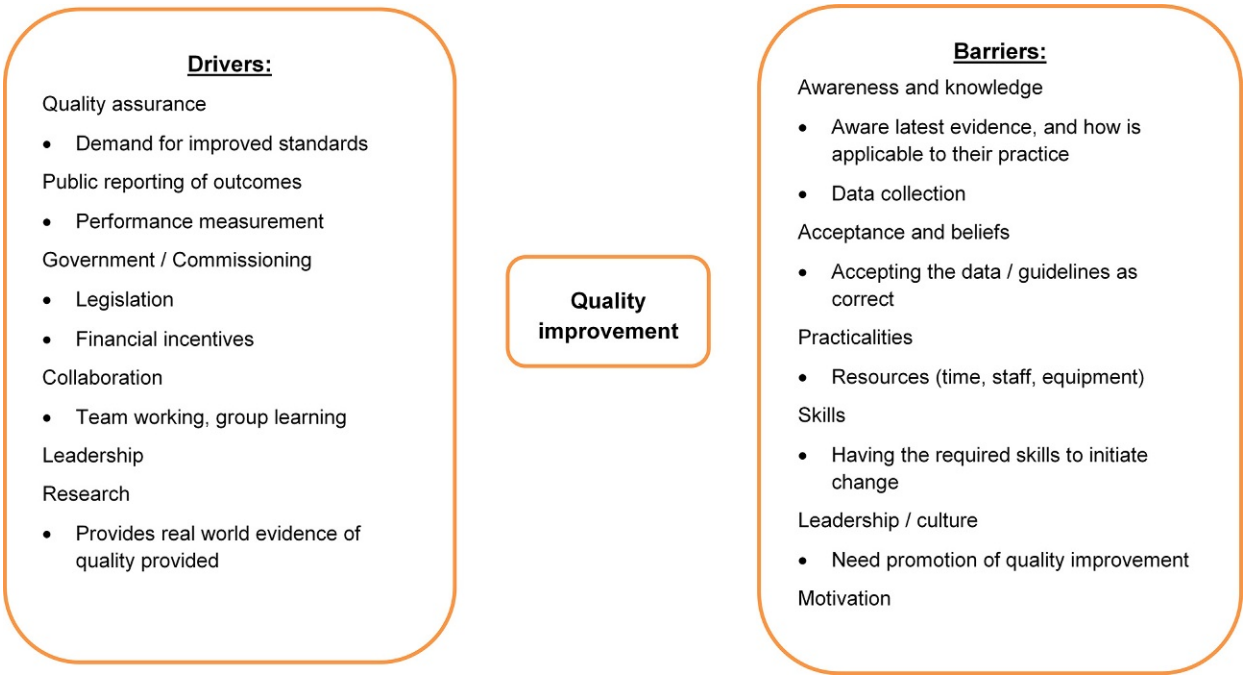

Fig. 2 Schematic representing the drivers and barriers to achieving quality improvement.

There remains a delay for the adoption of new interventions within quality indicators for example there were several randomized control trials demonstrating the benefit of clopidogrel for acute coronary syndrome patients published between 2001 and 2005 (Yusuf et al., 2001; Sabatine et al., 2005; Chen et al., 2005). Nevertheless in their 2008 paper the Canadian society remained unsure of the evidence (Tu et al., 2008), whilst the Americans only considered it as a test measure (Krumholz et al., 2008). For quality indicators to be effective they need to be updated regularly to ensure they are evaluating the most contemporary care interventions (Fig. 2).

Steps for Implementation

For the successful implementation of quality indicators there needs to be accurate and timely recording of the data required in their calculation. Given the increasing use of electronic health records in the modern world, this at face value seems like it should be straightforward. Clinical registries would therefore be an obvious choice for determining levels of performance. However, it is not as simple as it would appear, registries collect patient sensitive information and this must be handled appropriately and confidentiality. The data that registries collect must be both valid and accurate, if the data is to be used to assess and report quality. The collection and subsequent reporting of this data must also be timely to allow identification of underperforming services and allow steps for improvement to be implemented.

One exemplar of successful implementation is the American "Get with the Guidelines" initiative and the ACTION registry. For a hospital to be included in the "get with the guidelines" initiative, they have to pay for participation in the registry. In return, such hospitals receive detailed quarterly risk adjusted reports including both quality indicators and metrics in order to improve their standards (GWTG). In addition they have a hospital profile on the American College of Cardiology patient website highlighting their performance. This approach has been shown to lead to an improvement in guideline adherence that was higher than to be expected from the improvements seen with public reporting of results alone (Lewis et al., 2008). The success of this method, however, heavily depends on the nature of the healthcare system. In America, in a health insurance environment there is strong competition between providers based on hospital performance due to the patient's ability to choose the best performing hospitals. In a nationalized healthcare system, such an implementation strategy would not be possible.

When considering how to integrate the role of registries within quality improvement the 2015 statement by the ACC/AHH provides a framework (Bhatt et al., 2015). They demonstrate how registries can fit within the cycle of quality. (The cycle of quality was proposed by Califf et al. in their paper which considered how quality should be introduced into the therapeutic development cycle (Califf et al., 2002).) Registries can influence every stage of this cycle both by providing the data for analysis, identifying areas of discrepancy, and in time showing an improvement following an intervention. Traditionally registries have had short term or limited outcomes recorded, however, by linking to other data sources, such as the United Kingdom Myocardial Ischaemia National Audit Project and the Office of National Statistics for evaluation of mortality, or insurance claims data in the United States, allows for broader and longer term outcomes to be investigated (Fig. 3).

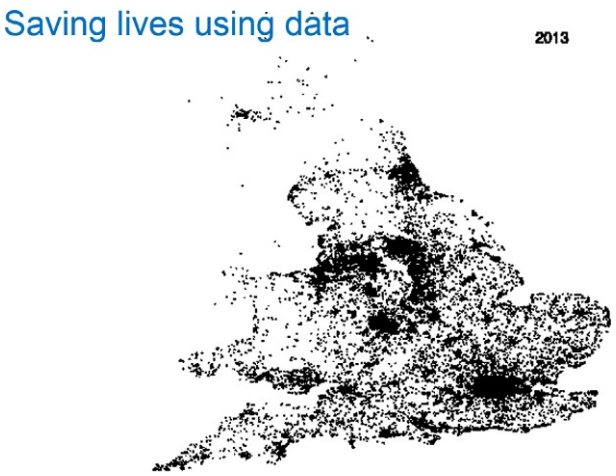

Fig. 3 A graphical representation of 5% of the cases within MINAP from 2003 to 2013, where each dot represents a case of acute myocardial infarction. MINAP is a mandatory registry for all cases of acute myocardial infarction within England and Wales.

Registries can and have been used for both international and within-country analyses of performance and also to assess temporal trends in performance (Chung et al., 2015; Hall et al., 2016; Saunderson et al., 2014; Dondo et al., 2016a,b). However, the measures used for comparison have varied. The existence of quality indicators does not automatically mean that each country collects the necessary data for their assessment. If quality indicators were designated as the standard gauge of quality then not only should it drive improvements in data collection as each registry/country would have to collect the same data, but also allow easier comparison both within and between countries. This in turn leads to more accurate and trustworthy reporting to drive further improvement in quality of care and outcomes.

The Future

Measuring quality is now recognized as a fundamental component of healthcare systems, as they strive for improved care and outcomes. Quality indicators provide a method for assessing the quality of care provided across the full spectrum of domains of patient care. They allow comparison, both within and between countries and the identification of variation can be a driver for change and improvement to lead to reductions in the premature mortality from coronary artery disease.

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Recreational Drugs: Effects on the Heart and Cardiovascular System

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Introduction

"Recreational drugs" are defined as substances "used without medical justification for. . .psychoactive effects often in the belief that occasional use of such a substance is not habit-forming or addictive" (Recreational Drug, 2017).

They can be delineated into three main subtypes: stimulants, depressants, and psychotropics. This chapter will seek to describe the cardiovascular, myocardial, electrophysiological, and valvular effects of the more commonly used recreational drugs in the world. These include ethanol, marijuana, cocaine, methamphetamine, opioids, psychedelics, and MDMA, along with other substances such as toluene, khat, and anabolic steroids.

Ethanol

Ethanol, known chemically as ethyl alcohol and in vernacular speech as *alcohol*, is a commonly used recreational drug used by millions in the United States. It is most commonly ingested as a liquid beverage. According to studies from the National Institute of Health, 87.6% of people over the age of 18 have used ethanol at some point in their lifetime (Center for Behavioral Health Statistics and Quality, 2014a). Heavy drinking and binge drinking have been proven to result in systemic arterial hypertension, coronary artery disease, cardiac arrhythmia, and sudden cardiac death (Husain et al., 2014).

Ethanol impacts the heart via several mechanisms: it disrupts cardiovascular metabolism by decreasing the amount of oxygen supplied to the heart, reducing levels of adenosine triphosphate and leading to a decrease in aerobic and an increase in anaerobic metabolism. Ethanol's metabolites, acetaldehyde and reactive oxygen species (ROS), decrease protein synthesis and expression; and ethanol increases expression of the *c-myc* gene, which contributes to apoptosis (Walker et al., 2013).

Ethanol has been documented to have both long- and short-term impact on blood pressure. Ethanol exhibits a dose-dependent increase in systolic blood pressure. The acute ingestion of ethanol can cause tachycardia and lower-than-average stroke volume (Guzzo-Merello et al., 2014). Long-term use of ethanol causes multiple pathologic occurrences including impairment of baroreceptors, increased sympathetic activity, and increased cortisol levels in the blood. Ethanol can also cause increased calcium in the blood, stimulation of the RAAS syndrome, and causes oxidative injury in the endothelium resulting in decreased endothelium-related nitric oxide production.

Ethanol also has a direct effect on the myocardium. Detrimental effects are found especially in the cases of heavy chronic ethanol users (over 80 g per day over at least 5 years). For reference, the National Institutes of Health defines one "standard" drink as containing 14 g of pure ethanol, equivalent to 12 oz of beer, 5 oz of wine, or 1.5 oz of hard liquor (Kerr and Stockwell, 2012). Ethanol causes alterations of the excitation–contraction coupling cascade, the structure and function of cardiac mitochondria and

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sarcoplasmic reticulum, and decreases contractile proteins. This can result in increased end-systolic and diastolic LV volumes, posterior wall thickening, and a decrease in ejection fraction (Kerr and Stockwell, 2012). Traditionally, such patients develop a dilated cardiomyopathy. However, this does not occur in every person who abuses ethanol.

Retrospective studies have looked at the prevalence of ethanol abuse in those who develop a dilated cardiomyopathy. While not all ethanol users develop a cardiomyopathy, recent studies show a prevalence of 0.43% in women and 0.25% in men who abuse ethanol, compared to a general population prevalence of 0.03%–0.05% (Guzzo-Merello et al., 2014). With cessation of ethanol and prompt treatment of the cardiomyopathy with beta blockers, angiotensin converting enzyme inhibitors, and diuretics, this cardiomyopathy can often be reversed. However, with continued use of ethanol, mortality figures as high as 42% over 3 years have been reported (George and Figueredo, 2010).

Ethanol has a well-established effect on nutritional deficiencies, especially in those with chronic usage. Especially common are deficiencies in magnesium and potassium, which can predispose patients to ventricular arrhythmias (Mahmoud et al., 2007). Magnesium deficiency is associated with ventricular tachycardia, torsades de pointes, and sudden cardiac death. Magnesium deficiency can also result in refractory hypokalemia, again causing ventricular tachycardias such as torsades de pointes.

Ethanol has a strong relationship to supraventricular tachycardias, specifically, atrial fibrillation.

Meta-analysis of numerous studies exploring the connection between ethanol and atrial fibrillation has noticed an increased risk of atrial fibrillation in those with daily heavy ethanol ingestion (approximately 42 g of ethanol a day). The pathologic mechanisms of ethanol affect the myocardium, creating a substrate that is highly proarrhythmic (Samokhvalov et al., 2010). Similarly, binge drinking of ethanol has long been recognized to be a cause of supraventricular tachycardias, even in those without a chronic ethanol habit, resulting in the so-called holiday heart syndrome. This appears to be a more common finding in those with underlying cardiac disease or cardiac comorbidities (Tonelo et al., 2013).

Marijuana—Natural and Synthetic Variations

Marijuana, derived from the plant *cannabis*, is the world's most commonly used illicit drug; the World United Nations Office on Drugs and Crime reporting approximately 3.8% of the world's population used the drug in 2014. Based on this figure, somewhere near 200 million people used cannabis in 2014, representing a 27% increase in users since 1998 (United Nations Office on Drugs, 2014).

In the United States, marijuana is a federally prohibited substance. It is classified as a schedule I substance in the Controlled Substances Act (United States Code, n.d.). In 2012 Colorado and Washington became the first states to legalize marijuana for recreational use by persons aged 21 and older. Since then Alaska, Oregon, Maine, Massachusetts, Nevada, California, and the District of Columbia have followed suit and adopted more expansive laws legalizing recreational marijuana use. Including the aforementioned states, a total of 29 states have adopted the use of medicinal marijuana (ProCon.org., 2017).

Marijuana is made from the drying of the leaves and flowers of the cannabis plant. The drug goes by many names including pot, weed, herb, and ganja. The active substance in marijuana is delta 9-tetrahydrocannabinol, or THC. THC works at the cannabinoid 1 receptor in the brain, adipose tissue, myocardium, vascular endothelium, and sympathetic nerve terminals and the cannabinoid 2 receptor in the lymphatic tissue, macrophages, and the periphery, ultimately producing its psychoactive effects (Steffens and Mach, 2006). The mechanism of delivery is most often inhalation either via combustion or vaporization, though ingestion is also a popular mode of delivery. The majority of data regarding the adverse effects of cannabis on the pulmonary, psychiatric, and cardiovascular system are associated with inhalation of the drug. Interestingly, noninhalational administration of cannabinoids has been shown to have some beneficial effects on the modulation of atherosclerotic processes in animal models (Singla et al., 2012).

More recently, considerable attention has come out about the medical use and potential benefits of cannabinoids. There is strong evidence to support their use in those with chronic pain and spasticity. There is less convincing evidence to support their use to treat nausea and vomiting secondary to chemotherapy and in anorexia secondary to HIV and AIDS (Whiting et al., 2015). Other papers have described the utility of cannabinoid therapy in treating the effects of PTSD, and it has shown some utility in treating symptoms of depression and anxiety; there are currently ongoing randomized placebo-controlled trials to study their efficacy.

Interestingly, although cannabis is the most commonly used illegal psychoactive substance, its use only constitutes a small proportion of the global disease burden relative to other substances. Large cross-sectional epidemiological studies suggest that of the approximately two million total disability adjusted life years lost to substance use disorders (excluding tobacco) marijuana only represented 5.5%, ranking 4th after alcohol (47%), opioids (24.3%), and amphetamines (7%) (Degenhardt et al., 2013). Given the current push for decriminalization, it is likely that this number may increase in the coming years.

Several hemodynamic effects of smoking marijuana have been well described. There is a dose-dependent increase in heart rate, peaking 10–30 min after inhalation. The magnitude of heart rate increase seems to be highly variable amongst users; however it has been shown to range from 20% to 100%. Postural hypotension after smoking marijuana has been well described and there seems to be a decrease in supine blood pressure as well. Onset of symptoms after ingestion of marijuana is typically longer, approximately 1 h, with peak plasma concentrations in the blood after 4–6 h (Huestis, 2007).

One study demonstrated that among patients with chronic stable angina, the anginal threshold is acutely decreased after smoking one marijuana cigarette. In this trial, smoking a marijuana cigarette decreased exercise time to angina by 48%. This represents a significantly greater effect relative to a tobacco cigarette (23%) and placebo (8.6%) (Aronow and Cassidy, 1974).

In the prospective Determinants of Myocardial Infarction Onset Study, 3886 adult in patients with MI were followed. In this cohort, 3.2% of patients had smoked marijuana in the past year. The marijuana user group were more likely to be male (94% versus 67%, $P<.001$), current cigarette smokers (68% versus 32%, $P<.001$), and obese (43% versus 32%, $P=.008$). Interestingly, marijuana users were less likely to have angina or hypertension. Using a case controlled analysis the risk of myocardial infarction onset was elevated 4.8 times over baseline (95% confidence interval, 2.4–9.5) in the 60 min after marijuana use. The elevated risk rapidly decreased thereafter (Mittleman et al., 2001).

In addition to myocardial infarction, marijuana use has been implicated in pathologic disease in other arterial beds. There have been multiple case reports of ischemic stroke during marijuana use in heavy users. Epidemiologic studies have looked at this association in an attempt to demonstrate causality based on the temporal relationship of marijuana use, exclusion of other risk factors for stroke, and recurrence of stroke with resumption of marijuana use (Hackam, 2015). A large cross-sectional survey of patient with acute ischemic stroke found that among younger adults, recreational marijuana use is independently associated with a 17% increased likelihood of hospitalization for acute ischemic stroke (odds ratio 1.17, 95% CI 1.15, 1.20) (Rumalla et al., 2016).

There is an increasing number of case reports describing a link between marijuana use and atrial fibrillation, especially in young people (Korantzopoulos et al., 2008; Krishnamoorthy et al., 2009). The mechanism of this is unclear although it is thought to be related to adrenergic stimulation and related autonomic alterations (Jones, 2002).

Cocaine

Cocaine, a derivative of the *coca* plant, is another commonly used illicit drug with adverse cardiovascular effects. Cocaine is legitimately utilized in some minor medical procedures, such as achieving hemostasis in ENT and nasal surgeries. As an illegal substance, cocaine is either ingested, insufflated, smoked, or injected into the veins. Cocaine can also be altered in a chemical reaction with sodium bicarbonate over heat to produce "crack" cocaine, a potent "free-base" form of cocaine that, when smoked, produces a rapid-onset high (Jeffcoat et al., 1989).

Cocaine is a commonly used stimulant which induces an intense feeling of increased energy and euphoria, due to its effect in the neurotransmitter synapses in the central nervous system. In the cardiovascular system, cocaine operates as an alpha-1 and beta-1 agonist, causing increased heart rate, increased myocardial oxygen uptake, and increased cardiac contractility (Liaudet et al., 2014).

The effect of cocaine in raising systolic blood pressure is due to the stimulatory effect on alpha-1 receptors in the periphery (Schwartz et al., 2010), as well as stimulating the release of endothelin I, a potent vasoconstrictor, from endothelial cells and inhibiting the production of nitric oxide (Schwartz et al., 2010). It also deregulates receptors on the endothelium of blood vessels, making vessels more sensitive to sympathetic stimulation. Additionally, cocaine acts as a platelet activator, increasing levels of von-willebrand factor and fibrinogen, promoting thrombosis (Schwartz et al., 2010).

Cocaine has a direct effect on both the myocardium and conduction system of the heart. The pro-thrombotic state induced by cocaine can result in intra-coronary thrombosis, causing myocardial infarction. Cocaine increases blood pressure, increases heart rate, and increases cardiac contractility, all of which increase myocardial oxygen demand in the setting of vasospasm of the coronary arteries, may result in ischemia and possible infarction (Hollander, 2008).

Cocaine also operates similarly to a class IC antiarrhythmic, blocking sodium and potassium channels and prolonging the effective refractory period, the PR, QRS, and QT intervals, and predisposing patients to arrhythmia (Hoffman, 2010). Commonly, reentry supraventricular tachycardia is seen, and also ventricular tachycardia which can in some cases degrade into ventricular fibrillation, causing cardiac arrest. Atrial fibrillation after cocaine use has also been reported.

Cocaine has no direct effects on the valves of the heart, though those that inject cocaine are more at risk for endocarditis secondary to the effect of contaminated needles and poor hygiene. Chronic cocaine usage has been shown to result in a dilated cardiomyopathy; the mechanism behind this is thought to possibly be due to repeated catecholamine surges and therefore repeated episodes of subendocardial ischemia (Frustaci et al., 2015).

Interestingly, the concurrent metabolism of cocaine and ethanol in the liver by transesterification of cocaine creates a metabolite called cocaethylene, which is structurally similar to cocaine, but has a different effect on central and peripheral neurotransmitters than cocaine alone. Cocaethylene causes a profound decrease in ventricular contractile function and stroke volume, and also has a strong negative inotropic effect (Herbst et al., 2011). In fact, the co-administration of these two substances in an animal trial resulted in a prolonged cardiac depression and also increased the prevalence of ventricular tachycardia.

Methamphetamine/Stimulants

Methamphetamine and related "stimulants" are the second most commonly used illicit drug in the world, second only to cannabis. Methamphetamine is medically indicated for use in patients to treat narcolepsy, attention-deficit hyperactivity disorder, and obesity. Methamphetamine itself is a synthetic derivation of ephedrine, which comes from the Ephedra genus of Chinese shrubs (Won et al., 2013).

Methamphetamine like compounds are derived from the B-phenylethylamine core structure. They easily cross the blood–brain barrier and resist biotransformation and release monoamine neurotransmitters from nerve endings. Different compounds are derived by substitutions at the aromatic ring and the alpha and beta carbons of the aliphatic chain and amine terminal. These substitutions create different characteristics in each compound, some with greater psychomotor stimulation, anorectic, or hallucinogenic effects (Carvalho et al., 2012).

Methamphetamine acts physiologically to release serotonin, dopamine, norepinephrine, and epinephrine into the neuronal synapses. This results in increased alertness, euphoria, energy, and decreased appetite. Cardiovascularly, it can increase both heart rate and blood pressure. It is usually smoked, injected, or insufflated (Won et al., 2013).

Methamphetamine has been strongly associated with both hypertrophic and dilated cardiomyopathies. Histologically, fibrosis and necrosis of cardiac myocytes have been documented. These effects are likely multifactorial in origin. A proposed physiologic mechanism for methamphetamine-associated cardiomyopathy has been catecholamine excess, coronary vasospasm, effects of the generation of reactive oxygen species (Lord et al., 2010).

Interestingly, some methamphetamine derivatives, most notably a diet suppressant called fenfluramine–phentermine (Fen-Phen), have been associated with valvular thickening and dysfunction. This is thought to be due to fibroplasia of the endocardium of the right heart valves, brought by serotonin-rich blood coming back to the right side of the heart following metabolism in the liver. Additional effects of methamphetamine derivatives on the left-sided heart valves have also been described (Connolly et al., 1997). Fenfluramine and other methamphetamine derivatives have also been shown to cause pulmonary hypertension resulting in right heart failure (Mark et al., 1997). It was for its detrimental effects on cardiac valves that the Centers for Disease Control ordered a withdrawal of the sale and distribution of Fen-Phen in 1997 (Centers for Disease Control, 1997).

Limited retrospective studies have shown an increased prevalence of QTc prolongation in subjects with methamphetamine usage, in relation to the general population (Haning and Goebert, 2007).

Opioids

The term opioid refers to natural or synthetic substances that act at the mu, kappa, or delta receptor sites whose biochemical structure is derived from the naturally occurring alkaloids produced by the opium poppy plant (*Papaver somniferum*). Delta and kappa receptors are found in the central and peripheral nervous system and are mainly responsible for analgesic and sedative effects, while stimulation of mu receptors results in respiratory depression and reduction in GI motility (Chen and Ashuburne, 2015).

Their use and abuse go back millennia and were described as far back as 1500 BC when grains of the poppy plant were used to form a paste and administered to prevent "excessive crying in children" as described in the *Ebers Papyrus*. In 1806, the active ingredient in opium was isolated by Serturner, who named the substance Morphine after the god of dreams, Morpheus (Brownstein, 1993). Codeine was isolated from opium several years later. While incredibly effective as analgesics, both of these agents had as much potential for abuse as opium and the search for safer, less addictive alternatives started in the 19th century and continues still. The first of these "safer" opiates was heroin, which was produced in 1898 by the Bayer Company, who pronounced it to be more potent than morphine and initially thought to be free from abuse liability.

By the 1920s, doctors were aware of the highly addictive nature of opioids and tried to avoid treating patients with them. Heroin became illegal in 1924. Methadone is a synthetic compound derived in 1946 as a structurally unrelated compound to morphine with similar pharmacologic properties. Several other synthetic opioids have been engineered since then, none of which have been free of addictive potential. Both the natural and synthetic forms have different onsets and durations of action. For instance, morphine takes approximately 15 min to reach near-full effectiveness, and its analgesic effect lasts approximately 1.5–7 h. Fentanyl, a synthetic opioid more potent than morphine, has an onset of action of 5 min, but duration of action of only 30 min (Vardanyan and Hruby, 2014).

Opioid abuse and addiction is one of our top national health challenges in the United States. Although opioids usually are prescribed to control pain, diminish cough, or relieve diarrhea, they also produce feelings of euphoria, tranquility, and sedation that ultimately lead to abuse. Overall, an estimated 400,000 persons have used heroin in the past month and 4 million have reported nonmedical use of prescription pain relievers in our country (Center for Behavioral Health Statistics and Quality, 2014b).

Heroin and prescription opioids share many routes of administration beyond ingestion. Intravenous is the most rapid route but also the most dangerous to the user while intranasal, subcutaneous injection, and inhalation remain popular routes of delivery for abusers. The cardiovascular effects of opioids are in part related to route of administration, with infective endocarditis being a major problem among intravenous drug users (IVDU) and electrophysiologic consequences present regardless of route of administrations. The incidence of infective endocarditis in injection drug users is thought to be 1.5–3.3 cases per 1000 patient years in the United States (Baddour et al., 2005). Some opioids have been associated with inhibition of the hERG protein which regulates the I_{kr} repolarization channel, responsible for returning the heart to its resting voltage potential during diastole. Theoretically, this causes prolongation of the QT interval. There is a small amount of observational evidence showing a dose-dependent relationship between amount of oxycodone (an opiate derivative) and prolongation of the QT interval.

Methadone is a commonly used replacement therapy for those attempting detoxification from opioid abuse. It is a chiral opioid with primarily mu receptor agonist properties, and influences the release of other neurotransmitters (acetylcholine, norepinephrine, substance P, and dopamine) (Lusetti et al., 2016). It can be taken orally and has a half-life of over a day due to its lipophilicity and ability to remain in the tissues longer than other formulations of opioids. It has been discovered to result in increased prevalence of prolonged QT and incidence of torsades de pointes in patients undergoing methadone replacement therapy, especially within the first two weeks of starting treatment. This is again thought to be due to the effect that opiates seem to have on the hERG protein. Importantly, those of the female gender, those with underlying heart disease, and those with metabolic derangements such as hypokalemia are at an especially high risk of developing prolonged QT from methadone usage (Wedam and Haigney, 2016; Vieweg et al., 2013).

Psychedelics: LSD and Psilocybin

Lysergic acid diethylamide (LSD) and psilocybin are recreational substances that can induce feelings of euphoria, mind alteration, paranoia, and visual and auditory hallucinations. LSD is a derivative of the ergot fungus, originally synthesized by Albert Hoffman in 1938. Psilocybin is the psychedelic compound active in "magic mushrooms." Both of these psychedelics are usually taken by mouth to induce their effects.

The psychedelics chemically resemble serotonin. LSD has agonist, partial agonist, and antagonist effects at serotoninergic, dopaminergic, and adrenergic receptors, in contrast, psilocybin has serotonergic, but no dopaminergic effects (Borowiak et al., 1998). LSD is synthetically derived, and is approximately 100 times more potent than psilocybin. Ingestion of LSD can cause visual and auditory hallucinations, along with episodes of euphoria and paranoia. LSD can induce a mild sympathetic response, though not to the degree that cocaine or other stimulants cause. The effect on adrenergic receptors in the body can lead to tachycardia and hypertension (Ghuran and Nolan, 2000).

Both LSD and psilocybin do not appear to have well-established effects on the myocardium. However, there have been isolated case reports describing psilocybin usage as the possible instigator of myocardial infarction, though this appears exceedingly rare (Borowiak et al., 1998).

MDMA

3,4-Methylenedioxymethamphetamine (MDMA) is a commonly used recreational drug, and is especially popular among the "rave" club culture. It generally promotes a feeling of euphoria, increased self-confidence, increased sociability, and arousability. It is also known by other names including "ecstasy" and "molly." Figures from the 2011 National Survey on Health and Drug use estimated that approximately 14.5 million people in the United States above the age of 14 have tried ecstasy at least once (Badon et al., 2002). It is most commonly ingested by insufflation or by mouth. It has a 30-min onset of action and has a half-life of approximately 6–8 h.

Structurally, it is a ring-substituted amphetamine that mainly induces release of serotonin (SERT), and to a lesser degree, dopamine (DAT) and norepinephrine as well. It mainly acts on the SERT and DAT receptors, modulating serotonin and dopamine reuptake channels, respectively (Hagino et al., 2001).

Cardiovascularly, MDMA has been studied in both human and animal models. Increases in heart rate and blood pressure after administration of MDMA have been documented in rat models (Bexis and Docherty, 2006). In humans, it increases the systolic and diastolic blood pressure, and also increases heart rate, especially when dosed to over 1 mg/kg in human subjects (Dumont and Verkes, 2006).

There has been increasing research into the effects of MDMA on the cardiac tissue itself. It appears as though MDMA has a detrimental effect on the myocardial antioxidant defense system and can cause oxidative stress which disrupts calcium homeostasis in myocytes. In animal models, contraction band necrosis was noticed after only 6 h after MDMA administration (Daniela et al., 2008). MDMA is a modulator of the SERT reuptake channel, and inhibits serotonin from being taken up by the presympathetic innervating cell. Recent research has established a connection between SERT and its modulation of cardiac and smooth muscle contractility, platelet aggregation, and cell mitogenesis, and appears to increase myocardial cell metabolism (Ni and Watts, 2006). These effects increase risk of both myocardial infarction and pulmonary hypertension. MDMA does appear to have in-vitro proliferative effects on human cardiac valvular cells, similar to fenfluramine. It can cause a similar effect of prolonged mitogenic responses indicative of processes similar to heart valve fibroplasia (Setola et al., 2003).

Limited studies have also shown a relationship between MDMA and cardiac arrhythmia, mainly supraventricular tachycardia variants, especially when "binging" on MDMA (Badon et al., 2002). This is thought to be due to the effect that MDMA has on cardiac cell homeostasis and possible sympathetic hyperstimulation.

Other Recreational Drugs

Toluene

Toluene is an aromatic hydrocarbon. It is a mono-substituted benzene derivative, consisting of a CH_3 group attached to a phenyl group in chemical structure. It is a clear and colorless solvent that is often added to various chemical compounds including gasoline. It is also used as a key ingredient in paint thinners and adhesives. Toluene vapor is exhaled unchanged, while the rest enters the bloodstream via absorption from the alveoli. Toluene dose-dependently and reversibly inhibits NMDA receptors and enhances GABA by increasing the intracellular calcium concentration in the presynaptic terminal (Beckley and Woodward, 2013). It is especially popular with teenagers in developing countries, owing to its easy accessibility and relatively low cost. In rat models, high levels of administered toluene resulted in bradycardia and eventual asystole, while subacute levels resulted in repolarization disorders and arrhythmia (Morvai et al., 1976). In case reports of human insufflation, toluene has been reported to cause myocardial infarction. The exact mechanism of this has not been proven, but it appears that toluene causes an increased sensitivity to catecholamines, resulting in tachycardia, coronary vasospasm, and resultant infarction. Toluene also appears to play a role in inhibiting sodium channels in cardiac myocytes which are responsible for depolarization (Daniel, 2008). During acute intoxications of toluene, EKG changes such as bundle branch blocks and prolonged QRS complexes are common, which can lead to ventricular arrhythmias and sudden death.

Khat

Khat is a commonly used recreational substance in the Middle East and the horn of Africa. It is made from the leaves of the *catha edulis forsk* plant, and is structurally related to amphetamine, noradrenaline, and ecstasy (MDMA). The main ingredients of the Khat plant are cathinone and cathine. It is chewed or ingested for its euphoric effects, and is also used to improve performance (Ali et al., 2010). It has central nervous system stimulatory effects derived from the cathinone found in the leaves. It appears to increase the amount of noradrenaline in synapses in the peripheral vasculature, which causes increased stimulation of alpha receptors and amine-associated receptors (Al-Motarreb et al., 2010). Cardiovascularly, khat increases diastolic blood pressure and also increases inotropic and chronotropic effects (Al-Motarreb, 2004). Those who chew khat for longer than 6 h each day are at an increased risk for acute myocardial infarction. There does not appear to be a direct effect of khat on the myocardium, but it is thought that the relationship between khat's vasoconstrictive effects and sympathetic effects may mediate cardiomyopathy and MI as previously described (El-Menyar et al., 2015).

Similar to amphetamine, cathinone causes coronary artery vasospasm, and it also causes a prothrombotic state that can lead to infarction. There are also associations between khat usage and increased prevalence of cardiac arrhythmia. A study done in Yemen, the country with the highest percentage of khat users, used Holter monitoring to track for occurrences of arrhythmia in khat users, with and without underlying cardiac disease. The study revealed a higher prevalence of ventricular tachyarrhythmias (mostly NSVT) in both populations on days where khat was ingested (Jayed and Al-Huthi, 2016). The researchers involved in this particular study even went as far as to suggest prophylactic low dose beta-blocker to prevent arrhythmia in heavy users of khat.

Anabolic and Androgenic Steroids

Exogenous anabolic and androgenic steroids (AAS) are synthetic derivatives of testosterone originally developed in the late 1930s. The chemical structure is derived from cholesterol that is synthesized in the liver, and then metabolized in the adrenal glands and testes to AAS. It is estimated that more than 3 million individuals in the United States abuse AAS. This abuse led Congress to add AAS to Schedule 3 of the Controlled Substances Act (Achar et al., 2010). AAS can be administered orally, parenterally, and transdermally, and act by binding to the nuclear androgen receptor in the cytoplasm, and then translocate into the nucleus. This binding results in sequential conformational changes of the receptor affecting the interaction between receptors and proteins. The basic structure of all AAS is a perhydrocyclopentano phenanthrene ring. Modification of structures side chains allows for production of different designer drugs within this class (Frati et al., 2015).

AAS were also noted as drugs of abuse as early as the mid-1950s, when it was discovered that a championship weightlifting team from the Soviet Union were taking these supplements to contribute to their skeletal muscle strength and weightlifting ability (Golestani et al., 2012). Since that time, AAS have continued to be used drugs of abuse, especially in competitive athletes. It is the most commonly found prohibited substance found in drug tests across the spectrum of different sports. AAS have a number of proven cardiovascular side effects. They have been shown to increase resting diastolic and systolic blood pressures (Grace et al., 2003). AAS also cause a dose-dependent increase in low-density lipoproteins (LDL) and a dose-dependent decrease in cardioprotective high-density lipoproteins (HDL) (Baggish et al., 2010). Supratherapeutic doses of AAS also seem to contribute to decreased ability of coronary arteries to vasodilate, and may promote platelet aggregation through increased expression of thromboxane A2 receptors.

Exogenous AAS have also been shown to contribute to increased left ventricular hypertrophy (LVH) (Baggish et al., 2010). This is thought to be not only by increasing blood pressure, but also through direct stimulation of androgen receptors on cardiac myocytes that cause LVH. Additionally, postmortem studies of those with a history of AAS abuse do show evidence of myocardial fibrosis and cardiac steatosis. This hypertrophy creates a substrate which is more conducive to cardiac arrhythmia with resultant sudden cardiac death (Payne et al., 2004).

Chart: Commonly used recreational drugs and main cardiovascular effects

Drug type	*Physiologic effects*	*Myocardium effects*	*Arrhythmia*	*Valvular*
Ethanol	Acutely: - high heart rate - low stroke volume Chronic usage: - impaired baroreceptor function- increased sympathetic activity - decreased endothelial nitric oxide production	- Increased end-systolic and diastolic LV volumes - Posterior LV wall thickening - Decrease in ejection fraction - Dilated cardiomyopathy	- With nutritional deficiencies, increased risk of ventricular tachycardia, torsades de pointes. - Ingestion of >3 alcoholic drinks a day linked to increased risk for atrial fibrillation	N/A

(*Continued*)

(Continued)

Drug type	*Physiologic effects*	*Myocardium effects*	*Arrhythmia*	*Valvular*
Marijuana	- Increased heart rate - Postural hypotension - Higher incidence of ischemic stroke	- Increased risk of myocardial infarction in time period directly after inhalation	Atrial fibrillation	N/A
Cocaine	- Increased blood pressure - Vasospasm - Platelet activation - Increased vWf activity - Increased thrombosis	- Increased cardiac contractility - Dilated cardiomyopathy - Higher risk for myocardial infarction	- Activity similar to IC antiarrhythmic. - Prolongs effective refractory period, prolongs PR, QRS, QT	- Endocarditis risk in users of intravenous cocaine
Stimulants	- Increased heart rate - Increased blood pressure	- Hypertrophic cardiomyopathy - Dilatory cardiomyopathy - Myocyte fibrosis and necrosis	- QTc prolongation	- Right heart valvulopathy, hyperplasia - Pulmonary artery hypertension
Opioids	- Decreased respiratory drive - Slowed heart rate	- Possibly cardioprotective effects postinfarction	- Prolonged QTc	- Endocarditis risk in users of intravenous opioids
MDMA	- Increased blood pressure - Increased heart rate	- Increased oxidative stress - Increased contraction band necrosis	- Supraventricular tachycardia	- Heart valve hyperplasia

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Relationship Between Vegetables and Fruits (Antioxidant Vitamins, Minerals, and Fiber) Intake and Risk of Cardiovascular Disease

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Cardiovascular disease (CVD) is the leading noncommunicable disease (NCD), and caused nearly half of deaths due to NCD (Mendis et al., 2011). To prevent CVD, it is necessary to improve lifestyles and constitutional risk factors such as hypertension, diabetes, and dyslipidemia. The guidelines for prevention of CVD recommend smoking cessation, increased physical activity, weight control, and dietary modifications. Dietary modifications include reduced intakes of total fat, saturated fat, and salt, and increased intakes of fruits and vegetables (World Health Organization, 2007). Diet is an important factor to prevent CVD because habitually diets influence various body metabolisms that are involved in the pathogeneses of CVD. The present review focuses on intakes of fruits and vegetables.

Several studies have shown that higher intakes of fruits and vegetables have been associated with lower risk of CVD, coronary heart disease (CHD), and stroke (**Table 1**). A metaanalysis has shown statistically significant inverse associations between intakes of fruits and vegetables and the risk of CHD in Americans and Europeans, indicating that the pooled relative risks (RRs) and 95% confidence intervals (95%CIs) of CHD for one-portion/day increment were 0.96 (0.93–0.99) in total fruit/vegetable intakes, 0.93 (0.89–0.96) in fruit intakes and 0.89 (0.83–0.95) in vegetable intakes (Dauchet et al., 2006). Two metaanalyses have also reported that intakes of fruits and vegetable were inversely associated with the risk of stroke in Americans, Europeans, and Asians (Dauchet et al., 2005; He et al., 2006), and a subgroup analysis for risk of stroke showed that intakes of total fruits/vegetables had significant protective effect on risk of both ischemic and hemorrhagic strokes; the pooled RRs (95%CIs) of subjects who took fruit and vegetable more than five servings per day were 0.72 (0.66–0.79) and 0.73 (0.61–0.87), compared with subjects who took them less than three servings per day, respectively (He et al., 2006). In a British survey, those who ate more than three portions of total fruits/vegetables daily were significantly at lower risk of mortality from CVD in a dose-response manner; the hazard ratios (HRs) (95%CIs) were 0.82 (0.70–0.95) for 3–5 portions, 0.80 (0.66–0.96) for 5–7 portions, and 0.69 (0.53–0.88) for 7+ portions compared with those who ate <1 portion per day (Oyebode et al., 2014). After further adjustment for intakes of other foods in two Asian cohort studies, the inverse association between intakes of fruits and vegetables with mortality from CVD was significant; HR (95%CI) was 0.74 (0.61–0.91) for the highest quartile of fruit and vegetable intake, compared with the lowest (Okuda et al., 2015), and 0.77 (0.67–0.88) for the highest quartile of fruit intake, compared with the lowest (Nagura et al., 2009). A subgroup analysis by fruits intake in the UK Women's Cohort Study showed that higher intakes of fresh fruit but not dried fruit or fruit juice was associated with

Table 1 Intakes of vegetables and fruits and the risk of cardiovascular disease

Published year study population	*Study name or setting name*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Dauchet et al. (2006) Pooled analysis of nine cohorts	ARIC, ATBC, BLSA, HPFS, NHANES, NHS, PHS, WHS, Mobile clinic of social insurance study	221,080 (91,379 men and 129,701 women)		Metaanalysis	5–19	Dietary intake by food record, diet history methods, or FFQ		For the risk of CHD, RRs = 0.96 (0.93–0.99) for total fruit and vegetable, 0.93 (0.89–0.96) for fruit, and 0.89 (0.83–0.95) for vegetable (each increment of 1 portion/day)
He et al. (2006) Pooled analysis of nine cohorts	ARIC, DDCH, HPFS, LSS, NHANES I, NHS, the Framingham study, the Zutphen study, cohort of Finnish male smokers	257,551		Metaanalysis	13	Dietary intake by FFQ, dietary history method, or 24-h dietary recall		For the risk of stroke, RRs = 0.89 (0.83–0.97) and 0.74 (0.69–0.79) for 3–4 and >5 servings of total fruit/vegetable compared to <3 servings For the risk of stroke, RRs = 0.71 (0.63–0.80) for total fruit and vegetable in men and 0.76 (0.69–0.83) in women (comparing >5 servings with <3 servings) For the risk of ischemic and hemorrhagic stroke, RRs = 0.72 (0.66–0.79) and 0.73 (0.61–0.87) for total fruit and vegetable (comparing >5 servings to <3 serving) For the risk of stroke, RRs = 0.72 (0.66–0.79) for fruit and 0.81 (0.72–0.90) for vegetable (comparing >5 servings to <3 servings)
Dauchet et al. (2005) Pooled analysis of seven cohorts	ARIC, HPFS, LSS, NHANES, NHS, the Framingham study, cohort of Danish men and women	90,513 men and 141,536 women	25–103	Metaanalysis	3–20	Dietary intake by food record, diet history methods, or FFQ		For the risk of stroke, RRs = 0.95 (0.92–0.97) for total fruit and vegetable, 0.89 (0.85–0.93) for fruit and 0.97 (0.92–1.04) for vegetable (each increment of 1 portion/day)
Oyebode et al. (2014) UK	The Health Survey for England	65,226	35+	Prospective study	7.7	Dietary intake of fruit and vegetable consumption on the previous day (24 h from midnightto midnight)	Age, sex, social class, cigarette smoking, BMI, physical activity, education, and alcohol intake Age, sex, social class, cigarette smoking, BMI, physical activity, education, and alcohol intake, and further portions of vegetables/ fruits consumption	For CVD mortality, HRs = 0.91 (0.78–1.05), 0.82 (0.70–0.95), 0.80 (0.66–0.96), and 0.69 (0.53–0.88) for total fruit and vegetable (comparing 1–<3, 3–<5, 5–<7 and 7+ portions to 0–<1 portions) HR = 0.82 (0.68–0.98) of CVD mortality for the highest intakes of fruit (comparing 4+ portion to 0–<1 portions) HR = 0.78 (0.60–1.01) of CVD mortality for the highest intakes of vegetable (comparing 3+ portions to 0–<1 portions)

Okuda et al. (2015) Japanese	The NIPPON DATA80 Study	9112 (4000 men and 5112 women)	30–79	Prospective study	24	Dietary intake by 3-day dietary records	Age, sex, BMI, smoking habit, drinking habit and sodium intake	For CVD mortality, HRs = 0.74 (0.61–0.90) for total fruit and vegetable, 0.78 (0.65–0.96) for fruit, and 0.80 (0.65–0.98) for vegetable (comparing the highest vs. lowest quintiles) For stroke mortality, HRs = 0.77 (0.57–1.05) for total fruit and vegetable, 0.68 (0.52–0.89) for fruit, and 0.79 (0.59–1.07) for vegetable (comparing the highest vs. lowest quintiles) For CHD mortality, HRs = 0.57 (0.38–0.87) for total fruit and vegetable, 0.91 (0.60–1.39) for fruit, and 0.63 (0.41–0.98) for vegetable (comparing the highest vs. lowest quintiles)
							Age, sex, BMI, smoking habit, drinking habit and sodium intake; and further intakes of meat, fish and shellfish, milk and dairy products and soybeans and legumes	For CVD mortality, HRs = 0.74 (0.61–0.91) for total fruit and vegetable, 0.79 (0.65–0.96) for fruit, and 0.81 (0.66–1.00) for vegetable (comparing the highest vs. lowest quintiles) For stroke mortality, HRs = 0.80 (0.59–1.09) for total fruit and vegetable, 0.72 (0.54–0.95) for fruit, and 0.81 (0.60–1.09) for vegetable (comparing the highest vs. lowest quintiles) For CHD mortality, HRs = 0.57 (0.37–0.87) for total fruit and vegetable, 0.89 (0.58–1.36) for fruit, and 0.65 (0.42–1.02) for vegetable (comparing the highest vs. lowest quintiles)
Nagura et al. (2009) Japanese	JACC	25,206 men and 34,279 women	40–79	Prospective study	12.7	Dietary intake by FFQ	Sex, age, BMI, smoking status, alcohol intake, hours of walking, hours of sleep, education years, perceived mental stress, cholesterol intake, SFA intake, n-3 fatty acids intake, sodium intake and histories of hypertension and diabetes	For CVD mortality, HRs = 0.75 (0.66–0.85) for fruit and 0.88 (0.78–0.99) for vegetable (comparing the highest vs. lowest quintiles) For stroke mortality, HRs = 0.67 (0.55–0.81) for fruit and 0.97 (0.81–1.16) for vegetable (comparing the highest vs. lowest quintiles) For CHD mortality, HRs = 0.74 (0.55–0.99) for fruit and 0.77 (0.58–1.00) for vegetable (comparing the highest vs. lowest quintiles)
							Sex, age, BMI, smoking status, alcohol intake, hours of walking, hours of sleep, education years, perceived mental stress, cholesterol intake, SFA intake, n-3 fatty acids intake, sodium intake and histories of hypertension and diabetes, and further vegetable/fruit and bean intakes	For CVD mortality, HRs = 0.77 (0.67–0.88) for fruit and 0.96 (0.84–1.10) for vegetable (comparing the highest vs. lowest quintiles) For stroke mortality, HRs = 0.65 (0.53–0.80) for fruit and 1.09 (0.90–1.33) for vegetable (comparing the highest vs. lowest quintiles) For CHD mortality, HRs = 0.79 (0.58–1.08) for fruit and 0.85 (0.64–1.14) for vegetable (comparing the highest vs. lowest quintiles)

(*Continued*)

Table 1 (Continued)

Published year study population	*Study name or setting name*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Lai et al. (2015) UK women	UKWCS	30,458 women	35–69	Prospective study	16.7	Dietary intake by FFQ	Age, BMI, physical activity, smoking status, socioeconomic status, alcohol intake, total vegetable intake, and mutual adjustments for fruits that are not in the exposure category	HR = 0.57 (0.34–0.95) of CVD mortality for the highest vs. lowest quintiles of total fruit intake HR = 0.70 (0.35–1.40) of stroke mortality for the highest vs. lowest quintiles of total fruit intake HR = 0.45 (0.21–0.97) of CHD mortality for the highest vs. lowest quintiles of total fruit intake For CVD mortality, HR = 0.56 (0.33–0.96) for fresh fruit, 0.85 (0.53–1.37) for dried fruit, and 0.81 (0.52–1.27) for fruit juice (comparing the highest vs. lowest quintiles) For stroke mortality, HR = 0.78 (0.38–1.59) for fresh fruit, 0.93 (0.48–1.81) for dried fruit and 0.67 (0.35–1.29) for fruit juice (comparing the highest vs. lowest quintiles) For CHD mortality, HR = 0.39 (0.18–0.87) for fresh fruit, 0.79 (0.40–1.54) for dried fruit and 0.99 (0.53–1.85) for fruit juice (comparing the highest vs. lowest quintiles)

ARIC, Arosclerosis Risk in Communities Study; *ATBC*, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; *BLSA*, Baltimore longitudinal study of aging; *BMI*, body mass index; *CHD*, coronary heart disease; *DDCH*, Danish Diet, Cancer and Health study; *FFQ*, food frequency questionnaire; *HPFS*, Health Professionals Follow-up Study; *HR*, hazard ratio; *IDF*, insoluble dietary fiber; *JACC*, Japan Collaborative Cohort Study; *LSS*, Life Span Study (Hiroshima/Nagasaki); *NHANES*, National Health and Nutrition Examination Survey; *NHS*, Nurses' health study; *PHS*, Physicians' health study; *RR*, relative risk; *SDF*, soluble dietary fiber; *TDF*, total dietary fiber; *UKWCS*, United Kingdom Women's Cohort Study; *WHS*, Women's Health Study.

lower mortality from CVD; the HRs (95%CIs) were 0.56 (0.33–0.96) for the highest versus lowest quintile of fresh fruits, 0.85 (0.53–1.37) for dry fruits, and 0.81 (0.52–1.27) for fruits juice (Lai et al., 2015). Therefore, higher intakes of fruits and vegetables might have a role in the prevention of CVD, stroke, and CHD.

Through antioxidant effects of vitamins, metabolism-control effect of minerals, and cholesterol-lowering effect of dietary fiber, vegetables and fruits have been postulated in many epidemiological and clinical studies as biological explanations for reducing the risk of CVD.

In this review, we summarize the results of epidemiological studies, which examined the associations between these nutrients intakes with the risk of CVD.

Antioxidant Vitamins

The preventive effects of vegetables and fruits on CVD events such as CHD and stroke have been potentially attributed to the reduced oxidative stress by antioxidants (Asplund, 2002). Carotenoids such as α- and β-carotene and vitamin C exert antioxidant functions by quenching singlet molecular oxygen or free radicals (Tapiero et al., 2004). Vitamin C also reverses endothelial vasomotor dysfunction in the circulatory system (Levine et al., 1996). Vitamin E is an important natural antioxidant which protects against the oxidation of biomembrane lipids, thereby maintaining the stability of the biomembranes (Burlakova et al., 1998). The most common and biologically active form of vitamin E is α-tocopherol, which accounts for 90% of total vitamin E in tissues (Engin, 2009). Here we focus on prospective observational studies that have discussed the associations of dietary and supplemental intakes of these vitamins with the risk of CVD. The interventional studies of vegetables and fruits are summarized in the other section.

Carotenoids

Prospective studies on associations between carotenoid intake and CVD are summarized in **Table 2**. A pooled analysis of nine cohort studies reported no association between intakes of total and specific carotenoid with risk of major CHD events (Knekt et al., 2004). Among eight prospective cohort studies on CHD mortality and incidence (Buijsse et al., 2008; Genkinger et al., 2004; Klipstein-Grobusch et al., 1999; Knekt et al., 1994; Kushi et al., 1996; Osganian et al., 2003; Rimm et al., 1996; Sahyoun et al., 1996), two studies have reported a statistically significant lower risk of CHD incidence for the highest versus lowest quintiles of dietary carotenoid intake; among US male health professionals; the RR (95%CI) for carotene intake was 0.71 (0.53–0.86) (Rimm et al., 1993); among US women; the RRs (95%CI) were 0.80 (0.65–0.99) for alpha-carotene intake and 0.74 (0.59–0.93) for beta-carotene intake. Another study among Dutch elderly men reported inverse associations between dietary carotenoid intake and risk of CVD mortality; the RRs (95%CI) for the highest versus the lowest tertile of intake were 0.82 (0.69–0.98) for total carotenoid, 0.81 (0.66–0.99) for alpha-carotene, and 0.80 (0.66–0.97) for beta-carotene (Buijsse et al., 2008). However, some other studies reported no associations for total or specific carotenoids intakes with the risk of CVD. Among eight prospective cohort studies on stroke mortality and incidence (Ascherio et al., 1999; Daviglus et al., 1997; Del Rio et al., 2011; Hirvonen et al., 2000; Keli et al., 1996Ross et al., 1997; Voko et al., 2003; Yochum et al., 2000), a couple of studies have reported a lower risk of stroke incidence with specific carotenoid intakes; reduced risk of incident ischemic stroke was observed for the highest versus lowest quartiles of dietary lutein intake among US health professionals (Ascherio et al., 1999); the RR (95%CI) was 0.63 (0.40–0.99). Another study among Finnish male smokers reported a lower risk of cerebral infarction incidence in the highest versus lowest quartiles of dietary beta-carotene intake and lycopene intake; the RRs (95%CIs) were 0.74 (0.60–0.91) for beta-carotene intake and 0.74 (0.59–0.92) for lycopene intake. Dietary lycopene was also associated with a lower risk of intracerebral hemorrhage incidence in the same study; the RR (95% CI) for the highest versus lowest quartiles was 0.45 (0.24–0.86) (Hirvonen et al., 2000).

Vitamin C

Prospective studies on the associations between vitamin C intake and CVD are summarized in **Table 3**. A pooled analysis of nine cohort studies reported positive association between risk of major CHD events and dietary vitamin C intake even after adjusting for various confounders; the RR (95%CI) for the highest quintile of intake to the lowest was 1.23 (1.04–1.45) (Knekt et al., 2004). Among 10 studies which reported association between vitamin C and heart disease incidence and mortality (Buijsse et al., 2008; Gale et al., 1995; Genkinger et al., 2004; Klipstein-Grobusch et al., 1999; Knekt et al., 1994; Kubota et al., 2011; Kushi et al., 1996; Osganian et al., 2003; Rimm et al., 1993; Sahyoun et al., 1996), only one study reported a statistically significant inverse association between vitamin C intake and incident CHD among women; the RR (95%CI) for the highest quintile to the lowest was 0.63 (0.41–0.97) (Kubota et al., 2011), while the others reported null association.

A metaanalysis of 12 prospective studies reported statistically significant inverse associations between risk of stroke and dietary vitamin C intake; the RR (95%CI) for the higher versus lower intakes of vitamin C was 0.81 (0.74–0.90) and the RR (95%CI) for an increment of 100 mg/day in dietary vitamin C intake was 0.83 (0.75–0.93) (Chen et al., 2013a). Among 10 prospective cohort studies on stroke mortality and incidence (Ascherio et al., 1999; Daviglus et al., 1997; Del Rio et al., 2011; Gale et al., 1995; Hirvonen et al., 2000; Keli et al., 1996; Kubota et al., 2011; Ross et al., 1997; Voko et al., 2003; Yochum et al., 2000), three studies have indicated statistically significant inverse associations; a significant lower risk of stroke mortality was found for the highest

Table 2 Carotenoid intake from foods and the risk of cardiovascular disease

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Rimm et al. (1993) US male health professionals	HPFS	39,910	40	Prospective cohort	4	Intake from foods by FFQ (carotene)	Age, smoking status, BMI, total calories, dietary fiber, alcohol consumption, reported hypertension, regular aspirin use, physical activity, parental history of myocardial infarction before age 60, profession, and intakes of vitamin C and E	RR = 0.71 (0.53–0.86) for coronary heart disease risk (comparing the highest vs. lowest quintiles of carotene intake)
Knekt et al. (1994) Finnish men and women	FMCS	5133	35	Prospective cohort	14	Dietary intake by diet history	Age, smoking, serum cholesterol, hypertension, BMI, and energy intake	For coronary heart disease mortality, RRs = 1.02 (0.70–1.48) for men, 0.62 (0.30–1.29) for women (comparing the highest vs. lowest tertiles of carotenoid intake)
Sahyoun et al. (1996) noninstitutionalized Massachusetts residents	Nutrition Status Survey	725	60	Prospective cohort	9	Intake from foods and supplements by dietary records	Age, sex, disease status, disabilities affecting shopping	RR = 0.64 (0.33–1.27) for heart disease mortality (comparing the highest vs. lowest tertiles of carotenoid intake)
Kushi et al. (1996) US postmenopausal women	IWHS	41,836	7	Prospective cohort	55	Dietary intake by FFQ	Age, total energy intake, BMI, waist-to-hip ratio, pack-years of cigarette smoking, hypertension, diabetes mellitus, oral contraceptive use, estrogen replacement therapy, physical activity, alcohol intake, marital status, and level of educational attainment	For CHD mortality, RRs = 1.22 (0.76–1.96) for vitamin A, 0.80 (0.50–1.28) for retinol, 1.03 (0.63–1.70) for carotenoids (comparing the highest vs. lowest quintiles)
Keli et al. (1996) Dutch men	ZES	552	50	Prospective cohort	15	Dietary intake by diet history	Age, average systolic blood pressure, serum cholesterol, energy intake, lifetime cigarette smoking exposure, fish consumption, and alcohol consumption habits	RR = 0.54 (0.22–1.33) for stroke, comparing the highest group (>1.37 mg/day) to the lowest (<1.01 mg/day) of beta-carotene intake
Daviglus et al. (1997) Middle-aged US men	Chicago Western Electric Study	1843	40	Prospective cohort	30	Dietary intake by diet history questionnaire	Age, systolic blood pressure, number of cigarettes smoked per day, body mass index, serum cholesterol, total energy intake, alcohol consumption, and diagnosis of diabetes	RR = 0.84 (0.57–1.24) for the risk of stroke (comparing the highest vs. lowest tertiles of beta-carotene intake)
Ross et al. (1997) Chinese men	Shanghai, People's	18,244	45	Prospective cohort	8		Education, marital status, body mass index, lifetime	RR = 1.0 (0.7–1.5) for stroke mortality (comparing the highest vs. lowest tertiles of carotene intake)

	Republic of China					Dietary intake by FFQ (total carotene)	cigarette use, lifetime ethanol intake, and history of hypertension	
Klipstein-Grobusch et al. (1999) Dutch men and women	The Rotterdam Study	5159	4	Prospective cohort	55	Dietary intake by FFQ	Age, sex, body mass index, pack-years, equivalent household income, education, alcohol intake, use of antioxidative vitamin supplements, and categories of energy-adjusted b-carotene, vitamin C, and vitamin E intakes	For the risk of myocardial infarction, RR = 0.55 (0.34–0.83) for beta-carotene (comparing the highest vs. lowest tertiles of intake)
Ascherio et al. (1999) Male health professional	HPFS	43,738	40	Prospective cohort	8	Dietary intake by FFQ	Age, smoking, hypertension, hypercholesterolemia, body mass index, physical activity, parental history of myocardial infarction, alcohol consumption, and total energy intake	For total stroke, RRs = 0.77 (0.54–1.08) for beta-carotene, 0.94 (0.66–1.34) for alpha-carotene, 0.70 (0.49–1.01) for lutein, 1.01 (0.65–1.57) for lycopene. For ischemic stroke, RRs = 0.86 (0.56–1.32) for beta-carotene, 1.11 (0.73–1.70) for alpha-carotene, 0.63 (0.40–0.99) for lutein, 0.96 (0.68–1.36) for lycopene For hemorrhagic stroke, RRs = 0.76 (0.36–1.60) for beta-carotene, 0.50 (0.22–1.13) for alpha-carotene, 0.88 (0.40–1.92) for lutein, 1.04 (0.52–2.07) for lycopene (comparing the highest vs. lowest quartiles of intake)
Yochum et al. (2000) Postmenopausal women	IWHS	34,492	55	Prospective cohort	N/A	Dietary intake by FFQ (vitamin A, retinol, carotenoids)	Age, total energy intake, BMI, waist-to-hip ratio, high blood pressure, diabetes, use of estrogen replacement therapy, alcohol intake, education, marital status, pack-years of smoking, physical activity level, and intakes of cholesterol, saturated fat, fish, vitamin E, vitamin C, dietary fiber, and whole grains	For stroke mortality, RRs = 1.09 (0.62–1.92) for vitamin A, 0.86 (0.52–1.41) for retinol, and 0.88 (0.50–1.54) for carotenoids (comparing the highest vs. lowest quintiles of intake)
Hirvonen et al. (2000) Finnish male smokers	ATBC	26,593	50	Prospective cohort	6.1	Dietary intake by diet history	Age, supplementation group, systolic and diastolic blood pressures, serum total cholesterol, serum HDL cholesterol, body mass index, height, smoking years, number of cigarettes daily, history of diabetes or coronary heart disease, alcohol intake, and education	For cerebral infarction, RRs = 0.74 (0.60–0.91) for beta-carotene, 0.81 (0.66–1.00) for lutein + zeaxanthin, 0.74 (0.59–0.92) for lycopene (comparing the highest vs. lowest quartiles) For subarachnoid hemorrhage, RRs = 0.67 (0.35–1.28) for beta-carotene, 0.47 (0.24–0.93) for lutein + zeaxantin, 0.63 (0.33–1.20) for lycopene (comparing the highest vs. lowest quartiles) For intracerebral hemorrhage, RRs = 0.66 (0.36–1.19) for beta-carotene, 0.81 (0.46–1.43) for lutein + zeaxantin, 0.45† (0.24–0.86) for lycopene (comparing the highest vs. lowest quartiles)

(*Continued*)

Table 2 (Continued)

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Voko et al. (2003) Dutch men and women	The Rotterdam Study	7983	55	Prospective cohort	6.4	Dietary intake by FFQ	Age, sex, total energy intake, smoking, pack-years of cigarettes smoked, hypertension, diabetes mellitus, history of coronary heart disease, transient ischemic attacks	For the risk of ischemic stroke, RRs = 0.78 (0.55–1.1) among total population, 0.81 (0.54–1.20) among nonsmokers, 0.75 (0.35–1.58) among smokers (comparing the highest vs. lowest tertiles)
Osganian et al. (2003) US women	NHS	73,286	34	Prospective cohort	15	Intake from foods and supplement by FFQ	Age, smoking, postmenopausal hormone use, parental history of myocardial infarction, history of high blood pressure, history of high cholesterol, diabetes, BMI, regular exercise, aspirin use, alcohol intake, total energy intake, saturated fat intake, polyunsaturated fat intake, trans unsaturated fat intake, cereal fiber intake, folate intake, dietary glycemic load, and vitamin B-6 intake	For the risk of coronary artery incidence, RRs = 0.80 (0.65–0.99) for alpha-carotene, 0.74 (0.59–0.93) for beta-carotene, 0.90 (0.72–1.12)) for lutein + zeaxanthin, 0.93 (0.77–1.14) for lycopene, 1.17 (0.94–1.44) for beta-cryptoxanthin, the highest vs. lowest quintiles
Knekt et al. (2004) Pooled analysis of nine cohorts	AHS, ARIC, STBC, FMC, GPS, HPFS, IWHS, NHS(a), NHS(b), VIP	156,959	35	Pooled analysis	10	Intake from foods by FFQ or dietary records	Age, energy intake, smoking status, BMI, physical activity, education, alcohol intake, history of diabetes, hypercholesterolemia or serum cholesterol, hypertension or blood pressure, postmenopausal hormone use among the women, quintiles of intake of energy-adjusted saturated fatty acids, cholesterol, flavonoids, folate with supplements, vitamin B6 with supplements, and cereal fiber	For the risk of cardiovascular incidence, RRs = 0.90 (0.77–1.04) for alpha-carotene, 0.92 (0.79–1.06) for beta-carotene, 0.89 (0.75–1.04) for lutein, 0.99 (0.85–1.44) for lycopene, 0.94 (0.79–1.12) for beta-cryptoxanthin, 0.91 (0.78–1.06) for total carotene, the highest vs. lowest quintiles
Genkinger et al. (2004) Community dwelling US population	Odyssey Cohort	6151	13	Prospective cohort	N/A	Dietary intake by FFQ	Age, smoking status, body mass index, cholesterol concentration, and energy	HR = 0.97 (0.68–1.38) for the risk of CVD mortality for the highest vs. lowest quintiles of beta-carotene intake

Buijsse et al. (2008) Dutch elderly men	WES	559	65	Prospective cohort	15	Intake from foods by diet history,	Age, energy, BMI, physical activity, alcohol consumption, socioeconomic status, use of aspirin, use of anticoagulants, diet prescription, intake of fiber, beta-carotene, folate, saturated fatty acids, trans fatty acids, polyunsaturated fatty acids, and intake of vitamin E	For the risk of cardiovascular mortality, RRs = 0.82 (0.69–0.98) for total carotenoid, 0.81 (0.66–0.99) for alpha-carotene, 0.80 (0.66–0.97) for beta-carotene, 0.95 (0.81–1.12) for lutein, 0.91 (0.76–1.08) for lycopene, 0.86 (0.72–1.03) for beta-cryptoxanthin, 0.88 (0.70–1.10) for zeaxanthin for the highest vs. lowest quintiles of intakes
Del Rio et al. (2011) Italian men and women	EPIC-Italian	41,620	Not shown	Prospective cohort	7.9	Dietary intake by FFQ	Stratified by center, sex, and age. Adjusted for energy, hypertension, smoking status, education, nonalcohol energy intake, alcohol drinking, waist circumference, obesity, and physical activity	0.97 (0.65–1.42) for all types of strokes, 0.82 (0.49–1.36) for ischemic stroke, 1.51 (0.67–3.41) for hemorrhagic stroke (comparing the highest vs. lowest tertiles)

BMI, body mass index; *FFQ*, food frequency questionnaire; *NHS*, Nurses' health study; *RR*, relative risk; *AHS*, Adventist Health Study; *ARIC*, Arosclerosis Risk in Communities Study; *ATBC*, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; *EPIC*, European Prospective Investigation into Cancer and Nutrition; *FMC*, Finnish Mobile Clinic Health Examination Survey; *GPS*, Glostrup Population Study; *HPFS*, Health Professionals Follow-Up Study; *IWHS*, Iowa Women's Health Study; *NHS*, Nurses' Health Study; (a) 1980–1986; (b) 1986–1996; *VIP*, Vasterbotten Intervention Program; *ZES*, Zutphen Elderly Study.

Table 3 Vitamin C intake and the risk of cardiovascular disease

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Rimm et al. (1993) US male health professionals	HPFS	39,910	40	Prospective cohort	4	Intake from foods by FFQ	Age, smoking status, BMI, total calories, dietary fiber, alcohol consumption, reported hypertension, regular aspirin use, physical activity, parental history of myocardial infarction before age 60, profession, and intakes of carotene and vitamin E	RR = 1.25 (0.91–1.71) for coronary heart disease risk (comparing the highest vs. lowest quintiles)
Gale et al. (1995) British men and women	Eight areas in Britain	730	65	Prospective cohort	20	Dietary intake by 7 days dietary records	Sex and age in groups of 5 years	For stroke mortality, RRs = 0.4 (0.2–0.6), for CHD mortality, 0.8 (0.6–1.2), comparing the highest intake group (44.9 mg) to the lowest (27.9 mg)
Knekt et al. (1994) Finnish men and women	FMCS	5133	35	Prospective cohort	14	Dietary intake by diet history	Age, smoking, serum cholesterol, hypertension, BMI, and energy intake	For coronary heart disease mortality, RRs = 1.00 (0.68–1.45) for men, 0.62 (0.30–1.29) for women (comparing the highest vs. lowest tertiles)
Sahyoun et al. (1996) noninstitutionalized Massachusetts residents	Nutrition Status Survey	725	60	Prospective cohort	9	Intake from foods and supplements by dietary records	Age, sex, disease status, disabilities affecting shopping	RR = 0.38 (0.19–0.75) for heart disease mortality, comparing the highest vs. lowest tertiles
Kushi et al. (1996) US postmenopausal women	IWHS	41,836	7	Prospective cohort	55	Dietary intake by FFQ	Age, total energy intake, BMI, waist-to-hip ratio, pack-years of cigarette smoking, hypertension, diabetes mellitus, oral contraceptive use, estrogen replacement therapy, physical activity, alcohol intake, marital status, and level of educational attainment	For CHD mortality, RR = 1.49 (0.96–2.30) (comparing the highest vs. lowest quintiles)
Keli et al. (1996) Dutch men	ZES	552	50	Prospective cohort	15	Dietary intake by diet history	Age, average systolic blood pressure, serum cholesterol, energy intake, lifetime cigarette smoking	RR = 1.21 (0.55–2.66) for stroke (comparing the highest vs. lowest quartiles)

							exposure, fish consumption, and alcohol consumption habits	
Daviglus et al. (1997) Middle-aged US men	Chicago Western Electric Study	1843	40	Prospective cohort	30	Dietary intake by diet history questionnaire	Age, systolic blood pressure, number of cigarettes smoked per day, body mass index, serum cholesterol, total energy intake, alcohol consumption, and diagnosis of diabetes	RR = 0.71 (0.47–1.05) for the risk of stroke (comparing the highest vs. lowest tertiles)
Ross et al. (1997) Chinese men	Shanghai, People's Republic of China	18,244	45	Prospective cohort	8	Dietary intake by FFQ	Education, marital status, body mass index, lifetime cigarette use, lifetime ethanol intake, and history of hypertension	RR = 1.1 (0.7–1.6) for stroke mortality (comparing the highest vs. lowest tertiles)
Ascherio et al. (1999) Male health professional	HPFS	43,738	40	Prospective cohort	8	Dietary intake by FFQ	Age, smoking, hypertension, hypercholesterolemia, body mass index, physical activity, parental history of myocardial infarction, alcohol consumption, and total energy intake	RRS = 0.95 (0.66–1.35) for total stroke, 1.03 (0.66–1.59) for ischemic stroke, 0.82 (0.36–1.89) for hemorrhagic stroke (comparing the highest vs. lowest quartiles)
Klipstein-Grobusch et al. (1999) Dutch men and women	The Rotterdam Study	5159	4	Prospective cohort	55	Dietary intake by FFQ	Age, sex, body mass index, pack-years, equivalent household income, education, alcohol intake, use of antioxidative vitamin supplements, and categories of energy-adjusted b-carotene, vitamin C, and vitamin E intakes	For the risk of myocardial infarction, RR = 1.05 (0.65–1.67) (comparing the highest vs. lowest tertiles)
Yochum et al. (2000) Postmenopausal women	IWHS	34,492	55	Prospective cohort	55	Dietary intake by FFQ	Age, total energy intake, BMI, waist-to-hip ratio, high blood pressure, diabetes, use of estrogen replacement therapy, alcohol intake,	RR = 0.99 (0.58–1.72) for stroke mortality (comparing the highest vs. lowest quintiles)

(*Continued*)

Table 3 (Continued)

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
							education, marital status, pack-years of smoking, physical activity level, and intakes of cholesterol, saturated fat, fish, vitamin E, carotenoids, dietary fiber, and whole grains	
Hirvonen et al. (2000) Finnish male smokers	ATBC	26,593	50	Prospective cohort	6.1	Dietary intake by diet history	Age, supplementation group, systolic and diastolic blood pressures, serum total cholesterol, serum HDL cholesterol, body mass index, height, smoking years, number of cigarettes daily, history of diabetes or coronary heart disease, alcohol intake, and education	RR = 0.89 (0.72–1.09) for cerebral infarction, 1.16 (0.62–2.18) for subarachnoid hemorrhage, 0.39 (0.21–0.74) for intracerebral hemorrhage (comparing the highest vs. lowest quartiles)
Osganian et al. (2003) US women	NHS	85,118	36	Prospective cohort	15	Intake from foods by FFQ	Age, smoking, postmenopausal hormone use, parental history of myocardial infarction, history of high blood pressure, history of high cholesterol, diabetes, body mass index, regular exercise, aspirin use, alcohol intake, total caloric intake, saturated fat intake, polyunsaturated fat intake, transunsaturated fat intake, cereal fiber	RR = 0.86 (0.59–1.26) for CHD mortality (comparing the highest vs. lowest quartiles)

							intake, folate intake, dietary glycemic load, and vitamin B6, E and carotene intake	
Voko et al. (2003) Dutch men and women	The Rotterdam Study	7983	55	Prospective cohort	6.4	Dietary intake by FFQ	Age, sex, total energy intake, smoking, pack-years of cigarettes smoked, hypertension, diabetes mellitus, history of coronary heart disease, transient ischemic attacks	For the risk of ischemic stroke, RRs = 0.66 (0.46–0.93) among total population, 0.81 (0.55–1.20) among nonsmokers, and 0.27 (0.11–0.67) among smokers (comparing the highest vs. lowest tertiles)
Knekt et al. (2004) Pooled analysis of nine cohorts	AHS, ARIC, STBC, FMC, GPS, HPFS, IWHS, NHS(a), NHS(b), VIP	156,959	35–52	Pooled analysis	10	Intake from foods by FFQ or dietary records	Age, energy intake, smoking status, BMI, physical activity, education, alcohol intake, history of diabetes, hypercholesterolemia or serum cholesterol, hypertension or blood pressure, postmenopausal hormone use among women, quintiles of intake of energy-adjusted saturated fatty acids, cholesterol, flavonoids, folate with supplements, vitamin B6 with supplements, and cereal fiber	RR = 1.23 (1.04–1.45) of the risk of cardiovascular incidence for the highest vs. lowest quintiles
Genkinger et al. (2004) Community dwelling US population	Odyssey Cohort	6151	13	Prospective cohort	N/A	Dietary intake by FFQ	Age, smoking status, body mass index, cholesterol concentration, and energy	HR = 1.10 (0.79–1.52) for risk of CVD mortality for the highest vs. lowest quintiles

(*Continued*)

Table 3 (Continued)

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Buijsse et al. (2008) Dutch elderly men	ZES	559	65	Prospective cohort	15	Intake from foods by diet history,	Age, energy, BMI, physical activity, alcohol consumption, socioeconomic status, use of aspirin, use of anticoagulants, diet prescription, intake of fiber, beta-carotene, folate, saturated fatty acids, trans fatty acids, polyunsaturated fatty acids, and intake of vitamin E	RR = 1.02 (0.85–1.23) of the risk of cardiovascular mortality for the highest vs. lowest tertiles
Kubota Y et al. (2011) Japanese men and women	JACC	23,119 men and 35,611 women	40	Prospective cohort	16.5	Dietary intake by FFQ	Age, history of hypertension and diabetes, smoking status, alcohol consumption, body mass index, mental stress, walking, sports, education levels, dietary intakes of total energy, cholesterol, saturated fatty acids, n-3 fatty acids, and sodium	For the risk of stroke, HRs = 0.84 (0.62–1.13) among men and 0.70 (0.54–0.92) among women. For the risk of coronary heart disease, HRs = 0.86 (0.57–1.31) among men and 0.63 (0.41–0.97) among women. For the risk of total cardiovascular disease, HRs = 0.88 (0.72–1.07) among men and 0.79 (0.66–0.94) among women (comparing the highest vs. lowest quintiles)
Del Rio et al. (2011) Italian men and women	EPIC-Italian	41,620	Not shown	Prospective cohort	7.9	Dietary intake by FFQ	Stratified by center, sex, and age. Adjusted for energy, hypertension, smoking status, education, nonalcohol energy intake, alcohol drinking, waist circumference, obesity, and physical activity	HRs = 0.89 (0.60–1.32) for all types of strokes, 0.58 (0.34–0.99) for ischemic stroke, 1.93 (0.84–4.42) for hemorrhagic stroke (comparing the highest vs. lowest tertiles)

Chen et al. (2013a) 12 prospective studies (6 in Europe, 3 in the US, and 3 in Asia)	ATBC, CVDFACTS, EPIC-Italian, HPFS, IWHS, JACC, WES, the Rotterdam Study, the Zutphen study, cohort of men in Shanghai, China, cohort of men and women lining in eight areas in Britain, cohort of Finnish men and women	29,648	N/A	Metaanalysis	9.5–20	Intake (foods) by FFQ		RR = 0.81 (0.74–0.90) of the risk of stroke for the higher vs. lower intakes. RR = 0.83 (0.75–0.93) of the risk of stroke for an increment in dietary vitamin C intake of 100 mg/day

BMI, body mass index; *FFQ*, food frequency questionnaire; *NHS*, Nurses' health study; *RR*, relative risk; *AHS*, Adventist Health Study; *ARIC*, Arosclerosis Risk in Communities Study; *ATBC*, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; *CVDFACTS*, CardioVascular Disease risk FACtor Two-township Study; *EPIC*, European Prospective Investigation into Cancer and Nutrition; *FMC*, Finnish Mobile Clinic Health Examination Survey; *GPS*, Glostrup Population Study; *HPFS*, Health Professionals Follow-Up Study; *IWHS*, Iowa Women's Health Study; *JACC*, Japan Collaborative Cohort Study; *NHS*, Nurses' Health Study; (a) 1980–1986; (b) 1986–1996; *VIP*, Vasterbotten Intervention Program; *WES*, Western Electric Study; *ZES*, Zutphen Elderly Study.

intake group (44.9 mg) versus the lowest (27.9 mg) of dietary vitamin C among British men and women; the RR (95%CI) was 0.4 (0.2–0.6) (Gale et al., 1995); a significant lower risk of intracerebral hemorrhage incidence was found for the highest versus lowest quartiles of dietary vitamin C intake among Finnish male smokers; the RR (95%CI) was 0.39 (0.21–0.74) (Hirvonen et al., 2000); and a significant lower risk of ischemic stroke incidence was found for the highest versus lowest tertiles of dietary intake of vitamin C among Italian men and women; the RR (95%CI) was 0.58 (0.34–0.99) (Del Rio et al., 2011).

Vitamin E

Prospective studies on vitamin E intake and CVD are summarized in **Table 4**. A pooled analysis of nine cohort studies reported significant lower risk of major CHD events for the highest versus lowest quintiles of dietary vitamin E intake after adjusting for age and energy intake. After further adjustment for other confounders, the association remained statistically significant for women but not for men. Altogether, the association was marginally significant; the RR (95%CI) for the highest versus lowest quintiles of intakes was 0.84 (0.71–1.00) (Knekt et al., 2004). Among nine prospective cohort studies on heart disease mortality and incidence (Buijsse et al., 2008; Genkinger et al., 2004; Klipstein-Grobusch et al., 1999; Knekt et al., 1994; Kubota et al., 2011; Kushi et al., 1996; Rimm et al., 1993; Sahyoun et al., 1996; Stampfer et al., 1993), three studies reported statistically significant lower risks of CHD incidence and mortality for higher intakes of vitamin E (alpha-tocopherol); lower risk of CHD incidence was observed for the highest versus lowest quintiles among US health professionals (Rimm et al., 1993); the RR (95%CI) was 0.60 (0.44–0.81), and lower risk of CHD mortality was observed for the highest versus lowest quintiles of vitamin E intakes among Finnish women (Knekt et al., 1994) and US postmenopausal women (Kushi et al., 1996); the RRs (95%CIs) were 0.35 (0.14–0.88) for Finnish women and 0.38 (0.20–0.80) for US postmenopausal women.

Among eight prospective cohort studies on stroke mortality or incidence (Ascherio et al., 1999; Del Rio et al., 2011; Hirvonen et al., 2000; Keli et al., 1996; Kubota et al., 2011, Ross et al., 1997; Voko et al., 2003; Yochum et al., 2000), one study reported a statistically significant lower risk of stroke mortality for the highest versus lowest quintiles of dietary vitamin E intake among US postmenopausal women; the RR (95%CI) was 0.41 (0.20–0.80) (Yochum et al., 2000).

Comment

We reviewed the associations between antioxidant vitamin intakes and risk of CVD. Higher dietary intake of vitamin C and E tended to be inversely associated with risk of CVD. The evidence of protective effect of dietary carotenoid intake on risk of CVD is limited. Further epidemiological studies to clarify the associations between antioxidant vitamins and risk of CVD are needed.

Minerals

Dietary minerals, rich in vegetables and fruits, consist of major minerals and trace minerals. Major minerals include sodium, magnesium, phosphorous, sulfur, chlorine, potassium, and calcium, and trace minerals include iron, zinc, copper, and so on. Of them, iron and zinc account for large proportions of trace minerals in the human body.

In this section, we review the association between dietary minerals intake and risk of CVD.

Tables 5–11 include the summary of the associations between mineral intakes and the risk of CVD, CHD, and stroke.

Major Minerals

Magnesium

A recent metaanalysis showed that dietary magnesium intake was inversely associated with risk of CVD events including incidence and mortality; a 100 mg/day increment of dietary magnesium intake was inversely associated with risk of CVD events; the pooled RR (95%CI) was 0.91 (0.86–0.96) (Qu et al., 2013). However, the association between magnesium intake and the risk of CVD seemed not to be linear. The risk-lowering effect of magnesium intake seemed to be strong between subjects whose daily magnesium intake was low (between 150 and 400 mg) (Qu et al., 2013). As for CHD, two prospective studies have examined the association between dietary magnesium intake/urinary excretion and CHD incidence and mortality (Chiuve et al., 2013; Joosten et al., 2013). Dietary magnesium intake was inversely associated with mortality from CHD, but not with the incidence of CHD in American women (Chiuve et al., 2013). Urinary magnesium excretion, an index of magnesium intake, was inversely associated with the incidence of ischemic heart disease in the Netherlands (Joosten et al., 2013). As for stroke, dietary magnesium intake was inversely associated with the incidence of total stroke, but not of ischemic and hemorrhagic strokes (Adebamowo et al., 2015). Total magnesium intake was not associated with the incidence of stroke and its subtypes (Adebamowo et al., 2015). A possible threshold effect of magnesium intake was suggested for its association with risk of CHD and stroke (Chiuve et al., 2013; Joosten et al., 2013). In these studies, the mortality from CHD and the incidence of total CHD were almost 35% and 40% respectively higher in the lowest quintile of magnesium intake/excretion, while it was even in other higher intake categories from the second to the highest quintile.

Table 4 Vitamin E intake from foods and the risk of cardiovascular disease

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Stampfer et al. (1993) US women	NHS	87,245	35	Prospective cohort	8	Intake from foods by FFQ	Age, smoking	RR = 0.95 (0.72–1.23) for coronary heart disease risk, comparing highest quintile to the lowest
Rimm et al. (1993) US male health professionals	HPFS	39,910	40	Prospective cohort	4	Intake from foods by FFQ	Age, smoking status, BMI, total calories, dietary fiber, alcohol consumption, reported hypertension, regular aspirin use, physical activity, parental history of myocardial infarction before age 60, profession, and intakes of carotene and vitamin C	RR = 0.60† (0.44–0.81) for coronary heart disease risk, comparing highest quintile to the lowest
Knekt et al. (1994) Finnish men and women	FMCS	5133	35	Prospective cohort	14	Dietary intake by diet history	Age, smoking, serum cholesterol, hypertension, BMI, and energy intake	For coronary heart disease mortality, RR = 0.68 (0.42–1.11) for men, 0.35 (0.14–0.88) for women, comparing the highest tertile to the lowest
Sahyoun et al. (1996) Noninstitutionalized Massachusetts residents	Nutrition Status Survey	725	60	Prospective cohort	9	Intake from foods and supplements by dietary records	Age, sex, disease status, disabilities affecting shopping	RR = 0.75 (0.41–1.39) for heart disease mortality, comparing highest tertile to the lowest
Keli et al. (1996) Dutch men	ZES	552	50	Prospective cohort	15	Dietary intake by diet history	Age, average systolic blood pressure, serum cholesterol, energy intake, lifetime cigarette smoking exposure, fish consumption, and alcohol consumption habits	RR = 1.64 (0.54–4.97) for stroke, comparing the highest group ($\geq$21.3 mg/day) to the lowest ($<$14.4 mg/day)
Kushi et al. (1996) US postmenopausal women	IWHS	34,486	7	Prospective cohort	55	Intake from foods by FFQ	Age, total energy intake, BMI, waist-to-hip ratio, pack-years of cigarette smoking, hypertension, diabetes mellitus, oral contraceptive use, estrogen replacement therapy, physical activity, alcohol intake, marital status, and level of educational attainment	For coronary heart disease mortality, RR = 0.38 (0.20–0.80) (comparing highest quintile to the lowest)

(*Continued*)

Table 4 (Continued)

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Ross et al. (1997) Chinese men	Shanghai, People's Republic of China	18,244	45	Prospective cohort	8	dietary intake by FFQ	Education, marital status, body mass index, lifetime cigarette use, lifetime ethanol intake, and history of hypertension	RR = 0.8 (0.6–1.2) for stroke mortality (comparing highest tertile to the lowest)
Klipstein-Grobusch et al. (1999) Dutch men and women	The Rotterdam Study	5159	4	Prospective cohort	55	Intake from foods and supplements by FFQ	Age, sex, body mass index, pack-years, equivalent household income, education, alcohol intake, use of antioxidative vitamin supplements, and categories of energy-adjusted b-carotene, vitamin C, and vitamin E intakes	For the risk of myocardial infarction, RR = 1.21 (0.75–1.98) for beta-carotene (comparing highest tertile to the lowest)
Ascherio et al. (1999) Male health professional	HPFS	43,738	40	Prospective cohort	8	Intake from foods and supplements by FFQ	Age, smoking, hypertension, hypercholesterolemia, body mass index, physical activity, parental history of myocardial infarction, alcohol consumption, and total energy intake	RRs = 1.25 (0.88–1.78) or total stroke, 1.18 (0.77–1.82) for ischemic stroke, 1.31 (0.62–2.76) for hemorrhagic stroke (comparing the highest vs. lowest quartiles).
Hirvonen et al. (2000) Finnish male smokers	ATBC	26,593	50	Prospective cohort	6.1	Dietary intake by diet history	Age, supplementation group, systolic and diastolic blood pressures, serum total cholesterol, serum HDL cholesterol, body mass index, height, smoking years, number of cigarettes daily, history of diabetes or coronary heart disease, alcohol intake, and education	RRs = 0.86 (0.70–1.06) for cerebral infarction, 0.81 (0.44–1.50) for subarachnoid hemorrhage, 0.64 (0.36–1.15) for intracerebral hemorrhage (comparing the highest vs. lowest quartiles)
Yochum et al. (2000) Postmenopausal women	IWHS	34,492	55	Prospective cohort	N/A	Dietary intake by FFQ	Age, total energy intake, BMI, waist-to-hip ratio, high blood pressure, diabetes, use of estrogen replacement therapy, alcohol intake, education, marital	RR = 0.40 (0.20–0.80) for stroke mortality (comparing the highest vs. lowest quintiles)

							status, pack-years of smoking, physical activity level, and intakes of cholesterol, saturated fat, fish, vitamin C, carotenoids, dietary fiber, and whole grains	
Voko et al. (2003) Dutch men and women	The Rotterdam Study	7983	55	Prospective cohort	6.4	Dietary intake by FFQ	Age, sex, total energy intake, smoking, pack-years of cigarettes smoked, hypertension, diabetes mellitus, history of coronary heart disease, transient ischemic attacks, and polyunsaturated fatty acid intake	For the risk of ischemic stroke, RRs = 0.72 (0.42–1.25) among total population, 0.67 (0.35–1.26) among nonsmokers, 0.77 (0.24–2.39) among smokers (comparing the highest vs. lowest tertiles)
Knekt et al. (2004) Pooled analysis of nine cohorts	AHS, ARIC, STBC, FMC, GPS, HPFS, IWHS, NHS(a), NHS(b), VIP	156,959	35–52	Pooled analysis	10	Intake from foods by FFQ or dietary records	Age, energy intake, smoking status, BMI, physical activity, education, alcohol intake, history of diabetes, hypercholesterolemia or serum cholesterol, hypertension or blood pressure, postmenopausal hormone use among the women, quintiles of intake of energy-adjusted saturated fatty acids, cholesterol, flavonoids, folate with supplements, vitamin B6 with supplements, and cereal fiber	RR = 0.84 (0.71–1.00) of the risk of cardiovascular incidence for the highest vs. lowest quintiles
Genkinger et al. (2004) Community dwelling US population	Odyssey Cohort	6151	13	Prospective cohort	N/A	Dietary intake by FFQ	Age, smoking status, body mass index, cholesterol concentration, and energy	HR = 1.07 (0.79–1.46) for risk of CVD mortality for the highest vs. lowest quintiles

(*Continued*)

Table 4 (Continued)

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Buijsse et al. (2008) Dutch elderly men	ZES	559	65	Prospective cohort	15	Intake from foods by FFQ	Age, energy, BMI, physical activity, alcohol consumption, socioeconomic status, use of aspirin, use of anticoagulants, diet prescription, intake of fiber, beta-carotene, folate, saturated fatty acids, trans fatty acids, polyunsaturated fatty acids and intake of vitamin C	For the risk of cardiovascular mortality, RRs = 0.92 (0.76–1.12) for total vitamin E, 0.96 (0.82–1.12) for alpha-tocopherol for the highest vs. lowest tertiles
Del Rio et al. (2011) Italian men and women	EPIC-Italian	41,620	N/A	Prospective cohort	7.9	Dietary intake by FFQ	Stratified by center, sex, and age. Adjusted for energy, hypertension, smoking status, education, nonalcohol energy intake, alcohol drinking, waist circumference, obesity, and physical activity	RRs = 1.08 (0.68–1.72) for all types of strokes, 0.76 (0.40–1.42) for ischemic stroke, 2.94 (1.13–7.62) for hemorrhagic stroke (comparing the highest vs. lowest tertiles)
Kubota et al. (2011)	JACC	23,119 men and 35,611 women	40	Prospective cohort	16.5	Dietary intake by FFQ	Age, history of hypertension and diabetes, smoking status, alcohol consumption, body mass index, mental stress, walking, sports, education levels, dietary intakes of total energy, cholesterol, saturated fatty acids, n-3 fatty acids, and sodium	For the risk of stroke, HRs = 1.20 (0.78–1.85) among men and 0.71 (0.50–1.00) among women. For the risk of coronary heart disease, HRs = 0.98 (0.55–1.75) among men and 0.83 (0.48–1.44) among women. For the risk of total cardiovascular disease, HRs = 0.91 (0.69–1.22) among men and 0.82 (0.65–1.04) among women (comparing the highest vs. lowest quintiles)

BMI, body mass index; *FFQ*, food frequency questionnaire; *NHS*, Nurses' health study; *RR*, relative risk; *AHS*, Adventist Health Study; *ARIC*, Arosclerosis Risk in Communities Study; *ATBC*, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; *EPIC*, European Prospective Investigation into Cancer and Nutrition; *FMC*, Finnish Mobile Clinic Health Examination Survey; *GPS*, Glostrup Population Study; *HPFS*, Health Professionals Follow-Up Study; *IWHS*, Iowa Women's Health Study; *JACC*, Japan Collaborative Cohort Study; *NHS*, Nurses' Health Study; (a) 1980–1986; (b) 1986–1996; *VIP*, Vasterbotten Intervention Program; *ZES*, Zutphen Elderly Study.

Table 5 Magnesium intake/excretion and the risk of cardiovascular disease

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Qu et al. (2013) Pooled analysis of 13 cohorts	ATBC, ARIC, CVDFACTS, HHP, HPFS, JACC, NHS, WHS, the Cohort of Swedish Men, the Swedish Mammography Cohort	477,680 (men and women)	30–89	Metaanalysis	7–30	Dietary intake measured by 24-h dietary recall or FFQ	–	RR = 0.85 (0.78–0.92) of CVD incidence and mortality for the highest vs. lowest category of dietary magnesium intake RR = 0.91 (0.85–0.97) of the CVD incidence and mortality for 100 mg/day increment of dietary magnesium intake
Chiuve et al. (2013) American women	NHS	86,323 (women)	30–55	Prospective cohort	28 (median)	Dietary intake measured by FFQ	Age, calendar year, total calories, smoking, BMI, parental history of MI, alcohol intake, physical activity, menopausal therapy, multivitamin use, and intake of omega-3 fats, polyunsaturated: saturated fat, trans fat, dietary cholesterol, calcium, cereal fiber, potassium, vitamin D, and the development of possible intermediate diseases (hypertension, hypercholesterolemia, and diabetes) during follow-up	RR = 0.64 (0.46–0.87) of fatal CHD and RR = 1.02 (0.82–1.25) of nonfatal CHD for the highest vs. lowest quartiles of dietary magnesium intake
Joosten et al. (2013) Dutch men and women living in Groningen, the Netherlands	PREVEND Study	7664 (men and women)	Not shown	Prospective cohort	10.5 (median)	Urinary excretion measured by 24-h urine collection	Age, smoking status, sex, BMI, ratio of total to HDL cholesterol, parental history of IHD, and alcohol consumption, urinary calcium, sodium, potassium, and creatinine, C-reactive protein, systolic blood pressure, antihypertensive therapy, type 2 diabetes, urinary albumin, ischemic ST-T segment changes, and prolonged QTc interval	HR = 1.44 (1.07–1.92) of fatal and non fatal CHD for the middle quintile of urinary magnesium excretion compared to the lowest
Adebamowo et al. (2015) American women	NHS I and II	180,864 (women)	30–55: NHS I 25–42: NHS II	Prospective cohort	30: NHS I 22: NHS II	Dietary and supplemental intake measured by FFQ	Age, calendar year, BMI, parental history of heart disease, alcohol intake, physical activity, smoking, postmenopausal hormone therapy, oral contraceptive use, menopausal status, aspirin, multivitamin, history of hypertension, hypercholesterolemia, diabetes at baseline, thiazide use, and intakes of total calories, potassium and calcium	RR = 0.93 (0.79–1.08) of the incidence of total stroke for the highest vs. lowest quintiles of total (dietary + supplemental) magnesium intake RR = 0.82 (0.69–0.97) of the incidence of total stroke for the highest vs. lowest quintiles of dietary magnesium intake RR = 0.91 (0.71–1.15) of the incidence of ischemic stroke for the highest vs. lowest quintiles of dietary magnesium intake RR = 0.87 (0.59–1.29) of the incidence of hemorrhagic stroke for the highest vs. lowest quintiles of dietary magnesium intake

ARIC, Arosclerosis Risk in Communities Study; *ATBC*, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; *BMI*, body mass index; *CHD*, coronary heart disease; *CVD*, cardiovascular disease; *CVDFACTS*, CardioVascular Disease risk FACtor Two-township Study; *FFQ*, food frequency questionnaire; *HDL*, high-density lipoprotein; *HHP*, Honolulu Heart Program; *HPFS*, Health Professionals Follow-up Study; *HR*, hazard ratio; *IHD*, ischemic heart disease; *JACC*, Japan Collaborative Cohort Study; *MI*, myocardial infarction; *NHS*, Nurses' health study; *PREVEND*, Prevention of Renal and Vascular End-Stage Disease; *RR*, relative risk; *WHS*, Women's Health Study.

Table 6 Phosphorus intake and the risk of cardiovascular disease

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Chang et al. (2014) American men and women	NHANES III	9686 (men and women)	20–80	Prospective cohort	14.7 (median)	Dietary intake measured by 24-h dietary recall	Age, sex, race, ethnicity, poverty:income ratio, total energy intake, BMI, systolic blood pressure, current and former smoking, physical activity, non-HDL cholesterol, log albumin:creatinine ratio, estimated glomerular filtration rate, and low vitamin D concentration	HR = 1.02 (0.25–4.21) of CVD mortality for increment of 1 unit as ln(phosphorus intake) in subjects whose phosphorus intake was >=1400 mg/d, and HR = 0.99 (0.46–2.14) in subjects whose phosphorus intake was <1400 mg/d HR = 3.39 (1.43–8.02) of CVD mortality for increment of 0.1 mg/kcal of phosphorus in subjects whose phosphorus intake was ≥0.35 mg/kcal, and HR = 0.30 (0.13–0.73) in subjects whose phosphorus intake was <0.35 mg/kcal

BMI, body mass index; *CVD*, cardiovascular disease; *HDL*, high-density lipoprotein; *HR*, hazard ratio; *NHANES*, National Health and Nutrition Examination Survey.

Table 7 Potassium intake/excretion and the risk of cardiovascular disease

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Aburto et al. (2013) Pooled analysis of nine cohorts	ATBC, CVDFACTS, HPFS, JACC, NHANES I, NHS, ONTARGET and the TRANSCEND trials, the Rotterdam Study, population-based cohort of Southern California	Not shown	Not shown	Metaanalysis	4.7–19	Dietary intake measured by 24-hour dietary recall or FFQ, and estimated 24-hour urinary excretion	–	RR = 0.76 (0.66–0.89) of the risk of stroke incidence for higher potassium intake RR = 0.88 (0.70–1.10) of the risk of CVD incidence for higher potassium intake RR = 0.96 (0.78–1.19) of the risk of CHD incidence for higher potassium intake
Adebamowo et al. (2015) American women	NHS I and II	180,864 (women)	30–55: NHS I 25–42: NHS II	Prospective cohort	30: NHS I 22: NHS II	Dietary and supplemental intake measured by FFQ	Age, calendar year, BMI, parental history of heart disease, alcohol intake, physical activity, smoking, postmenopausal hormone therapy, oral contraceptive use, menopausal status, aspirin, multivitamin, history of hypertension, hypercholesterolemia, diabetes at baseline, thiazide use, and intakes of total calories, magnesium, and calcium	RR = 0.91 (0.78–1.06) of the incidence of total stroke for the highest vs. lowest quintiles of total (dietary + supplemental) potassium intake RR = 1.00 (0.84–1.18) of the incidence of total stroke for the highest vs. lowest quintiles of dietary potassium intake RR = 0.92 (0.72–1.18) of the incidence of ischemic stroke for the highest vs. lowest quintiles of dietary potassium intake RR = 0.82 (0.55–1.22) of the incidence of hemorrhagic stroke for the highest vs. lowest quintiles of dietary potassium intake

ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; *BMI*, body mass index; *CHD*, coronary heart disease; *CVD*, cardiovascular disease; *CVDFACTS*, CardioVascular Disease risk FACtor Two-township Study; *FFQ*, food frequency questionnaire; *HPFS*, Health Professionals Follow-up Study; *JACC*, Japan Collaborative Cohort Study; *NHANES*, National Health and Nutrition Examination Survey; *NHS*, Nurses' health study; *ONTARGET*, Ongoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial; *RR*, relative risk; *TRANSCEND*, Telmisartan Randomized Assessment Study in ACE Intolerant Subjects With Cardiovascular Disease.

Table 8 Calcium intake and the risk of cardiovascular disease

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Wang et al. (2012) Pooled analysis of nine cohorts	ATBC, HHP, HPFS, IWHS, JACC, JPHC, NHS, the population-based cohort of Finnish, the cohort of Dutch civil survants	355,619 (men and women)	40–99	Metaanalysis	8–28	Dietary intake measured by 24-hour dietary recall or FFQ	–	RR = 0.86 (0.69–1.06) of the risk of stroke incidence and mortality in the highest vs. lowest category of dietary calcium intake, and RR = 0.80 (0.63–1.01) in the highest vs. lowest category of calcium supplement use RR = 0.92 (0.80–1.07) of the risk of CHD incidence and mortality in the highest vs. lowest category of dietary calcium intake, and RR = 1.01 (0.78–1.30) in the highest vs. lowest category of calcium supplement use
Adebamowo et al. (2015) American women	NHS I and II	180,864 (women)	30–55: NHS I 25–42: NHS II	Prospective cohort	30: NHS I 22: NHS II	Dietary and supplemental intake measured by FFQ	Age, calendar year, BMI, parental history of heart disease, alcohol intake, physical activity, smoking, postmenopausal hormone therapy, oral contraceptive use, menopausal status, aspirin, multivitamin, history of hypertension, hypercholesterolemia, diabetes at baseline, thiazide use, and intakes of total calories, magnesium and potassium	RR = 1.04 (0.92–1.17) of the incidence of total stroke for the highest vs. lowest quintile of total (dietary + supplemental) calcium intake RR = 0.99 (0.88–1.12) of the incidence of total stroke for the highest vs. lowest quintiles of dietary calcium intake RR = 0.92 (0.77–1.10) of the incidence of ischemic stroke for the highest vs. lowest quintiles of dietary calcium intake RR = 1.15 (0.86–1.54) of the incidence of hemorrhagic stroke for the highest vs. lowest quintiles of dietary calcium intake

ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; *BMI*, body mass index; *CHD*, coronary heart disease; *FFQ*, food frequency questionnaire; *HHP*, Honolulu Heart Program; *HPFS*, Health Professionals Follow-up Study; *IWHS*, Iowa Women's Health Study; *JACC*, Japan Collaborative Cohort Study; *JPHC*, Japan Public Health Center-based study; *NHS*, Nurses' health study; *RR*, relative risk.

Table 9 Iron intake and the risk of cardiovascular disease

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Hunnicutt et al. (2014) Pooled analysis of eight cohorts	EPIC, HPFS, IWHS, JACC, MESA, NHANES I, the Rotterdam Study, the population-based cohort of Finnish	80,162 for incidence 142,842 for mortality	20–99	Metaanalysis	4–15	Dietary total, heme, and nonheme iron intake measured by FFQ	–	RRs = 0.85 (0.73–0.999) of the incidence of CHD for the highest vs. lowest quintiles of total iron intake, and 1.57 (1.28–1.94) for the heme iron intake, and 0.77 (0.54–1.10) for the nonheme iron intake RR = 0.91 (0.69–1.19) of the mortality of CHD for the highest quintile of total iron intake compared to the lowest, and RR = 1.37 (0.86–2.18) for the heme iron intake, and RR = 0.98 (0.82–1.17) for the nonheme iron intake
Bates et al. (2011) British men and women	The British National Diet and Nutrition Survey	1054 (men and women)	76.6 (mean)	Prospective cohort	Not shown	Dietary heme, and nonheme iron intake measured by weighed dietary record of 4 days	Age, sex	HR = 1.06 (0.94–1.20) of the vascular disease mortality for the 1 mg/day increment of heme iron intake HR = 0.93 (0.82–1.07) of the vascular disease mortality for the 1 mg/day increment of nonheme iron intake
Kaluza et al. (2013) Swedish men	The cohort of Swedish men	38,859 (men)	45–79	Prospective cohort	11.7 (mean)	Dietary heme and nonheme iron intake measured by FFQ	Age, education, smoking status and pack-years of smoking, BMI, total physical activity, history of hypertension, high blood cholesterol level, diabetes mellitus, aspirin use, family history of myocardial infarction, alcohol intake and intake of dietary fiber, vitamin E, β-carotene, vitamin C, and calcium. Heme and nonheme iron were mutually adjusted	HRs = 1.16 (1.03–1.31) of the incidence of total stroke for the highest vs. lowest quintiles of heme iron intake, and 0.99 (0.85–1.14) of nonheme iron intake HRs = 1.15 (1.00–1.31) of the incidence of cerebral infarction for the highest versus lowest quintile of heme iron intake, and 0.95 (0.81–1.12) of nonheme iron intake HRs = 1.06 (0.76–1.46) of the incidence of intracerebral hemorrhage for the highest vs. lowest quintiles of heme iron intake, and 1.29 (0.88–1.90) of nonheme iron intake

BMI, body mass index; *CHD*, coronary heart disease; *EPIC*, European Prospective Investigation into Cancer and Nutrition; *FFQ*, food frequency questionnaire; *HPFS*, Health Professionals Follow-up Study; *HR*, hazard ratio; *IWHS*, Iowa Women's Health Study; *JACC*, Japan Collaborative Cohort Study; *MESA*, Multi-Ethnic Study of Arosclerosis; *NHANES*, National Health and Nutrition Examination Survey; *RR*, relative risk.

Table 10 Zinc intake and the risk of cardiovascular disease

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*adjusted variables*	*Relative risk*
de Oliveira Otto et al. (2012) American men and women	MESA	5285 (men and women)	61.8 ± 10.3	Prospective cohort	6.2 (median)	Dietary intake measured by FFQ	Age, sex, energy intake, race, ethnicity, education, study center, alcohol intake, physical activity, BMI, fiber intake, cigarette smoking, dietary supplement use, ratio of polyunsaturated fat intake:saturated fat intake, magnesium, nonheme iron, antioxidant intake, and heme iron intake	HRs = 1.12 (0.66–1.90) of the incidence of CVD for the highest vs. lowest quintile of zinc intake, 1.66 (1.10–2.49) of zinc intake from red meat, and 0.68 (0.35–1.30) of zinc intake from other sources
Lee et al. (2005) American women	IWHS	34,492 (postmenopausal women)	55–69	Prospective cohort	15	Dietary intake measured by FFQ	Age, energy intake, BMI, waist-hip ratio, physical activity, cigarette smoking, alcohol consumption, hormone replacement therapy, high blood pressure, heme iron, nonheme iron, saturated fat, trans fat, polyunsaturated fat, folate, beta-carotene, vitamin E, and vitamin C	HRs = 1.00 (0.69–1.44) of the cardiovascular mortality for the highest vs. lowest quintiles of zinc intake among subjects whose alcohol intake was 0-9 g/day, and 0.37 (0.13-–1.06) among subjects whose alcohol intake was >= 10 g/day, and 0.21 (0.04–1.16) among subjects whose alcohol intake was >= 30 g/day
Bates et al. (2011) British men and women	The British National Diet and Nutrition Survey	1054 (men and women)	76.6 (mean)	Prospective cohort	Not shown	Dietary intake measured by weighed dietary record of 4 days	Age, sex	HR = 0.84 (0.71–0.99) of the vascular disease mortality for the 1 mg/day increment of zinc intake

BMI, body mass index; *CVD*, cardiovascular disease; *FFQ*, food frequency questionnaire; *HR*, hazard ratio; *IWHS*, Iowa Women's Health Study; *MESA*, Multi-Ethnic Study of Arosclerosis; *RR*, relative risk.

Table 11 Copper intake and the risk of cardiovascular disease

Published year study population	*Study name or setting*	*Sample size*	*Age at entry*	*study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Bates et al. (2011) British men and women	The British National Diet and Nutrition Survey	1054 (men and women)	76.6 (mean)	Prospective study	Not shown	Dietary copper intake measured by weighed dietary record of 4 days	Age, sex	HR = 0.92 (0.78–1.10) of the vascular disease mortality for the 1 mg/day increment of copper intake

HR, hazard ratio.

Phosphorous

The evidence between phosphorous intake and risk of CVD is very limited. A prospective study of Americans has shown a U-shaped relation between dietary phosphorous density (mg/kcal) and mortality from CVD (Chang et al., 2014).

Sulfur

There are no epidemiological studies that have examined the association between sulfur intake and risk of CVD so far.

Potassium

A metaanalysis showed that dietary potassium intake was not associated with incidence of CVD or CHD, while dietary potassium intake was inversely associated with incidence of stroke (Aburto et al., 2013). The pooled RR (95%CI) of the protective effect of the higher versus lower potassium intakes on risk of incident stroke was 0.76 (0.66–0.89) (Aburto et al., 2013). A recent report for the prospective study of American women showed that, compared with the lowest quintile of dietary potassium intake, the highest quintile had the lower multivariable-adjusted risk of total and ischemic strokes (Adebamowo et al., 2015); the pooled RRs (95% CIs) for the highest versus lowest quintiles of dietary potassium intake were 0.88 (0.79–0.98) for total stroke and 0.86 (0.74–1.00) for ischemic stroke. However, the association was no longer significant after further adjustment for magnesium and calcium intake (Adebamowo et al., 2015).

Calcium

A metaanalysis showed that dietary and supplemental calcium intakes were not associated with incidence and mortality of CHD or total stroke (Wang et al., 2012). A prospective study of American women showed no significant association between dietary and total calcium intake with incidence of total, ischemic, and hemorrhagic strokes (Adebamowo et al., 2015). However, several prospective studies of Asians, whose dietary calcium intake was lower than that of Westerners, showed that dairy calcium intake was inversely associated with incidence and mortality of stroke (Abbott et al., 1996; Umesawa et al., 2006, 2008). For Westerners, a prospective study of American women showed an inverse association between dairy calcium intake and incidence of stroke; the multivariable HR (95%CI) of total stroke for the highest versus lowest quintiles of dairy calcium intake was 0.70 (0.51–0.97) (Iso et al., 1999), while a prospective study of American men did not show a significant association between them (Ascherio et al., 1998).

A metaanalysis, which examined an effect of calcium supplementation on the risk of CVD incidence and mortality, showed unexpectedly that calcium supplements without coadministered vitamin D were positively associated with the risk of myocardial infarction (MI); the pooled RR (95%CI) of incident MI for calcium supplements versus no supplement was 1.27 (1.01–1.59) (Bolland et al., 2010).

Trace Minerals

Iron

Dietary iron consisted of heme and nonheme irons. Heme iron is rich in meat and fish while nonheme iron is rich in plant foods and eggs. According to a metaanalysis (Hunnicutt et al., 2014), dietary total iron intake was inversely associated with risk of incident CHD; the pooled RR (95%CI) of CHD for the highest versus lowest quintiles of dietary total iron intake was 0.85 (0.73–0.999) (Hunnicutt et al., 2014), while dietary heme iron intake was positively associated with risk of incident CHD; the pooled RR (95% CI) of CHD for the highest versus lowest quintiles of dietary heme iron intake was 1.57 (1.28–1.94) (Hunnicutt et al., 2014). Heme iron, nonheme iron, and total iron intakes were not associated with fatality from CHD (Hunnicutt et al., 2014). A prospective study of British showed that dietary heme iron and nonheme iron intakes were not associated with mortality from CVD (Bates et al., 2011). For stroke, a prospective study of Swedish men showed that dietary nonheme iron intake was not associated with the

incidence of total, ischemic, and hemorrhagic strokes, while dietary heme iron intake was positively associated with the incidence of total and ischemic strokes (Kaluza et al., 2013).

Zinc

A cross-sectional study of Indians showed that low dietary zinc consumption was positively associated with the prevalence of coronary artery disease (Singh et al., 1998). A prospective study, the Multi-Ethnic Study of Atherosclerosis, examined the association between dietary zinc intake and the incidence of CVD (de Oliveira Otto et al., 2012). In that study, dietary zinc intake was not associated with the incidence of CVD, while zinc intake from red meat was positively associated with it; the multivariable-adjusted HR (95%CI) for the highest versus lowest quintiles of zinc intake from red meat was 1.42 (1.07–1.89). However, the association with zinc from red meat was attenuated greatly after adjustment for total heme iron intake; the multivariable HR was 1.36 (0.85–2.17; $P=0.10$) (de Oliveira Otto et al., 2012). As for CVD mortality, a prospective study of British men and women showed that dietary zinc intake was inversely associated with mortality from CVD (Bates et al., 2011); the age and sex-adjusted HR (95%CI) of CVD mortality for the 1-SD increment of dietary zinc intake per day (men: 2.86 mg, women: 2.56 mg) was 0.84 (0.71–0.99). A prospective study of American women showed no association between dietary zinc intake and mortality from CVD (Lee et al., 2005).

Other trace minerals

A metaanalysis showed that serum selenium concentrations were inversely associated with the risk of CHD incidence and mortality, but selenium supplementation was not associated with it (Flores-Mateo et al., 2006).

A prospective study of British men and women showed that dietary copper intake was not associated with mortality from CVD (Bates et al., 2011).

Comment

We reviewed the associations between mineral intakes and risk of CVD incidence and mortality. Magnesium and potassium intakes may be inversely associated with risk of CVD, while heme iron intake has been suggested to be positively associated with risk of CVD. The evidence between trace mineral intake and risk of CVD is limited.

Dietary Fiber

Dietary fiber consists of water-soluble and water-insoluble fibers. The major components of water-soluble dietary fiber were water-soluble pectin and alginic acid, and those of water-insoluble dietary fiber were cellulose, lignin, and water-insoluble pectin.

In this section, we review the associations between dietary fiber intakes and risk of CVD.

Table 12 includes the summary of the associations between dietary fiber intakes and risk of CVD, CHD, and stroke.

Total Dietary Fiber

Three metaanalyses have shown that 7–10 g/daily increment in the intakes of total dietary fiber was associated with lower risk of total CVD, CHD, and stroke; the pooled RRs (95%CIs) were 0.91 (0.88–0.94), 0.91 (0.87–0.94), and 0.93 (0.88–0.98) per 7-g increment respectively, and 0.91 (0.88–0.94) of CVD and 0.89 (0.85–0.93) of CHD per 10-g increment (Kim and Je, 2016; Threapleton et al., 2013b,c). The risk of mortality from CVD, CHD, and stroke in the highest versus lowest category of total dietary fiber intakes was also significant; the pooled RRs (95%CIs) were 0.77 (0.71–0.84), 0.76 (0.67–0.87), and 0.87 (0.77–0.99), respectively (Kim and Je, 2016; Chen et al., 2013b), and when stratified by region, the pooled RRs (95%CIs) of risk of mortality from CVD for the highest versus lowest category intakes of total dietary fiber were 0.71 (0.59–0.86) in Europe, 0.77 (0.65–0.91) in USA, and 0.83 (0.70–0.98) in other regions (Kim and Je, 2016). Furthermore, the significant inverse associations between intakes of total dietary fiber and the incidence of CVD were shown only in nonsmokers; HRs (95%CIs) for the highest quintile of intake to the lowest were 0.59 (0.38–0.90) in men and 0.61 (0.45–0.83) in women, but not in smokers; HRs (95%CIs) were 1.05 (0.79–1.40) in men and 0.58 (0.24–1.39) in women (Kokubo et al., 2011). Because these evidences indicated consistently that intakes of total dietary fiber were associated with lower risk of CVD, CHD, and stroke, increasing intakes of dietary fiber may greatly contribute to the prevention of CVD.

Water-Soluble and Water-Insoluble Dietary Fiber

A metaanalysis reported that highest versus lowest intakes of soluble dietary fiber were associated with reduced risk of mortality from CVD (the pooled RR = 0.85, 95%CI = 0.75–0.96) and CHD (the pooled RR = 0.73, 95%CI = 0.62–0.85), and those of insoluble dietary fiber were also associated with lower risk of mortality from CVD (the pooled RR = 0.77, 95%CI = 0.66–0.90) and CHD (the pooled RR = 0.66, 95%CI = 0.52–0.83) in Americans, Europeans, Australians, and Japanese (Kim and Je, 2016). Intakes of insoluble dietary fiber with 7-g increment were associated with lower risk of CVD (the pooled RR = 0.82, 95% CI = 0.70–0.96) and CHD (the pooled RR = 0.82, 95%CI = 0.68–0.99), respectively, but not significant for those of soluble dietary

Table 12 Intakes of dietary fiber and the risk of cardiovascular disease

Published year study population	*Study name or setting name*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Kim and Je (2016) Pooled analysis of 15 studies from 12 cohorts	ATBC, BMES, EPIC, JACC, NHANES I, NHS, NIH-AARP Diet & Health Study, PREDIMED study, UKWCS, Southern California population-based cohort, Whitehall II cohort, Zutphen Study		20–85	Metaanalysis	14.5	Dietary intake by FFQ, 24-h dietary recall, cross-check dietary history method or 7-day food diary		RR = 0.77 (0.71–0.84) of CVD mortality for TDF (comparing the highest vs. lowest category) RR = 0.76 (0.67–087) of CHD mortality for TDF (comparing the highest vs. lowest category) For CVD mortality, RRs = 0.71 (0.59–0.86) in Europeans, 0.77 (0.65–0.91) in Americans, and 0.83 (0.70–0.98) in others (comparing the highest vs. lowest category) For CHD mortality, RRs = 0.76 (0.67–0.86) in Europeans, 0.52 (0.28–0.98) in Americans, and 0.85 (0.73–1.00) in others (comparing the highest vs. lowest category) For CVD mortality, RRs = 0.77 (0.71–0.82) for cereal fiber, 0.95 (0.89–1.02) for vegetable fiber, 0.96 (0.83–1.11) for fruit fiber, and 0.89 (0.82–0.98) for legume fiber (comparing the highest vs. lowest category) For CHD mortality, RRs = 0.83 (0.76–0.92) for cereal fiber, 0.90 (0.79–1.02) for vegetable fiber, 0.68 (0.49–0.95) for fruit fiber, and 1.33 (0.65–2.72) for legume fiber (comparing the highest vs. lowest category) For CVD mortality, RRs = 0.85 (0.75–0.96) for SDF and 0.77 (0.66–0.90) for IDF (comparing the highest vs. lowest category) For CHD mortality, RRs = 0.73 (0.62–0.85) for SDF and 0.66 (0.52–0.83) for IDF (comparing the highest vs. lowest category) For CVD mortality, RR = 0.91 (0.88–0.94) for TDF intakes per 10-g increment For CHD mortality, RR = 0.89 (0.85–0.93) for TDF intakes per 10-g increment For CVD mortality, RRs = 0.94 (0.90–0.98) for cereal fiber, 0.92 (0.89–0.95) for vegetable fiber, 0.96 (0.90–1.01) for fruit

(*Continued*)

Table 12 (Continued)

Published year study population	*Study name or setting name*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
								fiber, and 0.87 (0.68–1.13) for legume fiber (each 5-g increment) For CHD mortality, RRs = 0.95 (0.89–1.01) for cereal fiber, 0.92 (0.86–0.98) for vegetable fiber, 0.89 (0.73–1.10) for fruit fiber, and 0.90 (0.78–1.03) for legume fiber (each 5-g increment) For CVD mortality, RRs = 0.87 (0.74–1.02) for SDF and 0.92 (0.85–1.00) for IDF (each 5-g increment) For CHD mortality, RRs = 0.83 (0.71–0.98) for SDF and 0.83 (0.71–0.98) for IDF (each 5-g increment)
Threapleton et al. (2013b). Pooled analysis of 22 studies (largely US or Europe, 2 in Japan and 1 in Australia)	ATBC, BMES, CHS, EPIC, EPIC-Heart, EPIC-Norfolk, HPFS, JACC, JPHC, MDCC, NHANES I, NHS, OVS, SHHS, UKWCS, WHS, ZES, NIH-AARP Diet and Health Study, Whitehall II			Metaanalysis	3+	Dietary intake by FFQ, recalls, diet histories or 7-day food diary		RR = 0.91 (0.88–0.94) of the risk of CVD for TDF intakes per 7-g increment RR = 0.91 (0.87–0.94) of the risk of CHD for TDF intakes per 7-g increment RR = 0.88 (0.75–1.03) of the risk of CVD for SDF intakes per 4-g increment RR = 0.89 (0.78–1.02) of the risk of CHD for SDF intakes per 4-g increment RR = 0.82 (0.70–0.96) of the risk of CVD for IDF intakes per 7-g increment RR = 0.82 (0.68–0.99) of the risk of CHD for IDF intakes per 7-g increment For the risk of CVD, RRs = 0.92 (0.84–1.00) for cereal fiber per 7-g increment, 0.96 (0.93–1.00) for fruit fiber per 4-g increment, and 0.92 (0.87–0.96) for vegetable fiber per 4-g increment For the risk of CHD, RRs = 0.84 (0.76–0.94) for cereal fiber per 7-g increment, 0.92 (0.83–1.01) for fruit fiber per 4-g increment, and 0.94 (0.89–1.00) for vegetable fiber per 4-g increment
Threapleton et al. (2013c). Pooled analysis of eight cohorts	ATBC, BMES, HPFS, JACC, JPHC, NHANES I, NHS, MDCC			Metaanalysis	8–19			RRs = 0.93 (0.88–0.98) of the risk of stroke for 7-g increment of TDF intake
Chen et al. (2013b) Pooled analysis of six cohorts	ATBC, HPFS, JACC, JPHC, MDCC, NHS	314,864		Metaanalysis	8–18			For the risk of stroke, RRs = 0.87 (0.77–0.99) for TDF in both sexes, 0.95 (0.83–1.08) in men, and 0.80 (0.68–0.96) in women (comparing the highest vs. lowest category)

Kokubo et al. (2011). Japanese	JPHC	86,387 (40,046 men and 46,341 women)	45–65	Prospective study	10.4	Dietary intake by FFQ	Age, sex, smoking, alcohol, BMI, history of diabetes, medications for hypertension and hypercholesterolemia, exercise, dietary intakes of fruits, vegetables, fish, sodium, isoflavone and energy, and public health center	For the incidence of CVD, HRs = 0.65 (0.48–0.87) for TDF, 0.74 (0.56–0.97) for SDF, and 0.64 (0.47–0.85) for IDF (comparing the highest vs. lowest quintiles) For the incidence of total stroke, HRs = 0.64 (0.46–0.88) for TDF, 0.78 (0.58–1.06) for SDF, and 0.62 (0.45–0.85) for IDF in women (comparing the highest vs. lowest quintiles) For the incidence of cerebral infarction, HRs = 0.73 (0.55–0.97) for TDF, 0.73 (0.47–1.14) for SDF, and 0.62 (0.40–0.98) for IDF in women (comparing the highest vs. lowest quintiles) For the incidence of intracerebral hemorrhage, HRs = 0.53 (0.28–0.97) for TDF, 0.71 (0.40–1.26) for SDF, and 0.55 (0.30–1.00) for IDF in women (comparing the highest vs. lowest quintiles) For the incidence of CHD, HRs = 0.68 (0.32–1.42) for TDF, 0.60 (0.24–1.21) for SDF, and 0.78 (0.48–1.27) for IDF in women (comparing the highest vs. lowest quintiles) For the incidence of CVD, HRs = 0.59 (0.38–0.90) and 0.61 (0.45–0.83) for TDF among men and women with nonsmoking, and 1.05 (0.79–1.40) and 0.58 (0.24–1.39) among men and women with smoking (comparing the highest vs. lowest quintiles)
Rimm et al. (1996). US men	HPFS	43,757 men	40–75	Prospective study	6	Dietary intake by FFQ	Saturated fat, vitamin E, age, BMI, physical activity, smoking, alcohol, hypertension, hypercholesterolemia, family history of myocardial infarction, and profession	RRs = 0.64 (0.47–0.87) of the incidence of myocardial infarction for the highest vs. lowest quintiles of TDF intake RRs = 0.81 (0.70–0.93) of the incidence of myocardial infarction for intakes of TDF per 10-g increment For the incidence of myocardial infarction, RRs = 1.07 (0.57–2.02) for SDF and 0.75 (0.59–0.94) for IDF (each 10-g increment) For the incidence of myocardial infarction, RRs = 0.73 (0.56–0.94) for cereal fiber, 0.84 (0.65–1.08) for vegetable fiber, and 0.82 (0.63–1.07) for fruit fiber (comparing the highest vs. lowest quintiles)

(*Continued*)

Table 12 (Continued)

Published year study population	*Study name or setting name*	*Sample size*	*Age at entry*	*Study design*	*Follow-up years*	*Exposure*	*Adjusted variables*	*Relative risk*
Threapleton et al. (2013a). UK women	UKWCS	31,036 women		Prospective study	14.3	Dietary intake by FFQ	Age, BMI, calories from carbohydrate, fat, and protein, ethanol intake, physical activity, smoking status, socioeconomic status	For CHD mortality, HRs = 0.76 (0.32–1.80) for SDF and 0.75 (0.33–1.71) for IDF (comparing the highest vs. lowest quintiles) For stroke mortality, HRs = 0.60 (0.25–1.43) for SDF and 0.78 (0.37–1.67) for IDF (comparing the highest vs. lowest quintiles) For CVD mortality, HRs = 0.68 (0.37–1.25) for SDF and 0.78 (0.45–1.36) for IDF (comparing the highest vs. lowest quintiles) For CVD mortality, HRs = 0.91 (0.56–1.48) for cereal fiber , 0.68 (0.43–1.06) for fruit fiber, 0.75 (0.49–1.17) for vegetable fiber, 1.03 (0.62–1.72) for legume fiber, and 0.61 (0.39–0.96) for fiber from nuts and seeds (comparing the highest vs. lowest quintiles) For stroke mortality, HRs = 0.77 (0.40–1.50) for cereal fiber, 0.79 (0.42–1.48) for fruit fiber, 0.58 (0.30–1.11) for vegetable fiber, 0.79 (0.37–1.67) for legume fiber, and 0.45 (0.23–0.85) for fiber from nuts and seeds (comparing the highest vs. lowest quintiles) For CHD mortality, HRs = 1.06 (0.52–2.15) for cereal fiber, 0.55 (0.28–1.06) for fruit fiber, 0.95 (0.52–1.74) for vegetable fiber, 1.33 (0.65–2.71) for ledume fiber, and 0.84 (0.44–1.59) for fiber from nuts and seeds (comparing the highest vs. lowest quintiles)
Eshak et al. (2010). Japanese	The Japan Collaborative Cohort Study for Evaluation of Cancer Risks (JACC)	58,730 (23,119 men and 35,611 women)	40–79	Prospective study	14.3	FFQ	Age, BMI, history of hypertension, history of diabetes, alcohol consumption, smoking, education level, hours of exercise, hours of	HRs = 0.83 (0.63–1.09) and 0.82 (0.57–0.97) of CVD mortality for TDF in men and women (comparing the highest vs. lowest quintiles) HRs = 0.81(0.61–1.09) and 0.80 (0.57–0.97) of CHD mortality for TDF in

	walking, perceived mental stress, sleep fish, SFA, (n-3) fatty acids, sodium, folate, and vitamin E	men and women (comparing the highest vs. lowest quintiles) For CVD mortality HRs = 0.81 (0.63–1.04) and 0.83 (0.53–1.02) for SDF, and 0.82 (0.65–0.98) and 0.69 (0.53–0.91) in men and women (comparing the highest vs. lowest quintiles) For CHD mortality, HRs = 0.71 (0.41–0.97) and 0.72 (0.43–0.99) for SDF, and 0.48 (0.27–0.84) and 0.49 (0.27–0.86) for IDF in men and women (comparing the highest vs. lowest quintiles) For CHD mortality, HRs = 0.86 (0.64–0.99) for cereal, 0.56 (0.35–0.90) for fruit and 0.92 (0.56–1.52) for vegetable in men, and 0.77 (0.59–0.98) for cereal, 0.40 (0.28–0.78) for fruit and 0.91 (0.55–1.49) for vegetable in women (comparing the highest vs. lowest quintiles)
	Age, BMI, history of hypertension, history of diabetes, alcohol consumption, smoking, education level, hours of exercise, hours of walking, perceived mental stress, sleep fish, SFA, (n-3) fatty acids, sodium, folate, and vitamin E, and further for other types of dietary fiber (cereal, fruit, and vegetable fibers).	For CHD mortality, HRs = 0.89 (0.65–1.01) for cereal fiber, 0.55 (0.32–0.96) for fruit fiber and 0.90 (0.54–1.51) for vegetable fiber in men, and 0.76 (0.59–0.97) for cereal fiber, 0.42 (0.33–0.81) for fruit fiber and 0.97 (0.58–1.62) for vegetable fiber in women (comparing the highest vs. lowest quintiles)

ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; *BMES*, Blue Mountains Eye Study; *BMI*, body mass index; *CHD*, coronary heart disease; *CHS*, Cardiovascular Health Study; *EPIC*, European Prospective Investigation into Cancer and Nutrition; *FFQ*, food frequency questionnaire; *HPFS*, Health Professionals Follow-up Study; *HR*, hazard ratio; *IDF*, insoluble dietary fiber; *JACC*, Japan Collaborative Cohort Study; *JPHC*, Japan Public Health Center-based study; *MDCC*, Malmo Diet and Cancer Cohort; *NHANES*, National Health and Nutrition Examination Survey; *NHS*, Nurses' health study; *NIH-AARP*, National Institutes of Health-American Association of Retired Persons; *OVS*, Oxford Vegetarian Study; *PREDIMED*, Prevencion con Dieta Mediterranea; *RR*, relative risk; *SDF*, soluble dietary fiber; *SHHS*, Scottish Heart Health Study; *TDF*, total dietary fiber; *UKWCS*, United Kingdom Women's Cohort Study; *WHS*, Women's Health Study; *ZES*, Zutphen Elderly Study.

fiber (the pooled RRs (95%CIs): 0.88 (0.75–1.03) of CVD and 0.89 (0.78–1.02) of CHD) (Threapleton et al., 2013b). Similarly in US men, each 10-g increment of insoluble but not soluble dietary fiber was inversely associated with the incidence risk of myocardial infarction (MI); the RRs (95%CIs) for the highest quintile of intake to the lowest were 0.75 (0.59–0.94) for insoluble dietary fiber and 1.07 (0.57–2.02) for soluble dietary fiber (Rimm et al., 1996). A cohort study reported that the highest versus lowest quintile of insoluble and soluble dietary fiber intakes were associated with a reduced incidence of CVD in Japanese women; the RRs (95%CIs) were 0.64 (0.47–0.85) for insoluble fiber and 0.74 (0.56–0.97) for soluble fiber, and only insoluble fiber was significantly associated with lower incidence of total stroke (RR = 0.62, 95%CI = 0.45–0.85) and cerebral infarction (RR = 0.62, 95%CI = 0.40–0.98) in women (Kokubo et al., 2011). However in UK women, intakes of both insoluble and soluble were not significantly associated with mortality from CVD, CHD, or stroke (Threapleton et al., 2013a). Accordingly, intakes of soluble or insoluble dietary fiber were likely to associate with lower risk of CVD, CHD, and stroke, but it would be necessary to accumulate more evidence.

Dietary Fiber From Food Source

Two metaanalyses including dietary fiber from different food sources showed that 4–5 g increasing intakes of dietary fiber from vegetables were associated with lower risk of CVD and CHD; the pooled RRs (95%CIs) of CVD were 0.92 (0.87–0.96) per 4 g and 0.92 (0.89–0.95) per 5 g, and those of CHD were 0.94 (0.89–1.00) per 4 g and 0.92 (0.86–0.98) per 5 g), and those from cereals had similar trend for the risk of CVD and CHD; the pooled RRs (95%CIs) of CVD were 0.94 (0.90–0.98) per 5 g and 0.92 (0.84–1.00) per 7 g, and those of CHD were 0.84 (0.76–0.94) per 5 g and 0.84 (0.76–0.94) per 7 g in Americans, Europeans, Australians, and Japanese (Kim and Je, 2016; Threapleton et al., 2013b). In addition, the highest quintile of dietary fiber intakes from nuts and seeds were associated with lower mortality from CVD (RR = 0.61, 95%CI = 0.39–0.96) and stroke (RR = 0.45, 95%CI = 0.23–0.85) in the UK women, when compared to the lowest intakes (Rimm et al., 1996). Two metaanalyses showed that intakes of fiber from fruits per 4–5 g/day increase are likely to be associated with insignificant lower risk of CVD (the pooled RRs (95%CIs): 0.96 (0.93–1.00) per 4-g increment and 0.96 (0.90–1.01) per 5-g increment and CHD (the pooled RRs (95%CI): 0.92 (0.83–1.01) per 4-g increment and 0.89 (0.73–1.10) per 5-g increment (Kim and Je, 2016; Threapleton et al., 2013b). In the highest versus lowest category of fruit fiber intakes, a recent metaanalysis showed significantly a lower risk of CHD mortality (Kim and Je, 2016), and a prospective study of Asians cohort also reported a lower risk of CHD mortality after further adjustment for other types of dietary fibers (cereal and vegetable fibers); the HRs (95%CI) were 0.55 (0.32–0.96) in men and 0.42 (0.33–0.81) in women (Eshak et al., 2010).

Comment

We reviewed the association between dietary fiber intake and the risk of CVD. Intakes of total dietary fiber may be associated with reduced risk of CVD. However the evidence for the associations between soluble and insoluble dietary fiber or dietary fiber from food sources and the risk of CVD needs to be accumulated.

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Remission and Recovery in Heart Failure

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Introduction

The syndrome of heart failure (HF) was once widely considered to be conceptually equivalent and equal to left ventricular systolic dysfunction with reduced left ventricular ejection fraction (LVEF) and compromised contractility. A growing number of patients however would have the bedside clinical syndrome with normal LVEF. This led to the partitioning of HF into two operational and physiological frameworks—HF with reduced ejection fraction (HF*r*EF) was joined by its new comparator, HF with preserved ejection fraction (HF*p*EF).

The HF*r*EF and HF*p*EF distinction is critical as both the underlying pathophysiological drivers of HF subtype and responses to clinical interventions appear distinct (Borlaug, 2011; Paulus and Tschöpe, 2013). The clinical arc of HF*r*EF is altered by neurohormonal blockade provided by oral agents like beta-blockers, angiotensin-converting enzyme inhibitors, aldosterone receptor blockers, neprilysin inhibitors, and mechanical therapies like cardiac resynchronization therapy (CRT) for those with dyssynchrony and concomitant left bundle branch block. By contrast, these therapies fail to confer this benefit to patients with HF*p*EF, leading to static mortality for HF*p*EF patients over time (Massie et al., 2008; Lee et al., 2004). Consequently, LVEF is imperative in driving clinical management in patients with HF.

It has become increasingly recognized that patients with low LVEF can recover and experience substantial improvements in LVEF, which has complicated the HF*r*EF and HF*p*EF dichotomy. This is addressed in the 2013 American College of Cardiology/American Heart Association guidelines, which state the following: "It has been recognized that a subset of patients with HF*p*EF previously had HF*r*EF. These patients with improvement or recovery in EF may be clinically distinct from those with persistently preserved or reduced EF. Further research is needed to better characterize these patients (Yancy et al., 2013)."

This article will summarize existing literature on LVEF recovery and potential clinical and therapeutic implications. Emerging evidence substantiates a distinct clinical arc among patients with LVEF recovery apart from HF*p*EF or HF*r*EF and suggests that HF with recovered LVEF may be a distinct phenotype. How this transitioning population should be classified and treated remains unknown.

Defining Cardiac Remodeling, Remission, and Recovery

The phenomenon whereby a patient with a reduced LVEF subsequently has a higher LVEF has been variably termed improved EF, better EF, and recovered EF (Punnoose et al., 2011; Stevenson, 2014). LVEF recovery highlights the dynamic nature of cardiac function and has reintroduced concepts old and new into the HF vernacular. Because LVEF recovery can be ascertained relatively easily, it has been the focus of most existing clinical studies. However, it is important to define the relationship between LVEF recovery, cardiac remodeling/reverse remodeling, and myocardial remission and recovery.

We define cardiac remodeling as those myocardial adaptations that change the shape, mass, and function of the ventricle. Cardiac remodeling can be both physiological, as seen in elite exercising athletes, and pathological as seen in hypertensive and postinfarction individuals. Reverse remodeling can then be used to describe the reversal of those adaptations, and in this article, we use it to describe the reversal of pathological remodeling. While LVEF recovery is one metric by which reverse remodeling can be

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ascertained, other structural abnormalities are not encompassed by LVEF alone, including LV hypertrophy, LV dilation, shape, and sphericity.

Hellawell and Margulies review how reverse remodeling can occur in specific cardiomyopathies from the improvements in LVEF seen in peripartum cardiomyopathy to the improvements that occur after alcohol cessation in patients with alcohol-related cardiomyopathy (Hellawell and Margulies, 2012). Reverse remodeling can occur spontaneously in some cases or in response to medical or device therapies as described in the section to follow. It has been recognized that reverse remodeling is associated with the normalization of underlying molecular and cellular changes that drive remodeling; however, it is important to acknowledge that myocardial recovery is not synonymous with reverse remodeling or LVEF recovery (Mann and Burkhoff, 2012). The term myocardial recovery is reserved for those who both normalize LVEF and appear to have a functional cure from HF by being free of future cardiovascular events. Indeed, in the sections to follow, we show that most patients with LVEF recovery likely carry with them the legacy of HF with residual risk of cardiovascular events and thus more appropriately can be considered to have myocardial remission, rather than myocardial recovery (Fig. 1).

Although LVEF oversimplifies its physiological underpinnings, in the current landscape, where it classifies and guides treatment in HF, it has inherent value in discussing myocardial remission and recovery. LVEF improvement implies the active unraveling of the adverse adaptations of cardiac remodeling that occur in the failing heart. The reversal of these adaptations in the heart with improvements in shape, size, and function, we refer to reverse remodeling. In patients with chronic systolic HF, LVEF improvement therefore is nearly forme fruste of myocardial reverse remodeling. For the remainder of this article, we therefore examine LVEF recovery, with the understanding that this likely implies myocardial reverse remodeling and myocardial remission. Given that these patients appear distinct from those with persistent HF*r*EF or HF*p*EF, we will also refer to those with LVEF recovery as having HF with recovered EF (HFrecEF).

Specific Therapies That Promote LVEF Improvement

Spontaneous Recovery

Although understudied, the spontaneous and unassisted improvement of LVEF has long been appreciated and described. Some of the earliest published works describe improvements in LVEF in nearly 40% of patients with acute myocarditis (Dec et al., 1985), in patients who used chronic alcohol (Francis et al., 1990), and in 27% of patients with recent-onset dilated cardiomyopathy referred for cardiac transplantation (Steimle et al., 1994). Other reversible myopathies extend to include stress cardiomyopathy, tachycardia-mediated cardiomyopathy (McLaran et al., 1985), alcohol-related cardiomyopathy (Nicolás et al., 2002), and peripartum cardiomyopathy.

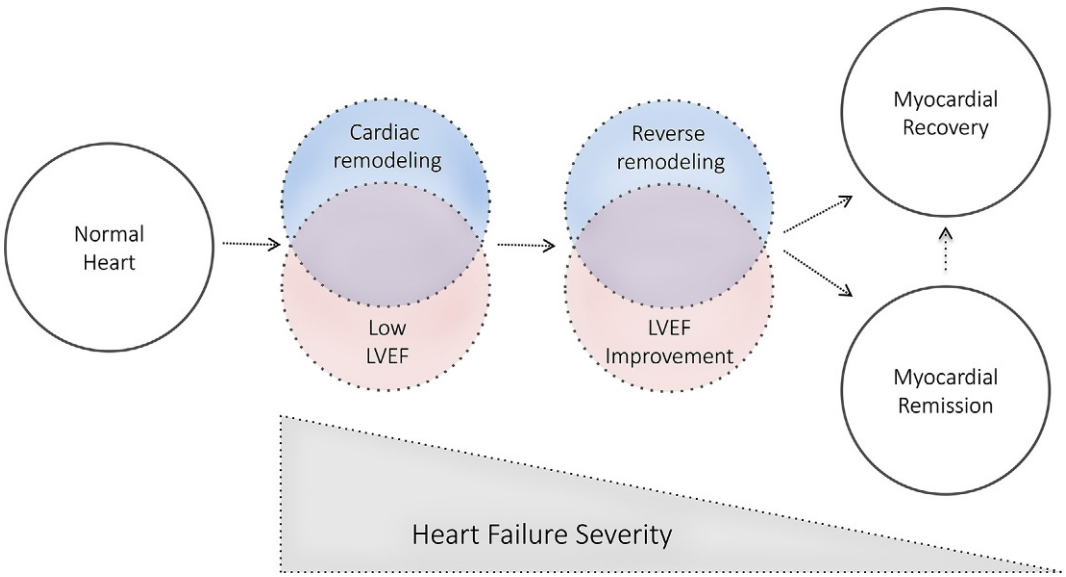

Fig. 1 The progression to recovery and remission. The normal heart, after incurring an insult, is left with impaired systolic function and low EF. Further deterioration occurs by way of pathological cardiac remodeling where the heart increases in mass and becomes dilated and spherical. Reverse remodeling, either spontaneously, or with either drugs or devices, engenders LVEF improvement (or recovered EF) and most often takes the path toward myocardial remission whereby the heart remains at risk of future cardiovascular events. It may however, take a path toward myocardial recovery and achieve functional cure whereby the heart is free from future cardiovascular events. Myocardial recovery is likely rare. After the initial insult and low EF state, reverse remodeling progressively attenuates the severity of heart failure.

Medical Therapy

It is known that medical therapy alone engenders improvements in clinical outcomes, promotes reverse remodeling, and improves LVEF. In a meta-analysis of 86 remodeling trials, the absolute change in LVEF associated with HF therapies was compared with placebo with an average follow-up time of 6 months (Kramer et al., 2010). Kramer and others would find that those drugs that engendered lower mortality also improved LVEF when compared with placebo. Classical mortality improving agents like the beta-blocker bisoprolol decreased end-diastolic volumes and improved LVEF on average +12% after pooling results (Kramer et al., 2010). Carvedilol had a mean increase in LVEF of +6.9%, metoprolol +4.5%, enalapril +2.7%, hydralazine–isosorbide dinitrate +2.9%, and spironolactone +3.0%. These guideline-recommended therapies were consistently observed to improve LVEF in patients with LV systolic dysfunction (Kramer et al., 2010). The principle here is that abrogation of the sympathetic/stress response and the activated neurohormonal cascade can lead to improved hemodynamic and neurohormonal profiles that promote reverse remodeling and overall incremental improvements in LVEF.

Device Therapy

In addition to medical therapy, device therapy is known to promote LVEF improvement. The best example is perhaps CRT or biventricular pacing. Among patients with HF*r*EF, wide QRS, and left bundle branch block, CRT has been shown to target the dyssynchrony seen in the myopathic heart and improve clinical outcomes of symptoms and survival. CRT improves both atrioventricular synchrony and the ventricular pattern of electrical activation that integrates the heart. These features, acting in concert, are thought to be the underlying authors that promote systolic augmentation (Leclercq and Kass, 2002). Kramer and others would show that CRT reduced both end-systolic and end-diastolic volumes (markers of reverse remodeling) and on average had an absolute improvement in LVEF by about +2.7% across four trials and with a mean of 21 weeks of follow-up.

Although these changes are modest, the CRT "super responder" or "hyper responder" has also been described who has marked improvements in LVEF after implantation and initiation of CRT. In a study of patients with nonischemic dilated cardiomyopathy and ejection fractions under 35% with end-diastolic diameters >60 mm, CRT was installed, and patients were followed for 6 months (Castellant et al., 2009). Of the 51 patients with nonischemic cardiomyopathy, 11 were found to be hyper responders who had a mean LVEF increase from an average of 26+/−9% to 59+/−6% ($P < 0.01$). The remaining 40 patients had no functional improvement and a mean LVEF improvement from 21+/−9% to 26+/−12% ($P = 0.03$). Patient characteristics that predict "super responders" have been examined in the Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy (MADIT-CRT) among other studies and include female sex, no prior myocardial infarction, left bundle branch block, QRS duration ≥150 ms, body mass index <30 kg/m^2, and smaller baseline left atrial volume index (Hsu et al., 2012). Not surprisingly, super response has been associated with improved outcomes when compared with hyporesponse to CRT.

A second notable example of LVEF recovery with device therapies is that of marked cardiac reverse remodeling observed with mechanical unloading of the heart with left ventricular assist devices (LVAD) (Westaby et al., 1997). Initial reports supported transient normalization of systolic function with LVAD unloading (Frazier, 1994; Levin et al., 1996). Since then, the possibility of LVAD explantation after LVEF recovery with a combinatorial approach of LVAD unloading and high-dose pharmacological therapy has been reported. In a prospective study of 20 patients with nonischemic cardiomyopathies, a strategy combining LVAD unloading with high-dose pharmacological therapy (carvedilol, digoxin, lisinopril, losartan, and spironolactone followed by the beta-2 agonist clenbuterol) led to LVAD explantation in 12 of 20 patients. The mean preexplant EF was 70%, and the mean postexplant EF was 58%. By comparison, previously published LVAD explantation rates ranged from 11% to 24% among patients with nonischemic cardiomyopathy enriched for acute myocarditis (Dandel et al., 2008; Maybaum et al., 2007; Simon et al., 2005).

Despite the excitement around the possibility of LVAD unloading and recovery, the ability to explant an LVAD still remains rare, with myocardial recovery in only a small percentage of patients with end-stage HF after LVAD therapy. (Mancini et al., 1998). With sufficient time, the vast majority of patients with chronic LV systolic dysfunction do exhibit reverse remodeling with LVAD unloading; however, in the INTERMACS registry, slightly <1% of patients were explanted because of LVEF recovery (Kirklin et al., 2015).

LVEF Recovery Among the HF Population: Determinants and Outcomes

Punnoose, Desai, and others were some of the first to hypothesize that improvements in LVEF occurred in patients with systolic HF and that this represented a clinical entity distinct from the HF*p*EF and HF*r*EF syndromes (Punnoose et al., 2011). They labeled this population as having HF with "recovered LVEF" and systematically studied the emerging phenomenon at their referral institution. They and others have collectively amassed a body of work that suggests that the recovered LVEF population may be phenotypically and prognostically distinct than the current HF*p*EF and HF*r*EF paradigm (Basuray et al., 2014; de Groote et al., 2014; Dunlay et al., 2012; Florea et al., 2016; Joyce et al., 2016; Kalogeropoulos et al., 2016; Lupón et al., 2017; Merlo et al., 2011; Punnoose et al., 2011; Teeter et al., 2012; Zhang et al., 2015).

It is important to acknowledge that LVEF is dynamic and that this dynamism is likely attributable to cardiac remodeling and reverse remodeling. In a health plan-based sample of 2413 patients with HF and ≥2 LVEF measurements, investigators examined transitions between HF*p*EF (classified as EF >40%), HF*r*EF (classified as EF <40%), and death. Over a mean follow-up time of 4.4

years, there were 8183 transitions, highlighting how dynamic LVEF can be (Clarke et al., 2013). Similarly, among 1233 HF patients in the Olmsted county cohort, the mean LVEF decreased by 5.8% among patients with HF*p*EF and increased by 6.9% over 5 years among patients with HF*r*EF (Dunlay et al., 2012). This suggests that patients are not longitudinally restricted to HF*r*EF or HF*p*EF status. In the section that follows, we examine studies focused on patients with HF*r*EF who show LVEF improvement over time. Rather than reclassifying this group as HF*p*EF, these studies substantiate the fact that this group may harbor a unique clinical trajectory; hence, we will refer to this group as HFrecEF.

Determinants of LVEF Recovery

What we know about the recovered EF population comes from a handful of hospital- and community-based cohorts that are summarized in Table 1. The definition of recovered LVEF varies from study to study with reduced ejection fraction having different echocardiographic thresholds that range from less than 35%, 40%, 45%, and 50%. This variability limits some of the interpretation of the studies although certain themes consistently emerge. In patients with HF*r*EF, a substantial number appear to experience LVEF recovery, with estimates ranging from as low as 9% to as high as 43% (Table 1). The true prevalence of the population remains uncertain but probably lies somewhere in between and needs more careful epidemiological study.

There appears to be a theme with regard to the clinical profile of patients with HFrecEF as compared with patients with persistent HF*r*EF. Indeed, patients who experience LVEF recovery tend to be younger, female, and have a nonischemic mechanism of their HF. Recovered EF patients tend to have a shorter duration of HF symptoms and a higher blood pressure, less likely to have LBBB, and more likely to have atrial fibrillation (Lupón et al., 2017; Punnoose et al., 2011). In addition to better renal function and being on higher doses of neurohormonal blockade, they also tend to have structurally more normal hearts with smaller LV end-diastolic dimensions (Florea et al., 2016).

The biochemical signatures of the recovered EF population are also distinct from the HF*r*EF population. A number of biomarkers seem to suggest persistent yet less severe neurohormonal cascade activation. For example, a study from the Val-HeFt trial showed that patients with recovered EF have lower levels of norepinephrine and natriuretic peptides (Florea et al., 2016). In the Penn Heart Failure Study, recovered LVEF patients also had lower levels of natriuretic peptides, troponin I, uric acid, and S-Flt-1 compared with HF*r*EF, although natriuretic peptides remained persistently elevated above the ninety-fifth percentile in about 30% of HFrecEF patients (Basuray et al., 2014). This suggests that despite LVEF recovery, continued neurohormonal activation can still be present.

When comparing HFrecEF with HF*p*EF patients, there a number of clinical factors that distinguish these two phenotypes (Table 1). Patients with LVEF improvement are again younger in age with less coronary disease. They also have less comorbidity like diabetes, CKD, and hypertension and are more likely to be on guideline-directed medical therapy.

Clinical Outcomes After LVEF Recovery

Outcomes for patients with HFrecEF are summarized in Table 1. The recovered LVEF population is consistently associated with improved survival when compared with the persistent HF*r*EF population. One study found that an EF improvement of 5% was associated with a 12% mortality reduction (Dunlay et al., 2012). In addition to lower all-cause mortality, HFrecEF is also associated with the lower risk of need for transplant or LVAD (Basuray et al., 2014) and lower risk of sudden cardiac death and ventricular arrhythmias (Merlo et al., 2011; Zhang et al., 2015). Patients with HFrecEF also have improved outcomes compared with HF*p*EF, with lower all-cause mortality, cardiovascular, HF-related, and sudden death (Lupón et al., 2017), although cardiac hospitalizations appear similar between HFrecEF and HF*p*EF in one study (Basuray et al., 2014). This favorable and improved clinical arc suggests that LVEF recovery (and HFrecEF) is indeed clinically distinct from persistent HF*r*EF or HF*p*EF.

While outcomes in patients with HFrecEF are improved compared with HF*r*EF and HF*p*EF as summarized earlier, there remains residual risk for recurrent and future cardiovascular events. Up to a quarter of patients with HFrecEF can have a subsequent LVEF decline (de Groote et al., 2014). Despite LVEF recovery, patients with HFrecEF also experience persistent HF symptoms and clinical events, including cardiovascular death, HF death, and sudden death (Basuray et al., 2014; Lupón et al., 2017). Another tangible example comes from CRT "super responders" who have marked normalization of EF to >50% after device initiation. Although long-term outcomes in this population are generally considered to be excellent, they continue to experience HF hospitalizations and appropriate CRT-D intervention despite EF normalization (Zecchin et al., 2014). This risk remains latent and can occur years after EF normalization (Zecchin et al., 2014).

Clinical Management of Patients With LVEF Recovery

Given the residual risk of cardiovascular events, how then are we to manage the patient who has achieved LVEF recovery but remains in myocardial remission with HFrecEF? Those with specific etiologic causes of cardiomyopathy require ongoing considerations specific to that etiology and have been reviewed (Basuray and Fang, 2016). With respect to general guideline-directed medical therapies for HF, it remains unclear whether such therapies should be continued, weaned, or discontinued in patients with LVEF recovery. Some studies have shown that discontinuation of beta-blocker or angiotensin-converting enzyme inhibitor therapy results in subsequent LVEF decline and recurrent clinical HF events (Moon et al., 2009; Waagstein et al., 1989). In this context, it is generally recommended that HF medications and lifestyle interventions be continued in those with

Table 1 Determinants and outcomes of LVEF recovery in heart failure populations

References	*Sample*	*Definition of HFrecEF*	*Percent of LVEF recovery*	*Correlates of HFrecEF*	*Outcomes of HFrecEF*
Lupón (2017)	Hospital-based cohort: 1057 patients with HF	LVEF <45% at baseline and ≥45% at 1 year	22	Compared with HFrEF: younger age, more women, nonischemic etiology, nondiabetic, more AF, less LBBB, shorter HF duration, and better NYHA class	Lower mortality (HR 1.77, 95% CI 1.20–2.61 in HFpEF vs. HFrecEF and HR 1.54, 95% CI 1.162.04 HFrEF vs. HFrecEF). Also lower CV and HF events. Lower cumulative incidence of CV- and HF-related hospitalizations
Kalogeropoulos et al. (2016)	Hospital-based cohort: 2166 outpatients with HF	LVEF >40% and any previous LVEF ≤40%	16	Compared with HFpEF: younger age, less CAD, DM, CKD, lower BP, more likely on GDMT, less likely ICD/CRT	Lower mortality at 3 years (HR 0.35, 95% CI 0.18–0.70 c/w HFrEF) but not at 1 year. Age- and sex-adjusted 3-year mortality 4.8% (vs. 13.2% in HFpEF and 16.3% in HFrEF)
Florea et al. (2016)	Val-HeFT trial: 3519 with HF, baseline LVEF <35%, and subsequent TTE at 12 months	LVEF <35% at baseline and >40% at 12 months	9	Compared with HFrEF: younger age, less ischemic, more hypertension, lower LVEDD index, higher eGFR, greater BB and ARB use. Biomarkers: less severe neurohormonal activation (lower NE, PRA, ETI, BNP, NT-proBNP). Independent correlates: sex, ischemic pathogenesis, BMI, DBP, LVEDD index, BB use, valsartan use	Survival higher in HFrecEF c/w HFrEF. Mortality rate 2.9 per 100 PY (95% CI 1.8–4.5) in HFrecEF versus 5.8 per 100 PY (95% CI 5.3–6.4) in HFrEF
Joyce et al. (2016)	Hospital-based cohort: 726 ambulatory HF	Prior LVEF <50% with subsequent LVEF ≥50% at study visit	19	Compared with HFpEF: better QOL and breathing by visual analog scale	Not examined
Zhang et al. (2015)	PROSE-ICD (systolic HF eligible for primary prevention ICD): 538 with subsequent LVEF assessment	Absolute increase in LVEF >5%	40	Compared with worse or unchanged LVEF: more likely to have a CRT-D (as opposed to ICD)	Lower mortality at mean follow-up of 2 years (HR 0.33, 95% CI 0.18–0.59) in HFrecEF versus unchanged EF. Also lower risk of appropriate shocks. Among patients with LVEF improvement >35%, four experienced appropriate shock
Basuray et al. (2014)	Hospital-based cohort (Penn Heart Failure Study): 1821 chronic HF	Prior LVEF <50% with subsequent LVEF ≥50% at study visit	10	Compared with HFpEF: younger, less CKD, less HTN, less symptoms, more GDMT. Compared with HFrEF: similar age, less ischemic origin, less CKD, similar HTN. Biomarker profiles: lowest in HFrecEF group, including BNP, troponin I, S-Flt-1, uric acid. However 1/3 of HFrecEF still had BNP >135 pg/mL, other abnormalities in biomarkers	Lower risk of combined death, transplant, or VAD (multivariable-adjusted HR 2.0, 95% CI 1.0–4.0 in HFpEF vs. HFrecEF and HR 3.4, 95% CI 2.0–5.7 in HFrEF vs. HFrecEF). Lower risk of cardiac hospitalization compared with HFrEF (HR 1.8, 95% CI 1.4–2.4 HFrEF vs. HFrecEF) but similar to HFpEF
de Groote et al. (2014)	Hospital-based cohort: 174 with recovered LVEF on beta-blocker	LVEF <45% with subsequent LVEF ≥45% after beta-blocker with ≥5% LVEF increase		No comparator group	Survival rates 90% at 5 years, 75% at 10 years similar to expected survival rates. Subsequent LVEF decline seen in a quarter
Dunlay et al. (2012)	Community-based cohort (Olmsted): 1233 participants with incident HF	LVEF ≥50% with previous LVEF <50%	39	Among HFrEF, LVEF increased by 5.8% on average over 5 years, greater improvement in women, younger patients, without CAD, on medications	Lower mortality: a 5% increase in LVEF was associated with a 12% reduction in mortality (adjusted HR 1.12, 95% CI 1.07–1.16)

(*Continued*)

Table 1 Determinants and outcomes of LVEF recovery in heart failure populations (Continued)

References	*Sample*	*Definition of HFrecEF*	*Percent of LVEF recovery*	*Correlates of HFrecEF*	*Outcomes of HFrecEF*
Teeter et al. (2012)	Hospital-based cohort: 224 with HF <6-month duration and LVEF <30%, 115 with follow-up TTE	LVEF <30% at baseline and subsequent LVEF ≥40%	43	Compared with no LVEF improvement: less ischemic etiology, higher BP, narrower QRS, greater wall thickness, and smaller LV dimensions at baseline, more likely to have achieved target beta-blocker dose	Not compared
Punnoose et al. (2011)	Hospital-based cohort: 358 stable HF outpatients	LVEF <40% and subsequent LVEF ≥40% with ≥5% absolute change	34	Compared with either HFpEF or HFrEF: younger, fewer HF hospitalizations, milder HF symptoms. Compared with HFpEF: less HTN, DM, or AF; lower BP; better eGFR. Compared with HFrEF: more women, less CAD, higher BP	Not examined
Merlo et al. (2011)	Tertiary referral center registry: 361 idiopathic DCM patients, 242 had follow-up TTE	LVEF increase ≥10% or follow-up LVEF ≥50% with baseline LVEF 45%–49% and decrease in LV end-diastolic diameter index ≥10%	37	Compared with nonrecovery: lower LVEF, higher restrictive filling pattern. Independent predictors of recovery—higher systolic BP (OR 1.23, 95% CI 1.01–1.53 per 10 mmHg increase) and the absence of LBBB (OR 2.47, 95% CI 1.25–4.87)	Better transplant-free survival (5- and 10-year survival 94% and 85% in HFrecEF compared with 81% and 63%, respectively, in nonrecovery). Multivariable-adjusted HR for death/transplant HR 0.44, 95% CI 0.25–0.78, for sudden death/ventricular arrhythmia HR 0.39, 95% CI 0.21–0.74

LVEF recovery (Basuray and Fang, 2016) although no clinical trials are available to substantiate this strategy. For those patients receiving unloading therapy with an LVAD, it remains unclear who merits explantation and whether medical therapy should be offered synchronously. Whether LVAD therapy might serve as a durable bridge to either remission or recovery remains to be seen (Drakos et al., 2012).

The management of patients with implantable cardioverter defibrillators (ICDs) and LVEF recovery is also uncertain. A recent study showed that among 126 patients with primary prevention ICDs and LVEF improvement to >35% after device implantation, the risk of appropriate shocks was decreased but not abrogated, with four patients receiving appropriate shocks (Zhang et al., 2015). It thus remains unclear whether routine replacement of ICD generators is warranted in patients with LVEF recovery and how the presence, absence, or frequency of appropriate or inappropriate device therapies inform this decision. The European Society of Cardiology guidelines advocate against routine replacement and for reassessment of patient goals. They write that "patients should be carefully evaluated by an experienced cardiologist before generator replacement, because management goals and the patient's needs and clinical status may have changed (Ponikowski et al., 2016)."

Given that EF normalization likely comes with myocardial remission and not functional cure or recovery, more study is required to define how best to follow these patients, how frequently to obtain EF assessments, and what to do with our therapies once EF has normalized.

Current Knowledge Gaps

There is much that we do not know about LVEF recovery and reverse remodeling. With this emerging population, questions surrounding the diagnosis, follow-up, and management of these patients will become more frequent. Many fundamental prognostic, diagnostic, and therapeutic questions still remain.

One fundamental question that remains is whether there exists a subset of patients with LVEF recovery that also have true myocardial recovery and "cure" from HF. Is it possible to transition from remission to recovery, and can these entities be more robustly predicted and promoted? Part of the difficulty in answering this question may be that LVEF, while simple and easy to use, is a summary metric that does not incorporate many important physiological elements—it is often conflated with contractility but in reality remains conceptually uncoupled (Konstam and Abboud, 2017). Because of these limitations, LVEF may not distinguish between patients who achieve myocardial remission versus myocardial recovery. In the future, more advanced imaging techniques,

including cardiac magnetic resonance imaging and echocardiographic strain, may offer the ability to more closely understand the extent to which reverse remodeling has occurred. Further, biomarker profiles may offer insights into which patients have achieved recovery in contrast to remission and may also uncover other underlying processes at play during reverse remodeling. For example, a specific circulating extracellular microRNA (miR-30d) was associated with LVEF improvement in response to CRT and also appears protective against cardiomyocyte apoptosis (Melman et al., 2015).

A second set of remaining questions centers on how we might tailor treatments to individual patients. The routine use of a given treatment paradigm seems antithetical to the emergence of personalized medicine. Questions still remain over what medications are best to use once LVEF is normalized, and which medications if any should be continued, discontinued, or merely weaned. This extends into the arena of device therapy, and one wonders given the compelling cost and epidemiology of HF whether there are gains and savings to be had in the thoughtful customization and de-escalation of therapy for this unique improving population. With up to 43% of patients experiencing LVEF improvements in some series, this is clearly an important area of further study.

In sum, LVEF improvement can be seen spontaneously or in response to specific medical or device therapies. While certain clinical characteristics predict LVEF improvement and outcomes appear improved when compared with HF*p*EF or HF*r*EF, the trajectory for those with HFrecEF appears most consistent with myocardial remission, rather than true recovery from HF. This population offers new insights into the dynamic and plastic nature of the heart. What is clear is that more study is required in the form of mechanistic fundamental science and future clinical trials to better understand the phenomena of remodeling, remission, and recovery.

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Remote Monitoring of Cardiovascular Implantable Electronic Devices

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Introduction

Cardiovascular implantable electronic devices (CIEDs) have undergone revolutionary changes in the last decade. These changes have permitted an increase in the use of both pacemakers (PM) and implantable cardioverter defibrillators, collectively known as CIEDs. PM were the first generation of CIEDs available for implantation in humans, as early as 1958. Implantable defibrillators in the current form (transvenous) have been available since 1997. CIEDs are increasing in use in North America (Kurtz et al., 2010; Mond et al., 2008; Uslan et al., 2008). These devices are primarily required in older populations, where conduction system disease and significant structural heart disease prevail. As the absolute and relative numbers of the elderly population increase, so do the use of CIEDs. The primary increase in CIED use is due to an increase in the implantation of prophylactic implantable cardioverter and defibrillators (ICDs), rather than PM. Increasing use of cardiac interventions (Cox, 1999), coupled with increasing survival post-MI, a growing number of heart failure survivors (Wijeysundera et al., 2010), and the burgeoning elderly population have all combined to increase the population of patients eligible for implantation of prophylactic ICDs (Moss et al., 2002; Bardy et al., 2005). It is projected that the heart failure population will double by the year 2025 such that the absolute number of patients eligible to receive an ICD for primary prevention will likely rise accordingly (Ross et al., 2006). Recent studies have further expanded indications for cardiac resynchronization therapy (CRT), which has been found to have significant reduction in mortality in mild-moderate heart failure (Tang et al., 2010). This will result in a significantly increased burden on the health care system, whose budget may not be expected to expand at the same rate. The resource implications are not the only consideration in the broader use for these devices.

Over the last decades, CIEDs have evolved in many different ways. Under this acronym are regrouped PM, ICD, and CRT devices. Battery longevity has increased while their volume shrunk, algorithms for detection of arrhythmias were refined, as well as programming strategies to either minimize the impact of pacing (PM) or maximize pacing efficiency (CRT). Likewise, strategies to reduce the rates of inappropriate therapies for ICDs were developed (Moss et al., 2012; Tan et al., 2014). During the life of these patients, many issues may arise, such as atrial or ventricular arrhythmias that may result in syncope, stroke or sudden death, need for increased monitoring resulting from device advisories, or minor programming adjustments to improve device performance, or simply the need for enhanced surveillance as the device battery depletes and replacement is anticipated. Among the improvements in device technology, remote monitoring (RM) capabilities are first and foremost. The main goal behind development of such technology was to allow physician to be promptly made aware of CIED malfunction or a significant change in the patient's clinical status.

With the advent of RM, a few hurdles had to be dealt with. From a physician perspective, liability issues were raised. From a device company and legal perspective, ownership and protection of the data collected had to be debated and organized. From a healthcare perspective, accessibility and costs related to widespread deployment of such technology needed to be assessed. Finally, from a patient perspective, understanding the technology and being able to use it properly at home were certainly the main challenges.

Nevertheless, all major device companies developed their proprietary systems which were integrated to their CIED systems and the technology was tested worldwide. Nowadays, all types of devices have RM capacity but the availability of the technology varies according to legislations, regulatory offices, and local management decisions. The use of RM is well established and supported by data gathered through single-center studies, multicenter randomized control trials, and large population-based registries.

Technology

In order to understand the technology, it is important to review the required tools for RM, the processes behind data collection, transmission, and management (Yee et al., 2013). First and foremost, in order to offer RM to a patient, the selected device must have the built-in capacity. Fortunately, all major companies now offer products with available RM features for PM, ICD, and CRT devices. The second component that is required is a company-specific transmission pod. The latter will transmit data over a phone landline

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or more recently over a cellular network. Newer devices have wireless communication with the transmission pod thereby forfeiting the requirement of applying a wand over the device.

RM generally involves three layers of data transfer. First, recorded and measured data must transfer from the device to the patient's pod. It is generally recommended that the patient places the pod in their main bedroom so transmissions may occur at night. Reasons for failure at this level include insufficient power (or outage) from either the device or the pod itself, or inadequate proximity between the patient and the pod. The second level of transmission involves transfer from the patient's home to a web-based server. This occurs in one of the two ways: via a landline or cellular network. In the first generations of RM devices and pods, only landlines allowed for proper transmissions. Therefore, patients living in remote regions or those who did not have a landline in their bedroom could not use the technology. With the advent of cellular transmissions, the technology is now available to more patients. The third level of transmission relates to information that is conveyed to device clinics or physicians. Once stored on a web-based network, alerts can be sent to clinics involved with a given patient and data can be securely accessed by device clinic specialists. Thereafter, how the acquired data is managed relies on individual clinics and local practices.

Data that is monitored, recorded, and relayed via RM pertains to device status and functionality as well as rhythm disturbances and other parameters. The information gathered is transferred through two types of transmissions: scheduled and triggered. *Scheduled* transmissions are either nominally programmed or they are setup by device clinics and care givers. Nominally scheduled transmissions vary from daily to 6 months depending on the device company proprietary system. *Triggered* transmissions are sent when prespecified parameters are reached. Most of these triggers are programmable and include device-specific measurements such as impedances and battery voltage, measured parameters (thresholds and sensing values), and arrhythmias (monitored or treated). As stated previously, for those transmissions to occur, the patient must be in the vicinity of the transmission pod. Therefore, the device of a patient who sustains a significant ventricular arrhythmia and is not physically near the pod will not transfer any data until the proximity requirements are met. Likewise, an arrhythmia that would occur below the predefined thresholds would not be transmitted.

Although relatively simple in design and straightforward in utilization, some hurdles are seen in the day to day use of RM. In essence, to benefit from RM, the ideal setup requires a combination of a patient who understands the technology and uses it properly and a dedicated device clinic that has integrated RM in its workflow.

Benefits of RM

Numerous groups have evaluated the safety and benefits of integrating RM to usual care. Briefly, in randomized control trials, RM proved to be superior in terms of time to detection and management of significant arrhythmic events or CIED malfunction but also in terms of reduction of inappropriate shocks, hospital visits, hospitalizations, and even mortality in one trial. The most relevant literature will be reviewed in the following sections (**Table 1**).

Mortality

Once a given technology or therapy is accepted by regulatory entities and is being used outside the context of a randomized controlled trial, it becomes important to validate in large and real-life cohorts if the benefits previously observed are still accurate. RM is no exception to this tendency and two large database cohort studies that have been published are discussed later.

ALTITUDE is a cohort study that compared patients with ICD and CRT devices who were either using RM or usual care between 2004 and 2009 (Saxon et al., 2010). Overall survival and shock therapies were analyzed in more than 185,000 patients living in the United States. In this large database, RM was associated with statistically significant reduction in 1-year and 5-year mortality rates in the United States. In a matched cohort, RM was associated with a 50% relative reduction in overall mortality (HR: 0.56 for ICD and 0.45 for CRT-D, $P < 0.0001$) (Saxon et al., 2010). Similarly, another cohort study was published in 2015 (Varma et al., 2015). In this second report from a US database, more than 269,000 patients who received PM, ICD, and CRT devices with RM capabilities were analyzed according to the degree to which they actually used RM. High RM users were defined as those patients who sent >75% of weekly transmission during the observation period. Nonusers were those who did not send a single transmission while low users were those who successfully transmitted at least one but no more than 75% of their weekly transmissions. As in the ALTITUDE study, patients using RM had a survival benefit over those not using it (HR 2.10 [2.04–2.16] $P < 0.001$). Furthermore, there was a gradient of increased survival that correlated the intensity at which the technology was being used. In this report, high users had a statistically significant survival benefit over low users (HR 1.32 [1.27–1.36] $P < 0.001$).

When analyzing overall mortality in retrospective cohorts, adjudication of events is usually not an issue. Only patients that were lost to follow-up or became ineligible might have an impact on the overall mortality of that cohort. However, overall mortality should not be seen as a surrogate of net clinical benefit since the relationship between the underlying cause of death and the technology evaluated cannot be ascertained. In ALTITUDE, the authors attempted to attenuate this factor by indicating that there were no differences between the groups in terms of baseline characteristics such as age, gender, type of device implanted, and its duration as well as economic and educational status. Likewise, to account for underlying confounding variables, in the study by Varma, the authors evaluated eight different socioeconomic factors to delineate adherence factors to RM that might have an impact on overall mortality. While there were differences in the intensity of RM usage and six of the eight factors, no relationship between

Table 1 Summary of remote monitoring randomized clinical trials

Study	N	*Device*	*Frequency of transmissions*	*Follow-up (months)*	*Results*
TRUST	1339	ICD	Daily	15	1. RM reduced total in-hospital device evaluations by 45% without affecting morbidity 2. RM: 85.8% of all 6-, 9-, and 12-month follow-ups were performed remotely only 3. Median time to evaluation was less than 2 days in the RM group compared with 36 days in the conventional group ($P=0.001$) for all arrhythmic events
ECOST	473	ICD	Daily	27	1. 38.5% of patients in the active and 41.5% in the control group experienced ≥ 1 major adverse event ($P<0.05$ for noninferiority) 2. Shocks delivered was significantly lower in the RM ($n=193$) than in the control ($n=657$) group ($P<0.05$) 3. Inappropriate shocks were 52% lower in the active ($n=11$) than in the control ($n=22$) group ($P<0.05$) 4. Battery longevity was longer in the active group because of a lower number of capacitor charges (499 vs. 2081)
EVATEL	2011	ICD	n/a	n/a	1. The noninferiority hypothesis with a safety margin of 5% between the two groups is not validated 2. The number of inappropriate therapies was lower in the remote group ($n=33$; 4.7%) as compared with the control group ($n=55$; 7.5%) ($P=0.03$)
COMPAS	2012	Pacemaker	Daily	18	1. 90 patients (18.2%) had experienced a major adverse event, including 43 patients (17.3%) in the active group and 47 (19.1%) in the control group (HR 0.90; 95% CI: 0.59–1.41; $P=0.63$), confirming the noninferiority of remote monitoring 2. 71% of interim follow-ups were noncontributory versus 38% in the RM group ($P<0.001$) 3. Mean 117-day gain in the event detection (95% CI: 49–184 days; $P<0.001$)
CONNECT	2010	ICD/CRT-D	Event-triggered or q 3 months	15	Median time from clinical event to clinical decision per patient was reduced from 22 days in the in-office arm to 4.6 days in the remote arm ($P=0.001$). The health care utilization data revealed a decrease in mean length of stay per cardiovascular hospitalization visit from 4.0 days in the in-office arm to 3.3 days in the RM arm ($P=0.002$)
EVOLVO	2012	ICD/CRT-D	Event-triggered or q 4 months	16	1. Over 16 months, visits were 35% less frequent in the remote arm (75 vs. 117); incidence density, 0.59 versus 0.93 events per year ($P=0.005$) 2. A 21% difference was observed in the rates of total healthcare visits for heart failure, arrhythmias, or ICD-related events (4.40 vs. 5.74 events per year; $P<0.001$) 3. The time from an ICD alert condition to review of the data was reduced from 24.8 days in the standard arm to 1.4 days in the remote arm ($P<0.001$) 4. The patients' clinical status, as measured by the Clinical Composite Score, was similar in the two groups, whereas a more favorable change in quality of life was observed from the baseline to the 16th month in the RM arm ($P=0.026$)
IN-TIME	664	ICD/CRT-D	Daily	12	At 1 year, 63 (18.9%) of 333 patients in the telemonitoring group versus 90 (27.2%) of 331 in the control group ($P=0.013$) had worsened composite score (OR 0.63, 95% CI 0·43–0.90). Ten versus 27 patients died during follow-up

ICD, implantable cardioverter defibrillator; *CRT-D*, cardiac resynchronization therapy defibrillator; *OR*, odds ratio.

those factors and survival was established. Indeed, other factors such as motivation to engage in other treatments and underlying comorbidities and (their severity) might have an impact that cannot be measured by this type of trial. In the end, whereas *association* is demonstrated in those studies, *causality* has yet to be established.

Nevertheless, it is plausible that RM has an impact on mortality given that arrhythmia and device malfunctions can be detected earlier as demonstrated in numerous RCTs as discussed later. For instance, both sustained ventricular arrhythmias and atrial fibrillation have been associated with worsening heart failure and even mortality (Dries et al., 1998; A.V.I.D. Investigators, 1997; Connolly et al., 2000). It seems reasonable to postulate that by identifying those arrhythmias earlier, physicians might intervene earlier and therefore, change the patient's trajectory of care. In turn, this might lead to the overall improved mortality. In fact, a single RCT revealed that mortality was reduced for RM patients: IN-TIME (Hindricks et al., 2014). This multicenter trial randomized 664 symptomatic heart failure patients (NYHA II or III) who received an ICD or a CRT-D device to RM or usual care. Patients had a reduced left ventricular ejection fraction (mean LVEF 26% in both groups), no permanent atrial fibrillation, and were on optimal drug treatment. At the end of the 12-month study period, 10 (3.4%) patients had died in the RM group compared with 27 (8.7%) in the control group, yielding a hazard ratio of 0.36 (95% CI 0.17–0.74) (log-rank $P=0.004$). The wide confidence interval reflects the overall small number of events. However, the difference in the point-estimate is considerable and the strength of association is striking. Furthermore, since biases and potential differences between patients in each group are reduced by randomization, findings in this trial reinforce the mortality benefits that were documented in registries. Again, causality has yet to be determined and all previous comments on overall mortality remain relevant. In search of a plausible hypothesis for the observed superiority of RM,

authors of IN-TIME argued that early detection of suboptimal device function such as reduced percentage of biventricular pacing and inappropriate shocks along with earlier detection of arrhythmias might have had an impact on the mortality benefit. All those arguments provide plausible explanations for the mortality benefit especially since they were all associated with improved outcomes in various trials (Moss et al., 2012; Poole et al., 2008; Ruwald et al., 2015).

Diagnosis Delay

Previous RCTs have evaluated RM in comparison with usual care globally yielding superiority or non inferiority of RM on variable outcomes. One of the first trials to be published in 2010 was TRUST, a multicenter RCT comparing RM to usual care (Varma et al., 2010). This trial evaluated a single RM system (Biotronik HomeMonitor®) and randomized 1339 ICD patients in a 2:1 block randomization fashion. The primary endpoint was reduction of total in-hospital visits which was clearly demonstrated with a 45% reduction in the number of visits in patients randomized to RM. Importantly, there were no differences in adverse event rates despite the reduced number of clinic visits. Moreover, in secondary analyses, when arrhythmias were documented by the ICD, the median time to evaluation was significantly shorter in the RM group (<2 vs. 36 days ($P<0.001$)). In other words, the conclusion that can be derived from those results is that RM enhances device clinics' efficiency. Indeed, given that routine follow-ups are less likely to translate into meaningful interventions, reducing the number of such visits can only increase clinic's efficiency. In this trial, more than 90% of the clinical visits were nonactionable meaning that the device interrogation did not provide any information that resulted in an intervention. In real-life setting, actionable events include modifying the device functional parameters, modifying the patient's medical therapy, ordering relevant tests, and scheduling lead or generator-related procedures. Therefore, device-detected arrhythmias are usually actionable and RM allowed for faster evaluations. This time difference was more than a month in TRUST. Somehow, one might rightfully postulate that reducing the number of contacts with medical personnel might result in an increased risk of unfavorable outcomes such as death and stroke rates. That assumption just could not be verified in this trial nor other alike trials (see later).

Based on that single RCT, safety and efficacy of RM was demonstrated but it is important to notice that Biotronik's RM system sent statutory daily transmissions whereas other companies had weekly (St.Jude Medical Merlin®), monthly (Boston scientific Latitude®), and quarterly (Medtronic Carelink®) statutory transmissions. Nevertheless, trigger-based transmissions when certain prespecified and programmable outcomes are met are sent with all RM systems thereby reducing the risk of missing meaningful device malfunction or arrhythmic events. Therefore, it is reasonable to assume that the results of any trial are generalizable to all RM systems.

In fact, CONNECT is another trial that was published in 2010 that showed superiority of RM compared with usual care (Crossley et al., 2011). In this trial, 1997 patients receiving an ICD or a CRT-D device were randomized in 1:1 ratio to either RM or usual care. The system being evaluated was Medtronic Carelink which sent statutory quarterly or event-triggered transmissions. Again, despite a different philosophy of transmission management, RM allowed faster actionable interrogations as the median time from a clinical event to the clinical decision was significantly reduced from 22 days in the usual care group to 4.6 days in the RM group ($P<0.001$). The two most common events detected were related to atrial tachycardia/fibrillation: its burden and the ventricular rate during AT/AF. In CONNECT, health care utilization was measured somewhat differently than in TRUST. The mean length of stay of hospitalization for cardiovascular reasons was reduced from 4.0 to 3.3 days ($P=0.002$) which resulted in a reduced cost of hospitalization by 1793 $US (95% CI [1644–1940 $US]). How RM impacted hospital stay remains debatable. As mentioned earlier, data acquired through device checks is not always useful. In CONNECT, physicians stated that valuable information was gathered in only 24% of the in-office visits compared with 62% in the RM arm. One last finding from this trial is very important to mention as it outlines the necessity to ascertain that patients understand the technology and that clinicians are aware of what information will be gathered and transmitted by the device. During the 15-month period of follow-up, 329 triggered-activated alerts occurred. However, only 55% resulted in an actual transmission to the clinic. The remaining 45% occurred with patients who had not properly set up and initiated their home monitor to adequately transmit data. Conversely, 246 clinical alerts were not transmitted at all because the specific alert was programmed off (7%) or the alert had not been reset after being previously triggered (93%). Those two findings support an important concept: proper education pertaining to RM technology is mandatory for proper and optimal use in other to enjoy the possible benefits.

Later, these benefits of RM as well as other advantages were also documented in different publications. Also in 2012, EVOLVO showed that patients receiving RM capable devices had a more favorable change in quality of life as measured by the Minnesota Living with Heart Failure Questionnaire over a 16-month period. In that trial, it is possible that some of the improvement on quality of life was related to the less frequent hospital and clinic visits and faster diagnostic time (Landolina et al., 2012). In the EVATEL study enrolling 1501 patients, there was no difference in the primary endpoint of major adverse events (death, hospitalization, inappropriate ICD therapy). (Presented by Mabo et al., European Society of Cardiology, 2011, Paris, France.) A significant reduction in inappropriate or ineffective ICD therapy (8.1% vs. 5.5%, $P=0.04$) was noted. In the ECOST study involving 433 patients, RM was found to be noninferior to in-office follow-up in terms of major safety events including death (Guedon-Moreau et al., 2013). However, RM decreased inappropriate ICD shocks by 52% (5.0% vs. 10.4%, $P=0.03$) and decreased hospitalizations by 72% (3 vs. 11, $P=0.02$). Cost-effectiveness analysis from ECOST reveals a significant cost-saving to the remote management strategy as compared with usual follow-up. Over a follow-up of 27 months, the mean nonhospital costs per patient-year were €1695 ± 1131 in the active, versus €1952 ± 1023 in the control group ($P=0.04$), a €257 difference mainly due to device management.

Pacemakers

Whereas most of the trials were designed to evaluate the efficacy of RM in ICD and CRT-D patients, one trial evaluated PM patients only. COMPAS randomized nonpacemaker dependant patients to RM or usual follow-up, the latter not being prespecified for the trial (Mabo et al., 2012). Interestingly, patients followed in the RM arm had only two scheduled visits: one at the beginning and one at the end of the18-month follow-up period. Accordingly, there were no scheduled visits during the course of the trial but clinicians were made aware of any adverse events to allow proper follow-up. Even though patients were not assessed in device clinics for more than a year, there were no significant differences in the composite endpoint of death, hospitalization for complications related to the pacing system, and hospitalization for an adverse cardiovascular event. During the trial, 43 patients (17.3%) in the active group and 47 (19.1%) in the control group experienced a major adverse event yielding a hazard ration of 0.90 that was statistically nonsignificant (95% CI: 0.59–1.41; $P=0.63$).

Based on all the available literature, it appears that RM benefits both patients and healthcare systems. Overall, studies have demonstrated that RM can be used safely in all device patient-population, with the exception of PM-dependent patients. In a broader perspective, it appears that RM allows for an increased efficiency in care delivery. Arrhythmias and malfunctions are recognized earlier, clinic visits are more efficient as nonactionable visits are reduced and more importantly, the technology does not bare any increased risk of major cardiovascular events. Although, as pointed out earlier, RM requires a comprehensive system that ensures that patients understand and use the technology properly and also that information gathered through remote transmissions are treated promptly and adequately by clinics and physicians. In its design, IN-TIME incorporated a prespecified response to any transmitted alert. Authors support that this protocolled response probably had an impact on the increased survival of patients enrolled in RM. Whereas this remains debatable, this underlies an important concept: in order to be useful, data gathered must be analyzed and treated appropriately otherwise remote technology is unlikely to yield any benefit.

Future

Now that RM is a well-established technology, the next logical step is to evaluate if remote programming is something that is feasible, safe, and beneficial. RPM-CIED is a Canadian three-step study that was designed to evaluate whether remote programming is likely to yield health and efficiency benefits on top of RM (NCT02585817). Yet to be published, the study is underway and beyond evaluating benefits of remote patient management, it will seek to evaluate logistical, legal, and behavioral barriers to implement remote programming.

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Right Heart Catheterization

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Introduction

Right heart catheterization (RHC; also called pulmonary artery (PA) catheter or Swan–Ganz catheter) assesses cardiopulmonary hemodynamics and is used in the evaluation of a variety of conditions. RHC allows for the direct measurement of right atrial (RA), right ventricular (RV), and PA pressures (Swan et al., 1970). Left atrial pressure can also be estimated by measurement of pulmonary capillary wedge pressure (PCWP). Cardiac output (CO) is assessed using automated thermodilution techniques or calculated using the Fick method. RHC can be placed in the intensive care unit, cardiac catheterization unit, or operating room. Options for the insertion sites include the internal jugular, subclavian, femoral, or antecubital (basilic or brachial) veins (Summerhill and Baram, 2005). If the catheter is going to remain in for continuous hemodynamic monitoring, the right internal jugular vein is preferable to allow the patient to sit up in bed rather than lie flat. If possible, the target vessel should be visualized with ultrasound to minimize complications. **Table 1** outlines the procedure for RHC insertion.

Indications and Contraindications

The first RHC in a human was performed in Germany by Werner Forssman in 1929. He self-catheterized using a urethral catheter through his antecubital vein into his right ventricle and confirmed placement with a X-ray (**Fig. 1**) (Meyer, 1990). The technique was modified, most notably by Jeremy Swan and William Ganz, allowing quick placement and the ability to remain in situ for prolonged periods of monitoring (Lategola and Rahn, 1953). This led to a surge in use and at their peak, RHC were being used in 45% of critically ill patients in America (Bayliss et al., 2009).

The widespread use of RHC despite some concerns over safety led to a number of studies evaluating their efficacy. Most failed to demonstrate any benefit in survival or reduced days in the hospital with regular RHC use in critically ill patients (Connors et al., 1996; Yu et al., 2003; Sandham et al., 2003; Binanay et al., 2005; Richard et al., 2003; Shah et al., 2005). For this reason, most societal guidelines no longer support their routine use. In a widely referenced observational study of ICU patients, RHC use was associated with increased 30-d mortality and increased resource utilization (Connors et al., 1996). The ESCAPE trial randomized 433 patients with heart failure to therapy guided by clinical assessment and a RHC or by clinical assessment alone (Binanay et al., 2005). There was no difference in mortality or length of hospital stay in patients with a RHC, but there was a higher incidence of in hospital adverse events. With little evidence supporting widespread use in critical care patients, there has been a trend toward decreased routine use in the United States (Wiener and Welch, 2007). Despite this, most experts believe that RHC placement can provide valuable information in the following select populations:

- Unclear volume status in the setting of shock
- Severe cardiogenic shock
- Evaluation for cardiac transplantation or left ventricular assist device

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Table 1 Right heart catheterization insertion technique

Obtain informed consent
Review laboratory values to ensure no coagulopathy, electrolyte, or acid–base abnormalities
Select insertion site (internal jugular, subclavian, femoral, or antecubital veins)
Sterilize wide procedural field with aseptic technique
Inject local anesthesia (1% or 2% lidocaine) to superficial insertion site and anticipated needle tract
Insert introducer using modified Seldinger technique
Confirm introducer placement in target vessel
● Visualize dark blood with no pulsatile flow
● Transduce pressure waveform
● Blood gas analysis
Prepare catheter by connecting and flushing all ports
Zero and reference system
Advance catheter under fluoroscopic guidance (preferred) or by guidance of pressure wave forms
● Once tip of catheter is in SVC, inflate balloon
● Catheter should be advanced slowly through RA, RV, and PA
● Once PCWP obtained, balloon should be deflated
Catheter should be left in place for as little time as possible
Catheter should always be removed with the balloon deflated

RA, right atrium; RV, right ventricle; PA, pulmonary artery; PCWP, pulmonary capillary wedge pressure.

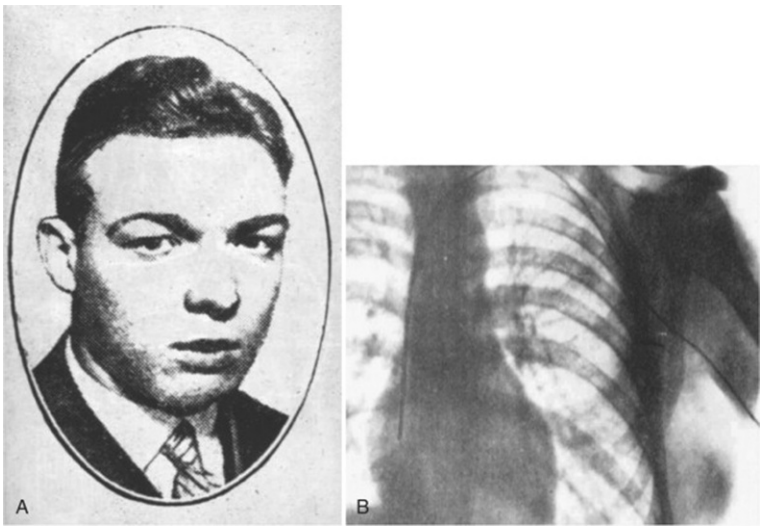

Fig. 1 (A) Werner Forssman. (B) "The catheter extends from the left cephalic vein down into the right atrium."

- Diagnosis and management of PA hypertension
- Severe cardiopulmonary disease patients undergoing corrective or other surgery

Absolute contraindications to RHC placement include lack of consent, significant infection at the insertion site, and the presence of a RV assist device. Coagulopathy and thrombocytopenia are relative contraindications. However, RHC is frequently performed without interruption of anticoagulation and may be safe in patients with an INR of up to 3.5 if done using internal jugular vein or antecubital vein access (Ranu et al., 2010). Severe electrolyte and acid–base disturbances increase the risk of life-threatening arrhythmias and should be corrected prior to RHC placement. Patients with pulmonary hypertension (PH) and Eisenmenger's syndrome are considered increased risk due to possible PA rupture. Other increased risk patients include those with defibrillator (risk of dislodgement), left bundle branch block (risk of complete heart block), prosthetic or stenotic tricuspid valve (risk of difficulty advancing catheter), and persistent left superior vena cava (risk of misplacement in left atrium). RHC can typically be placed safely in these populations by expert providers and with the use of fluoroscopy.

Complications

Though generally considered a safe procedure, several complications can occur due to RHC. Vascular access complications, including bleeding, arterial puncture, and pneumothorax, are the most frequently encountered. When passed through the right ventricle, the catheter commonly causes ventricular ectopy. This typically resolves with advancement of the catheter into the PA. Temporary right bundle branch block is observed in up to 5% of RHC (Putterman, 1989). The most feared and potentially catastrophic complication of RHC placement is PA rupture, which is rare occurring in 0.06%–0.2% of cases (Boyd et al., 1983; Shah et al., 1984). This may present with frank hemoptysis and risk factors include underlying PH, advanced age, mitral valve disease, hypothermia, and anticoagulation or coagulopathy. The risk of PA rupture can be decreased by avoiding distal catheter placement and over inflation of the balloon. Rapid identification and treatment of pulmonary rupture is critical as the mortality rate exceeds 30% (Kearney and Shabot, 1995). Initial treatment may include reinflation of the balloon to occlude the bleeding vessel and temporarily obstruct the feeding artery. If this does not stop the hemorrhage, emergent thoracotomy may be required.

Pressures

RA Pressure

The RA pressure is the "filling pressure" of the right heart. It reflects venous return to the RA and RV end-diastolic pressure. Normal RA pressure is 3–7 mmHg (**Table 2**). The classic RA waveform consists of three discrete positive and two negative deflections (**Fig. 2**). The "a wave" represents the pressure increase in the RA during atrial contraction. This is followed by the "x descent" reflecting the fall in pressure during atrial relaxation. The small "c wave" mirrors the pressure increase in the RA as a consequence of the tricuspid valve bulging into the RA during ventricular isovolumic contraction. Next, a "v wave" is seen reflecting increased atrial pressure from passive blood return while the tricuspid valve remains closed. Finally, the "y descent" is seen with the fall in atrial pressure after the tricuspid valve opens and blood rushes from the atria to the ventricle. Typically, the "a wave" is slightly larger than the "v wave." Several conditions are identified by their characteristic effect on RA pressure waveforms. Tricuspid regurgitation results in large "v waves" due to backflow of blood into the RA during ventricular systole (**Fig. 3**). In atrial fibrillation, there are no "a waves" seen due to the lack of coordinated atrial activity. Atrioventricular dissociation, as seen in complete heart block, ventricular tachycardia, or asynchronous ventricular pacing, may result in large "cannon a waves" (**Fig. 4**). These occur due to atrial contraction against a closed tricuspid valve.

RV Pressure

RV pressure is recorded at the maximal systolic pressure, minimal early diastolic pressure, and end-diastolic pressure (**Fig. 2**). Normal RV systolic pressure is 20–30 mmHg and normal diastolic pressure is 3–7 mmHg (**Table 2**). The RV waveform has a rapid upstroke and downstroke during systole. Ventricular diastole consists of three phases: early rapid filling, slow filling, and atrial systole. The end-diastolic pressure is measured as the pressure after atrial contraction and immediately before the onset of ventricular contraction. RV pressure can be elevated in PH, pulmonary embolism, and pulmonic stenosis.

PA Pressure

The PA waveform consists of a systolic pressure, dicrotic notch (representing closure of the pulmonic valve), and diastolic pressure (**Fig. 2**). Normal systolic PA pressure is 20–30 mmHg and normal diastolic pressure is 8–12 mmHg (**Table 2**). Elevated PA pressure is seen in decompensated heart failure and numerous conditions the result in elevated pulmonary vascular resistance. Distinguishing among etiologies of elevated PA pressure can be challenging and is discussed separately in the section on "**Hemodynamic Interpretation**."

Table 2 Normal pressure measurements

Chamber	*Average (mmHg)*	*Range (mmHg)*
Right atrium	5	±2
Right ventricle	25/5	±5/±2
Pulmonary artery	25/10	±5/±2
Left atrium (PCWP)	10	±2

PCWP, pulmonary capillary wedge pressure.



Fig. 2 Pressure waveforms.



Fig. 3 RA pressure in subject with a restrictive cardiomyopathy breathing spontaneously. The "v" wave (*downward arrow*) is broad, indicating tricuspid regurgitation, and so is the deep "y" descent (*upward arrow*), owing to the restrictive cardiomyopathy. The horizontal lines mark inspiration. The rhythm is atrial fibrillation.

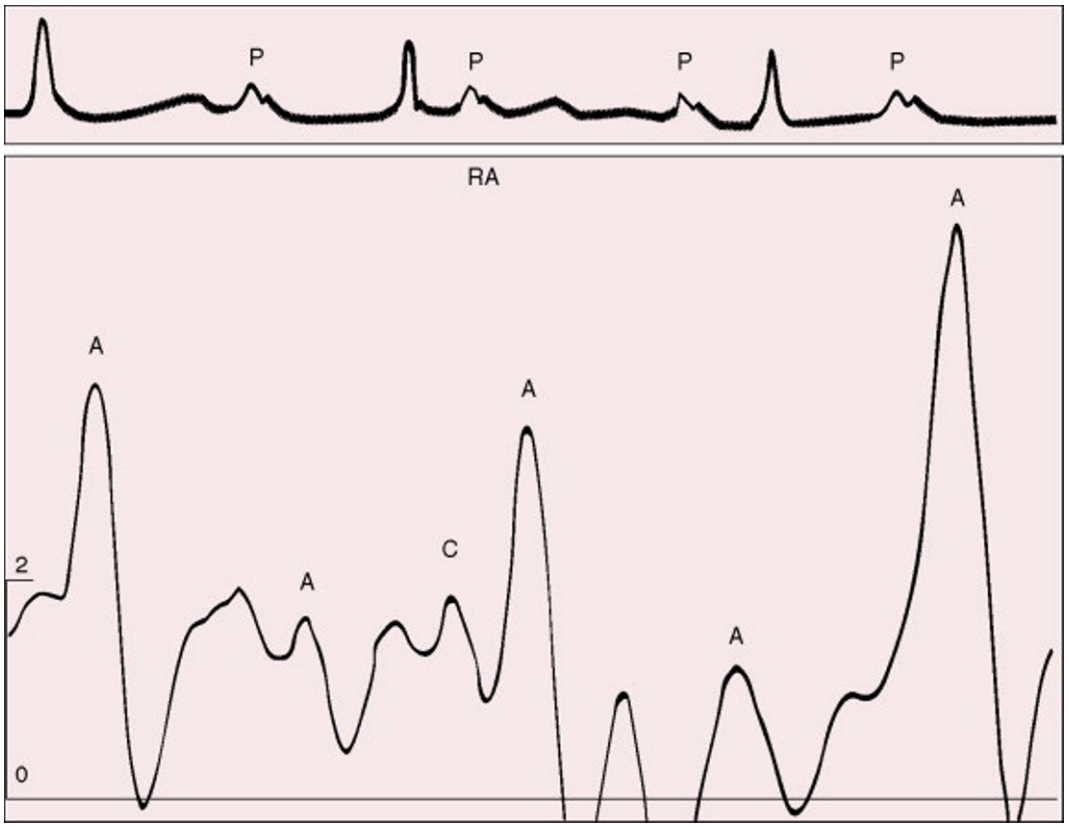

Fig. 4 Electrocardiogram and right atrial (RA) pressure pulse from a 13-year-old boy with isolated congenital complete heart block. The A waves intermittently increase (cannon waves) when a P wave falls between QRS complex and T wave. The increase occurs because the right atrium contracts against a closed tricuspid valve. The A waves are small when a P wave falls between T wave and QRS complex (i.e., when the tricuspid valve is open).

Pulmonary Capillary Wedge Pressure

The PCWP is obtained as an estimate of the left ventricular end-diastolic pressure (LVEDP) or left heart "filling pressure." With the catheter in the PA, a balloon at the distal end of the catheter is gently inflated and "wedged" into a pulmonary capillary. This obstructs blood flow creating a static column of blood between the catheter tip and left atrium. If there is no other anatomic obstruction to blood flow between the PA and left atrium, pressure measurement at the catheter tip estimates the left atrial pressure (Walston and Kendall, 1973; Lappas et al., 1973).

The normal PCWP is 8–12 mmHg (**Table 2**) and the waveform is similar to RA pressure (**Fig. 1**). Atrial contraction produces the "a wave" with the "x descent" seen as a fall in pressure during atrial relaxation. The "c wave," which is often not seen, is due to slight pressure increase from protrusion of the mitral valve into the left atrium during ventricular isovolumic contraction. With passive blood return to the atria with a closed mitral valve, a "v wave" is seen. Finally, the "y descent" occurs after the mitral valve opens and blood rushes from the atrium to the ventricle. In contrast to the RA waveform, the PCWP "v wave" is typically slightly larger than the "a wave." There can be significant respiratory variation in the PCWP measurement, and it should be recorded at end-expiration. The PCWP measurement should be taken at the "a wave" to avoid large "v waves" confounding interpretation. Numerous conditions can cause discordance between the PCWP and LVEDP. The PCWP can be lower than LVEDP in situations with decreased left ventricular compliance (diastolic dysfunction, positive pressure ventilation, cardiac tamponade, or myocardial ischemia) or in conditions such as aortic stenosis that result in premature mitral valve closure (Raper and Sibbald, 1986). Conversely, PCWP can be greater than LVEDP if there is hypoxia or conditions causing constriction of small pulmonary veins. Procedural errors in obtaining data (see "**Data Acquisition Errors**" section) such as over or under wedging of the catheter can also cause PCWP measurements that do not accurately reflect the true LVEDP, making confirmation of PCWP position essential to any hemodynamic assessment. This can be done by fluoroscopy, waveform inspection, and observing that the oxygen saturation of blood drawn distally to the inflated balloon is consistent with systemic arterial oxygen saturations. Several conditions can be identified by their characteristic effect on PCWP waveforms. The "a wave" can be elevated in conditions of increased resistance to LV filling, such as mitral stenosis, volume overload, or decreased LV compliance. Classically, mitral regurgitation may result in prominent "v waves." Prominent "v-waves" are neither sensitive nor specific for mitral regurgitation and their height is not reflective mitral regurgitation severity (Pichard et al., 1982; Fuchs et al., 1982; Schwinger et al., 1988; Snyder et al., 1994).

Cardiac Output

CO may be measured using the Fick or indicator dilution methods. The preferred expression of CO is the cardiac index, which is calculated by dividing the CO by the body surface area (**Table 3**). The PA saturation can also estimate CO.

The Fick method approximates CO using the amount of oxygen extracted by the body as measured by the arterial–venous oxygen difference (**Table 3**). The following formula calculates CO by the Fick method:

$$\text{Cardiac output (L/min)} = \frac{\text{Estimated oxygen consumption}}{10\ (\text{ateriovenous oxygen difference})}$$

While oxygen consumption can be measured directly through exhaled breath analysis, this method is cumbersome, so it is typically estimated from a nomogram based on age, sex, height, and weight. The ateriovenous oxygen difference is calculated as:

$$= (1.34)(\text{Hgb concentration})(\text{arterial oxygen saturation} - \text{mixed venous oxygen saturation})$$

In the presence of a left to right shunt present, the mixed venous oxygen saturation is calculated as [3 × SVC saturation + IVC saturation]/4.

The Fick method has a total error of 10%–15%. It is more accurate in low CO states since lower CO results in a higher ateriovenous oxygen difference. The Fick method is also more accurate in conditions where the heart rhythm is irregular, such as atrial fibrillation. The primary disadvantage of the Fick method is the reliance on estimated oxygen consumption due to the

Table 3 Commonly used calculations during right heart catheterization

Cardiac output (L/min)	$\frac{\text{Estimated oxygen consumption}}{10\ (\text{ateriovenous oxygen difference})}$
Ateriovenous oxygen difference (mL/100 mL)	(1.34) (Hgb concentration)(SaO2–MvO2)
Cardiac Index (L/min/m^2)	$\frac{\text{Cardiac output}}{\text{Body surface area}}$
Transpulmonary gradient (mmHg)	Mean PA pressure—PCWP
Pulmonary vascular resistance (Wood units)	$\frac{\text{TPG}}{\text{Cardiac output}}$
Systemic vascular resistance (Wood units)	$80 \times \frac{[\text{Mean arterial pressure (mmHg)} - \text{right atrial pressure (mmHg)}]}{\text{Systemic blood flow (cardiac output; L/min)}}$

SaO2, arterial oxygen saturation; MvO2, mixed venous oxygen saturation; Hgb, Hemoglobin; PA, pulmonary artery; PCWP, pulmonary capillary wedge pressure; TPG, transpulmonary gradient.

difficulty of direct measurement. Additionally, the Fick method cannot detect rapid changes in CO, so it is not useful in patients undergoing interventions such as valvuloplasty or cardiac surgery.

The indicator dilution method is performed by injecting a bolus of cold saline in the proximal port of the catheter (Ganz et al., 1971). There it mixes with blood lowering the temperature. When blood flows past the distal thermistor port, the temperature change over time is recorded as a temperature–time curve. The area under this temperature–time curve is inversely proportional to CO. The indicator dilution method has the advantage of being simple to perform and having the capacity to take repeated measures to evaluate reproducibility. It is more accurate in high CO states, and there are catheters available that allow for continuous indicator dilution measures. These correlate well with bolus methods (Yelderman et al., 1992) but are more expensive and have not been shown to improve outcomes (London et al., 2002). Serial indicator dilution measures should vary less than 10% to be considered reliable. Tricuspid regurgitation can falsely lower indicator dilution measures due to a prolonged, low amplitude curve produced as blood is recycled back and forth across the tricuspid valve. Indicator dilution measures may also be less accurate with irregular heart rhythms. Both right-to-left and left-to-right shunts can falsely elevate indicator dilution measures of CO. In left-to-right shunts, increased right heart volumes and dilution of the injected cold saline will attenuate the height of the temperature–time curve resulting in an overestimate of CO. In right-to-left shunts, the cold saline can be shunted to the left heart reducing PA cooling which also attenuates the height of the temperature–time curve leading to overestimated CO.

The saturation of PA blood (i.e., mixed venous saturation) is used as an overall estimate of CO. PA saturation less than 65% indicate low output and greater than 80% high output. Compared to the other methods of assessing CO which have multiple potential sources of error, PA saturation has the advantage of being a direct measurement. PA saturation may not be reliable in the setting of shunt, hypoxia, or profound anemia.

Hemodynamic Calculations

Several calculations based on RHC findings can assist in hemodynamic interpretation (**Table 3**). The transpulmonary gradient (TPG) is defined as the difference between the mean PA pressure and PCWP. The normal value is $\leq$12 mmHg and an elevated TPG is suggestive of pulmonary arterial hypertension. Once CO has been determined, vascular resistance can be estimated. Pulmonary vascular resistance (PVR) is calculated as the TPG divided by CO. The normal PVR value is <3 Wood units (or 240 dynes s cm^{-5}) and elevated values also suggest pulmonary arterial hypertension. Systemic vascular resistance is estimated as:

$$\text{SVR} = 80 \times \frac{[\text{Mean arterial pressure (mmHg)} - \text{right atrial pressure (mmHg)}]}{\text{Systemic blood flow (cardiac output; L/min)}}$$

The calculations of vascular resistance are dependent on both direct measures (i.e., pressures) and indirect measures (CO), each of which have sources of error, making them the least accurate of all RHC values.

Hemodynamic Interpretation

Clinicians who order and manage RHC placement should be able to reliably interpret the results. Misinterpretation of RHC data is common and may be a significant contributor to the lack of benefit seen in prior clinical trials (Squara et al., 2002). As fewer RHC are obtained, this may become more challenging as trainees and clinicians have less experience with data interpretation (**Table 4**).

Acute Heart Failure

Acute heart failure is characterized by elevated PCWP. Pulmonary pressure is typically elevated proportionally to PCWP. Likewise, RA pressure may be elevated proportionally to the PCWP. When RA pressure to PCWP ratio exceeds 0.67 it may indicate RV failure (Kormos et al., 2010). The CO may be normal or depressed depending on how advanced is the heart failure.

Table 4 Interpreting hemodynamics

			PH associated with HF	
	Heart failure	*PAH*	*"In proportion"*	*"Out of proportion"*
RA (mmHg)	Normal or ↑	Normal or ↑	Normal or ↑	Normal or ↑
RV (mmHg)	Normal or ↑	↑	↑	↑
Mean PA (mmHg)	Normal or ↑	≥25	≥25	≥25
PCWP (mmHg)	>15	≤15	>15	>15
CO (L/min)	Normal or ↓	Normal or ↓	Normal or ↓	Normal or ↓
TPG (mmHg)	Normal	>12	≤12	>12
PVR (Wood units)	Normal	>3	<3	>3

PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; RA, right atrium; RV, right ventricle; PA, pulmonary artery; PCWP, pulmonary capillary wedge pressure; CO, cardiac output; TPG, transpulmonary gradient; PRV, pulmonary vascular resistance.

Pulmonary Hypertension

RHC is the gold standard for the diagnosis of PH. Pulmonary arterial hypertension (PAH) is defined as mean PA pressure ≥25 mmHg and PCWP ≤15 mmHg. PH associated with heart failure presents a diagnostic challenge. In this case, it must be determined if the elevated pulmonary pressure is secondary to volume retention alone or due to adaptive changes in the pulmonary vasculature. The TPG ≤12 mmHg and PVR <3 Wood units is indicative of PH "in proportion" to heart failure where the elevated pulmonary pressure is a result of volume retention. In this setting, patients should be diuresed to a PCWP <15 with repeat hemodynamic measurements before considering PAH-specific pulmonary vasodilator therapy. In patients with PH and PCWP >15, a TPG >12 mmHg and PVR >3 Wood units suggest PH "out of proportion" to heart failure. In other words, the elevated pulmonary pressure cannot be fully explained by volume retention and may in part be due to changes in the pulmonary vasculature (Naeije et al., 2013). The diastolic pressure gradient (DPG) may be another measure to diagnose "out of proportion" PH. The DPG is calculated by difference between the pulmonary arterial diastolic pressure and PCWP. Both a TPG >12 mmHg and DPG >7 mmHg are associated with lower median survival in patients with PH due to left-sided heart disease (Naeije et al., 2013; Gerges et al., 2013). Patients with PH "out of proportion" to heart failure may benefit from PAH-specific therapy with guidance from a PAH specialist. In patients with PCWP >25 mmHg, PH should be considered "in proportion" to HF regardless of TPG and DPG. These patients should be completely diuresed before considering PAH-specific therapy.

Cardiac Transplantation

RHC is a critical component in the evaluation for cardiac transplantation. Irreversible PH is associated with posttransplant RV failure and subsequent increased morbidity and mortality (Costard-Jackle and Fowler, 1992). Elevated PVR >5 Wood Units and TPG >15 mmHg are potential significant contraindications to cardiac transplantation (Mehra et al., 2016). Because of the difficulty distinguishing the etiology of PH in heart failure, a vasodilator challenge (see "**Acute Vasodilator Testing**" section) is recommended as part of the transplant evaluation when PA systolic pressure is >50 mmHg and PVR is >3 Wood units, with a systolic blood pressure >85 mmHg (Mehra et al., 2016). For patients with secondary PH, ventricular unloading with a left ventricular assist device is often used to successfully bridge patients to transplantation. With LVAD support, significant reductions in TPG and PVR can be seen within 1 month of implantation (Kutty et al., 2013). RHC should be performed in all patients prior to transplant listing and as frequently as every 3–6 months while on the waitlist in patients with secondary PH or who are decompensating.

Cardiac Tamponade

The diagnosis of cardiac tamponade can be confirmed by RHC. In tamponade, the RA, PA diastolic pressure, and PCWP are all elevated and equal within 4–5 mmHg of each other (**Fig. 5**). The RA and PCWP generally have an attenuated or absent "y descent." CO will be reduced and the SVR is elevated. The definitive treatment involves pericardiocentesis to remove pericardial fluid.

Acute Vasodilator Testing

Acute vasodilator testing involves administration of a short-acting vasodilator in patients with PH followed by measurement of the hemodynamic response using a RHC. It can be performed using a variety of agents, including nitroprusside, nitroglycerin, inhaled nitric oxide, or IV epoprostenol. Acute vasodilator testing should be performed in patients with Group 1 PAH to determine potential responsiveness to calcium channel blocker therapy. A positive response in PAH is defined as reduction in mean PA pressure of >10 mmHg to reach a mean of <40 mmHg with increased or unchanged CO. Acute vasodilator testing is also useful to determine

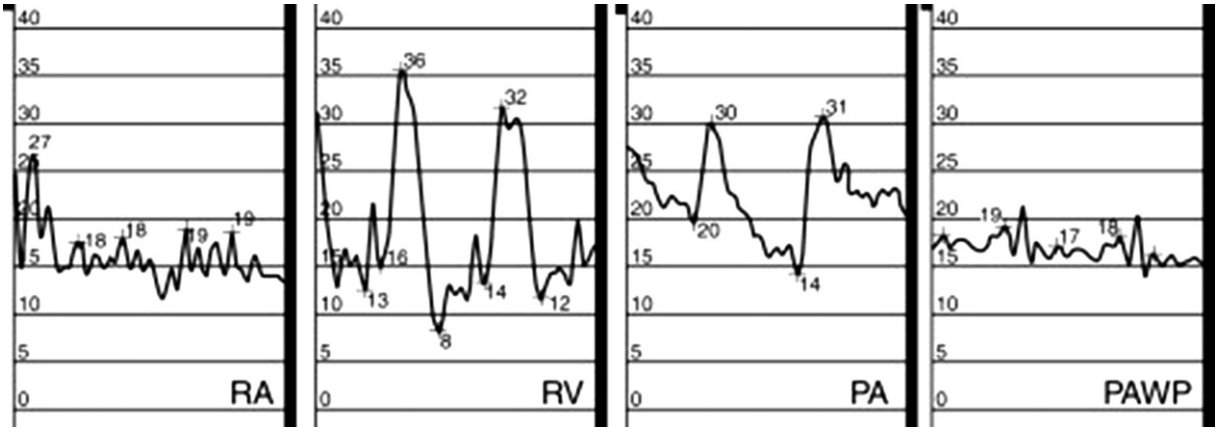

Fig. 5 Pressure tracings in cardiac tamponade. PA, Pulmonary artery; PAWP, pulmonary artery wedge pressure; RA, right atrium; RV, right ventricle. There is equalization of all diastolic pressures across all chambers.

the reversibility of elevated pulmonary pressures in heart failure. The foundation of acute vasodilator testing in heart failure is that PA pressures will rapidly fall (and CO increase) when the left heart is "unloaded." However, some patients with chronic elevations of their LVEDP can have remodeling of their pulmonary arteries resulting in irreversible PH. If there is inadequate response to an acute vasodilator challenge, the patient should be hospitalized with continuous hemodynamic monitoring. The PVR will often decline after 24–48 h of treatment some combination of inotropes, vasodilators, and diuretics. In patients where the PVR is reduced to <2.5, but the systolic blood pressure falls to <85 mmHg, the risk of right heart failure following transplantation remains high (Costard-Jackle and Fowler, 1992; Butler et al., 2005).

Data Acquisition Errors

There are several important sources of error in performing a RHC that physicians should be aware of. Pressure waveforms can become dampened, appearing as rounded contours with delayed upstrokes and downstrokes. This is often due to air bubbles within the catheter or tubing and can be corrected by flushing the system. Catheter "whip" can appear as an erratic waveform with highly variable pressures. This is also typically resolved by flushing the system or repositioning the catheter.

The RHC must be zeroed and referenced to obtain accurate readings. To zero, the system is opened to air to establish atmospheric pressure as zero. Referencing (or "leveling") is done to confirm the air–fluid interface of the transducer is at the level of the patient's heart. If the heart is lower than the transducer level, the pressure will be erroneously low. Conversely, if the heart is higher than the transducer the pressure measure will be falsely high.

Obtaining an accurate PCWP is critical, particularly since it will influence subsequent TPG and PVR calculations. Both over- and underwedging can confound the PCWP results. Overwedging results from excessive inflation of the balloon. It produces a dampened waveform and can lead to falsely elevated PCWP due to transmittal of pressure from the balloon. It usually corrects with partial deflation of the balloon. Overwedging also increases the risk of PA rupture. If the PA is incompletely occluded, underwedging can occur which can also result in artificial elevation of the PCWP. This frequently occurs in patients with high PA pressures due to poorly compliant pulmonary vasculature. Underwedging can be corrected by deflating the balloon and slowly reinflating until the catheter wedges more completely. The catheter can also be taken back into the main PA and guided into a different PA branch.

Summary

RHC remains important in the diagnosis and management of select cardiopulmonary disorders. Direct measure of RA, RV, PA, and PCWP and estimates of CO and vascular resistance can be obtained. These values are particularly useful in the management of advanced heart failure, PH, and in evaluating patients for heart transplantation. Misinterpretation of RHC data is common and clinicians performing and interpreting hemodynamics should understand how to decipher the results and be aware of common data acquisition errors. However, with fewer RHC now being performed this has become more challenging.

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Risk Factors for Cardiovascular Disease

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Introduction

Understanding the causes of heart disease is a relatively young field in medicine. In fact, heart disease was relatively rare prior to the early 1900s. With the advent of antibiotics and refrigeration, early deaths from infectious disease plummeted. In addition, the advent of automobiles and grocery stores decreased the need for sourcing our own food by farming, and thus more sedentary lifestyles. This combination leads to a rapid rise in heart disease. This transition from a society with the majority of mortality from infectious disease to that of cardiovascular disease follows socioeconomic progression. It can still be seen throughout third world and developing countries today (Yusuf et al., 2001).

But the relationships of diet, smoking, cholesterol, and the causes of heart disease that seem so obvious now were a long time coming (Fig. 1). The connection between cholesterol and heart disease was not made until Virchow found fat in atherosclerotic lesions in 1856, and Anitschkow showed in 1913 that feeding rabbits a cholesterol-rich diet promoted atherosclerosis (Anitschkow and Chatalov, 1913). The connection between smoking and heart disease came slowly with the accumulation of epidemiologic data during the 1940s and 1950s, but was not widely accepted until the report *Smoking and Health: Report of the Advisory Committee to the Surgeon General* was published in 1964.

In addition, determining what is causing heart disease (risk factors) and what is associated with heart disease (risk markers) continues to be debated. Risk factors are causative agents that lead to atherosclerosis and cardiovascular disease. Risk markers are signs of underlying atherosclerosis or factors that occur concurrently, for example, coronary calcification. There is likely crossover of many factors as well, such as obesity, which is likely a risk factor in itself, yet is associated with many other risk factors such as insulin resistance and sedentary lifestyle.

In this article, we will review the known major risk factors for the development of atherosclerosis and cardiovascular disease, and discuss some of the newer factors still being discovered.

Cholesterol

The link between cholesterol and atherosclerosis really developed in the 20th century. The strongest connections came from epidemiologic data such as the Framingham group and the Seven Countries Study. The Framingham group showed that cholesterol level in patients without prior coronary artery disease was linked to the development of coronary heart disease in the future (Castelli et al., 1992). These patients were controlled for other risk factors including hypertension, diabetes, and tobacco use, showing that cholesterol level was an independent risk factor. In 1976, Ancil Keys published the Seven Countries Study that was one of the first to suggest that diet had a significant influence on cholesterol and on cardiovascular risk of death (Keys, 1970). This landmark study followed the diets and cholesterol levels in cohorts of patients in seven different countries to determine if there was an influence on mortality. There was a clear link between a diet low in saturated fats and high in vegetables and reduced mortality. The favorable diet pattern came to be known as the Mediterranean diet. Despite clear evidence of this connection in 1976, the idea of diet to improve cardiovascular health has been slowly adopted in the United States.

Further evidence of cholesterol's contribution to atherosclerosis and coronary artery disease can be found in genetic population studies. Familial hypercholesterolemia (FH) is an autosomal dominant disorder with a prevalence in the United States of approximately 1:250 people (de Ferranti et al., 2016), though it is even more prevalent in certain subgroups, such as those of French Canadian ancestry (1:150–200). FH is actually a combination of genotypes that share the same phenotype of high low-density lipoprotein (LDL) cholesterol level. The most common are abnormalities of the LDL receptor that prevent it from clearing LDL from the bloodstream. There are over 1700 mutations in the LDL receptor alone, which accounts for 90% of the cases of FH (Hartgers et al., 2015). These mutations affect the function of the LDL receptor in one of five ways. Class 1 mutations result in a complete lack of LDL receptors. Class 2 mutations result in the LDL receptors not being transported from the endoplasmic reticulum to the Golgi apparatus. Class 3 mutations result in an LDL receptor that is unable to bind to LDL. The LDL receptor in a class 4 mutation binds LDL, but is unable to

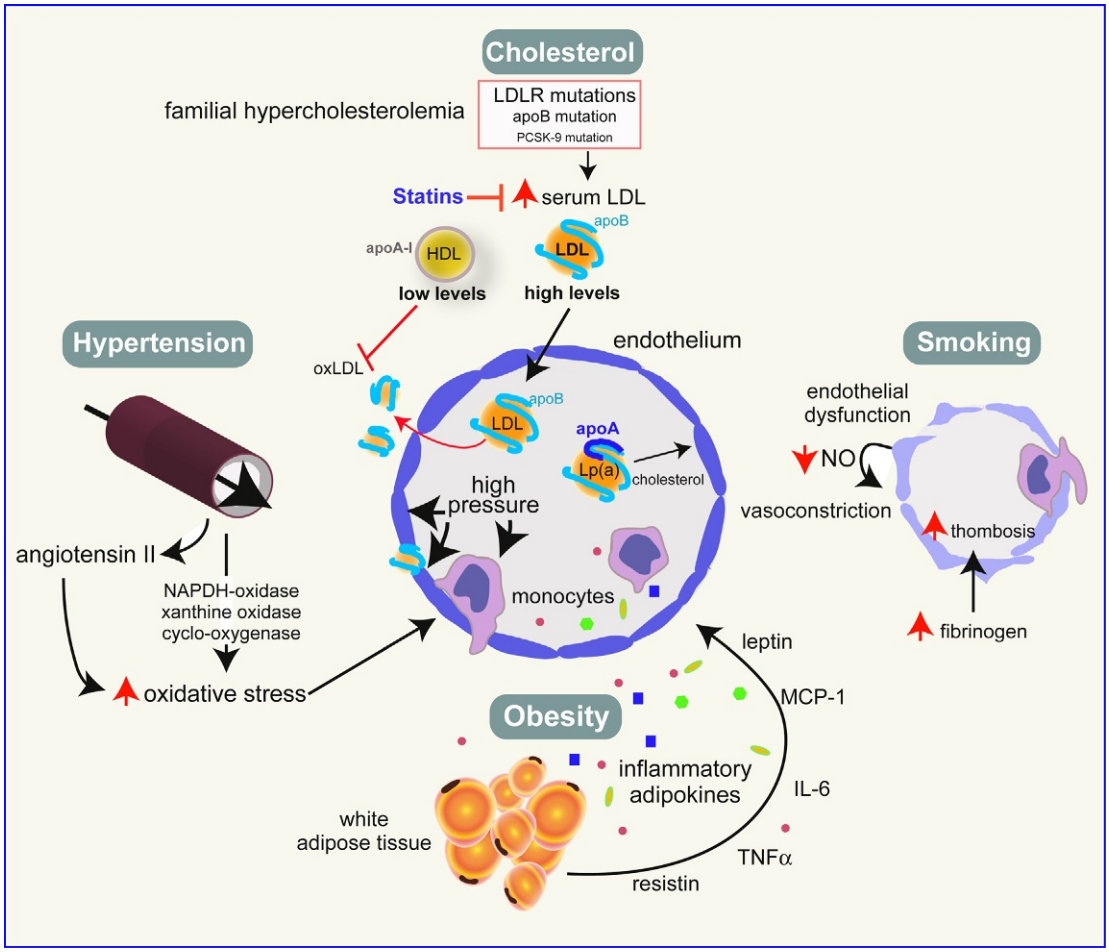

Fig. 1 Relationships of diet, smoking, cholesterol, and the causes of heart disease.

internalize it. Finally, class 5 mutations result in an inability of the cell to recycle the receptor back to the surface. The remaining 10% of genetically identified cases of FH result from abnormalities in two other genes. The first gene codes for apolipoprotein B, the protein portion of LDL that is responsible for facilitating attachment of the LDL molecule to the LDL receptor. The abnormal apolipoprotein B does not adequately bind to the LDL receptor, thereby preventing clearance of LDL from the bloodstream. This defect has a prevalence of roughly 1:1000 (Foody and Vishwanath, 2016). The second is an abnormality in the gene that codes for proprotein convertase subtilisin/kexin type 9 (PCSK-9) (Cohen et al., 2006). PCSK-9 is a protein that helps regulate the number of LDL receptors on the cell surfaces. The more circulating PCSK-9, the more LDL receptor degradation, and the less recycling of the receptors, resulting in higher serum levels of LDL (Cohen et al., 2006). Alternatively, patients with low circulating levels of PCSK-9, due to a loss of function mutation, have higher numbers of LDL receptors and very low cholesterol levels.

Multiple epidemiologic studies have shown an increase in coronary risk in patients with FH that is much higher than matched controls, suggesting that prolonged exposure to elevated blood levels of LDL over a lifetime is a risk factor for coronary heart disease (CHD). Indeed, this relationship is even higher for patients with homozygous FH, suggesting a dose-related response (Austin et al., 2004). Further proof of this concept can be seen in patients with a loss of function in the gene coding for PCSK-9 that results in very low LDL. These patients have a markedly reduced risk of developing CHD, suggesting that a lifetime of reduced LDL exposure is protective (Cohen et al., 2006).

Likewise, multiple randomized controlled clinical trials have shown that lowering serum LDL with medical therapy reduces both the risk of recurrent myocardial infarction (MI) (secondary prevention) and the risk of the development of cardiovascular disease (CVD) (primary prevention). Much of the data on statin therapy for lowering LDL cholesterol have been pooled and evaluated by the Cholesterol Treatment Trialists group, showing a clear correlation between LDL level and cardiac risk (Cholesterol Treatment Trialists' Collaborators, 2012).

Thus, we have data showing that lifetime exposure to high cholesterol increases risk for CVD in a dose-dependent manner, that lifetime exposure to low levels of LDL decreases risk, and that medically lowering LDL cholesterol also reduces risk. Is there molecular data showing a mechanistic explanation for this effect?

Indeed, there is a wealth of evidence showing that LDL movement into the subendothelial layer is one of the earliest phases of the development of atherosclerosis. Once the endothelial layer is disrupted or inflamed, markers on the surface of the endothelium will attract LDL molecules, which then move into the subendothelial layer, beginning the complex formation of the atherosclerotic lesion (Kwak et al., 2014; Libby, 2002b). In addition, research has shown that the higher the circulating level of LDL, the more molecules will travel through the endothelium and promote atherosclerosis (the mass transport theory) (Lusis, 2000; Olgac et al., 2008). Therefore, it is plausible that the lower the level of circulating LDL, the less promotion of atherosclerosis, the cause of vascular disease.

Other lipoproteins play a role in the development of atherosclerosis as well. Low levels of high-density lipoprotein (HDL) have been associated with higher risk of atherosclerosis in epidemiologic studies, though the association is complex (McQueen et al., 2008). HDL has an opposing relationship to LDL in that higher levels of HDL reduce the risk of atherosclerosis, while lower levels increase the risk (Castelli et al., 1992). However, the absolute level of HDL does not seem to have as clear a role in predicting future risk as does the HDL function. HDL plays a major role in reverse cholesterol transport, which is the process by which cholesterol is transported from the circulation to the liver. HDL molecules also reduce the amount of cholesterol in the atherosclerotic plaque by removing it via a mechanism known as cholesterol efflux. The extent to which this occurs is known as the cholesterol efflux capacity. HDL also has an antiinflammatory effect, and can decrease the oxidation of LDL in the subendothelial space, which is a key step in the progression of atherosclerosis (Ansell et al., 2005; Libby, 2002a). Paradoxically, in some situations, for instance during an inflammatory state such as an infection, HDL can be proinflammatory (Ansell et al., 2005).

Despite a wealth of epidemiologic data and clear mechanistic research on how HDL inhibits atherosclerosis, a variety of interventions to raise HDL have yet to show benefit. Though some early studies suggested benefit using niacin to target low HDL (Brown et al., 2001), subsequent larger trials have not been able to reproduce this effect (AIM-HIGH Investigators et al., 2011). A class of drugs called cholesterol ester transfer protein (CETP) inhibitors have been developed, which work to block the enzyme cholesterol ester transfer protein. CETP reduces circulating HDL cholesterol levels by transferring esterified cholesterol ester from HDL to larger lipoproteins, such as chylomicrons, VLDL, and LDL, in exchange for triglycerides. By blocking this enzyme, circulating HDL increases. However, no clinical trial of any of the CETP inhibitors brought to market thus far has shown a clinical benefit (Kingwell et al., 2014). This may in part be due to the finding that inhibiting CETP by some mechanisms also increases the number of small, dense LDL particles, which are atherogenic, thus offsetting the benefit of the increase in HDL (Durrington, 2012).

The connection between elevated serum triglycerides and atherosclerosis has been less clear. It is likely that elevated triglyceride level is more a marker of increased atherogenic risk rather than atherogenic itself. In the Emerging Risk Factors study, a meta-analysis of multiple prospective studies including data on over 300,000 individuals, there was a clear correlation between elevated triglyceride level and future development of cardiovascular disease. However, when the data was controlled for HDL cholesterol and non-HDL cholesterol, the correlation disappeared, suggesting that the association with elevated levels of LDL and low levels of HDL were the driving factors (Emerging Risk Factors Collaboration et al., 2009).

Elevated levels of triglycerides are frequently associated with the metabolic syndrome (increased waist circumference, insulin resistance, low HDL, high blood pressure, and elevated triglycerides) which has been linked to increased cardiovascular risk. Once again, this appears to be primarily an association rather than a causative factor. For this reason, the American Heart Association (AHA) statement on Triglycerides and Cardiovascular Disease suggests that the finding of elevated triglycerides in a patient should prompt aggressive lifestyle intervention including a diet low in simple carbohydrates and higher in omega-3 fats, weight loss, and increased physical activity. Medical therapy aimed at lowering triglycerides with fibrates should be reserved for patients at risk of triglyceride-induced pancreatitis (Miller et al., 2011).

There are other lipoprotein types that are atherogenic as well, such as lipoprotein(a) (Lp(a)). Lp(a) is essentially an LDL molecule that has an apolipoprotein A molecule attached to the apo B molecule. The level of Lp(a) is genetically determined and not influenced by diet. The LPA gene determines the amount of Lp(a) produced by the liver. Lp(a) is particularly atherogenic due to the similarity between apo a and plasminogen, which it competes with for the plasminogen receptor. Lp(a) does not have the fibrinolytic ability, however, so it is thereby somewhat prothrombotic by inhibiting some plasminogen-mediated fibrinolysis. In addition, it has a high affinity for injured endothelial cells, binding to deliver cholesterol. This may have been advantageous at one point in evolution as a cellular membrane repair function, but with our current lifestyles and other cardiovascular (CV) risk factors, it is now proatherogenic (Tsimikas and Hall, 2012).

Hypertension

Hypertension is one of the most prevalent risk factors for cardiovascular disease. The most current data from the National Health and Nutrition Examination Survey 2011–2014 showed a prevalence of 29.1%. The prevalence increases with age, from 7.3% among adults aged 18–39, 32.2% in those aged 40–59, and 64.9% in adults 60 and over (Yoon et al., 2015). The prevalence has not really changed over the last 10 years. Among adults with hypertension, only 53% had controlled hypertension. The prevalence of controlled hypertension increased significantly between 1999 and 2009 from 31.5% to 53.3%, but plateaued from 2009 to 2014 without any further improvement.

The connection between hypertension and vascular disease is well established in epidemiologic and clinical trials. The Framingham study showed a clear connection between hypertension and risk of the development of cardiovascular disease (Franklin et al., 2001). There seems to be a stronger correlation between diastolic hypertension at a younger age, while systolic hypertension and then pulse pressure become more correlated with age.

The treatment of hypertension in multiple large randomized controlled trials has shown clear reduction in event rates of MI, stroke, and death (Turnbull and Blood Pressure Lowering Treatment Trialists' Collaboration, 2003). The level to which blood pressure should be treated, however, has been controversial. Though epidemiologic studies show a graded increase in risk with increasing blood pressure as a continuum, randomized controlled trials have not demonstrated a significant reduction in cardiovascular risk with reductions in blood pressure below 140/90 mmHg (Rosendorff et al., 2015). In addition, some studies have suggested excess risk of aggressive blood pressure lowering in patients over 60 years of age (Bavishi et al., 2016). The most recent guideline released in 2014 therefore recommends target blood pressure lowering to <140/90 in individuals <60 and <150/90 in patients >60 years (Rosendorff et al., 2015). The recently completed SPRINT study, however, may influence future recommendations. This large, prospective, NIH-sponsored trial compared standard (<140 mmHg) vs intensive (<120 mmHg) BP lowering in patients without CVD or diabetes. They found a reduction in major adverse cardiovascular events in patients treated to the lower goal of <120 mmHg (The SPRINT Research Group et al., 2015). However, there has been some controversy on the SPRINT trial, for instance on the methods used to assess BP (Bavishi et al., 2016). Interestingly, another recent large trial (HOPE-3) that looked at treating intermediate risk patients with average systolic blood pressure of 138 failed to show a reduction in major adverse cardiovascular events with a combination of an angiotensin II receptor blocking agent and hydrochlorothiazide (Lonn et al., 2016). Though the exact target to lower blood pressure will likely remain controversial, the NHANES data above clearly shows that we have plenty of room to improve our treatment of hypertension in the United States with still 47% of patients not adequately treated and at least 25% of patients with diagnosed hypertension not taking any medication at all (Nwankwo et al., 2013; Yoon et al., 2015).

There are clear epidemiologic studies showing a connection between hypertension and CVD, and trials showing a reduction in CVD with the treatment of hypertension, but what about a biologic mechanism connecting hypertension and CVD? There are multiple mechanisms by which hypertension contributes to atherosclerosis. First is the increased vascular stress on the endothelium caused by the increased pressure. There is evidence that particularly in areas where there is nonlaminar flow, increased radial forces lead to changes in the endothelial layer that make it more susceptible to LDL movement into the subendothelium, as well as the attachment of monocytes, two of the earliest steps in the development of atherosclerosis (Kwak et al., 2014).

Another link between hypertension and CVD is a result of increased oxidative stress. Several different enzyme systems are activated in the setting of hypertension resulting in increased availability of superoxide anions that decrease the availability of nitric oxide and generate proinflammatory radicals. These enhanced enzyme systems include NADPH oxidase, xanthine oxidase, and cyclooxygenase as well as decreased activity of superoxide dismutase, which works as a detoxifying agent (Zaheer et al., 2016). Angiotensin II is also increased in hypertension and is another strong enhancer of oxidative stress (Luft, 2001). Increased oxidative stress and inflammation has been closely linked to the development of atherosclerosis through a variety of mechanisms including endothelial cell damage, increased expression of adhesion molecules, and activation of inflammatory proteins that promote atherosclerosis (Libby, 2002b).

Smoking

Cigarette smoking remains the single most preventable cause of early death in the United States. The 2014 Surgeon General report states that over 20 million premature deaths can be attributed to smoking over the last 50 years since the first surgeon general report was released. Of these, 7.8 million are attributed to cardiovascular disease in smokers and another 2.2 million are attributed to cardiovascular disease in people exposed to secondhand smoke (General, 2014). Between 2005 and 2009, 32% of all deaths from coronary heart disease were attributable to smoking, making it the strongest risk factor for coronary heart disease other than age. Despite advances in smoking cessation, it is predicted that, at current rates, 5.6 million current Americans younger than 18 years of age are projected to die prematurely of a smoking-related illness.

There are an estimated 1.3 billion smokers worldwide, of which 82% are in developing countries (Shafey et al., 2003). There have been great strides in reducing the number of smokers in the last 50 years since the original surgeon general's report. The prevalence of current cigarette smoking among adults has declined from 42% in 1965 to 18% in 2012. However, there has been a slowing in the rate of decline, particularly in the last 8 years, with the prevalence remaining relatively flat, though the number of cigarettes smoked has dropped, with fewer smokers in the >20 cigarettes per day, but an increase in the number of smokers using 1–10 per day.

There have been many studies looking at the effect of smoking on risk of CHD. The epidemiologic connection between smoking and heart disease was first noted in the 1950s, and gathered more and more data over the years. One of the largest of the more recent studies was the INTERHEART study—a prospective case control study in multiple sites around the world that looked at a variety of risk factors for heart disease, including smoking as well as secondhand smoke (Teo et al., 2006). They found an overall threefold increased risk of acute MI in patients who smoked. The risk was directly proportional to the number of cigarettes smoked, with odd ratios of 1.63 in subjects who smoked 1–9 cigarettes per day, 2.59 for those who smoked 19–20 per day, and 4.59 for the >20 cigarettes/day smokers, but escalate to 9.16 in those who smoked >40 cigarettes/day. These ratios were similar for men and women.

Secondhand smoke exposure was also associated with risk of MI in a graded manner. Compared with subjects reporting no secondhand smoke exposure, those with 1–7 h of exposure per week had an odds ratio of 1.24 for MI, while those with 15–20 h per week increased their risk almost twofold.

It was also interesting to see how quickly risk dropped in nonsmokers. Within the first 3 years after quitting, risk dropped in half, though there did continue to be some excess risk even 20 years after quitting (Teo et al., 2006).

Smoking leads to a variety of abnormalities in the vascular system that ultimately leads to atherosclerosis and CVD. One of the earliest is vasoconstriction due to endothelial dysfunction, as well as an inhibition of nitric oxide production, which further impairs vasodilation. Smoking also appears to contribute to LDL oxidation, another important factor in the early development of atherosclerosis. The general injury of the endothelium causes a change in the adhesive properties that increases the recruitment, adhesion, and migration of monocytes into the subendothelial space, the next step in the development of atherosclerosis. In addition to its role in the development of the atherosclerotic plaque, smoking has been shown to increase levels of fibrinogen, which increases thrombosis and can therefore contribute to the subsequent clot formation in the ruptured plaque that leads to an acute MI (Powell, 1998).

As the largest preventable cause of CVD in the world, there is appropriately much focus on eliminating smoking, like the US Healthy People 2020 initiative, which hopes to reduce the prevalence of cigarette smoking among adults to 12%. With the number still hovering around 16%–18%, and holding steady, this goal may be difficult to attain. The American Heart Association's Million Hearts program targets a variety of cardiovascular risk factors, including smoking, with a goal to save a million lives in 5 years. If we hope to continue to reduce the incidence of heart disease, smoking cessation will need to remain a top priority.

Obesity

Obesity has become a global epidemic that is rampant in the United States. Of US adults, 69% are either overweight or obese and 35% are obese body mass index (BMI >30) (Flegal et al., 2012). Obesity is clearly a risk marker for CVD, in that it is associated with multiple known risk factors such as diabetes, elevated cholesterol, hypertension, and sedentary lifestyle. However, several large trials have established that obesity is an independent risk factor for heart disease as well. The Prospective Studies Collaboration looked at 57 prospective studies with almost 900,000 participants. This study showed a clear correlation of obesity, as measured by body surface area, and risk of CVD adjusted for age, study, and smoking. They found that for each additional 5 kg/m^2 increase in BMI above 25 there was an additional 40% increase in risk of vascular mortality (hazard ratio (HR) 1.41) (Prospective Studies Collaboration et al., 2009). They found a U-shaped curve in the relationship between BMI and mortality in general with the ideal BMI from 22.5 to 25 kg/m^2. The inverse relationship between BMI and mortality below 22.5 was due predominantly to an increase in respiratory diseases and lung cancer.

The Emerging Risk Factors Collaboration looked at 58 prospective studies with data from 221,934 subjects in 17 countries to assess the relationship of BMI, waist circumference, and waist to hip ratio to determine which measure of obesity best correlated with risk of first-onset cardiovascular disease. They found a significant increased risk in CVD with all three measures of obesity when adjusted for age, sex, and smoking status without a clear improvement in correlation with any one. However, the correlation between BMI and CVD risk was significantly attenuated when additional adjustments for systolic blood pressure, history of diabetes, and total and HDL cholesterol were made (from a HR of 1.27 to 1.07) (Emerging Risk Factors Collaboration et al., 2011b).

These studies, and others, suggest that the majority of the connection between obesity and cardiac risk lies in the increase in associated risk factors like diabetes, cholesterol, and decreased activity. However, there are physiologic findings in obesity that promote atherosclerosis. Obesity is an inflammatory state, as adipose tissue is a source of proinflammatory adipokines including tumor necrosis factor (TNF) alpha, IL, monocyte chemoattractant protein-1 (MCP-1), resistin, and leptin. These adipokines have a variety of effects including stimulating vascular reactivity, promoting inflammation, and even promoting coagulation (Mandviwala et al., 2016).

Obesity is therefore both a risk factor and a risk marker for cardiovascular disease, and its rise over the last 20 years remains both a major health risk and an important target for intervention.

Sedentary Lifestyle

Sedentary lifestyle has become a growing concern as a risk factor for cardiovascular disease. Dr. James Levine, director of the Mayo Clinic-Arizona State University Obesity Solutions, has dubbed "sitting the new smoking."

The Australian Diabetes, Obesity and Lifestyle study measured sedentary activity by hours of television viewing. They adjusted for age, sex, waist circumference, and exercise, and found a significant correlation between hours of television watched and cardiovascular mortality. Compared with the baseline of <2 h/day, the HR for >2 to <4 was 1.19, and 1.8 for >4 h/day (Dunstan et al., 2010). Even in patients who exercise, the negative impact of more time spent sitting outweighs the benefit of exercise. This was evaluated in a large prospective study in the United States sponsored by the American Cancer Society that prospectively surveyed 123,216 subjects subsequently followed for 14 years. After adjustment for age, smoking, BMI, alcohol consumption, and comorbidity index (including hypertension, diabetes, and cholesterol), they found that time spent sitting >6 h/day versus <3 h/day was associated with a mortality RR of 1.17 in men and 1.34 in women (Patel et al., 2010). The relative risks were even higher in the group with the most sitting and least exercise combined. The associations were strongest for cardiovascular mortality.

Like obesity, being sedentary is clearly associated with an unhealthy lifestyle that increases cardiovascular risk factors; however, there are some physiologic changes that likely make sedentary behavior both a risk marker and a risk factor in itself. One study has

shown that lipoprotein lipase activity is decreased during sitting, which would result in less catabolism of triglycerides and increase levels of plasma triglycerides (Zderic and Hamilton, 2006). Another possibility is the decrease in endothelial nitric oxide expression caused by lower blood flow in the limbs with prolonged sitting (Stamatakis et al., 2011). Studies have shown that decreased nitric oxide and the subsequent decrease in vascular reactivity is a risk factor for atherosclerosis.

The American Heart Association and American College of Cardiology recommend 30 min of aerobic exercise 5 days a week at a minimum for a healthy lifestyle (Eckel et al., 2014). Many also now recommend limiting prolonged sitting and now recommend getting up and moving for at least 5 min out of every hour of sitting as a separate and equally important way to reduce cardiovascular risk.

Diabetes

Diabetes has long been known as a risk factor for the development of atherosclerosis. As one of the strongest risk factors for coronary artery disease and MI, patients with diabetes at age 50 will be expected to die 6 years earlier than nondiabetic matched controls (Emerging Risk Factors Collaboration et al., 2011a). The risk of vascular death is twice that of nondiabetics even when adjusted for age, sex, BMI, LDL, systolic blood pressure, smoking, and several other risk factors.

Prospective studies have also shown a relationship between elevated glucose at baseline with the development of vascular disease, even without the diagnosis of diabetes (Emerging Risk Factors Collaboration et al., 2010b). In addition, hemoglobin A1c, a marker of longer term glucose control, is correlated with increased risk for the development of coronary artery disease (Turner et al., 1998).

Much research has been done on the mechanisms of how diabetes leads to vascular disease. The mechanism is predominantly related to endothelial dysfunction. The endothelium is the monolayer of cells that lines all vascular beds and regulates the interaction between the vessel wall and the blood. One of the ways diabetes affects the endothelium is by inhibiting endothelial-derived nitric oxide (Beckman et al., 2002). Nitric oxide is the key mediator of vascular relaxation and plays a role in limiting inflammation. Insulin resistance also leads to increased availability of free fatty acids, which further inhibit nitric oxide-mediated vasodilation. Diabetes also increases plaque instability by inhibiting collagen synthesis by smooth muscle cells, affecting the stability of the fibrous cap. Through a variety of mechanisms, diabetes also enhances platelet activation, which, in addition to the augmented blood coagulability, further increases the risk of thrombotic occlusion with plaque rupture (Beckman et al., 2002).

Inflammation

Inflammation is one of the newly identified risk factors for cardiovascular disease. There has been controversy as to whether inflammation is a risk marker (a product of atherosclerosis) or a risk factor, though it appears to be both.

Atherosclerosis is clearly an inflammatory state. Leukocytes attracted to cell surface adhesion molecules, in turn stimulated by cytokines, begin the process of atherosclerosis. One of the best studied markers of inflammation is C-reactive protein (CRP). Studies, such as the Emerging Risk Factors Collaboration, have shown that CRP level is a strong predictor of vascular events in apparently healthy controls, independent of other conventional risk factors such as cholesterol, smoking, and hypertension. In fact, this study suggested the degree of predictive power was similar to that of total and HDL cholesterol (Emerging Risk Factors Collaboration et al., 2010a).

A large randomized controlled trial that compared the use of rosuvastatin vs placebo in patients with elevated CRP but relatively "normal" LDL cholesterol showed that treatment with the statin markedly reduced cardiac events and mortality (Ridker et al., 2008). This benefit held true even in patients that would have been "low risk" according to their conventional risk factors, attesting to the additional benefit of CRP in predicting risk.

Ongoing trials using antiinflammatory medications to reduce risk of cardiac events, such as the NIH-sponsored CIRT trial, should help determine if attenuating inflammation reduces cardiac events. This study is looking at the effect of low-dose methotrexate in patients with coronary artery disease to see if reducing inflammation will reduce recurrent coronary events. Inflammation modulation will certainly continue to be a target for treatments that hope to reduce the development of cardiovascular disease.

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Risk Prediction

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Overview

Two complementary strategies for prevention are the population approach and the high-risk approach. The high-risk preventive strategy identifies and targets individuals at elevated risk of developing cardiovascular disease (CVD) who would benefit from preventive therapies such as statins or aspirin as well as effective dietary and behavioral interventions. For this purpose, an algorithm using the Framingham equation for estimating 10-year risk of coronary heart disease (CHD) had been promoted by the Adult Treatment Panel-III (ATP-III) panel for the treatment of hyperlipidemia in the United States since 2001 (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), 2002). In 2013, the ACC/AHA risk-based approach using the Pooled Cohort Equations replaced the previous ATP-III algorithm for the primary prevention of atherosclerotic cardiovascular disease (ASCVD) (Goff et al., 2014). The principal assumption of these approaches is that the intensity of preventive therapies should match the level of absolute risk, which had previously been estimated by simply counting the number of risk factors. In this section, the definition, evaluation, application, and future directions of CVD risk prediction models are briefly introduced.

Definition

A CVD risk prediction model is a mathematical equation that combines multiple risk factors for an individual to estimate the absolute probability of developing CVD within a specific time period (Moons et al., 2012a). Correct interpretation of the estimated risk obtained from the model, say, a "10-year CVD risk of 10%," is that 10 out of 100 individuals with the same estimated risk would be expected to sustain a major ASCVD event by the end of 10 years (Lloyd-Jones, 2010). Procedures for the development of multivariable prediction models are described in detail elsewhere (Sullivan et al., 2004).

Framingham Risk Score

The Framingham risk function was the first CVD risk prediction model that was developed from the Framingham Heart Study in the 1970s (Kannel et al., 1976). The function has been updated by adding newer risk factors (Anderson et al., 1991), or employing risk factor categories (Wilson et al., 1998). The Framingham Heart Study website provides online risk calculators (The Framingham Heart Study, n.d.). The Pooled Cohort equations were developed in more racially and geographically diverse US population than Framingham Study alone in order to expand the utility and generalizability of the prediction model (Goff et al., 2014).

Assessment of Predictive Performance: Discrimination

Discrimination is the ability of a model to distinguish individuals who experienced the outcome from those who did not and is usually summarized via the c-statistic (Moons et al., 2012a). The c-statistic is the area under the receiver operating curve and can have values from 0.5 (no discriminative ability) to 1.0 (perfect discrimination). The c-statistics <0.70, 0.70 to <0.80, and >0.80 are judged to have poor, acceptable, and excellent discrimination, respectively (Lloyd-Jones, 2010). Changes in the c-statistics are often used to compare prediction models with or without a novel risk marker. However, achieving clinically meaningful increase in a c-statistic is often difficult even though a novel risk factor may add predictive value (Folsom et al., 2006; Cook, 2007). Instead, other

metrics have been proposed including net reclassification improvement (NRI) and integrated discrimination improvement (IDI) (Pencina et al., 2008).

Risk Reclassification Analysis

The NRI for adding a novel risk factor to a prediction model is the ratio of net increase in risk factor categories among those who developed disease to net decrease among those who did not develop disease (**Table 1**). IDI is another measure of model improvement and is defined as the integrated difference of sensitivity minus "one minus specificity" and is estimated using the difference in discrimination slopes, which is defined as a difference between mean predicted probabilities of an event for those with events and the corresponding mean for those without events. For example, in the Atherosclerosis Risks in Communities Study, adding cardiac troponin T to traditional risk factors improved CVD risk prediction, as indicated by an NRI of 10.1 and an IDI of 0.032 (Saunders et al., 2011; Folsom, 2013).

Calibration

Calibration assesses the ability of a model to predict the absolute level of risk. It is a measure of the model's goodness of fit and is often assessed visually by comparing the observed and predicted numbers of events in quantiles (e.g., deciles) using statistics such as the Hosmer–Lemeshow, Grønnesby–Borgan, or D'Agostino and Nam chi-squared tests (Demler et al., 2015). Calibration slope can be derived from the plot of the observed disease probability (y-axis) versus the expected probability (x-axis) in each decile for a prediction model. If the model is well calibrated, the slope of the plot would be expected to be close to 1 (a 45-degree line) and the constant to be close to 0. See references for examples of calibration figures (Dufouil et al., 2017; Riley et al., 2016).

Internal and External Validation

Because a prediction model is developed using a specific data sample from a source population, the model typically performs better in the development data sample than when applied to a different data sample even from the same population. This phenomenon is called "overfitting" or "optimism" and may be evaluated using only the data at hand by a process called internal validation. The most widely advocated technique for the internal validation is bootstrapping, a statistical method that aims to mimic the sampling process, that is, the development sample from the source population (Moons et al., 2012a). In each bootstrap sample, the data are analyzed as in the original study sample, repeating each step of the model development. The summary statistics (e.g., bootstrap-adjusted c-statistics) represent what can be expected when the model is applied in new individuals from the same source population.

In contrast, external validation applies the original model to a different data sample from the same or a different population using the predictor and outcome values for individuals in the new data sample to quantify the model's predictive performance (Moons et al., 2012b). A recent review of 363 CVD prediction models reported in 136 articles found that 22% were internally validated and 36% were externally validated (Damen et al., 2016).

Table 1 Definition of net reclassification improvement

People who developed disease

		New model		*Reclassification*	
Existing model	Low risk	Intermediate risk	High risk	Up (appropriate)	Down (inappropriate)
Low risk	No change	A	B	A+B+C	D+E+F
Intermediate risk	D	No change	C	Net increase	
High risk	E	F	No change	(A+B+C)-(D+E+F)	

People who did not develop disease

		New model		*Reclassification*	
Existing model	Low risk	Intermediate risk	High risk	Up (inappropriate)	Down (appropriate)
Low risk	No change	a	b	a+b+c	d+e+f
Intermediate risk	d	No change	c	Net decrease	
High risk	e	f	No change	(d+e+f)−(a+b+c)	

NRI=Net increase/Net decrease

Although the number of externally validated models is still low, there seems an abundance of CVD prediction models in the literature (Damen et al., 2016). Yet, many studies do not report fully the development and validation process of their prediction models. The transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) Initiative developed a set of recommendations, with a checklist of 22 items, to standardize reporting of prediction model studies (Collins et al., 2015).

Application

It has been advocated for decades that the intensity of risk factor management should be matched to the absolute risk of CVD (Califf et al., 1996). The number needed to treat to prevent a CVD event is lowest for those at highest risk. Clinical assessment of estimated 10-year risk was first recommended in the United States by the 2001 ATP-III in order to guide hyperlipidemia treatment (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), 2002). This risk-based approach for cholesterol management was adopted worldwide (NICE, 2017; Teramoto et al., 2013; Piepoli et al., 2016; Stone et al., 2014). Although direct evidence from clinical trials about the effectiveness of risk prediction models in clinical practice is limited and inconclusive (Karmali et al., 2017), it was estimated from Copenhagen General Population Study data that more CVD events would be prevented by treating fewer patients by using the 2013 ACC/AHA absolute risk-based approach than approaches based on cholesterol thresholds for inclusion/exclusion from randomized clinical trials (Mortensen et al., 2015).

CVD risk prediction estimates can also inform individuals about their personal risk of CVD so that they may be motivated to modify their behaviors and to adopt healthier lifestyles. Estimated risk can be a tool for shared doctor–patient decision-making regarding the management of risk factors.

Future Directions

Despite the rapid proliferation of literature on CVD risk prediction models, they are underutilized in the clinical setting (Fuster, 2017). Clinicians need to be better informed about when, how, and which model to use. It also would be useful to document whether using risk prediction tools leads to more appropriate initiation of pharmacotherapy or modification of patient behavior.

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Role of Coronary Artery Revascularization in Heart Failure

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Introduction

Coronary artery disease is still the most common cardiac pathology leading to heart failure in the industrialized world (Gheorghiade et al., 2006). Studies in the 1970s established the benefit of surgical revascularization in patients with angina and extensive coronary artery disease, but excluded patients with left ventricular (LV) dysfunction (Coronary Artery Surgery Study (CASS), 1983; The Veterans Administration Coronary Artery Bypass Surgery Cooperative Study Group, 1984; Varnauskas, 1988). However, recent observational studies are demonstrating that there may be a benefit for surgical revascularization in patients with ischemic cardiomyopathy (O'Connor et al., 2002; Cleland et al., 2011; Chareonthaitawee et al., 2005). To better understand the role of surgical revascularization in these patients, the Surgical Treatment for Ischemic Heart Failure (STICH) trial was designed. The focus of this article will be to examine the outcome of this trial, and subsequent STICH Extension Study (STICHES) to provide the reader with the most current information regarding the role of coronary artery bypass grafting (CABG) in patients with severe LV dysfunction (ejection fraction <35%) (Velazquez et al., 2011, 2016).

Assessment of Revascularization Potential in Ischemic Cardiomyopathy

All patients with heart failure by the time they arrive for surgical evaluation have had one if not multiple echocardiographic imaging documenting the degree of LV dysfunction. The next step in their evaluation will be to determine if they have coronary artery disease that is amenable for CABG, and then if the myocardium along the diseased coronary artery distribution is viable. If either of these parameters is not met the patient is deemed a poor candidate for CABG, and should be evaluated for a mechanical circulatory support device or heart transplantation (Fig. 1). Moreover, careful evaluation of comorbid conditions is exceedingly important. Patients with few risk factors have an acceptable Society of Thoracic Surgery (STS) predicted risk of death score making CABG compelling. The decision however to proceed with CABG becomes more difficult when risk factors cause a rise in the STS score.

Coronary Artery Disease Evaluation

Coronary angiography is the gold standard and most commonly obtained imaging modality for characterizing coronary artery disease and graft patency. It is a minimally invasive procedure where the right and left main coronary arteries are injected with dye via a peripherally placed catheter, and then X-ray motion pictures are obtained. Despite its invasiveness and associated risks including stroke, vessel dissection, acute myocardial infarction, arrhythmias, puncture site hematomas and pseudoaneurysm, it remains a very safe diagnostic method. Its lack of three-dimensional expositions however has led to the development of coronary computed tomography angiography or multidetector computed tomography (MDCT).

Multidetector Computed Tomography

This imaging modality has the same detection profile as angiography, but is three-dimensional, noninvasive, rapid, and painless. After injecting a nonionic contrast agent and allowing time for it to reach the arterial system, CT data is acquired in a single breath hold of 25–30 s. Further improvement in imaging quality is achieved by slowing the heart rate into the 60s with beta-blockers. Image completion is then facilitated by electrocardiographic gated reconstruction algorithms. In coronary vessels greater than 1.5 mm MDCT has sensitivity, specificity, positive predictive valve, and negative predictive value of 89%, 98%, 90%, and 98%, respectively. Overall, MDCT detected 78% of stenosis revealed by angiography (Bluemke et al., 2008). If the coronary vessels are heavily calcified, intramyocardial, or covered with excess fat newer MDCT scanners are better than coronary angiography at identifying these variations. This feature is especially helpful for minimally invasive coronary procedure where tactile sensation is limited.

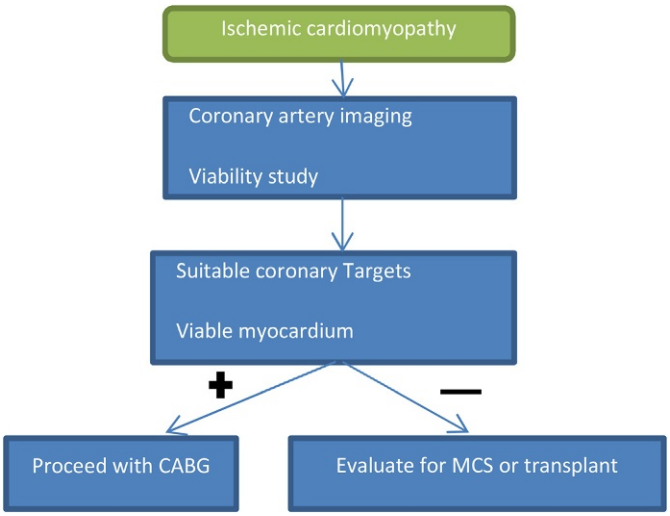

Fig. 1 Diagnostic and treatment algorithm for ischemic cardiomyopathy. *MCS*, mechanical circulatory support.

Myocardial Viability

The degree of coronary artery stenosis will cause varying degrees of myocardial damage. Nuclear imaging and cardiac magnetic resonance imaging (MRI) have emerged as the two well-validated techniques for uncovering what myocardium remains viable after ischemia. Glucose metabolism substitutes free fatty acid metabolism as the main energy source in ischemic but viable myocardium. The nuclear imaging provided by single photon emission computed topography utilized a glucose analog and tracer, F-fluorodeoxyglucose (F-FDG), to detect glucose metabolism in myocytes. Following detection of a fixed perfusion defect on perfusion imaging, preservation of F-FDG uptake in this region by PET scan is suggestive of viable or hibernating myocardium and therefore indicative of the beneficial effect of revascularization in patients with ventricular dysfunction (Selvanayagam et al., 2004; Kim et al., 1999).

Cardiac MRI lately has been gaining in popularity as a reliable diagnostic method for cardiac structure, and function after ischemia. Kim and colleagues observed that contrast MRI combined with cine MRI showed hyperenhancement and dysfunctional contractility with myocardial infarction, but dysfunctional contractility without hyperenhancement was seen in injured yet viable myocardium. Normal myocardium had no hyperenhancement and normal contractility. The same group went on to investigate the use of contract-enhanced MRI to predict improvements following revascularization in areas of abnormal ventricular contraction. Their data showed that improvement in regional contractility after revascularization decreased progressively as the transmural extent of the hyperenhancement before revascularization increased. The extent of LV tissue dysfunctional that is not hyperenhanced before revascularization was related to the degree of improvement in wall motion scores and ejection fraction after revascularization (Selvanayagam et al., 2004; Kim et al., 1999). Further confirmatory studies regarding enhanced MRI firmly established its ability to predict myocardial viability after surgery (Kim et al., 2000).

Treatment of Coronary Artery Disease With Concomitant Ventricular Dysfunction

Treatment of coronary artery disease in patients with normal LV function has been well established for the past 3 decades. However, the management of coronary artery disease patients with severe LV dysfunction is not as firmly established. Varying data highlights the difficulty in managing this complex group of patients. At the present time the American Heart Association and American College of Cardiology guidelines give coronary revascularization in patients with severe LV dysfunction a level IIb recommendation. In other words, it "may be considered." Conclusions from the recent STICH and STICHES trials have been at odds with this recommendation.

Prior to the STICH trial data regarding coronary artery revascularization in the face of LV dysfunction seemed to support surgical management of these patients. In 1984 the Coronary Artery Surgery Study (CASS) essentially showed that patients with moderate LV dysfunction did not benefit from coronary revascularization while the subgroup of patients with severe LV dysfunction had an improved survival with surgical therapy. Bounous et al. had similar findings in their 1988 study. The major drawback of these and many subsequent studies was the lack of randomization. The retrospective nature of these investigations invariably presented bias in the equation. Moreover, some have argued that medical therapy has improved significantly over the past two decades thus rendering the risks of undergoing surgical therapy in the face of severe LV dysfunction unnecessary. Clearly a large randomized trial comparing the two modalities was necessary.

The STICH trial was a large multi-national study sponsored by the National Institute of Health involving over 1200 patients. All patients had an ejection fraction <35%, which would qualify as severe LV dysfunction. Patients were randomized into either the medical management arm or the surgical management arm; all-cause mortality was the primary outcome tracked. This study was specifically designed to identify the potential benefits of surgical revascularization in this complex group of patients with severe LV dysfunction. Outcomes at 10 years were analyzed in the follow-up STICHES trial.

The 5- and 10-year all-cause mortality were determined to be lower in the patients who underwent CABG, indicating a durable benefit derived from surgical revascularization in the setting of LV dysfunction. CABG was, however associated with a perioperative mortality rate of 3.6%, which was overcome after 2 years of follow-up. Interestingly survival within the two cohorts continued to diverge after the initial perioperative period (Fig. 2). The CABG cohort also showed a 26% relative risk reduction of all-cause mortality at 5 years. After 10 year follow-up this number increased to 28% (Fig. 2). Therefore, overall it seems CABG in the setting severe LV dysfunction is associated with improved survival when compared to medical therapy alone.

While the major conclusion of the STICH trial is obvious, there are numerous trends that become evident upon closer examination of the data. First of all, 10-year all-cause mortality was even higher in younger patients treated with medical therapy alone. In other words, CABG is particularly beneficial in younger patients with ischemic cardiomyopathy and severe LV dysfunction. Second, a substudy found that results of cardiac PET-CT and dobutamine stress echo did not predict mortality in the cohort undergoing CABG. Third, the reduction in cardiovascular mortality was consistent in the CABG group across all ages; however the reduction in all-cause mortality was not.

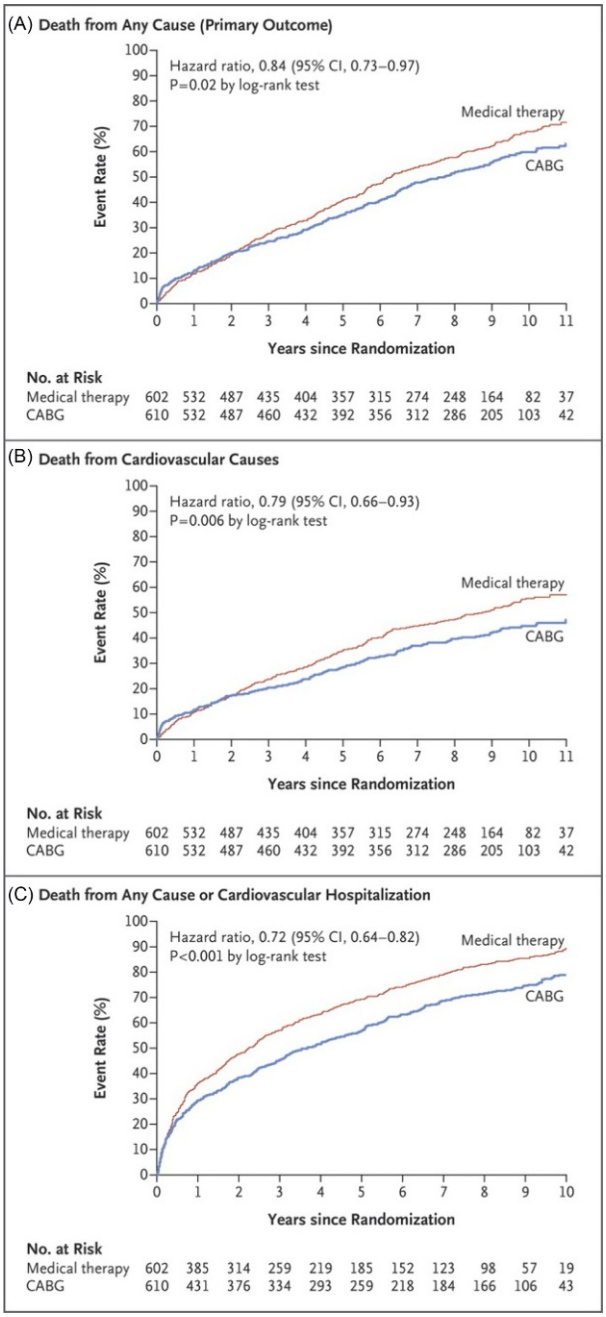

Fig. 2 Kaplan–Meier estimates of the rates of (A) death from any cause, (B) death from cardiovascular causes, and (C) death from any cause or hospitalization for cardiovascular causes. Reproduced from Velazques, E. J., Lee, K. L., Jones, R. H., et al. (2016). Coronary-artery bypass surgery in patients with ischemic cardiomyopathy. *New England Journal of Medicine* **374**, 1511–1520, with permission.

Mitral valve repair/replacement in patients with left ventricular dysfunction and ischemic MR remains a controversial topic. The severity of MR requiring intervention as well as the technique for addressing MR are currently not agreed upon. While it did not offer robust guidelines regarding ischemic mitral valve disease, the STITCH trial did uncover certain trends. First, patients with even mild ischemic mitral regurgitation were at increased risk of poor outcomes versus those without MR. Second, patients with severe left ventricular dysfunction as well as ischemic MR should undergo concomitant mitral valve surgery (repair vs replacement). Third, in this cohort, mitral valve replacement may be the preferred strategy. The management of moderate mitral regurgitation in this group requires further investigation.

Surgical Ventricular restoration (SVR) was also investigated in the STITCH trial. Data prior to this trial were mixed with regards to mortality after SVR and CABG versus CABG alone. Zhaohua and colleagues found one, five, and ten year mortality rates of 95%, 86% and 80% respectively after CABG combine with SVR and mitral valve repair in 61 patients. Shen and colleagues found early improvement in NYHA functional class in patients undergoing SVR plus CABG. However, these differences did were nonexistent in long term followup. No significant differences in survival or major adverse cardiac events were found in mid to long term follow up between the two cohorts. The STITCH data showed similar findings. There was also no difference in quality of live between CABG/SVR patients and isolated CABG patients. This lead authors to conclude that SVR does not add significant clinical benefit while it does add healthcare costs.

It is quite clear that the STICH trial lends support to CABG in patients with severe LV dysfunction; however it did not compare survival in patients undergoing PCI. Some studies have found increased mortality in patients undergoing PCI but to date there are no large randomized trials comparing PCI and CABG in patients with severe LV ejection fraction.

In conclusion a number of retrospective studies have examined the benefit of coronary artery revascularization in patients with ischemic cardiomyopathy-related LV dysfunction; however, prior to the STICH trial there was a lack of large randomized studies examining this topic. The results drawn for the STICH trial support surgical revascularization in patients with severe LV dysfunction. There are numerous caveats. First, perioperative mortality risk must be considered when evaluating this complex group of patients. Those with an excessively high risk of perioperative mortality may not show survival benefit with CABG. Second heart failure patients must have viable myocardium and suitable targets for bypass in order to be considered for surgical revascularization. Third, it is imperative that cardiologists and surgeons work in concert when managing patients with ischemic cardiomyopathy and severely depressed LV ejection fraction. In this manner risk of surgery, as well as alternative options, can be considered in these patients.

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Role of Digoxin in Heart Failure

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Introduction

Digoxin is a cardiac glycoside isolated from the foxglove plant *Digitalis purpurea*. Its effects on the heart were first described by Withering (1785). Over the 230 years since this first description, digoxin is still used in the management of cardiovascular diseases, including heart failure. Specifically, digoxin is recommended by the American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) and the European Society of Cardiology (ESC) to decrease hospitalizations for heart failure, as multiple trials have shown its ability to improve symptoms, quality of life, and exercise tolerance (Dobbs et al., 1977; The Digitalis Investigation Group, 1997; Lee et al., 1982; The Captopril-Digoxin Multicenter Research Group, 1988; Guyatt et al., 1988; DiBianco et al., 1989; Uretsky et al., 1993; Packer et al., 1993; Yancy et al., 2013; McMurray et al., 2012). However, unlike beta-blockers and renin–angiotensin inhibitors, digoxin has not been shown to reduce mortality. This has likely been a contributing factor in the reduction of digoxin use in the management of heart failure, where it is currently reserved for patients with heart failure with reduced ejection fraction (HFrEF) who remain symptomatic despite the use of disease-modifying therapies (Patel et al., 2016; Hussain et al., 2006; Yancy et al., 2013; Goldberger and Alexander, 2014).

Mechanism of Action

Digoxin is a positive inotrope and negative chronotrope with primarily three (3) mechanisms of action—hemodynamic, neurohormonal, and electrophysiological (Eichhorn and Gheorghiade, 2002; Ziff and Kotecha, 2016).

Hemodynamic

Digoxin inhibits the membrane-bound alpha-subunit of the sodium–potassium ATPase pump in cardiac myocytes (Fig. 1; Smith, 1988). This pump is responsible for maintaining the resting membrane potential of the cell by pumping three (3) sodium ions out of the cell and two (2) potassium ions into the cell in a process that consumes energy in the form of adenosine triphosphate. With inhibition of the pump, the intracellular sodium concentration rises. Due to this rise in intracellular sodium, transmembrane sodium–calcium exchangers reach an equilibrium that favors calcium entry into myocytes and a decrease in calcium extrusion from myocytes—the net result is an increase in intracellular calcium. This rise in intracellular calcium increases the force-generating interactions between actin and myosin during myocyte contraction. The result is an increase in contractility—that is, positive inotropy—and, ultimately, an increase in cardiac output.

Neurohormonal

The body responds to heart failure with neurohormonal activation, resulting in the release of hormones such as norepinephrine and angiotensin. Digoxin inhibits the release of such hormones (Gheorghiade and Ferguson, 1991). While the exact mechanism is currently unknown, digoxin induces vagal activation that augments parasympathetic tone and reduces sympathetic outflow (Ziff and Kotecha, 2016). One possible mechanism includes the attenuation of carotid sinus baroreceptor activity by digoxin, resulting in a decrease in efferent sympathetic tone and subsequent decrease in neurohormonal activation (Schobel et al., 1991).

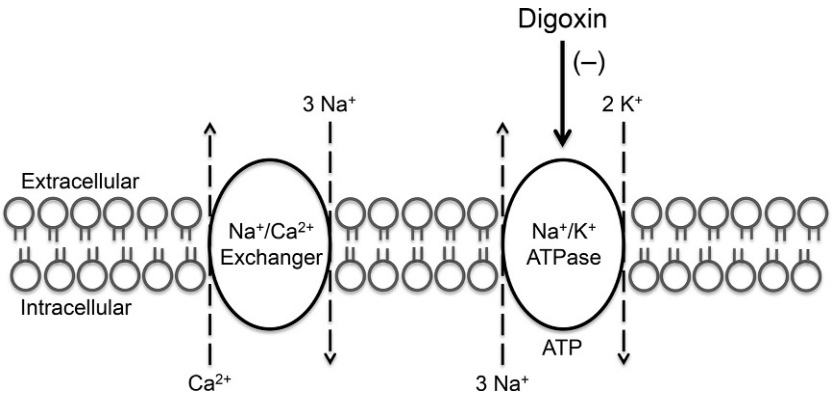

Fig. 1 Mechanism of action of digoxin. Digoxin inhibits the membrane-bound alpha-subunit of the sodium–potassium ATPase pump in cardiac myocytes. As a result, intracellular sodium concentration increases. Due to this rise in intracellular sodium, transmembrane sodium–calcium exchangers reach an equilibrium that favors calcium entry into myocytes and a decrease in calcium extrusion from myocytes—the net result is an increase in intracellular calcium. This causes an increase in myocyte contractility and, ultimately, an increase in cardiac output.

Electrophysiological

Digoxin's induction of the vagal response is associated with a parasympathomimetic effect on atrial myocardium, resulting in a slowing of conduction through the AV node and prolonging refractoriness (Smith, 1988). This ultimately results in a reduction in heart rate or a negative chronotropic effect. Unfortunately, this same mechanism contributes to the arrhythmias associated with digoxin use.

Clinical Use

The ACCF/AHA and ESC recommend the use of digoxin in patients with HFrEF in order to decrease hospitalizations for heart failure (Yancy et al., 2013; McMurray et al., 2012). These guidelines are largely based on the Digitalis Investigation Group (DIG) trial, a randomized controlled trial that suggested digoxin use reduced hospitalizations, but did not demonstrate a decrease in mortality (The Digitalis Investigation Group, 1997). These findings contribute to the use of digoxin in patients with HFrEF who remain symptomatic despite the use of therapies that have shown to reduce mortality, such as beta-blockers, ACE inhibitors, ARBs, and aldosterone receptor antagonists. As reflected by the major society guidelines above, current guidelines dictate the use of beta-blockers and renin–angiotensin inhibitors in the initial management of HFrEF. This is demonstrated in clinical practice by the recent downward trend in the use of digoxin for HFrEF (Patel et al., 2016; Ziff and Kotecha, 2016).

HFrEF With Atrial Fibrillation

Given its properties as both a positive inotrope and negative chronotrope, it seems reasonable that digoxin would be useful in patients with HFrEF and atrial fibrillation with rapid ventricular response. Unfortunately, the benefit of digoxin in this population is not clear. Although the utility of digoxin has been cited in literature, evidence suggesting that it may lead to increased mortality in heart failure patients with atrial fibrillation also exists (Khand et al., 2003; Shah et al., 2014; Vamos et al., 2015). As such, current ACCF/AHA guidelines recommend beta-blockers for rate control in heart failure patients with atrial fibrillation, but acknowledge that digoxin may have a role as an adjunct therapy (Yancy et al., 2013).

Women

A post hoc analysis of data from the DIG trial suggested that digoxin use among women with HFrEF was associated with an increase in mortality (Rathore et al., 2002). A subsequent study, again using data from the DIG trial, evaluated whether serum digoxin levels impacted outcomes in women with heart failure. This study found that low serum concentrations of digoxin were associated with a reduction in hospitalization and no effect on mortality, while higher serum concentrations were associated with an increase in mortality (Adams et al., 2005).

Right Heart Failure

While data are limited, digoxin may have a role in the management of right heart failure secondary to primary pulmonary hypertension (PPH). A prospective study of PPH patients with isolated right ventricular dysfunction showed that short-term administration of digoxin resulted in a modest increase in cardiac output and a significant reduction in plasma norepinephrine (Rich et al., 1998).

Dosing

Digoxin has a narrow therapeutic window. Because higher concentrations—that is, greater than 1.2 ng/mL—are associated with an increase in mortality, the optimal serum digoxin concentration for both men and women with HFrEF appears to be 0.5–0.9 ng/mL, although there have been no prospective, randomized trials to evaluate this (Rathore et al., 2002; Adams et al., 2005). The ACCF/AHA currently recommends daily doses of 0.125–0.25 mg with no loading doses. The lower dose of 0.125 mg is favored in those that are older than 70 years, with renal impairment, or with low body mass, as serum concentrations may rise more in proportion to the dosage (Yancy et al., 2013).

Adverse Effects

The most common side effects associated with digoxin involve the gastrointestinal, cardiovascular, and central nervous systems. In particular, digoxin may cause nausea, vomiting, anorexia, blurred vision, and cardiac arrhythmias. While it is reasonable to simply discontinue digoxin to ameliorate side effects, severe toxicity should be treated with F(ab) fragments of anti-digoxin antibodies (Kelly and Smith, 1992).

In addition to side effects and toxicities, clinical deterioration is seen with the withdrawal of digoxin therapy (The Captopril-Digoxin Multicenter Research Group, 1988; The Digitalis Investigation Group, 1997; Adams et al., 2016). While neurohormonal antagonists such as beta-blockers and renin–angiotensin inhibitors are the preferred treatment for HFrEF, these medications should be added to digoxin monotherapy (Yancy et al., 2013). That is, a patient with HFrEF on digoxin monotherapy should not have this therapy discontinued in favor of starting neurohormonal antagonists—the latter should be added on.

Summary

- While neurohormonal antagonists are preferred for their disease-modifying properties, digoxin is recommended by major societies to decrease hospitalizations for HFrEF.
- Digoxin has hemodynamic, neurohormonal, and electrophysiological mechanisms of action.
- Evidence is unclear on the role of digoxin in HFrEF with atrial fibrillation.
- There was previous concern for an increase in mortality with digoxin use among women with HFrEF; however, more recent evidence suggests that lower serum drug concentrations have no effect on mortality.
- Lower serum digoxin concentrations—that is, 0.5–0.9 ng/mL—are recommended.
- Withdrawal of digoxin therapy has been associated with clinical deterioration.

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Role of Echocardiography in Selection, Implantation, and Management of Left Ventricular Assist Device Therapy

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Introduction

Life-saving circulatory support by ventricular assist devices is an established treatment for end-stage heart failure (HF) and especially left ventricular assist devices (LVADs) have emerged as a valid therapeutic option primarily designed as a bridge to heart transplant (HTx), bridge to transplant candidacy, or as destination therapy for patients who cannot undergo HTx. In a limited number of patents LVADs may also become a bridge to cardiac recovery allowing successful device explantation.

Larger studies and recent guidelines emphasize the important role of echocardiography in LVAD candidate selection including patient screening for cardiac abnormalities with negative impact on LVAD therapy, planning of implant surgery, guiding intraoperative device placement, pump optimization and monitoring of RV function, postoperative surveillance of LVAD and RV function, as well as evaluation of native myocardial recovery with corresponding adjustment of LV unloading, selection of potential weaning candidates, and decision-making in favor of LVAD explantation.

The chapter reviews the knowledge about the role of echocardiography for managing LVAD therapy. Special attention is focused on echo assessment of right ventricular (RV) function before LVAD implantation and the reliability of echo parameters for preoperative prediction of postoperative RV function, as well as on the particular role of echocardiography for weaning decisions in patients showing unloading-promoted LV reverse remodeling with improvement of cardiac function.

Preoperative Role of Echocardiography

In addition to documenting baseline cardiac structure and function, preoperative transthoracic and transesophageal echocardiography (TTE and TEE) are indispensable for LVAD candidate selection and their screening for cardiac abnormalities with negative impact on LVAD therapy. Echocardiography is also necessary for RV assessment and decision-making on the necessity of a mechanical support also for the RV.

Candidate Selection for LVAD Therapy

Proper candidate selection is paramount for successful LVAD therapy but also challenging because congestive HF due to primary impaired LV function is a complex syndrome with many specific risk factors for adverse outcome after LVAD implantation (Cohen et al., 2015; Feldmann et al., 2013; Lund et al., 2010; Stainback et al., 2015). The criteria for the use of LVAD therapy are therefore based on a wide variety of clinical, hemodynamic, laboratory, and cardiac-imaging data. An important part of information necessary for LVAD candidate selection is provided by TTE and TEE.

Evaluation of LV dysfunction and screening for cardiac abnormalities with negative impact on LVAD therapy

Echocardiography is mandatory for evaluation of LV dysfunction in LVAD candidates and also the first-line imaging modality used to screen candidates for cardiac and aortic abnormalities that increase the risk for complications, or may represent contraindications to LVAD implantation (Stainback et al., 2015).

Although LV ejection fraction (LVEF) is by no means the only parameter used for decision-making to implant an LVAD, accurate LVEF measurement is still necessary. It is recommended to use three-dimensional (3D) echocardiography for LVEF measurements (Stainback et al., 2015). The use of a microbubble contrast agent to enhance endocardial definition can improve LVEF measurement (Stainback et al., 2015).

Comprehensive preoperative TTE and/or TEE is particularly useful for the diagnosis of possible intra-cardiac shunting and thrombi, valvular abnormalities, endocarditis, and diseases of the aorta, because their detection before implantation surgery can be decisive for the planned LVAD therapy. Thus, because patent foramen ovale (PFO) and other interatrial communications should be closed at the time of LVAD implantation, attention must be focused on signs which suggest their existence. For detection of intra-cardiac shunting, TEE-color-Doppler scanning of the fossa ovalis margins at low Nyquist-limit plus intravenous agitated saline combined with an appropriately performed Valsalva maneuver are necessary because elevated atrial pressure may reduce the inter-atrial pressure gradient and prevent detection of the defects by color-Doppler imaging alone (Feldmann et al., 2013; Stainback et al., 2015). Congenital or post-infarction septum defects (VSDs) must also be excluded by color-Doppler interrogation because VSDs should be closed during LVAD implantation in order to prevent right-to-left shunting with consecutive hypoxemia and risk of paradoxical embolization during LVAD support (Cohen et al., 2015; Patangi et al., 2013; Rasalingam et al., 2011). In patients with risk for LV thrombus formation (decreased LVEF, LV aneurysm), the use of a micro-bubble contrast agent should also be considered (Cohen et al., 2015; Stainback et al., 2015). Exclusion of relevant aortic valve insufficiency (AI) and mitral stenosis (MS) before LVAD implantation is also necessary. Aortic regurgitation (AR) and MS of $\geq$ moderate degree can importantly impair LVAD therapy and must therefore be considered for correction during LVAD implantation surgery (Cohen et al., 2015; Feldmann et al., 2013; Stainback et al., 2015). However, AR quantification in LVAD candidates is challenging due to the reduced stroke volume (SV) and the low AR velocities (low pressure gradients between the aorta and the LV because of the high LV diastolic pressure and the low diastolic pressure in the aorta). Therefore, the AR volume can also be relatively low despite a high regurgitant fraction and the degree of AI can be underestimated. Accordingly, both Doppler-derived LV outflow-tract (LVOT) SV and regurgitant fraction should be calculated routinely in LVAD candidates (Stainback et al., 2015). Careful search for relevant AI is particularly necessary in patients with aortic root dilatation, aortic valve (AV) degeneration, eccentric AR (especially if associated with a bicommissural AV), or an AV prosthesis (Stainback et al., 2015). Severe AV stenosis (AS) can be tolerated, but patients may have insufficient forward flow in the event of obstructive LVAD failure, even if residual LV function is present (Stainback et al., 2015). In patients with a bio-prosthetic AV, AR of $\geq$ moderate degree requires bio-prosthesis replacement. Also relevant stenoses of prosthetic mitral valves require replacement with a bio-prosthesis at LVAD implantation (Stainback et al., 2015). TTE has important limitations for the assessment of prosthetic valves and therefore, adjunctive TEE is necessary. TR of $>$moderate degree is a potentially ominous finding and recent guidelines recommend that surgical valve repair should also be considered at the time of LVAD implantation (Stainback et al., 2015). Moderate pulmonary regurgitation (PR) may be well tolerated by LVAD recipients with optimal LV unloading. However, PR of $\geq$ moderate degree would require repair in the event of additional RVAD implantation (Feldmann et al., 2013; Stainback et al., 2015). More than mild pulmonary valve stenosis increases the risk for RVF after LVAD implantation (Cohen et al., 2015; Stainback et al., 2015).

Exclusion of endocarditis (contraindication for LVAD implantation) is mandatory. Diseases of the aorta (aneurisma, atheroma, coarctation, dissection) can complicate or might be even contraindications for LVAD implantation and their preoperative detection is therefore necessary (Feldmann et al., 2013; Stainback et al., 2015).

Selection of a particular LVAD for an individual patient is a complex decision-making process where echocardiography is helpful to confirm the etiology and severity of HF and especially to assess the potential reversibility of HF, thus facilitating also the choice between temporary and long-term LVAD support (Saffarzadeh and Bonde, 2015; Stainback et al., 2015).

Echocardiography is also necessary before the decision to use a percutaneous temporary LVAD like Impella or TandemHeart system. Thus, detection of LV thrombi, AS or AI of $\geq$ moderate degree, and aortic abnormalities are contraindications for the use of an Impella system, whereas detection of a $\geq$ moderate AS or a VSD is contraindication for the use of a TandemHeart system (Saffarzadeh and Bonde, 2015).

Decision on the necessity of mechanical support also for the RV

LVADs are more reliable and safer for patients and also provide a better quality of life than biventricular assist devices (BiVADs), but end-stage HF often involves both ventricles, even if its initial cause was left-sided heart disease (Dandel et al., 2015a; Dang et al., 2006; Matthews et al., 2008; Ochiai et al., 2002). Although LVADs can facilitate RV improvement, about 25% of LVAD recipients develop severe RV failure (RVF) associated with increased patient morbidity and mortality even after later implantation of a mechanical support also for the RV (Dandel et al., 2015a; Dang et al., 2006; Matthews et al., 2008; Ochiai et al., 2002). A temporary RVAD in long-term LVAD recipients with high risk for postoperative RVF can avoid permanent BiVAD support. Thus, patients who need a temporary or long-term BiVAD support should already be identified preoperatively or at the latest intra-operatively.

RVF after LVAD implantation appeared related to preoperative RV geometry, contractile function, and TR which are highly load sensitive parameters (Dandel et al., 2013, 2015a; Dandel and Hetzer, 2016; Kukucka et al., 2011a; Potapov et al., 2008). Due to its load dependency, the RV function usually either remains unchanged or will even improve after reduction of pulmonary vascular

resistance (PVR) subsequent to LVAD implantation, as long as the postoperative outcome is not impaired by irreversibly poor end-organ function and LV unloading is properly optimized to avoid excessive septum shifting from right to left (Dandel and Hetzer, 2015a, 2016; Dandel et al., 2013; John and Boyle, 2012; Kukucka et al., 2011a; Potapov et al., 2008).

RVF after LVAD implantation can be classified into three major types:

a. RVF due to preoperatively underestimated irreversible RV alterations (preoperatively often predictable)
b. RVF which becomes manifest only intra-operatively or early postoperatively (less predictable)
c. RVF which develops late after LVAD implant (preoperatively less predictable).

Preoperative detection of risk factors for post-LVAD RV failure

Echocardiography is indispensable for preoperative distinction between patients with and without the potential to remain free from RVF after LVAD implantation, because patients with different post-LVAD course of RV function reveal already preoperatively significant differences in echo-derived right-sided heart morphological and functional parameters (Aissaoui et al., 2015; Atluri et al., 2013; Drakos et al., 2010; Fitzpatrick et al., 2008; Fukamachi et al., 1999; Kato et al., 2013; Vivo et al., 2013). Thus, those with postoperative RVF show before LVAD implantation, significantly larger RV end-diastolic diameters (RVEDD) with higher short/long (S/L)-axis ratios, higher RV/LV diameter ratios, lower RV fractional area change (FAC_{RV}) and EF, lower tricuspid lateral annulus peak systolic amplitude and velocity of displacement (TAPSE and TAPS′, respectively), lower RV free wall peak systolic longitudinal strain and strain rate (PSSL and PSSrL, respectively), higher degree of TR and higher systolic pressure gradients between the RV and right atrium ($\Delta P_{RV\text{-}RA}$) (Aissaoui et al., 2015; Atluri et al., 2013; Dandel et al., 2013; Dang et al., 2006; Drakos et al., 2010; Fitzpatrick et al., 2008; Fukamachi et al., 1999; Grant et al., 2012; Kato et al., 2013; Kavarana et al., 2002; Kukucka et al., 2011a,b; Matthews et al., 2008; Porter et al., 2015; Potapov et al., 2008; Raina et al., 2013; Topilsky et al., 2011a; Vivo et al., 2013). Several preoperative echo-derived measures like RVEDD >55 mm, RV end-diastolic S/L axis ratio >0.57, FAC_{RV} <31%, TAPS′ (also known as S′) <8 cm s^{-1}, PSSL <−9.6%, PSSrL <0.6 s^{-1}, TR >2nd degree, $\Delta P_{RV\text{-}RA}$ <35 mmHg, and RV/LV diameter ratio ≥0.75 were revealed as significant risk factors for RVF after LVAD implantation (**Table 1**) (Aissaoui et al., 2015; Atluri et al., 2013; Dandel et al., 2013; Grant et al., 2012; Kato et al., 2013; Kukucka et al., 2011b; Matthews et al., 2008; Potapov et al., 2008; Raina et al., 2013; Topilsky et al., 2011a; Vivo et al., 2013). However, these variables were not identified in all studies as significant risk factors for RVF (Grant et al., 2012; Raina et al., 2013; Karogelopoulos et al., 2016; Kiernam et al., 2015; Lang et al., 2015; Puwanant et al., 2008; Shiga et al., 2012). This might be mainly due to differences in defining RVF and selection criteria for VAD support, but probably also due to certain limitations of echocardiography for RV assessment (Dandel et al., 2015a; Lang et al., 2015).

Preoperative prediction of post-LVAD RV function

Proper decision-making in favor or against the necessity for implantation of a mechanical support also for the RV is paramount for a successful VAD therapy in patients with congestive HF. However, with few exceptions, the numerous preoperative risk factors for post-LVAD RVF, which were identified by echocardiography, appeared alone unable to predict the necessity of mechanical support also for the RV (Dandel et al., 2013; Neyer et al., 2016; Raina et al., 2013). As shown in **Tables 2** and **3**, at certain cut-off values, several echo-derived parameters can predict freedom from post-LVAD RVF, but only the global PSSrL appeared able to predict also the development of RVF after LVAD implantation (Dandel et al., 2013).

There are particular challenges and limits in RV echo assessment with important impact on the diagnostic and prognostic value of echocardiography (**Table 4**) (Anavekar et al., 2007; Dandel and Hetzer, 2009; Focardi et al., 2015; Kurtz, 2012; Rudski et al., 2010). However, due to the distinct load dependency of RV size, geometry, and function, the main limitation for the clinical value of individual echo-derived RV measurements is their possible alteration already before any alteration in myocardial contractility. This might explain the relative low predictive value of RV echo measurements, which otherwise proved to be relevant risk factors for RVF after LVAD implantation. Especially RVEF, $FAC_{RV,}$ and TAPSE, but also RV systolic wall motion and myocardial deformation velocity, can decrease with increasing PVR even if myocardial contractility remains unaltered. Also TR can induce RVEF, $FAC_{RV,}$ and TAPSE changes, which can be misleading for assessment of RV contractile function (Dandel and Hetzer, 2016; Dandel et al., 2015b). Thus, without the interpretation of parameter values in relation to PVR, PAP, or $\Delta P_{RV\text{-}RA}$, echo assessment of RV function may become unreliable and this could explain its limited ability to predict preoperatively the presence of severe RVF after LVAD implantation (Dandel and Hetzer, 2016; Dandel et al., 2013).

Integrative approaches using combinations of parameters, which include RV afterload, appeared more reliable for evaluation of RV function (Cameli et al., 2012; Dandel et al., 2013, 2015a,b; Di Maria et al., 2015; Frea et al., 2012; Sharma et al., 2005). Thus, the RV stroke work index (SWI_{RV}) obtained from right heart catheterization (RHC) measurements appeared more useful than the currently used standard echo variables for evaluation of RV function in end-stage congestive HF (Cameli et al., 2012). However, because estimation of RA and mean PAP by echocardiography are challenging, echo-derived SWI_{RV} is rarely used for assessment of RV function. Recently, two simplified composite echo-derived variables were identified as easy calculable surrogates for the SWI_{RV} (Di Maria et al., 2015; Frea et al., 2012). One of them, the "simplified RV contraction-pressure index (sRVCPI)," incorporates TAPSE and load, and is derived as $sRVCPI = TAPSE \times \Delta P_{RV-RA}$, where ΔP_{RV-RA} is the pressure gradient between RV and RA (Frea et al., 2012). sRVCPI showed a close correlation with the RHC-derived SWI_{RV} plus a high sensitivity and specificity to predict depressed SWI_{RV} (Frea et al., 2012). The other echo-derived surrogate for the RHC-derived $SWI_{RV,}$ the "RV stroke work (RVSW)," incorporates SV and load and is derived as $RVSW = 4 \times (\text{peak TR jet velocity})^2 \times (\text{pulmonary valve area} \times \text{VTI})$, where VTI is the velocity-time integral of the systolic trans-pulmonary jet and

Table 1 Relevance of the main echocardiography-derived variables generally recognized as preoperative risk factors for RV failure after LVAD implantation

Statistical method	*Selected echocardiographic variables*	*Odds RVF*	*95% CI*	P *value*
Univariate logistic regression	RV end-diastolic diameter (RVEDD) (Dandel et al., 2013; Kato et al., 2013)	1.25	1.13–1.38	<0.01
		1.05	0.93–1.21	0.44
	RV end-diastolic short-/long-axis ratio (Potapov et al., 2008)	4.4	1.4–13.7	0.01
	RV/LV end-diastolic diameter ratio (Grant et al., 2012)	2.75	0.24–33.8	0.42
	RV ejection fraction (RVEF) (Dandel et al., 2013; Grant et al., 2012)	0.87	0.80–0.95	<0.01
		1.01	0.93–1.11	0.75
	RV fractional area change (FAC_{RV}) (Grant et al., 2012; Kato et al., 2013)	0.94	0.88 –1.00	0.53
		0.96	0.92–1.01	0.09
	Tricuspid annulus peak systolic excursion (TAPSE) (Dandel et al., 2013; Grant et al., 2012)	0.98	0.82–1.00	<0.01
		0.26	0.06–1.05	0.06
	Tricuspid annulus peak systolic velocity (S′) (Kato et al., 2013)	0.22	0.09–0.46	<0.01
	Lateral RV peak longitudinal strain (Grant et al., 2012; Kato et al., 2013)	2.26	1.10–1.48	<0.01
		0.86	0.76–0.96	<0.01
	RV myocardial performance index (MPI_{RV}) (Topilsky et al., 2011a)	0.63	0.44–0.85	<0.01
	Peak early diastolic velocity of the lateral tricuspid annulus (E′) (Kato et al., 2013)	0.11	0.03–0.31	<0.01
	RV peak early filling velocity per free wall diastolic velocity (E/E′) (Kato et al., 2013)	1.34	1.12–1.81	<0.01
	RV/LV end-diastolic diameter ratio (RVEDD/LVEDD) >0.72 (Kukucka et al., 2011b)	11.4	3.0–41.6	<0.01
	Left ventricle end-diastolic diameter (LVEDD) (Shiga et al., 2012)	0.91	0.84–0.98	0.02
	LVEDD ≤62 mm (Shiga et al., 2012)	12.6	1.38–115.4	0.02
	Tricuspid regurgitation (TR) severity ≥ grade III (Potapov et al., 2008)	4.70	1.26–17.6	0.01
	> grade II (Dandel et al., 2013)	2.40	1.13–5.13	<0.01
	≥ grade II (Grant et al., 2012)	1.04	0.69–1.56	0.84
	TR flow time corrected for heart rate (TRDc) (ms) (Topilsky et al., 2011a)	0.83	0.74–0.91	<0.01
	RV global peak longitudinal strain (%) (Kato et al., 2013)	1.26	1.10–1.48	<0.01
Multivariate analyses	RVEDD >55 mm (Aissaoui et al., 2015)	1.97	1.2–3.2	<0.01
	Lateral RV peak longitudinal strain (%) <−9% (Grant et al., 2012)	0.84	0.75–0.92	<0.01
	Left ventricle end-diastolic diameter (LVEDD) ≤62 mm (Shiga et al., 2012)	12.8	1.39–118.4	0.03
	MPI_{RV} (Topilsky et al., 2011a)	0.64	0.40–0.93	0.02
	RV/LV end-diastolic diameter ratio (Vivo et al., 2013)	5.5	2.4–12.4	0.01
	Left atrium (LA) volume index (Raina et al., 2013)	0.77	0.63–0.95	0.02
	TTE echo-score (FAC_{RV} + LA volume index + estimated PAP) (Raina et al., 2013)	1.66	1.06–2.62	<0.01
	Severe TR (Atluri et al., 2013)	4.1	1.4–12.4	0.01
	TR flow time corrected for heart rate (TRDc) (ms) (Topilsky et al., 2011a)	0.82	0.69–0.93	<0.01
	RV end-diastolic volume index[a] (RVEDVI) (Kiernam et al., 2015)	1.16	1.00–1.33	0.04
	RV end-systolic volume index[a] (RVESVI) (Kiernam et al., 2015)	1.14	1.01–1.28	0.03

[a]Measured by 3-dimensional echocardiography; RV, right ventricle; RVF, right ventricular failure.

showed also a close correlation with the RHC-derived SWI_{RV} (Di Maria et al., 2015). However, the potential usefulness of these two echo-derived composite variables for RV assessment in LVAD candidates remained to be established.

Also recently, composite echo variables which incorporate either longitudinal displacement and load (i.e., TAPSE/systolic PAP and TAPSE/PVR) or velocity of myocardial shortening and load (i.e., "afterload corrected PSSrL") were found suited for assessment of RV contractile function (Dandel et al., 2013, 2015b; Guazzi et al., 2013; Lopez-Candales et al., 2014). The TAPSE/systolic PAP ratio is a simplified approach to assess RV contraction by plotting fiber longitudinal shortening vs. the force generated for overcoming the imposed load (Guazzi et al., 2013). Its limitations are related to the potential limitations of TAPSE and systolic PAP echo measurements. Nevertheless, this index appeared to be a strong and independent predictor of mortality in patients with HF due to primary impaired LV function (Topilsky et al., 2011a). Because PVR is a critical determinant of RV systolic function, its inclusion into echo-derived composite variables can also be beneficial for assessment of the relationship between increased afterload and RV function (Lopez-Candales et al., 2014). The echo-derived "RV ejection efficiency (RVEe)," defined as RVEe = TAPSE/PVR, is such a composite variable which reflects that relationship in a simplified manner (Lopez-Candales et al., 2014). Using TAPSE as a surrogate for RV ejection and echo-derived PVR (PVR = TR peak velocity/RV outflow tract velocity-time integral) as a surrogate for the RHC-derived PVR, the echo-derived RVEe is easily calculable and might be feasible for evaluation of RV systolic performance (Abbas et al., 2013; Lopez-Candales et al., 2014). However, future studies are necessary to determine if echo-derived RVEe might be able to predict post-LVAD RV function.

Two composite echo-derived variables for evaluation of RV function had already confirmed the assumption that the predictive value of certain echo-derived RV parameters for post-LVAD RVF can be improved if RV loading conditions are also considered (Dandel et al., 2013). One of them is the right ventricular "afterload-corrected PSSrL," which is based on the relationship between

Table 2 Predictive value of preoperatively measured most relevant echocardiographic parameters and indexes for postoperative RV failure in patients who underwent LVAD implantation

		Prediction of RV failure			
Parameters and indexes	*Cut-off*[a]	*Sensitivity % (CI)*[b]	*Specificity % (CI)*[b]	*NPV*[c] *% (CI)*[b]	*PPV*[d] *% (CI)*[b]
RV-S/L_{ED} (Dandel et al., 2013)	0.57	84 (72–93)	74 (70–76)	94 (90–94)	46 (40–52)
LA volume index (Raina et al., 2013)	38 mL/m^2	86	56	90	52
$\Delta P_{RV\text{-}RA}$ (Dandel et al., 2013)	35 mmHg	84 (73–92)	93 (89–95)	96 (92–98)	76 (66–83)
TAPS′ (Dandel et al., 2013)	8 cm s^{-1}	84 (73–92)	90 (93–98)	95 (92–98)	70 (61–77)
RV lateral PSSL (Grant et al., 2012)	−9.6%	68	76	76	68
RV global PSSL (Kato et al., 2013)	−14.4%	83	66	92	51
RV global PSSrL (Dandel et al., 2013)	0.6 s^{-1}	80 (70–85)	98 (95–99)	92 (81–98)	95 (92–96)

RV, right ventricle; *S/L_{ED}*, end-diastolic short-/long-axis ratio; *LA*, left atrium; *$\Delta P_{RV\text{-}RA}$*, systolic pressure gradient between RV and right atrium; *TAPS′*, tricuspid annulus peak systolic velocity; *PSSL*, peak systolic longitudinal strain; *PSSrL*, peal systolic longitudinal strain rate.
[a]Optimal cut-off derived from ROC analysis.
[b]95% confidence interval.
[c]Negative predictive value = prediction for freedom from RV failure.
[d]Positive predictive value = prediction for development of RV failure.

Table 3 Overview of the risk discrimination capacity for right ventricular failure (RVF) of echo-derived composite variables which incorporate major risk factors for RV failure after left ventricular assist device (LVAD) implantation

				RVF prediction (%)			
						Predictive value %	
Variables (units)	*Components of the combined variables*	*AUC (95% CI)*	*Cut-off values*	*Sensitivity %*	*Specificity %*	*Positive*	*Negative*
RV load-corrected PSSrL (Dandel et al., 2013) PSSrL · $\nabla P_{RV\text{-}RA}$ (mmHg/s)	Peak systolic global longitudinal strain rate (PSSrL), pressure gradient between RV and RA ($\nabla P_{RV\text{-}RA}$)	0.98					
(0.97–0.99)	24	89 (79–95)		96 (93–98)	97 (84–99)	87 (77–93)	
Load adaptation index (LAI) (Dandel et al., 2013)	TR velocity time integral; RV end-diastolic area and long-axis length	0.97					
(0.97–0.99)	14	91 (80–97)		95 (92–97)	83 (73–88)	97 (95–99)	
RV echocardiographic score (Shiga et al., 2012)	RV peak systolic global longitudinal strain (PSSL), TR grade, LV systolic diameter	0.88					
(0.75–1.00)	–	87 (60–98)		87 (66–97)	81 (54–96)	91 (71–99)	
RV E/E′ (Aissaoui et al., 2015)	Peak early tricuspid filling velocity (E) RV free wall peak early diastolic velocity at the tricuspid annulus (E′)	0.72	10.2	83.3	56.8	–	–
TTE score [points (p)] (Raina et al., 2013)	FAC_{RV} >31%; RAP (estimated) >8 mmHg; LA volume index <38 mL/m^2	0.73	≥5	63	78	75	64
ARVADE score [points (p)] (Aissaoui et al., 2015)	LV E/Em ≥18.5; RVEDD ≥50 mm; INTERMACS level	0.87	>3	89	74	–	–

AUC, area under the curve; *RV*, right ventricle; *RVF*, right ventricular failure; *RA*, right atrium; *TR*, tricuspid regurgitation; *RAP*, right atrial pressure; *FAC_{RV}*, RV fractional area change; *LV*, left ventricle; *LA*, left atrium; *RVEDD*, right ventricular end-diastolic diameter.

Table 4 Major challenges and limits in echo assessment in RV assessment (Anavekar et al., 2007; Dandel et al., 2015a; Dandel and Hetzer, 2016; Dandel et al., 2013; Dandel and Hetzer, 2009; Estep et al., 2014a; Feldmann et al., 2013; Focardi et al., 2015; Kassi and Estep, 2016; Kurtz, 2012; Lam et al., 2009; Rich et al., in press; Rudski et al., 2010; Stainback et al., 2015; Cohen et al., 2015; Abbas et al., 2013)

Echo measurements	*Challenges*	*Limitations*
RV volumes and EF	Complex three-dimensional RV shape Limited definition of RV endocardial surface and restricted echo-imaging window due to RV retrosternal position. Dedicated views of the RV can be insufficient for reliable assessment. Measurements require high spatial resolution	2D-echo-derived EF calculation requires geometric assumptions Only 3D-echo-derived measurements are recommended for clinical use Afterload dependency Misleading RVEF changes inducible by TR
RV fractional area-change (FAC_{RV})	Suboptimal RV endocardial definition (especially in systole) Measurements require high spatial resolution	High inter- and intra-observer variability Load dependency Misleading changes inducible by TR.
Tricuspid annulus peak systolic excursion (TAPSE)	Angle dependence of measurements	Describes displacement of single wall segment Poor correlation with MRI-derived RVEF Load dependency of measurements Influenced by overall heart motion and LV function Misleading changes inducible by TR.
Tricuspid lateral annulus peak systolic velocity S′ (TAPS′)	Angle dependence of measurements	Load dependent (less than EF, FAC or TAPSE) Blindness to systolic dysfunction in parts of the RV remote from the tricuspid annulus
TR-derived RV dp/dt	Requires a complete TR spectral Doppler envelope and parallel alignment with the TR jet	Angle and load dependency of measurements Lack of sufficiently validated normative data
Isovolumic contraction peak velocity (IVC_V) and Isovolumic acceleration (IVA)	Angle dependence of measurements	Negative impact of TR Measurements are moderately load-dependent and also dependent on patient age and heart rate RV wall motion during its isovolumic contraction can be affected by the simultaneous LV contraction Lack of recommendable reference values
RV longitudinal strain and strain rate	TDI-derived measurements are angle dependent; acquisition is technically challenging because the thin RV free wall yields a poor signal-to-noise ratio High image quality is required	Relatively low temporal resolution and segmental reproducibility of measurements (especially the 3D-speckle-tracking approach) Influence of RV loading conditions especially on strain values.
Doppler-derived parameters of RV diastolic function (E,A, E/A, E/E′)	Respiration can induce misleading diastolic flow changes	TR, heart rate, and preload changes can induce misleading diastolic parameter changes
RV myocardial performance index (MPI_{RV})	Normal values differ on the basis of whether pulsed or tissue Doppler was chosen for its calculation Pulsed Doppler data necessary for its calculation cannot be obtained from one cardiac cycle	Possible pseudo-normalization in severe RVF RV hypertrophy can shorten the IVRT leading to misleading reduction of the MPI_{RV} Afterload dependence Alteration of its values by severe TR Unreliable when RA pressure is elevated Not recommend by the ASE as the sole quantitative method for evaluation of RV function

RV, right ventricle; *EF*, ejection fraction; *2D*, two dimensional; *3D*, three dimensional; *TR*, tricuspid regurgitation; *LV*, left ventricle; *RVF*, RV failure; *TDI*, tissue Doppler imaging; *RA*, right atrium; *E*, early diastolic trans-tricuspid flow velocity; *A*, late diastolic trans-tricuspid flow velocity; *E′*, lateral tricuspid annulus early diastolic peak velocity; *IVRT*, isovolumic relaxation time; *ASE*, American Society of Echocardiography.

RV myocardial shortening velocity and RV load, the other is the "load adaptation index (LAI_{RV})," based on the relationship between RV load and RV dilation, also taking the RA pressure into account. These easily obtainable variables showed high predictive values for both, post-LVAD RVF and freedom from RVF, during LVAD support (**Tables 2** and **3**) (Dandel et al., 2013, 2015a). Both variables appeared also predictive for RVF developing during the next 12 months in patients with end-stage precapillary pulmonary hypertension, which confirmed their usefulness for assessment of RV function and its responsiveness to loading conditions (Dandel et al., 2015b).

The "afterload-corrected PSSrL" is defined as $PSSrL \times \Delta P_{RV\text{-}RA}$ (**Fig. 1A** and **D**) (Dandel et al., 2013, 2015a,b). Because myocardial shortening velocity is load dependent, the PSSrL will decrease with the increase in RV systolic pressure. Nevertheless, as long as RV contractile function remains stable, due to the $\Delta P_{RV\text{-}RA}$ increase, the RV load-corrected PSSrL will remain relatively stable. However, if the afterload increase overwhelms RV ability to adapt its contractile function correspondingly (afterload mismatch), in addition to PSSrL reduction, there will be also a reduction in $\Delta P_{RV\text{-}RA}$ (due to increase of RA pressure), even before the RV systolic pressure

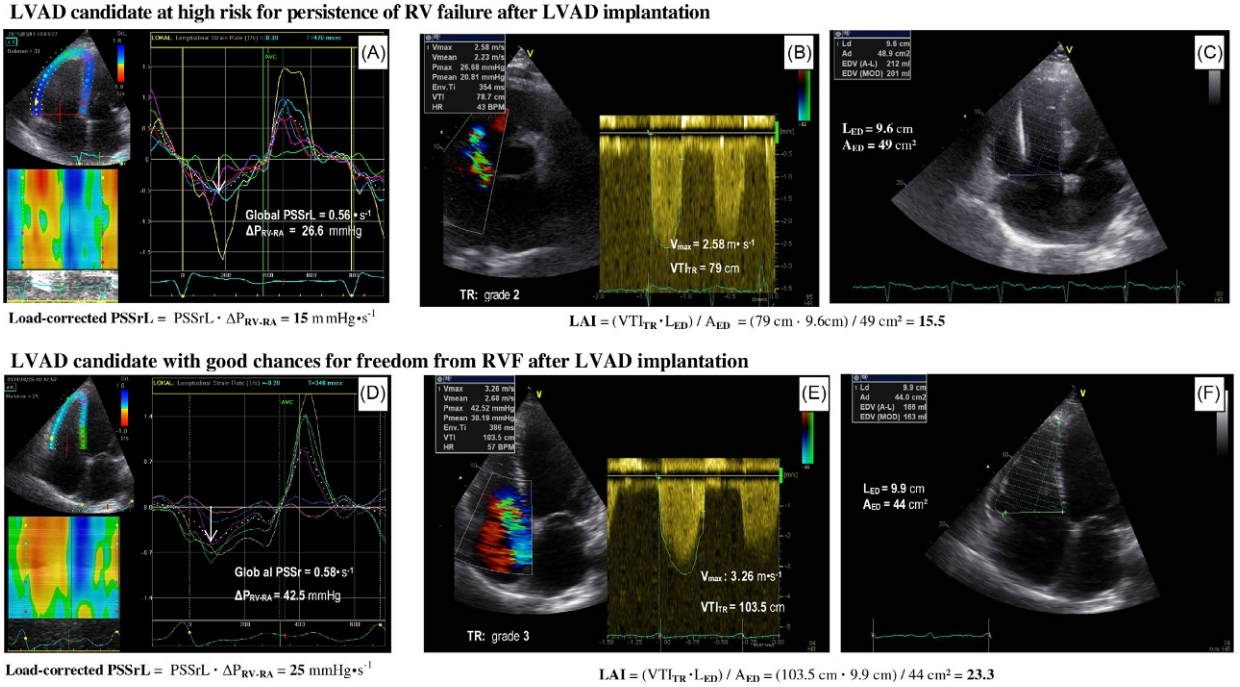

Fig. 1 Right ventricular (RV) load-corrected global peak systolic longitudinal strain rate (PSSrL) and load adaptation index (LAI) in two patients with end-stage congestive heart failure due to primary impaired left ventricular (LV) function. The low LAI and load-corrected PSSrL in the first patient (images A, B, and C) indicate a high risk for persistence of RV failure after LVAD implantation (possible need for temporary or even permanent mechanical support also for the RV). In the second patient (images D, E, and F), the lesser RV dilation (smaller end-diastolic area and less altered geometry) and the higher velocity of RV myocardial shortening (higher global PSSrL), despite higher afterload, yield a 50% and 66% higher LAI and load-corrected PSSrL, respectively, which indicates a high potential for recovery during LVAD support. *TR*, tricuspid regurgitation; VTI_{TR}, TR velocity-time integral; V_{max}, maximum (peak) velocity; L_{ED}, end-diastolic long-axis length; A_{ED}, RV end-diastolic area; $\Delta P_{RV\text{-}RA}$, pressure gradient between RV and right atrium.

finally also becomes lower due to progressive impairment of RV contractility. The load-corrected PSSrL can be therefore more useful for evaluation of RV adaptability to loading conditions than the PSSrL alone.

A distinctly different approach for assessment of RV ability to improve after reduction of its afterload is provided by the LAI_{RV} which is reflected by the ratio between $\Delta P_{RV\text{-}RA}$ and RV end-diastolic volume per long-axis length (EDV/L_{ED}):

$$LAI_{RV} = \frac{\Delta P_{RV-RA}}{EDA/L_{ED}} \approx \frac{VTI_{TR}}{A_{ED}/L_{ED}} = \frac{VTI_{TR}(cm) \times L_{ED}(cm)}{A_{ED}(cm^2)}$$

Thus, using TR velocity-time integral (VTI_{TR}) instead of $\Delta P_{RV\text{-}RA}$ and the easily measurable RV end-diastolic area (A_{ED}) instead of the RV end-diastolic volume (EDV) a dimensionless index of similar value for RV evaluation can be obtained (**Fig. 1**) (Dandel et al., 2013, 2015a,b). Because $\Delta P_{RV\text{-}RA}$ is calculated from the mean velocity of the TR jet, the use of VTI_{TR} instead of $\Delta P_{RV\text{-}RA}$ is unrestricted possible and has the advantage to include also the duration systolic loading. Because the regurgitant jet velocity recorded by continuous wave Doppler is used for the calculation of $\Delta P_{RV\text{-}RA}$ by means of the Bernoulli equation, the replacement of $\Delta P_{RV\text{-}RA}$ by the VTI_{TR} is possible without any restriction and has the advantage to also include the duration systolic loading. The use of A_{ED} instead of EDV is justified by the good correlation between the echo-derived RV-A_{ED} and the MRI-derived RV-EDV (Shiran et al., 2014). Thus, a small RV area relative to long-axis length (size and geometry unaltered) in a patient with high VTI_{TR} (i.e., high RV systolic pressure and relatively low RA pressure) yields a high LAI_{RV} which indicates good RV adaptation to load (ability to increase systolic pressure without dilation and also without RA pressure increase) suggesting a good RV contractile function and the potential of RV to improve its performance after reduction of loading conditions. A large area relative to long-axis length (spherical dilation) despite a relatively low VTI_{TR} (more increase in RA pressure than in systolic RV pressure) yields a low LAI_{RV} which indicates poor adaptation to load (disproportionate RV dilation with limited ability to raise RV systolic pressure in response to increased afterload, consequently also progressive TR and RA pressure rise), suggesting a reduced myocardial contractility and low probability for RV improvement during LVAD support. It was found that LAI_{RV} values <15 indicate low RV adaptability to load which will be insufficient to prevent RVF, even at normal PVR (Dandel et al., 2013, 2015a).

Prediction of Patient Outcome With LVAD Therapy

A major source of LVAD recipients' morbidity and mortality relates to RVF. RV reverse remodeling and functional improvement after LVAD implantation depend not only on the reversibility of RV myocardial alterations but also on the reversibility of pathologic

circulatory and metabolic changes induced by imbalanced neurohumoral/inflammatory reactions to the low cardiac output and congestive HF-related end-organ failure (especially kidney and liver). Conversely, the reversibility of pathologic neurohumoral activation and end-organ failure during LVAD support depend on RV function. Prediction of both RV improvement and outcome during LVAD support is therefore highly complex and is a challenging issue and it is not surprising that the majority of scoring systems which are currently used for decision-making between LVAD and BiVAD implantation are based rather on variables which reflect the preoperative severity of HF-related multi-organ dysfunction than on measurements directly related to RV contractile performance including its potential reversibility (Atluri et al., 2013; Drakos et al., 2010; Fitzpatrick et al., 2008; Matthews et al., 2008). This might explain the different risk discrimination capacities for post-LVAD RVF found in different studies using the same scoring system not containing data on RV size, geometry, and function.

Certain risk scores for post-LVAD RVF with and without incorporated echo-data appeared also able to stratify the risk for death after LVAD implantation (Aissaoui et al., 2015; Cowger et al., 2016; Lietz et al., 2007; Matthews et al., 2008; Miller and Russell, 2012; Potapov et al., 2011a; Drakos et al., 2010; Topilsky et al., 2011a). The highest ability to identify patients at higher risk for postoperative death was shown by the complex scoring systems that incorporate preoperative clinical, hemodynamic, and laboratory data reflecting mainly end-organ dysfunction which can remain a high risk for mortality even after improvement of RV function during LVAD support (Drakos et al., 2010; Lietz et al., 2007; Matthews et al., 2008; Miller and Russell, 2012; Potapov et al., 2011a). However, additional inclusion of certain echo-derived parameters for evaluation of RV function into established risk scores based only on variables reflecting the preoperative severity of HF-related multi-organ dysfunction can improve prediction of post-LVAD RVF and patient outcome (Aissaoui et al., 2015; Grant et al., 2012; Kato et al., 2013).

Role of Echocardiography at LVAD Implantation Surgery

Intraoperative TEE is helpful for cannula placement, assessment, and optimization of LV unloading, assessment of RV function, final evaluation of cardiac valve function, and final exclusion of a PFO that may have been masked by increased left atrial (LA) pressure.

Assessment of Inflow and Outflow Cannula Position and Blood Flow

Blood flow at both cannulas can be evaluated by color flow, pulsed-wave (PW) and continuous-wave (CW) Doppler. Acoustic shadowing (reverberation artifact from the pump) can impede or limit Doppler interrogation. The inflow cannula (IC) should be parallel to the septum and directed toward the mitral valve (MV) and not abutting any ventricular wall (Gelzinis, 2012; Magunia et al., in press; Stainback et al., 2015). An excessive degree of IC deviation toward the ventricular septum may necessitate surgical revision. Correct position of the IC can be confirmed during short-term reduction of LV volume by increasing the pump rate (Kirkpatrick, 2012). Real-time 3D echocardiography may be used for better visualization of the terminal portion of the IC within the LV (Stainback et al., 2015). For continuous flow (CF) devices, the normal color-flow Doppler pattern should consist of unidirectional laminar flow with systolic augmentation (peak velocity: between 1 and 2 m s^{-1}, depending on preload and intrinsic LV function) (Chumnanvej et al., 2007; Gelzinis, 2012). Regurgitation suggests pump malfunction, turbulence/high velocity suggests obstruction (thrombus or intermittent obstruction by LV wall) (Cohen et al., 2015). At the outflow cannula (OC), the normal flow is laminar and unidirectional (peak velocity: 0.5 and 2 m s^{-1}). OC flow patterns can be affected by the insertion angle of the cannula into the aorta. IC and OC peak flows are usually higher for pulsatile devices, but not beyond 2.3 m s^{-1}. Examination of OC anastomosis is also necessary (Chumnanvej et al., 2007; Cohen et al., 2015).

Optimization of LV Unloading and Evaluation of RV Function

The main echo parameters for assessment and monitoring of LV unloading by an LVAD are ventricular and atrial septum position, LVEDD, and AV opening (Chumnanvej et al., 2007). TEE is also mandatory for intraoperative monitoring of RV size, geometry, and function plus close observation of possible changes in TR and/or RA size (Dandel et al., 2015a; Kukucka et al., 2011a,b). After LVAD initiation, acute RV geometry changes during sudden excessive LV unloading can induce RV dysfunction (up to acute RVF). RV overdistension must therefore be strictly avoided. TEE-confirmed neutral ventricular and atrial septum position is essential for avoidance of RV and tricuspid ring-geometry alterations (Chumnanvej et al., 2007; Dembitsky and Naka, 2012; Marzec and Ambardekar, 2013). Worsening of RV function immediately after LVAD initiation, associated with absence of AV opening, leftward septum-shift with extreme LV diameter decrease (± suction events), and TR increase indicate excessive LV unloading (**Fig. 2**) (Dandel et al., 2015a).

Preexisting RV dysfunction is often reversible by LV decompression-induced reduction of RV afterload.

However, if LV unloading shows no effect on RV size and function, the additional implantation of a temporary RVAD can be beneficial because early RVAD implantation improves survival over-delayed placement (Fitzpatrick et al., 2009). After LVAD implantation, reduction of RV afterload is usually followed by reduction of TR. However, leaving greater than moderate TR can induce worsening of RV function with further worsening of TR, which will later need surgical correction (Dembitsky and Naka, 2012; Fitzpatrick et al., 2009; Marzec and Ambardekar, 2013). TV repair performed simultaneously with LVAD implantation in

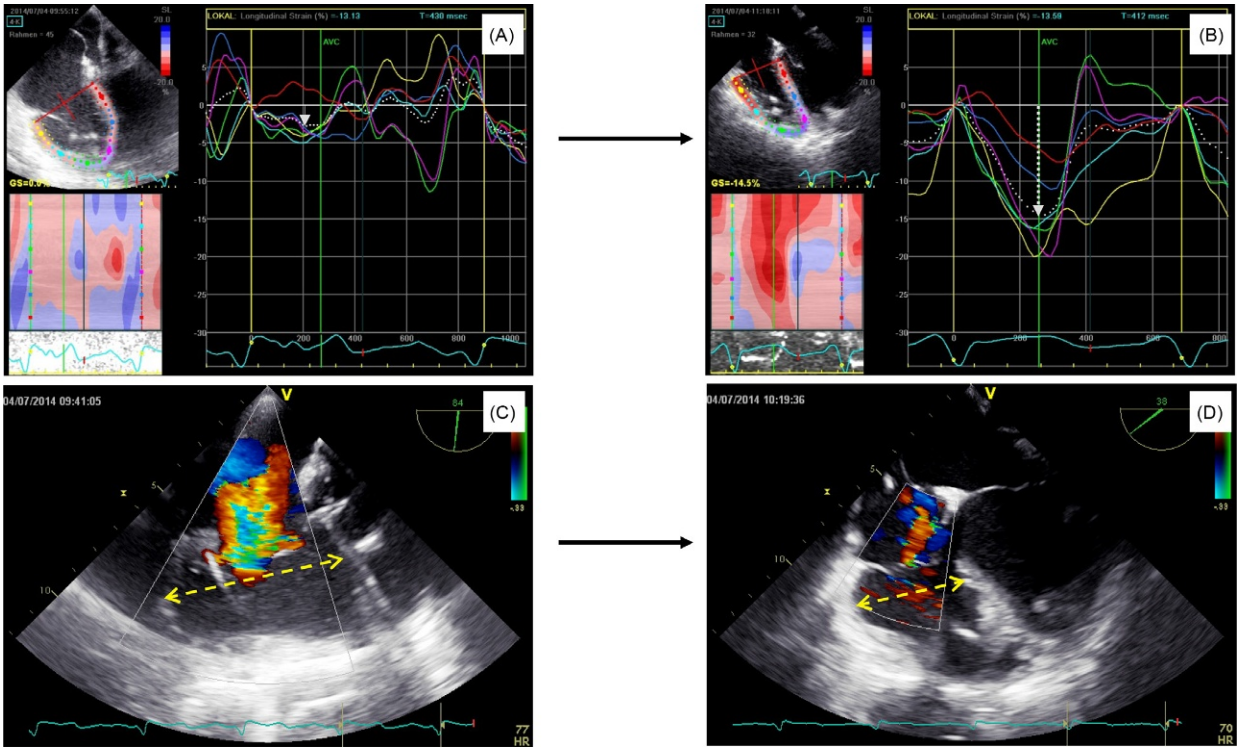

Fig. 2 Intraoperative transesophageal echo images of the right ventricle (RV) during acute severe RV failure induced by excessive unloading of the failing LV shortly after initiation of the implanted LVAD (images A and C) and after reversal of RV failure (B and D) induced by optimization of LVAD support. Image A shows massive RV and right atrial (RA) dilation with ventricular and atrial septum shift to the left as well as extremely altered RV myocardial global and regional longitudinal strain values. In addition to the extremely low global peak longitudinal systolic strain (PSSL), the regional strain curves indicate high asynchrony and dyssynergy of both systole and diastole. Image C also shows a severe TR. Images B and D recorded 1 h after optimization of LVAD function show normalization of RV size and geometry, normal septum position, normal RA size, and striking reduction of TR. PSSL increase from 2.3% to 14.5% plus improvement of synchrony and synergy indicate massive improvement of RV myocardial contractile function.

patients with intraoperative TR ≥ grade 3 associated with preoperative RVF can promote RV reverse remodeling and spare them from permanent BiVAD (Maltais et al., 2012; Potapov et al., 2011b).

After LVAD initiation, TEE is also necessary to reassess AV coaptation, because the decrease in LV pressure increases the transvalvular pressure gradient and therefore aggravates AR, which preoperatively is often underestimated (Dandel et al., 2015a; Rao et al., 2001). AR can further be exacerbated if the OC position is too close to the AV producing valvular distortion and local pressure increase with aortic root dilation and increase of the transvalvular pressure gradient (Dembitsky and Naka, 2012; Rao et al., 2001). Because AR impairs LV unloading and can lead to progressive congestive HF in LVAD recipients, patients with AR ≥ grade 2, especially those who have thin leaflets, require AV repair, closure, or bioprosthesis (Dembitsky and Naka, 2012; Feldmann et al., 2013).

LVAD initiation may create a pressure gradient from the RA to the LA, with exaggerated right-to-left shunting through a PFO. To reveal shunting by TEE it is useful to induce a short-term shifting of atrial septum to the left by increasing the LVAD flow (Dembitsky and Naka, 2012). Intraoperative TEE after LVAD initiation is mandatory because PFO should be surgically closed before leaving the operating room (Dembitsky and Naka, 2012; Patangi et al., 2013). After LVAD initiation, TEE is necessary for guiding pump speed adjustment to optimize LV unloading without any septum shift and if possible with maintaining an AV opening at least once every three heart beats (Cohen et al., 2015; Patangi et al., 2013; Topilsky et al., 2011b). Although maximum achievable LVAD support might be beneficial for patient exercise capacity and LV reverse remodeling, submaximal support in order to keep a near physiologic AV function is preferred in outpatients, because full support can lead to more suction alarms, AV leaflet fusion, aortic rood thrombosis, and development or aggravation of AR by increasing the pressure gradient over the closed AV (Topilsky et al., 2011b).

Role of Echocardiography in the Postoperative Management of LVAD Recipients

Echocardiography is the major tool for routine surveillance of LVAD recipients. It is required for assessment and optimization of LV unloading, for monitoring of RV function, and for early detection of cardiac abnormalities which can impair LVAD therapy. It is also

the mainstay for evaluation of unloading-promoted cardiac improvement and decision-making about the possibility to wean certain patients from their LVAD.

Monitoring and Optimization of LV Unloading

Maintenance of proper LV unloading is paramount for long-term outcome after LVAD implantation, and echocardiography is the imaging modality of choice for surveillance of LV unloading. Abnormal LVAD parameters and/or clinical symptoms of HF need immediate TTE evaluation.

Insufficient or impaired LVAD support causes LV overfilling with all its consequences. Major echo findings during LV overfilling are AV opening associated with increasing LVEDD, rightward septum shift, LA enlargement, increased spontaneous "echo-contrast" in the LV and LA, new appearance or increase of mitral regurgitation (MR), RV and RA diameter increase, TR and PAP increase, and dilated inferior vena cava (Chumnanvej et al., 2007; Kassi and Estep, 2016). Few studies also aimed to define parameters which might be used as echo surrogates for LV filling pressure. In one study, the mitral deceleration index and LA pressure estimated on the basis of diastolic position of atrial septum showed significant correlation with pulmonary capillary wedge pressure (PCWP) (Topilsky et al., 2011b). Other studies showed that prolongation of mitral deceleration time, decrease in trans-mitral early diastolic flow velocity (E), decrease of the ratio between E and the tissue Doppler-derived mitral annular early velocity (e′), and decrease in LA size can be used in LVAD recipients as echo surrogates of decreased LV filling pressure (Lam et al., 2009; Topilsky et al., 2011c). The slope of the anterior mitral leaflet closure after atrial phase of LV diastolic filling obtained from M-mode TTE was also found to be a possible echo surrogate for LV filling pressure (Martinez et al., 2014). In a larger study, an echo-derived algorithm developed for evaluation of LV filling pressures in patients with systolic HF supported by an LVAD appeared able to distinguish normal from elevated LV filling pressures (Estep et al., 2014a). The parameters selected for that algorithm were: trans-mitral early (E) and late (A) diastolic flow-velocity ratio (E/A), systolic PAP, E/e′ ratio, LA volume index, and RAP. Because in CF pumps, changes in PCWP during stepwise pump-speed changes (ramp studies) correlated only weakly with changes in LVEDD, LVEDD alone is not a reliable echo-derived surrogate for PCWP (Jung et al., 2015).

LVEDD, septal position, and AV opening are most often used to optimize LVAD settings (Kirkpatrick et al., 2010; Shah et al., 2012). For CF-LVADs, the rotor speed should be adjusted until the septum is neutrally positioned and the AV opens to a small degree for a brief period of time and/or a reduced frequency at the lowest possible LVEDD (Kirkpatrick, 2012; Kirkpatrick et al., 2010). In patients with unloading-promoted LV improvement, proper adjustment of LVAD function is necessary to prevent excessive unloading. Major echo signs for over-pumping are shrinking of LV cavity size with leftward septum shift (± suction events), no opening of the AV (± spontaneous echo contrast in the aortic root), altered RV geometry with TR increase, reduction in the peak velocity of the TR jet, and worsening of RV function (Kirkpatrick, 2012; Stainback et al., 2005).

Serial assessment of AV opening is mandatory for evaluation and optimization of LV unloading. Because AV opening depends on the aortic-to-LV pressure difference during LV systole, the degree and frequency of openings will increase with LV recovery or when LVAD malfunction leads to high LV pressures. Conversely, AV openings will decrease or even stop with aggravation of LV dysfunction or after excessive LV decompression by the LVAD (LV pressure decrease and aortic pressure increase).

Recently it was shown that 3D echocardiographic assessment of LV and RV volumes and shape can help optimization of LV unloading in LVAD recipients (Addetia et al., 2017). It was also found that adding 3D echocardiographic assessment of the RV to the clinical echo-ramp study may better optimize LVAD speed (Addetia et al., 2017).

Search for Abnormalities Which May Impair LVAD Therapy

Several circumstances and cardiac abnormalities like cannula obstruction, tamponade, AR, intracardiac thrombi, and vegetations, which impair LVAD therapy, are detectable by echocardiography. Early detection of cannula obstruction (partial, intermittent, or complete), which can result from malposition, kinking, thrombosis, or vegetations, can be of vital importance for the patient. LV shrinking by excessive unloading which facilitates IC obstruction by cardiac structures ("suck down" of septum, trabeculations, papillary muscles, chordal structures) and reduction of preload by tamponade or hypovolemia, which also predispose to obstruction of the IC orifice, are other abnormalities easily detectable by follow-up echo controls (Cohen et al., 2015; Kirkpatrick, 2012). In LVAD recipients with progressive LV improvement, with the increasing contribution of the LV to the CO, the initially optimal LV unloading can turn into excessive unloading necessitating adjustment of pumping rates or rotor speed to prevent suction events. High velocity aliased flows with turbulence at the cannulas orifices (for pulsatile devices: >2.3 m s^{-1} peak inflow and >2.1 m s^{-1} peak outflow; for CF- devices: >2 m s^{-1} peak inflow and outflow) suggest obstruction (Kirkpatrick, 2012). Low velocities at the IC may indicate thrombosis inside the cannula. Reduced diastolic flow velocity and increased systolic/diastolic flow velocity ratio suggest thrombosis-induced pump malfunction (Fine et al., 2014). Regurgitant flow at the IC suggests LVAD malfunction. Whereas in pulsatile LVADs, failure of the one-way valve leads to regurgitation, in CF devices regurgitation occurs when pumps are unable to overcome the systemic vascular resistance. Also intermittent low peak velocity flow may suggest LVAD malfunction if a misalignment between flow and ultrasound beam direction or a relevant LV-SV can be excluded. At the IC, PW-Doppler can be used to calculate device SV, but it is challenging to obtain reliable measurements. For the IC the most optimal Doppler angle is usually

found in apical views but interrogation can be impaired by reverberation artifacts from the pump. The OC in the ascending aorta is usually best visualized from a right parasternal view but often difficult to image by TTE (Kirkpatrick, 2012; Stainback et al., 2005).

Echocardiography is also necessary for detection and surveillance of AV dysfunction because significant AR can arise also a month after LVAD implantation and acquired AS due to cusp fusion is a relevant complication of LVADs (Kirkpatrick, 2012; Grinstein et al., 2016; Pak et al., 2010). Because patients without AV opening are at high risk for thrombotic events, follow-up echo controls should be also focused on the search for LV and aortic thrombi (Dobarro et al., in press; Pak et al., 2010). In an LVAD-supported heart, AR can occur also during LV systole if the aortic pressure exceeds LV pressure. Traditional quantification of AI severity using vena contracta, proximal isovelocity surface area, and jet width/LVOT diameter ratio do not take into consideration the pancyclic nature of AR jets in LVAD recipients and may therefore underestimate the severity of AR (Grinstein et al., 2016). Newly developed echo-derived variables like OC diastolic acceleration and systolic-to-diastolic peak velocity ratio (S/D-ratio) appeared more reliable for AR quantification in LVAD recipients (Grinstein et al., 2016).

Assessment of changes in LVEDD, AV opening time, and mitral inflow deceleration time during pump speed-change testing (ramp-tests) appeared also useful in diagnosing CF-LVAD malfunction in the setting of pump thrombosis (Estep et al., 2014b). However, AR or elevated mean arterial pressure can generate false positive ramp tests, whereas a small thrombus can induce false negative results (Adatya et al., 2013).

Surveillance of RV Function

Although LV unloading often induces already intraoperatively acute reduction of the PVR and long-term LVAD support further reduces PVR facilitating RV reverse remodeling and functional improvement, both early and late RVF after LVAD implantation are not always avoidable (Dandel et al., 2015a; Rich et al., in press). Serial follow-up assessment of RV size, geometry, and function is therefore mandatory and TTE is the method of choice for early detection of right-sided heart dysfunction. RVEDD, RA volume, and TR increase are major signs for imminent or already existing RVF and additional signs for insufficient or excessive LV unloading suggest that the alteration of right-sided heart function might be related to the disadvantageous LVAD support. No regression of TR and persistent dilation of RV and RA, after optimization of LV unloading, indicate either reduced RV myocardial contractility or RV overloading or both. Main causes for RV overloading despite optimal LVAD support are pressure overload by increased precapillary vascular resistance (irreducible by LV unloading) and/or volume overload, especially due to severe TR or renal dysfunction. Normal or reduced PVR in such patients indicates that reduced contractile function is mainly responsible for RV dysfunction. Reduction of LAI_{RV} values can also confirm the impairment of RV contractile function.

Evaluation of Unloading-Promoted Cardiac Improvement

Echocardiography is the cornerstone for assessment of cardiac recovery in LVAD recipients allowing both selection of weaning candidates and decision-making in favor of or against LVAD removal. Repeated TTE screenings with normal LVAD function are necessary for selection of potential weaning candidates (i.e., patients with signs of reverse remodeling who also seem to have their contractile function improved to levels deserving further evaluation). Potential weaning candidates are those with LVEDD $<$55 mm (or 55–60 mm at BSA $\geq$1.8 m^2) and fractional shortening $>$15%, no or $\leq$grade 1 MR and/or AR, no RV dilation and no or $\leq$grade 2 TR during LVAD support (Dandel et al., 2011). Repeated short-term interruption of LV unloading (off-pump or turn-down trials) is mandatory for assessment of recovery, regardless of whether the assessment is performed at rest or during exercise. Whereas pulsatile LVADs allow optimal assessment of heart function during complete pump stops (off-pump trials), complete stops of CF pumps lead to retrograde blood flow into the LV which induces misleading LVEDD increase (volume overload) and reduction of the diastolic arterial pressure that, by reducing the LV afterload, can generate overestimations of LV systolic function. The misleading retrograde flow into the LV during off-pump trials is a major negative factor that can interfere with successful LVAD explantation. For such pumps, rotor-speed reduction (turn-down trials) to values resulting in close to zero flow in one cardiac cycle is therefore better than complete pump stop (Birks and Miller, 2012; Cohen et al., 2015; Dandel et al., 2005; Dandel et al., 2011).

Before the first off-pump trial, it is useful to perform stepwise pump-rate reductions under TTE monitoring, to verify whether complete interruption of unloading is possible. If unloading reduction already provokes symptoms (dizziness, sweating, etc.), any turn-down to zero unloading is senseless and risky. If the patient remains asymptomatic but the LVEDD increases beyond 60 mm, or the right-sided heart revealed morphological and functional instability (increasing TR, RV dilation with reduced output), any unloading interruption trials are also senseless, because such a patient is not yet a weaning candidate.

Echo assessment of recovery in weaning candidates is usually based on the results of repeated short-term off-pump or turn-down to zero-unloading trials (Birks and Miller, 2012; Dandel et al., 2005; Dandel et al., 2011; Dandel and Hetzer, 2015a; Dandel et al., 2012; George et al., 2004; George et al., 2007; Müller et al., 1997). After months of continuous LVAD support, even short periods of physiological loading by interruption of LVAD support may represent a serious challenge for a possibly incompletely recovered LV. It therefore appears reasonable to avoid (at least initially) any risk of myocardial exhaustion, which

might interfere with possibly still ongoing recovery. Therefore, all adults and children who were weaned since 1995 in the Berlin Heart Center from long-term VADs underwent assessment of recovery exclusively at rest (Dandel et al., 2011; Dandel et al., 2012). Although at rest the conclusive value of assessments is limited by the lack of information about inotropic reserves and cardiac adaptation to stress, the weaning results obtained in Berlin were not relevantly affected by that, insofar as the results reported by groups who additionally used stress echocardiography and/or exercise testing were not better (Birks and Miller, 2012; Dandel et al., 2011; Dandel et al., 2012; Khan et al., 2001). Nevertheless, stress echocardiography can provide valuable information on inotropic reserves and adaptation to stress, which might be useful for weaning decisions. An absolute EF increase by 5% during dopamine infusion appeared able to indicate preservation of contractile reserve (Birks and Miller, 2012). A possible limitation of dobutamine stress echocardiography (DSE) can be, at least theoretically, the risk of myocardial exhaustion with negative impact on an ongoing myocardial recovery process. Further studies are therefore necessary to establish the real value of DSE for weaning decisions.

In principle, echo assessment of recovery should be as comprehensive as possible, including Tissue Doppler imaging (TDI) and speckle-tracking-derived strain echocardiography (STE) (Dandel et al., 2011; Dandel et al., 2012; Gupta et al., 2014). The advantages of STE are its ability to differentiate between active and passive movement of myocardial segments, the angle independency of myocardial deformation measurements, and the possibility to quantify intraventricular asynchrony and dyssynergy plus to evaluate components of myocardial function, such as longitudinal myocardial shortening, that cannot be visually assessed (Dandel and Hetzer, 2009; Dandel et al., 2014; Formica et al., 2010). The major limitation of STE is its dependence on image quality. **Figs. 3** and **4** show the usefulness of TDI and 2D-STE for assessment of recovery after LVAD implantation. However, not all parameters with impact on weaning decisions can be reliably measured in all patients because of poor image quality in some LVAD recipients, especially if off-pump Doppler and STE measurements had to be performed during rotor speed reduction instead of complete pump stop.

The cornerstones for weaning decisions are echocardiography and RHC (Birks and Miller, 2012; Dandel et al., 2011; Dandel and Hetzer, 2015a; Formica et al., 2010). The main criteria for LVAD removal are shown in **Table 5**. Off-pump LVEF $\geq$45% plus normal LVEDD at rest are weaning requirements and their stability after maximum improvement over the next 2–4 weeks during and between additional off-pump tests appeared predictive for long-term post-explant outcome (Birks and Miller, 2012; Dandel et al., 2011; Dandel et al., 2012; Dandel and Hetzer, 2015b). Other off-pump echo data on LV geometry and function (including TDI and strain data) are useful for weaning decisions especially if their stability after maximum remission is also considered. In borderline cases, STE off-pump data including intraventricular synchrony and synergy of contraction can be useful for final decisions. After maximum improvement, stability of LV size, geometry, and function also during moderate loading by pump reduction for several days can facilitate weaning decisions. Stable normal hemodynamics during the final off-pump trial is required before weaning, but alone it is insufficient predictive for long-term cardiac stability after VAD removal (Dandel et al., 2011). In patients with normal off-pump RHC data and <5 years duration of LV dysfunction before LVAD implantation, echo-derived off-pump measurements obtained at rest revealed high predictive values for post-explant cardiac stability during the next 5 years even in patients with chronic cardiomyopathy as the underlying cause for LVAD implantation (Dandel et al., 2014). The major risk factors for HF recurrence after LVAD explantation which are detectable by pre-explant off-pump trials are shown in **Table 6**.

Conclusions and Future Directions

Echocardiography is the first-line imaging modality to guide the management of LVAD recipients and of major importance for long-term successful LVAD therapy. It is required for preoperative patient selection and detection of cardiac abnormalities which must be corrected at the time of LVAD implantation, timing of LVAD implant surgery, perioperative assessment of RV function, and decision-making about the necessity for a temporary mechanical support also for the RV, intraoperative optimization of LVAD function, postoperative surveillance, and optimization of LVAD function plus evaluation of native myocardial recovery and decision-making about the possibility to wean certain patients from the LVAD.

Because reliable assessment of RV function in LVAD candidates and prediction of the natural history of RV dysfunction after LVAD implantation remains one of the major preoperative challenges, one future goal should be the validation of the more recently introduced echo-based composite variables which focus strictly on RV adaptability to loading conditions and to include also echo-derived parameters for evaluation of RV function into the established risk scores, based only on variables reflecting the preoperative severity of HF-related multi-organ dysfunction, in order to improve their predictive value for RVF after LVAD implantation.

The low number of studies on the usefulness of TDI and myocardial strain imaging for optimization of LVAD therapy and the high degree of methodological heterogeneity are probably the main causes for the underuse of these extremely important diagnostic tools. The use of strain imaging might have additionally been affected by the continuous progression of the technique and device update, which would also explain the inconsistency of certain preliminary findings. Thus, future studies aimed to standardize the methodology for myocardial velocity and deformation assessment before, during, and after LVAD implantation are absolutely necessary.

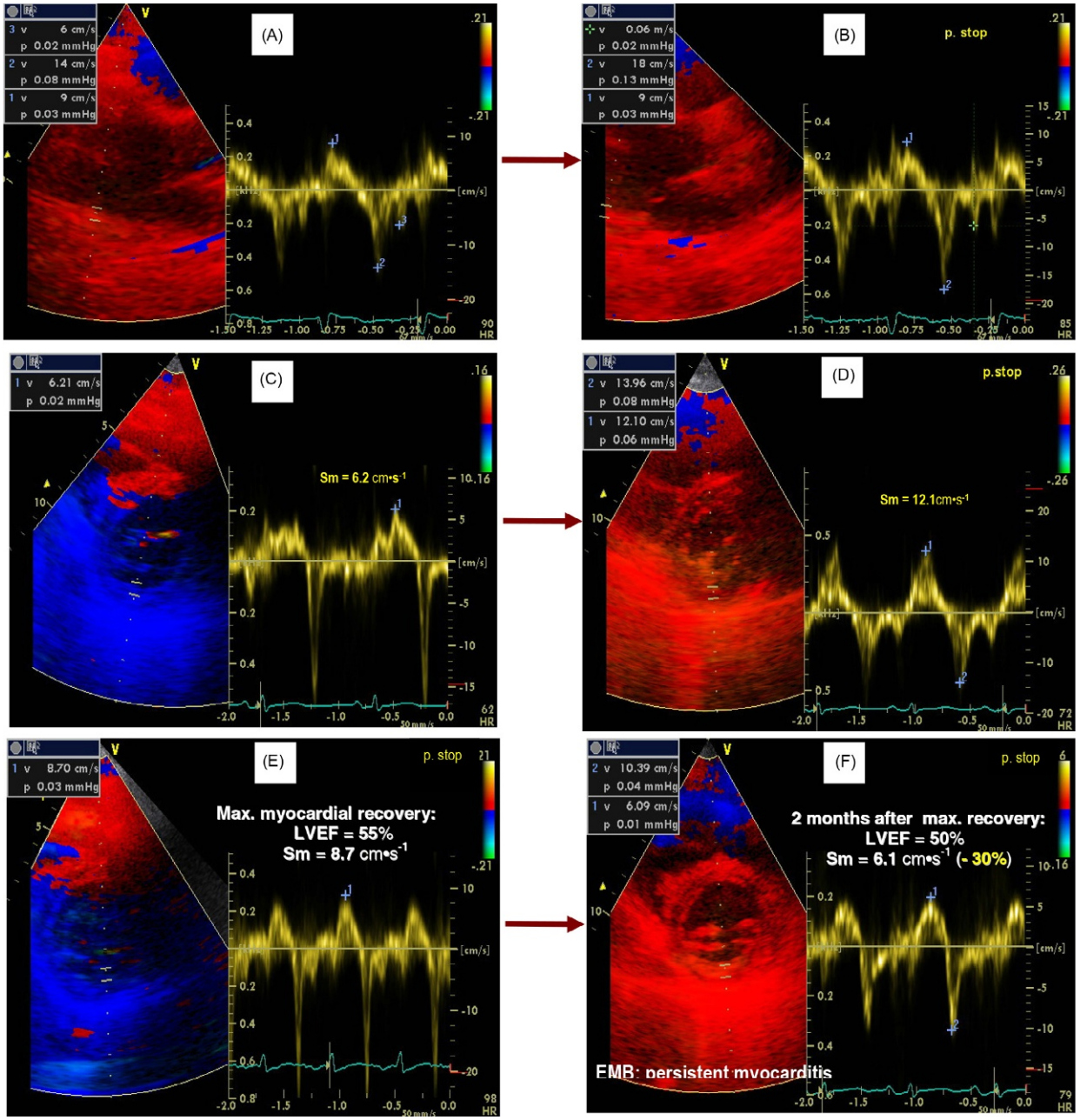

Fig. 3 Usefulness of tissue-Doppler imaging (TDI) for assessment of left ventricular (LV) myocardial improvement in 3 different LVAD recipients. Images A and B show identical peak systolic radial wall motion velocities (Sm) of 9 cm s^{-1} at the LV basal posterior wall (parasternal long-axis view) during full LVAD support (A) and during an off-pump trial. Weeks later, although LVEF remained unchanged at 50%, the off-pump Sm reached a maximum of 11 cm s^{-1}. The LV function remained thereafter stable, also after LVAD explantation. Images C and D recorded from another patient show a striking increase of radial Sm at the LV basal posterior wall (parasternal short axis view) during the off-pump trial from 6.2 to 12.1 cm s^{-1}. The low Sm during full LVAD support was induced by excessive unloading of an already recovered LV with an increasing output through the aortic valve. The LV function remained optimal and stable after LVAD explantation. Images E and F show two off-pump TDI recordings obtained from another patient who showed relatively high off-pump LVEF values, which misleadingly suggested a nearly complete recovery from a giant-cell myocarditis. At the time of maximum improvement (E), the patient had off-pump LVEF and Sm values of 55% and 8.7 cm s^{-1}, respectively. Thereafter, although off-pump EF did not fall below 50%, the Sm dropped continuously during the next 2 months reaching finally only 6.1 cm s^{-1} (F). The suspicion of a recurrence or persistence of myocarditis was finally confirmed by endomyocardial biopsy (EMB). Thus, LVAD explantation was contraindicated despite nearly normal LVEF values.

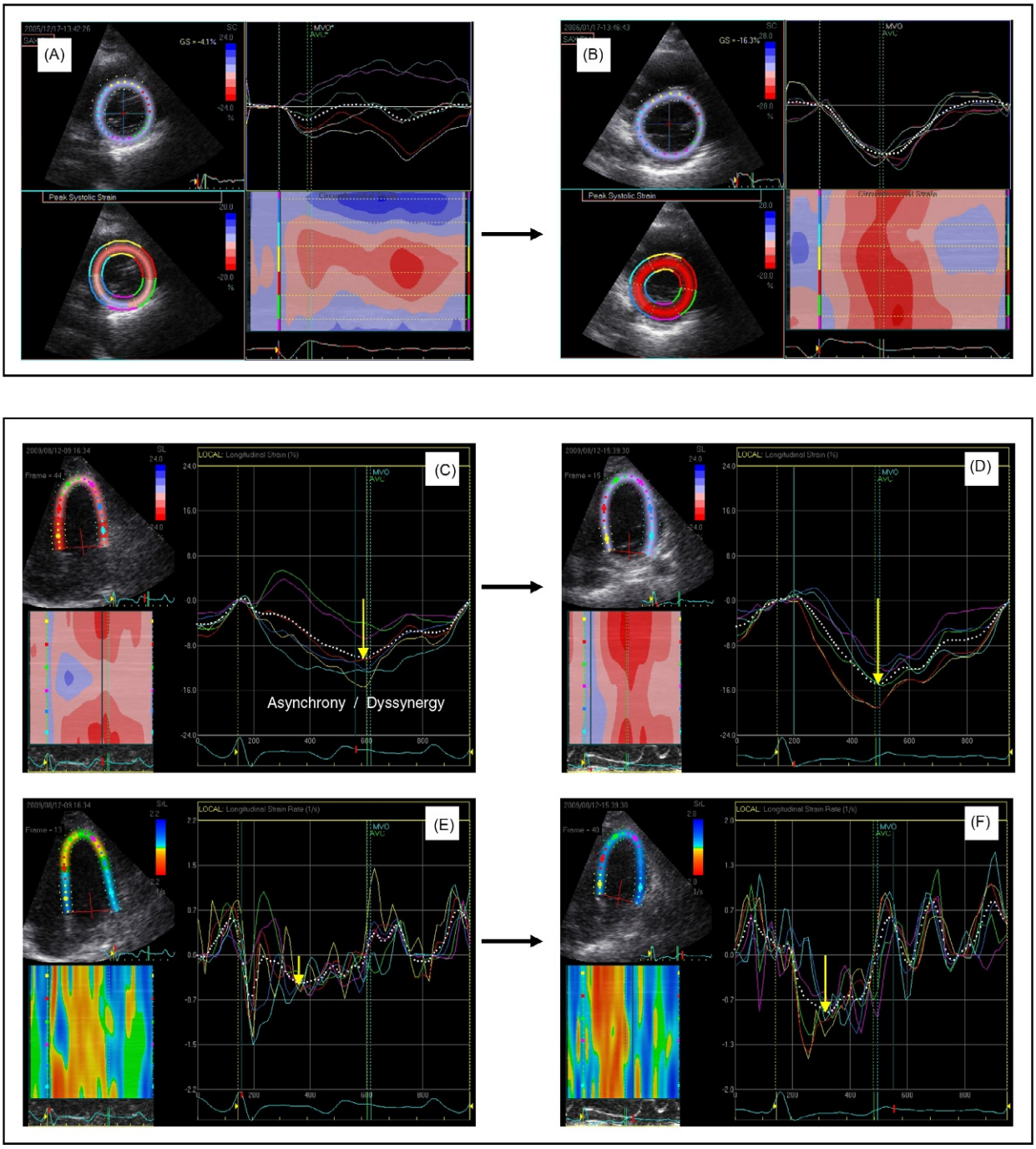

Fig. 4 Usefulness of speckle tracking-derived myocardial strain and strain-rate measurements for assessment of LV improvement during LVAD support. Images A and B show circumferential strain images recorded in a patient before LVAD implantation (A) and 1 month later during an off-pump trial for assessment of myocardial improvement (B). The circumferential peak global strain increased massively from (−) 4.1% to (−) 16.3% and the severe asynchrony and dyssynergy tuned into nearly uniform circumferential shortening during LV systole. Images C, D, E, and F show the longitudinal strain (C and D) and strain-rate (E and F) images recorded in another patient during full pump support (C and E) and during the off-pump trial (D and F). The relevant increase of the global strain (D) and strain rate (E) values with evident improvement of both synchrony and synergy of myocardial deformation during systole and diastole confirms the necessity for a complete interruption of LV unloading during the assessment of LV myocardial recovery.

Table 5 Main Criteria for Possible Explantation of a Left Ventricular Assist Device (LVAD) in Adult Patients with Unloading-Promoted Myocardial Reverse Remodeling and Improvement of Cardiac Function (Birks and Miller, 2012; Dandel et al., 2011; Dandel et al., 2012; Dandel et al., 2014; Dandel and Hetzer, 2015b)

Examination	*Parameters and parameter-derived measurements during pre-explant off-pump trials at rest, without inotropic support*	*Predictive value for ≥5 years cardiac stability*
Echocardiography[a]	LV ejection fraction (LVEF) ≥45% during the final off-pump trial	79%
	Stable pre-explant LVEF ≥45% after maximum improvement and during the final off-pump trial	86%
	LV end-diastolic diameter (LVEDD) ≤55 mm at BSA ≤1.8 m^2 during the final off-pump trial	LVEF ≥45% + LVEDD ≤55 mm: 86%
	Stable pre-explant LVEDD after maximum improvement and during the final off-pump trial	Stable LVEF ≥45% + stable LVEDD ≤55 mm: 94%
	Sm ≥8 cm/s, stable after maximum improvement and during the final off-pump trial	LVEF ≥45% + stable Sm ≥8 cm/s: 87%
	LV end-diastolic relative wall thickness (RWT) ≥0.38	LVEF ≥45% + RWT ≥0.38: 87%
	Stable VTI (PW-Doppler) in the LVOT during the final off-pump trial (stable stroke volume)	Alone not predictive
	No or ≤grade I MR and/or AR	Alone not predictive (requested before weaning)
	No RV dilation (RVOT diameter <35 mm, end-diastolic short-/long-axis ratio <0.6)	Alone not predictive
	Stable RV size and geometry during the final off-pump trial	Alone not predictive
	No, or ≤grade II TR and/or PR during the final off-pump trial	Alone nor predictive
RH Catheterization	Cardiac index (CI) >2.6 L min^{-1}/m^2 BSA (stable during the final off-pump trial)	Alone not predictive (requested before weaning)
	Pulmonary capillary wedge pressure <13 mmHg (stable during the final off-pump trial)	Alone not predictive (requested before weaning)
	Right atrial pressure (mean) <10 mmHg (stable during the final off-pump trial)	Alone not predictive (requested before weaning)
Electrocardiography	Sinus rhythm, heart rate (HR) <90/min and less than 25% HR increase during off-pump trials	Alone not predictive (requested before weaning)
Arterial pressure	Systemic mean arterial pressure ≥65 mmHg	Alone not predictive

[a]In patients with borderline echo data, 2D speckle-tracking imaging parameters, including also their pre-explant stability after maximum improvement and during the final off-pump trial, can be taken into consideration for weaning decisions (Dandel and Hetzer, 2009; Dandel et al., 2011; Dandel et al., 2012; Dandel and Hetzer, 2015b). *Sm*, peak systolic wall motion velocity at the basal posterior wall; *LVOT and RVOT*, left and right ventricular outflow tract. *MR, AR, TR and PR*, mitral, aortic, tricuspid, and pulmonary regurgitation, respectively; *VTI*, velocity-time integral; *PW*, pulsed wave.

Table 6 Pre-explant echocardiographic risk assessment for post-explant heart failure recurrence (Birks and Miller, 2012; Dandel et al., 2011; Dandel and Hetzer, 2015a; Dandel et al., 2012; Dandel et al., 2014)

Echo-approach	*Pre-explant echo parameters*	*Predictive value for HF recurrence during the first 3 post-explant years*
Off-pump standard TTE at rest	LVEF low and/or unstable[a]	89% for LVEF <45%[b]
		100% for LVEF <45% if history length of HF is >5 years
		90% for LVEF ≥45% with pre-explant alteration of >10% of best value
		88% for LVEF <50% with pre-explant alteration of >10% of best value
		89% for LVEDD >55 mm[b] at BSA ≤1.8 m^2
	LVEDD large and/or unstable[a]	90% for LVEDD >55 mm[b] at BSA ≤1.8 m^2 and LVEF <50%
		82% for RWT_{ED} <0.38[b]
	LV end-diastolic RWT reduced and/or unstable[a]	83% for RWT_{ED} <0.38 and LVEF ≤50%
		87% for RWT_{ED} reduction of >8%[b]
		85% for left ventricle S/L_{ED} increase of >10%[b]
	S/L_{ED} unstable[a]	Risk factor but no data on predictive value
	Trans-mitral restrictive flow-profile (accentuation or new appearance)	
	VTI reduction in the LVOT during the off-pump trials	Risk factor but no data on predictive value
	TR (new appearance or accentuation) and/or TR-jet velocity increase during off-pump trials	Risk factor but no data on predictive value
	RVEDD increase ± RV geometry alteration	Risk factor but no data on predictive value
Off-pump TDI and 2D-STE at rest	Sm low and/or unstable[a]	83% for Sm <8 cm s^{-1}[b]
	TAPS′ reduction during off-pump trials	90% for Sm pre-explant alteration of >10%
	LV myocardial asynchrony and/or dyssynergy during off-pump trials	Risk factor; no data on predictive value
Off- pump TTE after exercise with dobutamine	LVEF <50% after 6 MW	Risk factor; not sufficient data on predictive value
	No relevant LVEF increase during DSE	Risk factor; not sufficient data on predictive value

HF, heart failure; *TTE*, trans-thoracic echocardiography; *LVEF*, LV ejection fraction; *LVEDD*, LV end-diastolic diameter; *BSA*, body surface area; *VTI*, velocity-time integral; *S/L_{ED}*, end-diastolic short-/long-axis ratio; *LVOT*, LV outflow tract; *TDI*, tissue Doppler imaging; *2D-STE*, two-dimensional speckle-tracing echo imaging; *RWT_{ED}*, end-diastolic relative wall thickness ([posterior wall thickness + ventricular septum thickness]/LVEDD); *Sm*, systolic peak wall motion velocity measured with the pulsed-wave tissue Doppler at the basal posterior wall (parasternal short- or long-axis view); *6 MW*, 6 minutes walk; *DSE*, dobutamine stress echocardiography.

[a]Unstable: parameter changes during the time between maximum improvement and left ventricular assist device (LVAD) explantation and/or during the final off-pump trial

[b]Unstable during the final off-pump trial.

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Salt and Blood Pressure

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Introduction

Salt (sodium chloride) is an essential element for life, not only for humans, but also for animals, and even for many plants. Humans have had a long, close, and complex relationship with salt. For instance, salt served as money; salt was the instigator of warfare; salt created trades and cultures; salt was thought to facilitate sexual potency and fertility; and salt was a symbol of loyalty, friendship, truth, and wisdom (because the essence of salt has never changed). Now, we are facing a new challenge: excess salt intake may be hazardous to our health. The salt–human relationship appears to be delicate. This article provides an overview of the current understanding of the relationship between sodium intake and blood pressure (BP), including the prevalence, pathophysiology, and treatment.

Salt and BP

Animal experiments, epidemiological studies, and clinical trials have provided compelling evidence that high sodium intake increases BP. The INTERSALT Study (Elliott et al., 1996; Intersalt Cooperative Research Group, 1988), a standardized, worldwide epidemiologic study of large sample size ($n = 10{,}079$ men and women aged 20–59 years from 32 countries) assessed the relationships between 24-h urine sodium excretion and BP both within and across populations, and positive associations were found in many, but not all, populations. The estimated effects of reduced sodium intake on BP are that a 100 mmol day^{-1} (2300 mg day^{-1} sodium or 6 g day^{-1} salt) lower sodium intake is estimated to reduce systolic BP (SBP) by 1–6 mmHg and for diastolic BP (DBP) by 0.03–2.5 mmHg (Elliott et al., 1996; Intersalt Cooperative Research Group, 1988). BP and age were positively associated and BP was further elevated by higher sodium consumption. However, in populations with very low sodium intake (0.2–6 mmol day^{-1}), BP was not found to increase with age. In Western countries, BP tends to rise with age, and the prevalence of hypertension is 60–90% in older adults (Aronow et al., 2011; Benetos et al., 2016; Vasan et al., 2001). Given the findings from the INTERSALT Study, this phenomenon may be partly attributable to high sodium intake. Unfortunately, recent studies have revealed a significant increase in BP among children and adolescents over the last decade, and the BP was positively associated with sodium intake (**Fig. 1**) (Falkner, 2015; Rosner et al., 2013; Yang et al., 2012). Specifically, each 43 mmol day^{-1} (1000 mg day^{-1}) increase of sodium intake, estimated from multiple 24-h dietary recall measures, was associated with $\approx$1.0 mmHg increase in SBP, with a greater effect noted in overweight/obese children (i.e., SBP increase by 1.5 mmHg per each 1000 mg day^{-1} increase of sodium intake). The adjusted odds ratio for prehypertension/hypertension risk in the highest (vs. lowest) quartile of sodium intake was 2.0 (95% confidence intervals (CIs): 0.95–4.1) in overall (Yang et al., 2012), whereas, the odds ratio when comparing the highest to lowest quartile of sodium intake in overweight/obese children was 3.5 (95% CIs: 1.2–9.2).

Measuring Sodium Intake in Populations

Accurate and reliable measurement of individual sodium intake is extremely difficult (Cogswell et al., 2016). Consequently, the methodological challenge may cause inconsistent findings in the relationship between sodium intake and human health. Estimating sodium intake using dietary surveys is inaccurate. Spot urine tests overestimate individual intake at low levels of consumption

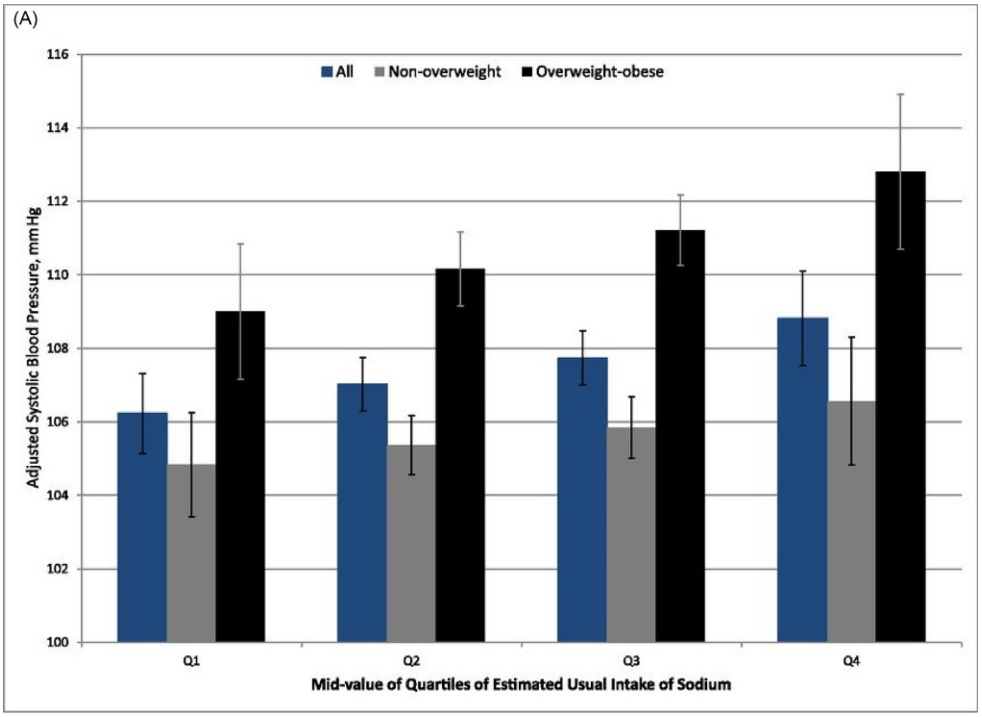

Fig. 1 Sodium intake and SBP among US children and adolescents (NHANES 2003–2008). Bars show adjusted mean (95% CIs) SBP levels by sodium intake quartile and weight status among American children and adolescents 8–18 years of age. Adjustment factors included age, sex, race, and total energy. *NHANES*, National Health and Nutrition Examination Survey; *SBP*, systolic blood pressure; *CIs*, confidence intervals. Reproduced from Yang, Q., Zhang, Z., Kuklina, E. V., Fang, J., Ayala, C., Hong, Y., Loustalot, F., Dai, S., Gunn, J. P., Tian, N., Cogswell, M. E. and Merritt, R. (2012). Sodium intake and blood pressure among US children and adolescents. *Pediatrics* **130**(4), 611–619.

and underestimate intake at high levels (Brown et al., 2013; Mente et al., 2014). 24-hour urine collection is considered as the gold standard for estimating sodium intake (Cobb et al., 2014). However, because of challenges collecting urine in free-living persons, day-to-day variability in sodium intake, biological variability in urine sodium excretion, and unmeasured extrarenal sodium loss, even single 24-h urine collection may be not accurate. In a strictly controlled environment, 24-h urine samples taken every other day correctly classified a 3-g difference in salt intake for only 49% (Lerchl et al., 2015). By increasing the observations up to three consecutive 24-h collections, the estimation was improved to 75% (**Fig. 2**). Therefore, multiple 24-h urine collections (e.g., weeks) are the most accurate for estimating individual sodium intake estimation (Oh et al., 2016). We need to consider these methodological challenges when we interpret current available observational studies relating sodium intake to health.

Salt Sensitivity

BP response to sodium intake is heterogeneous across individuals, partly because of other components of the diet including potassium and calcium. The relationship between sodium intake and BP becomes stronger if the urinary sodium–potassium ratio rather than the sodium excretion rate itself was considered (Intersalt Cooperative Research Group, 1988). Salt sensitivity is another determinant of individual BP response to sodium intake. In those with salt sensitivity, BP increases when excess sodium was consumed, whereas BP reduces with a low-sodium diet. The trait is reproducible (Elijovich et al., 2016). Although the definition of salt sensitivity has been inconsistent across studies, most defined it as a proportional change ($\geq$3% to $\geq$10%) or an absolute change ($\geq$3 to $\geq$10 mmHg) in mean arterial pressure following low to high sodium intake (Oh et al., 2016). However, these classifications may be arbitrary, since BP response to sodium intake is continuous, with a normal distribution within populations (He et al., 2009). Although it depends on the definition and measurement methods, the proportion of salt sensitivity within populations has been estimated to be 25–50% in normotensives and 40–75% in hypertensives (Oh et al., 2016). Particularly, salt sensitivity is prevalent in African Americans, older persons, and individuals with diabetes, low-renin hypertension obesity (e.g., metabolic syndrome), and chronic kidney disease (Elijovich et al., 2016). BP response to sodium intake substantially increases with age (Weinberger and Fineberg, 1991), suggesting that environmental factors and age-related decline in organ function (kidney in particular) play a crucial role in the pathogenesis of salt sensitivity.

The molecular mechanisms underlying salt sensitivity remain unclear and continue to be debated. Genetic factors may account for 30–50% of the variation in BP response to sodium intake in Asians (Gu et al., 2007) and 75% in African Americans (Svetkey et al., 1996). The heritability estimates of BP change between random-sodium and low-sodium diet among white US

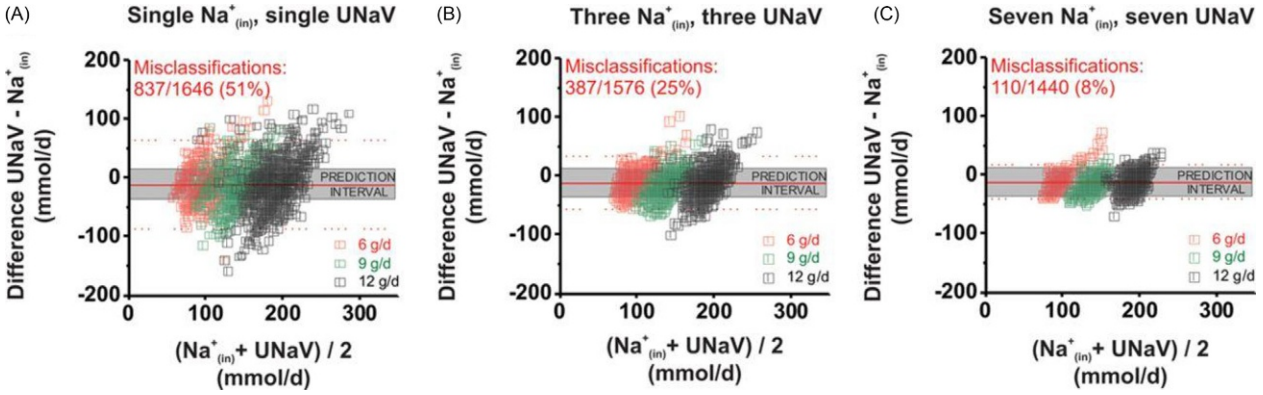

Fig. 2 Analyses of agreement between sodium intake and excretion in Bland–Altman plots. (A) Bland–Altman plot to test the agreement between single recorded 24-h sodium intakes and single UNaV. The prediction interval to accurately predict sodium intake by UNaV is defined as ±25 mmol day^{-1} of the mean difference between sodium intake and UNaV. (B) Analysis of agreement between three consecutively recorded sodium intakes and three UNaV collections. Multiple collections reduce the variability and thereby improve the predictive value of UNaV. (C) Seven consecutive collections reduce the number of misclassifications of UNaV to <10%. *Red solid line* indicates regression line; and *red dotted line*, upper and lower confidence level. UNaV, 24-h urine collection for sodium excretion. Reproduced from Cowley AW Jr (1992) Long-term control of arterial blood pressure. *Physiological Reviews* **72**, 231–300.

families were higher in monozygotic twins versus sibling (0.6–0.7 vs. 0.3–0.5) (Miller et al., 1987). DNA polymorphisms associated with renal sodium handling (e.g., α-adducin molecule and renal isoenzyme of 11-beta-hydroxysteroid dehydrogenase) correlate with salt sensitivity (Bianchi et al., 2005; Lovati et al., 1999). Asians tend to have genetically salt sensitivity. For example, the frequencies of DNA polymorphisms associated with renal sodium handling (e.g., Gly460Trp polymorphism of α-adducing gene; T(−344) C polymorphism of the aldosterone synthase gene (*CYP11B2*); T235 allele of the angiotensinogen gene) are higher in Asian compared with white populations (Katsuya et al., 2003; Kokubo, 2014). In addition, monogenic forms of salt-sensitive human hypertension, which are not applicable to general populations though, are characterized by gene mutations encoding molecules involved in renal sodium handling (Hall, 2016; Lifton et al., 2001). Collectively, these results suggest that kidney may play a critical role in salt–BP association (Hall, 2016). As suggested by Guyton, if natriuresis is impaired in the kidney (i.e., pressure natriuresis is shifted to higher BP) by intrarenal disturbances, extrarenal factors (e.g., increased sympathetic nervous and renin–angiotensin–aldosterone system activity), excessive antinatriuretic hormones, or deficit of natriuretic hormones, BP tends to rise by salt intake (Cowley, 1992; Guyton, 1980; Hall, 2016). Conversely, other studies revealed no sodium retention after salt loading in those with salt sensitivity compared with normal (i.e., salt resistant) individuals (Morris et al., 2016; Schmidlin et al., 2007). Instead, vasodilation to salt loading was impaired in those with salt sensitivity (Laffer et al., 2016; Morris et al., 2016; Schmidlin et al., 2007). This is an alternative view to Guyton's theory, claiming that vasculature rather than kidney dysfunction is a key initiator of salt-induced hypertension. It should be noted, however, that most of the studies used short-term (days or weeks) salt loading or deprivation from salt, meaning the long-term effect of salt intake/reduction on vasculature in those with salt sensitivity is unknown. The underlying mechanisms behind vascular dysfunction in salt sensitivity are not well understood, but may include multiple factors, such as impaired nitric oxide bioactivity, sympathetic nerve activation, activated circulating, and local renin–angiotensin–aldosterone system (Laffer et al., 2016; Morris et al., 2016; Schmidlin et al., 2007, 2011). Increased concentration of sodium or chloride stored in the interstitial space of skin or other tissues (e.g., the vasculature and brain) may also contribute to vascular dysfunction in those with salt sensitivity via inflammation (Machnik et al., 2009). The effects in human, and determination of whether and how altering the distribution of salt and body fluids leads to long-term BP regulation merit further investigation. Salt sensitivity is associated with target organ damage and adverse cardiovascular outcomes, independently—at least in part—of BP level and the occurrence of hypertension (Elijovich et al., 2016; Morimoto et al., 1997). Therefore, unraveling the mechanisms of salt sensitivity would provide promising avenues for therapeutics against salt-induced increases in BP. Another challenge includes difficulty implementing the paradigm of salt sensitivity into clinical practice or population-based interventions due to a lack of accurate assessment of the salt sensitivity phenotype. Ambulatory BP monitoring (Castiglioni et al., 2011), beat-to-beat BP and pulse rate variability (Coruzzi et al., 2005), and urinary biomarkers (e.g., renal tubular cells and exosome miRNome analyses) (Gildea et al., 2013a,b) have been proposed as clinical measures of salt sensitivity, which need to be further replicated and validated.

Effects of Reduced Sodium Intake on BP

Meta-analyses of randomized controlled trials in both adults and children demonstrate that reduced sodium intake led to BP reduction in a dose-dependent fashion (Aburto et al., 2013; He and MacGregor, 2006; Midgley et al., 1996). A reduction of 100 mmol day^{-1} in sodium (2300 mg day^{-1} sodium) intake was associated with SBP/DBP reduction of 7.1/3.9 mmHg in hypertensives and 3.6/1.7 mmHg in normotensives (He and MacGregor, 2002). The effects on BP of reduced sodium intake were

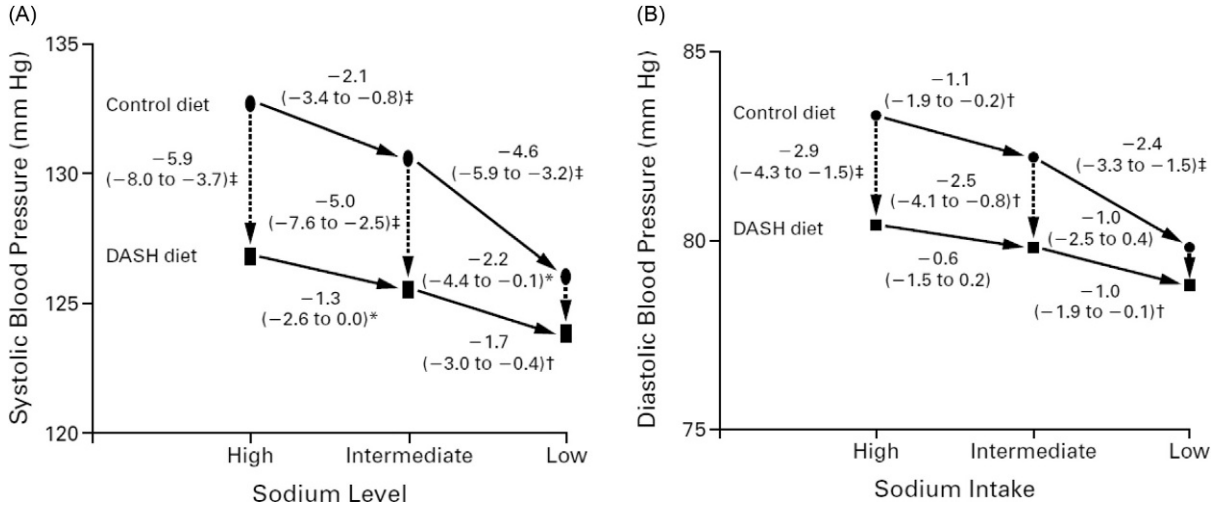

Fig. 3 The effect on SBP (A) and DBP (B) of reduced sodium intake and the DASH diet. The mean changes in BP are shown for various sodium levels *(solid lines)*, and the mean differences in BP between the two diets at each level of sodium intake are shown. Unidirectional arrows are used for simplicity, although the order in which participants were given the sodium levels was random with a crossover design. The numbers next to the *dotted lines* connecting the data points are the mean changes in BP. The 95% CIs are given in parentheses. Asterisks ($P<0.05$), daggers ($P<0.01$), and double daggers ($P<0.001$) indicate significant differences in BP between groups or between dietary sodium categories. *DASH*, Dietary Approaches to Stop Hypertension; *SBP*, systolic blood pressure; *CIs*, confidence intervals. Reproduced from Sacks, F. M., Svetkey, L. P., Vollmer, W. M., Appel, L. J., Bray, G. A., Harsha, D., Obarzanek, E., Conlin, P. R., Miller, E. R. 3rd, Simons-Morton, D. G., Karanja, N., Lin, P. H., DASH-Sodium Collaborative Research Group (2001). Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *New England Journal of Medicine* **344**(1), 3–10.

greater in hypertensives (vs. normotensives) and African Americans or Asians (vs. whites) (Graudal et al., 2012). Another meta-analysis including 37 randomized controlled trials observed that: (1) reduced sodium intake led to SBP reduction by 3.4 mmHg (95% CIs: 2.5–4.3) and DBP reduction by 1.5 mmHg (95% CIs: 0.98–2.1 mmHg); (2) when sodium intake was <2000 mg day^{-1} (vs. ≥ 2000 mg day^{-1}), SBP was reduced by 3.5 mmHg (95% CIs: 0.8–6.2 mmHg) and DBP reduction by 1.8 mmHg (95% CIs: 0.5–3.1 mmHg); and (3) reduced sodium intake had no adverse effect on blood lipids, catecholamine levels, or renal function (Aburto et al., 2013). The 37 trials included all allocated at least one group of participants to reduced sodium intake and other groups to higher sodium intake; had a duration of intervention lasting 4 or more weeks (excluding acute salt restriction trials); achieved an sodium intake difference of 40 or more mmol day^{-1} between intervention and control; and had 24-h urinary sodium excretion data (Aburto et al., 2013). A meta-analysis including children and adolescents under 18 years of age observed that reduced mean salt intake by 42% (interquartile range: 7–58%) led to SBP reduction by 1.2 mmHg (95% CIs: 0.6–1.8 mmHg) and DBP reduction by 1.3 mmHg (95% CIs: 0.7–1.9 mmHg) (He and MacGregor, 2006).

The combined effects of a reduced sodium intake with weight loss or the Dietary Approaches to Stop Hypertension (DASH) diet on BP reduction are greater than the effects of either intervention alone (Sacks et al., 2001; Whelton et al., 1998). In the Trial of Nonpharmacologic Interventions in the Elderly that recruited older hypertensives 60–80 years of age ($n=953$), change in mean SBP/DBP by reduced sodium intake alone (80 mmol day^{-1} (1800 mg day^{-1}) or less) was −3.4/−1.9 mmHg, whereas by sodium intake reduction plus weight loss (≥ 4.5 kg (10 lb)) was −5.3/−3.4 mmHg (Whelton et al., 1998). The DASH diet is rich in vegetables, fruits, and low-fat dairy products (e.g., whole grains, fish, and nuts). In the DASH-sodium study, a total of 412 participants were randomly assigned to sodium intake (high: 150 mmol (3450 mg)/day; intermediate: 100 mmol day^{-1}; or low: 50 mmol (1150 mg)/day for 30 days each) with or without consumption of the DASH diet. Reduced sodium intake from the high to the intermediate level reduced SBP/DBP by 2.1/1.1 mmHg during the control diet and by 1.3/0.6 mmHg during the DASH diet. Reduced sodium intake from the intermediate to the low level caused additional SBP reductions of 4.6/2.4 mmHg during the control diet and 1.7/1.0 mmHg during the DASH diet (**Fig. 3**). As compared with the control diet with the high sodium level, randomization to the DASH diet with the low sodium level led to a mean SBP that was 7.1 mmHg lower in those without hypertension, and 11.5 mmHg lower in participants with hypertension. The BP-lowering effect in hypertensives appears to be equal to or greater than a single antihypertensive-drug therapy.

Conclusion

The World Health Organization currently recommends consuming 2000 mg sodium/day (Drewnowski et al., 2015). The American Heart Association recommends a sodium intake <1500 mg day^{-1} for all Americans (Lloyd-Jones et al., 2010). The US Department of Agriculture and US Department of Health and Human Services joint 2010 Dietary Guidelines for Americans endorse

recommendations to reduce sodium intake below 1500 mg day^{-1} in African Americans, those >51 years of age, and those with hypertension, diabetes mellitus, or chronic kidney disease, with a recommendation for sodium intake <2300 mg day^{-1} for all others (US Department of Agriculture and US Department of Health and Human Services, 2010). However, implementation of sodium intake reductions is a grand challenge. Despite BP-lowering effect of reduced sodium intake is obvious, sodium intake remains high in many countries. Nearly half of Americans are trying to reduce their sodium intake, and most prefer consuming low-sodium food (Elijovich et al., 2016; Jackson et al., 2016). Nevertheless, the current mean sodium consumption of Americans is approximately 3400 mg day^{-1} (8.5 g of salt), with 77% of the sodium coming from packaged, processed, and restaurant foods (Centers for Disease Control and Prevention, 2010). How do we bridge this gap between what amount of sodium is preferred and what amount is actually consumed? Efforts to reduce sodium intake should rely both on consumers' selection of low-sodium food, and perhaps more important, on the reduced sodium environment that requires cooperation of manufactures, food-service operators, and policy decision makers (Cogswell et al., 2016). To reduce sodium intake at the population level, reformulation of processed foods should be operated either through negotiation with industry or through regulation by law (Cappuccio, 2007). Such a nationwide salt reduction strategy will lead to cardiovascular disease prevention at the community level and ultimately reduce health care costs substantially (Bibbins-Domingo et al., 2010).

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Sex and Gender Differences in Cardiovascular Disease

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Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide for men and women. Although the risk factors for CVD are similar in men and women, the impact of some of the risk factors differs by sex, and there are gender and sex differences in the clinical presentation and disease pathophysiology. Sex differences reflect biological influences dictated by the sex chromosomes, genetic polymorphisms, and/or single-nucleotide polymorphisms on the sex chromosomes and autosomes, sex-steroidal modulation of gene expression, and epigenetic modulation resulting from environmental factors. Gender differences relate to societally constructed roles of men and women and can also influence the phenotypic presentation and outcomes of CVD (Fig. 1). This chapter focuses on the biological basis for sex differences and documented sex differences in presentation, treatment, and outcomes of CVD. A better understanding of these differences could ultimately lead to a more personalized management of CVD.

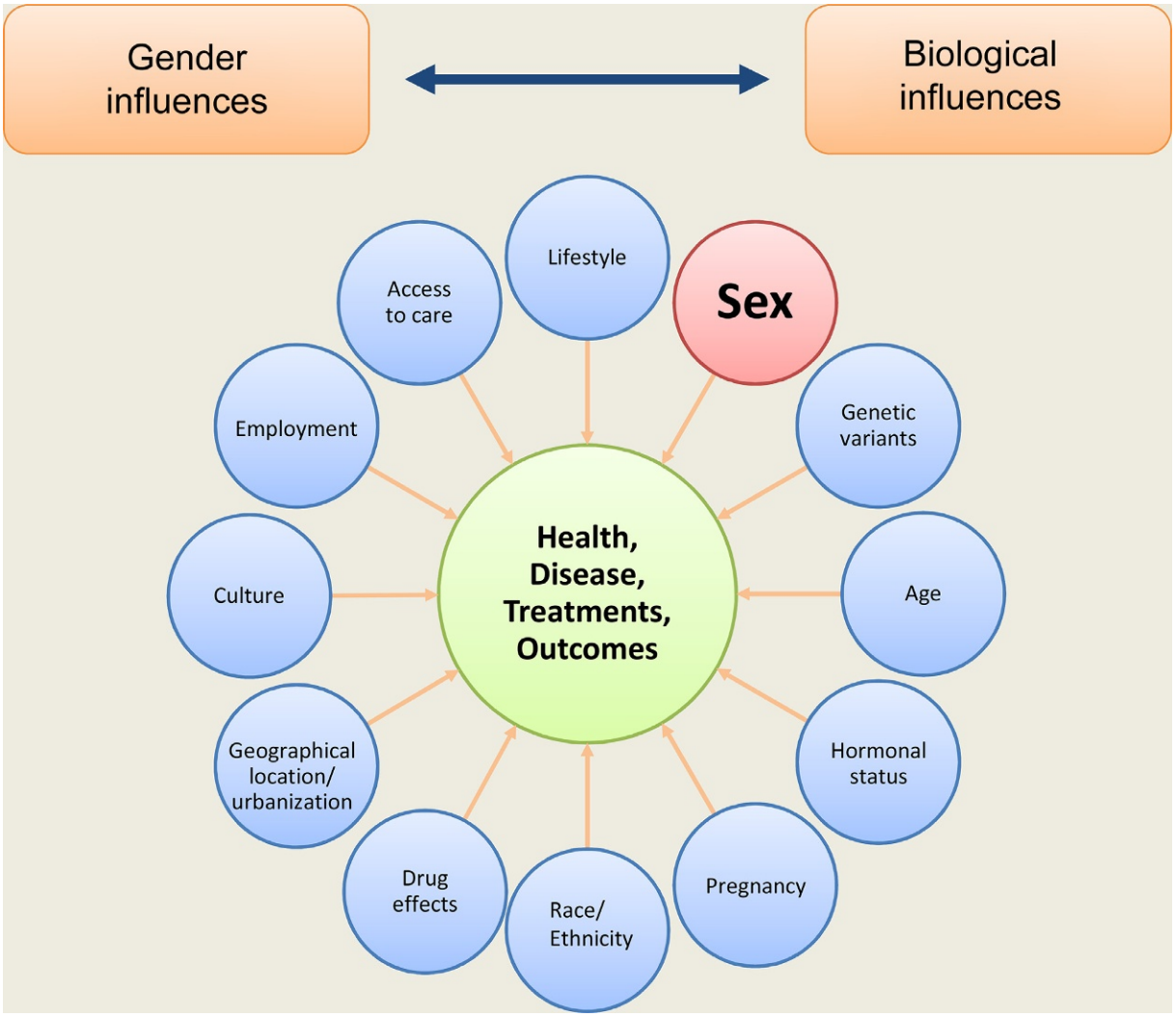

Fig. 1 Influence of biology and gender on sex differences in cardiovascular disease. Sex differences in the individual risk and phenotypic presentation of disease reflects the interactions of biological influences dictated by the sex chromosomes, genetic polymorphisms and/or single nucleotide polymorphisms on the sex chromosomes and autosomes, sex-steroidal modulation of gene expression, and epigenetic modulation resulting from environmental factors. In addition gender differences from societally constructed roles of men and women influences the phenotypic presentation and outcomes of cardiovascular disease.

Biological Basis for Sex Differences in CVD

CVDs involve complex traits. Therefore, sex differences in the individual risk and phenotypic presentation of disease reflect the interactions of genetic differences dictated by the sex chromosomes, genetic polymorphisms, and/or single-nucleotide polymorphisms on the sex chromosomes and autosomes, sex-steroidal modulation of gene expression, and epigenetic modulation resulting from environmental factors (Paynter et al., 2010; Winham et al., 2015).

Genes

In humans, biological sex is determined by the sex chromosomes (X and Y). Females have two copies of the X chromosome; males have one copy of the X and Y chromosome in every cell of the body, except for the gametes. There are some rare exceptions: XXY (Klinefelter's syndrome), X monosomy (Turner's syndrome), XXX, and XYY. In females, to reduce the dose effect of two X chromosomes, one X undergoes incomplete inactivation, which varies by tissue and may change throughout the course of life. It is unclear at this time how patterns of X-inactivation are regulated or how these patterns influence susceptibility or etiology of disease (Berletch et al., 2011; Agrelo and Wutz, 2010; Wu et al., 2014).

Historically, the genes on the Y chromosome, in particular those in the sex-determining region (SRY), were considered primarily involved with sexual differentiation. However, with newer genetic analyses, SRY is identified as a transcription factor that may regulate autosomal genes (Bellott et al., 2014). In addition, a common European lineage group of genes on the Y chromosome is

Haplogroup 1. Men who are carriers for Haplogroup 1 may be at risk for atherosclerosis as these genes regulate functions associated with progression of the disease including inflammation, autoimmunity, and adaptive immunity and resistance to infection (Bloomer et al., 2014; Bloomer et al., 2013). Nongonadal functions associated with the X chromosomes that are important for development of CVDs include coagulation, innate immunity, synthesis of norepinephrine, energy metabolism, blood pressure, and adiposity (Kanehisa et al., 2017). An analysis of RNA levels of 683 genes on the X chromosome in patients who had experienced an ischemic stroke showed a difference in X-related gene expression with upregulation of these genes in females compared to males. These results identify cellular pathways regulated by the X chromosome that may provide future sex-specific therapeutic targets (Winham et al., 2015).

Sex Steroid Hormones

The sex chromosomes induce development of the gonads that synthesize sex steroid hormones. The major sex steroids are testosterone, estrogen, and progesterone. These hormones are synthesized from cholesterol in gonadal and in some extragonadal tissues in both males and females. Testosterone is converted to estrogen by the enzyme aromatase, the activity of which varies by sex and age in men and women.

Sex hormones initiate structural or transient changes in tissues. Structural changes can persist when the hormones are absent, whereas the transient effects, called "activational effects," require the presence of the hormones. The most dramatic activational effects of the sex hormones are observed in women during pregnancy with modulation of functions of multiple organ systems that reverse once the fetus is delivered (e.g., breast development, skeletal adjustments, increases in blood volume and cardiac output, renal function, and nurturing behavior). Sex steroids initiate changes in cellular function through activation of steroid receptors on the cell surface (G-protein coupled receptors, GPR30), and through receptors within the cytoplasm and nucleus that belong to a family of nuclear binding receptors. Tissue-specific responses to sex steroids reflect differences in binding of the hormone–receptor complex to response elements on various genes. The gene for the androgen receptor is on the X chromosome; therefore, variations in this gene will more likely influence androgen insensitivity in males who carry only one copy of the X chromosome. Some cellular functions modulated by sex steroids and related to CVD risk include production of nitric oxide, regulation of angiotensin-converting enzyme (ACE), expression of angiotensin receptors, production of extracellular matrix, regulation of cellular pathways associated with apoptosis and cell survival, mitochondrial energy metabolism, production of oxygen-derived free radicals, and DNA methylation (Gaignebet and Kararigas, 2017; Regitz-Zagrosek and Kararigas, 2017; Miller and Duckles, 2008). The contribution of genetic variants in genes for the estrogen receptors in the etiology of CVD is difficult to define most likely due to the complex nature of CVD in general. However, a man with a mutation in the gene for estrogen receptor-α developed accelerated atherosclerosis, which was related, in part, to the loss of production of nitric oxide from the endothelial cells that line the arterial wall (Sudhir et al., 1997a,b). Other studies of how genetic variants in steroid receptors affect CVD are mixed, most likely reflecting the complex nature of CVD and may be related to metabolic components of cardiovascular risk (Clegg et al., 2017; Winham et al., 2015; Weng et al., 2016).

Sex and Gender Differences in Development and Susceptibility to Risk Factors for CVD

Risk factors can be divided as "conventional" traditionally accepted factors, "nonconventional" risk factors and factors specific to women as they result from the impact of reproductive hormones or disorders. **Table 1** summarizes some of the sex/gender differences associated with these factors.

Conventional Risk Factors

Factors affecting development of CVD can be classified as those that are modifiable (behaviors such as smoking, diet, activity, and exposure to environmental pollutants) and those based on the biology of being male or female. Based on a large population study, a group of "conventional" risk factors for CVD were identified and included in the Framingham Risk Score: age, sex, smoking, systolic blood pressure, use of antihypertensive therapy, dyslipidemia, body mass index, and diabetes (Kannel et al., 1961). Risk for CVD increases with age and the age-associated risk varies by sex (Benjamin et al., 2017). Smoking increases risk for CVD in both men and women. The global prevalence of smoking is lower in women than in men (World Health Organization, 2016a,b); however women who smoke may have a higher relative risk of myocardial infarction (MI) compared to men (Huxley and Woodward, 2011). This greater risk may be more prominent in heavy smokers as shown in an Asian Pacific cohort where hazard ratios for CVD and stroke were significantly higher for women compared to men who smoked more than 20 cigarettes per day (Woodward et al., 2005). High blood pressure is responsible for a large proportion of CVD and develops at younger ages in men than in women. However, on a population level, there is no difference in the risk for CVD between men and women per unit increase in blood pressure (Peters et al., 2013). Cardiovascular risk per equivalent increase in total cholesterol is similar for men and women. Lipid changes occur primarily during the later phases of menopause, with menopause-related changes being similar in magnitude to the impact attributable to aging (Derby et al., 2009). Statin therapy is effective in modulating dyslipidemia in both men and women, but the risk and side effects of these medications may vary by sex (Mosca, 2015). The increased risk of cardiac mortality from excess weight is similar for men and women despite differences in the distribution of body fat around the waist or excess weight on the hips and thighs (Emerging Risk Factors et al., 2011). Increases in weight are associated with type II diabetes mellitus, high blood pressure, and an increase in inflammation, oxidative stress, and impaired endothelial function (King and Grant, 2016). However, type II

Table 1 Sex and gender differences in the development and susceptibility to risk factors for cardiovascular disease (Makkar et al., 1993)

Conventional risk factors	*Sex differences in the relative association of conventional cardiovascular risk factors*
Age	Men develop atherosclerotic disease at earlier ages than women however in both sexes there is a constant linear association of cardiovascular risk with age (Tunstall-Pedoe, 1998; Kannel et al., 1961)
Overweight and obesity	The relative risk of future cardiovascular disease among overweight and obese individuals is similar for both sexes. Menopause is associated with changing body composition with increased visceral adipose tissue (Benjamin et al., 2017; Singh et al., 2013; Lovejoy et al., 2008)
Race and ethnicity	Black men and women have twofold higher rates of fatal coronary heart disease events compared to white men and women. Black men have lower rates of incident nonfatal coronary heart disease events compared to white men, however black women have higher rates of nonfatal coronary heart disease compared to white women (Safford et al., 2012)
Smoking	There is a higher prevalence of smoking in men compared to women. However women smokers have a 25% greater risk of coronary heart disease compared to male smokers (Huxley and Woodward, 2011; World Health Organization, 2016a,b)
Hypertension	The prevalence of hypertension is similar between men and women, and the association with cardiovascular disease does not significantly differ by sex (Benjamin et al., 2017; Peters et al., 2015)
Dyslipidemia	Premenopausal women have higher HDL-cholesterol, and lower LDL-cholesterol and triglycerides compared to men. However, after menopause, the lipid profile changes in women such that LDL-cholesterol and triglycerides increase and HDL-cholesterol decreases (Wang et al., 2011; Derby et al., 2009)
Diabetes mellitus	Prevalence of type II diabetes is higher in men than women. However, diabetes confers a greater risk for stroke, coronary artery disease and heart failure in women compared to men (Peters et al., 2015)
Risk factors that are more predominant in women	*Sex differences in nonconventional cardiovascular risk factors*
Autoimmune disease	Autoimmune and inflammatory disease is strongly associated with cardiovascular disease. There is a strong predominance of autoimmune and inflammatory disease in women compared to men including rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis (Ngo et al., 2014)
Psychosocial factors (stress and depression)	Women have higher prevalence of psychosocial stressors such as depression and stress compared to men. Women are disproportionately susceptible to the effects of psychosocial stressors on cardiovascular risk (Xu et al., 2015; Vaccarino and Bremner, 2016)
Pregnancy related risk factors	*Evidence for an independent association and existing knowledge gaps*
Hypertensive disorders of pregnancy (preeclampsia [PE] and gestational hypertension [GH])	Hypertensive disorders of pregnancy, particularly PE doubles the risk of future coronary artery disease, stroke, and death, and results in a 4-fold risk of future heart failure (Wu et al., 2017) Controversy remains as to whether PE is an independent risk factor or acts through the increased risk of developing traditional risk factors such as hypertension (Heida et al., 2016)
Spontaneous preterm delivery	Spontaneous preterm delivery is associated with a two-fold increase in future cardiovascular disease. Evidence is limited on whether it is an independent and modifiable risk factor (Heida et al., 2016)
Gestation diabetes (GDM)	Gestational diabetes is associated with the future development of diabetes and metabolic syndrome. There is also an association between GDM and future cardiovascular risk independent of diabetes and metabolic syndrome
Small for gestational age (SGA) infant	There is a positive association between SGA infant and maternal cardiovascular disease, however, evidence is limited and there are no current specific recommendations for management (Heida et al., 2016)
Reproductive health related risk factors	*Evidence for an independent association and existing knowledge gaps*
Early Menarche	Menarche occurring $<$11 years (early) or $>$17 years (late) is associated with a modest increase in future cardiovascular disease risk (Canoy et al., 2015). Early menarche is associated with metabolic syndrome, thus, whether early menarche is independent of metabolic risk factor remains unclear
Natural Menopause	Natural menopause is associated with adverse lipid profile including increased LDL and lower HDL, and changes in body fat composition. There is no evidence of an independent association with future cardiovascular disease Although menopausal hormone therapy is not recommended solely for the primary prevention of cardiovascular disease, such treatments are effective in reducing vasomotor symptoms of menopause and if started soon after menopause in women with low metabolic risk profile may reduce risk; transdermal products carry less risk of venous thromboembolism than oral products (Baber et al., 2016; Canonico et al., 2007; Wild et al., 2013; Miller and Manson, 2013)
Oophorectomy	Bilateral and unilateral oophorectomy before the age of natural menopause carries and increased risk of cardiovascular mortality with is reduced with hormone replacement therapies (Shuster et al., 2010; Rivera et al., 2009)
Premature ovarian insufficiency (POI)	POI is associated with a 1.61 RR of developing or dying from cardiovascular disease. Studies show it is an independent risk factor after adjusting for conventional risk factors. Women with POI may benefit from hormone replacement therapy for symptom management and prevention of adverse cardiovascular effects until they reach the natural age of menopause (Faubion et al., 2015)
Polycystic ovarian syndrome (PCOS)	PCOS is associated with higher risk of developing diabetes, hypertension, dyslipidemia, and obesity. However after adjustment for traditional risk factors, there is no evidence that it is an independent risk factor for developing CVD (Heida et al., 2016)

diabetes confers a greater risk for CVD and stroke in affected women compared to men (Huxley et al., 2006; Peters et al., 2014). The reason for this sex difference in CVD risk with type II diabetes is not known but may reflect discrepancies in the management and treatment of the diabetes (Kramer et al., 2012).

Although these established risk factors have been integrated into various CVD risk calculators used to guide prevention and treatment of CVD, they typically underestimate risk for women, thus there is a need for development of other methods and models to account for conditions specific to women (Mosca et al., 2011; Cook and Ridker, 2014; Goff et al., 2014; Bushnell et al., 2014).

Nonconventional Risk Factors

Autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematous show a female predominance and can lead to accelerated atherosclerosis and premature coronary disease, particularly in younger women (Fairweather et al., 2008). Psychological stress significantly affects the risk of CVD in men and women (Dimsdale, 2008). However, acute stress leading to stress cardiomyopathy, or Takotsubo cardiomyopathy shows a clear predilection in women (Steptoe and Kivimaki, 2012).

Impact of Reproductive Factors on CVD Risk in Women

Ovarian function and estrogen production influence a woman's CV health throughout her life. The risk of CVD is higher in women who reach menopause at an early age such as in those with primary ovarian insufficiency or loss of menstrual periods due to excessive exercise or stress (Muka et al., 2016a). Although polycystic ovarian syndrome is associated with insulin resistance and dyslipidemia, there is ongoing debate as to whether these women have an increased risk of CVD (Shaw et al., 2008; Zhao et al., 2016). Oophorectomy before the natural age of menopause and early natural menopause (<46 years of age) is associated with increased risk of CVD (Rocca et al., 2016; Rivera et al., 2009; Wellons et al., 2012). However, some studies suggest that if estrogen therapy is initiated soon after oophorectomy in women <45 years of age or within 5 years of natural menopause, risk of CVD may be reduced (Rocca et al., 2016; Hodis et al., 2016; Hsia et al., 2006). Women who experience vasomotor symptoms during menopause may also have an increased risk of CVD (Thurston et al., 2017). Estrogen therapies are recommended for treatment of menopausal symptoms, but although such treatments might have associated effects on CVD risk reduction, they are not recommended for primary prevention of CVD (Baber et al., 2016; Salpeter et al., 2006; Mikkola et al., 2015; Boardman et al., 2015; Salpeter et al., 2009). There is a risk of venous thromboembolism with use of exogenous estrogen products; however, this risk is reduced with the use of transdermal compared to oral estrogen compounds (Canonico et al., 2007) (**Fig. 2**).

Regarding parity, there is a trend toward increased risk of CVD in nulliparous women (Simons et al., 2012). However, pregnancy-related complications such as gestational diabetes mellitus predispose women to development of type 2 diabetes later

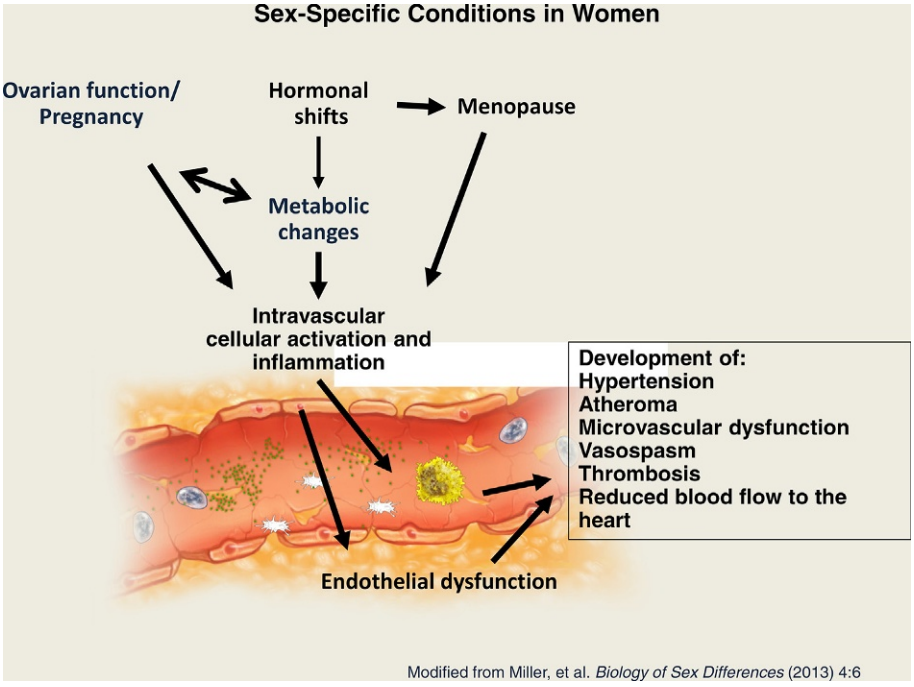

Fig. 2 Ovarian function specifically sex hormone changes at pregnancy, menopause and other states of hormone flux influence a woman's CV health throughout her life. Sex hormones initiate changes in cellular function through activation of steroid receptors on the cell surface, cytoplasm and nucleus. Tissue-specific responses to sex steroids reflect differences in binding of the hormone-receptor complex to response elements on various genes. Ultimately sex hormones modulate tissue responses to various traditional and sex specific risk factors.

in life. Hypertensive disorders of pregnancy (i.e., gestational hypertension, preeclampsia, eclampsia, and HELP syndrome) increase the risk for future CVD as well (Bellamy et al., 2007; Garovic and August, 2013).

Sex and Gender Differences in Ischemic Heart Disease

Definition

Ischemic heart disease (IHD) is a heterogeneous syndrome with many etiologies. It is characterized by myocardial ischemia from decreased oxygen supply to the heart usually as a result of conditions that cause decreased blood flow and/or increased oxygen demand to the myocardium. IHD is also referred to as coronary heart disease and coronary artery disease (CAD). Myocardial ischemia can occur in the absence of atherosclerotic occlusive CAD. Conditions that lead to prolonged ischemia result in myocyte cell death termed MI and the appearance of cardiac-specific proteins, of which troponin is the marker of choice (Thygesen et al., 2007). High sensitivity troponin, a new and more sensitive assay that can detect subclinical myocardial damage, is associated with long-term mortality, but measurement thresholds and prognostic values differ by sex (Everett et al., 2016).

Clinical Presentation

The clinical presentation of IHD varies depending on the chronicity of the underlying disease. One presenting symptom of IHD is substernal chest pain also called *angina pectoris*. However the spectrum can vary from no symptoms (silent MI) to more severe presentations with heart failure, arrhythmias, and sudden death. The vast majority of both men and women presenting with suspected IHD have angina pectoris (Vaccarino et al., 2013). However, there is a higher incidence of women presenting with atypical symptoms such as fatigue, sweating, dizziness, difficulty sleeping, anxiety, palpitations, indigestion, and arm discomfort (Canto et al., 2012; Khan et al., 2016). More men than women present with sudden cardiac death (SCD) from all causes; however, younger women with IHD have a higher incidence of SCD than men (Lewis et al., 2016). Sex and gender differences in the presentation of IHD could reflect differences in the underlying biology of IHD, modulation of the perception of pain by sex hormones, and sociocultural norms resulting in misattribution of symptoms by women (Bairey Merz et al., 2006; Craft, 2007; Lichtman et al., 2015).

Epidemiology

IHD is one of the leading causes of morbidity and mortality in the United States in both men and women (Benjamin et al., 2017). It is estimated that 16.5 million Americans over the age of 20 have IHD. In 2017 it is estimated that about 695,000 Americans will have a new MI or die due to IHD and 325,000 will have a recurrent MI (Benjamin et al., 2017). And while the overall mortality rates from IHD have declined in certain populations in the United States over the last 2 decades, individuals younger than 55 years of age, and young women in particular, have not had similar reduction in mortality rates (Wilmot et al., 2015; Benjamin et al., 2017).

Short- and long-term mortality rates after MI are worse in women, particularly in younger women, than in men (Jneid et al., 2008; Canto et al., 2012; Wilmot et al., 2015; Vaccarino et al., 1999). Sex and gender disparities in mortality rates from MI are multifactorial and include delays in recognition of symptoms and sex differences in the diagnosis, institution of therapy, comorbidities, as well as underlying differences in vascular biology including smaller arteries, diffuse atherosclerosis, and greater coronary microvascular dysfunction in women (Bairey Merz et al., 2006; Khan et al., 2016; Jneid et al., 2008).

Causes of Ischemic Heart Disease

Atherosclerosis and Ischemic Heart Disease

Atherosclerosis is a progressive disease marked by an accumulation of lipids and other fibrous material in the coronary arteries known as atheroma (or plaque) (Libby, 2002). Atherosclerosis is the central mechanism of the majority of IHD presentations. The innermost single layer of cells that line vessels, the vascular endothelium, plays a key role in maintaining homeostasis between vasomotor tone, hemostasis, inflammation, and oxidative stress (Iantorno and Weiss, 2014). Impaired endothelial function due to damage by various risk factors leads to atherosclerosis, with inflammation characterized by activated leukocytes adhering and penetrating into the endothelium causing progression of atherosclerosis (Libby, 2002).

Traditional risk factors as well as risk factors unique to women have different effects on the vascular endothelium and the progression of atherosclerosis (cross reference risk factor section and **Table 1**).

Acute presentations of IHD result from disruption of the cellular layer covering atheroma and exposure of lipid-rich core to intraluminal blood leading to thrombosis resulting in partial or complete occlusion of the coronary arteries, and cardiomyocyte death (Libby, 2008; Ross, 1993). Plaque rupture leading to coronary thrombosis is more common in men than in women, whereas plaque erosion seems to be the major contributor of acute presentations of IHD in women (Virmani et al., 2000; Yahagi et al., 2015). On presentation men have higher burden of plaque, more eccentric atheroma with more obstructive coronary arteries compared to women, who have a greater prevalence of nonobstructive CAD and/or normal coronaries on presentation (Han et al., 2008; Schulman-Marcus et al., 2016; Shaw et al., 2006; Shaw et al., 2009).

Coronary Microvascular Dysfunction

The vascular endothelium through expression of adhesion molecules and release of anticoagulant substances maintains vascular homeostasis (Iantorno and Weiss, 2014). Nitric oxide (NO) is a critical molecule produced by the endothelium that inhibits adhesion of cells to the endothelial surface and causes the underlying smooth muscle of the blood vessel to dilate. Loss of production of NO is sometimes referred to as "endothelial dysfunction." Endothelial function is an early marker of atherosclerosis (Ross, 1993). Coronary microvascular dysfunction (CMD) is defined as having microvascular endothelial and/or nonendothelial dysfunction that results in reduced perfusion leading to myocardial ischemia (Bairey Merz et al., 2017; Bairey Merz et al., 2006). Even with no obstructive epicardial CAD, CMD is associated with ischemic symptoms and is a potent predictor of future cardiovascular events above what might be predicted based on traditional risk factors (Reriani et al., 2016; von Mering et al., 2004; Pepine et al., 2010). CMD, previously thought to be more predominant in women, is present in both men and women and is associated with adverse outcomes in both sexes (Murthy et al., 2014; Reriani et al., 2016).

MI With Nonobstructive Coronary Arteries

MI with Nonobstructive Coronary Arteries (MINOCA) (Beltrame, 2013) is defined as MI without obstructive epicardial stenosis (no epicardial vessel with $\geq$50% stenosis) and is now recognized as a common cause of IHD in individuals, and particularly in women, presenting with IHD symptoms (Bairey Merz et al., 2017; Pasupathy et al., 2015; Planer et al., 2014; Beltrame, 2013). In the Women's Ischemia Syndrome Evaluation (WISE) study, about two-thirds of women who presented with IHD symptoms had nonobstructive CAD (Bairey Merz et al., 2006, 2017), now called Ischemia and No Obstructive Coronary Artery Disease (INOCA). Despite having mild coronary atherosclerosis, adverse CVD event rates and morbidity rates were significantly higher at 10 years in these women compared to asymptomatic women (Sharaf et al., 2013; Shaw et al., 2006; Jespersen et al., 2012; Gulati et al., 2009).

There are several potential etiologies for MINOCA including epicardial coronary artery and microvascular vasospasm, coronary microvascular dysfunction, plaque rupture and/or erosion with spontaneous fibrinolysis, coronary emboli, myocarditis, and spontaneous coronary artery dissection acting alone or in combination (Lanza et al., 2007; Reynolds et al., 2011; Camici and Crea, 2007; Galiuto et al., 2010; Mauricio et al., 2016; Bairey Merz et al., 2017). Both the vascular effects of sex hormones and risk factors unique to women may contribute to the higher predominance of MINOCA in women (Ng, 2007; Muka et al., 2016b). Identifying subtle vascular wall abnormalities in patients with possible MINOCA can be difficult with conventional invasive coronary angiography but can be better elucidated with Intravascular Ultrasound (IVUS) or Optical Coherence Tomography (OCT) (Takahashi et al., 2016).

There are no randomized clinical trials comparing secondary prevention treatment strategies for INOCA and MINOCA. Current treatment strategies are extrapolated from evidence-based treatment of obstructive CAD. However observational and small pilot studies suggest benefit from antiplatelet therapy, angiotensin receptor blockers, beta-blockers, aldosterone antagonists, and statins for both men and women (Camici and Crea, 2007; Lindahl et al., 2017; Bairey Merz et al., 2017).

Nonatherosclerotic Causes of IHD

Nonatherosclerotic causes of IHD are rare in the general population, but they are an important cause in younger people and women in particular (Waller, 1989; Saw et al., 2014). Appropriate diagnosis of these syndromes has critical implications on therapy and prognosis.

Spontaneous Coronary Artery Dissection

Spontaneous coronary artery dissection (SCAD) is a rare cause of MI but has a relatively high incidence in younger women suffering from MI, often related to pregnancy (Saw et al., 2016). Incidence rates are reported to be 22%–43% based on case series of women under age 50 presenting with MI but are likely higher when considering that these numbers miss cases that present with SCD, or younger women who are less likely referred for coronary angiography (Saw et al., 2014; Nakashima et al., 2016; Alfonso et al., 2016). Two proposed mechanisms for SCAD are tears in the intimal layer of the coronary arteries resulting in the accumulation of blood within the tear, and spontaneous rupture of the vasa vasorum leading to hemorrhage into the vessel wall (Saw et al., 2016; Angelini, 2007). Both mechanisms lead to accumulation of intramural hematoma and luminal obstruction leading to myocardial ischemia. There are several conditions that may predispose to SCAD by being associated with abnormal vessel wall integrity including connective tissue disorders, vasculopathies (such as fibromuscular dysplasia (FMD)), and sex hormones (Henkin et al., 2016).

FMD is a rare disorder of the medium-sized arteries that predominantly affects women. A diagnosis of FMD is present in about 72%–86% of women presenting with SCAD (Saw et al., 2016). Approximately 5% of women with SCAD present with acute MI during pregnancy (Saw et al., 2016). A combination of progesterone-related changes in the elastic fibers of the arterial wall weakening the media, estrogen-related prothrombotic effects, and hemodynamic changes associated with pregnancy may also contribute to SCAD (Saw et al., 2016). SCAD is also associated with other states of sex hormone flux including use of oral contraceptive and hormone replacement therapy (Saw et al., 2016). In clinically stable individuals, SCAD is managed conservatively as percutaneous coronary intervention (PCI) risks propagation of the arterial dissection flap, while more severe or unstable cases can

be treated with PCI or coronary artery bypass grafting (CABG) (Tweet et al., 2014). A diagnosis of SCAD is critical to guide acute management, to prognosticate on future events as well as guide further evaluation of extra coronary manifestations such as FMD, cerebral aneurysms, and increased risk in relatives (Saw et al., 2013, 2016).

Stress Cardiomyopathy

Stress cardiomyopathy, also known as Takotsubo cardiomyopathy, is a syndrome of transient reduction in left ventricular cardiac contractility, typically in the left ventricular apex, associated with a drop in ejection fraction, hypotension, electrocardiographic changes, and elevated cardiac enzymes consistent with MI but usually in the absence of angiographic evidence of stenosis (De Caterina et al., 2011). It is often described as being precipitated by sudden and intense emotional or physical stress (Wittstein et al., 2005). It most often (over 80% of cohorts) affects postmenopausal women; however in men it is more frequently related to physical triggers such as extreme exercise and trauma (Templin et al., 2015). The exact pathophysiology is not fully elucidated. One mechanism proposed is that a sudden catecholamine surge leads to myocyte toxicity and apoptosis from excessive stimulation of the β-adrenoreceptors (El-Battrawy et al., 2016; Lyon et al., 2008). Other mechanisms proposed to play a role in stress cardiomyopathy include diffuse epicardial vasospasm, coronary thrombi that spontaneously lyse, and diffuse microvascular dysfunction (Crea et al., 2015). Sex differences in the release of catecholamines by the adrenal medulla to stress, variations in the metabolism of catecholamines, and the loss of vascular protective effects of estrogenic modulation of the microvascular endothelium occurring at menopause may explain the higher incidence in older postmenopausal women (Lyon et al., 2008). Despite only having transient and reversible myocardial dysfunction, stress cardiomyopathy is still an important cause of morbidity and mortality. Thirty-day mortality rates are estimated to be similar to that of acute MI (Kurowski et al., 2007; Templin et al., 2015). In addition, other associated conditions including chronic heart failure, arrhythmias, and thromboembolic events account for significant morbidity from this condition (El-Battrawy et al., 2016; Templin et al., 2015). Acute therapy in stress cardiomyopathy is focused on hemodynamic support, optimization of heart failure therapy, and anticoagulation to prevent thromboembolism, while chronic management can include ACE inhibitors and beta-blockers.

Diagnostic Modalities Ideal for Assessing Nontraditional Causes of Ischemia

The differential diagnosis for ischemic chest pain in women is broad and encompasses atherosclerotic and nonatherosclerotic mechanisms. Recognition of the causes of ischemic chest pain is vital in instituting the appropriate therapy promptly and future secondary prevention strategies. The differential diagnosis and likelihood of the various possible diagnoses vary across women's lifespan (Fig. 3).

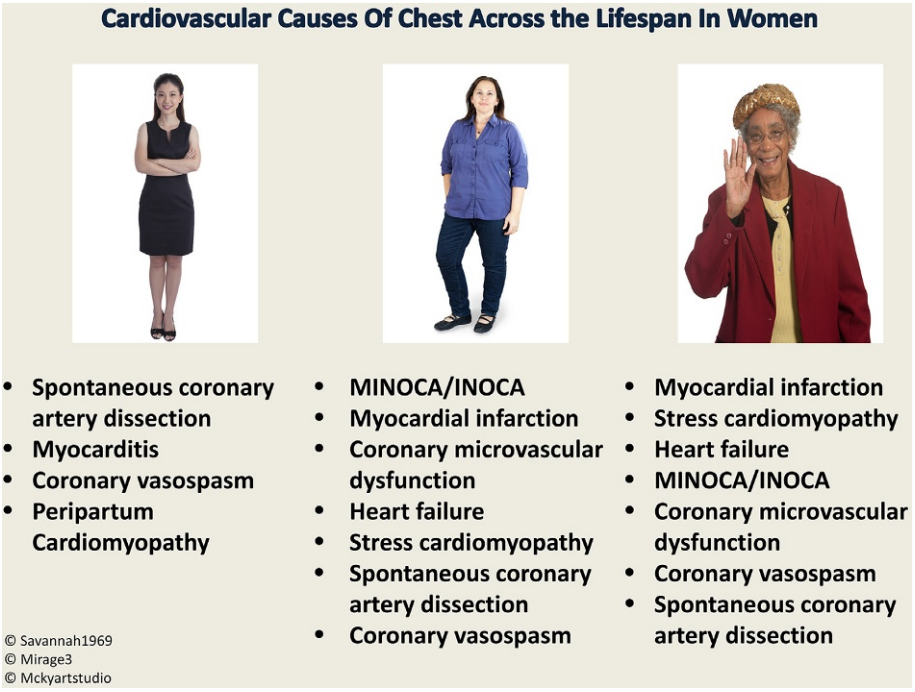

Fig. 3 Differential diagnosis of the cardiovascular causes of chest pain by age group and in order of prevalence. Savannah1969|Dreamstime.com—https://www.dreamstime.com/stock-photo-happy-senior-lady-waving-image23745740#res18337753 Happy Senior Lady Waving Photo. Mirage3| Dreamstime.com. Mckyartstudio | Dreamstime.com—https://www.dreamstime.com/stock-photo-portrait-happy-young-business-woman-isolated-confident-xwoman-standing-full-length-black-suit-businesswoman-real-estate-image42922496#res18337753 Portrait of Happy Young Business Woman Isolated Photo.

Noninvasive testing is the cornerstone of diagnosis of obstructive CAD and differences in body habitus, breast attenuation, smaller vessels, and predominance of coronary microvascular disease can lead to differences in diagnostic capabilities in women. Invasive coronary angiography, while allowing diagnosis of epicardial coronary stenosis, may be inadequate for diagnosis of MINOCA, INOCA, and CMD. Evolving technology such as optical coherence tomography OCT, coronary computed tomography (CCT), positron emission tomography (PET), and magnetic resonance imaging (MRI) to measure myocardial blood flow may enhance diagnostic capabilities in women who present with IHD (Takahashi et al., 2016; Schindler et al., 2010; Hays et al., 2012).

Prevention and Treatment Strategies

Etiologies of IHD are varied in pathophysiology (atherosclerotic and nonatherosclerotic) and anatomy (obstructive, nonobstructive, and coronary microvascular dysfunction). While landmark clinical trials have guided development of many therapies for obstructive CAD with improvements in mortality, women and especially minority women have been underrepresented in major cardiovascular trials (Ouyang et al., 2016). There is limited knowledge on the optimal management for etiologies of IHD including MINOCA, INOCA, and CMD (Bairey Merz et al., 2017). Current therapeutic strategies are extrapolated from knowledge from primary and secondary prevention clinical trials including antiplatelet therapies, statins, angiotensin receptor blockers, ACE inhibitors, as well as therapies targeted at specific conditions such as coronary vasospasm including nitrates and calcium channel blockers in CMD (Bairey Merz et al., 2017). Further research into roles that sex hormones and sex-specific risk factors contribute to the pathogenesis of IHD in women can potentially lead to successful therapeutic choices.

Heart Failure

Definition

Heart failure (HF) is a clinical syndrome defined by symptoms of fatigue, dyspnea, and fluid retention (Yancy et al., 2013). The causes of HF are diverse, but all lead to structural and/or functional impairment of ventricular filling or ejection of blood (Yancy et al., 2013). The syndrome of HF can result from diseases of the myocardium, pericardium, endocardium, or heart valves, but the majority of people with HF have a disorder of the left ventricular (LV) myocardium (Yancy et al., 2013). Historically HF was characterized by disorders in LV systolic function (currently termed heart failure with reduced ejection fraction, or HFrEF). However, it is recognized that heart failure despite preserved ejection fraction (HFpEF) accounts for nearly half of all HF hospital admissions, with morbidity and mortality rates comparable to HFrEF (Sharma and Kass, 2014).

Epidemiology

The annual incidence of HFrEF has stabilized over the past decade; however the incidence of HFpEF and hospitalizations for HFpEF have continued to rise (Owan et al., 2006; Bhatia et al., 2006). HFpEF has been reported to be more common in women than in men; women with HFpEF present at a later age and with more comorbidities than their male counterparts (Owan et al., 2006; Bhatia et al., 2006; Sharma et al., 2015; Steinberg et al., 2012).

Risk Factors

Sex and gender differences exist in the pathophysiology, presentation, and response to therapy for various etiologies of HF. Some risk factors for HF confer differential risk by sex and gender. Hypertension results in a threefold increased risk of HF in women compared to men, potentially due to differential adaptive mechanisms (Levy et al., 1996). HF risk is fivefold higher in women with diabetes versus twofold higher in men with diabetes (Kannel and McGee, 1979), potentially due to differential LV remodeling in setting of hyperglycemia (Galderisi et al., 1991). Obesity likewise confers a higher risk of HF in women compared to men (Kenchaiah et al., 2002). Other risk factors such as tobacco use, alcohol, and decreased physical activity have not shown significant interaction between sex and HF risk (Kenchaiah and Vasan, 2015).

Heart Failure With Reduced Ejection Fraction

HFrEF is defined as the clinical syndrome of HF and LV ejection fraction $\leq$40% (Yancy et al., 2013). Important risk factors for HFrEF range from traditional cardiovascular risk factors such as hypertension, obesity, IHD, valvular disease, to genetics, infections, infiltrative diseases, and toxins (Yancy et al., 2013). Men have a higher risk of HFrEF compared to women (Ho et al., 2013). However there are two important HF etiologies that are unique in women, peripartum cardiomyopathy, or show a marked preponderance in women, stress cardiomyopathy. Stress cardiomyopathy is described under nonatherosclerotic causes of IHD.

Peripartum Cardiomyopathy (PPCM)

Peripartum cardiomyopathy (PPCM) is defined as heart failure with reduced ejection fraction (≤45%) that presents within the last month of pregnancy or within five months after delivery; lack of an identifiable etiology of HF; and the absence of preexisting structural heart disease (Hibbard et al., 1999; Demakis and Rahimtoola, 1971; Sliwa et al., 2010b; Sharma, 2015). The incidence in the United States is 1/1000 live births; however there are substantial geographic and ethnic variations with reports of 1/300 in Haiti and 1/100 in parts of Nigeria suggesting a genetic and/or environmental component (Arany and Elkayam, 2016; Sliwa et al., 2010b; Sharma, 2015). Risk factors for PPCM include older age, black race, preeclampsia, and multiple gestations (Sliwa et al., 2010b; Arany and Elkayam, 2016). Proposed mechanisms leading to PPCM include viral myocarditis, malnutrition, and microchimerism (persistence of fetal cells in the maternal circulation) (Bultmann et al., 2005; Arany and Elkayam, 2016).

PPCM may also result from a loss of function of signal transducer and activator of transcription 3 (STAT 3), a transcription factor that upregulates antioxidant enzymes and modulates the activity of prolactin, a protein secreted in late pregnancy that is associated with oxidative stress and myocyte cell death (Halkein et al., 2013; Hilfiker-Kleiner et al., 2007). The dopamine 2-receptor agonist bromocriptine inhibits the release of prolactin, and in small-randomized controlled trials has shown benefit in the recovery of LV function, NYHA functional class, and reduction in mortality (Sliwa et al., 2010a; Haghikia et al., 2013). However, side effects, which include cessation of lactation, and concerns for teratogenicity, limit its use (Arany and Elkayam, 2016). Therapeutic targets based on novel cellular pathways described earlier remain to be explored (Arany and Elkayam, 2016; Arany et al., 2008).

Therapeutic Interventions for Heart Failure With Reduced Ejection Fraction

There are no clear sex differences in response to therapy in HFrEF. However, women are underrepresented in clinical trials of HF therapies, so sex differences may be missed (Westerman and Wenger, 2016). In addition women are significantly underrepresented in referrals for guideline-directed, device-based therapies including implantable cardioverter-defibrillators (ICD) (Hernandez et al., 2007) and cardiac resynchronization therapy (CRT-D) (Alaeddini et al., 2008; Hernandez et al., 2007). Probing the underlying reasons for less than optimal referrals for CRT-D and ICD may guide interventions to improve the numbers of these life-saving therapies offered to women.

Heart Failure With Preserved Ejection Fraction

Heart failure with preserved ejection fraction (HFpEF) is a syndrome that includes signs and symptoms of HF, an LV ejection fraction ≥50%, and the absence of valvular heart disease (Yancy et al., 2013). The hallmark of HFpEF is both abnormal relaxation and increased stiffness of the myocardium, typically called diastolic dysfunction (Redfield, 2016). Despite normal LV ejection fraction at rest, individuals with HFpEF are unable to augment LV systolic function appropriately to stress (Redfield, 2016).

Impaired myocardial relaxation has been associated with oxidative stress, depressed β-adrenergic signaling, intracellular calcium handling, and microvascular endothelial dysfunction (Sag et al., 2013; Norman et al., 2011; Sharma and Kass, 2014; Kranias and Bers, 2007). In addition, myocardial stiffness and fibrosis may result from inflammation, neurohormonal activation, and altered cell signaling (Sharma and Kass, 2014; Westermann et al., 2011; Glezeva and Baugh, 2014; van Heerebeek et al., 2008; Redfield, 2016). Animal models show estrogen protects against abnormal LV remodeling through activation of a cellular pathway involving cyclic guanosine monophosphate, protein kinase G, and nitric oxide (cGMP/PKG/NO pathway) (Sasaki et al., 2014). Others have shown that patients with HFpEF have lower myocardial PKG activity and lower cGMP associated with higher myocardial oxidative stress (van Heerebeek et al., 2012; Sharma and Kass, 2014). Better understanding of sex differences in humans is important as this pathway may be a potential therapeutic target of phosphodiesterase (PDE) inhibitors, which have shown therapeutic potential in animal models and in human tissue samples from HFpEF patients (Matyas et al., 2017; van Heerebeek et al., 2012; Takimoto et al., 2005).

Therapeutic Interventions in Heart Failure With Preserved Ejection Fraction

Traditional HF therapies including ACE inhibitors, angiotensin receptor blockers, aldosterone antagonists, and β-adrenergic receptor-blockers have not reduced mortality in HFpEF. Research into novel therapies is underway. These therapies include PDE inhibitors, neprilysin (a neutral endopeptidase) inhibitors, and inorganic nitrates (Bonderman et al., 2014; Boon et al., 2013; Tanaka et al., 2016; Westermann et al., 2011; Chiao et al., 2012; Sabbah et al., 2016; Zamani et al., 2015; Redfield et al., 2015; Borlaug et al., 2015; Borlaug et al., 2016).

In summary, much remains to be learned about the pathophysiology of HFpEF and the associated sex differences. Interventions aimed at HFpEF are further challenged by the clinical and mechanistic heterogeneity of the syndrome. Efforts are underway to further classify patients by symptoms, clinical presentation, and underlying mechanism of disease in order to target therapies based on identified clinical and molecular phenotypes (Shah et al., 2015).

Arrhythmias

Sudden Cardiac Death

Sex differences exist with regard to the frequency and characteristics of SCD. In general, women are more likely to present with SCD at home, have a lower frequency of an initial shockable rhythm, and are less likely to have a witnessed SCD (Bougouin et al., 2015). However, in the young (<40 years of age), two-thirds of SCD occur in men with the remaining one-third in women (Winkel et al., 2017). Further, sports-related SCD is significantly more common in males. Myocarditis and dilated cardiomyopathy appear to contribute to SCD equally between men and women, whereas atherosclerotic CAD, Brugada syndrome, and hypertrophic cardiomyopathy are more common in men and spontaneous coronary dissection (SCAD) and mitral valve prolapse are more common causes in young women (Basso et al., 2015). In middle-aged adults, men are still predominant with higher rates of obesity, sleep apnea, and seizure disorders contributing to the etiology of their SCD than women (Noheria et al., 2013). With regard to prognosis, women are more likely to survive to hospital discharge than men (Bougouin et al., 2015). Women with Brugada syndrome have a more favorable prognosis than men, with a less severe clinical presentation and less spontaneous type 1 Brugada syndrome (Sieira et al., 2016). Further, in arrythmogenic right ventricular dysplasia, men have a higher risk of ventricular fibrillation than women, while women have a higher risk of heart failure or cardiac transplantation (Kimura et al., 2016). With regard to therapy, women have a lower proportion of implantable cardiac defibrillators (ICDs) but once implanted have a similar mortality and less appropriate ICD shocks compared to men (Santangeli et al., 2010).

Endogenous and Exogenous Hormones and the QT Interval

A prolonged QT interval, the measure of the time between the start of the Q wave and the end of the T wave in the cardiac cycle, is a marker for an increased risk of ventricular tachyarrhythmias and SCD (Algra et al., 1991). Sex differences exist in the QT interval between men and women and during puberty; the QT interval in males shortens by 20 ms, whereas the QT of females remains unchanged (Rautaharju et al., 1992). These sex differences in the QT interval are due to sex hormone effects on the expression of ion channels, and direct effects on the activity of ion channels (Kurokawa et al., 2016). In particular, estrogen decreases potassium channel current and may lengthen the QT interval through this mechanism; whereas, progesterone decreases calcium channel current and increases potassium channel current and therefore shortens the QT interval. Both endogenous and exogenous sex hormones affect the QT interval (Zhang et al., 2011). Endogenous testosterone and progesterone shorten the action potential (Rodriguez et al., 2001) while estrogen lengthens the QT interval (Nakagawa et al., 2006). This results in a 6% shorter QT interval in men compared to women. Regarding menopause hormone therapy (MHT) there appears to be a differential effect of estrogen-alone therapy (ET) compared to estrogen plus progesterone therapy (EPT) on the QT interval. Women on ET have longer QT intervals compared with women who were never treated with MHT (Kadish et al., 2004). In contrast, women on EPT show no significant changes in the QT interval (Carnethon et al., 2003). Few studies have investigated effects of oral contraception on QT interval, particularly as QT changes may depend upon formulation. For example, oral contraceptives with antiandrogenic progestins may increase the QT interval (Sedlak et al., 2013). Females have a higher rate of developing drug-related long QT and related arrhythmias (Makkar et al., 1993). Women have a higher rate of developing drug-related long QT and related arrhythmias including life-threatening dysrhythmias such as drug-induced torsades de pointes (Makkar et al., 1993; Trinkley et al., 2013).

Atrial Fibrillation

The prevalence of atrial fibrillation is lower in women compared to men in all age categories; however the risk of subsequent morbidity including stroke and heart failure is higher in women (Rane and Patton, 2015). Further, the risk of thromboembolism is greater in women not treated with oral anticoagulation (Fang et al., 2005). Women are prescribed oral anticoagulants less often than men and when they are treated with anticoagulation, have higher bleeding complications (Rane and Patton, 2015). Women tend to be treated less and treated later and more conservatively than men with less catheter ablation procedures despite equivalent postoperative and long-term outcomes (Forleo et al., 2007).

Conclusion

Sex and gender differences are evident when considering the broad areas of CVD risk factors, myocardial ischemic syndromes, heart failure, and arrhythmias. These differences may be related to sex differences in underlying biological pathways, the impact of genes, epigenetic factors, sex hormones, behavioral and societal factors, or distinct subphenotypes of individuals. This chapter provides a background for students and researchers interested in better understanding these differences. However there are additional areas of importance that we have not addressed, including the impact of gender and transgender on behavioral and societal aspects related to CVD (**Fig. 4**). It is important to consider the multiple levels at which sex and gender affect an individual's susceptibility to disease, its presentation, and how they impact approaches to care. Improving our understanding of these differences has the potential to significantly improve our ability to effectively prevent disease and improve outcome for all patients.

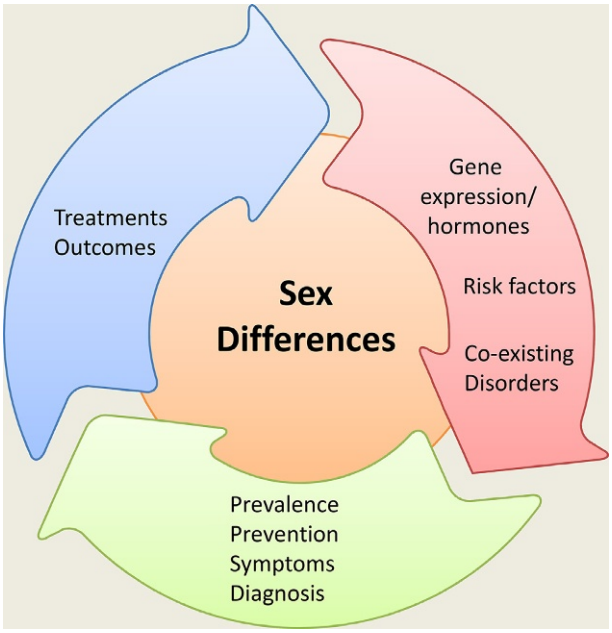

Fig. 4 Sex and gender differences in cardiovascular disease are influenced by genetic, sex hormone, and societal factors. In turn these differences have profound effects on the prevalence of the disease, the symptoms at presentation, the diagnostic capabilities, modalities for primary and secondary prevention and treatment outcomes.

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Sex Differences in the Physiology and Pathology of the Aging Heart

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Introduction

The incidence and prevalence of cardiovascular disease (CVD) rise dramatically with age in both sexes. This is perhaps not surprising as there is growing evidence that age itself causes distinct changes in the structure and function of the heart, even in individuals with no obvious signs of CVD. This suggests that age-dependent remodeling may lay the foundation for the development of CVDs later in life. Structural and functional changes associated with cardiac aging have been reported in hearts from humans and animal models of aging although there are characteristic differences between the sexes.

This article reviews the major structural, cellular, and functional changes in the aging heart. Changes at both the macroscopic and microscopic levels are described, and the functional abnormalities that arise from these modifications are discussed. We also highlight key differences in cardiac aging between males and females. Both human and animal studies are considered, as similar changes are seen in both models of cardiac aging. Finally, we discuss how these maladaptive changes affect clinical outcomes and may predispose older men and women to develop characteristic CVDs in old age.

Age-Associated Structural Changes in the Heart

The aging process is characterized by prominent changes in the structure of the heart. Structural changes in the heart involve atrial and ventricular remodeling with variations in wall thickness, dilatation, hypertrophy, fibrosis, and calcification of specific regions, such as the aortic valve, as summarized in **Table 1**. These changes have been identified both in older humans, typically older than 65 years of age, and in various animal models of aging. Most studies of cardiac aging in animals have used approximately 24-month-old mice and rats to model human aging. These rodents have an approximately 50% mortality rate at 24 months of age (Turturro et al., 1999), which is comparable to approximately 85-year-old in humans (Grundy, 2003).

With advancing age there are significant modifications in the gross morphologic structure of the heart. Marked calcification of the aortic valve leaflets with age has been observed in both older humans (New and Aikawa, 2011) and aging rats (Roosens et al., 2012). Whether the prevalence and extent of calcification differs between the sexes is unclear. Roosens et al. (2012) have suggested that mechanical stress and inflammation could be possible mechanisms for aortic valve calcification in rats. Atrial hypertrophy and dilatation also occur commonly in older adults, likely as a consequence of increased diastolic filling pressure (**Table 1**). Boyd et al. (2011) concluded that atrial dilatation becomes significant in older humans after the age of 75 years. Studies in animals have shown similar results, with a marked age-associated increase in left atrial dimensions in older mice and rats (Dai et al., 2009; Lau et al., 2013; Medrano et al., 2015). Whether there are male–female differences in atrial hypertrophy and dilatation has not been clearly investigated in animal models although there is no evidence for such differences in human work (Boyd et al., 2011).

Atrial and ventricular fibrosis also increase with age both in humans (Mirza et al., 2012; Dzeshka et al., 2015; Lam et al., 2012; Ling et al., 2012) and in animals (Lau et al., 2013; Lindsey et al., 2005; Horn et al., 2012). This increase in fibrosis may arise as a consequence of myocyte loss plus changes in loading that promote fibroblast proliferation. Animal studies have not yet addressed whether there is a sex difference in fibrosis with aging although there is no evidence for male–female differences in human work. There is a prominent increase in left ventricular (LV) wall thickness with age, and this increase is much greater in women than in men (Hees et al., 2002; Chen et al., 2016). LV wall thickness also increases with age in mice and rats, but whether this differs between the sexes is not known (Lindsey et al., 2005; Medrano et al., 2015; Fannin et al., 2014). This age-dependent increase in LV wall thickness could be caused by a combination of factors, including myocyte hypertrophy, increased fibrosis, and higher LV afterload due to age-dependent stiffening of the vasculature (Lakatta and Levy, 2003). By contrast, whether LV mass changes with age is controversial although LV mass-to-volume ratio increases with age in both sexes (Chen et al., 2016; Gebhard et al., 2013; Eng et al., 2015; but c.f. Hees et al., 2002). Recently, Chen et al. (2016) reported that women exhibit a greater increase in LV mass than men although this has not been seen in all studies (Hees et al., 2002). LV mass also appears to increase with age in animal models

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Table 1 Age-associated structural changes in the heart

Structural change[a]	*Model*	*Aged group*[b]	*Sex difference*	*Possible causes/mechanism*	*References*
Aortic valve calcification	Human	65 years+	No evidence [c]	Calcification due to mechanical stress and/or inflammation	(New and Aikawa, 2011)
	Rats	24–30 months	Not known[c]		(Roosens et al., 2012)
Atrial hypertrophy and dilatation	Human	20–80 year	No evidence	Increased diastolic filling pressure	(Boyd et al., 2011)
	Mouse	15–31 months	Not known		(Dai et al., 2009; Medrano et al., 2015; Lau et al., 2013)
	Rat	15–23 months			
Atrial fibrosis	Human	65 years+	No evidence	Myocyte loss/change in loading promotes fibroblast proliferation and fibrosis	(Mirza et al., 2012; Dzeshka et al., 2015)
	Rats	12–15 months	Not known		(Lau et al., 2013)
Increased LV mass	Human	21–96 years	More prominent in females	Myocyte hypertrophy, increased fibrosis plus vascular changes that increase LV afterload	(Chen et al., 2016; Gebhard et al., 2013; Eng et al., 2015; but *c.f* Hees et al., 2002)
	Mouse	16–29 months	May be more prominent in males		(Dai et al., 2009; Koch et al., 2013; Shinmura et al., 2011)
	Rats	29 months			
Increased LV wall thickness	Human	21–96 years	More prominent in females	Myocyte hypertrophy, increased fibrosis plus vascular changes that increase LV afterload	(Hees et al., 2002; Chen et al., 2016)
	Mouse	15–31 months	Not known		(Lindsey et al., 2005; Medrano et al., 2015; Fannin et al., 2014)
	Rat	26–30 months			
LV fibrosis	Human	75 years+	No evidence	Myocyte loss/change in loading promotes fibroblast proliferation and fibrosis	(Dzeshka et al., 2015; Mirza et al., 2012; Lam et al., 2012; Ling et al., 2012)
	Mouse	15–23 months	Not known		(Lindsey et al., 2005)
	Sheep	>8 years			(Horn et al., 2012)

[a] *LV*, left ventricle.
[b] Compared to young adult humans or animals.
[c] No evidence indicates that studies have included both sexes and found no difference between sexes. Not known indicates that studies have not investigated sex differences.

(Dai et al., 2009; Shinmura et al., 2011). One mouse study showed that LV mass increases with age in male rodents more than females (Koch et al., 2013), whereas the opposite is seen in humans, so more work in this area is required.

These studies demonstrate that age-related changes in the heart occur at the macroscopic level and that there are potentially some differences between the sexes. Still, most studies in animal models have used exclusively male animals, so the question of male–female differences has rarely been addressed. Some of the structural changes described in **Table 1** have cellular correlates, as outlined in the next section.

Age-Associated Cellular Changes in the Heart

It is evident from the preceding discussion that the aging process affects the gross morphological structure of the heart in both humans and animal models. There are also characteristic age-dependent changes in the heart at the microscopic level, as summarized in **Table 2**. The number of pacemaker cells in the sinoatrial node (SAN) of the heart declines markedly with age in humans although it is unclear whether this differs between the sexes (Lakatta, 1993; Mirza et al., 2012). Whether the number of pacemaker cells also declines in aging animals has not yet been investigated. The expression of genes involved in sinoatrial node function does decline with age in rats, but whether these rats were male, female, or both was not specified (Tellez et al., 2011). Studies in humans and nonhuman primates report that the number of ventricular myocytes declines with age, and there is evidence that this cell loss is more prominent in males than in females in both models (Olivetti et al., 1991, 1995; Zhang et al., 2007). The loss of myocytes in aging has been attributed to apoptosis, necrosis, autophagy, and potentially a limited ability of stem cells to regenerate (Sheydina et al., 2011; Dai et al., 2012; Leon and Gustafsson, 2015; Hariharan and Sussman, 2015). Interestingly, apoptosis increases with age in hearts from male but not female monkeys (Zhang et al., 2007). This may help explain the marked ventricular myocyte loss seen in older male hearts although additional experiments in this area would be informative.

The loss of ventricular myocytes with age would be expected to increase the mechanical burden on surviving myocytes and promote their hypertrophy. This has been investigated experimentally in both humans and animal models of aging (**Table 2**). Olivetti and colleagues used morphometric techniques to investigate the impact of age and sex on the human heart (Olivetti et al.,

Table 2 Age-associated cellular changes in the heart

Cellular change[a]	*Model*	*Aged group*[b]	*Sex difference*	*Possible causes/ mechanism*	*References*
Decreased number of SAN (pacemaker) cells	Human	>60 years	Unclear	Unclear	(Mirza et al., 2012; Lakatta, 1993)
Reduced number of ventricular myocytes	Human	16–95 years	More prominent in males	Apoptosis, necrosis, autophagy	(Olivetti et al., 1991, 1995)
	Monkey	17–26 years	More prominent in males		(Zhang et al., 2007)
Ventricular myocyte hypertrophy	Human	16–95 years	More prominent in males	Reactive hypertrophy due to myocyte loss	(Olivetti et al., 1991, 1995)
	Rats	24–31 months	More prominent in males		(Feridooni et al., 2015)
	Mice	24–31 months			
	Guinea pigs	96 months+			
	Rabbits	26 months			
	Monkey	17–26 years			
Increased number of fibroblasts in atria and ventricles	Human	54–93 years	More prominent in males	Replacement fibrosis due to myocyte loss	(Liu et al., 2013)
	Rodents	24 months	No clear sex difference in animal models		(Horn and Trafford, 2015; Horn et al., 2012; Jugdutt et al., 2010)
	Sheep	>8 years			
	Dogs	up to 10 years			

[a]*SAN*, sinoatrial node.
[b]Compared to young adult humans or animals.

1991, 1995). Their studies showed that ventricular myocyte volume increases with age and that this increase is much greater in men than in women (Olivetti et al., 1991, 1995). A number of different laboratories have investigated ventricular myocyte hypertrophy in aging by measuring the length, width, and cross-sectional area of cells isolated from various aging animal models. These studies have shown that age-associated ventricular myocyte hypertrophy occurs in aging male rats, mice, guinea pigs, rabbits, and monkeys (reviewed by Feridooni et al., 2015). Only a few studies have explored the impact of age on ventricular myocyte morphology in female animals, and the results are less clear. Some have reported an age-related increase in cell length, width, and/or cross-sectional area in females while others have not (Mellor et al., 2014; Howlett, 2010; Grandy and Howlett, 2006; Dibb et al., 2004). This suggests that concentric hypertrophy (lateral growth of ventricular myocytes) and eccentric hypertrophy (longitudinal growth) may occur in hearts from older males and perhaps also females. However, whether age-dependent ventricular myocyte loss and hypertrophy occurs at different rates and through different mechanisms in male and female hearts has not been firmly established.

While the number of ventricular myocytes declines with age, there is strong evidence for proliferation of cardiac fibroblasts in the aging heart (Horn and Trafford, 2015), as summarized in **Table 2**. Fibroblasts are the cells that produce both extracellular matrix and collagen, and their proliferation leads to interstitial fibrosis in the atria, sinoatrial node, and ventricles of older adults (Mirza et al., 2012; Dzeshka et al., 2015). Interestingly, studies in humans have shown that this age-dependent increase in fibrosis is more prominent in men than in women, at least in the ventricles (Liu et al., 2013). Studies in animal models including rodents, sheep, and dogs have reported that fibroblast proliferation, collagen accumulation, and interstitial fibrosis increase with age in both the atria and the ventricles although whether this differs between the sexes has not yet been investigated (Horn and Trafford, 2015; Lau et al., 2013; Horn et al., 2012; Jugdutt et al., 2010). These macroscopic and microscopic changes in the structure of the heart with age are thought to be maladaptive and may adversely affect myocardial function, as explored in more detail in the next section.

Age-Related Changes in Heart Function

The previous sections have discussed structural and cellular changes in the heart with age, which along with age-related changes in calcium handling (Feridooni et al, 2015) can lead to changes in cardiac function. Age-associated functional changes are observed in both the electrical and contractile function of the heart at the cellular and organ level. These changes are summarized in **Table 3** and include reduced heart rate variability, slowed conduction, increased action potential duration in atrial myocytes, reduced systolic function, smaller contractions in ventricular myocytes, reduced early diastolic filling of the LV, and increased late diastolic filling. Although some of these changes have been shown in animal studies to be more prominent in males, sex differences in most of these age-related functional changes have not been investigated (**Table 3**).

Table 3 Age-related changes in heart function

Functional changes[a]	*Model*	*Aged group*[b]	*Sex difference*	*Possible causes/mechanism*	*References*
Impaired ability to ↑ HR upon standing from the supine position, lower maximal HR in exercise	Human	19–89 years	No evidence[c]	↓ responsiveness to ß-adrenergic stimulation, ↓ number of pacemaker cells, reduced ion channel expression in SAN cells	(Nes et al., 2013; Mirza et al., 2012; Ferrara et al., 2014; Lakatta and Levy, 2003)
	Rats	24 months	Not known[c]		(Tellez et al., 2011)
Prolongation of the QRS complex and slowed conduction	Human	65 years+	No evidence	↓ expression of connexion-43; leads to fewer cell-to-cell connections	(Mirza et al., 2012)
	Mouse	24 months	Not known		(Bonda et al., 2016)
Atrial myocytes are depolarized, with ↑ APD	Rat	24–28 months	Not known	Changes in underlying Ca^{2+} and K^{+} currents	(Dun and Boyden, 2009)
	Dog	>8 years			
Depressed systolic function (↓ stroke volume, ↓ ejection fraction)	Humans	35–94 years	May be more prominent in males	↓ number of ventricular myocytes, ↓ ability of ventricular myocytes to contract	(Claessens et al., 2007 (but c.f. Eng et al., 2015)
	Rats	26–30 months	More prominent in males		(Fannin et al., 2014; Shinmura et al., 2011; Koch et al., 2013; Dai et al 2009; Dai et al., 2014)
	Mice	12–31 months			
Smaller, slower contractions in isolated ventricular myocytes	Rats	24 months	More prominent in males	↓ peak Ca^{2+} current, ↓ Ca^{2+} release from sarcoplasmic reticulum stores, smaller Ca^{2+} transient	(Howlett, 2010; Grandy and Howlett, 2006; Feridooni et al., 2015)
	Mice	24 months			
Slowed rate of early LV filling in diastole, diastolic dysfunction	Humans	20–80 years	No evidence	Increased LV fibrosis, increased myocyte stiffness mediated by titin, ↓ rate of decay of Ca^{2+} transient	(Boyd et al., 2011; Strait and Lakatta, 2012; Fleg and Strait, 2012)
	Rats	26–30 months	Not known		(Fannin et al., 2014; Medrano et al., 2015; Shinmura et al., 2011; Koch et al., 2013; Dai et al., 2009; Dai et al., 2014)
	Mice	12–31 months			
Increased atrial contraction and ↑ late diastolic filling	Human	20–85 years	No evidence	Slow LV filling ↑ diastolic filling pressure, causes atrial dilation plus hypertrophy and ↑ atrial contraction	(Strait and Lakatta, 2012; Fleg and Strait, 2012)

[a]*APD*, action potential duration; *HR*, heart rate; *LV*, left ventricle; *SAN*, sinoatrial node.

[b]Compared to young adult humans or animals.

[c]No evidence indicates that studies have included both sexes and found no difference between sexes. Not known indicates that studies have not investigated sex differences.

Age does not affect resting heart rate in studies of aging humans and rodents of both sexes (Lakatta and Levy, 2003; Fannin et al., 2014; Medrano et al., 2015; Koch et al., 2013). Aging is associated, however, with an impaired ability of the heart rate to increase when a person moves from a supine to standing position (Lakatta and Levy, 2003), and a reduced maximal heart rate during exercise (Nes et al., 2013). Potential mechanisms for this include a decline in pacemaker cell number (Mirza et al., 2012) and reduced ion channel expression in pacemaker cells in old age (Tellez et al., 2011). Additionally, an impaired responsiveness to ß-adrenergic stimulation with aging (Ferrara et al., 2014) could contribute to this reduced heart rate response. In younger people, activation of the sympathetic nervous system (by exercise, for example) results in an increase in both heart rate and the force of contraction, through the release of catecholamines that activate β-adrenergic receptors. However, in both aging humans and animals there is evidence of a decline in responsiveness to ß-adrenergic stimulation (Ferrara et al., 2014; Farrell and Howlett, 2008; Brodde and Leineweber, 2004; White et al., 1994), which may contribute to a decline in heart rate response. Interestingly, one study in male and female monkeys found an age-related decline in responsiveness to ß-adrenergic stimulation in males but not in females (Takagi et al., 2003). Overall however, as most studies have not explored sex differences, and the majority investigated used only male, the effect of sex on heart rate response in old age and the potential mechanisms involved still remains to be investigated.

Changes in the electrical function of the heart occur in old age. There is an age-associated slowing of conduction, and prolongation of the QRS complex, in humans and animal models (Bonda et al., 2016; Mirza et al., 2012). A recent animal study in aging male mice identified that a potential mechanism for this may be reduced cell-to-cell connections as a result of lower expression of connexion-43 (Bonda et al., 2016). Animal studies have also investigated changes in the electrophysiological properties of atrial myocytes with old age. These have shown that in older animals, compared to young animals, right atrial myocytes are depolarized and have longer action potentials (Dun and Boyden, 2009). These changes are associated with changes in underlying ion currents, including an increase in potassium currents and a decrease in calcium currents with old age (Dun and

Boyden, 2009). There are limited clinical studies using atrial myocytes, so whether these same changes occur in older humans is not yet clear. Potential sex differences in atrial myocyte electrophysiology or changes to conduction speed in old age are not known, and further mechanistic studies including females are needed.

Systolic function has historically thought to be preserved in aging, with no change in measures such as stroke volume and ejection fraction, between younger and older people (Lakatta and Levy, 2003). Recent evidence, however, suggests a decline in systolic function, at least in men, with aging (Claessens et al., 2007; Eng et al., 2015). The extent to which this decline occurs in women is less clear, with one clinical study finding that cardiac contractility is well preserved in women (Claessens et al., 2007), and another showing that stroke volume decreased in men and women to the same extent (Eng et al., 2015). The difference in these findings may be due to the different age ranges of the studies (35–55 years vs 54–94 years, respectively). Interestingly, animal studies also find that measures of systolic function, including stroke volume and ejection fraction, decline with age in males, but not in females (Koch et al., 2013; Shinmura et al., 2011; Dai et al., 2009, 2014; Fannin et al., 2014).

Changes in ventricular myocyte contractile function are also seen with aging. Ventricular myocytes isolated from aged rodents have smaller, slower contractions than cells from young animals (Howlett, 2010; Grandy and Howlett, 2006). Furthermore, consistent with the work cited above in hearts in vivo, the decrease in contractile function in ventricular myocytes is greater in males than in females (Howlett, 2010; Grandy and Howlett, 2006). Changes in calcium handling in aged isolated myocytes provide a potential mechanism for this, with decreases in peak calcium transients in cells isolated from aged male mice (Howlett, 2010; Grandy and Howlett, 2006). There is also a reduction in peak calcium currents, which may result in less calcium influx in myocytes from aged male rodents (Feridooni et al., 2015). In cells isolated from aged female rodents however, there is no change in peak calcium transients or calcium currents (Feridooni et al., 2015) and cells from aged female sheep in fact display an increase in these measures (Dibb et al., 2004). It remains to be confirmed whether these changes in myocyte contractility and calcium handling are seen in humans of both sexes.

Diastolic dysfunction, and a reduction in the heart's ability to relax, is a commonly seen change in the aging heart. Impairment of ventricular relaxation results in left ventricular filling of older hearts that is both slower, and occurs later, than in young adult hearts (Fleg and Strait, 2012; Lakatta and Levy, 2003; Strait and Lakatta, 2012; Boyd et al., 2011). Problems with relaxation, known as diastolic dysfunction, are also seen in both male and female aging animal models (Shinmura et al., 2011; Fannin et al., 2014; Dai et al., 2009, 2014; Medrano et al., 2015; but c.f. Koch et al., 2013). Potential mechanisms for diastolic dysfunction in old age have been investigated in both human and animal studies. Increased LV fibrosis (Horn and Trafford, 2015) may contribute to reduced ventricular relaxation and thus reduced passive filling of the LV (Loffredo et al., 2014). Myocyte stiffness, as a result of age-related changes to the sarcomeric protein titin, may also contribute to ventricular stiffness (Hamdani et al., 2013). There is also a slower calcium transient decay seen in ventricular myocytes isolated from older animals, compared to younger animals (Feridooni et al., 2015). This results in prolonged availability of cellular calcium, which causes ongoing activation of contractile filaments and may delay ventricular relaxation (Loffredo et al., 2014; Dai et al., 2012). As a result of slow early LV filling in diastole, there is increased late diastolic filling, accompanied by an increased force of atrial contraction (Strait and Lakatta, 2012). The contribution of the atria to LV filling in old age is thus much greater than in younger people. This age-related decrease in LV filling speed also results in an increase in diastolic filling pressure, which can result in atrial dilation and hypertrophy (Fleg and Strait, 2012). Although diastolic dysfunction and changes to LV filling appear to occur in both sexes, any changes in underlying causes are unclear as few mechanistic studies have used females, and more studies are needed.

The implications of changes in cardiac function, and any sex differences in these changes, on clinical outcomes and associations with cardiovascular diseases are explored in the next section.

Sex Differences in Clinical Cardiac Outcomes With Aging

Many of the cellular, structural, and functional changes described in the previous sections are maladaptive and can lead to increased risk of serious clinical outcomes in aging patients. Clinical outcomes that are associated with significantly increased risk in old age are summarized in **Table 4** and include heart failure with reduced ejection fraction (HFrEF), heart failure with preserved ejection fraction (HFpEF; characterized by diastolic dysfunction), bradycardia, and the need for pacemaker implantation and atrial fibrillation. Clinical studies have also identified significant differences in risk, prevalence, and outcomes of these clinical cardiac complications between males and females.

Aortic valve calcification increases in old age. This age-associated change in the heart can obstruct outflow from the LV and may lead to the development of heart failure (New and Aikawa, 2011). This risk appears to be higher for men than for women (Milin et al., 2014). The decline in myocyte number, decreased size of myocyte contraction and overall depressed systolic function, associated with old age can also lead to an increased risk of HFrEF. There is some evidence to suggest that each of these mechanisms occurs more in males than in females (Zhang et al., 2007; Olivetti et al., 1991, 1995; Howlett, 2010; Grandy and Howlett, 2006) although most of this research has only been completed in male animals. Nonetheless, these differences may explain why older men are more likely than older women to develop HFrEF (Dunlay and Roger, 2012).

Old age is also associated with an increased risk of HFpEF, which is characterized by diastolic dysfunction with no significant reduction in ejection fraction (Kaila et al., 2012; Dunlay and Roger, 2012). Potential mechanisms for this adverse clinical outcome include slow passive LV filling and slowed relaxation, increased LV fibrosis and thickness and ventricular myocyte changes such as increased stiffness and slowed calcium removal. Overall, these age-associated maladaptive changes can cause the atria to make a

Table 4 Sex differences in clinical cardiac outcomes with aging.

Age-related clinical outcome[a]	*Sex difference*	*Potential mechanisms*	*References*
Increased risk of HFrEF	Risk is higher for men than for women	Aortic valve calcification, obstruction of LV outflow	(New and Aikawa, 2011; Milin et al., 2014)
Increased risk of HFrEF	Increased risk in men	Reduced numbers of ventricular myocytes, and myocyte hypertrophy Decreased myocyte contraction, and decreased Ca^{2+} transients Decreased systolic function	(Dunlay and Roger, 2012)
Increased risk of HFpEF	More prevalent in women but associated with worse outcomes in men	Slow passive LV filling, diastolic dysfunction Increased left ventricular fibrosis, stiffness, and wall thickness Slowed Ca^{2+} removal, increased diastolic Ca^{2+}, and increased myocyte stiffness (titin changes)	(Dunlay and Roger, 2012; Kaila et al., 2012; Loffredo et al., 2014; Greiten et al., 2014; Lam et al., 2012)
Increased risk of bradycardia and pacemaker implantation	Not known	Decreased expression of SAN node channels, SAN dysfunction	(Mirza et al., 2012)
Increased risk of atrial fibrillation	More prevalent in men but associated with worse outcomes in women	Atrial hypertrophy and fibrosis Remodelling of ion channels in atrial myocytes	(Pancholy et al., 2014)

[a]*HFrEF*, heart failure with reduced ejection fraction; *HFpEF*, heart failure with preserved ejection fraction LV left ventricle; *SAN*, sinoatrial node.

significantly larger contribution to ventricular filling, which reduces cardiac output and predisposes older people toward the development of HFpEF (Loffredo et al., 2014; Kaila et al., 2012). The risk factors associated with HFpEF and the risks associated with a diagnosis differ between men and women. Myocardial ischemia is an important risk factor in men, while hypertension is a greater risk factor in women (Greiten et al., 2014). Interestingly, although HFpEF is more common in older women (Dunlay and Roger, 2012; Greiten et al., 2014), it is associated with worse outcomes in men, including increased risk of hospitalization and mortality (Lam et al., 2012). HFpEF represents a significant clinical problem in the older population, with an increasing prevalence, and a lack of treatment options (Greiten et al., 2014; Loffredo et al., 2014).

Age-related dysfunction of the sinoatrial node, including reduced numbers of pacemaker cells and reduced expression of ion channels in these cells is associated with an increased risk of bradycardia and pacemaker implantation in old age (Mirza et al., 2012). Potential sex differences in the risk of developing bradycardia in old age are not known. There are important sex differences, however, in the increased risk of atrial fibrillation with aging. Although there is a higher risk for developing atrial fibrillation amongst men, it is associated with worse outcomes such as stroke or systemic embolization in women (Pancholy et al., 2014). Mechanisms for the increased risk of atrial fibrillation in old age in both sexes include atrial hypertrophy and fibrosis and changes in ion channel expression of atrial myocytes although the potential sex differences in these underlying mechanisms are not yet known.

Overall, there is an increased risk of HFrEF, HFpEF, bradycardia, and atrial fibrillation in old age. There are some important sex differences in both the risk of these clinical outcomes and the associated risk of poor outcomes with these clinical diagnoses. More research is needed to identify potential sex differences in the underlying structural and functional changes that result in these clinical outcomes.

Summary

The age-related changes in the heart at the cellular, structural, and functional level and the clinical implications of these changes for both men and women are summarized in **Fig. 1**. For some of these age-related cardiac changes there is either no evidence of any sex-related differences or no research to date exploring sex differences. Age-related changes without any clear known sex effect include a reduction in sinoatrial node cells, aortic valve calcification, atrial hypertrophy and fibrosis, LV fibrosis, a decrease in maximal heart rate, an increased QRS complex and slowed conduction, prolonged action potential duration in atrial myocytes, diastolic dysfunction, and an increase in atrial contraction force. Additionally, older males and females are at increased risk of bradycardia and pacemaker implantation. There are some clear sex differences, however, in some of the changes that occur to the heart with age. Older males appear to have a greater reduction in myocytes, more significant myocyte hypertrophy, and a greater increase in fibroblasts than older females. These cellular changes may contribute to functional changes seen in older males, such as a more prominent decrease in myocyte contractions and reduced systolic function, when compared to older females. Furthermore, these

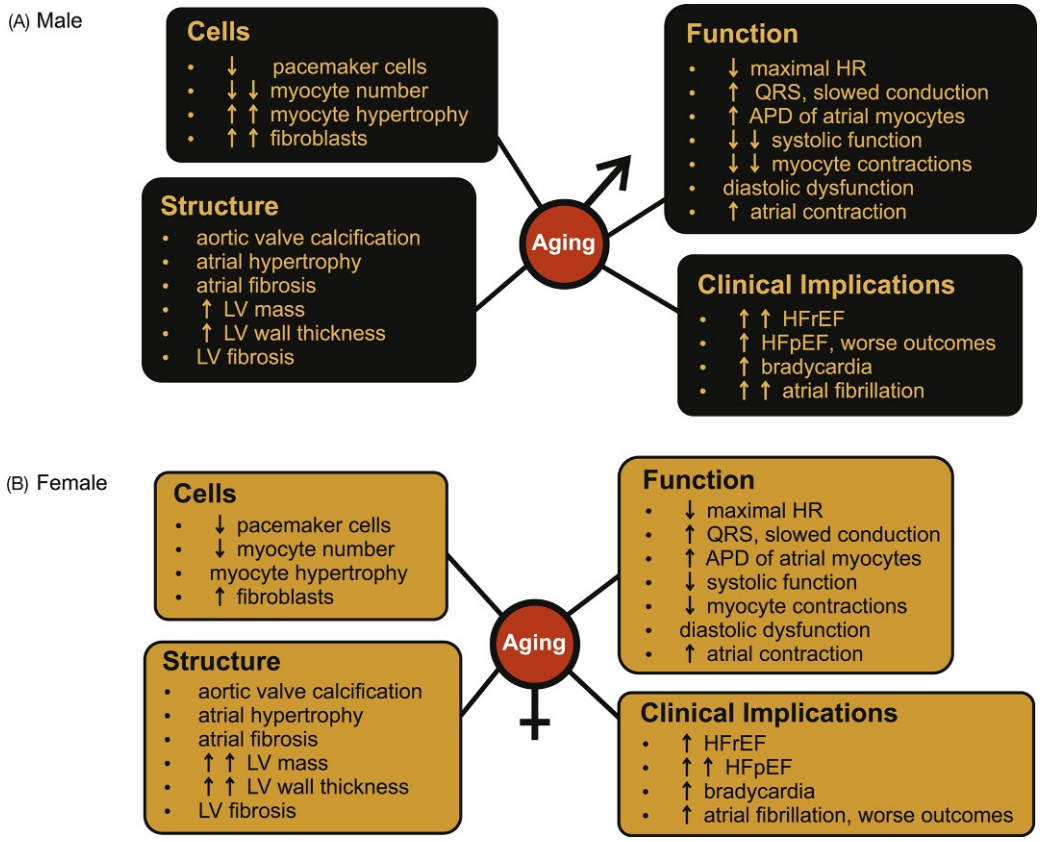

Fig. 1 Major age-related cellular, structural, functional, and clinical changes in the heart. (A) Characteristic cardiac aging changes in the male heart. (B) Changes that typically occur as a function of age in the female heart. HR, heart rate; *HFrEF*, heart failure with reduced ejection fraction; *HFpEF*, heart failure with preserved ejection fraction; *LV*, left ventricle; *APD*, action potential duration.

changes result in differences in clinical outcomes for older males including an increased risk of HFrEF and atrial fibrillation, and worse outcomes associated with HFpEF. Older females, on the other hand, have more significant increases in LV mass and wall thickness, than older males, which may contribute to the increased risk of HFpEF in older females. Older females also have worse outcomes associated with atrial fibrillation. Overall, there are some important sex differences in cardiac changes with aging that result in different clinical outcomes for males and females. More research is needed however, especially in female animals, to further understand these clinical differences, their implications, and their structural and functional mechanisms.

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Signaling in Cardiac Physiology and Disease

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Introduction

Cardiovascular disease (CVD) is a broad spectrum pathology that involves deleterious signaling mechanisms leading to remodeling of blood vessels and the heart manifesting in a disease state. CVD includes coronary artery diseases (CAD), stroke, heart failure, hypertensive heart disease, heart arrhythmia, congenital heart disease, myocarditis, aortic aneurysm, and peripheral artery disease. CVD is a major cause of death in the world, causing almost 17.3 million fatalities per year. Nearly one out of seven deaths in the United States occur due to heart failure, and the majority of the deaths area associated primarily with socioeconomic status. Increasing evidence shows that the African-American population has a higher susceptibility toward manifestation of CVD compared to other ethnicities in the United States (American Heart Association). CVD and stroke account for 80% deaths in males and 75% in females (World Health Organization 2011) with significant occurrence in older adults.

Development/progression of CVD pathologies classically involve the narrowing of vessels that in turn exert mechanical overload on the heart to pump more forcefully to keep the supply intact. Consequently, the vessels undergo remodeling in response to the increased force of flow leading to a cycle of bidirectional remodeling that ultimately underlies heart attack/stroke. Thus, manifestation for each of the CVD pathologies is associated with ongoing chronic remodeling and occurs due to altered signaling mechanisms driven potentially by dysregulated receptor signaling. There are several underlying conditions that can affect CVD pathology and underlying heart health, including diet, physical activity, tobacco consumption, body weight/obesity, high-cholesterol levels, and diabetes (Kenchaiah et al., 2002; Hippisley-Cox and Coupland, 2016a). Despite the plethora of underlying conditions that contribute to the CVD, the majority of current treatments target receptors like angiotensin and/or beta-adrenergic receptors for maintaining cardiac function, while other major mechanisms causing CVD are under intense investigation.

Cardiac Receptor Signaling in Physiology and Failure

Beta-adrenergic receptor (βAR) signaling plays a critical role in the maintenance of cardiac function by integrating cardiovascular and sympathetic inputs (Leimbach et al., 1986; Koch, 2004). Sympathetic overdrive occurs as an adaptive response that initiates cardiac hypertrophic signaling following βAR stimulation. This stimulation leads to receptor activation and series of downstream signals that convert the chemical stimulus to an elevation in physical contractile response. Importantly, at the molecular level, increased βAR activation and signaling leads to myocardial hypertrophy as an adaptive process, improving ventricular function to balance workload. However, sustained cardiac hypertrophy associated with ensuing increase in work load consequently activates sympathetic drive. Chronic sympathetic overdrive in addition to activating βARs also subsequently inactivates or desensitizes the

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receptors as a feedback mechanism, resulting in reduced cardiac contraction and a greater risk for stroke and heart failure (Leimbach et al., 1986; Koch, 2004; Esler et al., 1997).

Beta-Adrenergic Receptors (βARs)

βARs are prototypical members of the G-protein-coupled receptor (GPCR) family. GPCRs are the largest family of cell surface receptors and are key regulators of cellular processes including cardiac function. They are able to recognize cues from the extracellular milieu and transmit those signals across the membrane into the cell to mediate a response. Given their key role in regulating multiple process, they are also major targets for drugs, and currently >30% of marketed drugs target GPCR function either activating/inhibiting their function in pathology (Stevens et al., 2013). Structurally, GPCRs are membrane proteins characterized by seven transmembrane spanning domains interconnected by three intracellular and three extracellular loops. Despite GPCRs binding to a plethora of agonists, they structurally have a classically conserved core where agonist binds, an extracellular amino terminal (N-terminal) that plays a role in agonist binding, and an intracellular carboxyl terminal (C-terminal) region key for regulating receptor signaling (Madamanchi, 2007). GPCRs are activated following extracellular stimuli such as hormones (epinephrine/norepinephrine), light, sound, and odor that transmit the stimuli into the cell (Rockman et al., 2002). In this context, βARs are the key sensors for epinephrine and norepinephrine and mediate cellular responses through three receptor subtypes: β1, β2, or β3ARs (Xiao, 2001). β2AR is the most ubiquitously expressed receptor subtype, while β1AR is the most abundant subtype in the heart accounting for 75%–80% of βARs. β3AR is expressed almost exclusively in adipose tissues (Madamanchi, 2007; Xiao, 2001). Activation of the βARs subtypes leads to a conformational change in the receptor, allowing interaction with heterotrimeric G-proteins.

Heterotrimeric G-proteins

The heterotrimeric G-proteins are composed of three subunits, α, β, and γ, which are localized to the inner leaflet of the plasma membrane. Conformational changes that occur following activation of the receptor lead to interaction with G-proteins, and dissociation of the heterotrimeric G-protein into Gα and βγ subunits. The dissociation of the Gα from the βγ subunits exchanges GDP with GTP, leading to activation of downstream signals primarily by coupling to effectors like adenylyl cyclase, phosphodiesterases, phospholipase C, and ion channels (Wettschureck and Offermanns, 2005b). Activated effectors in turn tightly regulate the intracellular concentration of downstream secondary messengers like cAMP, diacylglycerol, sodium or calcium cations that titrate the physiological response through posttranslational signaling mechanism for acute responses, and gene transcription/translation for long-term chronic remodeling responses (Wettschureck and Offermanns, 2005a; Wettschureck et al., 2005). Hydrolysis of the GTP-bound Gα subunit to GDP results in reassociation with Gβγ subunits, completing a cycle and terminating the signal initiated by the activated receptors. The length of the G-protein signal is determined by the duration of the GTP-bound Gα-subunit, which could be regulated by regulator of G-protein signaling (RGS) proteins or by covalent modifications of the G-protein subunits (Sato et al., 2006; Sjogren and Neubig, 2010). There are four subtypes of Gα-subunits Gαs, Gαi, Gαq, and Gα12, enabling diversity in responses (Madamanchi, 2007). Gαs is stimulatory while Gαi is inhibitory in the context of adenylyl cyclase activation and cAMP generation following βAR interaction with epinephrine or norepinephrine (Lefkowitz, 1998; Communal et al., 1999). Classically, the Gβγ subunits of the G-proteins were thought to be the brakes on G-protein signaling as their reassociation with Gα leads to termination of signaling. However, increasing evidence shows that the Gβγ subunits themselves also mediate downstream signaling, independent of Gα-subunits, that has significant physiological and pathological implications. The Gβγ subunits are always complexed together and tethered to the membrane by posttranslational prenylation of the γ subunit (Kisselev et al., 1995). Although the role of different isoforms of Gα subunits in regulating downstream signaling is well understood, the role of multiple isoforms of the G β and γ subunits in signaling is being appreciated only recently have studies have shown that the isoform combination of Gβγ subunits can provide diversity in signaling and response to extracellular stimuli (Khan et al., 2013).

βAR Downstream Signaling

Engagement of βAR with agonist classically leads to G-protein activation in the cardiac system, which couples to the stimulatory Gαs G-protein subunit leading to activation of adenylyl cyclase. Consequently, adenylyl cyclase mediates generation of cAMP, activating PKA that in turn through a series of subsequent steps results in increased cardiac contractility (Movsesian, 1998; Zaccolo, 2009). Simultaneously, the dissociated Gβγ subunit of the heterotrimeric G-protein recruits G-protein-coupled receptor kinase 2 (GRK2) that phosphorylates βAR, allowing for β-arrestin binding, which terminates the signals from GPCRs, resulting in desensitization (Wolfe and Trejo, 2007). β-Arrestin is a cytosolic scaffolding protein. However, recent studies have shown that while β-arrestin terminates signals from activated GPCRs, it also simultaneously initiates β-arrestin-dependent signaling (Dorn, 2009b; DeWire et al., 2007). These observations lead to the concept that GPCRs can initiate G-dependent signaling and G-protein independent, β-arrestin-dependent signaling (Dorn, 2009b) (**Fig. 1**). Increasing evidence suggests that β-blockers that traditionally block G-protein signaling may mediate unique beneficial effects via G-protein-independent mechanisms. In addition, stimulation of GPCR leads to unique signals that allow for cross-talk and subsequent activation of traditional receptor tyrosine kinases (epidermal

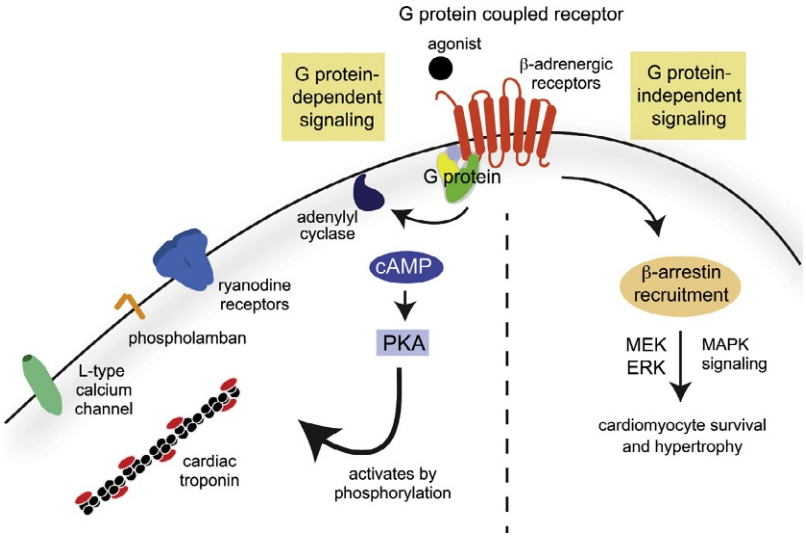

Fig. 1 Illustration of beta-adrenergic receptor (βAR) downstream signaling: G-protein-dependent and G-protein-independent signaling pathways.

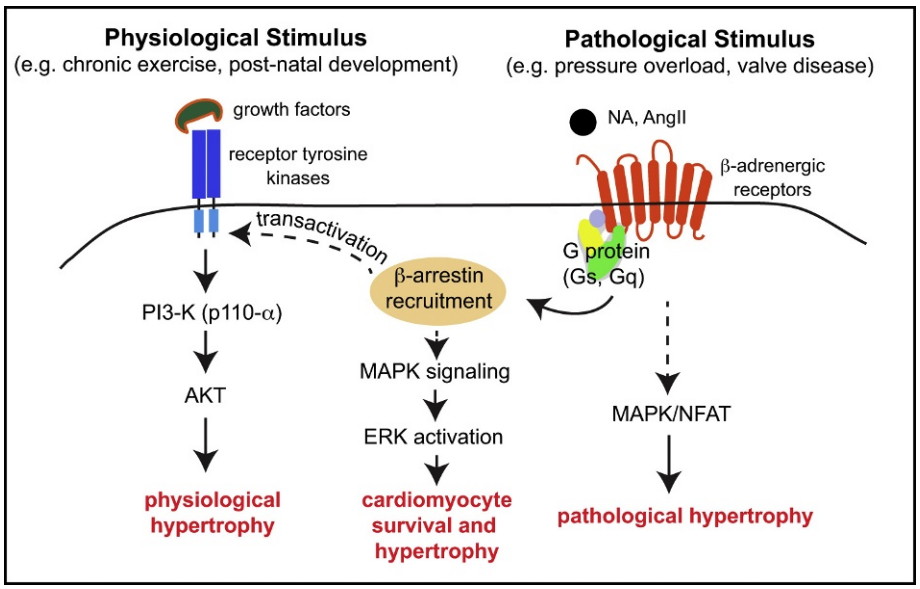

Fig. 2 Illustration showing mechanisms of cardiac remodeling.

growth factor receptor (EGFR), platelet-derived growth factor receptor), resulting in transactivation (Fig. 2). These signaling pathways provide multiple mechanisms by which GPCRs can regulate, titrate, and mediate responses to diverse sets of extracellular stimuli.

G-Protein-Dependent Signaling

Stimulation of β1 and β2AR leads to coupling to stimulatory G-protein Gαs that activates adenylyl cyclase, generating the cAMP that initiates signals mediating inotropic effects. However, persistent stimulation of β2ARs leads to switching of coupling from stimulatory Gαs to inhibitory Gαi resulting in inhibition of adenylyl cyclase activation, reducing cAMP and inotropic effects (Boknik et al., 2009). Interestingly, β3ARs mediate negative inotropic effects by activation of protein kinase G through cGMP pathways showing how a hormonal epinephrine or norepinephrine stimulation response can be diversified in a cell to provide an appropriate contractile response. A primary role of cAMP is to activate PKA, which mediates phosphorylation of a set of regulatory proteins in cardiomyocytes resulting in positive inotropy (increased contraction) and also concomitant increased relaxation (lusitropy) (Zaccolo, 2009; Movsesian, 1998). PKA mediates phosphorylation of proteins in the sarcoplasmic reticulum (SR) that regulate both inotropic and lusitropic effects. PKA phosphorylation of phospholamban releases its inhibitory effect on SR Ca^{2+}

ATPase-2 (SERCA2a), accelerating Ca^{2+} uptake increasing the available pool of Ca^{2+} for the next contraction (Bers, 2002). Similarly, there is increased phosphorylation of the ryanodine receptor, contributing to a faster onset for contraction following βAR stimulation (Maltsev et al., 2006; Tilley and Rockman, 2006). In addition to regulation of SR proteins, PKA also plays a critical role in regulating positive inotropy by phosphorylating the L-type Ca^{2+} channel, increasing the influx of Ca^{2+} (Shaw et al., 2009). Finally, PKA also phosphorylates cardiac troponin I (cTnI), reducing Ca2+ sensitivity to enhance cardiac relaxation (Wattanapermpool et al., 1995). These observations show how G-protein-dependent signaling is a key player underlying the process of cardiac contraction mediated via increased βAR stimulation due to elevated sympathetic drive in response to mechanical demands (Bers, 2002). Although PKA is primarily activated by Gαs-G-protein due to βAR, the cardiac systems also respond to mechanical needs by activating the Gαq-G-protein via angiotensin or endothelin receptors (Rosano and Bagnato, 2009). Gαq initiates phospholipase C (PLC)-mediated hydrolysis of phosphatidylinositol 4,5-bis-phosphate (PIP_2) to diacyl glycerol (DAG) and inositol trisphosphate (IP_3) which acts as second messengers similar to cAMP. DAG activates protein kinase C (PKC), while IP_3 regulates Ca2+ channels in the SR (Carrasco et al., 2004). PKC activation leads to phosphorylation of numerous substrates including activation of the MAPK signaling pathway that primarily drive cell growth responses resulting in cardiac hypertrophy (Kehat and Molkentin, 2010) (Dorn and Force, 2005). In addition, IP_3-mediated Ca^{2+} release regulates calmodulin, which in turn mediates hypertrophic response through calcineurin and Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII) (Kehat and Molkentin, 2010; Chen et al., 2011). These observations show the critical role G-protein-dependent signaling plays in dynamically regulating cardiac responses to the ever changing needs of the body.

G-Protein Independent Signaling

The concept of G-protein independent signaling has evolved only recently, as small molecules have been developed that can generate unique GPCR confirmations to selectively activate specific downstream signaling pathways (Carrasco et al., 2004). This concept came from studies showing unconventional angiotensin receptor desensitization in response to modified peptide agonists (Feng et al., 2005). The findings from this study suggested that agonist binding of the receptor could lead to different confirmations with each of the confirmations potentially coupling to a diverse downstream components including activation of G-protein-independent signaling. β-Arrestins are recruited to the receptor following phosphorylation, initiating signals that are independent of G-proteins, as its recruitment terminates G-protein signaling (Lefkowitz, 1998). The role of β-arrestin 1 and 2 in G-protein-independent signaling is only being appreciated recently, in contrast to their classical role in desensitization and receptor internalization (Shenoy and Lefkowitz, 2011a; Kendall et al., 2011). β-Arrestin 2 forms a signalosome initiating activation of extracellular regulated kinase 1/2 that in turn mediates phosphorylation of ribosomal S6 kinase, mediating proliferation in neonatal cardiomyocytes (Cuello et al., 2011; Ahn et al., 2009). In addition, β-arrestin 1 interacts with Rho GTPase-activating protein that in turn activates Rho, mediating stress fiber formation (Barnes et al., 2005; Anthony et al., 2011). Given the role of β-arrestin as a scaffold that can mediate G-protein-independent signaling, proteomic studies have shown that β-arrestins interact with a large set of key molecules involved in signal transduction that have clear implications in manifestation of the phenotypes. For example, β-arrestins can scaffold exchange protein activated by cAMP (EPAC) and CAMKII to potentially modulate cardiac contractility (Mangmool et al., 2010). Similarly, interaction with actin, cofilin, and myosin could alter actin remodeling and stress fiber formation (Xiao et al., 2007, 2010; Christensen et al., 2010), while interaction with the myosin-binding subunit of myosin phosphatase could be an integral regulator of cardiac contractility (Kooij et al., 2010; Godin and Ferguson, 2010). These observations provide an understanding of the broader scale of β-arrestin activity in mediating cardiac contraction, cytoskeleton remodeling, and proliferation.

β-Arrestin-dependent signaling pathways have attained prominence due to their persistent recruitment to GPCRs following GRK mediated phosphorylation, indicating that they play a key role in G-protein-independent signaling (Dorn, 2009b). GPCRs can be classified into Class A or Class B receptors based on their internalization and strength of interaction with β-arrestin. Class A receptors tend to have preferential binding to β-arrestin 2, which undergoes faster recycling as β-arrestin falls off the receptor complex during internalization of receptors such as β1AR, β2AR, or vasopressin receptors (Luttrell and Gesty-Palmer, 2010). In contrast, Class B receptors undergo internalization but have slower recycling rates because β-arrestin forms a more stable complex with the receptor in the endosome in the case of the angiotensin receptor 1 (AT1R) (Pierce and Lefkowitz, 2001). Interestingly, Class B GPCRs do not have a preference for β-arrrestin 1 or 2 (Zhang et al., 1999; Noor et al., 2011). The formation of the complex with the receptor allows for prolonged cytosolic endosomal signaling via ERK activation (DeWire et al., 2007), which can regulate cardiomyocyte survival and hypertrophy (Tohgo et al., 2002; Aplin et al., 2007). However, release of β-arrestin from Class A receptors including βARs allows for the β-arrestin signals to be more widespread, allowing for increased signals within the cell compared to Class B receptors. This is important given that β-blockers that bind βARs to block G-protein coupling mediate G-protein-independent signaling, which underlies some of the benefits associated with β-blocker treatment.

Transactivation

In addition to the G-protein-dependent and -independent signaling mediated by GPCR ligands, GPCR can also mediate cell survival signal through transactivation of EGFR. It is important to note that β-arrestin plays a key role in the process of the cross-talk between GPCR and EGFR (Heitzler et al., 2009; Cattaneo et al., 2014; Maudsley et al., 2000; Noma et al., 2007; Tilley et al., 2009). Studies have shown that many GPCRs induce EGFR transactivation, wherein ERK1/2 is significantly activated, mediating a cardiac

hypertrophy response (Noma et al., 2007). Importantly, it is known that β1AR-mediated EGFR transactivation is a key pathway in mediating cardiac hypertrophic response, resulting in dilated cardiomyopathy (Toker and Cantley, 1997; Esposito et al., 2011; Kim et al., 2009; Zhai et al., 2006). Although multiple studies have shown the role of β-arrestin in the transactivation pathway, there are also studies that have described the role of G-proteins in the process of transactivation (Cattaneo et al., 2014; Heitzler et al., 2009). This brings forth the idea that depending on the agonist and the GPCR, the GPCR could engage in G-protein-dependent as well as -independent pathways to mediate the downstream effects. However, studies have shown that the cardio-protective effects of β-blocker are mediated by transactivation of EGFRs, which is primarily regulated through β-arrestin (Toker and Cantley, 1997; Kim et al., 2008).

Regulation of βAR Activation/Signaling

It is important for GPCRs like βARs to be activated in response to hormones to initiate signals. On the other hand it is also critical to tightly control termination of signals to prevent overstimulation. This process is choreographed as the activated βARs are phosphorylated by GRKs (Lymperopoulos et al., 2012; Lefkowitz, 1998; Monto et al., 2012; Aguero et al., 2012; Zhu et al., 2012; Belmonte and Blaxall, 2011), resulting in β-arrestin binding and termination of G-protein coupling/signaling. β-Arrestin binding to the phosphorylated βAR leads to endocytosis into endosomes (Lefkowitz, 1998; Shenoy and Lefkowitz, 2011b; Shukla et al., 2011; Lymperopoulos, 2011; Patel et al., 2009; Rajagopal et al., 2010). Resensitization of βARs occurs by dephosphorylation of the receptors by protein phosphatase 2A (PP2A) in the endosomes before being recycled back to plasma membranes (Ferguson, 2001; Rockman et al., 2002; Krueger et al., 1997; Drake et al., 2006).

Desensitization

Epinephrine or norepinephrine binding to βARs results in activation of G-protein and dissociation of Gαs and Gβγ subunits. Agonist bound receptor is then phosphorylated by PKA, protein kinase C, and GRKs to uncouple from the G-proteins, initiating classical desensitization (Walker et al., 2011). In this context, Gαs through cAMP activates PKA, and Gβγ through Ca^{2+} activates PKC, both of which phosphorylate GPCRs indiscriminately, desensitizing the receptors independent of the agonist (Lefkowitz, 1998). Such a phenomenon is termed heterologous desensitization or agonist-independent desensitization, wherein stimulated and unstimulated GPCRs are phosphorylated (Rockman et al., 2002; Penn et al., 1998). In contrast, GRK-mediated phosphorylation and desensitization is an agonist-specific response mediated by GRKs. There are seven GRKs of which GRKs 2, 3, 5, and 6 are ubiquitously expressed, while GRK 1 and 7 are rhodopsin kinases and GRK 4 is expressed primarily in the testis (Sallese et al., 1997, 2000; Virlon et al., 1998; Kearney et al., 2005; Hall et al., 2012; Sato et al., 2015; Pronin et al., 1998). GRK 2, 3, and 5 are abundantly expressed in the heart and play a critical role in regulating βAR function. GRK 2 and 3 are cytosolic proteins and are recruited to the βARs at the plasma membranes by the dissociated Gβγ subunits, while GRK5 is tethered to the membrane by high-affinity association with phospholipids (Pitcher et al., 1998; Pronin et al., 1998). Thus, GRK-mediated phosphorylation of the C-terminal tail of the agonist-bound βAR results in binding of the receptor to β-arrestin (Madamanchi, 2007). In addition to mediating G-protein-independent signaling, β-arrestin acts as an adaptor protein for recruiting molecules in receptor endocytosis, leading to internalization of desensitized βARs (Lefkowitz and Shenoy, 2005).

Oxidative stress and inflammation can also trigger βAR dysfunction potentially by altering desensitization by a distinct mechanism from the classical desensitization pathway. Proinflammatory cytokines such as TNFα, IL-1β, and IL-13 significantly predispose βARs toward desensitization in cardiomyocytes as well as in human airway smooth muscle cells (Prabhu, 2004; Gulick et al., 1989). Reactive oxygen species (ROS) also play an important role in mediating adrenergic function (Gupta et al., 2006; Donoso et al., 2011). In this context, several studies have provided evidence for oxidative stress-mediated modification in the components of the βAR desensitization pathway that underlies the altered receptor response (Whalen et al., 2007; Ozawa et al., 2008). For example, nitrosylation of GRK2 has been shown to attenuate βAR phosphorylation, thereby reducing desensitization and downregulation of βARs (Ozawa et al., 2008; Whalen et al., 2007). Given these observations, proinflammatory cytokines and ROS-dependent oxidative–nitrosylative switches may play an important role in shifting the regulation of kinases, thereby significantly impacting βAR desensitization.

Resensitization

Resensitization is a process of restoration of receptor responsiveness in the presence or absence of a stimulus (Vasudevan et al., 2011). Resensitization of βARs takes place following desensitization by phosphorylation of βARs by GRKs and PKA in response to sympathetic overdrive (Lymperopoulos et al., 2012; Lefkowitz, 1998; Monto et al., 2012; Aguero et al., 2012; Zhu et al., 2012; Belmonte and Blaxall, 2011). Following phosphorylation, receptors are internalized into the endosomes via β-arrestin binding (Lefkowitz, 1998; Shenoy and Lefkowitz, 2011b; Shukla et al., 2011; Lymperopoulos, 2011; Patel et al., 2009; Rajagopal et al., 2010). Once in the endosomes, the phosphorylated βARs are dephosphorylated by protein phosphatase 2A (PP2A). This process is thought to be dependent on the acidification of the endosomes (Gardner et al., 2007). Once βARs are dephosphorylated by PP2A, the naïve receptors are recycled back to the plasma membrane. PP2A is a heterotrimeric holoenzyme characterized by a scaffolding A, regulatory B, and catalytic subunit C (Sontag, 2001; Cohen, 1989). Despite the regulation of the PP2A holoenzyme,

resensitization, which is a key step in reestablishing receptor signaling potential, until now has been considered to be a passive homeostasis process. However, recent studies have shown that resensitization is not a passive process but a tightly regulated mechanism mediated by phosphoinositide 3-kinase γ (PI3Kγ). PI3Kγ regulates a known endogenous modulator of PP2A function called inhibitor of PP2A (I2PP2A) (Oliver and Shenolikar, 1998; Li et al., 1995). PI3Kγ phosphorylates I2PP2A on residues 9 and 93, resulting in enhanced binding of I2PP2A to PP2A, blocking PP2A activity (Nienaber et al., 2003; Naga Prasad et al., 2000). Thus, resensitization mechanisms are only coming to be appreciated, and further studies are required to identify novel regulatory mechanisms that modulate this key process. In addition, there is evidence that βARs can be activated for signaling within endosomes (Ferguson, 2001), outside of the typical pathway of recycling to the plasma membrane. These exciting observations indicate that more studies are needed to comprehensively understand resensitization and suggest a changing paradigm of resensitization mechanisms (Ferguson, 2001).

Receptor Endocytosis and Trafficking

Phosphorylation of βARs in one of the key steps that initiates the process of receptor endocytosis. β-Arrestin binding to phosphorylated βARs not only mediates βAR desensitization and initiation of β-arrestin-dependent signaling but also plays a key role in recruiting molecules critical for endocytosis (Bhatnagar et al., 2001). β-Arrestin mediates its multiple activities by adopting altered confirmations with differential affinity to various proteins. Binding of β-arrestin to phosphorylated βAR results in a conformational change that exposes the C-terminal tail of β-arrestin to allow interaction with the endocytic machinery, including clathrin and adaptor subunit of AP2 (Anthony et al., 2011; Edeling et al., 2006). These interactions drive the βAR/β-arrestin complex toward clathrin-coated pits and subsequent endocytosis. It is important to note that clathrin-mediated endocytosis is a major mechanism by which GPCRs are endocytosed and trafficked in the cell. GPCRs may also use alternative pathways like caveolae for endocytosis of the receptor complex from the cell surface (Rapacciuolo et al., 2003). Although β-arrestin recruitment to the receptor complex is a key determinant in βAR endocytosis, other regulators alter the dynamics of this process. For example, proteins like β-arrestin and AP2 bind to phosphatidylinositol 4,5 *bis*-phosphate (PIP2) to be stabilized (Hinrichsen et al., 2006). However, β-arrestin and AP-2 have log fold higher affinity toward PIP3 (Gaidarov et al., 1999) generated by PI3K, and expression of a kinase-dead PI3K leads to significant impairment in βAR endocytosis (Patrucco et al., 2004). Once the receptor-enriched clathrin-coated vesicle is formed at the plasma membrane, it is pinched off by a scission GTPase dynamin, which is a critical step in the beginning of endosomal trafficking (Edeling et al., 2006; Warren et al., 1998; Santini et al., 1998; Orsini et al., 1999; Signoret et al., 1998). Endosomal trafficking is mediated by significant actin remodeling to provide the rails on which vesicle transport is facilitated either for recycling or lysosomal degradation. The fate of these vesicles was classically considered to be controlled by Rab GTPase family members (belonging to the family of Ras monomeric G-proteins), wherein enrichment of vesicles with specific members were sorted to lysosomes or recycled. For example enrichment of vesicles with Rab5 seem to lead to effective recycling (Seachrist et al., 2002; Zerial and McBride, 2001). However, in addition to Rab family members, receptor ubiquitination determines the fate of the endocytosed βAR (Shenoy et al., 2001, 2008). Ubiquitination is the addition of ubiquitin to a substrate protein. Agonist stimulation of βAR leads to rapid ubiquitination by the E3 ubiquitin ligase Nedd4 (neuronal precursor cell-expressed developmentally downregulated 4). Although Nedd4-directed ubiquitination directs the βAR toward lysosomal compartments (Shenoy et al., 2001, 2008), this process is dynamically regulated by deubiquitinases such as USP20 and USP33, which reverse βAR ubiquitination (Berthouze et al., 2009). The reversal of ubiquitination impairs lysosomal trafficking and shifting the process toward βAR resensitization and recycling. It is important to point out that even though βARs primarily engage and utilize β-arrestin-dependent mechanisms for endocytosis, there is evidence that other GPCRs like protease-activated receptor-1 or 5-hydroxy-tryptamine 2A serotonin receptor undergo endocytosis using β-arrestin-independent pathways (Bhatnagar et al., 2001).

Cardiac Remodeling

The heart functions as a single unit performing cardiac contraction from the time of its inception to death and is composed of multiple cell types that are in perfect synchrony. A major cell type in the heart is represented by cardiomyocytes, which are the primary drivers of contraction and supported by nonmyocytes including fibroblasts, endothelial cells, mast cells, vascular smooth muscle cells, and extracellular matrix providing elasticity to the contracting heart. A key aspect of this elasticity is the innate ability of the heart to change its contractile capacity in response to increasing mechanical load either due to physiological needs like exercise or narrowing arteries in cardiovascular disease. In this context, the mechanical load on the heart could increase due to various stimulus including but not limited to stress, growth, increase in the pressure or volume, mutations in proteins, or loss of contractile tissue (Frey et al., 2004). Therefore, in order to generate greater force to supply the same amount of blood to the body, the heart undergoes remodeling. In the classic response, the heart undergoes cardiac hypertrophy, which is thickening of the myocardium resulting in decrease in size of chambers (atria and ventricles). Selective enlargement of cardiomyocytes is associated with transcriptional upregulation of contractile proteins and their accumulation in the myocytes. The left ventricle is a critical chamber in generation of contractile pumping force and also senses mechanical load/systemic pressure and may respond by left ventricular hypertrophy. Hypertrophy is one of the most common signs of cardiac remodeling and can occur in response to physiological as well as pathological responses (Gradman and Alfayoumi, 2006). The hypertrophic responses of the left ventricle are empirically classified into physiological and pathological hypertrophy (**Fig. 2**) (Gradman and Alfayoumi, 2006; Levy et al., 1988; Frey et al., 2004).

Physiological Hypertrophy

Left ventricular hypertrophy in response to growth signals and/or chronic exercise is considered physiological hypertrophy. A key classical component of such a hypertrophic response is its reversal following the removal of stress. For example, left ventricular changes in athletes are remodeled back to the basal state when the athlete retires. Physiological hypertrophy is associated with an increase in myocyte volume and formation of new sarcomeres (McMullen and Jennings, 2007). At the molecular level, strenuous exercise or regulation of organs during postnatal growth is associated with significant release of insulin-like growth factor 1 (IGF1) (Conlon and Raff, 1999; Serneri et al., 2001). IGF1 acts via its receptor IGF1R, which mediates a series of downstream signals that activate PI3Kα, which enhances antiapoptotic Akt/PKB (protein kinase B) signaling (Schiaffino and Mammucari, 2011). Akt/PKB activates of mTOR (target of rapamycin) signaling, which mediates cell growth and a hypertrophic response (Schiaffino and Mammucari, 2011). Consistent with activation of these pathways, cardiomyocytes-specific overexpression IGFR1 or PI3Kα leads to physiological hypertrophy in the absence of features of apoptosis. Thus, physiological hypertrophy is considered to be beneficial and provides efficient adaptation of cardiac function in response to increasing demands of the body to growth.

Pathological Hypertrophy

Pathological hypertrophy is also characterized by left ventricular hypertrophy and occurs in response to stresses including systemic hypertension, ischemia, and mutations in contractile proteins leading to increased load on myocytes. However, it is not known whether this hypertrophy occurs due to the presence of intermittent unique signals, or a result of chronic stress signals (Frey et al., 2004). One of key outcomes of pathological hypertrophy is that despite management of the stress, ventricular hypertrophy is maintained, and with time can result in dilation and heart failure. In contrast to physiological hypertrophy, pathological hypertrophy is a part of an adaptive response termed "adaptive hypertrophic response" and continues to transition to "maladaptive hypertrophic response" leading to dilation characterized by cardiomyocytes apoptosis (Kehat and Molkentin, 2010; Dorn, 2009a). "Adaptive" and "maladaptive" hypertrophy are on a physiological continuum, and hearts may transition between the two, which are difficult to distinguish. Although there could be molecular signatures identifying these two seemingly similar phenotypes, comprehensive studies are still needed to define these signatures. However, a recurrent observation with pathological hypertrophy is the observation of fibrosis, apoptosis, and necrosis of myocytes (McMullen and Jennings, 2007). It is well known that many GPCRs are activated in response to cardiovascular stress, including both Gαs and Gαq pathways (Smrcka et al., 1991; Taylor and Exton, 1991; Taylor et al., 1991). The Gαq initiates PLCB-mediated phospholipid hydrolysis of PIP_2 to diacyl glycerol (DAG) and inositol trisphosphate (IP_3) (Smrcka et al., 1991; Taylor et al., 1991; Taylor and Exton, 1991). DAG activates PKC-dependent pathways, leading to the activation of ERK, JNK, and p38. The IP_3 generated also induces calcium increase, thereby activating calmodulin. Stress-induced PLC signaling also actively contributes to the development of left ventricular hypertrophy. However, a key question that remains is whether pathological responses observed in the heart due to chronic versus intermittent stress would a) manifest in similar phenotypes and b) activate different molecular signatures. In this context, studies by Perrino et al. suggest differential phenotypic and molecular responses (Perrino et al., 2006), supporting the idea that hearts could respond differently to these insults to remodel appropriately.

Signaling in Heart Failure

Sympathetic overdrive is one of the key factors involved in increased cardiac output, but chronic sympathetic activation also simultaneously leads to phosphorylation of βARs. In addition, increase in catecholamines not only leads to βAR phosphorylation but is also associated with increased GRK2 expression. Thus the concomitant increase in the agonist and elevation in GRK2 results in enhanced β-arrestin binding and loss of G-protein coupling (Lefkowitz, 1998). Increased βAR phosphorylation is a key driving force for cardiac dysfunction and ensuing heart failure (Rockman et al., 2002; Bristow and Ginsburg, 1986). Therefore, impaired G-protein coupling (desensitization) and loss of surface βARs (downregulation) due to elevated GRK2 are key hallmarks of heart failure (Rockman et al., 2002; Hamdani and Linke, 2012; Bristow, 2000; Bristow et al., 1982, 1986; El-Armouche and Eschenhagen, 2009; Murphy et al., 2010; Bristow and Ginsburg, 1986). It is important to note that there is a substantial decrease in the levels of β1AR compared to β2AR in heart failure (Freedman and Lefkowitz, 2004).

In addition to the classic sympathetic overdrive that underlies heart failure, many comorbid conditions such as hypertension, dyslipidemia, obesity, and diabetes are associated with significant proinflammatory cytokines including TNFα (Katsuki et al., 1998; Kenchaiah et al., 2002; Hippisley-Cox and Coupland, 2016b). TNFα is a known cardiac-depressant, and its overexpression in the rodent heart results in deleterious cardiac remodeling and heart failure (Kubota et al., 1997; Feldman et al., 2000). TNFα-dependent effects are thought to be mediated by altered Ca2+, sphingolipid mediators, arachidonic acid, and nitric oxide (Prabhu, 2004). In addition, a recent study showed that elevated TNFα results in increased GRK2 expression, which may noncanonically regulate βAR function by phosphorylating and desensitizing the receptor (Vasudevan et al., 2011). Thus, such a process could alter the canonical response of βARs to sympathetic hormones like epinephrine/norepinephrine. These observations explain the potential mechanistic pathways that underlie the risk factors for heart failure in comorbid conditions. Consistently, β-blockers are contraindicated in diabetes and obesity (Lee et al., 2011), supporting the idea that βARs may be desensitized through noncanonical mechanisms that need better understanding to develop therapeutic strategies in response to increased proinflammatory cytokines like TNFα.

Drug Targets and Impact in Clinical Medicine

Targeting of GPCRs constitutes over a third of all marketed drugs for the treatment of hypertension, heart disease, cancer, Alzheimer's disease, and asthma. β-Blockers have been one of the most clinically proven classes of therapeutics in cardiovascular medicine and are used as a first line of therapy following myocardial infarction and in control of hypertension, arrhythmia, and angina (Rutten et al., 2010; De Caterina and Leone, 2011). β-Blockers bind to βARs and block endogenous catecholamines from binding, thereby regulating inotropic and chronotropic effects through β1ARs in the heart (Rohrer et al., 1996). However, use of β-blockers may impinge on the function of β2ARs in the lungs (Jacobsen, 2011) and in maintaining vascular tone (Pourageaud et al., 2005), causing deleterious side effects. Given the complexity of βAR signaling in different organs, more refined approaches are necessary to specifically target individual aspects of GPCR function. In this context, pharmacological dissection of signal transduction pathways activated by GPCRs becomes an important component as revealed by in-depth studies being performed using "biased agonists" (Marti-Solano et al., 2013). Consistent with the diverse functions mediated by GPCRs, use of biased agonist will become important as they could selectively activate or block a subset of downstream signals allowing for harnessing appropriate signaling repertoire for a specific response (Marti-Solano et al., 2013). Use of these biased agonists would allow for specific targeting of unique GPCR responses without generating off-target signals that could have adverse effects. Further, these biased agonists could selectively target a downstream signaling pathway to provide beneficial clinical outcomes.

Conclusion

This article provides an overview of the current state of understanding of GPCR signaling using βARs as prototypical receptor in the context of desensitization, resensitization, and its significance in the heart. It is evident from many studies that homeostasis of βAR signaling is of utmost importance in cardiac function. Given that βAR activity/function relies on the delicate balance between desensitization and resensitization, i.e., the kinase and the phosphatase arms of maintaining receptor function, it becomes imperative to study the underlying signaling components and mechanisms as we move toward the use of biased agonists.

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Sinus Tachycardias: Inappropriate Sinus Tachycardia and Postural Tachycardia Syndrome

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Introduction

Sinus tachycardia is a normal physiological phenomenon that is described as an appropriate heart rate (HR) increase exceeding 100 beats per minute (bpm) while in sinus rhythm. This normally occurs in response to stress that increases sympathetic nervous system activity. In contrast, there are multiple syndromes in which sinus tachycardia occurs in response to minimal stress and is deemed inappropriate in nature; these include postural tachycardia syndrome (POTS) and inappropriate sinus tachycardia (IST).

POTS is defined by chronic symptoms of orthostatic intolerance that are worse with standing in conjunction with an increase in HR of $\geq$30 bpm within 10 min of standing (Sheldon et al., 2015). Additionally, orthostatic hypotension must be ruled out as a potential cause of symptoms. IST is defined by the presence of sinus tachycardia ($\geq$100 bpm) at rest or a mean 24 h HR $\geq$90 bpm (Sheldon et al., 2015) and results in symptomatic palpitations among other symptoms. To date, there is no known mortality from either of these conditions, but each condition can negatively impact quality of life.

In this article, we will discuss the clinical approach to the patient with symptomatic sinus tachycardia of unknown cause, as well as the epidemiology, pathophysiology, and management of POTS and IST.

Epidemiology

Postural Tachycardia Syndrome

POTS is identified as a syndrome and not a disease with a single discrete pathophysiological mechanism. Its overall prevalence is approximately 0.2% (Sheldon et al., 2015), with an estimated 500,000–3,000,000 individuals affected in the United States alone (Robertson, 1999; Garland et al., 2007). The majority of patients that present with POTS are female, with a female to male ratio of approximately 5:1. The age of onset is typically between the ages of 13 years and 40 years. Moreover, it has been reported that 12.5% of patients have a family history of orthostatic intolerance (Thieben et al., 2007). Little is known about the long-term outcomes of POTS; it is perceived as a chronic condition with no known mortality, and with at least partial improvement in many, but not all, patients (Sheldon et al., 2015).

Inappropriate Sinus Tachycardia

Including both symptomatic and asymptomatic patients, the prevalence of IST is estimated to be approximately 1.2% in a middle aged population (Still et al., 2005). There is also a recognized association between IST and health care workers (Krahn et al., 1995). It is not clear if this is representative of the overall IST population, or if this reflects a subgroup that is more likely to seek medical attention for these symptoms (Pellegrini and Scheinman, 2016). Similar to POTS, patients with IST are primarily young women (Brady et al., 2005), although it has also been reported in older individuals as well (Lopera et al., 2003). There is no known mortality associated with IST (Sheldon et al., 2015).

Associated Conditions

Chronic fatigue syndrome

Chronic fatigue syndrome (CFS) is characterized by persistent or recurrent unexplained fatigue and related symptoms for at least 6 months (Garland et al., 2015). According to the Centers for Disease Control and Prevention (CDC) criteria, CFS is defined as a case-defining fatigue associated with at least four of the following symptoms: impaired short-term memory or concentration, sore throat, tender lymphadenopathy, muscle pain, joint pain, headaches, sleep disturbance, and postexercise malaise (Okamoto et al., 2012). Similar to POTS, it is a clinical syndrome that is more commonly observed in females.

There is a substantial overlap between POTS and CFS. POTS is associated with several symptoms seen in CFS patients, including fatigue, lightheadedness, neurocognitive deficits, and exercise intolerance; it has been identified in 19%–70% of patients with CFS (Hoad et al., 2008; Schondorf et al., 1999; Stewart et al., 1999). Moreover, there is a high prevalence of fatigue (48%–77%) and CFS diagnosis (17%–23%) in POTS patients (Lewis et al., 2013). When POTS patients were grouped according to whether or not they met the criteria for a CFS diagnosis, there were no differences between groups in the magnitude of orthostatic tachycardia or orthostatic hypotension, supine and upright plasma norepinephrine values, or plasma volume (Okamoto et al., 2012). However, CFS in POTS was associated with increased sympathetic tone when supine and greater increases in sympathetic activity during standing. The lack of distinguishing features between POTS patients with and without CFS indicates that they may not be distinct clinical entities. While almost all patients with POTS experience chronic fatigue, a subset of these patients will also meet criteria for CFS. This might represent a distinct subset of the larger CFS population. IST is also speculated to be associated with CFS (Brady et al., 2005). To date there is limited research examining this relationship.

Ehlers–Danlos syndrome—hypermobility type

Ehlers–Danlos syndrome (EDS) is a heterogeneous disorder that presents in several forms (Castori et al., 2017), and is linked to sequence variations in genes encoding for fibrillary proteins and/or collagen processing enzymes (Wallman et al., 2014). It is characterized by fragile connective tissue, skin hyperextensibility, and joint hypermobility. The presence of EDS has been observed to be significantly higher in patients with POTS (18%) compared to autonomic patients without POTS (4%) and the general population (0.02%) (Wallman et al., 2014).

In particular, patients with hypermobile EDS (hEDS) frequently experience symptoms of autonomic dysfunction that are prevalent in POTS patients (Hakim et al., 2017; Tinkle et al., 2017), such as lightheadedness, nausea, sweating, and chest tightness (Gazit et al., 2003). Blood pressure and HR responses consistent with POTS have been observed to be the most typical responses to tilt in patients with hEDS (De Wandele et al., 2014). In addition to standing, POTS-like symptoms and orthostatic intolerance can be triggered by physical exercise, heavy meals, and a warm environment. hEDS is the subtype that is most commonly associated with POTS (Mathias et al., 2011), although the exact mechanisms connecting the two syndromes are unknown.

Vasovagal syncope

Vasovagal syncope (VVS) is a form of neurally mediated (or reflex) syncope that usually occurs with upright posture held for more than 30 s, or with exposure to emotional stress, pain, or medical settings (Sheldon et al., 2015). It is associated with hypotension

and relative bradycardia. POTS is a common confounder of VVS and presyncope. About 25% of patients who fulfill the diagnostic criteria for POTS develop VVS during tilt table testing (Carew et al., 2009). Despite the overlap, these disorders can have distinct hemodynamic patterns during tilt table testing (Nwazue and Raj, 2013). Patients with VVS hold a steady BP for several minutes (often >10 min) prior to developing symptoms and experiencing a rapid drop in BP. In contrast, during head-up tilt, POTS patients experience an excessive increase in their HR often without a decrease in BP. VVS patients typically experience symptoms after prolonged sitting or standing, while POTS patients experience symptoms shortly after a body position change, which is then sustained. Moreover, POTS typically affects individuals between the ages of 13–50 years, while VVS can occur at any age, with the first syncopal episode usually in the second or third decade of life (Nwazue and Raj, 2013; Sheldon et al., 2006). Although both disorders predominantly affect females, POTS does so to a greater extent (85%–90%) compared to VVS (60%).

Psychiatric illnesses

Given the presenting symptoms of POTS and IST, it is not uncommon for these patients to be initially mislabeled as having psychiatric disorders such as anxiety or depression. There is no increased prevalence of major depressive disorders or anxiety disorders in POTS patients in relation to the general population (Raj et al., 2009b). Symptoms of depression and anxiety do exist in this population group, but these are attributed to the underlying disease processes leading to increased sympathetic nervous system activity and sinus tachycardia rather than a primary psychiatric illness (Khurana, 2006; Masuki et al., 2007). Mental clouding, often described as "brain fog," is a particularly common symptom described by POTS patients and may be attributed to deficits in selective attention and cognitive processing (Karas et al., 2000; Arnold et al., 2015). Sleep disturbances and fatigue are also common findings that likely contribute to the decreased quality of life that many of these patients experience (Bagai et al., 2011; Benrud-Larson et al., 2002). For patients with IST, any proposed or theoretical relationship between IST and psychiatric illness has not been well described to date.

Pathophysiology

Postural Tachycardia Syndrome

The characteristic orthostatic tachycardia observed in POTS is known to be a "final common pathway" of multiple pathophysiologic processes; therefore, POTS is more appropriately referred to as a clinical syndrome reflecting several heterogeneous disorders rather than a disease (Garland et al., 2015; Jones et al., 2016). While the exact mechanisms that underlie POTS remain unknown, specific subtypes have been described. Due to the overlapping nature of these subtypes, it is often difficult to identify the clinical distinctions between them in individual patients.

Hypovolemia

A deficit in total blood, plasma, and red cell volume is a fairly common finding in POTS patients, occurring in up to 70% of patients (Sheldon et al., 2015; Raj et al., 2005a). Normally, plasma renin activity and angiotensin II (Ang II) are expected to increase in response to hypovolemia in order to promote blood volume expansion. Compared to healthy subjects, POTS patients have significantly lower serum levels of aldosterone and inappropriately low levels of plasma renin activity (Raj et al., 2005a). It has also been reported that circulating Ang II levels are elevated without a parallel increase in angiotensin (1–7) (Stewart et al., 2009; Mustafa et al., 2011). One possible mechanism for the elevated Ang II in these POTS patients is decreased Ang II degradation as a result of blunted angiotensin-converting enzyme 2 (ACE2) activity. The cause of decreased ACE2 activity and the exact mechanism by which elevated plasma Ang II contributes to POTS remain unclear. There also seems to be blunted angiotensin II receptor type 1 (AT2R) activity, as POTS patients were found to have low levels of aldosterone despite the high levels of Ang II (Mustafa et al., 2011), and had a reduced vasopressor response to an Ang II infusion (Mustafa et al., 2012). These abnormalities in the renin–angiotensin–aldosterone system may contribute to hypovolemia and impaired sodium retention in POTS patients.

Partial autonomic neuropathy

A subset of POTS patients is described to have small fiber neuropathy, usually in the lower limbs (Jacob et al., 2000). This causes sympathetic denervation and an inability to adequately vasoconstrict in response to orthostasis, leading to decreased venous return and stroke volume. This can result in a secondary increase in sympathetic tone and tachycardia in an effort to maintain cardiac output and blood pressure. The small fiber neuropathy in POTS patients is usually painless, and does not present with classic symptoms of a typical length-dependent neuropathy (Gibbons, 2014). It is estimated that approximately 50% of POTS patients may have distal small fiber neuropathy (Peltier et al., 2010; Thieben et al., 2007; Haensch et al., 2014).

Hyperadrenergic state

POTS patients may have elevated levels of plasma norepinephrine, suggestive of a hyperadrenergic state. A hyperadrenergic state in POTS is observed in up to 50% of patients, and is characterized by a systolic BP increase of $\geq$10 mmHg while standing upright for 10 min, and norepinephrine levels $\geq$600 pg/mL while standing (Sheldon et al., 2015). Patients experience similar or greater HR increases than nonhyperadrenergic POTS, but have more symptoms of sympathetic activation, including palpitations, anxiety, tachycardia, and tremulousness (Low et al., 2009). This elevated sympathetic tone can be a primary underlying problem for POTS patients, although it is often secondary to a partial dysautonomia or hypovolemia (Raj, 2013). In addition, prior studies have

shown that an increased phase IV overshoot of the Valsalva maneuver can be seen in this hyperadrenergic state (Gibbons et al., 2013).

Autoimmune autoantibodies

A subset of POTS patients report an acute "viral-like" illness a few weeks to a few months prior to the onset of symptoms, suggesting a potential immune-mediated pathophysiology (Jones et al., 2016). A potential relationship has been identified between circulating autoantibodies and POTS. A low titer of ganglionic acetylcholine receptor (AChR) antibodies was detected in approximately 15% of POTS patients evaluated at the Mayo Clinic between 1993 and 2003 (Thieben et al., 2007), although this might be an overestimate by current standards (Vernino et al., 2016). More recently, autoantibodies (AAb) to α1-adrenergic receptors (α1AR) and β1/2-adrenergic receptors (β1AR/β2AR) receptors have been reported (Li et al., 2014). Both α1AR and βAR AAb can have a direct stimulatory effect on adrenergic receptors. They can also exert an allosteric-mediated positive modulatory effect upon β1AR (pure agonist) and a negative modulatory effect on α1AR activity (partial agonist–antagonist) (Fedorowski et al., 2016). It is postulated that partially blocked α1AR could cause impaired vasoconstriction in response to norepinephrine (the natural ligand), resulting in greater initial hypotension with standing (**Fig. 1**) (Li et al., 2014). This may lead to an exaggerated sympathoneural response (with increased norepinephrine) to achieve adequate vasoconstriction. This may lead to the orthostatic tachycardia that is characteristic in POTS, as the price for BP preservation. Furthermore, the presence of β1AR/β2AR-activating AAb could further contribute to the marked tachycardia seen in POTS patients (Li et al., 2014).

Autoantibodies targeting cardiac lipid raft-associated proteins have also been found in patients with POTS (Wang et al., 2013). Targets include proteins regulating cellular processes such as signaling, metabolism, chaperones, and transcription. The interaction between these autoantibodies and proteins may trigger alterations in signaling pathways, producing cardiovascular abnormalities associated with POTS. However, the specific involvement of such proteins in the pathogenesis of POTS remains unknown.

Norepinephrine transporter deficiency

A rare form of hyperadrenergic POTS is caused by a loss of function mutation in the gene encoding for the norepinephrine transporter (NET) (Shannon et al., 2000). This mutation has only been found in one family. However, it has been observed that a broader group of POTS patients have decreased NET protein expression (from vein biopsies) compared to healthy subjects (Lambert et al., 2008). This deficiency increases synaptic norepinephrine through diminished clearance, and results in greater sympathetic nerve activation. Pharmacological inhibition of NET can also occur with many antidepressant and attention deficit medications, particular those in the serotonin-norepinephrine reuptake inhibitor (SNRI) class (Raj, 2006). Pharmacological NET inhibition can worsen tachycardia and symptom burden in POTS patients when standing (Green et al., 2013).

Mast cell activation disorder

A subset of POTS patients has mast cell activation without an overt trigger. These patients present with severe episodic flushing during tachycardia, have abnormal increases in urine methylhistamine concentrations, and often have hyperadrenergic features accompanying orthostatic hypertension (Jones et al., 2016). It remains unclear whether sympathetic stimulation triggers mast cell activation in these patients, or whether primary mast cell activation induces the release of vasodilators and compensatory sympathetic stimulation occurs (Shibao et al., 2005).

Gravitational deconditioning

Due to their disability, many POTS patients have restrictions on their activity, which can result in physical deconditioning (Garland et al., 2015). Even if this is not the primary cause of their POTS, it is often a contributing factor to their symptoms and functional limitation. When upright, patients exhibit typical indicators of deconditioning, including persistent tachycardia, reduced stroke volume, reduced left ventricular mass, and reduced peak oxygen uptake (Fu et al., 2010). Orthostatic tachycardia improves with a structured exercise program. In contrast, some patients continue to experience symptoms, suggesting that deconditioning may be a secondary mechanism in the development of POTS (Parsaik et al., 2012). For example, patients can become physically deconditioned due to another illness, or with prolonged bed rest, and develop POTS-like symptoms (Garland et al., 2015).

Although it remains unclear whether deconditioning is a primary or secondary phenomenon to POTS, a program of aerobic exercise and resistance training can improve exercise tolerance and ameliorate various features of POTS (Mathias et al., 2011). When POTS patients participated in 3 months of progressive exercise training, maximal oxygen uptake increased by 11%, indicating an increase in physical fitness (Fu et al., 2010). Left ventricular mass, end-diastolic volume, blood and plasma volumes also increased after training. Most importantly to the patients, their quality of life improved if they completed the exercise-training program.

Inappropriate Sinus Tachycardia

The mechanisms underlying IST are not well defined, but they are likely multifactorial and complex. Several associated pathophysiological changes can potentially result in the syndrome, including increased sinus node automaticity, beta-adrenergic receptor hypersensitivity, decreased parasympathetic activity, and impaired neurohumoral modulation (Sheldon et al., 2015; Chiale et al., 2006). The fundamental tension is between whether the underlying pathophysiology of IST results from abnormal autonomic regulation, abnormal sinus node function, or a combination of both (Olshansky and Sullivan, 2013; Nwazue et al., 2014).

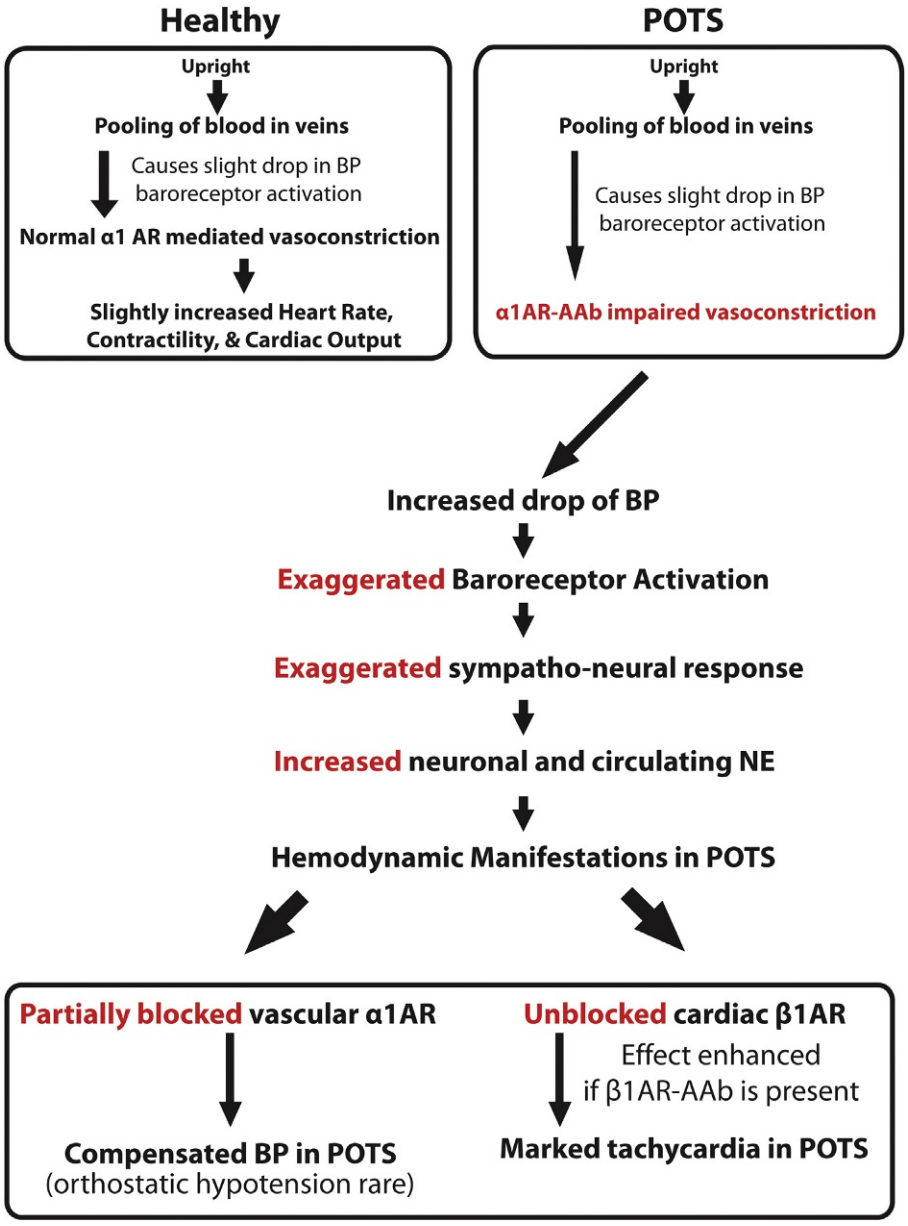

Fig. 1 Postulated mechanisms for α1-adrenergic receptor and β1/2-adrenergic receptor autoantibodies in the pathophysiological development of postural tachycardia syndrome. Serum autoantibodies may have a negative modulatory effect on α1AR activity and a positive modulatory effect on β1/2AR activity. The compensatory responses to these receptor blockades could contribute to hemodynamics of postural tachycardia syndrome. *α1AR*, α1-adrenergic receptor; *β1AR/β2AR*, β1/2-adrenergic receptor; *POTS*, postural tachycardia syndrome. Reproduced from the *Journal of the American Heart Association*, Autoimmune Basis for Postural Tachycardia Syndrome, 2014, H. Li *et al.*, with permissions from Wiley Blackwell.

While both IST and POTS patients appear to have abnormal autonomic modulation, with elevated sympathetic tone and diminished parasympathetic tone, it is more marked in IST patients (**Fig. 2**) (Nwazue et al., 2014). This may indicate a stronger autonomic influence in the pathophysiology of IST compared to POTS. The exaggerated sympathetic tone in IST patients may reflect an increase in antibodies to β-adrenergic receptors. Moreover, there are no significant differences in intrinsic HR (the HR after pharmacological autonomic blockade with propranolol and atropine) between IST, POTS, and healthy subjects (Nwazue et al., 2014). This suggests that abnormal sinus node automaticity may not play an important pathophysiological role in either disorder.

Clinical Evaluation

Sinus tachycardia is a normal response to exertion, but is also a common finding in clinical practice, and can be an important vital sign to help assess whether a patient may be acutely ill. Acute pathologies such as infection or dehydration can result in sinus

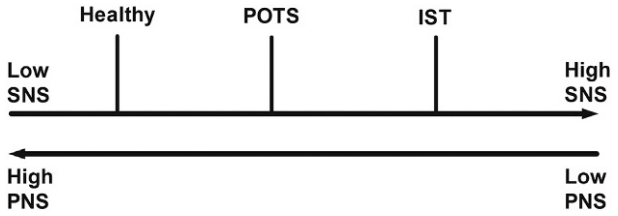

Fig. 2 Spectrum of sympathetic and parasympathetic autonomic nervous system activity in postural tachycardia syndrome and inappropriate sinus tachycardia. Healthy subjects have an appropriate balance of high parasympathetic tone and low sympathetic tone. In contrast, both inappropriate sinus tachycardia and postural tachycardia syndrome patients appear to have abnormal autonomic modulation, with elevated sympathetic tone and diminished parasympathetic tone. This effect is more marked in inappropriate sinus tachycardia patients. *IST*, inappropriate sinus tachycardia; *POTS*, postural tachycardia syndrome. Reproduced from the *Journal of the American Heart Association*, Postural Tachycardia Syndrome and Inappropriate Sinus Tachycardia: Role of Autonomic Modulation and Sinus Node Automaticity, 2014, V.C Nwazue *et al.*, with permissions from Wiley Blackwell.

Table 1 Diagnostic criteria for postural tachycardia syndrome and inappropriate sinus tachycardia

Postural tachycardia syndrome
1. Frequent, chronic (>6 months duration), symptoms that occur and worsen with standing
2. An increase in HR of ≥30 bpm (≥40 bpm in patients <18 years of age) associated within 10 min of a position change from supine to standing (held for more than 30 s)
3. The absence of orthostatic hypotension (a sustained decline in BP ≥20 mmHg systolic or ≥10 mmHg diastolic within 3 min of standing)
Inappropriate sinus tachycardia
1. Sinus tachycardia (>100 bpm at rest or a mean 24-h HR >90 bpm)
2. The absence of a primary cause of tachycardia
3. Distressing and disabling symptoms associated with the palpitations

bpm, beats per minute.

Definition adapted from Sheldon, R. S., Grubb, B. P., 2nd, Olshansky, B., Shen, W. K., Calkins, H., Brignole, M., Raj, S. R., Krahn, A. D., Morillo, C. A., Stewart, J. M., Sutton, R., Sandroni, P., Friday, K. J., Hachul, D. T., Cohen, M. I., Lau, D. H., Mayuga, K. A., Moak, J. P., Sandhu, R. K. and Kanjwal, K. (2015). 2015 heart rhythm society expert consensus statement on the diagnosis and treatment of postural tachycardia syndrome, inappropriate sinus tachycardia, and vasovagal syncope. *Heart Rhythm 12*, e41–e63.

tachycardia and will often be fairly obvious on history and examination. In contrast, those patients who presents in sinus tachycardia with no acute cause are more challenging to assess and properly diagnose. In order to know when to suspect a diagnosis of POTS or IST, it is important to first understand the diagnostic criteria for these conditions.

Diagnostic Criteria

The diagnostic criteria for POTS and IST are displayed in **Table 1** (Sheldon et al., 2015; Freeman et al., 2011; Garland et al., 2015). POTS is defined by three criteria: (i) an increase of HR of at least 30 bpm in response to a change in posture from supine to standing within 10 minutes; (ii) symptoms of orthostatic intolerance; (iii) orthostatic hypotension must be absent. Importantly, in order for a diagnosis of POTS to be reached, symptoms should be chronic (≥6 months) in duration. In pediatric patients (age <19 years), a larger HR increase (≥40 bpm) is required to make the diagnosis due to physiological orthostatic tachycardia seen in children and adolescents (Singer et al., 2012).

The symptoms of orthostatic intolerance associated with POTS can both cardiac and noncardiac in nature (Raj, 2013). Cardiac symptoms include palpitations, lightheadedness, chest pain, and shortness of breath (Thieben et al., 2007; Raj, 2013). These symptoms should be worse with standing. Noncardiac symptoms include mental clouding or "brain fog," headache, nausea, exercise intolerance, fatigue, tremulousness, blurred vision, and sleep disturbances (Raj, 2013; Thieben et al., 2007).

IST is defined by: (i) the presence of sinus tachycardia (≥100 bpm) when awake at rest or a 24-h mean HR of ≥90 bpm; (ii) symptomatic palpitations that the patient finds distressing (Sheldon et al., 2015). The symptoms associated with the tachycardia are required for the diagnosis of this syndrome. Symptoms share overlap with POTS patients and include palpitations, chest pain, shortness of breath, fatigue, myalgias, and lightheadedness (Olshansky and Sullivan, 2013; Femenía et al., 2012). In order for a diagnosis of IST to be made, other primary causes for the sinus tachycardia should be ruled out (Sheldon et al., 2015).

Differential Diagnosis

There are a number of primary causes (**Table 2**) for appropriate sinus tachycardia that must be considered and excluded prior to considering POTS or IST as the underlying etiology (Olshansky and Sullivan, 2013; Peyrol and Levy, 2016; Femenía et al., 2012). These primary causes can include acute physiological stimuli, medications, dietary indiscretions, and some other medical conditions. Physiological stimuli such as exercise, panic attacks, pain, and physical deconditioning can cause an increase in HR both at

Table 2 Primary causes of sinus tachycardia

Physiological	Autonomic
Dehydration	Orthostatic hypotension
Exercise	Postural tachycardia syndrome
Emotion	Cardiac
Pain	Acute myocardia ischemia/infarction
Pregnancy	Cardiomyopathy
Medications	Myocarditis
Anticholinergics	Pericarditis
Alcohol	Infection
Caffeine	Metabolic
Sympathomimetics	Anemia
Tobacco	Cushing's disease
	Hyperthyroidism
	Pheochromocytoma
	Psychiatric
	Anxiety/panic attacks
	Respiratory
	Chronic obstructive pulmonary disease
	Pulmonary embolism
	Shock

rest and while standing. There are also multiple classes of medications that can increase HR and present with sinus tachycardia, including sympathomimetics, anticholinergics, and rebound tachycardia from beta-blocker withdrawal (Olshansky and Sullivan, 2013). Dietary intake of alcohol and caffeine can also increase HR. Numerous acute and chronic medical conditions can lead to sinus tachycardia, including relatively common conditions such as hyperthyroidism, anemia, and dehydration (Raj, 2013; Olshansky and Sullivan, 2013).

History and Physical Examination

A detailed history and physical examination are important in the diagnosis of POTS and IST in order to help exclude more common causes of sinus tachycardia. Both patients with POTS and IST should have chronicity to their symptoms, which can be a helpful distinguishing factor from more acute causes of sinus tachycardia. It is important to ask about aggravating and alleviating factors for symptoms. This can be useful for discriminating between POTS and IST. Symptoms associated with IST often worsen in response to physiological or emotional stimuli that increase HR (Sheldon et al., 2015). In contrast, POTS symptoms should primarily be worse in response to standing or orthostatic stress (Sheldon et al., 2015). Additionally, POTS symptoms also tend to be worse in response to alcohol, poor sleep, heat, and exercise (Raj and Sheldon, 2016).

Syncope is another clinical presentation that can have symptoms that are similar to those described by patients with both POTS and IST. Importantly, only a minority of patients with POTS describe a history of fainting (Raj, 2013). Although the prevalence of syncope in patients with IST is currently unknown, it should not be a predominant feature of the syndrome. A history in which fainting is a major complaint may warrant further exploration for other potential etiologies.

A detailed review of past medical history can be very helpful to identify other associated conditions with POTS and IST. POTS is associated with hEDS, and gastroparesis (Garland et al., 2015; Benarroch, 2012). Both POTS and IST may share some overlap with CFS (Brady et al., 2005). The exact nature of the relationships between POTS, IST, and other associated conditions are still being understood.

As part of a review of systems, it is important to ask questions about symptoms of autonomic dysfunction. This should include assessment for autonomic neuropathy, which can be present in neuropathic POTS patients. This should include inquiring about gastrointestinal involvement including symptoms of constipation and irritable bowel syndrome (Sheldon et al., 2015; Wang et al., 2015). An assessment of autonomic function can be helpful for distinguishing these conditions from other autonomic disorders.

Similar to history, the physical exam may be helpful to distinguish POTS and IST from other primary causes of sinus tachycardia. Importantly, a clinical volume assessment and examining for any findings suggestive of hyperthyroidism can be of value. Overall, there are few physical findings in POTS or IST to be found on exam. Approximately 50% of POTS patients will have a dependent acrocyanosis present in their legs; this appears as a red-blue discoloration that is cold to touch (Raj, 2013). A neurological examination is useful to perform to examine for signs of small fiber neuropathy, which may be present in some POTS patients (Jones et al., 2016). There are no reported specific physical findings to be identified in a patient with IST; its diagnosis remains one of exclusion (Olshansky and Sullivan, 2013).

The most valuable part of the physical examination is to obtain a set of orthostatic vital signs in patients presenting with sinus tachycardia. This can be performed most simply by means of a supine to standing maneuver. Ideally, the patient will lie supine for a few minutes before baseline measurements of supine BP and HR. At this point, the patient quickly transitions to an upright position on their own accord and maintains a standing position for up to 10 min with BP and HR measurements made at minutes 1, 3, 5,

and 10 of standing. The HR responses to orthostatic stress can be an important way to distinguish between POTS and IST (Fig. 3). In POTS patients, HR responses are helpful to illustrate whether or not the patient meets the HR criteria for a diagnosis of POTS. Typically, POTS patients will have more severe orthostatic tachycardia in the morning compared to later in the day (Brewster et al., 2012). Importantly, patients with IST can have an orthostatic tachycardia in addition to their resting tachycardia. Unlike in POTS patients, standing is not usually the predominant cause of symptoms in IST. Additionally, this test can be helpful in ruling out orthostatic hypotension as a possible cause for symptoms.

Investigations

The investigative approach to the patient with sinus tachycardia is displayed in Table 3. In addition to a complete history and physical exam, a baseline 12-lead electrocardiogram is essential to distinguish sinus tachycardia from other possible atrial tachyarrhythmias. A basic panel of blood work is useful to help eliminate common causes of sinus tachycardia. This should include a complete blood count with a hematocrit (to exclude anemia) and a thyroid stimulating hormone level (to exclude hyperthyroidism). These are class IIa recommendations (benefit likely to exceed harm) by the Heart Rhythm Society (Sheldon et al., 2015). Other laboratory studies should only be done on a case-by-case basis depending on the clinical suspicion of other primary causes of sinus tachycardia.

A 24-hour Holter monitor can be a useful investigation, particularly for the diagnosis of IST (Sheldon et al., 2015). It can be of use to investigate the trend and fluctuations of HR over an extended period of time, including the relationship between HR and symptoms as well as the responses to stimuli such as physical exertion (Femenía et al., 2012). Additionally, the results of the 24-h Holter may provide objective HR findings that meet diagnostic criteria (24-h HR of $\geq$90 bpm with symptoms) for IST. A Holter may also be of use in both POTS and IST patients if there are ongoing concerns that a transient nonsinus tachyarrhythmia may be contributing to symptoms (Raj, 2013). However, a Holter monitor in the absence of orthostatic vital signs is not adequate for diagnosing POTS as the position/activity during the HR increases are not known.

Other investigations to consider include an exercise stress test and a transthoracic echocardiogram. These tests can be of use to help screen for other primary cardiovascular or systemic pathologies, but should only be considered in select patients in which there is some index of suspicion (Sheldon et al., 2015). An echocardiogram can be useful to rule out structural heart disease, particularly in patients in whom a good cardiac examination is not technically possible. In patients with IST, an exercise stress test may show enhanced HR responses (Morillo et al., 1994). The benefits of this test for the diagnosis of POTS and IST have not been widely reported.

Autonomic Function Testing

Autonomic function testing is not generally needed for a diagnosis of POTS or IST. It should be reserved for cases where there is suspected involvement of the autonomic nervous system and where testing may be of helpful to distinguish from POTS, IST, and other autonomic disorders. A detailed examination of autonomic function can include a head-up tilt test, Valsalva maneuver, supine and upright plasma norepinephrine levels, and an assessment of sudomotor function. The details of these tests are beyond the scope of this article and will not be discussed here.

Head-up tilt test

Head-up tilt testing is appropriate for patients in whom orthostatic vitals do not meet diagnostic criteria for POTS, but there is a strong clinical suspicion. It can be helpful to better characterize the orthostatic tachycardia and BP responses during standing. Passive standing (such as with a head-up tilt table test) can be associated with a greater orthostatic tachycardia than active standing,

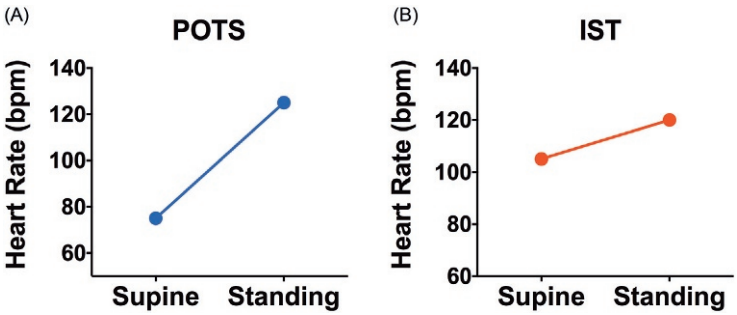

Fig. 3 Heart rate responses during supine and standing positions in patients with postural tachycardia syndrome (A) and inappropriate sinus tachycardia (B). When orthostatic vitals are taken as part of a physical exam, patients with postural tachycardia syndrome should have a normal heart rate while supine, but experience an excessive heart rate increase upon standing. In contrast, patients with inappropriate sinus tachycardia will have sinus tachycardia even while supine. These patients may often have an orthostatic increase in heart rate, but it is not usually as profound as for a patient with postural tachycardia syndrome. POTS, postural tachycardia syndrome; IST, inappropriate sinus tachycardia.

Table 3 Utility of investigations for assessing a patient with sinus tachycardia of unknown cause

Investigation	*Utility*	*Comment*
Orthostatic vitals	Essential	Assess whether patient meets diagnostic criteria for POTS. Rule out OH
Blood work	Essential	Helpful to rule out primary causes of sinus tachycardia
ECG	Essential	Assess for nonsinus tachyarrhythmias
Holter monitor	Most cases	Assess whether patient meets diagnostic criteria for IST. Rule out nonsinus tachyarrhythmias
Echocardiogram	Select cases	Rule out structural heart disease
Exercise stress test	Select cases	Assess heart rate responses to exercise
Head-up tilt test	Select cases	Useful in patients with an unclear diagnosis in which other autonomic disorders need to be ruled out
Valsalva maneuver	Select cases	Useful to differentiate between POTS, IST, and other autonomic disorders. POTS patients with mast cell activation or neuropathic subtypes may have abnormal responses
Plasma norepinephrine levels	Select cases	Elevated upright and absolute change from supine support a hyperadrenergic POTS subtype
Sweat testing	Select cases	Useful in POTS patients suspected of having a neuropathic subtype

POTS, postural tachycardia syndrome; *OH*, orthostatic hypotension; *IST*, inappropriate sinus tachycardia.

although the difference is not that great within the first 10 min (Plash et al., 2013). It can also be useful to distinguish between POTS, IST, and other types forms of orthostatic intolerance, such as VVS (**Fig. 4**).

Valsalva maneuver

A Valsalva maneuver provides an assessment of cardiovagal and adrenergic function. This test can be valuable to help differentiate POTS and IST from other autonomic disorders. In patients with POTS and IST, these responses should be intact and frequently normal compared to healthy subjects (**Fig. 5**) (Palamarchuk et al., 2016). POTS patients that have co-existing mast cell activation may show an exaggerated response, with an overshoot in late phase II and a heightened phase IV overshoot (Shibao et al., 2005). In contrast, patients with a neuropathic subtype of POTS have been found to have blunted phase IV overshoot in comparison to control subjects and patients with nonneuropathic POTS (Gibbons et al., 2013).

Plasma norepinephrine levels

Plasma norepinephrine levels can be obtained while supine and while standing (after at least 5–10 min); the baseline levels as well as absolute change can be helpful toward assessing the functioning of adrenergic terminals and the baroreflex (Low et al., 2013; Low, 2015; Goldstein et al., 1989). In POTS patients, the supine norepinephrine levels are usually normal, whereas absolute change and upright values can be elevated (usually >600 pg/mL) (Raj, 2006; Wagoner et al., 2016; Okamoto et al., 2012). This finding can be supportive of an underlying hyperadrenergic pathophysiological state that may be contributing to symptoms (Benarroch, 2012). Plasma norepinephrine levels are not normally performed in the workup of IST.

Sweat testing

Sweat testing is performed to assess sympathetic sudomotor axon function, and can be helpful for identifying patients with lower limb sympathetic denervation that can be characteristic in POTS patients with a neuropathic pathophysiological subtype (Low et al., 2013; Benarroch, 2012; Gibbons et al., 2013).

Management

There is no gold-standard therapy available for the management of POTS or IST. Both conditions must be managed in a graded approach that should first involve conservative nonpharmacological therapies, followed by pharmacological management as needed. Overall, strong evidence-based recommendations for management are lacking (Lau et al., 2016). A multidisciplinary approach is recommended in the management of POTS, involving different types of health care team members including nursing, physiotherapy, psychology, and physicians support (Kizilbash et al., 2014). This approach can certainly be of benefit for patients with IST as well (Brady et al., 2005; Shen, 2005).

Postural Tachycardia Syndrome

Management options for patients with POTS are displayed in **Table 4**.

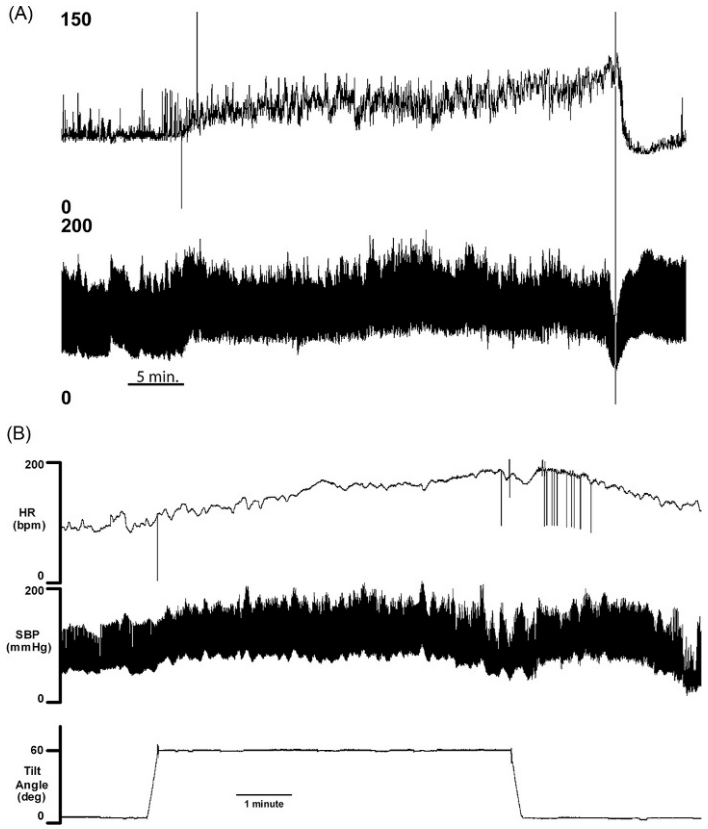

Fig. 4 Blood pressure and heart changes during head-up tilt testing for a patient with (A) vasovagal syncope and (B) postural tachycardia syndrome. Beat-to-beat blood pressure and heart rate responses are shown through the duration of testing. The patient with vasovagal syncope (A) exhibits a slight rise in heart rate over the upright phasing of testing (~35 min). In contrast, the patient with postural tachycardia syndrome (B) has an exaggerated heart rate response within minutes of being tilted upright. Blood pressure is well maintained for both patients through the majority of testing. *HR*, heart rate; *BP*, blood pressure. Reproduced from Electrophysiological Disorders of the Heart: Expert Consult, Chapter 73, 2012, S.R. Raj & R.S. Sheldon, with permission from Elsevier.

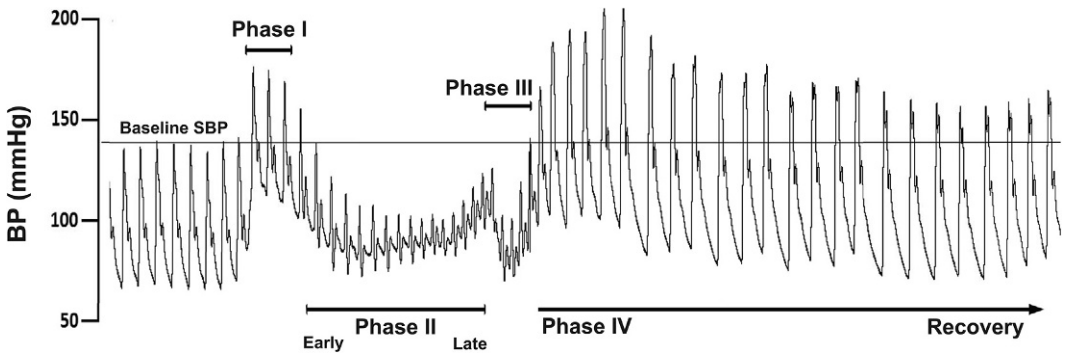

Fig. 5 Valsalva maneuver in a healthy subject. The test is performed by having the patient exhale against a closed glottis generating 40 mmHg of pressure for 15 s. The resulting blood pressure and heart rate waveforms can be broken down into four phases. Phase I represents a transient rise in blood pressure that occurs during the forced expiration. Increased intrathoracic pressure forces blood out of the pulmonary circulation, resulting in increased stroke volume and blood pressure. Phase II is commonly divided into an early (II_E) and late (II_L) interval. As intrathoracic pressure remains high during forced expiration, venous return decreases. This results in reduced stroke volume and blood pressure and represents the II_E component. The reduced blood pressure that occurs during phase II_E triggers a baroreceptor mediated increase in sympathetic tone causing a rise in heart rate and blood pressure that represents phase II_L. Phase III occurs upon termination of forced expiration, resulting in a decrease in intrathoracic pressure. This allows for increased venous return and causes a sudden decrease in blood in the systemic circulation resulting in a further drop in blood pressure. This drop in blood pressure causes another baroreceptor mediated increase in sympathetic tone resulting in a blood pressure overshoot that represents phase IV of Valsalva maneuver.

Table 4 Management of postural tachycardia syndrome

Nonpharmacological	*Comment*
Education	Critical
Exercise training	Very important; not easy
Increase fluid intake (2–3 L/day)	
Increase dietary salt intake (10–12 g/day)	
Pharmacological	
Volume expansion	
1–2 L bolus of normal saline IV	Only for use for acute worsening of symptoms
Fludrocortisone (0.05–0.2 mg/day PO once daily)	
Desmopressin (0.2 mg PO)	Only for occasional use
Antiarrhythmic medications	
Propranolol (10–20 mg PO QID)	Not well tolerated at higher doses
Ivabradine (5–7.5 mg PO once or twice daily)	
Pressor agents	
Midodrine (2.5–10 mg PO q4 hours three times daily)	Should not be taken within 4 h of bed
Clonidine (0.05–0.2 mg twice daily)	Can be associated with rebound hypertension and tachycardia
Methyldopa (125–250 mg PO twice daily)	
Other agents	
Pyridostigmine (30–60 mg PO three times daily)	
Modafinil (100 mg PO twice daily)	

Nonpharmacological

Education is at the forefront of conservative management options for POTS. Patients and families should be reassured that although POTS is a chronic condition, there are no reported cases of mortality from POTS. Additionally, even though the long-term outcomes from POTS are somewhat unknown, in many patients symptoms do seem to improve over time (Sheldon et al., 2015; Kizilbash et al., 2014). Patients should also be educated to limit or avoid triggers or activities that make symptoms worse (Benarroch, 2012).

Exercise-training program

Exercise is encouraged in patients with POTS, but should be undertaken in a gradual, graded approach given that it can also be an aggravating factor for symptoms, especially initially. Several studies have shown benefits from a short-term (3-month) exercise program in which patients first begin exercising in a recumbent or semirecumbent position (Fu et al., 2011; Fu and Levine, 2015). Cycling, swimming, and a rowing machine are activities that can be performed from these positions. These studies used an initial dose of 20–30 min of exercise (with a 5-min warm up and cool down), three times a week, at an intensity of 75% maximal target HR for their age (Fu and Levine, 2015). Additionally, these studies had patients perform strength training for 15–20 min, one to two times per week (Fu and Levine, 2015). Both cardio and strength sessions were increased as subjects became more fit, primarily in the second and third months. These studies found that exercise helped increase peak VO_2 as well as showing echocardiographic findings of significant cardiac remodeling (Fu and Levine, 2015). Another study found that a 3-month exercise program significantly improved quality of life and decreased HR responses to standing (George et al., 2016). In fact, 71% of patients no longer met POTS HR criteria at the end of the program (George et al., 2016).

An important issue raised from studies examining the therapeutic effects of exercise in POTS patients is nonadherence to therapy (Fu et al., 2010; George et al., 2016). In this regard, there are valuable clinical pearls clinicians should know to help patients be successful. Firstly, selecting an activity that can be performed in the recumbent position, such as rowing, swimming, or a cycling is important to minimize the effects of symptoms made worse in the upright position, especially initially (Raj, 2016). Secondly, patients must understand that it will take time (~4–6 weeks) before the benefits of exercise being to take effect (Raj, 2016). In fact, it is not unusual for patients to feel more fatigued upon starting an exercise program. Finally, it is important to educate patients that three months of training is not a "cure" for POTS. Similar to any patient, regular physical activity should be encouraged as a lifelong activity. Overall, the Heart Rhythm Society consensus statement for the management of POTS gives exercise a class IIa recommendation (Sheldon et al., 2015).

Fluids

Patients should be encouraged to increase both fluid (2–3 L) and dietary salt intake (10–12 g) per day if hypovolemia is suspected (Sheldon et al., 2015). POTS patients have been shown to have deficits in their circulating plasma volume (Raj et al., 2005a). It is believed that improved effective blood volume will help reduce the intensity of the reflex tachycardia associated with standing that may be contributing to symptoms.

Avoid potentially deleterious medications

Common medications that worsen symptoms should be avoided. These include nitrates or other diuretics, venodilators, and SNRI medications (Nwazue and Raj, 2013). Diuretics can decrease effective blood volume, and worsen symptoms driven by hypovolemia. Venodilators act to decrease preload and subsequently blood pressure leading to worsening reflex tachycardia. Selective NET inhibitors decrease the clearance of norepinephrine from synaptic terminals, which can lead to increased sympathetic activity resulting worsening symptoms secondary to increased HR.

Pharmacological

There are no drugs currently approved by the US Food and Drug Administration specifically for the treatment of POTS. All agents used are done so "off-label" and few have rigorous randomized clinical trials to support their use. Additionally, there is no "shotgun" first line agent to use for the pharmacological management of POTS. It is very important to consider the clinical features of the POTS and the possible pathophysiology that is leading to symptoms. In this way, medications can be selected that will best treat these deficits. Many of the pharmacological options to treat POTS can be broadly categorized into agents that target volume expansion, antiarrhythmic medications to slow HR, and pressor agents to improve peripheral vasoconstriction.

Blood volume expansion

In a presentation of a POTS patient with acute worsening of their symptoms, an intravenous bolus of 1–2 L of normal saline has been shown to acutely improve orthostatic HR and symptoms (Jacob et al., 1997; Gordon et al., 2000). For a patient with known POTS presenting in an acute care setting, like the emergency department, a normal saline infusion can be an effective option in such a scenario. This treatment modality acts to reduce hypovolemia in these patients. Essentially it follows a similar mechanism, but makes a much more acute impact, as increasing dietary fluid and salt intake. The long-term use of normal saline infusions as a means of treatment is not recommended (Sheldon et al., 2015). Significant risks include complications associated with central intravenous line insertion such as thrombosis and infection.

Fludrocortisone is a modified corticosteroid, which, at low doses (0.05–0.2 mg PO once daily), has predominantly mineralocorticoid activity resulting in sodium and water retention. This can be considered an adjunct to help boost plasma volume in addition to increasing dietary salt and water intake. There are no randomized controlled trials testing the use of fludrocortisone in POTS patients. A trial in patients with CFS and orthostatic intolerance did not find a significant improvement in symptoms, however this study focused on global symptoms rather than those secondary to orthostatic stress (Rowe et al., 2001). In patients with VVS, fludrocortisone may offer benefit in syncope recurrence in patients who reach a stabilized dose (Sheldon et al., 2016). Potential side effects of fludrocortisone include hypertension, fatigue, nausea, and hypokalemia (Lee and Krahn, 2016). The Heart Rhythm Society consensus statement suggests that despite limited evidence for the use of fludrocortisone in POTS patients, the potential benefits likely outweigh the risks (Sheldon et al., 2015).

Desmopressin (DDAVP) is a synthetic version of antidiuretic hormone and promotes free water retention. This has been tested in POTS patients with the hypothesis of eliciting blood volume expansion for POTS patients who are hypovolemic. One study found that DDAVP improved standing HR and symptoms in POTS patients upon acute administration (Coffin et al., 2012). Potential effects of its administration include hyponatremia (especially with the increased water intake advised for POTS patients), headache, and peripheral edema (Nwazue and Raj, 2013).

Antiarrhythmic medications

Propranolol, when used at low doses (10–20 mg PO four times daily), reduces supine and standing HR, and improves symptoms acutely (Raj et al., 2009a). Higher doses were found to make symptoms worse. Quality of life after three months of exercise training has been found to provide superior improvement in quality of life in comparison to long-acting propranolol despite both providing significant reductions in HR (Fu et al., 2011). To date, other beta blockers have not actively studied (Sheldon et al., 2015). Similar to fludrocortisone, the Heart Rhythm Society suggests propranolol can be considered for the treatment of POTS in the absence of evidence contraindicating its use (Sheldon et al., 2015). Some centers consider propranolol as first line treatment for POTS (Garland et al., 2015).

Ivabradine is a selective funny current (I_f) channel blocker that can be used to slow HR (DiFrancesco, 2010). It has been more extensively tested in the treatment of IST, but a retrospective case series of POTS patients prescribed ivabradine found that 60% of patients reported a symptomatic improvement with its use (McDonald et al., 2011). The major downside to this medication is a lack of availability in some countries, although this may change in the near future (Sheldon et al., 2015). Dosing is recommended at 5–7.5 mg once or twice daily.

Pressor agents

Midodrine is a pro-drug that is an alpha-1 adrenergic receptor agonist when converted to its active form. It can increase vascular resistance and venous return through peripheral constriction of arteries and veins. The improved venous return is believed to reduce the reflex tachycardia that accompanies standing. Midodrine may be more useful with the peripheral neuropathy seen in neuropathic POTS, where peripheral vasoconstriction may be impaired (Ross et al., 2014). Several studies in POTS patients have found the effects of midodrine to decrease supine and upright HR and improve symptoms acutely (Jacob et al., 1997; Gordon et al., 2000). Midodrine has several challenges associated with its use including the need for frequent dosing, its brief effect (~4 h), and

side effect of supine hypertension (Raj and Sheldon, 2016). As such, it is not recommended to be taken within 4–5 h of sleep (Jones et al., 2016). Other side effects of midodrine that may contraindicate its use include urinary retention in older men, and possible teratogenic effects when used in pregnant females (Raj and Sheldon, 2016). Expert opinion suggests that midodrine can be attempted for the management of POTS given the absence of contraindications against its use (Sheldon et al., 2015).

Clonidine and methyldopa are centrally acting sympatholytic agents that may be useful in patients with hyperadrenergic POTS. Clonidine is an alpha-2 receptor agonist that may be helpful in reducing symptoms in patients with prominent hyperadrenergic features (Gaffney et al., 1983). Methyldopa is a false neurotransmitter that exudes a similar effect, and has also been suggested for use in patients with a hyperadrenergic state (Shibao et al., 2005). It is thought to be easier to titrate in POTS patients in comparison to clonidine due to its longer half-life (Jacob and Biaggioni, 1999). The tolerability of these medications may be limited due to side effects including worsening drowsiness, fatigue, and mental clouding, all of which can be presenting complaints in POTS patients (Raj, 2013). Additionally, both medications may be less beneficial in POTS patients with significant neuropathic features, although data is lacking.

Droxidopa is a synthetic amino acid precursor for norepinephrine, and acts to increase synaptic levels of the neurotransmitter, enhancing the effects of vasoconstriction. It is postulated to act as an alternative to midodrine in POTS patients with symptoms refractory to treatment. One retrospective study found droxidopa to improve symptoms in POTS patients, but induced no significant reduction in standing HR and did not seem to improve quality of life in these patients (Ruzieh et al., 2017). Given that droxidopa is a norepinephrine precursor, it has the potential side effect of increasing sympathetic activity resulting in further increased HR, possibly worsening symptoms. Overall, there is a fundamental lack of data assessing the efficacy of droxidopa in POTS patients.

Other pharmacological agents

Pyridostigmine is a peripherally acting acetylcholinesterase inhibitor. The mechanism of action of pyridostigmine is to increase transmission of acetylcholine at synaptic junctions equipped with nicotinic or muscarinic AChR. This results in increased cardiovagal tone, helping reduce HR. It has been shown to reduce tachycardia and improve symptoms acutely in POTS patients (Raj et al., 2005b). Unfortunately, there are significant gastrointestinal side effects that limit the efficacy of this medication including severe cramping, nausea, and diarrhea in about 20% of POTS patients (Kanjwal et al., 2011). In patients who can tolerate the drug long-term, it does continue to help control tachycardia and symptoms (Kanjwal et al., 2011); the Heart Rhythm Society provides a modest recommendation for its use (Sheldon et al., 2015).

In patients in whom mental clouding is a predominant symptom, modafinil has anecdotally been used to improve concentration and alertness in POTS patients (Raj, 2013). It is a psychostimulant with similar actions to amphetamine and methylphenidate (Taneja et al., 2004). Additionally, it does not appear to worsen symptoms of orthostatic tachycardia when tested in POTS patients (Kpaeyeh et al., 2014).

Inappropriate Sinus Tachycardia

The treatment of IST is a challenge. A rate control strategy to eliminate the presence of the sinus tachycardia does not always eradicate symptoms (Olshansky and Sullivan, 2013; Sheldon et al., 2015). Additionally, there is little concrete evidence in the literature to develop strong recommendations for the management of IST (Sheldon et al., 2015). With that being said, conservative management with good patient education is key in the initial management. Patients can often be in great distress about their symptoms. Similar to the treatment of POTS, it is important to reassure patients that the disease course is thought to be benign (Abed et al., 2016) and that no known mortality is known to be caused from this condition (Sheldon et al., 2015; Olshansky and Sullivan, 2013).

The use of beta blockers or other negative chronotropic agents for the treatment of IST has been proposed as a means of reducing HR. Experts believe that symptoms often do not disappear while on beta blockers (Sheldon et al., 2015; Olshansky and Sullivan, 2013). Nondihydropyridine calcium channel blockers and amiodarone have also been suggested as potential therapeutic agents, but the benefits of HR reduction are likely outweighed by the adverse effects of these medications (Ptaszynski et al., 2013). Finally, no prospective studies or randomized controlled trials have been conducted to show any benefit for the use of these medications.

Ivabradine has been tested as an off-label product for the treatment of IST (Annamaria et al., 2015). It has been the most vigorously tested medication in the treatment of IST, including having one randomized control trial testing its efficacy. This trial found ivabradine was well tolerated, eliminated 75% of baseline symptoms in its cohort ($n = 21$) and fully eliminated symptoms in approximately 50% of patients (Cappato et al., 2012). In comparison to the beta blocker metoprolol, ivabradine exerts a similar decrease in HR, is better tolerated, and is more effective at relieving symptoms associated with IST (Ptaszynski et al., 2013). A dose of 5–7.5 mg twice daily can slow HR by 14%–31% of baseline (Annamaria et al., 2015).

Sinus node ablation or modification has been explored as a therapeutic option to treat patients with IST. Both radiofrequency and surgical techniques have been tried (Olshansky and Sullivan, 2013). Sinus rate can be significantly improved by these techniques, but there is limited evidence that clinical symptoms are vastly improved (Shen et al., 2001; Olshansky and Sullivan, 2013). Additionally, there are rather significant potential complications secondary to these permanent procedures including surgical risks and making the patient pacemaker dependent (Lee et al., 1995; Man et al., 2000). The Heart Rhythm Society consensus statement strongly recommends against the use of these treatments for patients with IST (Sheldon et al., 2015).

Conclusions

POTS and IST represent etiologies for a patient presenting in sinus tachycardia without any clear primary cause. Neither condition is believed to be linked to an increased risk of mortality, but each can substantially negatively impact a patient's quality of life if not treated. There is no gold-standard treatment for POTS or IST, and there is limited high quality evidence for management of these conditions. Experts agree that a multidisciplinary approach that considers the individual patient's presentation, underlying pathophysiology of the disease, and associated conditions allows for available treatment strategies to be selected and tailored to the patient at hand. Much remains unknown about these conditions and considerable effort is still required to fully understand the pathophysiology and optimal treatment of these conditions.

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Skeletal Muscle in Heart Failure

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Heart Failure

Congestive heart failure (CHF) is defined as "a pathophysiological state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate commensurate with the requirements of the metabolizing tissues (Francis et al., 2011)." In other words, the heart is unable to adequately perfuse peripheral tissue unless cardiac filling pressures are abnormally high (Lilly and Harvard Medical School, 2011). In the United States, the incidence of CHF is 550,000 cases per year and the prevalence is 5.8 million cases (Lilly and Harvard Medical School, 2011). CHF, which mostly affects older adults, is the most common diagnosis of hospitalized patients 65 years and older (Lilly and Harvard Medical School, 2011; Rich et al., 2016). The prevalence is expected to increase significantly due to associations with age and interventions that increase survival after CHF (Lilly and Harvard Medical School, 2011).

CHF presents as a clinical syndrome (Lilly and Harvard Medical School, 2011). One of the primary features of CHF is exercise intolerance (Fletcher et al., 2011). Patients also present with fatigue, volume overload, shortness of breath, dyspnea, impaired urine output, paroxysmal nocturnal dyspnea, orthopnea, nocturnal cough, dulled mental status, and edema (Lilly and Harvard Medical School, 2011). Patients are progressively restricted in daily activities (Minotti et al., 1992). The severity of physical limitations correlates more closely with prognosis than hemodynamics (Mettauer et al., 2006).

CHF may be classified as either heart failure with reduced ejection fraction (HFrEF) or heart failure with preserved ejection fraction (HFpEF). Most patients diagnosed with CHF have decreased systolic function, which is a form of HFrEF (Sarma and Levine, 2015). Research has historically focused on exercise intolerance in patients with HFrEF (Maurer and Schulze, 2012). The most common type of CHF in older patients, however, is HFpEF (Sarma and Levine, 2015). HFpEF is also more common in women (Sarma and Levine, 2015; Taub, 2016). Patients with HFpEF have similar mortality and rehospitalization rates (Sarma and Levine, 2015). With growing recognition of patients with HFpEF and a lack of specific therapies, researchers are now working to further characterize peripheral abnormalities in HFpEF (Maurer and Schulze, 2012).

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Skeletal Muscle in Heart Failure

In CHF, many bodily responses to maintain circulatory function ultimately become maladaptive, contributing to progression over time (Francis et al., 2011). Decreased cardiac performance leads to problems in peripheral circulation, the kidney, and many other organs (Francis et al., 2011).

As in other chronic conditions, such as chronic pulmonary or renal disease, CHF leads to skeletal muscle abnormalities (Zizola and Schulze, 2013). Skeletal muscle changes are clearly identifiable from an early stage in CHF and contribute to disease progression (Tomono et al., 2016; Georgiadou and Adamopoulos, 2012). The changes impact both peripheral and ventilatory muscles (Georgiadou and Adamopoulos, 2012). Alterations are present at rest, but they are exacerbated during exercise (Georgiadou and Adamopoulos, 2012).

Even though changes in CHF are induced by cardiac abnormalities, symptoms like exercise intolerance are often independent of central hemodynamic alterations and reduced blood flow to muscle (Georgiadou and Adamopoulos, 2012; Cicoira et al., 2001). Similarly, hemodynamic improvement does not necessarily reverse symptoms (Georgiadou and Adamopoulos, 2012). These observations suggest that circulatory changes are not primarily responsible for changes in skeletal muscle metabolism. This is in contrast to healthy patients, in whom exercise tolerance is related to cardiac output (Georgiadou and Adamopoulos, 2012). Instead, exercise tolerance in CHF patients with HFrEF and HFpEF is more related to biochemical, metabolic, and histologic changes in skeletal muscle that lead to skeletal muscle dysfunction (Georgiadou and Adamopoulos, 2012; Cicoira et al., 2001; Bowen et al., 2015). Consequently, training improves exercise tolerance through peripheral adaptations without impacting central hemodynamics. This implies that peripheral skeletal muscle plays a significant role in CHF symptoms. Researchers have suggested that the role of circulation may be dependent on the disease stage, with peripheral alterations playing greater roles later in the progression (Piepoli et al., 2001).

Studies have shown that peripheral factors play a role in patients with HFrEF and HFpEF. Many of these factors, which will be explained in depth in this paper, are shown below (**Fig. 1**, **Tables 1** and **2**). Patients with HFrEF and HFpEF have similar levels of exercise intolerance and dyspnea (Sarma and Levine, 2015). A study using soleus tissue in animal models suggested, however, that skeletal muscle alterations are exacerbated in HFrEF compared to HFpEF (Seiler et al., 2016). Diaphragm muscle, on the other hand, was not significantly different between the two groups (Seiler et al., 2016).

Skeletal Muscle Tissue and Muscle Fiber Changes

Skeletal muscle tissue is characterized by strong, quick, discontinuous, and voluntary contractions. The structural unit of skeletal muscle tissue is called a sarcomere. The composition of muscle fibers depends on activity, mechanical load, hormones, and age (Mettauer et al., 2006). Cardiac tissue, on the other hand, is characterized by continuous and involuntary contractions. Significant

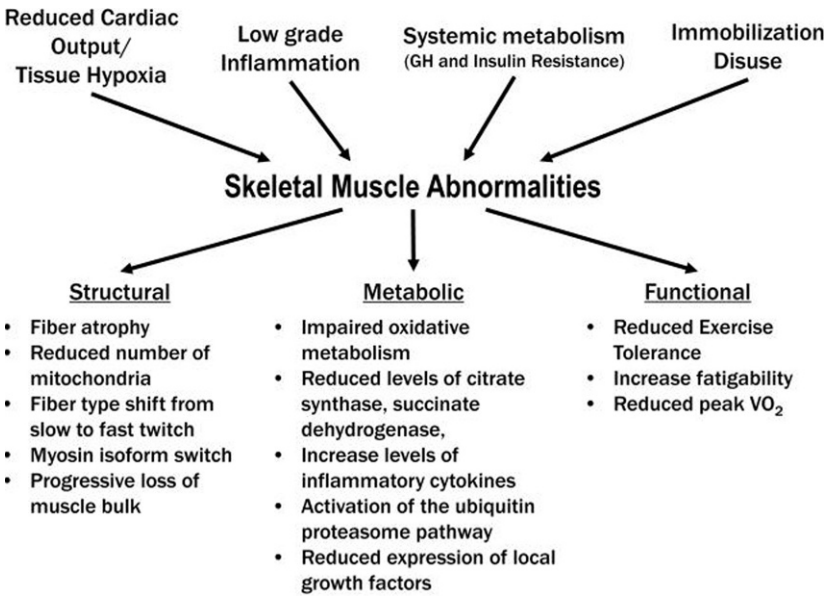

Fig. 1 Skeletal muscle abnormalities in CHF (Zizola and Schulze, 2013).

Table 1 Skeletal muscle changes in heart failure (Piepoli et al., 2001)

Function	↑ Weakness
	↑ Fatigability
Morphology	1. Quantity: Loss of muscle mass (bulk)
	2. Quality: Atrophy, damage and/or necrosis
Blood flow	↓ Capillary density (endothelial cell/fiber)
	↓ mL min^{-1}
	↓ Vasodilatation
Metabolism	↑ Glycolytic
	↓ Oxidative
	↑ Phosphocreatinine depletion
	↑ Intracellular acidosis
Ultrastructural	↓ Type I oxidative slow-twitch
1. Fiber type	↑ Type IIb glycolytic fast-twitch
2. Mitochondrial	↓ Volume density
3. Endothelial dysfunction	↓ Surface density of cristae

Table 2 Skeletal Muscle Abnormalities in Heart Failure (HF) (Liu and Marcinek, 2016)

Author, year (no.)	*Population character*	*Key measurement*	*Outcome and conclusion*
Mancini et al. (1988)	HF and age-matched healthy control male (~54 years)	HF peak VO_2 13.6 ± 5 mL/min/kg In vivo P31-NMR	Lower leg muscle mass loss in HF Calf metabolic abnormality part due to muscle atrophy During exercise Pi/PCr, HF > control Recovery time post Ex, HF > control Impaired metabolic profile suggests intrinsic changes not only due to muscle loss
Southern et al. (2015)	HF and age-matched control (~64 years)	Near-infrared spectroscopy	Oxidative capacity lower in HF and exercise training improved oxidative capacity only in control, suggested reduced oxidative capacity and impaired training adaptation in HF
Mancini et al. (1994)	HF and control (~58 years)	Ventilatory capacity: progressive isocapnic hyperpnea respiratory muscle deoxygenation near-infrared spectroscopy	Reduced respiratory ventilator capacity without respiratory muscle deoxygenation in HF
Wiener et al. (1986)	HF and age-matched control (~60 years)	Forearm blood flow with plethysmography Metabolism ^{31}P NMR	Forearm blood flow was similar, but increased Pi/PCr upon exercise suggested that abnormal metabolism in HF was not due to decreased muscle blood flow rather intrinsic changes in mitochondria or substrate utilization
Mancini et al. (1989)	HF and age-matched control (~57 years)	^{31}P NMR; Pi/PCr and PH changes In vitro muscle biopsy; enzyme activity fiber type	HF fiber shift to increased fast, glycolytic type IIb fibers; decreased fatty acid oxidation enzyme activity; ^{31}P NMR also showed higher Pi/PCr; no correlation between Pi/PCr to VO_2 and enzyme activity suggested intrinsic changes not due to metabolic response (Pi/PCr changes)
Toth et al. (2012)	HF and control, age and physical activity matched	18-Week resistance training (RE) Muscle biopsy, in vitro analysis	Larger but fewer mitochondria per fiber without difference in content between HF and control; no difference in mitochondria enzyme activity and mRNA level of cytochrome oxidase RE increased mitochondrial transcription factor A and muscle strength for both HF and control but did not alter mitochondrial size/content, enzyme, or transcriptional regulator
MiddleKauff et al. (2013)	HF and control (~50 year, peak VO_2 11.6 mL/kg/min)	Muscle biopsy and in vitro biochemistry analysis; fiber typing and mitochondria enzyme activity	HF fibers shift to fast twitch; no difference in vascular index or many of mitochondrial enzyme activities; clonidine revealed no impact for HF suggested skeletal myopathy is not caused by neuroendocrine disuse
Zizola et al. (2015)	Myocardial infarction induced mice	In vitro muscle biopsy: fiber typing and enzyme activity assay; ATP content	Muscle dysfunction associated with impaired PPARδ signaling; upon treating MI mice with PPARδ agonist corrected diminished oxidative capacity and FA metabolism (CPT1) and improved exercise capacity
Schrepper et al. (2012)	Pressure overload hypertrophy in rat heart	In vitro assay from muscle biopsy; isolation of fresh mitochondria mRNA and protein assay	Complexes I and II early increase and then later decline (I, II, and III) in respiratory capacity not due to differences in gene expression or supercomplex assembly
Dai et al. (2011)	Ang II induced cardiomyopathy in mice treated with SS-31	Flow cytometric access MitoSOX and DCFDA signal	SS-31 reduced Ang II-induced ROS signal (mitochondrial superoxide and total cellular ROS)
Kitzman et al. (2014)	HF with preserved ejection fraction (HFpEF)	Peak VO_2 Fiber type Capillary-to-fiber ratio	Reduced type I fiber and capillary to fiber ratio contribute to exercise intolerance among HF

similarities and differences between skeletal and cardiac muscle tissue are shown below (**Table 3**). Skeletal muscle tissue, along with vessels, nerve fibers, and connective tissue, forms the skeletal muscle organ. Skeletal muscle accounts for 40%–45% of body weight, which is more than any other organ of the body (Mohrman and Heller, 2014). Based on functional demands, muscles may be composed of different types of fibers with different capabilities (**Table 4**). Skeletal muscle has high capacity for regeneration and remodeling, making it a good target for therapeutic approaches (Rich et al., 2016).

In patients with CHF, muscle fibers are characterized by abnormalities. Changes are related to different isoforms of myosin heavy chain (MHC), which is the motor protein in the muscle thick filament myosin (Mettauer et al., 2001; Nicoletti et al., 2003). Muscle biopsies and ultrastructural analyses have shown that there are fewer slow fibers and more fast fibers in patients with CHF. The change is due to an increase in the relative content of MHC 2X from active healthy patients to sedentary healthy patients to CHF patients (Mettauer et al., 2001). In other words, patients with CHF have a higher proportion of fast-twitch, type II fibers relative to type I fibers (Georgiadou and Adamopoulos, 2012). These changes in muscle fibers have been confirmed in patients with HFpEF as well (Maurer and Schulze, 2012). This has been supported by observation of splitting of the inorganic phosphate (Pi) peak in patients with CHF, which implies the presence of two types of muscle fibers (Minotti et al., 1992).

Some studies have suggested that no significant fiber difference exists between sedentary controls and CHF patients (Mettauer et al., 2001). This would support the idea that fiber type changes are due to muscle disuse rather than specific effects of CHF. This was supported in studies that showed no fiber type shift compared to deconditioned controls with similar VO_2 max (see the "**Exercise**" section for a further discussion on VO_2 max) (Nicoletti et al., 2003). It has been shown, however, that angiotensin-converting enzyme inhibitor (ACEi) treatment normalizes myosin isoform abnormalities in muscle fibers in CHF patients (Mettauer et al., 2006). This may account for discrepancies between studies.

Muscle fiber type directly impacts metabolism (see "**Metabolic Changes**" section for further discussion). Mitochondrial respiration in active controls favors slow-oxidative muscle (Mettauer et al., 2001). This is characterized by high oxidative capacity, low sensitivity to external adenosine diphosphate (ADP), and control by mitochondrial creatine kinase (CK) (Mettauer et al., 2001). Respiration in sedentary controls and CHF patients, on the other hand, is closer to fast-glycolytic muscle in rodents (Mettauer et al., 2001). Some groups showed that muscle fibers in patients with CHF show decreased activity of oxidative enzymes while activity of glycolytic enzymes is maintained (Minotti et al., 1992). Type II fibers, which have higher oxygen and adenosine triphosphate (ATP) consumption, transition to anaerobic metabolism sooner (Vescovo et al., 2000). Muscle biopsies in patients with CHF showed reduced activity of both lipolytic and oxidative enzymes (Zizola and Schulze, 2013) .Fiber changes cause metabolic signaling to favor replenishment of energy stores instead of modification of energy production based on demands (Mettauer et al., 2006). The fibers also lead to a lower pH during exercise (Minotti et al., 1992).

Muscle fibers in patients with CHF may also be characterized by atrophy, decreased maximal strength, and reduced electromyographic activity (see "**Muscle Atrophy**" section for further discussion) (Georgiadou and Adamopoulos, 2012). Some studies have shown that all fibers have reduced size, whereas some studies suggest that only type II fibers have reduced diameter (Georgiadou and Adamopoulos, 2012). In skeletal muscle from a mouse model of dilated cardiomyopathy, the shift to more fatigable fibers was associated with activation of Wnt (Okada et al., 2015). Through β-catenin, Wnt activates Forkhead box O (FoxO) (Okada et al., 2015). FoxO signaling plays a critical role in the development of skeletal myopathy in CHF (Okada et al., 2015). Overexpression of FoxO1 increases shifts to fatigable fibers, whereas suppression of FoxO1 activity inhibits these changes (Okada et al., 2015). This supports the role of Wnt signaling as a potential target for new therapies against skeletal myopathy in CHF (Okada et al., 2015).

Clinically, muscle fiber changes in CHF are related to exercise intolerance and shortness of breath. There is a positive correlation between the percentage of MHC I and VO_2, ventilator threshold, and oxygen pulse (Nicoletti et al., 2003). Similarly, there is a negative correlation between MHC II and these measures of exercise capacity (Nicoletti et al., 2003). These relationships have been demonstrated in patients with HFpEF as well (Kitzman et al., 2014). Exercise may reverse fiber composition abnormalities, increasing slow-twitch fibers and decreasing fast-twitch glycolytic fibers in skeletal muscle (Piepoli et al., 2001). It may also increase muscle fiber size (Piepoli et al., 2001).

Various other changes in muscle fibers have been identified in patients with CHF. Fibers may be altered due to changes in regulatory components of thin filaments, including troponin, and proteins involved in calcium transport, including Ca^{2+}-ATPase (Georgiadou and Adamopoulos, 2012). There is also a reduction in capillary-to-fiber ratio (Kitzman et al., 2014). Researchers have suggested that there is a decrease in capillary density, although these findings have been controversial (Sarma and Levine, 2015; Mettauer et al., 2001; Georgiadou and Adamopoulos, 2012; Minotti et al., 1992; Mettauer et al., 2006). The decreased capillary density around skeletal myofibrils is related to decreased peak VO_2 (Sarma and Levine, 2015).

Metabolic Changes

Mitochondrial Content

Mitochondria are altered in skeletal muscle in patients with CHF. One study suggested that there is some parallelism between cardiac and skeletal muscle mitochondrial alterations in patients with CHF, and that these alterations begin before the major clinical Framingham criteria are installed (Guzman Mentesana et al., 2014). Ultrastructural analysis showed fewer, smaller mitochondria in patients compared to controls (Guzman Mentesana et al., 2014). Specifically, there are fewer mitochondria per muscle fiber (Zizola and Schulze, 2013). Mitophagy may be enhanced by hypoxia, inflammation, and muscle inactivity (Zizola and Schulze, 2013). In older patients with HFpEF, mitochondrial reductions in skeletal muscle have been demonstrated based on levels of porin expression (Molina et al., 2016). Morphometric analysis of vastus lateralis muscles from patients with CHF showed reduced volume

Table 3 Similarities and differences between skeletal and cardiac muscle (Kennelly and Murray, 2015)

Skeletal muscle	*Cardiac muscle*
Striated	Striated
No syncytium	Syncytial
Small T tubules	Large T tubules
Sarcoplasmic reticulum well developed and Ca^{2+} pump acts rapidly	Sarcoplasmic reticulum present and Ca^{2+} pump acts relatively rapidly
Plasmalemma contains few hormone receptors	Plasmalemma contains a variety of receptors (e.g., α- and β-adrenergic)
Nerve impulse initiates contraction	Has intrinsic rhythmicity
Extracellular fluid Ca^{2+} not important for contraction	Extracellular fluid Ca^{2+} important for contraction
Troponin system present	Troponin system present
Caldesmon not involved	Caldesmon not involved
Very rapid cycling of the cross-bridges	Relatively rapid cycling of the cross-bridges

Table 4 Similarities and differences between type I and type II muscle fibers (Kennelly and Murray, 2015; Kibble and Halsey, 2015)

Characteristic	*Slow twitch (type I)*	*Fast twitch (type II)*
Color	Red (myoglobin)	White (low myoglobin)
Metabolism	Oxidative	Glycolytic
Mitochondria	Abundant	Few
Glycogen content	Low	High
Duration	Prolonged	Short
Fatigability	Low	High
Myosin ATPase	Low	High
Energy utilization	Low	High
Contraction rate	Slow	Fast

density of mitochondria (Mettauer et al., 2006). In a rat model of right ventricular heart failure induced by monocrotaline (MCT) injection, MCT treatment was related to lower mitochondrial volume density and quality (Wust et al., 2012). Remaining mitochondria have fewer cristae (Mettauer et al., 2006; Zizola and Schulze, 2013). It has been noted, however, that skeletal muscle has less clear mitochondrial changes compared to cardiomyocytes (Mettauer et al., 2006). Moreover, a study comparing 13 CHF patients with 14 age- and activity-matched controls suggested that mitochondrial changes in CHF are primarily related to muscle disuse and other disease-related factors (Toth et al., 2012).

Exercise training is typically thought to alleviate quantitative and structural mitochondrial changes in patients with CHF. Exercise has been shown to increase the number of mitochondria in skeletal muscle (Maurer and Schulze, 2012). It also significantly increases the total volume density of mitochondria (Nicoletti et al., 2003). In one study, however, resistance training did not alter mitochondrial size and content in patients with CHF, leading the authors to suggest that impaired oxidative capacity is not related to mitochondrial dysfunction (Toth et al., 2012).

Oxidative Capacity

In addition to structural changes, mitochondria in skeletal muscles from patients with CHF also have impaired function. As in many diseases, mitochondrial alterations contribute to a reduction in oxidative metabolic capacity. Oxidative capacity is defined as the maximal rate at which oxidative phosphorylation may be performed (Leermakers and Gosker, 2016). Reduced oxidative capacity contributes to decreased skeletal muscle oxygen consumption (Sarma and Levine, 2015). Reduced oxygen consumption is further caused by impaired cardiac function in patients with HFrEF and by impaired oxygen diffusion (Sarma and Levine, 2015). Despite these changes, muscles typically are adequately supplied with oxygen during abnormalities, suggesting an intrinsic cause within skeletal muscle (Zizola and Schulze, 2013).

As oxidative metabolism fails, there is a shift to anaerobic, glycolytic energy production. This shift occurs in both patients with HFrEF and HFpEF (Kitzman et al., 2014). Increased anaerobic metabolism causes an earlier increase in the concentration of lactate in the blood, especially during exercise (Schulze et al., 2002). This occurs even in patients with chronic CHF with normal leg blood

flow (Nicoletti et al., 2003). The shift to glycolytic metabolism has been demonstrated in patients with CHF using ^{31}P magnetic resonance spectroscopy (Zizola and Schulze, 2013). Glycolytic metabolism also leads to earlier intramuscular acidosis (Georgiadou and Adamopoulos, 2012). In a rat model of CHF, skeletal muscle metabolic capacity was reduced in both oxidative and glycolytic muscles (Mettauer et al., 2006).

Quantitative, structural, and metabolic changes in mitochondria contribute to exercise intolerance. Mitochondrial alterations affect factors such as peak aerobic exercise capacity (Zizola and Schulze, 2013). In one study, levels of porin and mitofusin-2 (Mfn2) expression were positively correlated with peak VO_2 and 6-min walk distance (see "**Exercise**" section for further discussion) (Molina et al., 2016). Mitochondrial volume density and cristae reductions are proportional to declines in peak VO_2 (Mettauer et al., 2006).

Exercise may restore oxidative capacity in patients with CHF. Mitochondrial improvements from exercise training are significantly correlated with increased peak VO_2 (Piepoli et al., 2001). Exercise also improves metabolic function through slowing utilization of muscle glycogen, increasing reliance on fat oxidation and utilization, and decreasing production of lactate during exercise (Nicoletti et al., 2003). In one study, however, wrist-flexor training induced a 50% improvement in oxidative capacity in patients without CHF but did not induce improvement in patients with CHF (Southern et al., 2015). The authors suggested that CHF patients have impaired oxidative adaptations to endurance exercise compared to healthy controls (Southern et al., 2015).

Changes in oxidative capacity are controversial. Some studies suggest that oxidative capacity in patients with CHF is similar to oxidative capacity in sedentary controls (Mettauer et al., 2001). Further research is required to determine the contributions from atrophy, fibrosis, and inflammation (Zizola and Schulze, 2013). Moreover, mitochondrial abnormalities, reduced blood flow, and decreased oxygen extraction often reverse after administration of ACEi (Minotti et al., 1992). The more recent application of ACEi and β-blocker treatments may account for older studies observing intrinsic skeletal muscle mitochondrial defects while newer studies observe normal mitochondrial function (Mettauer et al., 2006).

Enzyme Abnormalities

In patients with CHF, impaired CK function in skeletal muscle is likely related to abnormal production and handling of high-energy phosphates (Mettauer et al., 2006; Schulze et al., 2002; Hambrecht et al., 1999). CK is an enzyme that adds a phosphate from ATP to creatine to produce phosphocreatine (PCr) (Mettauer et al., 2006). PCr functions as an energy buffer for ATP homeostasis in skeletal muscle (Greenhaff, 2001). Three isoforms of muscle CK are the dimers MM-CK, BB-CK, and MB-CK (Mettauer et al., 2006). There is also a mitochondrial isoform (mi-CK) (Mettauer et al., 2006). In patients with CHF, levels of total cytosolic CK, MM-CK, and mi-CK decrease (Mettauer et al., 2006). Reduced CK function is exacerbated by reduced oxidative capacity, which increases use of PCr as a source of energy. Many studies using ^{31}P magnetic resonance spectroscopy have shown that patients with CHF have increased rates of PCr utilization (Zizola and Schulze, 2013; Schulze et al., 2002; Hambrecht et al., 1999). PCr depletion is accompanied by increased levels of Pi (Minotti et al., 1992). Moreover, a study using ^{31}P magnetic resonance spectroscopy revealed that patients with HFpEF have longer recovery times to regenerate PCr compared to healthy, sedentary, age-matched controls (Sarma and Levine, 2015). Long recovery of high-energy phosphates is proportional to the severity of CHF (Mettauer et al., 2006). In addition, during exercise in patients with CHF, levels of ADP increase faster while pH falls significantly more compared to healthy controls (Nicoletti et al., 2003). Abnormal CK function is independent of peripheral blood flow, histologic changes, muscle mass, and hypoxia (Zizola and Schulze, 2013; Hambrecht et al., 1999). Impaired CK function and lower levels of ATP contribute to early fatigue and muscle intolerance in patients with CHF (Piepoli et al., 2001; Hambrecht et al., 1999). Since these changes may occur prior to atrophy, patients often present with fatigue prior to decreased contractile force (Schulze et al., 2002).

Reduced enzyme activity in the Krebs cycle and electron transport chain is related to energy depletion in patients. In a rat model of CHF induced by MCT injection, succinate dehydrogenase (SDH, complex II) activity and maximal respiratory rate were 15% lower in all muscle fibers (Wust et al., 2012). In patients with HFrEF but not HFpEF, soleus tissue muscle showed a reduced SDH to lactate dehydrogenase (LDH) ratio, which is further impacted by reduced levels of LDH in patients with CHF (Seiler et al., 2016). In the rat model, complex I/IV activity was also 20% lower (Wust et al., 2012). In general, the percentage of mitochondria stained for cytochrome oxidase (complex IV) is reduced in patients with CHF but improves with exercise (Mettauer et al., 2006). Changes in citrate synthase levels are more variable. Some studies have suggested that citrate synthase activity is reduced in skeletal muscle from patients with CHF, including older patients with HFpEF. Other studies, however, state that levels of citrate synthase are unchanged (Mettauer et al., 2001, 2006; Zizola and Schulze, 2013; Molina et al., 2016).

Other proteins are also affected. Reductions in fatty acid metabolism are characterized by low concentrations of skeletal muscle long chain acylcarnitine, increased lipid droplets, and decreased activity of β-hydroxyacyl coenzyme A dehydrogenase, an enzyme involved in β-oxidation of fatty acids (Nicoletti et al., 2003). Reductions in Mfn2 and porin, both of which are proteins that are present on the outer mitochondrial membrane, are correlated to measures of exercise tolerance in patients with HFpEF (Molina et al., 2016). Peroxisome proliferator-activated receptor-γ expression was reduced in soleus tissue from patients with HFrEF but not HFpEF (Seiler et al., 2016). Deactivation of the PPARα/AMPK pathway, which consequently downregulates many enzymes for glucose and free fatty acid metabolism, is also related to skeletal muscle metabolic dysfunction (Sente et al., 2016a).

Exercise may improve enzymatic abnormalities. In an animal model of CHF, exercise training decreased PCr depletion and increased levels of ATP (Piepoli et al., 2001; Nicoletti et al., 2003). In patients with CHF, ^{31}P nuclear magnetic resonance spectroscopy after submaximal exercise showed reduced acidosis, reduced PCr depletion, and increased levels of ADP (Minotti et al., 1992). Moreover, exercise reduced the recovery half-time for PCr (Schulze et al., 2002). Needle biopsies from patients after exercise have shown a significant increase in oxidative enzyme activity along with reduced or unaltered glycolytic enzyme activity

(Piepoli et al., 2001). In a study on rats with CHF, exercise training increased spinotrapezius muscle citrate synthase activity more than it did in sedentary counterparts (Hirai et al., 2014). Exercise has also been shown to increase the concentration of enzymes involved in lipid metabolism (Fletcher et al., 2011).

Oxidative Stress

Oxidative stress is defined as an excess production of reactive oxygen species (ROS) relative to antioxidant defense (Tsutsui et al., 2011). Sources of ROS include mitochondria, NAD(P)H oxidase, xanthine oxidase, and uncoupled nitric oxide synthase (NOS) (**Fig. 2A**; Guzman Mentesana et al., 2014; Tsutsui et al., 2011; Koba et al., 2009). Levels of ROS may also increase due to decreased antioxidative capacity, which may be caused by decreased function of radical scavenger enzymes including superoxide dismutase (SOD) and catalase (**Fig. 2B**; Koba et al., 2009). Increased ROS in mitochondria will damage mitochondrial DNA, which impairs mitochondrial function and further augments generation of ROS (Tsutsui et al., 2011). ROS may also mediate apoptosis (Tsutsui et al., 2011).

Heart failure increases oxidative stress. In patients, levels of ROS, such as superoxide, are increased (Koba et al., 2009). One study, however, found significant changes in markers of oxidative stress, including increased nicotinamide adenine dinucleotide phosphate oxidase and decreased antioxidative enzyme activity, in soleus tissue from patients with HFrEF but not HFpEF (Seiler et al., 2016). Superoxide may partially contribute to symptoms by sensitizing muscle afferents involved in the mechanoreflex (see the "**Neurologic Response and Muscle Reflexes**" section for further discussion) (Koba et al., 2009). Patients with CHF may maintain some antioxidant defense mechanisms. An nitrous oxide (NO)-dependent antioxidant defense may increase skeletal muscle resistance to catabolic processes (Okutsu et al., 2014). In a genetic mouse model of CHF, muscle CK extracellular superoxide dismutase (MCK-EcSOD) significantly attenuated cachexia and exercise intolerance (Okutsu et al., 2014). It also ameliorated MAFbx/Atrogin-1 mRNA expression, loss of mitochondria, and reduction of capillary density in skeletal muscle (Okutsu et al., 2014).

Oxidative stress contributes to the CHF syndrome in many ways. It is related to cachexia, insulin resistance, and exercise intolerance. ROS impair contractile function through modification of proteins related to excitation–contraction coupling (ECC) (see "**ECC and Calcium**" section for further discussion) (Tsutsui et al., 2011). Although muscle contraction increases ROS production in healthy subjects, active muscle in patients with CHF releases higher levels of cyclooxygenase products and bradykinin (BK), which are a source of superoxide generation (Koba et al., 2009). ROS also decrease peripheral perfusion due to endothelial dysfunction (Koba et al., 2009). Clinically, increased oxidative stress in the skeletal muscle is related to fatigue and dyspnea in patients with CHF (Guzman Mentesana et al., 2014). Exercise training may decrease oxidative stress in skeletal muscles (Maurer and Schulze, 2012; Cunha et al., 2012).

ECC and Calcium

Skeletal muscle function relies on intact ECC. The normal steps of this process are shown below (**Fig. 3**). In patients with CHF, ECC in skeletal muscle is abnormal.

Abnormalities in ECC may be due to problems in the neuromuscular junction. In lower limbs of patients with CHF, extension and flexion time-to-peak torque was longer (Brunjes et al., 2016). Furthermore, acceleration and deceleration times in the lower limbs were prolonged (Brunjes et al., 2016). CHF patients had increased adiposity, decreased lean muscle mass, decreased circulating unsaturated fatty acids, and increased ceramides (Brunjes et al., 2016). The researchers suggested that delayed torque development indicates skeletal muscle impairments that may reflect abnormal neuromuscular functional coupling (Brunjes et al., 2016).

There may also be abnormalities with intracellular calcium handling, causing an "intracellular calcium-overload state (Zizola and Schulze, 2013)." In CHF, the muscle-specific type 1 ryanodine receptor (RyR1), which exists on the sarcoplasmic reticulum (SR), becomes leaky (Zizola and Schulze, 2013). This occurs because chronic adrenergic stimulation results in

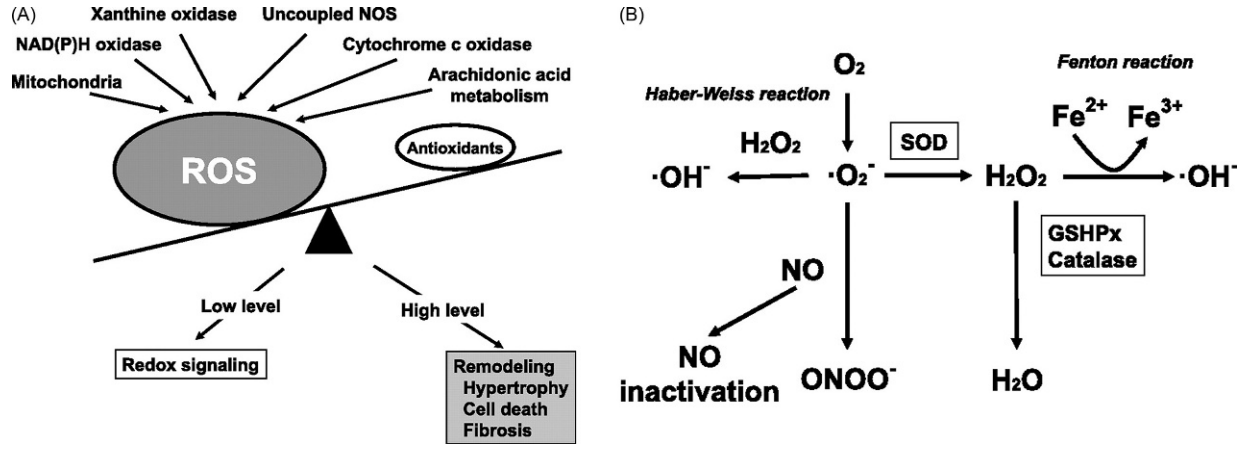

Fig. 2 (A) Sources of ROS (Tsutsui et al., 2011); (B) reactions underlying generation and degradation of ROS (Tsutsui et al., 2011).

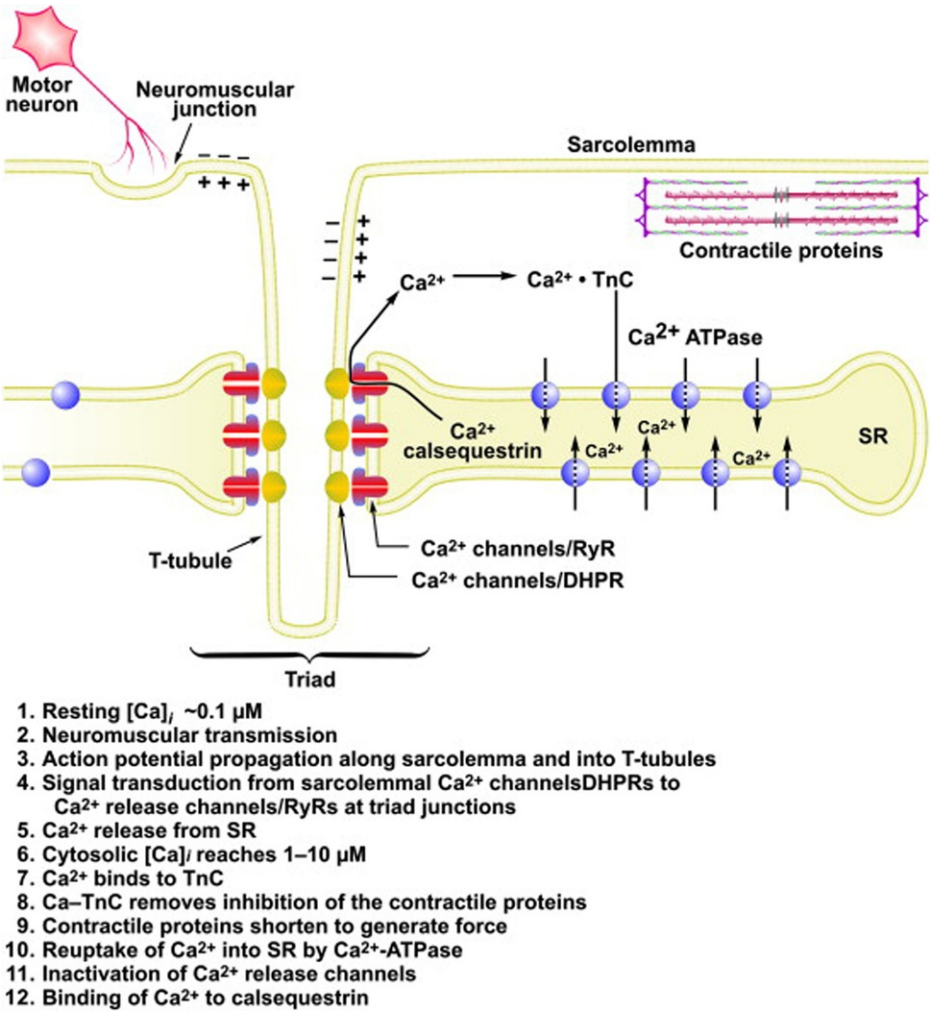

Fig. 3 Excitation–contraction coupling (Heiny and Meissner, 2012).

hyperphosphorylation of the channel (Zizola and Schulze, 2013). Channel hyperphosphorylation causes dissociation of calstabin-1, which is necessary to maintain the closed state of the RyR1 channel (Zizola and Schulze, 2013). A study of eight sedentary HF patients compared to seven age-matched, healthy but sedentary controls further showed that skeletal muscle RyR1 from human heart failure is posttranslationally modified (Rullman et al., 2013). Modifications included excessive phosphorylation, *S*-nitrosylation, and oxidation (Rullman et al., 2013). RyR1 from HF patients had depleted levels of calstabin1 (Rullman et al., 2013). Changes in RyR1 in CHF impair muscle function and exercise tolerance, most significantly via altered SR Ca^{2+} pumping in slow-twitch muscles (Zizola and Schulze, 2013).

A leaky SR with decreased Ca^{2+} content may impair ECC through altered action potential (AP)-evoked Ca^{2+} release, which may be called a "calcium spark (Zizola and Schulze, 2013; DiFranco et al., 2014)." In a study of four patients with CHF, excitability mechanisms seemed to be intact since single APs did not vary between CHF patients and healthy controls (DiFranco et al., 2014). AP-evoked Ca^{2+} release flux in single fibers, however, was significantly reduced compared to fibers from healthy controls (DiFranco et al., 2014). In a different study, skeletal muscle from animals with heart failure exhibited increased Ca^{2+} spark frequency, decreased Ca^{2+} spark amplitude, and increased Ca^{2+} spark duration (Zizola and Schulze, 2013).

Skeletal muscle expression of SR Ca^{2+}-ATPase (SERCA) 1a, the skeletal muscle specific isoform, differ in relation to muscle fiber composition, the degree of functional impairment, and the type of CHF model (Zizola and Schulze, 2013). Expression of SERCA was reduced in rats with post-infarction heart failure, which may explain abnormal calcium handling and accelerated muscle fatigue in CHF (Piepoli et al., 2001). The sarcolemma may also be abnormal (Maurer and Schulze, 2012).

Inflammation and Cytokines

Immune responses and inflammation play a significant role in CHF. Proinflammatory factors involved in CHF include tumor necrosis factor α (TNF-α), interleukin (IL) 1β (IL-1β), IL-1, IL-6 and interferon γ (IFN-γ) (Sente et al., 2016a). Levels of chemokines,

such as IL-8, also increase (Sente et al., 2016a). Biopsy-derived primary skeletal muscle myoblasts from patients with HFrEF also showed loss of antiinflammatory and proliferative activity (Sente et al., 2016b). Cytokines are associated with poor prognosis and recurrent hospitalization (Schulze et al., 2002).

Low-level systemic inflammation in CHF may cause changes in skeletal muscle (Zizola and Schulze, 2013). Plasma levels of circulating inflammatory cytokines differ between patients with HFrEF and HFpEF (Seiler et al., 2016). In HFrEF, plasma concentration of TNF-α was significantly greater (Seiler et al., 2016). In HFpEF, on the other hand, plasma concentrations of IL-1β and IL-12 were significantly greater (Seiler et al., 2016). This may partially explain differences between changes in skeletal muscle in HFrEF and HFpEF (Seiler et al., 2016). Even when circulating concentrations of cytokines are unaltered, CHF increases levels of TNF-α and IL-1β and decreases levels of antiinflammatory IL-10 in skeletal muscle and other tissue (Poole et al., 2012). Cytokines influence the expression of proteins in muscle (Schulze et al., 2002). For example, IL-1β may decrease expression of SERCA and phospholamban mRNA and protein in neonatal myocytes (Schulze et al., 2002). This prolongs the calcium transient (Schulze et al., 2002). TNF-α may initiate a similar effect (Schulze et al., 2002).

In advanced CHF, systemic cytokines may significantly upregulate skeletal muscle expression of inducible nitric oxide synthase (iNOS, NOS II). Increased iNOS was confirmed in skeletal muscle biopsies from patients with CHF (Gielen et al., 2003). NOS II is a calcium-independent enzyme that is expressed in immunologically activated cells in response to TNF-α, IFN-γ, and IL-1β (Riede et al., 1998). These cytokines work through activation of NF-κB (Zizola and Schulze, 2013). NOS II is a source of intracellular NO (Hambrecht et al., 1999). Toxic levels of NO may inhibit enzymes related to oxidative phosphorylation, such as cytochrome c oxidase (COX), and reduce peak oxygen uptake (Gielen et al., 2003). This process may attenuate skeletal muscle contraction and mediate muscle wasting through altered metabolism (Gielen et al., 2003). As a result, increased expression of NOS II is inversely correlated with oxidative capacity, functional work capacity, and exercise tolerance in patients with CHF (Hambrecht et al., 1999). Local inflammation in skeletal muscle with increased levels of IL-1β, TNF-α, and NOS II might further contribute to the progressive deterioration of skeletal muscle structure, function, and metabolism (Maurer and Schulze, 2012).

Exercise training for 6 months was shown to reduce skeletal muscle concentration of IL-1β by 27% and TNF-α by 28%, consequently decreasing iNOS expression by 32% (Adams et al., 2001; Schulze et al., 2001). A separate study on 20 male patients with stable CHF showed that exercise training did not affect serum levels of TNF-α, IL-6, and IL-1β, but did decrease skeletal muscle TNF-α, IL-6, and IL-1β (Gielen et al., 2003). In this study, exercise training reduced skeletal muscle iNOS expression by 52%. Exercise training also restores levels of the antiinflammatory mediator IL-10 (Gielen et al., 2003).

Disuse-induced atrophy promotes skeletal muscle inflammation through multiple pathways (see "**Muscle Atrophy**" section for further discussion). Skeletal muscle inflammation reduces O_2 delivery, decreases local insulin-like growth factor-1 (IGF-1) levels, and increases oxidative stress (Zizola and Schulze, 2013). Muscle inflammation induces cytokines, including IL-6 and TNF-α, which contribute to muscle wasting through activation of the NF-κB pathway (Zizola and Schulze, 2013). In rats, IL-6 increases skeletal and diaphragmatic muscle atrophy (Zizola and Schulze, 2013). It also activates the JAK-STAT and cAMP activated protein kinase (AMPK) pathways (Zizola and Schulze, 2013). Skeletal muscle inflammation increases FoxO activity, causing muscle protein degradation and atrophy through the ubiquitin-proteasome pathway (Zizola and Schulze, 2013). Systemic inflammation may also contribute to muscle atrophy. Local antiinflammatory effects of exercise may attenuate catabolic wasting associated with the progression of CHF (Gielen et al., 2003).

In patients with HFrEF, increased inflammation corresponds with increased levels of adiponectin (Sente et al., 2016a). It has been shown that increased adiponectin has a protective role through antiinflammatory mechanisms (Sente et al., 2016a).

Neurologic Response and Muscle Reflexes

The autonomic nervous system may be divided into the sympathetic and parasympathetic branches. In healthy subjects, exercise generally reduces parasympathetic activity and augments sympathetic activity (Fletcher et al., 2011). Sympathetic activation causes the release of norepinephrine and increases plasma concentrations of epinephrine (EPI) (Fletcher et al., 2011). In patients with CHF, there is abnormally high sympathetic activation and a withdrawal of parasympathetic activity at rest (Piepoli et al., 2001).

Skeletal muscle receptors that are stimulated by work are called ergoreceptors. The two types of ergoreceptors are mechanoreceptors and metaboreceptors (Georgiadou and Adamopoulos, 2012). Mechanoreceptors are myelinated type III afferents that respond to mechanical stimuli (Georgiadou and Adamopoulos, 2012). Metaboreceptors are chemically activated unmyelinated type IV afferents that respond to metabolites (Georgiadou and Adamopoulos, 2012). Metabolites that may stimulate metaboreceptors include acidosis, prostaglandins, BK, potassium, adenosine, and lactate (Georgiadou and Adamopoulos, 2012). One study, however, suggested that $K+$ and phosphate stimulate muscle afferents while $H+$ and lactate do not (Khan and Sinoway, 2000). Some studies have challenged the distinction between the two types of ergoreceptors. The response initiated by ergoreceptors is called the ergoreflex (Georgiadou and Adamopoulos, 2012). Specifically, during exercise, the response may be called the exercise pressor reflex (Wang et al., 2012).

Ergoreceptors are the basis of the muscle theory of sympathetic drive in CHF (Georgiadou and Adamopoulos, 2012). In normal patients, muscle contraction stimulates thin fiber afferents to evoke a change in blood pressure (BP) (Koba et al., 2009). The overall ergoreflex is more strongly stimulated in patients with CHF (Koba et al., 2009). Some studies have shown that responses of both mechanoreceptors and metaboreceptors are increased in CHF (Georgiadou and Adamopoulos, 2012). Others, however, have suggested that the metaboreceptor contribution to increased muscle sympathetic nerve activity is minimal or decreased in CHF

(Koba et al., 2009). Even if metaboreceptor responses are reduced, higher accumulation of metabolites due to slower oxygen uptake, lower VO_2, and other changes will still stimulate the metaboreceptors (Poole et al., 2012). In a study on rats with heart failure induced by coronary artery ligation, it was shown that continuous contraction stimulates both mechanically and metabolically sensitive muscle afferents whereas short, intermittent contraction stimulates predominantly mechanosensitive afferents (Koba et al., 2009).

The ergoreflex causes global sympathetic activation (Poole et al., 2012). This includes activation of cardiovascular pathways in the central nervous system (Koba et al., 2009). Increased responses cause neurohumoral derangement, altered autonomic state, exaggerated ventilatory responses, increased circulatory responses, and exercise intolerance (Georgiadou and Adamopoulos, 2012). Specifically, sympathetic activation reduces blood flow to resting muscle to less than one-fourth of the normal value (Mohrman and Heller, 2014). Since there is usually more blood flow to skeletal muscle at rest than necessary, resting tissue metabolic processes may not be impacted by this (see "**Blood Flow to Skeletal Muscle**" section for further discussion) (Mohrman and Heller, 2014). Due to the large amount of skeletal muscle tissue in the body, however, changes in blood flow due to sympathetic activity play a large role in regulation of BP (Mohrman and Heller, 2014).

Although changes are induced to meet metabolic needs of skeletal muscle, they become maladaptive in CHF (Francis et al., 2011). The decrease in blood flow to the periphery further increases skeletal muscle abnormalities (Francis et al., 2011). Enhanced sympathetic activity may be a factor in skeletal muscle breakdown. This shows that the reflex contributes not only to initiation of symptoms but also to CHF progression.

Neural alterations of vasomotor tone impact blood flow to exercising muscle (Fletcher et al., 2011). These changes are partially induced by the ergoreflex. In healthy subjects, exercise induces sympathetic activation (Fletcher et al., 2011). Sympathetic activation causes most vascular beds to constrict to restrict blood flow to nonexercising muscles (Fletcher et al., 2011). Though sympathetic activation may cause vascular beds for exercising muscle to constrict, the effect is outweighed by vasodilatation due to metabolites (Fletcher et al., 2011). Sympathetic activation serves to prevent peripheral vasculature from dilating beyond the capabilities of maximal cardiac output in order to maintain BP (Khan and Sinoway, 2000).

In patients with CHF, earlier and increased acidification and accumulation of exercising muscle metabolites increase involvement of the ergoreflex (Poole et al., 2012). Increased neurohumoral activation has been confirmed by higher plasma levels of catecholamines, renin activity, and aldosterone in patients with CHF (Piepoli et al., 2001). This increases peripheral vasoconstriction, thereby decreasing blood flow to skeletal muscle. Ergoreflex activation is significantly correlated with exercise capacity through variables including peak VO_2 and the ventilation/carbon dioxide production ratio (Nicoletti et al., 2003). The correlations suggest that increased muscle afferent activity contributes to reduced exercise tolerance (Nicoletti et al., 2003).

One study suggested that muscle reflexes in CHF are different in rhythmic exercise compared to static exercise (Khan and Sinoway, 2000). Using vascular resistance in the calf as a measure of sympathetic activation, it was shown that CHF patients have increased calf vascular resistance and BP during post-ischemia exercise, whereas values in controls decreased to baseline levels (Khan and Sinoway, 2000). The magnitude of the ventilatory response was also greater in patients with CHF (Khan and Sinoway, 2000). Patients with CHF had increased muscle acidosis and accumulation of Pi, which may cause increased activation of the muscle metaboreflex (Khan and Sinoway, 2000).

Data also suggest that purinergic P2X receptors of muscle afferent nerves contribute to enhanced sympathetic activation and BP changes during static exercise in patients with CHF (Xing and Li, 2016). In CHF, there is increased P2X current activity and increased expression of P2X3 and kinin B2 receptors (Xing and Li, 2016). BK at least partially causes modulation of the P2X-driven responses (Xing and Li, 2016). In accordance with this, in rats with heart failure, injection of BK into the arterial blood supply of hindlimb muscles more greatly heightened responses induced by P2X activation (Xing and Li, 2016).

Exercise may benefit patients with CHF by reducing the magnitude of the ergoreflex response. Positive effects may be mediated through a direct mechanism or through indirect effects on concentrations of stimulatory metabolites. In rats with CHF, exercise training prevented the sensitization of group III responses to contraction and stretch and partially prevented the decreased group IV responses to contraction and capsaicin (Wang et al., 2012). Conditioning also increases blood flow to skeletal muscle and increases the capacity for aerobic skeletal muscle metabolism, thereby decreasing anaerobic metabolism (Khan and Sinoway, 2000). As a result, acidification and concentrations of metabolites that stimulate the ergoreflex are decreased (Khan and Sinoway, 2000). These changes reduce ventilatory drive, vasoconstriction, and sympathetic activation (Piepoli et al., 2001). With exercise, the ergoreflex response in patients with CHF may be attenuated toward the level of activation in healthy subjects (Khan and Sinoway, 2000). Exercise training should be started early in CHF progression to maximally limit changes in the ergoreflex.

Over-activation of the sympathetic nervous system from skeletal muscle afferents may contribute to inflammation and cytokine activation. Increased levels of TNF-α and insulin resistance may contribute to catabolic muscle wasting (Nicoletti et al., 2003). These changes may also reduce anabolic function (Nicoletti et al., 2003).

Muscle Atrophy

Atrophy and Cardiac Cachexia

As in many systemic diseases, skeletal muscle atrophy may be observed in patients with CHF. Atrophy occurs early in the progression of CHF prior to documented loss of weight (Georgiadou and Adamopoulos, 2012). Atrophy is indicated by decreased fiber cross-sectional area (CSA) and decreased muscle bulk, especially in the quadriceps (Nicoletti et al., 2003). One paper noted

controversy regarding the effect of clinical status on muscle atrophy in CHF (Zizola and Schulze, 2013). Many studies have suggested that muscle protein metabolism is not impaired in stable patients, suggesting that initiation and progression of atrophy are related to events of disease exacerbation (Zizola and Schulze, 2013). Atrophy probably only plays a minor role in impairment of skeletal muscle energetics in CHF (Mettauer et al., 2006).

Changes in skeletal muscle occur due to suppression of whole-body protein synthesis and augmentation of myofibrillar protein degradation (Francis et al., 2011). In patients with systolic heart failure, skeletal muscle expression of the atrophy-promoting genes FoxO1 and FoxO3 was increased compared to controls (Forman et al., 2014). However, expression of other atrophy-related genes (atrogin-1 and MuRF-1), growth promoting genes (IGF-1), and inflammatory cytokines (TNF-α, C-reactive protein, IL-1β, and IL-6) was similar between groups (Forman et al., 2014). In one study, soleus tissue from patients with HFrEF, but not patients with HFpEF, showed a significant increase in markers of muscle atrophy (MuRF1, calpain, and ubiquitin proteasome) (Seiler et al., 2016). An additional mechanism may include selective atrophy of oxidative fibers (Francis et al., 2011).

Eventually, atrophy transitions to the severe muscle wasting that is characteristic of cardiac cachexia. Cachexia is defined as "non-intentional documented weight loss of at least 7.5% of a previously normal weight (Francis et al., 2011)." Skeletal muscle wasting is a well-known feature of late-stage heart failure (Minotti et al., 1992). Muscle wasting in cardiac cachexia affects all tissue compartments, but it may be more apparent in the legs (Francis et al., 2011; Mettauer et al., 2006). Clinically, muscle wasting is associated with exercise intolerance. Limb and respiratory muscle weakness contribute to increased morbidity and mortality (Mangner et al., 2015). Various factors are involved in skeletal muscle atrophy and wasting.

Sarcopenia

Sarcopenia refers to the normal progressive decline in muscle mass, strength, and function that occurs with aging (Maurer and Schulze, 2012). In research, it may be defined as appendicular skeletal mass (ASM) two standard deviations below the mean of a healthy reference group (Saitoh et al., 2016). As a systemic skeletal muscle disease, it impairs the function of limb skeletal muscles and respiratory muscles (Kinugasa and Yamamoto, 2016). There is a high prevalence of sarcopenia among the elderly and women (Sarma and Levine, 2015).

Sarcopenia is characterized by changes involving skeletal muscle dysfunction, oxidative protein damage, cytokines, and IGF-1 (Maurer and Schulze, 2012). Since these problems are also present in CHF, sarcopenia has been identified as a serious comorbidity in patients with CHF (Dos Santos et al., 2016). Sarcopenia is an independent risk factor for mortality in patients with cardiovascular diseases (Onoue et al., 2016). With this in mind, it was shown that a sarcopenia screening test involving age, grip strength, and calf circumference can predict future adverse events in patients with CHF (Onoue et al., 2016). A Kaplan–Meier curve showed that CHF event-free survival rate was significantly lower in patients with sarcopenia (Onoue et al., 2016).

In regard to CHF, sarcopenia is most often associated with HFpEF, especially in frail patients (Kinugasa and Yamamoto, 2016). Sarcopenia may contribute to the development of HFpEF through different metabolic and endocrine changes (Kinugasa and Yamamoto, 2016). Compared to symptomatic HFpEF patients without sarcopenia, those with sarcopenia performed worse during 6-min walk testing and showed lower absolute peak oxygen consumption (Bekfani et al., 2016). ASM was independently associated with reduced distance during the walk testing (Bekfani et al., 2016). Higher muscle strength and ASM values were associated with higher quality of living (Bekfani et al., 2016).

Deconditioning

Physical deconditioning due to decreased physical activity likely contributes to skeletal muscle wasting in patients with CHF. Deconditioning is typically associated with muscle disuse. Overall, changes in CHF are similar to changes due to deconditioning. It has been observed that biochemical and histological alterations in skeletal muscle in CHF are similar to those caused by deconditioning (Minotti et al., 1992). Specifically, both involve atrophy, metabolic alterations with decreased oxidative enzymes, sympathetic over-activation, and decreased muscle fiber size (Nicoletti et al., 2003). In terms of exercise intolerance, deconditioning and CHF are correlated with decrements in peak systemic VO_2, muscle strength, and endurance (Minotti et al., 1992). Exercise training improves both conditions. Since benefits of exercise training are nonspecific, however, improvements from exercise do not prove that deconditioning is the primary mechanism of skeletal muscle changes in CHF (Minotti et al., 1992). The similarities between CHF and deconditioning are summarized below (**Table 5**).

Although deconditioning may contribute to skeletal muscle alterations in CHF, it does not fully explain changes in muscle structure, metabolism, and function. Some components of the CHF syndrome suggest a generalized metabolic myopathy caused by CHF (Mettauer et al., 2006). In experimental models of CHF, all muscles including the diaphragm are characterized by abnormal enzymatic and mitochondrial function (Mettauer et al., 2006). In deconditioning, only postural oxidative muscles are affected (Mettauer et al., 2006). Moreover, enzymatic changes involving CK and glycolytic systems are present in CHF but not deconditioning (Mettauer et al., 2006). Disuse atrophy causes a shift toward the slow type of MHC, which is the opposite of what occurs in patients with CHF (Nicoletti et al., 2003).

Further studies are needed to fully determine the extent to which deconditioning impacts the syndrome of CHF. Regardless, localized and systemic exercise training and reconditioning may significantly alleviate changes in patients with CHF (Mettauer et al., 2006).

Table 5 Similarities between muscular deconditioning and heart failure (Piepoli et al., 2001)

	Deconditioning	*Heart failure*
Hemodynamics		
Vascular resistance	↑	↑
Resting heart rate	↑	↑
Max A-VO_2 differences	↓	↓/↔
Function		
Exercise tolerance	↓	↓
VO_2 max	↓	↓
Neuroendocrine/autonomic		
Renin/angiotensin	↓	↓
Sympathetic	↑	↑
Vagal activity	↑	↑
Baroreflex sensitivity	↓	↓
HRV	↓	↓
Skeletal muscle		
Muscle mass/bulk	↓	↓
Mitochondrial enzymes		
Oxidative	↓	↓
Glycolytic	↑	↑
Fiber type IIb/IIa ratio	↑	↑
Psychological		
Well-being	↓	↓
Activity scores	↓	↓

Muscle Cell Apoptosis

Apoptosis often occurs in skeletal muscle of patients with CHF. This has been confirmed in skeletal muscle biopsies (Hambrecht et al., 2002). Ultrastructural analysis reveals a higher number of apoptotic myocytes (Nicoletti et al., 2003). Though there are over 100 nuclei in each muscle fiber, only a minority of the nuclei display DNA fragmentation, which indicates cell death (Schulze et al., 2002). Impaired endothelial nitric oxide synthase (eNOS) expression is one factor associated with increased skeletal muscle apoptosis (Georgiadou and Adamopoulos, 2012). Apoptosis leads to decreased muscle bulk, decreased contractility, and increased muscle atrophy (Schulze et al., 2002). As a result, it is also associated with reduced exercise tolerance. It has been suggested, however, that apoptosis may minimally contribute to decreased muscle mass (Mettauer et al., 2006).

Many genes that regulate apoptosis are altered in CHF. Oncogenes including c-myc and bcl2 are reduced (Nicoletti et al., 2003). Tumor suppressor genes, including p53, and other factors that increase apoptosis, including NOS, nitric oxide, caspase-3, and ubiquitin, are increased (Nicoletti et al., 2003).

Growth Hormone/IGF-1 Axis

Many factors influence expression of IGF-1 in skeletal muscle. Serum levels of IGF-1 and growth hormone (GH) influence skeletal muscle expression of IGF-1. Nonhumoral stimuli may also stimulate IGF-1 expression (Schulze et al., 2002). One study showed that 4 days of muscle stretch, the strongest nonhumoral stimulus, induced upregulation of IGF-mRNA as soon as 12 h later (Schulze et al., 2002). Skeletal muscle IGF-1 levels are modified by muscle use (Schulze et al., 2002). In neonatal rats, muscle unloading by zero gravity caused decreased expression of IGF-1 in skeletal muscle (Schulze et al., 2002). The GH/IGF-1 axis is a regulator of normal growth, hypertrophy, and atrophy (Hambrecht et al., 2002). Binding of IGF-1 to receptors induces cellular proliferation and protection from apoptosis. It also prevents TNF-α induced cell death (Hambrecht et al., 2002).

Patients with CHF may have increased levels of GH with unusually normal or low serum levels of IGF-1 (Hambrecht et al., 2002). These findings suggest the development of GH resistance in CHF patients (Hambrecht et al., 2002). Local levels of IGF-1 are reduced before systemic changes are apparent (Hambrecht et al., 2002). Skeletal muscle biopsies from noncachectic patients with CHF had downregulated skeletal muscle expression of IGF-1 but normal serum concentrations of IGF-1 (Schulze et al., 2002). Changes in the GF/IGF-1 axis contribute to the loss of muscle bulk, muscle wasting, and eventually clinically recognizable cachexia (Hambrecht et al., 2002).

Pathophysiological changes related to alterations in the GH/IGF-1 axis in patients with CHF have been characterized. Insulin resistance in CHF may be caused by proinflammatory cytokines and oxidative stress (Georgiadou and Adamopoulos, 2012). Combined with decreased levels of IGF-1, this decreases activation of protein kinase B (Akt) in skeletal muscle (Georgiadou and Adamopoulos, 2012). Decreased Akt activation decreases phosphorylation of mammalian target of rapamycin (mTOR) and

glycogen synthase kinase, thereby decreasing protein synthesis (Georgiadou and Adamopoulos, 2012). Moreover, decreased Akt activation upregulates FoXO transcription factors (Georgiadou and Adamopoulos, 2012). FOXO transcription factors activate the ubiquitin proteasome pathway of protein degradation by activating ubiquitin-protein ligase atrogin-1/MAFbx (Georgiadou and Adamopoulos, 2012). Decreased levels of IGF-1 also activate caspase-3, which also contributes to protein degradation through the ubiquitin proteasome (Georgiadou and Adamopoulos, 2012). In a murine heart failure model with whole body insulin resistance, serine phosphorylation of Akt and glucose transporter 4 (GLUT4) in skeletal muscle is impaired by NAD(P)H oxidase-derived superoxide (Tsutsui et al., 2011). NAD(P)H oxidase inhibitor alleviated insulin resistance and signaling deficiencies in skeletal muscle (Tsutsui et al., 2011). Similarly, in skeletal muscle from post-infarction mice, it was shown that insulin-stimulated serine-phosphorylation of Akt decreased by 57% and GLUT4 translocation decreased by 69% (Fukushima et al., 2016). In this study, aliskiren, a direct renin inhibitor, alleviated changes, inhibited plasma renin, and decreased angiotensin II (Ang II) levels (Fukushima et al., 2016). Increased levels of Ang II suppress IGF-1 signaling through the Akt/mTOR/p70S6k pathway (Zizola and Schulze, 2013). Ang II also activates caspase-3, actin cleavage, ubiquitination, and apoptosis (Zizola and Schulze, 2013).

Renin inhibitors may reduce insulin resistance (Fukushima et al., 2016). It has not yet been determined if ACEi improve skeletal muscle in patients with CHF through the IGF-1 pathway or through direct effects on vascular endothelial function (Zizola and Schulze, 2013).

Skeletal muscle changes due to alterations in the GH/IGF-1 pathway have also been characterized. In noncachectic patients, low levels of systemic IGF-1, which occur in advanced stages of CHF, are associated with decreased leg muscle CSA and strength (Hambrecht et al., 2002). Even when serum IGF-1 levels are not significantly altered in patients with CHF, quadriceps skeletal muscle may have reduced IGF-1 mRNA expression and increased IGF-1 receptor mRNA expression (Hambrecht et al., 2002). Increased receptor expression, however, is unable to compensate for IGF-1 deficits that lead to atrophy (Hambrecht et al., 2002). Atrophy is accompanied by fatigability and reduced total muscle CSA that closely correlates with local IGF-1 expression (Hambrecht et al., 2002).

Exercise training, which serves as a natural cause of muscle stretch, may alleviate CHF symptoms through the GH/IGF-1 pathway (Schulze et al., 2002). One study showed that local IGF-1 expression more than doubled after 6 months of exercise training in patients with CHF (Schulze et al., 2002). Specifically, long-term aerobic exercise training (AET) has been recommended as an intervention for the IGF-1 deficient, catabolic state (Schulze et al., 2002). In mice, AET activated the IGF-1/Akt/mTOR pathway (Bacurau et al., 2016). This reduced exercise intolerance, soleus atrophy, and 26S proteasome hyperactivity (Bacurau et al., 2016). It also decreased levels of myostatin and Smad2 while preventing reduction of IGF-1, pAkt, p4E-BP1, and pP70 (Bacurau et al., 2016). Rapamycin treatment prior to AET limited beneficial effects of AET, showing that AET-mediated benefits may involve the IGF-1 pathway (Bacurau et al., 2016). Alternatively, therapies may focus on reducing GH resistance in patients with skeletal muscle wasting (Nicoletti et al., 2003). Cytokine antagonists, catabolic hormone antagonists, and amino acid supplementation may all reduce problems related to the GH/IGF-1 axis (Pasini et al., 2003).

Biochemical Pathways

Impaired energy metabolism contributes to skeletal muscle wasting. In mice, CK deficiency induced skeletal muscle wasting through activation of AMP-activated protein kinase (Mettauer et al., 2006). In a Wistar Kyoto rat model of CHF, muscle wasting was related to changes in antioxidative capacity (Mangner et al., 2015). In the quadriceps, antioxidative capacity was reduced, as shown by reduced glutathione peroxidase and manganese SOD activity (Mangner et al., 2015). COX activity decreased and LDH activity increased (although other studies have shown that LDH activity decreases—see "**Metabolic Changes**" section for further discussion) (Mangner et al., 2015). On the contrary, in the diaphragm, antioxidative capacity increased, as shown by increased glutathione peroxidase and manganese SOD activity (Mangner et al., 2015). Metabolic enzymes in the diaphragm were unaltered (Mangner et al., 2015). In both quadriceps and the diaphragm, catabolic responses and myopathy were increased, as shown by increased protein expression of the E3 ligase muscle ring finger 1 and proteasome activity (Mangner et al., 2015). Long-term exercise training may improve skeletal muscle COX activity (Schulze et al., 2002).

Protein degradation pathways play a role in muscle wasting. One major protein degradation pathway is the ubiquitin-proteasome pathway. In this pathway, proteins tagged with ubiquitin are degraded through a 26S proteasome in an ATP-dependent manner (Zizola and Schulze, 2013). The ubiquitin-proteasome pathway may be activated by factors in CHF, including Ang II and cytokines (TNF-α and IL-1) (Zizola and Schulze, 2013). A study on skeletal muscle in 4-week old Wistar rats with heart failure induced by MCT showed that the ubiquitin-proteasome and macroautophagolysosome pathways were activated even before morphological changes were present in skeletal muscle (Fujita et al., 2015). Skeletal muscle from mice with left ventricular dysfunction revealed activation of the ubiquitin-proteasome pathway with increased protein ubiquitination, FoxO transcription factor activation, increased expression of muscle-specific atrogenes and overall muscle atrophy (Zizola and Schulze, 2013). Increased activation of the ubiquitin-proteasome system in skeletal muscle was further shown in a sympathetic hyperactivity mouse model of CHF (Cunha et al., 2012). In this study, AET normalized levels of lipid hydroperoxides, carbonylated proteins, and E3 ligase mRNA (Cunha et al., 2012). Moreover, it restored chymotrypsin-like proteasome activity, which has also been observed in human skeletal muscle (Cunha et al., 2012). Studies suggest that physical exercise, especially prior to recognizable skeletal muscle atrophy, should be implemented to reduce over-activation of protein degradation pathways (Fujita et al., 2015). In CHF, increased PGC-1α, which is related to mitochondrial function and moderation of FoxO activity, may also reduce declines due to the ubiquitin pathway (Forman et al., 2014).

Circulating Factors

Patients with advanced CHF and cachexia have increased Ang II levels (Yoshida and Delafontaine, 2016). Ang II, via its type 1 receptor, causes muscle protein breakdown and apoptosis (Yoshida and Delafontaine, 2016). It also inhibits satellite cell proliferation and muscle regeneration (Yoshida and Delafontaine, 2016). Via the type 2 receptor (AT2R), however, Ang II potentiates muscle regeneration (Yoshida and Delafontaine, 2016). In a mouse model of CHF, muscle regeneration was reduced and AT2R expression was slightly increased (Yoshida and Delafontaine, 2016). The researchers suggested that an intronic enhancer (specifically AT2R intron 2) that regulates AT2R expression during stem cell (SC) differentiation is suppressed in CHF, thereby decreasing muscle regeneration (Yoshida and Delafontaine, 2016).

Skeletal muscle biopsies from patients with HFrEF indicate increased local concentrations of adiponectin in addition to circulating levels (Sente et al., 2016a). In patients with HFrEF, circulating adiponectin levels increase relative to the severity of the disease (Sente et al., 2016a). Adiponectin resistance in skeletal muscle may be related to increased free fatty acid availability and skeletal muscle wasting (Sente et al., 2016a).

Various other factors may be involved. Studies have shown an elevated cortisol to dehydroepiandrosterone ratio in patients with CHF (Schulze et al., 2002). In male patients with CHF, deficiencies in anabolic hormones, such as testosterone and dehydroepiandrosterone, correlate with a poor prognosis (Zizola and Schulze, 2013). Studies have also shown high levels of catecholamines, which would influence resting metabolic rate (Schulze et al., 2002; Hambrecht et al., 2002). One abstract focusing on 53 CHF patients with severely impaired exercise intolerance suggested that significantly increased serum concentrations of leptin, a hormone related to energy expenditure and weight loss, may be involved in catabolic alterations in patients with severe left ventricular dysfunction (Adams et al., 2001; Schulze et al., 2001) Many studies have shown that increased levels of circulating myostatin, a factor secreted by skeletal muscle, may contribute to muscle atrophy in CHF through antianabolic and antihypertrophic mechanisms (Zizola and Schulze, 2013). Studies related to changes in the concentration of circulating myostatin in patients with CHF, however, show conflicting results. Alterations involving aldosterone, renin, melanocortins, neuropeptide Y, and ghrelin have been observed (Georgiadou and Adamopoulos, 2012). Finally, sympathetic activation and cytokines (TNF-α, IL-6, IL-1β) contribute to muscle wasting.

Nutrition

Skeletal muscle plays an important role in metabolism. It exchanges amino acids with the liver (Pasini et al., 2003). These amino acids are used to produce glucose through gluconeogenesis during malnutrition (Pasini et al., 2003). Malnutrition has been shown to be related to muscle wasting and cachexia in patients with CHF. One study showed that 24% of patients with CHF are malnourished, whereas 68% have muscle atrophy (Pasini et al., 2003). Another study involving 130 ambulatory patients with CHF showed that poor nutritional status is related to poor clinical outcomes (Saitoh et al., 2016). In the study, patients with muscle wasting had significantly lower values of peak VO_2, 6-minute walk distance, and Mini-Nutritional Assessment-Short Form (MNA-SF) score (Saitoh et al., 2016). In multivariate analysis, MNA-SF remained an independent predictor of muscle wasting and mortality, leading the researchers to suggest that nutritional screening should be included as a fundamental part of patient assessment (Saitoh et al., 2016). Exogenous amino acid supplementation may serve as an alternative therapy for patients with CHF and poor nutritional status (Pasini et al., 2003). Amino acids may increase anabolic processes and reduce catabolic processes (Pasini et al., 2003).

Clinical Muscle Weakness

Reduced skeletal muscle mass, especially at the stage of cachexia, contributes to exercise intolerance in patients with CHF. Patients consistently show a reduction in muscle bulk and endurance. In one study, static and dynamic endurance were markedly decreased in patients with CHF (Minotti et al., 1992). There was a relationship between dynamic endurance and peak systemic VO_2 in patients with CHF (Minotti et al., 1992). In another study, CHF patients underwent localized unilateral arm training (Minotti et al., 1992). The training doubled or tripled wrist flexor endurance although the muscle size did not change (Minotti et al., 1992). The changes were associated with improvements in muscle oxidative metabolism, as shown by a slower increase of Pi and decrease of PCr (Minotti et al., 1992).

Some studies have suggested that muscle strength is not impaired in patients with CHF, although others have reported reduced muscle strength (Nicoletti et al., 2003). A study on patients with severe CHF showed that patients only had 55% of the predicted strength (Minotti et al., 1992). A different preliminary study comparing quadriceps strength and endurance in nine patients with CHF to five sedentary controls found that isometric strength was similar between the groups, although they suggested that this might be due to a wide range of clinical severity (Minotti et al., 1992). After normalizing work load to an individual's maximal strength, a group showed that the maximum load in patients with CHF was 30% lower compared to controls (Minotti et al., 1992).

Muscle contractility may also be altered by atrophy. One study focused on contractility in the soleus because it is a primary site of muscle atrophy (Panizzolo et al., 2016). Compared to age- and activity-matched controls, CHF patients had reduced passive soleus force at the same relative levels of muscle stretch (though the results were not statistically significant) (Panizzolo et al., 2016). The reduction was eliminated when normalized by CSA, showing that force output reduction may be most strongly associated with muscle size (Panizzolo et al., 2016). Passive force was also significantly higher in CHF patients at an absolute muscle length, which

could potentially be explained by shorter muscle slack lengths (Panizzolo et al., 2016). A second study related to muscle shortening showed decreased maximal force, speed, and power (Coggan and Peterson, 2016). This study, however, suggested the changes may be related to decreased NO availability (Coggan and Peterson, 2016).

Blood Flow to Skeletal Muscle

Blood flow to tissue is related to the level of metabolic activity. Cardiovascular hemodynamics are impacted by skeletal muscle due to its large mass (Mohrman and Heller, 2014). At rest, skeletal muscle receives 15%–20% of cardiac output (Fletcher et al., 2011; Mohrman and Heller, 2014). This is partially due to a high basal vascular tone (Mohrman and Heller, 2014). Resting skeletal muscles normally extract only 25%–30% of the oxygen delivered through blood flow (Mohrman and Heller, 2014). This means that, although blood flow to skeletal muscle is relatively low, it is still significantly greater than the flow necessary for metabolic requirements (Mohrman and Heller, 2014). During strenuous exercise, skeletal muscle may receive greater than 80%–85% of cardiac output (Fletcher et al., 2011; Mohrman and Heller, 2014). Specifically, this may be a change from 4–7 mL/min of blood per 100 g of skeletal muscle during rest to 50–75 mL/min of blood per 100 g of muscle during exercise (Fletcher et al., 2011). Blood flow in active skeletal muscle is mostly directed to oxidative portions rather than glycolytic portions (Fletcher et al., 2011).

Patients with CHF have reduced blow flow to skeletal muscle. Due to reduced cardiac output in CHF, circulatory regulation must decrease blood supply to exercising skeletal muscle in order to prevent dangerous changes in BP (Poole et al., 2012). Reduced blood flow impairs peripheral perfusion, causing reduced oxygen supply to skeletal muscle despite increased oxygen demands (Poole et al., 2012). Researchers are unsure if skeletal muscle changes in CHF are related to a CHF-specific myopathy or secondary effects of reduced blood flow in exercising muscle (Nicoletti et al., 2003). Changes secondarily affect respiratory, vascular, and locomotor muscle function (Poole et al., 2012).

Skeletal muscle in patients with CHF has changes related to capillaries. Many studies have shown that capillary density is reduced in the skeletal muscle. In a rat model of heart failure induced by MCT injection, capillary-to-fiber ratio was lower in the oxidative plantaris region but not in the glycolytic region (Wust et al., 2012). Some researchers suggest that, due to fiber atrophy, the number of capillaries per fiber area is unchanged (Mettauer et al., 2006). Post-capillary resistance is increased, which impairs blood flow (Poole et al., 2012). The combination of slowed capillary hemodynamics and reduced blood flow velocity helps to maintain normal levels of oxygen extraction (Mettauer et al., 2006). As a result, arteriovenous oxygen difference is typically normal during exercise in patients with CHF (Mettauer et al., 2006).

Changes in skeletal muscle blood flow are strongly related to alterations in vascular tone. Patients with CHF have increased peripheral vasoconstriction along with defective vasodilatation. Increased vasoconstriction may be caused by increased plasma concentrations of catecholamines, renin, Ang II, vasopressin, and endothelin-1 (Poole et al., 2012). Peripheral chemoreceptors and metaboreflexes also contribute to vasoconstriction through increased sympathetic activation of α-adrenergic tone (Poole et al., 2012). Experimental and clinical studies suggest that decreased vasodilatation, on the other hand, may be caused by vascular stiffness and resistance from alterations in arterial wall sodium content (Minotti et al., 1992). Vasodilatation is impaired by salt and water retention and improved by diuresis (Poole et al., 2012). In a study comparing dynamic, single leg knee-extensor exercise in patients with HFpEF compared to healthy controls, HFpEF patients showed significant attrition during more intensive exercise related to a marked reduction in leg blood flow and leg vascular conductance that was not attributed to disease-related changes in central hemodynamics (Lee et al., 2016). This suggested that impaired vasodilatation in exercising skeletal muscle vasculature impacts exercise intolerance in patients with HFpEF (Lee et al., 2016). Lower vasodilatation capability particularly impacts exercise tolerance in patients with sarcopenia (Dos Santos et al., 2016). Altered vascular tone also decreases flow of nutrients to skeletal muscle during exercise (Mettauer et al., 2006). This decreases oxygen and substrate supply to myocytes and reduces the increase in oxygen uptake at the beginning of exercise (Mettauer et al., 2006).

Vasodilatation may also be impaired through endothelial dysfunction. A study in 228 patients with CHF and sarcopenia confirmed the presence of impaired endothelial function (Dos Santos et al., 2016). Endothelial dysfunction may be augmented by endothelial cell damage and decreased capacity for repair due to low circulating levels of endothelial progenitor cells (Poole et al., 2012). In patients with CHF, there is reduced production and release of endothelium-derived vasodilator factors, such as NO, in response to acetylcholine (Piepoli et al., 2001). Basal release of NO, however, may be preserved or enhanced as a compensatory mechanism (Francis et al., 2011). NO has general antiatherogenic and antithrombotic effects (Fletcher et al., 2011). Decreased NO levels reduce the ability to oppose α-adrenergic vasoconstriction and reduce vasodilatation caused by shear stress (Poole et al., 2012). This causes oxygen delivery-utilization mismatch, in which microvascular oxygenation falls faster during increased metabolic demand (Hirai et al., 2014). It also reduces exercise tolerance in patients with HFrEF (Hirai et al., 2017). Endothelial dysfunction may be partially related to deconditioning, and it may be reversed through exercise (Francis et al., 2011).

Exercise may alleviate changes in skeletal muscle blood flow in patients with CHF. Exercise may increase muscle capillarity in patients with CHF through preservation of vascular endothelial growth factor pathways (Poole et al., 2012). In a study comparing stable patients with CHF to healthy controls, it was shown that acute exercise affects microcirculation in peripheral, nonexercising muscles in a different way in patients with CHF (Tzanis et al., 2016). Specifically, patients with CHF had lower muscle oxygen saturation (StO_2) and reperfusion rate at rest (Tzanis et al., 2016). Oxygen consumption rate increased after exercise in both patients with CHF and healthy controls (Tzanis et al., 2016). StO_2 decreased significantly after maximal exercise in patients with CHF, whereas it returned to preexercise values in healthy subjects (Tzanis et al., 2016). In a study on rats with CHF, exercise training

slowed the overall fall in microvascular oxygenation during contractions (Hirai et al., 2014). This effect was not abolished by *N*(G)-nitro-L-arginine methyl ester (Hirai et al., 2014). Moreover, sodium nitroprusside increased mean response time in both groups, thereby slowing kinetics in trained CHF rats (Hirai et al., 2014). The researchers concluded that improved NO-mediated function is not obligatory for training-induced improvements in skeletal muscle microvascular oxygenation in rats with CHF (Hirai et al., 2014). In a third study, it was shown that exercise training enhances endothelium-dependent coronary and peripheral arterial vasodilatation in patients with CHF (Fletcher et al., 2011). Exercise may improve vascular function through increasing arterial wall shear stress, which promotes enhanced expression and activation of eNOS through Akt-dependent phosphorylation (Fletcher et al., 2011). Exercise may also improve vascular endothelial function through elevation of circulating endothelial progenitor cells and improved endothelial repair (Poole et al., 2012).

Exercise

Exercise intolerance is one of the primary features of CHF. It is often accompanied by fatigue. Patients are often categorized based on results from cardiopulmonary exercise testing (Fletcher et al., 2011). Treadmill exercise time with the Naughton protocol is an alternative method of testing that is simple and accessible (Fletcher et al., 2011). Exercise intolerance is highly related to mortality in patients with CHF (Mettauer et al., 2001). Exercise intolerance is not correlated with left ventricular ejection fraction, which suggests that it may be instead related to peripheral abnormalities including blood flow to muscles and skeletal muscle dysfunction (Francis et al., 2011; Maurer and Schulze, 2012; Nicoletti et al., 2003). As mentioned throughout this paper, many factors contribute to reduced exercise capacity. Many maladaptive changes related to exercise intolerance are reversible.

Systemic exercise capacity is most commonly described in terms of peak oxygen uptake (VO_2 max). In healthy subjects, exercise capacity is related to cardiac output and peripheral oxygen delivery in response to metabolic demands (Minotti et al., 1992). At maximal exercise, VO_2 max is achieved and oxygen uptake plateaus (Minotti et al., 1992). In patients with CHF, however, there is only a weak relationship between VO_2 max and ejection fraction (Minotti et al., 1992). Skeletal muscle CSA, though, is an independent predictor of VO_2 max (Harrington et al., 1997). A study showed that patients with HFpEF had reduced VO_2 max and 6-min walk distances (Kitzman et al., 2014). In another study, addition of arm exercise to maximal leg exercise increased VO_2 max, showing that the decline in VO_2 max is not related to decreased cardiac output (Mettauer et al., 2006). In CHF patients, fatigue occurs prior to reaching VO_2 max (Minotti et al., 1992). These observations suggest that VO_2 max alterations, like other factors in exercise intolerance, are due to skeletal muscle abnormalities.

Exercise capacity may also be characterized by other variables. VO_2 kinetics are related to the speed of adjustments in oxygen uptake (Poole et al., 2012). Kinetics are slowed to several minutes in patients with CHF (Poole et al., 2012). This means that, for any metabolic transition, patients with CHF incur a greater oxygen deficit and more extreme intracellular perturbation of high-energy phosphagens (Poole et al., 2012). Slowed kinetics also accelerate glycogenolysis and contribute to fatigue and exercise intolerance (Poole et al., 2012). Anaerobic threshold (AT), also called lactate threshold, is the level of exercise beyond VO_2 max at which increased anaerobic metabolism results in lactate accumulation (Minotti et al., 1992). Typically, AT occurs at 50%–60% of peak exercise (Tomono et al., 2016). In patients with CHF, however, AT occurs at low absolute VO_2 levels (Poole et al., 2012). This means that patients with the most compromised oxygen delivery to muscle may have the highest oxygen requirements during exercise (Poole et al., 2012). In a study on 194 stable, optimally treated patients with CHF, the ratio of AT to VO_2 max (%AT/peak) showed a significant negative correlation with VO_2 max but not AT (Tomono et al., 2016). Although this showed that decreased VO_2 max is the primary mechanism of decline, AT is still a well-known factor in evaluating patients (Tomono et al., 2016). Lastly, patients with CHF have an increased ventilation response (VE/V_{CO2}) (Cicoira et al., 2001). This means that patients ventilate at a higher level for a given rate of carbon dioxide production (Cicoira et al., 2001). Ventilation is partially stimulated by the muscle metaboreflex. A study showed that skeletal muscle mass independently predicts VE/V_{CO2} (Cicoira et al., 2001). Changes in ventilation may be related to alterations in respiratory muscle (Nicoletti et al., 2003).

Appropriate exercise training is safe in patients with CHF. It may improve exercise tolerance through noncardiac factors including skeletal muscle structure and function, metabolic changes, peripheral blood flow, oxygen utilization, and endothelial function. In a study on patients with CHF, isolated quadriceps training improved maximal exercise capacity (Esposito et al., 2011). Exercise training also increases VO_2 max. In a meta-analysis of trials by the European Heart Failure Training Group, exercise improved VO_2 max by up to 2 $mL/kg/min^{-1}$, which is similar to the most effective pharmaceutical treatments, such as ACE inhibitors (Schulze et al., 2002). In a study on 20 male patients with stable CHF, training improved VO_2 max by 29% (Gielen et al., 2003). Improvements in all of these factors positively contribute to quality of life. Some of the benefits of exercise are summarized below (**Tables 6** and **7**).

Exercise training increases skeletal muscle bulk and CSA in patients with CHF (Piepoli et al., 2001). In male Wistar rats with ascending aortic stenosis that induced CHF, exercise prevented catabolic processes and muscle wasting (Souza et al., 2014). It did not, however, impact expression of anabolic factors (Souza et al., 2014). Similar changes are observed in patients with CHF. Improvements in muscle bulk and CSA lead to improved function in terms of strength, peak work rate, endurance, and fatigability (Piepoli et al., 2001).

Although aerobic exercise is the most often suggested form of exercise, other forms of exercise may have specific effects and benefits. Resistance training may target muscle hypertrophy and lengthening through generation of new sarcomeres (Panizzolo et al., 2016). This was confirmed in one study on patients with CHF with reduced soleus size and passive force

Table 6 Exercise training in CHF (Schulze et al., 2002)

Exercise training in chronic heart failure	
Skeletal muscle	*Endothelial function*
Oxidative enzymes ↑	Agonist-mediated endothelium-dependent peripheral vasodilation ↑
Fiber type shift (type II–type I)	Afterload reduction
Local IGF-1 expression ↑	
iNOS expression ↓	
Skeletal muscle mass ↑?	

Table 7 Skeletal muscle benefit of exercise training (Piepoli et al., 2001)

1. Morphology	↑ Cross sectional area
2. Function	↑ Peak work rate
	↑ Strength
	↑ Endurance
	↓ Fatigability
3. Blood flow	↑ Leg oxygen delivery
	↓ Leg vascular resistance
	↑ O_2 arterio-venous differences
4. Metabolism	↑ Oxidative metabolism
	↑ Adenosine triphosphate re-synthesis
	↓/↔ Anaerobic metabolism
	↓ Lactate accumulation, acidosis
	↓ Phosphocreatinine depletion
5. Ultrastructural	↑ Mitochondria size
	↑ Aerobic enzyme activity
	↑ Fiber size
	↑ Capillary density
	↑ Endothelium function
	↑ NO release

(Panizzolo et al., 2016). In another study, nondominant wrist flexor training improved oxidative capacity in skeletal muscles during submaximal work load (Volaklis and Tokmakidis, 2005). Further studies have shown that resistance training increases CSA of the quadriceps femoris, muscle fiber area, and oxidative capacity (Volaklis and Tokmakidis, 2005). Improvements due to resistance training are related to functional improvements demonstrated in 6-min walking tests (Volaklis and Tokmakidis, 2005). High-intensity interval training (HIIT) is a specific form of AET. In one study on 13 males with CHF, HIIT increased type I fibers, fiber CSA, capillary to fiber ratio, and expression of IGF-1 and insulin-like growth factor binding protein (IGFBP)-3 (Tzanis et al., 2016). In a second study on 26 patients with CHF, 12 weeks of HIIT at 85%–95% of peak VO_2 improved maximal exercise capacity and exercise hemodynamics (Spee et al., 2016). Improvements in recovery were related to attenuated skeletal muscle deoxygenation rather than cardiac output (Spee et al., 2016). HIIT may specifically reduce skeletal muscle myopathy and improve microvascular delivery and utilization (Spee et al., 2016).

Other Approaches to Treatment

This section will briefly list various programs, treatments, and potential therapies that have been suggested in addition to exercise.

Cardiac rehabilitation is increasingly applied to patients with CHF (Taub, 2016). It is newly indicated for patients with stable CHF with an ejection fraction below 35% (Taub, 2016). It may also be helpful in cases of HFpEF, which has limited treatment options (Taub, 2016). Exercise training is still a primary focus in cardiac rehabilitation, but it is combined with nutrition, BP management, and lipid management (Taub, 2016). Two intensive 72-h cardiac rehabilitation approaches include the Ornish and Pritikin programs (Taub, 2016). The Ornish program involves a 100% plant-based diet and live instructors (Taub, 2016). Patients in this program showed regression in coronary atherosclerosis (Taub, 2016). The Pritikin program involves some lean meat and fish, inclusion of video instruction and customizable steps (Taub, 2016). Patients in this program showed improvement in biomarkers like HbA1c (Taub, 2016). Cardiac rehabilitation improves mitochondrial function and nitric oxide function while also inducing antiinflammatory effects (Taub, 2016). Patients who participate in these programs have decreased levels of all-cause mortality, risk of reinfarction, and readmission (Taub, 2016).

A combination of physical training and certain drug therapies, such as administration of ACEi and Ang II-receptor blockers, may better improve exercise tolerance and characteristics of skeletal muscles (Georgiadou and Adamopoulos, 2012). One study showed than irbesartan, an Ang II type I receptor blocker, prevents myocyte apoptosis, muscle atrophy, and changes in MHCs in experimental CHF (Nicoletti et al., 2003). The researchers suggested that irbesartan limits activity of TNF-α and Ang II to reduce levels of apoptosis (Nicoletti et al., 2003). Beta blockers may also be used to reduce sympathetic activation in patients with CHF. It is yet to be determined if pharmacologic interventions including beta blockers and inhibition of the renin-angiotensin-aldosterone system have an impact on maladaptive changes in skeletal muscle (Maurer and Schulze, 2012).

Patients with CHF have elevated GH but reduced IGF-1 and IGFBP-3 (Khawaja et al., 2014). It has been shown that IGF-1 inversely modulates key atrophy induced genes via the PI3K/Akt/mTOR pathway, meaning this pathway may be potentially useful as a target for antiatrophic therapies (Georgiadou and Adamopoulos, 2012). In one study of patients with advanced heart failure, ventricular assist device (VAD) implantation increased skeletal muscle expression of IGF-1 10-fold and IGFBP-3 5-fold (Khawaja et al., 2014). Moreover, implantation enhanced oxidative gene expression (CD36, CPT1, and PGC1α) and oxidation rates (Khawaja et al., 2014). VAD implantation also increased the oxidative muscle fiber proportion, fiber CSA, and hand grip strength (Khawaja et al., 2014).

Studies on the impact of a nitrate-rich diet in patients with CHF have had varied results. In one study on patients with HFrEF, even though nitrate-rich supplementation resulted in a significantly higher plasma nitrite concentration, there was no significant difference in the time to exercise intolerance between nitrate and placebo groups (Hirai et al., 2017). Moreover, there were no significant differences in central hemodynamics, arterial BP, pulmonary oxygen uptake kinetics, skeletal muscle oxygenation, or blood lactate concentrations (Hirai et al., 2017). Other studies, however, have endorsed positive effects of a nitrate-rich diet. One study suggested that dietary nitrate supplementation improves skeletal muscle vascular function during exercise in rats with CHF (Ferguson et al., 2016). Another study on patients with CHF suggested that acute ingestion of nitrate-rich beetroot juice, a source of NO via the NO synthase-independent enterosalivary pathway, increased maximal muscle speed and power (Coggan and Peterson, 2016). A third study on nine patients with systolic heart failure showed that acute dietary nitrate intake is well tolerated, enhances NO bioavailability, and increases muscle power. The treatment did not alter BP or incite adverse clinical events. These three studies support nitrate-rich diet supplementation, especially with beetroot juice, as a novel therapeutic modality for the treatment of patients with CHF (Coggan et al., 2015).

Other potential therapies include enhanced external counterpulsation (Melin et al., 2016), therapeutic ultrasound to modulate inflammation (Rossato et al., 2015), autologous transplantation of satellite cells to reduce myopathy (Castellani et al., 2013), and activation of peroxisome proliferator-activated receptor delta (Myers and Yoshioka, 2015).

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Sleep and Circadian Cardiovascular Medicine

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Sleep Disturbance and Cardiovascular Disease

Recent studies have demonstrated that sleep disturbance is closely associated with hypertension, metabolic disease, and various types of cardiovascular disease (CVD) (**Fig. 1**) (Shimamoto et al., 2014; Kario, 2015a, 2016; St-Onge et al., 2016; Grandner et al., 2016). Sleep disturbance includes sleep deprivation, shift-working, insomnia, obstructive sleep apnea syndrome (OSAS), restless leg syndrome, narcolepsy, etc.

Obstructive Sleep Apnea Syndrome

OSAS is the most common sleep-related breathing disorder. In OSAS, the throat narrows or collapses repeatedly during sleep, causing apnea events; in addition, intermittent hypoxia and hypercapnia, recurrent arousals, and increase in respiratory efforts lead to secondary sympathetic activation, an increase in intrathoracic pressure, oxidative stress, and systemic inflammation (**Fig. 1**) (Kario, 2009, 2016; Lévy et al., 2015; Malhotra et al., 2015; Bradley and Floras, 2009). OSAS is associated with metabolic syndrome, hypertension, and CVDs including stroke, coronary artery disease (CAD), arrhythmias, heart failure (HF), and aortic dissection. Several population-based studies have demonstrated an increased incidence of baseline and future hypertension in subjects with OSAS (Lévy et al., 2015; Malhotra et al., 2015; Bradley and Floras, 2009). In addition, OSAS is the most common secondary cause of resistant hypertension.

Sleep Deprivation and Insomnia

There is increasing evidence that poor quantity and/or quality of sleep due to causes other than SAS, such as poor sleep hygiene (sleep deprivation, irregular falling asleep time, shift working) and insomnia, are closely associated with cardiometabolic risk factors, hypertension, and various CVDs and poor prognosis (**Fig. 1**) (Shimamoto et al., 2014; Kario, 2015a; St-Onge et al., 2016; Grandner et al., 2016). Sleep deprivation has become an important health problem in our modern 24-h society, with evidence showing that people in Western countries are sleeping on average only 6.8 h per night, 1.5 h less than a century ago (Nagai et al., 2010). Chronic insomnia is disrupted sleep that occurs at least three nights per week and lasts at least 3 months. People with insomnia tend to have difficulty falling asleep (onset) or staying asleep (maintenance), and/or they wake up too early in the morning. People with insomnia can feel dissatisfied with their sleep and usually experience one or more of the following symptoms: fatigue, low energy, difficulty concentrating, mood disturbances, and decreased performance in work or at school. Chronic

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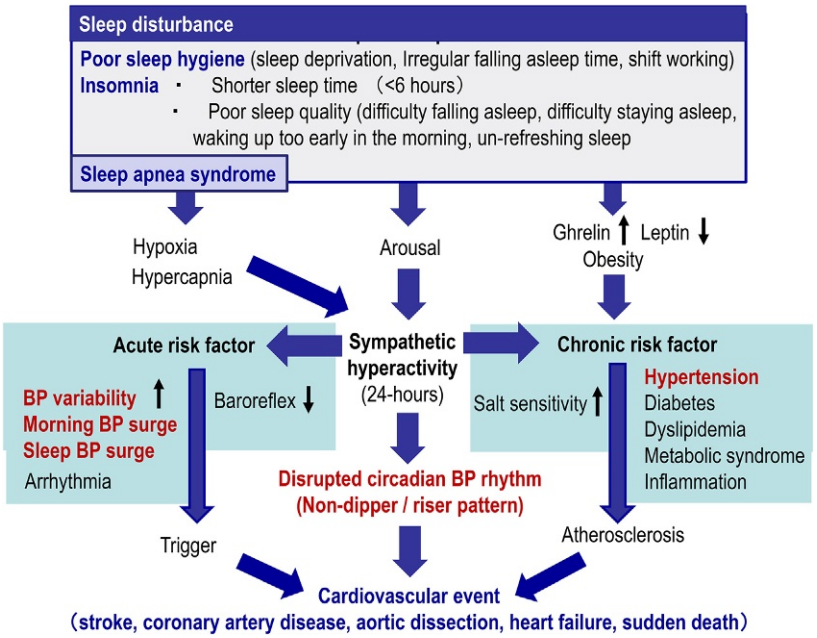

Fig. 1 Sleep disturbance and its relation to the mechanism of cardiovascular disease. *BP*, blood pressure.

insomnia disorders can have many causes. Changes in the environment, unhealthy sleep habits, shift work, other clinical disorders, and certain medications can lead to a long-term pattern of insufficient sleep.

Recent guidelines and/or statements from health organizations stress the importance of sleep hygiene for the management of hypertension and CVD prevention (Shimamoto et al., 2014; St-Onge et al., 2016).

Sleep Duration and Cardiovascular Disease

Both shorter and longer sleep durations are associated with the risk of future CVD events.

In a cross-sectional study of 30,397 individuals $\geq$18 years of age who responded to the 2005 National Health Interview Survey, the adjusted odds ratios (ORs) of CVD were 2.20, 1.33, 1.23, and 1.57 for a sleep duration of $\leq$5, 6, 8, and $\geq$9 h compared with a sleep duration of 7 h (referent). Thus, compared with sleep duration of 7 h, shorter and longer sleep durations are independently related to CVD (Sabanayagam and Shankar, 2010).

In a metaanalysis of multiple prospective studies (24 cohorts; $n=474684$; follow-up: 6.9–25 years) that included 16,067 events (4169 CAD, 3478 stroke, and 8420 CVD events), self-reported short sleep duration was associated with a greater risk of CAD (relative risk 1.48) and stroke (1.15), but not total CVD (1.03). Long sleep duration has also been associated with a greater risk of CAD (1.38), stroke (1.65), and total CVD (1.41) (Cappuccio et al., 2011).

In a case-control study of middle-aged men, long working hours and short sleep duration contributed independently to the risk of CVD in men. Men whose average number of working hours was $\geq$60 h/week were found to have significantly increased risks for CAD (OR 2.2) as compared to those with weekly working hours in the range of 40–48 h, and those with daily sleep duration <6 h were found to have increased risks for CAD (OR 3.0) as compared to those with sleep duration in the range of 6–9 h (Cheng et al., 2014).

In a metaanalysis of population-based cohort studies (27 cohorts; >70,000 elderly individuals aged $\geq$60 years), long and short sleep duration were associated with increased risk for all-cause mortality. Long sleep duration has also been associated specifically with CVD mortality (da Silva et al., 2016).

In a prospective cohort study of 392,164 adults aged $\geq$20 years who attended a health checkup program, when compared to those who slept 6–8 h per night, the risk of CAD death was increased by 34% and 35% in those who slept <4 h and >8 h, respectively. Thus, adequate sleep duration should be considered an important component of a healthy lifestyle (Strand et al., 2016).

In our Jichi Medical School Cohort Study, a nationwide population-based prospective study of 11,367 Japanese with an average follow-up of 10.7 years, the incidences of CVD for individuals sleeping <6 h and $\geq$9 h were 2.14 and 1.33 in men, and 1.46 and 1.28 in women, respectively, relative to those who reported sleeping 7–7.9 h per day (Amagai et al., 2010).

Short sleep is associated with particularly high risk of developing HF in men with CVD, and daytime napping is also associated with greater HF risk in older men. In a population-based prospective study of general practices in 24 British towns, which enrolled men aged 60–79 years without prevalent HF followed for 9 years ($n=3723$), self-reported daytime sleep duration >1 h was

independently associated with significantly greater HF risk (hazard rate (HR) 1.69) than in those who reported no daytime napping. Self-reported shorter nighttime sleep duration <6 h was significantly associated with HF risk in men with preexisting CVD (HR 2.91, vs. nighttime sleep of 7 h) (Wannamethee et al., 2016).

In a metaanalysis of a prospective study including 9692 stroke-free participants aged 42–81 years, there was a higher stroke risk among those who reported persistently long sleep or a substantial increase in sleep duration over time, compared to those reporting persistently average sleep. These findings were compatible with the pooled HRs from an updated metaanalysis, which were 1.15 for short and 1.45 for long sleep durations, respectively. Prolonged sleep may thus be a potentially useful marker of increased future stroke risk in an apparently healthy aging population (Leng et al., 2015).

Shorter sleep duration has been shown to be an independent risk for future incidence of stroke events in hypertensives, especially those with silent cerebral infarcts (SCI). In our study on 1268 elderly hypertensives (mean age 70.4 years), a shorter sleep duration of <7.5 h was independently associated with the risk of stroke (HR 2.21, $P=0.003$). When the subjects were divided into an SCI (+) group and SCI (−) group, the short sleep duration was a significant predictor for incident stroke only in the SCI (+) group (HR 2.52, $P=0.001$) (Eguchi et al., 2010).

Sleep Quality and Cardiovascular Disease

Not only sleep duration but also sleep quality is associated with CVD.

In a Dutch population-based cohort study in 20,432 men and women aged 20–65 years without any CVD history, short sleepers, especially those with poor sleep quality, had an increased risk of CVD and CAD. Sleep duration and sleep quality were assessed by a self-administered questionnaire. During 10–15 years of follow-up, short sleepers (≤6 h) had a 15% higher risk of total CVD (HR 1.15) and a 23% higher risk of CAD (HR 1.23) compared to normal sleepers (7 h). Short sleepers with poor sleep quality had a 63% higher risk of CVD (HR 1.63) and a 79% higher risk of CAD incidence (HR: 1.79) compared to normal sleepers with good sleep quality (Hoevenaar-Blom et al., 2011).

Insomnia is highly prevalent among HF patients. A recent large prospective Norwegian cohort, the Nord-Trøndelag Health study (HUNT) (54,279 men and women aged 20–89 years with a mean follow-up of 11.3 years), demonstrated that self-reported insomnia symptoms (including difficulty initiating sleep, difficulty maintaining sleep, and having nonrestorative sleep) were associated with a risk of future HF. There was a dose-dependent association between the number of insomnia symptoms and HF risk. The adjusted HRs were 0.96, 1.35, and 4.53 for people with one, two, and three insomnia symptoms, compared with those with no insomnia symptoms ($P=0.021$) (Laugsand et al., 2014).

Both SAS and non-SAS sleep quality are associated with atrial fibrillation (AF). In participants of the Multi-Ethnic Study of Atherosclerosis (MESA) Sleep Study who underwent polysomnography (PSG) ($n=2048$), a higher apnea–hypopnea index (AHI) was associated with increased odds of AF. In addition, lower odds of AF associated with longer duration of slow-wave sleep (SWS) (OR 0.66 per SD (34 min), $P=0.01$) was found, and this significance persisted after additionally adjusting for AHI (OR 0.68, $P=0.01$) (Kwon et al., 2015).

Sleep and Subclinical Cardiovascular Disease

Abnormal daily sleep duration and quality are associated with subclinical measures of CVD.

High BP, short sleep duration (≤5 h), poor sleep quality, and insomnia are associated with atherosclerosis risk leading to CVD in the elderly. In a cross-sectional study on 86 elderly aged ≥65 years (mean 74 years), carotid intima-media thickness (IMT) evaluated using ultrasonography was significantly greater in subjects with actigraphy-measured total sleep time (TST) ≤5 h than those with TST >7 h (1.3 vs. 0.9 mm; $P=0.009$). Multiple regression analysis revealed that systolic BP, TST, and the Pittsburgh Sleep Quality Index (PSQI) were significant contributing factors for increased IMT (Nakazaki et al., 2012). In the Coronary Artery Risk Development in Young Adults (CARDIA) Study, 1 h of longer sleep duration, as evaluated by wrist actigraphy monitors, was associated with 0.026 mm less carotid IMT among men ($P=0.02$) and 0.001 mm less carotid IMT among women ($P=0.91$) (Sands et al., 2012).

Longer measured sleep is associated with lower calcification incidence independent of examined potential mediators and confounders. Coronary artery calcification (CAC) is a subclinical predictor of CAD. In an observational cohort of 495 healthy middle-aged participants (35–47 years) from the CARDIA cohort Chicago site, CAC incidence was evaluated by computed tomography after 5-year follow-up. Longer sleep duration (evaluated by wrist actigraphy) was significantly associated with reduced 5-year CAC incidence (OR 0.67 per hour, $P=0.01$) (King et al., 2008). In a cross-sectional study of adults who underwent a health checkup examination, including assessment of sleep duration and quality coupled with either CAC ($n=29,203$) or brachial-ankle pulse wave velocity (PWV) ($n=18,106$), the multivariate-adjusted CAC score ratios comparing sleep durations of ≤5, 6, 8, and ≥9 h with 7 h of sleep were 1.50, 1.34, 1.37, and 1.72, respectively ($P=0.002$). The corresponding average differences in brachial-ankle PWV were 6.7, 2.9, 10.5, and 9.6 cm/s, respectively ($P=0.019$). Poor subjective sleep quality was associated with CAC in women but not in men, whereas the association between poor subjective sleep quality and brachial-ankle PWV was stronger in men than in women. These results underscore the importance of an adequate quantity and quality of sleep to maintain cardiovascular health (Kim et al., 2015).

In a recent systematic review of 32 studies, most of the included studies concluded that sleep duration and quality affect the burden of subclinical CVD in subjects free of clinical CVD. Subclinical CVD measures included coronary artery calcium (CAC) measured by electron beam computed tomography, carotid IMT measured by high-resolution B-mode ultrasound on carotid arteries, endothelial/microvascular function measured by flow-mediated dilation, peripheral arterial tone, iontophoresis or nailfold capillaroscopy, and arterial stiffness measured by PWV or ankle brachial index (Aziz et al., 2016).

Short sleep duration was associated with worse markers of white matter integrity in midlife, indicating a link between poor sleep and risk of dementia and stroke. White matter hyperintensities (WMH) have been associated with an increased risk of stroke and cognitive dysfunction. In a cross-sectional analysis of 514 elderly hypertensives (mean 72 years), short sleep duration was a significantly positive determinant for WMH, especially in those without chronic kidney disease (Nagai et al., 2015). In a prospective cohort of 613 black and white adults (mean 45 years) enrolled in the CARDIA Study, short sleepers ($\leq$6 h) had an elevated ratio of WMH to normal tissue in the parietal region (OR 2.31) compared to moderate sleepers (>6 to $\leq$8 h) after adjusting for confounding factors (Yaffe et al., 2016).

In a prospective cohort study of 4238 participants from the Nurses' Health Study, self-reported sleep duration was significantly associated with a decline in renal function over an 11-year period. Compared with sleeping 7–8 h per night, the adjusted ORs for a rapid decline in renal function (30% or greater reduction in the estimated glomerular filtration rate) were a significant 1.79 for <5 h sleep per night, a significant 1.31 for 6 h sleep per night, and an insignificant 0.88 for $\geq$9 h. Thus, shorter sleep duration was prospectively and independently associated with faster decline in renal function (McMullan et al., 2016).

These data are consistent with the hypothesis that the CVD consequences of short sleep may begin as early as adolescence and persist until older age.

Sleep and Diabetes

Sleep loss is associated with obesity, metabolic syndrome, and diabetes. An increasing number of epidemiological studies show an association between adverse metabolic traits, particularly obesity and type 2 diabetes, and each of short sleep duration, sleep disturbances, and circadian desynchronization of sleep (Schmid et al., 2015; Kawakami et al., 2004; Gottlieb et al., 2005; Tasali et al., 2008; Chao et al., 2011).

U-shaped patterns have been observed for the relationship between sleep duration and diabetes. In conclusion, both short and long sleep durations have been independently associated with newly diagnosed diabetes. In a total of 3470 adults recruited from a health checkup center, the multinomial regression model demonstrated that both short (<6.0 h) and long ($\geq$8.5 h) sleepers had a higher risk of newly diagnosed diabetes, with ORs of 1.55 and 2.83, respectively, compared with normal sleepers (6.0–8.49 h). However, sleep duration was not found to relate to prediabetes (Chao et al., 2011).

Shorter sleep duration may impair insulin sensitivity and beta-cell function in nondiabetic white men, possibly contributing to later type 2 diabetes and CVD. Support for this conclusion was provided by a study in 224 nondiabetic community volunteers (mean 44.5 years), which a 180-min, intravenous glucose tolerance test was administered, followed by laboratory measurement of insulin sensitivity (Si), acute secretion (AIRg), glucose effectiveness (Sg), and disposition index (Di) (Wong et al., 2015).

In addition, both acute total and short-term partial sleep deprivation have been shown to result in elevated high-sensitivity CRP concentrations, a stable marker of inflammation that has been shown to be predictive of CVD (Meier-Ewert et al., 2004).

Sleep and Sympathetic Nervous System

Sleep-associated alterations in the autonomic nervous system may partly account for the association of short sleep duration and poor sleep quality with CVD outcomes.

In an association study of 48 nonsleepy middle-aged volunteers (mean 59 years), recurrent arousal (arousal index) detected by overnight PSG was the single strongest predictor of muscle sympathetic nerve activity burst incidence. This result indicated that frequent arousals during sleep induce sympathetic nervous activity over a 24-h period (**Fig. 1**) (Taylor et al., 2016).

Increased sympathetic activity as measured by 24-h urinary catecholamines might play a critical role in the pathogenesis mediating the relationship of insufficient sleep (quantity and quality) with subsequent CVD and metabolic complications. In a 3-year follow-up study of 114 healthy middle-aged adults, cross-sectional analysis showed that objective poor sleepers (3-day actigraphy sleep efficiency <85%) had a higher 24-h urinary norepinephrine (NE) level (206 vs. 162 nmol/d, $P=0.03$) than good sleepers. However, the linear regression results showed that shorter time in bed, actual sleep time, longer sleep onset latency, and lower sleep efficiency were correlated with higher 24-h urinary NE but not salivary cortisol. The effect of poor sleep quality on 24-h urinary NE was stronger in males than in females (Zhang et al., 2011).

Short sleep duration, low sleep efficiency, and the combination of insomnia plus short sleep duration are associated with markers of autonomic tone that indicate lower levels of cardiac parasympathetic tone and/or higher levels of sympathetic tone. In a cross-sectional analysis of actigraphy-defined sleep duration and efficiency in 527 adults of the MESA, subjects who slept <6 h had a higher baseline heart rate (HR) and greater HR orthostatic reactivity compared to those who slept $\geq$7 h. Participants who slept 6 h to <7 h/night had a lower baseline high-frequency HR variability (HF-HRV) than those who slept $\geq$7 h. Participants with low sleep efficiency had lower baseline HF-HRV than those with higher sleep efficiency (Castro-Diehl et al., 2016).

Sleep loss may increase cardiovascular risk by dysregulating stress physiology. Sleep deprivation has been shown to amplify systolic BP increases in response to psychological stress. In a study in which two standardized psychological stress tasks (a Stroop color-word naming interference task and a speech task) were administered in 20 healthy young adults, the psychological stress-induced pressor effect was greater under the sleep deprivation condition (Franzen et al., 2011).

Sleep and Blood Pressure

The BP profiles related to sleep disturbance are high BP, exaggerated BP variability, and nocturnal hypertension with diminished nocturnal BP fall (**Fig. 1**). These characteristics may be partly explained by increased sympathetic nervous activity over a 24-h period, resulting in increased salt sensitivity and impaired baroreflex (**Fig. 1**) (Kario, 2012; Gangwisch, 2014).

Sleep Duration

Adults

Short sleep duration could be a significant risk factor for hypertension. In the longitudinal analyses of the first National Health and Nutrition Examination Survey ($n=4810$) conducted over an 8- to 10-year follow-up period, sleep durations of $\leq$5 h per night were associated with a significantly increased risk of hypertension (HR 2.10) in subjects between the ages of 32 and 59 years, and this association remained significant even after controlling for obesity and diabetes (Gangwisch et al., 2006). Such an association between short sleep duration and hypertension was also found in a study of 1046 French adults aged >40 years (mean 55.5 years) in a primary care health setting. Compared to the group sleeping 7 h, individuals sleeping <5 h had an increased adjusted OR for the prevalence of hypertension (OR 1.80) (Faraut et al., 2012).

Elderly

However, sleep duration might have less impact on hypertension in the elderly. In the population-based Rotterdam Study, there was no significant association between sleep duration and hypertension in a cross-sectional study of 5058 participants aged 58–98 years (van den Berg et al., 2007).

Sex-difference

Sleep deprivation may produce pressor effects in women. In a cross-sectional analysis of British civil servants aged 35–55 years at baseline ($n=5766$), short duration of sleep ($\leq$5 h per night) was associated with higher risk of hypertension compared with normal duration of sleep (7 h per night) in women (OR 1.72), independent of confounders, with an inverse linear trend across decreasing hours of sleep ($P=0.037$). No association was detected in men (Cappuccio et al., 2007). In a metaanalysis of 13 papers involving 347,759 participants, a U-shaped change in pooled ORs for hypertension due to the change of sleep duration was observed. The unadjusted OR for hypertension of individuals who slept $\leq$5 h vs. 7 h was 1.61; that for individuals who slept $\geq$9 h vs. 7 h was 1.29. Women deprived of sleep (sleep duration $\leq$5 h vs. 7 h; OR 1.68) had a higher risk of hypertension than men (OR 1.30). Thus, excessively longer and shorter sleep may both be risk factors for high BP, and these associations are stronger in women than in men (Wang et al., 2015).

Adolescents

The association between short sleep duration and higher BP and incident hypertension is found not only in adults but also in adolescents. In a study enrolling 246 healthy adolescents (mean 16 years), shorter, actigraphy-assessed sleep duration over 1 week was related to higher 48-h ambulatory BP and higher nighttime BP. Shorter sleep duration was also related to a higher systolic BP sleep/wake ratio. These results were independent of age, race, sex, and body mass index. Follow-up analyses by race revealed that associations between sleep duration and BP were largely present in white, but not black, adolescents (Mezick et al., 2012). In a more precise study using a school-based sample of 366 adolescents aged 11–16 years, ambulatory BP was measured every 30 min for 24 h on a school day; actigraphy was used to measure sleep duration. Longer sleep duration was significantly associated with lower ambulatory BP in adolescents. The mean sleep duration was 6.8 h at night, and 7.2 h over 24 h. Controlling for duration of sleep during the day and covariables, each additional hour of nighttime sleep was associated with a reduction in SBP (-0.57; $P<0.0001$); controlling for nighttime sleep duration and covariables, each additional hour of daytime sleep was associated with a reduction in systolic BP (-0.73; $P<0.001$) and also a reduction in diastolic BP (-0.50; $P<0.001$). These findings have potential implications for cardiovascular health in this age group (Meininger et al., 2014). However, in a study in healthy 8-year-old children without sleep-disordered breathing, neither quantity nor quality of sleep was related to 24-h ambulatory BP ($n=231$) or cardiovascular reactivity ($n=265$) as assessed by the Trier Social Stress Test for Children (Martikainen et al., 2011).

Thus, poor sleep may be associated with an overt, age-associated unhealthy cardiovascular phenotype.

Sleep Quality

In addition to short sleep duration, poor sleep quality may contribute to hypertension.

Chronic insomnia with short sleep duration is associated with an increased risk for incident hypertension to a degree comparable to that of sleep-disordered breathing defined as an obstructive AHI ≥ 5. In the Penn State Cohort study, which was performed using 8-h PSG in a general population sample of 1395 adults followed up for 7.5 years, compared with normal sleepers who slept ≥ 6 h, the highest risk for incident hypertension was in chronic insomniacs (defined as those with a complaint of insomnia lasting ≥ 1 year) with short sleep duration defined as the median polysomnographic percentage of sleep time (i.e., <6 h) (OR 3.8) (Fernandez-Mendoza et al., 2012).

In a multicenter prospective study known as the Outcomes of Sleep Disorders in Older Men Study, which included 784 community-dwelling men aged ≥ 65 years (mean 75 years) who did not have hypertension in an in-home PSG sleep analysis performed at baseline, 243 subjects newly developed hypertension after a mean follow-up of 3.4 years. Incident hypertension was associated with increased hypoxemia, increased sleep stages N1 and N2, and decreased stage N3 (SWS). After adjustment for age, nonwhite race, study site, and body mass index, the only sleep index to remain significantly associated with incident hypertension was the SWS percentage (OR for the lowest to highest quartile of SWS: 1.83). The percentage time in SWS was inversely associated with incident hypertension, independent of sleep duration and fragmentation, and sleep-disordered breathing. Therefore, selective deprivation of SWS may contribute to adverse BP in older men (Fung et al., 2011).

The association between poor sleep quality and a slight increase in BP is found even in adolescents. In a cross-sectional analysis of 238 adolescents, all without SAS or severe comorbidities, after adjustment for sex, body mass index, and socioeconomic status, the odds of prehypertension ($\geq$90th percentile for age, sex, and height) increased 3.5-fold for actigraphy-defined low sleep efficiency (low sleep efficiency $\leq$85%) and 2.5-fold for short sleep ($\leq$6.5 h). Adjusted analyses showed that adolescents with low sleep efficiency had on average a 4.0-mmHg higher systolic BP than other children ($P < 0.01$) (Javaheri et al., 2008).

Li and colleagues performed a study on 219 chronic insomniacs and 96 normal sleepers who underwent one night of in-laboratory PSG testing followed by a Multiple Sleep Latency Test (MSLT), which is the standard test of sleepiness/alertness. After controlling for variables, they found that insomnia combined with hyperarousal (MSLT >14 min) increased the odds of hypertension by 300% (OR 3.27) compared with the risk in normal sleepers with an MSLT $\leq$14 min. Thus physiological hyperarousal was shown to be an underlying mechanism of a higher risk of hypertension in chronic insomniacs (**Fig. 1**) (Li et al., 2015).

Poor sleep quality is significantly associated with resistance to treatment in hypertensive women. In a cross-sectional cohort study in 270 hypertensive patients (mean 57 years) consecutively recruited at the Outpatient Hypertension Unit, University of Pisa, Italy, poor sleep quality evaluated by the PSQI was shown to be an independent predictor of resistant hypertension (defined as office BP $\geq$ 140/90 mmHg with $\geq$3 antihypertensive drugs or controlled BP with $\geq$4 drugs) in women (OR 5.3), but not in men (Bruno et al., 2013).

Sleep and Circadian Rhythm

The sleep and circadian rhythm of the cardiovascular system have physiological effects on the diurnal variation of hemodynamics, and they are also thought to effect the pathophysiology of CVD.

Circadian Rhythm

The onset of CVD shows a circadian rhythm, and the incidence of CVD events such as stroke and myocardial infarction is greater in the early morning than at other times of day. BP also shows a circadian rhythm, decreasing by 10%–20% during sleep. This circadian rhythm in BP is the result of modifications in the clock gene-regulated endogenous sleep–wake rhythm with a cardiovascular response to living environment-based mental and physical activities (Kario, 2015a, 2016). Short-term BP variabilities such as morning BP surge, physical or psychological stress-induced changes in daytime BP, and nocturnal BP surge triggered by hypoxic episodes in OSAS modulate this circadian rhythm of BP, leading to individual differences in the circadian variability of 24-h ambulatory BP and cardiovascular risk (Kario, 2015b; Kario et al., 2003).

Nocturnal Blood Pressure During Sleep

Sleep is the major determinant of the nocturnal dipping status of BP. The sleep architecture (sleep stage) is closely associated with the nocturnal BP level (**Figs. 2** and **3**). Nocturnal BP gradually decreases along with the advance in non-REM sleep stages (from N1, N2, to N3) (Somers et al., 1993). Nocturnal BP surges with an exaggerated peak are frequently found during REM sleep. The basal BP, which is the lowest BP level with the minimal amplitude and frequency of BP surges, is usually found during Stage N3 (SWS) (**Fig. 2**). The basal BP is predominantly determined by the circulating blood volume and cardiovascular structure without any effect of neurohumoral activation (Kario, 2015a). In addition to the BP surges generated during REM sleep, microarousals (unconscious arousals) detected by PSG induce BP surges during non-REM sleep. At the time of apnea episodes, arousals frequently occur. Spontaneous non-SAS arousals increase with aging, and these events induce BP surge independently of apnea (**Fig. 4**). Arousals experimentally induced by auditory tones (range: 40–80 dB; duration: 0.5 s) increase BP by 15%–20% (Bangash et al., 2008).

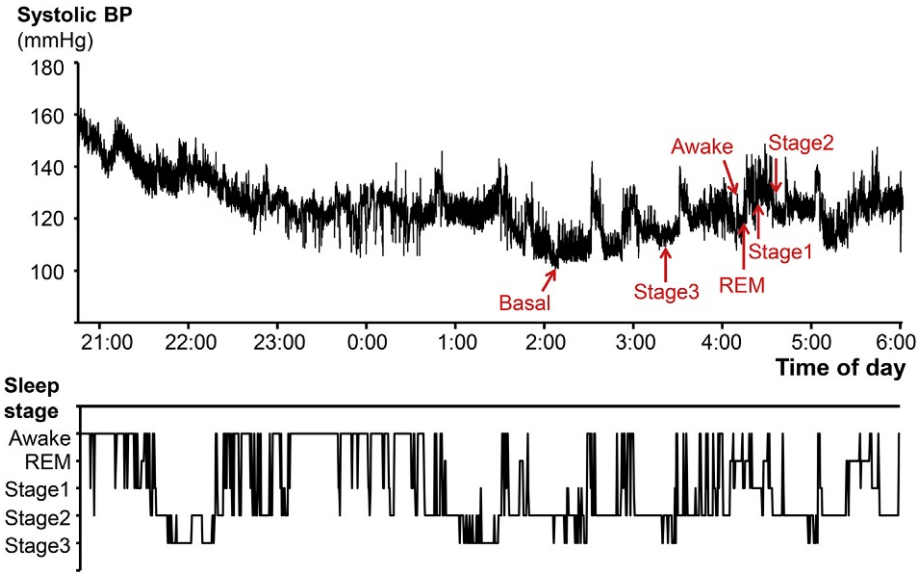

Fig. 2 Beat-by-beat sleep blood pressure continuously monitored by the newly developed tonometry-type wrist surge pressure monitoring-1 (WSP-1) system (The prototype model which is jointly developed by Omron Healthcare Co., Ltd. and Jichi Medical University School of Medicine*) in different sleep stages defined by simultaneous polysomnography. The data were obtained from 43-year-old healthy woman. *REM*, rapid-eye movement. *This research is partially supported by the Research and development of supportive device technology for medicine using ICT from Japan Agency for Medical Research and development, AMED.

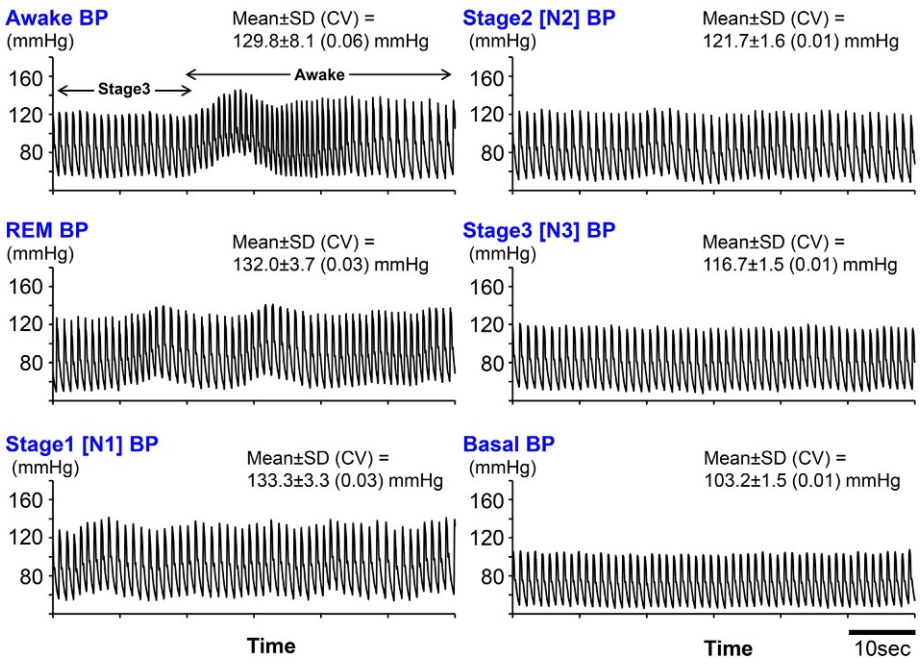

Fig. 3 Beat-by-beat short-term sleep blood pressure variability continuously monitored by the newly developed tonometry-type wrist surge pressure monitoring-1 (WSP-1) system (The prototype model which is jointly developed by Omron Healthcare Co., Ltd. and Jichi Medical University School of Medicine*) in the different sleep stages shown in **Fig. 2**. *REM*, rapid-eye movement. The blood pressure surges during awake or REM sleep are more pronounced than those during slow-wave sleep (N3). The basal BP, the lowest BP, is usually found during N3 sleep. *This research is partially supported by the Research and development of supportive device technology for medicine using ICT from Japan Agency for Medical Research and development, AMED.

Disrupted Circadian Rhythm in Blood Pressure

Disrupted circadian BP rhythm (i.e., the absence of a nocturnal-BP fall in a nondipper, or a nocturnal BP greater than daytime BP in a riser) (Fig. 5) is linked to a cardiovascular risk independent of a high BP level. Abnormality in the circadian rhythm of BP can be evaluated by 24-h ambulatory BP monitoring (ABPM) or home nocturnal BP monitoring (Kario, 2015a, 2016; Kario et al., 2015a).

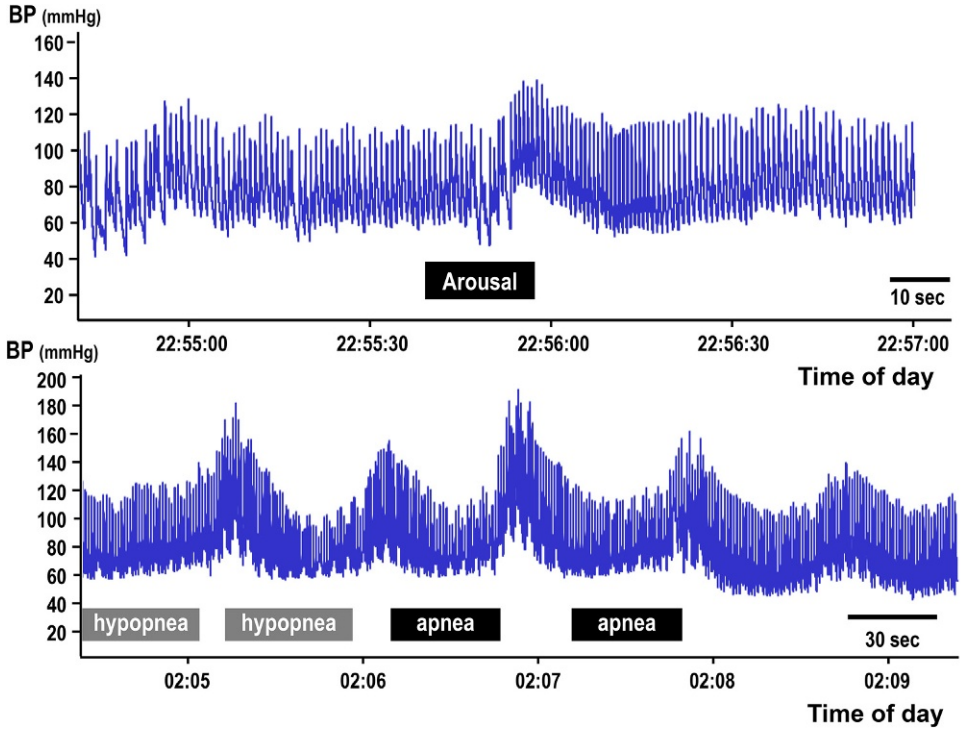

Fig. 4 Nocturnal blood pressure surges triggered by arousal (upper panel: data from 36-year-old healthy man) and apnea/hypopneas (lower panel: data from 50-year-old man with severe obstructive sleep apnea). Beat-by-beat sleep blood pressure was continuously monitored by the newly developed tonometry-type wrist surge pressure monitoring-1 (WSP-1) system (The prototype model which is jointly developed by Omron Healthcare Co., Ltd. and Jichi Medical University School of Medicine*). *This research is partially supported by the Research and development of supportive device technology for medicine using ICT from Japan Agency for Medical Research and development, AMED.

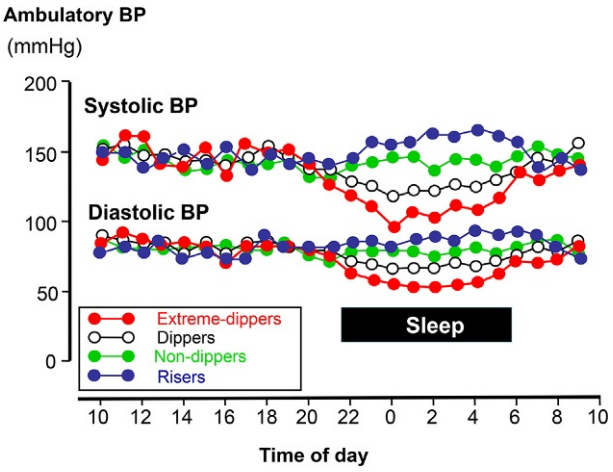

Fig. 5 Four different dipping statuses of nocturnal blood pressure in hypertension. The data shown were evaluated by ambulatory blood pressure monitoring. Reproduced from Kario K, Pickering TG, Matsuo T, Hoshide S, Schwartz JE, and Shimada K (2001) Stroke prognosis and abnormal nocturnal blood pressure falls in older hypertensives. Hypertension 38: 852–857.

Although the fall in nocturnal BP tends to disappear with aging, our ABPM studies in elderly patients with hypertension revealed that risers have a risk of developing SCI or symptomatic stroke (Kario et al., 1996, 2001). The riser pattern is an independent predictor of prognosis in HF patients with a preserved ejection fraction (Komori et al., 2017). A rise in nocturnal BP and being a nondipper/riser are also linked not only to cardiovascular risk but also to brain atrophy (Nagai et al., 2008) and decreased cognitive function/physical activity in elderly patients with hypertension (Yano et al., 2011). On the other hand, although the heart rate also decreases during nocturnal sleep, heart rate nondippers exhibit higher plasma BNP levels than heart rate dippers (Oba et al., 2017). In addition, the risk of stroke in nondippers who show neither a decrease in BP nor a decrease in heart rate during the nighttime

reached 8.9 times that in normal dippers for both parameters, and the risk increased synergistically (Kabutoya et al., 2010). In nondipper/riser subjects with a normal 24-h BP below 125/80 mmHg in a community-dwelling population, increased plasma blood BNP level and increased left ventricular mass on echocardiography were observed with progressive concentric cardiac hypertrophy (Hoshide et al., 2003). Even in normotensive young adults, disrupted circadian rhythms in BP (both a nondipper/riser pattern and an extreme-dipper pattern) are closely associated with the presence of CAC detected by coronary CT after a 10–15 year follow-up period (Viera et al., 2012). The cardiovascular risk of risers may be synergistically augmented by shorter sleep duration. In our prospective study on 1255 elderly hypertensive patients (mean 70.4 years), short sleep duration (<7.5 h (20th percentile)) was associated with incident CVD (HR 1.68), and the combination of a riser pattern in nocturnal BP and short sleep duration was most strongly predictive of future CVD (stroke, fatal, or nonfatal myocardial infarction, or sudden cardiac death) (HR 4.43), independent of ambulatory BP levels (Eguchi et al., 2008). These results indicate that disrupted circadian rhythms of BP or heart rate in relation to sleep are directly linked to hypertensive organ damage and cardiovascular events.

The BP nondipper/riser pattern can be caused by various mechanisms other than sleep disturbance, such as an increased circulating blood volume due to increased salt sensitivity, excessive salt intake, or autonomic nervous dysfunction, and there is a possibility that a clock gene abnormality and hyposecretion of melatonin are involved in these mechanisms (Kario, 2011). In particular, sleep disturbance directly modifies nocturnal BP. In a human intervention study, sleep deprivation was shown to cause nondipper nocturnal hypertension (Tochikubo et al., 1996). In addition, selective SWS deprivation cut the lowest nocturnal BP during sleep, resulting in increased nocturnal BP (Sayk et al., 2010). The patient with OSAS will become a nondipper/riser with an increased nocturnal BP, and sleep apnea episodes may induce nocturnal BP surges (**Fig. 4**) (Kario, 2009, 2015a, 2016). Exaggerated nocturnal BP surge may trigger sleep-onset cardiovascular events in OSAS patients (Kario, 2009, 2015a, 2016; Yoshida et al., 2016). Periodic leg movements induce repetitive nocturnal BP surges (on average, systolic BP 22 mmHg, diastolic BP 11 mmHg), suggesting a risk of CVD in patients with restless legs syndrome, especially in the elderly. The nocturnal BP surges were greater when the leg movements were accompanied with microarousal (Pennestri et al., 2007).

Office and Home BP Variability

In addition to diurnal BP variability, there are various other BP variabilities with different time phases, which could be detected by office and home monitorings (Kario, 2015b; Parati et al., 2013). Recent studies demonstrated that visit-to-visit office BP variability and day-by-day home BP variability are associated with cardiovascular risk independently of the average of BP levels (Rothwell et al., 2010; Johansson et al., 2012). Sleep quality is closely associated with office and home BP variability, especially with morning BP (Kario, 2015a). Uncontrolled morning hypertension is a major independent risk factor for CVD (Kario et al., 2014a, 2016a; Hoshide et al., 2015).

In our prospective study on 201 elderly individuals (80 years old) with one or more cardiovascular risks, long sleep duration and persistent insomnia were significantly associated with higher visit-to-visit BP variability (expressed as a coefficient of variation) and δ (maximum–minimum) BP based on 12 visits, long sleep duration, and persistent insomnia each had synergetic interactions with carotid artery stiffness and with visit-to-visit BP variability (Nagai et al., 2013).

Duration and quality of sleep are associated with great variability in home BP, which is known to have a cardiovascular prognostic value independently of the average of home BP levels. In the 1908 Finn-home study, adult participants aged 41–74 years, morning–evening, day-by-day (morning and evening), and first–second home BP variability variables during 7 consecutive days were significantly higher in participants with persistent insomnia than in those without insomnia. Systolic morning–evening, day-by-day, and morning day-by-day variables of home BP variability were significantly higher in long sleepers, and systolic morning day-by-day, diastolic day-by-day, and diastolic first–second measurement of home BP variability variables were higher in short sleepers than in the reference group. Insomnia combined with short sleep duration further increases home BP and heart rate variability (Johansson et al., 2011).

The Trigger Technique, a Novel Method of Nocturnal Blood Pressure Monitoring at Home

The clinical practice of sleep medicine is changing rapidly, with novel diagnostics and treatments that have established a home-based (rather than laboratory-based) management approach. The diagnostic approach for OSAS is transitioning from the gold standard of PSG in the sleep laboratory to home sleep testing (Malhotra et al., 2015). We have developed a trigger nighttime BP monitoring (TNP) method, which was based on the automated fixed interval-measurement technique with an added trigger function that initiates BP measurement when oxygen desaturation falls below a variable threshold continuously monitored by pulseoximetry (Kario, 2013, 2015a, 2016; Kario et al., 2014b; Kario and Hamasaki, 2015; Kuwabara et al., 2017). The home TNP can detect specific marked nocturnal BP surges triggered by hypoxic episodes in OSAS patients (**Fig. 6**). In addition, the heart rate-trigger function added to TNP is able to determine the "basal BP" based on the circulating volume and structural cardiovascular system without any increase in sympathetic tonus. This double-trigger, nighttime BP monitoring approach is a novel concept for evaluating the pathogenic pressor mechanism of nighttime BP. **Fig. 6** shows the nighttime BP parameters obtained from TNP (Kario et al., 2014b). In the case of nocturnal hypertension that exhibits a similar average of nighttime BPs, there may be a different mechanism: peak nighttime BPs measured by hypoxia trigger are attributed to the sympathetic overdrive, while basal nighttime BPs

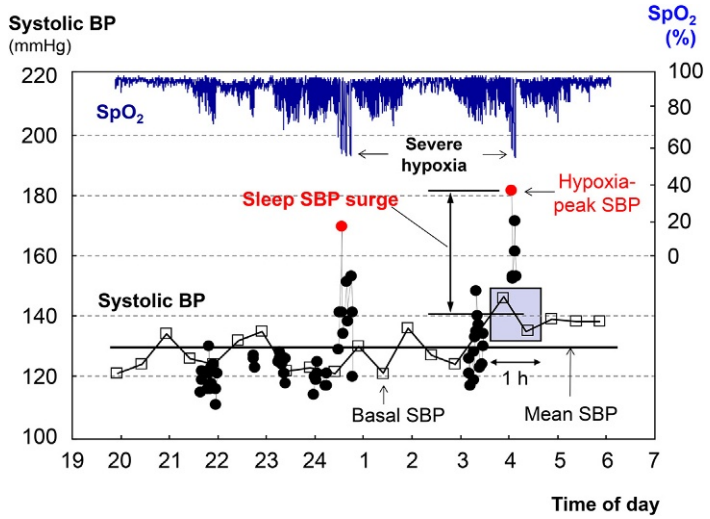

Fig. 6 Definition of nocturnal blood pressure parameters obtained by trigger nocturnal blood pressure monitoring (TNP). *BP*, blood pressure; *SBP*, systolic BP. Reproduced from Kario, K., Kuwabara, M., Hoshide, S., Nagai, M. and Shimpo, M. (2014). Effects of nighttime single-dose administration of vasodilating vs. sympatholytic antihypertensive agents on sleep blood pressure in hypertensive patients with sleep apnea syndrome. *Journal of Clinical Hypertension***16**, 459–466.

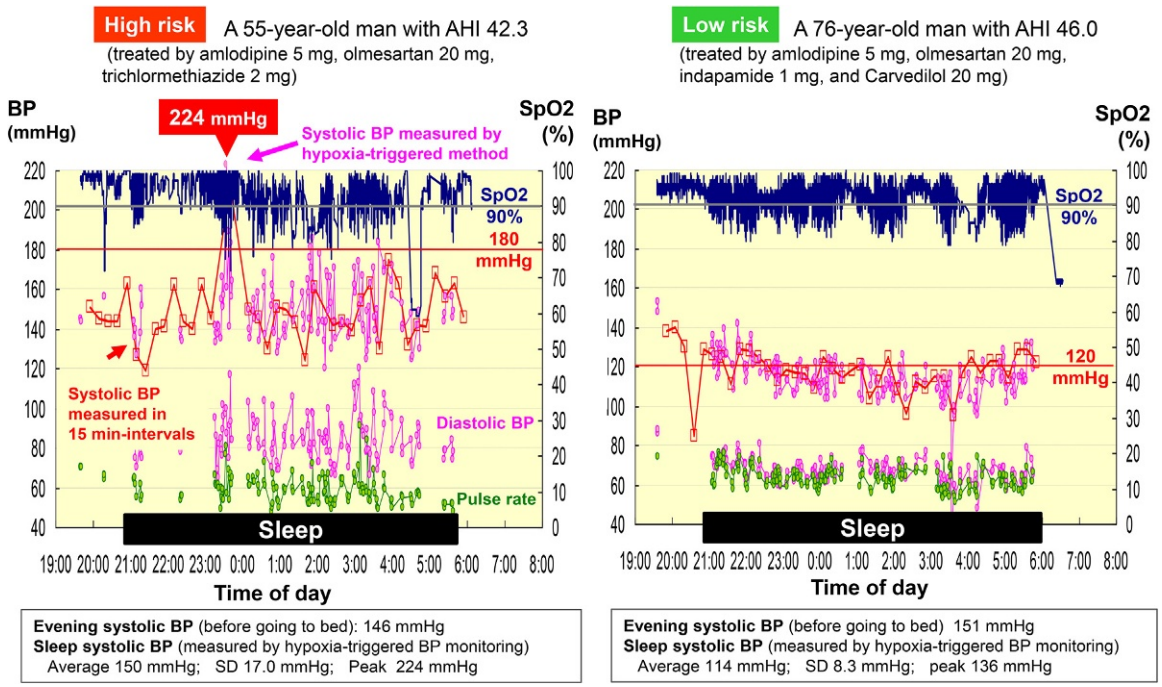

Fig. 7 Different nocturnal blood pressure surges in two patients with resistant hypertension and obstructive sleep apnea syndrome. *BP*, blood pressure; *AHI*, apnea–hypopnea index. Reproduced from Kario, K. (2013). Proposal of a new strategy for ambulatory blood pressure profile-based management of resistant hypertension in the era of renal denervation. *Hypertension Research 36*, 478–484.

measured by the lowest heart rate trigger are attributed to the circulating volume and vascular structure-related hypertension (Kario, 2015a). The former BP component of nocturnal hypertension may be suppressed by nighttime dosing of sympatholytic drugs, while the latter BP component may be suppressed by diuretics, calcium channel blocker, and/or RAS inhibitors.

The degree of nocturnal BP surge is markedly different among different OSAS patients even with similar AHI. The difference in the individual nocturnal BP surge may be partly due to the difference in altered chemoreflex control of sympathetic activity, and/or in impaired baroreflex due to vascular disease. Fig. 7 demonstrates the nighttime BPs detected by TNP in two cases with drug-resistant hypertension and SAS (Kario, 2013). Although the patients had comparable OSAS severity (as measured by the AHI), the trigger function of TNP revealed quite different nighttime BP surges between these patients. It is well known that cardiovascular events occur more frequently during sleep in patients with OSAS. The hypoxia-induced nighttime BP surge would trigger the

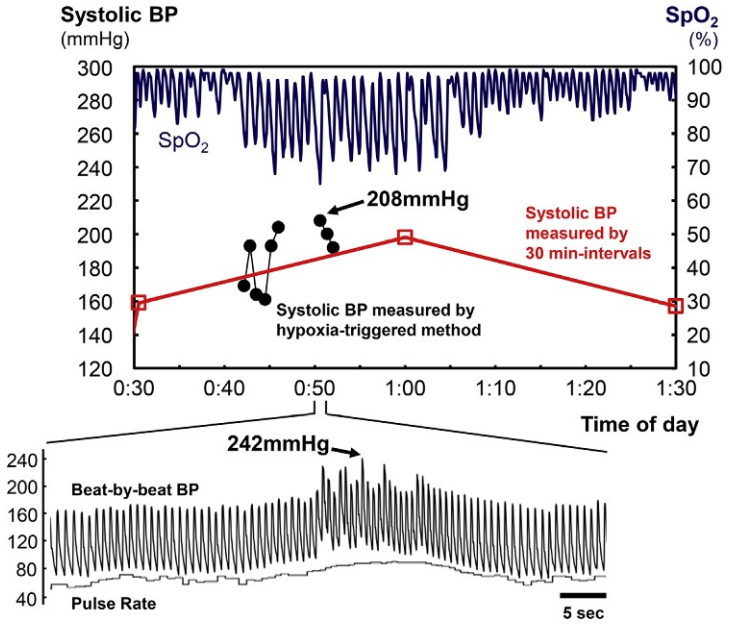

Fig. 8 Accurate absolute value of peak of sleep apnea-triggered nocturnal blood pressure surge measured by simultaneous blood pressure monitoring using both trigger nocturnal blood pressure monitoring (TNP) and beat-by-beat tonometry-type wrist surge pressure monitoring-1 (WSP-1) system (both of which monitoring are jointly developed by Omron Healthcare Co., Ltd. and Jichi Medical University School of Medicine*). Data are obtained from 53 year-old men with obstructive sleep apnea. *This research is partially supported by the Research and development of supportive device technology for medicine using ICT from Japan Agency for Medical Research and development, AMED.

sleep-onset cardiovascular events in OSAS patients. Using this TNP device, we could specifically identify the SAS patients at high-risk for sleep-onset cardiovascular events.

Finally, we have recently developed an IT-based nocturnal BP monitoring system (ITNP) with oxygen and heat-rate triggers and a 3G Web cloud computing system (Omron Healthcare Co., Ltd., Kyoto, Japan) (Kario, 2015a, 2016). The ITNP system is a cloud computing-based composite management and analysis system for the data sent from the BP device in the patient's home. This system can detect repeated and day-to-day variability in nocturnal and morning BP, and the nighttime BP surges associated with sleep apnea episodes, the degree of which can be affected by daily environmental changes. A single day PSG in the hospital under an alcohol-prohibited condition may underestimate the severity of OSAS and miss subjects with moderate OSAS. However, repeated assessment of OSAS using ITNP in a real-life setting would increase the sensitivity of the diagnosis of OSAS and related nighttime BP surge. ITNP could be used to evaluate the efficacy of continuous positive airway pressure (CPAP). ITNP is thus expected to contribute to the detection of high-risk OSAS with nocturnal hypertension and/or nighttime BP surge, and to the assessment of the quality of BP control during CPAP and/or antihypertensive treatment in OSAS patients. Simultaneous blood pressure monitoring using trigger nocturnal blood pressure monitoring (TNP) and beat-by-beat tonometry-type wrist surge pressure monitoring-1 (WSP-1) system accurately measured the highest absolute value of the peak blood pressure triggered by sleep apnea episode (Fig. 8).

Sleep Hygiene and Medication

Strict BP control over a 24-h period, including nighttime BP and hypoxia-induced peak, would effectively suppress the development of organ damage and cardiovascular events (Kario, 2015a, 2016). CPAP treatment with good adherence is effective for reducing BP, almost eliminating nighttime BP surge in patients with OSAS (Huang et al., 2015). However, in many patients with OSAS, adherence to the CPAP regimen is relatively poor over the long term. For OSAS patients with poor adherence to CPAP treatment, or those who decline to use CPAP, bedtime dosing of sympatholytic agents (Kario et al., 2014b), renal denervation (Kario et al., 2015b, 2016b,c), or vasodilators is effective to reduce nocturnal BP and hypoxia-induced nocturnal BP surge. In non-SAS patients with insomnia, hypnotics such as the GABAergic-agonist zolpidem, the melatonin MT1/MT2 receptor-selective-agonist ramelteon (Viera et al., 2012), or the orexin receptor-antagonist suvorexant have potential to reduce nocturnal BP, especially in those with drug-resistant nocturnal hypertension. However, randomized placebo-controlled clinical trials will be needed to confirm the efficacy of these and other hypnotics.

In clinical practice, the assessment of sleep disturbances, such as sleep deprivation, insomnia, and sleep apnea, and the promotion of sleep hygiene to maintain a sleep duration of >6 h and good sleep quality are essential components in the reduction of BP and cardiovascular risk.

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Sleep-Disordered Breathing and Heart Failure Interactions and Controversies

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Introduction

Every day over 2200 Americans die of cardiovascular disease (Mozaffarian et al., 2016). It has been estimated that up to 287,000 annual deaths may be attributable to heart failure (HF) (Mozaffarian et al., 2016). Sleep-disordered breathing (SDB), a term that denotes all respiratory disorders of sleep, is broadly categorized into obstructive sleep apnea (OSA) and central sleep apnea (CSA). SDB is independently associated with increased mortality and morbidity in HF patients (Khayat et al., 2012, 2015). The prevalence of SDB is estimated to exceed 60% in patients with systolic HF (heart failure with reduced left ventricular ejection fraction) (Khayat et al., 2009; Oldenburg et al., 2007). The association between SDB and increased mortality and readmission in HF patients supports the need for surveillance and expedited treatment of SDB cases in patients with known HF.

Definitions

SDB is defined by the presence of five or more abnormal respiratory events per 1 h of sleep. These respiratory events are generally divided into apneas and hypopneas (Berry et al., 2017). Apneas indicate absence of airflow defined as a period >10 s with >90% drop in oronasal thermal sensor flow (Berry et al., 2017). The oronasal sensor is placed over the mouth during a polysomnogram (PSG) or sleep study. Apneas are further differentiated into obstructive and central apneas (CA). Obstructive apneas (OA) are associated with persistent effort to breathe as measured by chest and abdomen effort belts that are ineffective in overcoming the upper airway collapse. In CA, there are absent inspiratory efforts caused by a temporary failure of the pontomedullary pacemaker (Javaheri and Dempsey, 2013). In hypopneas, there is a drop of >30% of the nasal pressure signal for that lasts longer than 10 s and there is a $\geq$4% desaturation from baseline (Berry et al., 2017).

Pathophysiology—Cause and Effect

There are multiple mechanisms of abnormal physiology in SDB that are deleterious to the HF patient including intermittent hypoxia, sympathetic overdrive, and endothelial dysfunction accompanied by oxidative stress. HF patients have instability in their respiratory control centers that can cause both obstructive and CSA (Dempsey, 2005). During both central and OA, there are periods of hypoxemia and hypercapnia that cause sympathetic activation. Particularly at the end of these apneic events, there are arousals where there are surges in the systolic and diastolic blood pressure of approximately systolic 20 mmHg and diastolic 15 mmHg (Morgan et al., 1985) accompanied by a rebound in oxygenation as patients frequently desaturate during these events. Not only are these sympathetic activations greater at night when the apneas are occurring, but also the elevated sympathetic tone persists into the waking hours (Somers et al., 1995). Stimuli from these hypoxemic and hypercapnic events change the sensitization of peripheral and central chemoreceptors triggering changes in how the body responds with sympathetic tone and ventilation (Smith and Muenter, 2000; Peng et al., 2003). Aside from considerations of oxygenation, OSA is correlated with oxidant-mediated vascular endothelial dysfunction (Varadharaj et al., 2015). This endothelial dysfunction has been linked to atherosclerosis and also felt to play a role in stroke (Varadharaj et al., 2015).

Although CSA and OSA present differently, they are both associated with a similar pattern of recurrent respiratory events and intermittent hypoxia during the night. Sympathetic activation and blood pressure surges occur similarly in these two types of SDB (Morgan et al., 1993).

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Presentation and Risk Factors of Sleep-Disordered Breathing

Patient with SDB may or may not be symptomatic upon presentation. However, symptoms may include excessive daytime sleepiness due to poor sleep quality from respiratory events interrupting sleep. Patients may also present with insomnia as the patient has frequent awakenings from respiratory events and is unable to go to sleep. Querying the bedpartner is relevant as the patient may have been witnessed to have apneas which support the presence of SDB. Snoring is an important symptom that is often reported by bedpartners. known risk factors for OSA include obesity, neck circumference >17 in. (43 cm) in men or >15 in. (38 cm) in women, narrowed airway, male gender, age, family history, alcohol use, tobacco use, and nasal congestion. Further, there are risk stratification tools that can help predict the patient's risk of OSA, such as the Flemmons score. This simple tool uses three risk factors: neck circumference in centimeter, presence of hypertension (four points), and witnessed apneas/gasping most nights (three points) (Flemons, 2002). If the cumulative score is >48, then the patient is high probability for sleep apnea, or 20 times as likely as a low risk patient (<43 cm) to have sleep apnea (Flemons, 2002). CSA, on the other hand, does not have defining symptoms but patients are typically older, have worse cardiac function, and tend to be predominantly males (Oldenburg et al., 2007).

Diagnostic Testing

The diagnosis of SDB is best performed by an attended or in-lab PSG. In the sleep laboratory, electroencephalogram, limb movements, electrocardiogram, and several respiratory parameters are simultaneously recorded during sleep for one night. The main advantage is being able to tell when the patient is definitively asleep and have a technologist available in the lab monitoring data acquisition to be able to fix any issues that arise overnight as well as treat the patient with positive airway pressure if their respiratory events are significant. However, the field is continually moving to more inexpensive and convenient portable home sleep studies, often referred to as unattended studies. Depending on the manufacturer, these can collect any number of variables but often have a chest best, and abdominal belt, as well as a nasal pressure sensor and a continuous pulse oximeter. The unattended sleep study is best reserved for those patients that are considered by risk factors to be high risk for OSA. As well, since the test does not have good negative predictive value, it is recommended that those at high risk for OSA and a negative unattended study have an attended PSG if their home study is negative.

Treatment of SDB Including OSA

Treatment of SDB improves a range of parameters related to HF including a reduction in sympathetic outflow, improvement in blood pressure control, decrease in morbidity from CAD, and is also associated with the nocturnal timing of sudden cardiac death. Regarding hypertension, in a trial of 194 patients receiving continuous positive airway pressure (CPAP) or no CPAP with a mean AHI of 40 on a mean of 3.8 antihypertensive drugs/patient, CPAP treatment for 12 weeks achieved a greater decrease in 24-h mean blood pressure (3.1 mmHg [95% CI, 0.6–5.6]; $P=0.02$) and 24-h diastolic blood pressure (3.2 mmHg [95% CI, 1.0–5.4]; $P=0.005$). However, 24-h systolic blood pressure was not significantly changed (3.1 mmHg [95% CI, −0.6 to 6.7]; $P=0.10$) compared with the control group (Martinez-Garcia et al., 2013). From an arrhythmia perspective, the prevalence of atrial fibrillation is three times higher in patients with OSA than in normal controls (Guilleminault et al., 1983). In a meta-analysis, treatment with CPAP resulted in 42% decreased risk of atrial fibrillation (Qureshi et al., 2015). There is also a beneficial effect on the treatment of SDB on CAD. The treatment of SDB has also been shown to reduce to improve the morbidity of patients with coronary artery disease. The incidence of CAD is higher in patients with OSA, and in one study this was a fivefold increase in risk even when controlling for other variables (Peker et al., 2006). Men in the Sleep Heart Health Study from 40 to 70 years of age with an AHI of ≥30 were 68% more likely to develop CAD than those without OSA (Gottlieb et al., 2010). Further, in patients with OSA treated with CPAP, their risk of developing CAD was reduced (Peker et al., 2006). (Ongoing studies for this NCT01335087, NCT00519597.) There is also a relationship between SDB and sudden cardiac death. In a retrospective review of patients with prior PSG that had suffered sudden cardiac death, it was observed that patients with OSA have peak deaths during the early morning hours between midnight and 6 a.m., whereas otherwise these sudden cardiac deaths occur around 6 a.m. in patients without OSA (Gami et al., 2005).

Treatment of OSA and CSA Including Controversies in Treatment of CSA

The treatments of OSA differ based on the severity of the OSA. For mild OSA, behavioral modifications like weight loss and positional therapy may be effective. In positional therapy, patients are kept out of the supine position in which most of their respiratory events occur. CPAP maintains the patency of the airway during sleep eliminating most of the apneas and hypopneas of OSA. CPAP is the treatment of choice for patients with more moderate to severe OSA (those who have more than 15 events per hour of sleep). Also, patients with milder cases (<15 events per hour) but who have sleepiness can benefit from CPAP. Sleepiness is often

quantified using questionnaires like the Epworth Sleepiness Scale. Also, for symptomatic patients with less severe cases (<15 events per hour), non-CPAP therapies like mandibular advancement using oral appliances can be considered (Epstein et al., 2009).

The treatment of CSA is slightly different from that of OSA. First, the prevalence of CSA is less than that of OSA in the general population. However, in a HF population, there is up to 40% prevalence of CSA (Oldenburg et al., 2007). Cheyne–Stokes breathing is a crescendo–decrescendo breathing style which is seen in patients with HF during sleep. There are CA between these periods of crescendo–decrescendo breathing. It can also be seen while the patient is awake and during periods of exercise (Poletti et al., 2009). Cheyne–Stokes breathing has been associated with higher levels of serum cathecholamines as well as correlates with worse NHYA functional status, and NT-proBNP levels (Poletti et al., 2009). The treatment of choice for OSA in HF patients is CPAP. However, in patients with CSA, CPAP partially treats the abnormality (Javaheri, 2000). It has previously been shown that by adding ASV (adaptive servo ventilation) to patients with HF and CSA incompletely treated with CPAP that both the AHI was improved as well as there were lower amounts of urinary cathecholamines (Kasai et al., 2013). Further, the group that received ASV had an improvement in ejection fraction compared to patients treated with CPAP alone (Kasai et al., 2013). Treatment of CSA in HF is based on CANPAP—The Canadian Continuous Positive Airway Pressure Trial for Congestive Heart Failure (Bradley et al., 2005). CANPAP showed that although CPAP decreased the severity of CSA, increased ejection fraction, improved nocturnal oxygenation, and increased 6 min walk distance, it did not affect the survival of patients enrolled (Bradley et al., 2005). ASV then came in vogue as being the treatment of choice for those with CSA and Cheyne–stokes respirations based on a small study of 25 patients with stable congestive HF that were treated with CPAP or ASV (Philippe et al., 2006). ASV was observed in this study to more fully eradicate SDB events and was the only intervention able to get the AHI to $<10\ h^{-1}$. ASV also showed a significant increase in LVEF and an improvement in quality of life (Philippe et al., 2006).

Recently, a study evaluated 2717 patients with moderate to severe OSA with known coronary or cerebrovascular disease to receive both standard care or CPAP treatment and standard care. Treatment with CPAP for a mean follow-up of 3.7 years did not prevent cardiovascular or cerebrovascular events compared with standard therapy (McEvoy et al., 2016). Recently, the results of this study were overturned by the much larger SERVE-HF study which evaluated 1325 patients with systolic HF and mild and moderate sleep apnea. This trial showed that the use of ASV in HF not only did not improve LVEF, also was associated with increased cardiovascular and all-cause mortality (Cowie et al., 2015).

SDB and Cardiac Surgery

Sleep apnea evaluation prior to cardiac surgery has also been recognized as being increasingly important as recent studies that those with OSA are independently associated with longer postoperative recovery, higher use of postoperative pacemaker use, and higher rate of respiratory insufficiency based on recent data (Ding et al., 2016). The dataset used for this most recent investigation consisted of 290 patients in a single Chinese center, which overturned the suggestion in smaller prior studies that there were not adverse outcomes in those receiving cardiac surgery with SDB, showed a prevalence of OSA in 16.2% of patients and CSA in 22.7% of patients (Ding et al., 2013). Interestingly, those with predominantly CSA had a higher first dose of dobutamine and a higher rate of pacemaker use postoperatively. It remains unclear if intervening by treating the SDB can improve the outcomes in this population.

Summary

SDB is highly prevalent comorbidity in patients with cardiovascular disease. In particular, HF patients have the highest prevalence of SDB. Identification and treatment of SDB cases in these patients can be associated with improved outcome. Treatment of SDB using CPAP is highly effective and well tolerated. Treatment of SDB is associated with significant functional improvement, including resolution of sleepiness and improved alertness.

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Socioeconomic Factors and CVD

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Introduction

There are numerous studies that have examined the relationship between socioeconomic status (SES) and cardiovascular mortality. Strong associations between low SES and poor health have been observed in many studies (Kaplan and Keil, 1993; Albert et al., 2006; Havranek et al., 2015; Danaei et al., 2010; Lynch et al., 1996). The term "socioeconomic status" includes various measures such as education, income, occupation, and living conditions. The Whitehall study clarified that the lower the job status, the higher the prevalence of coronary heart disease (Marmot et al., 1978). Furthermore, the difference in ischemic heart disease (IHD) prevalence was threefold between the lowest and highest levels of SESs (Gonzalez et al., 1998). Other studies also clarified the inverse relationship between SES and IHD, especially among men in the Western world (Andersen et al., 2003). The main reasons for this phenomenon are differences in lifestyles and exposure to hazardous environments among the social classes (Kaplan and Keil, 1993). Previous studies have shown that health-related behaviors are strongly shaped by social factors such as income, education, and employment (Boniface et al., 2001; Wardle et al., 2002; Laaksonen et al., 2005; Hanson and Chen, 2007). Jemal et al. studied the 2001 US death data and concluded that "potentially avoidable factors associated with lower educational status account for almost half of all deaths among working-age adults in the U.S." (Jemal et al., 2001).

Causal pathways that link SES to cardiovascular diseases (CVS) have been studied extensively (**Fig. 1**). The high prevalence of CVD associated with low SES can be explained by conventional risk factors such as high blood pressure, diabetes, or poor cholesterol profiles through unhealthy lifestyles (e.g., smoking, low physical inactivity, excessive alcohol, etc.). Furthermore, socioeconomic disadvantages may adversely affect cardiovascular risk through its impact on mental health. A vast body of literature has documented associations between emotional states and CVD risk. In particular, depression and elevated depressive symptoms are associated with an increased risk of morbidity, adverse cardiovascular outcomes, and mortality among patients with CVD, even after controlling the other risk factors (Lynch et al., 1996; Owen et al., 2003; Petersen et al., 2008; Jousilahti et al., 2003).

Recently, in addition to the behavioral and psychological pathways, particular attention has been given to the pathophysiological conditions that link stress from the SES to the cardiovascular system. Psychological stress elicits measurable changes in the sympathetic-parasympathetic balance and the tone of the hypothalamic-pituitary-adrenal axis and may negatively affect the cardiovascular system both acutely (by precipitating myocardial infarction, left-ventricular dysfunction, or severe arrhythmia) and chronically (by accelerating the atherosclerotic process) (Brotman et al., 2007). In addition to these processes, inflammation also plays a crucial role in the pathogenesis of atherosclerotic heart disease. We will review two commonly proposed pathways of SES's effect on CVD development: the pathway through lifestyle-related diseases (which includes behavioral and environmental issues) and the pathway via pathophysiological factors (a fairly direct pathway to CVD development from SES as the stressor).

SES and CVD via Lifestyle-Related Diseases (Conventional Risk Factors)

Socioeconomically disadvantaged populations suffer from a variety of Framingham risk factors (Daviglus et al., 2012; Safford et al., 2012; Yusuf et al., 2004). Individuals with a low SES are more likely to be exposed to hazardous environments and tend to engage in unhealthy personal behaviors. Smoking, poor diet, inactivity, obesity, and poor adherence to medication tend to be more prevalent among individuals of low SES, which is observed in many studies such as in the Whitehall study where obesity was more prevalent among individuals with low-status jobs (Havranek et al., 2015; Marmot et al., 1978; Mullie et al., 2010; Giskes et al., 2010; Gilman et al., 2003). In addition, low neighborhood SES was related to a higher incidence of myocardial infarction and heart disease mortality (Chaix et al., 2007). This data implies that socially disadvantaged individuals face environmental stressors, such as poor working conditions or neighborhoods, which, coupled with the lack of knowledge and limited resources, drive these individuals to low-cost, high-calorie fast-food options, resulting in obesity, metabolic syndromes, and hypertension, which are CVD risk factors (Roux, 2001; Chow et al., 2009). Furthermore, smoking was the risk factor that varied the most among the job categories for increased morbidity and mortality, and the prevalence of smoking was also high among the disadvantaged groups compared to the high income and education groups (Laaksonen et al., 2005; Hiscock et al., 2012). However, effective interventions for the lower socioeconomic groups are sparse, while discrepancies in smoking and diabetes worsened in these 30 years in the United States

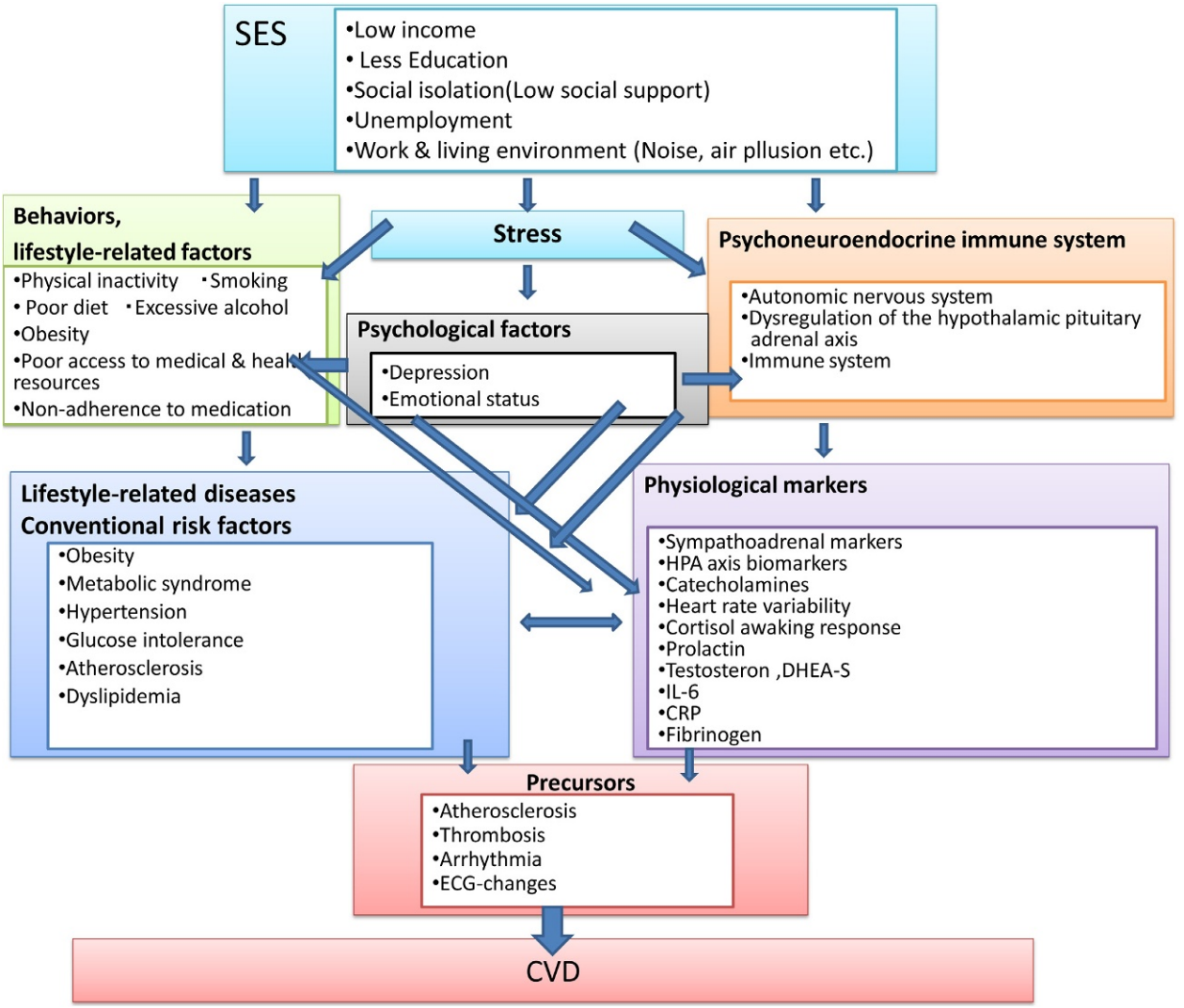

Fig. 1 Pathways to CVD from SES.

(Hiscock et al., 2012; Kanjilal et al., 2006). For individuals with a low SES, the incidence of smoking is twice as high, and the attempts to quit are less likely to be successful. Studies have suggested that this may be the result of reduced social support for quitting, low motivation to quit, stronger addiction to tobacco, increased likelihood of not completing courses in pharmacotherapy or behavioral support sessions, psychological differences, such as lack of self-efficacy, and marketing influences from the tobacco industry (Hiscock et al., 2012).

SES and CVD via the Pathophysiological Pathway From Stress

Based on previous studies, differences in smoking status, obesity, physical activity, blood pressure, and plasma cholesterol level can only partly explain differences in mortality rates due to CVD and imply that there are other aspects beyond the established CVD risk factors (Braveman and Gottlieb, 2014). SES influences psychological and physical experiences and is linked to the function of the limbic system and stress response. A substantial amount of literature has documented the relationship between adverse social position, life stress, low rank in social hierarchies, racism, and disadvantaged living and working conditions and the function of the limbic system and stress response. Exposure to these stressors may directly cause the disease (Brotman et al., 2007; Maria Glymour et al., 2014). Several studies have investigated the relationship among biomarkers, SES, and CVD. For example, the study in Finland clarified the relationship among SES, CRP, serum amyloid A, and fibrinogen levels, where mean levels of these inflammatory markers decreased significantly with increasing SES (Jousilahti et al., 2003). It was also evaluated in the Whitehall II cohort participants, which found high CRP levels were associated with low SES (Owen et al., 2003). Petersen et al. (2008) found that the midlife adults living in less advantaged neighborhoods exhibit higher levels of circulating proinflammatory markers, both IL-6

and CRP, than residents of more affluent areas. The sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis activation are associated with platelet activation, endothelial dysfunction, hypertension, suppression of the immune system, inflammation, and changes in cardiac cellular electrophysiology. Repeated exposure to stressors associated with socioeconomically disadvantageous factors has been hypothesized to eventually overwhelm the body's ability to recover from threatening episodes and lead to the accumulation of an "allostatic load" that increases CVD risk. An allostatic load is a multicomponent construct that reflects the physiological changes across different biological regulatory systems in response to chronic social and environmental stress (Seeman et al., 2010; McEwen and Seeman, 1999; McEwen, 2004). Examples include the induction of proinflammatory responses, such as the production of IL-6 and CRP, due to stress, and the effects of lower income and educational achievements that lead to the direct or the eventual contribution (i.e., through hypertension and poor cholesterol profiles) to CVD.

Perspectives and Recommendations for Preventing CVD by SES

Despite considerable evidence indicating the significant effects of socioeconomic factors on the development of CVD, not every individual exposed to low SES develops the disease. Protective social factors, such as social support, self-esteem, and self-efficacy, may mitigate the deleterious effects of adverse social conditions (Matthews et al., 2010). Income may have less of a health impact when there is less social stigma associated with having limited economic means. Health behaviors may also mediate the link between adverse social environments and cardiovascular risk. Social capital factors, such as social support and cohesion, are associated with health behaviors and CVD risk (e.g., obesity and hypertension) and vary across the socioeconomic spectrum (Havranek et al., 2015; Sundquist et al., 2006; Gallo et al., 2009). In recent years, income disparities are increasing in Japan as well, and there have been concerns regarding the widening health inequalities due to socioeconomic conditions (Kagamimori et al., 2009). Moreover, there is evidence that regardless of the individual's income, living in an area with considerable income disparities raises health risks. To improve these disparities, the Ministry of Health, Labour and Welfare in Japan is promoting "The second term of the National Health Promotion Movement in the twenty-first century (Health Japan 21 (the second term))" from the 2013 to the 2022 fiscal year (Japan, 2011). The goals in implementing the National Health Promotion are to "extend the healthy life expectancy and to reduce health disparities." Furthermore, the development of social capitals in the community has been recommended to reduce health inequalities and extend the healthy life expectancy (Japan, 2011; Kabayama et al., 2014).

Approaches to narrow these disparities should be promoted and implemented in the regional health plans of local governments as well as in the occupational safety, education, and health activities of all ages. Medical and health professionals are expected to cooperate in and support these initiatives from their professional standpoints. Future studies are required to further investigate the associations among neighborhood characteristics, health behaviors, and CVD risk to establish a population-based prevention strategy for the lower SES groups.

Conclusion

We systematically reviewed the literature that highlighted the importance of socioeconomic factors on the development of CVD as well as plausible pathways and mechanisms that may explain their effects. Numerous studies have shown the crucial relationship between socioeconomic factors and CVD. Furthermore, due to the involvement of multiple mechanisms, some may only manifest decades after exposure (Braveman and Gottlieb, 2014). Studies have also indicated that medical care is not enough to prevent CVD, suggesting the importance of individual's SES improvement and narrowing the health disparities. These results highlight the need for strategic approaches to prevent CVD, which focus on the equity of social and physical environments, including psychosocial and physical stress, and social isolation, with population- wide and high-risk interventions (Bell et al., 2012; Clark et al., 2009).

Since social conditions are difficult to change and may take months, years, or decades to modify substantially, public health practitioners can be key resources for local and national policy makers to resolve the crucial issue of health equity (Braveman and Gottlieb, 2014). Through clinical and public health interventions, we need to advance these understandings and overcome challenges to prevent CVD.

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Stage A Heart Failure: Identification and Management of Heart Failure Risk Factors

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Introduction

Heart failure (HF) is an important and growing public health problem with a prevalence in the United States that is expected to increase from 6.5 million adults currently to 8 million by 2030 (Benjamin et al., 2017). Indeed, HF affects the health of many individuals; approximately one in five persons will develop HF during their lifetime (Lloyd-Jones et al., 2002), and HF is listed as a cause of death on one in eight death certificates (Benjamin et al., 2017). The cost to the US health-care system is similarly staggering with an estimated annual cost of ~30 billion dollars (Benjamin et al., 2017). Recognizing the substantial population, individual, and financial burden of HF, recent US (Yancy et al., 2013; Schocken et al., 2008) and international (Ponikowski et al., 2016) HF guidelines have emphasized the importance of disease prevention.

HF is a progressive condition in which risk factors lead to adverse cardiac structural and functional remodeling and eventually to overt congestive HF (Fig. 1). This sequence of events is represented by the American College of Cardiology (ACC)/American Heart Association (AHA) HF stages (Hunt et al., 2001), which were designed to emphasize the progressive nature of HF development. Stage A represents individuals who are at "high risk" for HF but do not have structural heart disease or symptoms of HF, stage B characterizes individuals with structural heart disease but without signs or symptoms of HF, stage C denotes individuals with structural heart disease and current or prior HF symptoms, and stage D is refractory HF (Hunt et al., 2001). This schematic of HF progression highlights the importance of disease prevention as two of the four ACC/AHA HF stages consist of individuals free of symptomatic HF. Hence, the ACC/AHA HF stages highlight that prevention of HF must occur both when risk factors are identified (stage A) and after these risk factors have led to adverse cardiac changes (stage B) in order to prevent overt congestive HF (stage C).

In the following article, we will review the risk factors that comprise stage A HF, summarize the evidence linking these risk factors with incident HF, and evaluate the available data supporting the treatment of these risk factors to prevent new-onset HF.

Prevalence of Stage A HF

Stage A HF is defined by the presence of HF risk factors, several of which are extremely common in the general population. Estimates of the prevalence of stage A HF vary according to the population studied but range from ~33% in a sample from National Health

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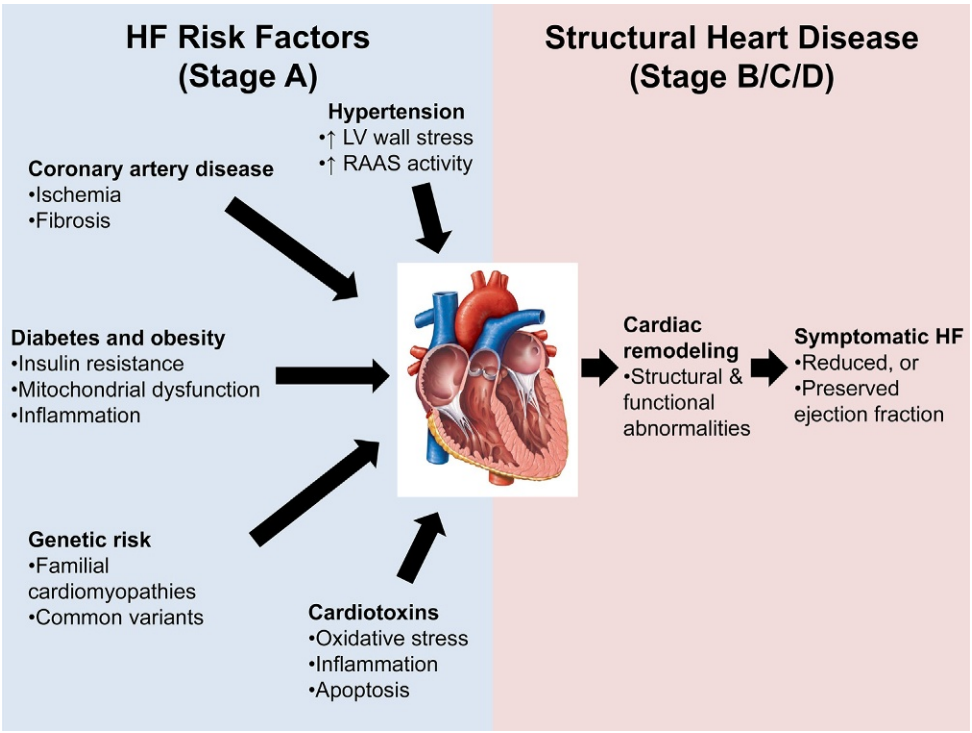


Fig. 1 Schematic representation of heart failure progression.

and Nutritional Examination Survey (NHANES) with a mean age of 47 years (Kovell et al., 2015) to 52% in a sample of older adults ranging in age from 67 to 91 years from the Atherosclerosis Risk in Communities Study (Shah et al., 2017).

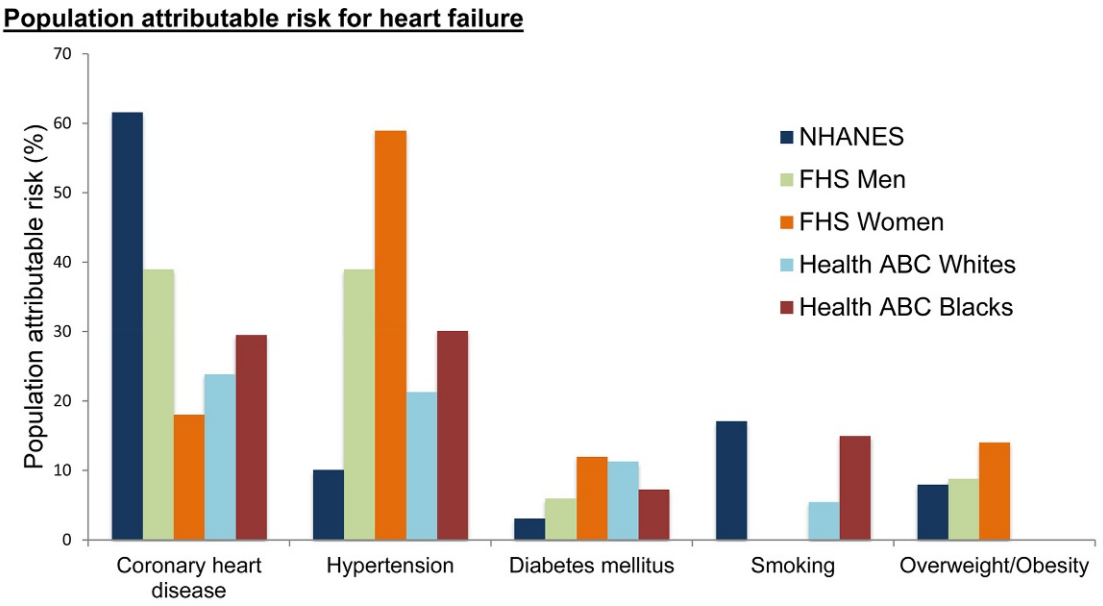
The population attributable risk for HF risk factors has been evaluated in several community-based samples (Dunlay et al., 2009; He et al., 2001; Levy et al., 1996; Kalogeropoulos et al., 2009). While the specific attributable risk estimates vary by sex, race, and cohort characteristics, coronary heart disease (CHD), hypertension, obesity, diabetes, and smoking are consistently demonstrated to be responsible for a majority of incident HF cases (Fig. 2).

Hypertension

Hypertension is a key modifiable risk factor for developing HF. In the Framingham Heart Study (FHS), 91% of individuals with HF had antecedent hypertension (Levy et al., 1996). Indeed, the lifetime risk of HF is largely driven by the burden of hypertension; across age groups, the risk of HF is approximately twofold for individuals with systolic blood pressure $\geq$160 mmHg or diastolic blood pressure $\geq$100 mmHg versus those with blood pressures of $<$140/$<$90 mmHg (Lloyd-Jones et al., 2002). The robust association of hypertension with new-onset HF is consistent across racial and ethnic groups (He et al., 2001; Huffman et al., 2013). In fact, the impact of hypertension may be especially pronounced in African Americans (Bahrami et al., 2008) in whom hypertension is more prevalent, and achievement of treatment targets is less common (Bibbins-Domingo et al., 2009). Hypertension is responsible for a higher population attributable risk in women versus men (Fig. 2; Levy et al., 1996). Investigations of lifetime hypertension exposures suggest that the chronicity (Lee et al., 2007) and severity (Levy et al., 1996) of hypertension are important considerations in estimating future HF risk.

The results from a number of clinical trials have proved that medical therapy for hypertension reduces the risk of developing HF. In a meta-analysis of several trials, treatment of hypertension was observed to reduce the relative risk of HF (40%–46%) more than other cardiovascular outcomes (Psaty et al., 2003). In the recent systolic blood pressure intervention trial (SPRINT), individuals at high risk for cardiovascular disease experienced a 38% relative risk reduction for incident HF with intensive (target systolic blood pressure $<$120 mmHg) versus standard (target systolic blood pressure $<$140 mmHg) antihypertensive treatment (The SPRINT Research Group, 2015). Again, the relative risk reduction for HF was higher than that observed for other cardiovascular outcomes including myocardial infarction and stroke.

The choice of medication to achieve blood pressure lowering does appear to impact the effectiveness of drug treatment. In the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT), blood pressure lowering with doxazosin was associated with an increased risk of HF, while amlodipine, lisinopril, and chlorthalidone were associated with similar reductions in risk (ALLHAT Collaborative Research Group, 2000). One potential explanation for the pronounced effect of antihypertensive treatment on HF risk is that diuretics were used as antihypertensives in many of these studies (including SPRINT) (Psaty et al., 2003;



Multivariable-adjusted relative risk of heart failure

Risk Factor	NHANES	FHS Men	FHS Women	Health ABC Whites	Health ABC Blacks
Coronary heart disease	8.11 (6.95-9.46)			2.72 (1.89-3.90)	3.31 (2.26-4.85)
Myocardial infarction		6.34 (4.61-8.72)	6.01 (4.37-8.28)		
Hypertension	1.40 (1.24-1.59)	2.07 (1.34-3.20)	3.35 (1.67-6.73)	1.80 (1.27-2.55)	1.95 (1.33-2.84)
Diabetes mellitus	1.85 (1.51-2.28)	1.82 (1.28-2.58)	3.73 (2.71-5.15)	2.08 (1.35-3.22)	1.37 (0.88-2.14)
Smoking	1.59 (1.39-1.83)			2.04 (1.15-3.64)	2.08 (1.37-3.16)
Overweight	1.30 (1.12-1.52)	1.68 (1.25-2.27)	1.17 (0.86-1.61)		

Fig. 2 Population attributable and relative risks of select heart failure risk factors. (Adapted from Shenoy, M., Chapman, C., Nawaz, Z. and Sweitzer, K. (2006). Diagnosis and management of stage A heart failure. *Congestive Heart Failure* **12** (3), 146–152.) Population attributable risks for heart failure are displayed for individuals from National Health and Nutrition Examination Survey (NHANES; data collection 1982–92), Framingham Heart Study (FHS; data collection 1970–91); Health, Aging, and Body Composition Study (Health ABC Study; data collection from 1997 to approximately 2005). Information included from the following sources: He, J., Ogden, L. G., Bazzano, L. A., et al. (2001). Risk factors for congestive heart failure in US men and women: NHANES I epidemiologic follow-up study. *Archives of Internal Medicine*, **161** (7), 996–1002; Levy, D., Larson, M. G., Vasan, R. S., et al. (1996). The progression from hypertension to congestive heart failure. *JAMA* **275** (20), 1557–1562; Kalogeropoulos, A., Georgiopoulou, V., Kritchevsky, S. B., et al. (2009). Epidemiology of incident heart failure in a contemporary elderly cohort: the health, aging, and body composition study. *Archives of Internal Medicine* **169** (7), 708–715.

The SPRINT Research Group, 2015). Instead of preventing the development of HF, diuretics may simply delay the diagnosis of HF by preventing fluid retention. Diuretics may thus serve to delay phenotype detection without altering the disease course (Kjeldsen et al., 2016). Nevertheless, there is robust evidence to support the treatment of hypertension to prevent the development of symptomatic HF, while the optimal medications and blood pressure targets continue to be debated.

Atherosclerotic Vascular Disease

Atherosclerotic vascular disease contributes to a significant proportion of HF cases and is responsible for the largest population attributable risk among the different risk factors in several studies (Fig. 2). In NHANES, CHD accounted for 61.6% of HF cases (He et al., 2001). The population attributable risk estimates are somewhat lower in other cohorts, such as the FHS, where CHD accounted for 39% of HF cases in men and 18% in women (Lloyd-Jones et al., 2002). The observation that CHD accounts for a higher proportion of HF cases among men versus women is consistent across cohorts (He et al., 2001; Levy et al., 1996; Dunlay et al., 2009). Furthermore, the risk of HF in individuals with CHD is quite high with ~36% of individuals in Rochester Minnesota with CHD from 1979 to 1994 eventually developing HF (Hellermann et al., 2003). While the incidence of future HF in individuals with CHD is likely to be lower in more contemporary populations with higher rates of early revascularization, CHD and myocardial infarction continue to be among the dominant causes of new-onset HF in cohorts with more recent exposures (Kalogeropoulos et al., 2009). CHD causes myocardial damage and cell death, which leads to scar formation, ventricular remodeling, and eventually overt HF. However, atherosclerotic vascular diseases that do not directly affect the heart, such as peripheral arterial disease, have also

been associated with future HF risk (Gupta et al., 2014). While the mechanisms for this association are less direct, vascular dysfunctions, such as increased vascular stiffness and altered ventricular–arterial coupling, are likely to partially explain these relations.

Evidence from clinical trials suggests that treatment of individuals with CHD can prevent the future development of HF. In the Scandinavian Simvastatin Survival Study (4S), individuals with established CHD were randomized to treatment with simvastatin versus placebo. Over a mean follow-up of 5 years, a 21% reduction in incident HF was observed for individuals randomized to simvastatin treatment (Kjekshus et al., 1997). Similarly, in the treating to new targets trial, there was lower incidence of HF hospitalization with high-dose versus low-dose atorvastatin (LaRosa et al., 2005). Significant reductions in HF hospitalizations were also observed with the combination of clopidogrel and aspirin versus aspirin alone after non-ST elevation myocardial infarction in the clopidogrel in unstable angina to prevent recurrent events trial (Yusuf et al., 2001). Hence, secondary prevention of CHD with lipid-lowering therapy or intensification of antiplatelet therapy results in reduced incident HF events. In the setting of an acute myocardial infarction, early revascularization, which has become the standard of care over the last decades, results in less myocardial injury and a lower risk of developing HF (Lystash et al., 1995). Further improvements in primary and secondary prevention of atherosclerotic vascular disease would therefore also be expected to reduce the burden of HF.

Diabetes Mellitus

Diabetes mellitus (Kannel et al., 1974; He et al., 2001; Chen et al., 1999) and insulin resistance (Ingelsson et al., 2005) have long been known to increase the risk of HF. The presence of diabetes is estimated to increase the risk of developing new-onset HF fourfold (Nichols et al., 2004). A linear association between glycemic control and HF risk has been described (Iribarren et al., 2001), and the association of diabetes and incident HF persists after accounting for CHD (Kannel et al., 1974). Dysglycemia is theorized to lead to overt HF through its detrimental effects on cardiac structure and function (Devereux et al., 2000; Rutter et al., 2003; Battiprolu et al., 2013) and through decrements in endothelial and vascular function.

The interaction between medical treatment of diabetes and HF risk has proved complicated. Observational data do support enhanced cardiac structure and function with improvement of cardiometabolic traits (von Bibra, 2004). However, better glycemic control has not consistently resulted in lower HF risk. Indeed, most randomized clinical trials have failed to demonstrate improved cardiovascular outcomes with tighter glycemic control in type 2 diabetes mellitus (Patel et al., 2008; Duckworth et al., 2009; Action to Control Cardiovascular Risk in Diabetes Study Group, 2008). Furthermore, different drug classes appear to have differential effects on HF risk in diabetics (Hippisley-Cox and Coupland, 2016). This observation has become increasingly relevant as a number of recently developed glucose-lowering agents, such as the thiazolidinediones (Komajda et al., 2010; Fitchett et al., 2016b) and dipeptidyl peptidase-4 inhibitors (Fitchett et al., 2016b; Scirica et al., 2014), have been found to increase this risk of HF. On the other hand, in the EMPA-REG OUTCOME trial, empagliflozin (an inhibitor of sodium–glucose cotransporter 2) led to a 35% relative risk reduction in HF hospitalizations in individuals at high cardiovascular risk, regardless of the presence of HF at baseline (Fitchett et al., 2016a; Zinman et al., 2015). The diuretic effect of empagliflozin, via glycosuria, is theorized to be one of the primary mechanisms for the robust effect on HF outcomes with empagliflozin. Whether this reduction in HF events is a class effect observed for other inhibitors of the sodium–glucose cotransporter 2 will be determined after the publication of two ongoing trials (Fitchett et al., 2016b).

In addition to treating hyperglycemia, addressing comorbidities is an important component of cardiovascular risk reduction in type 2 diabetes mellitus. In particular, the use of angiotensin receptor blockers in patients with proteinuria resulted in a 32% relative risk reduction for first HF hospitalizations in the RENAAL and losartan intervention for endpoint reduction studies (Brenner et al., 2001; Lindholm et al., 2002). Angiotensin converting enzyme inhibitors or angiotensin receptor blockers should therefore be considered in all patients with type 2 diabetes and proteinuria to reduce the risk of developing HF.

Obesity

Obesity is directly and robustly related to HF risk. In the FHS, each one-point higher BMI was associated with 5% and 7% higher risk for HF in men and women, respectively (Kenchaiah et al., 2002). Obesity is also known to be associated with adverse cardiac structural and functional remodeling (Bello et al., 2016; Cheng et al., 2010). Despite the strong evidence that obesity is associated with incident HF and potentially causes it (Hagg et al., 2015), the effect of weight-loss interventions on HF risk remains uncertain. As weight loss in patients with established (stage C) HF has been reported to paradoxically lead to worse outcomes (Zamora et al., 2016), the impact of weight loss on the development and progression of HF requires further study.

Dyslipidemia

Individuals with suboptimal lipid profiles are at increased risk of developing HF (Dhingra et al., 2008). In the FHS, lower high-density lipoprotein (HDL) and higher non-HDL cholesterol were associated with incident HF independent of myocardial infarction (Velagaleti et al., 2009), but these data have not been replicated in other cohorts. Higher triglycerides have also been suggested to be

associated with increased HF risk in diabetics (Ebong et al., 2013). The association of dyslipidemia and HF is theorized to be primarily attributable to the contribution of dyslipidemia to epicardial coronary artery disease and microvascular dysfunction. As discussed earlier in section "Atherosclerotic Vascular Disease," lipid-lowering treatment with statins leads to a reduced risk of HF (Kjekshus et al., 1997; LaRosa et al., 2005). Dyslipidemia should therefore be treated according to clinical practice guidelines in order to prevent adverse cardiovascular outcomes including HF.

Smoking

Smoking (both past and current) has been associated with incident HF after adjustment for interim coronary events (Gopal et al., 2012). Although data supporting a reduction in HF risk following smoking cessation are lacking, abstinence from cigarette smoking is a vital component of cardiovascular health promotion and should therefore be routinely emphasized.

Cardiotoxins

Chemotherapy

A number of chemotherapy drugs—including both novel targeted therapeutics and traditional cytotoxic agents—increase the risk of developing HF. The classic example is anthracyclines, which were first described to cause HF symptoms 40 years ago (Von Hoff et al., 1977). HF risk from anthracycline agents is dose-dependent, occurring in 8.8% of patients receiving a cumulative doxorubicin exposure of 250 mg/m^2 and 53.9% of individuals treated with 500 mg/m^2 (Swain et al., 2003). Anthracyclines cause cardiomyocyte damage via the increased production of reactive oxygen species and mitochondrial dysfunction (Zhang et al., 2012). This toxicity usually results in irreversible left ventricular systolic dysfunction. Although anthracycline cardiotoxicity is most commonly seen within the first year of treatment, it can present up to 20 years post treatment (Larsen and Mulvagh, 2017). Trastuzumab, a monoclonal antibody against the human epidermal growth factor receptor 2, which is used in select patients with breast cancer, has been reported to further potentiate the risk of HF in individuals receiving anthracyclines (Chen et al., 2012). A number of other established agents such as cyclophosphamide, 5-fluorouracil, and sunitinib have been linked with increased HF risk (Finet, 2017). The field of targeted cancer therapies is progressing rapidly with many new agents under development. One such class is immune checkpoint inhibitors. These drugs have shown tremendous potential in a number of cancers, but fulminant myocarditis resulting in cardiogenic shock has also been reported as a rare toxicity (Johnson et al., 2016). As new agents are developed, the list of chemotherapies with potential cardiotoxicities is likely to grow.

Several strategies are routinely employed to minimize HF risk in individuals receiving cardiotoxic chemotherapy, particularly anthracyclines. Preventive principles include using the lowest necessary doses and considering alternative agents in patients with cardiovascular risk factors. Several small studies have evaluated the benefit of prophylactic administration of standard HF therapies such as beta-blockers, angiotensin converting enzyme inhibitors, or angiotensin receptor blockers in patients receiving cardiotoxic chemotherapy and showing an attenuation of left ventricular systolic dysfunction (Bosch et al., 2013; Gulati et al., 2016). Larger trials with longer follow-up are needed to determine if these interventions might reduce the risk of incident HF.

Chest Radiation

Precise estimates of the risk of future HF in individuals receiving chest radiation are limited by the long delay between the radiation exposure and the development of HF. Nevertheless, the risk of HF is estimated to be increased fivefold after 30 years in individuals receiving chest radiation (Aleman et al., 2007). Radiation can contribute to the development of HF by promoting coronary artery disease, pericardial disease, interstitial fibrosis and diastolic dysfunction, and complex valvular lesions (Finet, 2017). Careful surveillance of individuals who received chest radiation is therefore necessary to identify individuals at high risk for developing HF (Groarke et al., 2014).

Alcohol

While the precise dose relationships have not been elucidated, the association between alcohol consumption and HF risk is theorized to constitute a J-curve relationship. Modest alcohol consumption (generally defined as 1–2 drinks per day) has been associated with a reduced risk of new-onset HF in the FHS and Atherosclerosis Risk in Communities cohorts (Walsh et al., 2002; Gonçalves et al., 2015). By contrast, heavy alcohol consumption (defined as 100 g (~7 drinks) per day for men or 80 g (~5–6 drinks) per day for women) increases the risk of developing HF (Fernández-Solà, 2015). Heavy alcohol use frequently causes a dilated cardiomyopathy, which is often reversible with decreasing or ceasing alcohol consumption (Nicolás et al., 2002).

Other Cardiotoxins

A number of other drugs—both medications and drugs of abuse—have been linked with cardiotoxicities. In particular, amphetamines and cocaine are reported to lead to left ventricular dysfunction and overt HF (Subzposh et al., 2014; Neeki et al., 2016).

A careful medication and drug history should therefore be collected on all patients, and counseling to avoid potential cardiotoxins should be routinely performed.

Family History and Genetic Risk

HF is theorized to be a heritable condition, with approximately 18% of the HF burden in the FHS attributable to a parental history of HF (Lee et al., 2006). Furthermore, a parental history of HF resulted in a 70% higher individual risk of developing HF.

Family history of HF likely influences HF risk through both direct and indirect mechanisms. Shared genetic risk for associated conditions, such as hypertension, coronary artery disease, and obesity, might explain some of the observed heritability (Lloyd-Jones et al., 2004; Murabito et al., 2005).

While many single-gene mutations have been discovered that lead to specific (familial) cardiomyopathies, such as hypertrophic cardiomyopathy and dilated cardiomyopathy, the genetic architecture of common HF risk remains mostly unknown (Tayal et al., 2017). One example of a genetic variant that has been associated with incident HF is the transthyretin variant valine-to-isoleucine substitution at position 122 (V122I), which is found in 4% of African Americans and is involved in transthyretin cardiac amyloidosis. Recently, carriers of these mutations were found to have a 47% higher risk of incident HF (Quarta et al., 2015). Therefore, this variant may represent a risk factor for HF in African Americans and warrants further study (Alexander and Falk, 2016).

Recognizing the importance of heritability in HF pathogenesis, the US HF guidelines recommend gathering a three-generation family history of HF to assess HF risk (Yancy et al., 2013). First-degree relatives of patients with familial cardiomyopathies should undergo surveillance echocardiography, genetic counseling, and potentially genetic testing (Yancy et al., 2013).

Other Conditions

A number of other conditions have been reported to predispose to HF but have not yet been included in HF guidelines as conditions that define stage A HF (Table 1). While accounting for less population attributable risk for HF, addressing these conditions may be an important component of HF risk reduction strategies for individual patients.

Table 1 Conditions comprising stage A heart failure

	Conditions
Risk factors included in US heart failure guidelines	Hypertension
	Coronary artery disease
	Diabetes
	Cardiotoxins
	Family history of cardiomyopathy
	History of rheumatic fever
	Thyroid dysfunction
	Smoking
	Metabolic syndrome
	Atherosclerotic vascular disease
Additional risk factors (*established*)	Obesity
	Dyslipidemia
	Kidney dysfunction
	Physical inactivity
	Obstructive sleep apnea
	Chronic right ventricular pacing
	Human immunodeficiency virus
Additional risk factors (*potential*)	Genetic risk
	Novel biomarkers
	Risk scores

Information included from the following sources: Hunt, S. A., Baker, D. W., Chin, M. H., et al. (2001). ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult: executive summary a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines. *Circulation* **104** (24), 2996–3007; Yancy, C. W., Jessup, M., Bozkurt, B., et al. (2013). 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. *Circulation* **128** (16), e240–e327.

Chronic Kidney Disease

Several measures of chronic kidney disease (CKD) including kidney dysfunction (low estimated glomerular filtration rate) and kidney injury (albuminuria) are associated with new-onset HF in the community (Köttgen et al., 2007; Lam et al., 2011; Dhingra et al., 2011; Fried et al., 2003; Chae et al., 2003; Ingelsson et al., 2007; Blecker et al., 2011; Nayor et al., 2017). Additionally, albuminuria and reduced eGFR have been associated with decrements in cardiac structure and function (Matsushita et al., 2015; Lieb, 2006; Park et al., 2012; Patel et al., 2009), which may underlie these associations.

Prevention of CKD may therefore represent an important consideration for reducing the burden of HF.

Physical Inactivity

Greater physical activity (Djoussé et al., 2009; Agha et al., 2014; Pandey et al., 2015) and less inactivity (He et al., 2001) are associated with lower risks of incident HF in community-based cohorts. In fact, higher physical activity and lower sedentary time have independent and complementary associations with a lower risk of new-onset HF (Young et al., 2014). In a longitudinal analysis from the FHS, reductions in physical activity over time were also associated with elevated HF risk (Kraigher-Krainer et al., 2013). Specific cut points for optimal physical activity or sedentary time to prevent HF have not been agreed upon, but the data do support recommendations to encourage regular physical activity to reduce HF risk.

Chronic Right Ventricular Pacing

Chronic right ventricular pacing results in dyssynchronous cardiac contraction, which can lead to adverse cardiac remodeling and, eventually, to overt HF (Sweeney, 2003; Thambo, 2004; O'Keefe et al., 2005). In one study of 177 patients randomized to right ventricular versus biventricular pacing, right ventricular pacing was associated with a 7% reduction in ejection fraction over the course of the study (Yu et al., 2009). Biventricular pacemakers, in comparison, attenuated ventricular remodeling. Similar benefits would be expected from other interventions to minimize right ventricular pacing such as using lower pacing backup rates and optimization of atrial pacing (Gillis, 2014). Hence, efforts should be made to identify and minimize a high burden of right ventricular pacing in order to mitigate HF risk.

Human Immunodeficiency Virus

Individuals with human immunodeficiency virus (HIV) infection are known to be at increased risk for HF, but the dynamics of this association are changing in the era of widespread use of antiretroviral therapy (Barnes et al., 2017). Previous reports of an HIV-associated cardiomyopathy in 10%–40% of HIV-positive individuals predated the routine use of antiretroviral therapy (Cerrato et al., 2013). Although the risk is certainly lower now, contemporary reports suggest that even on effective antiretroviral treatment, HIV-infected individuals have a higher risk of developing HF than the general population (Al-Kindi et al., 2016; Butt et al., 2011). While mechanisms underlying these observations are being investigated (Remick et al., 2014), increased surveillance and treatment of associated risk factors is important in this population.

Sleep Apnea

Obstructive sleep apnea is associated with an approximately twofold higher risk of developing HF (Shahar et al., 2001). A number of potential mechanisms to explain the association have been posited, including intermittent hypoxia, oxidative stress, systemic inflammation, exaggerated negative intrathoracic pressure, sympathetic overactivation, and hypertension (Kasai and Bradley, 2011). While treatment of sleep apnea with positive-pressure ventilation may improve daytime somnolence and quality of life, trials have generally failed to show that positive-pressure ventilation reduces cardiovascular risk in individuals with sleep apnea (Barbe et al., 2012; McEvoy et al., 2016).

Cardiovascular Health Score

Risk scores combining the assessments of numerous HF risk factors may also prove useful in evaluating and treating stage A HF. A cardiovascular health score can be derived from the presence or absence of optimal values for the seven behaviors and risk factors outlined in the American Heart Association's 2010 strategic planning statement outlining goals for 2020 (Life's Simple 7™) (Lloyd-Jones et al., 2010). Although no trial has been performed in testing this hypothesis, multiple observational studies have demonstrated that an ideal cardiovascular health score is directly associated with a lower risk of developing HF (Nayor et al., 2016; Shah et al., 2015). By combining multiple HF risk factors into one easily calculated metric, this (and similar approaches) might enable patients and clinicians to better gauge HF risk and prioritize lifestyle modifications or treatment goals.

Future Considerations for Stage A HF

The ACC/AHA HF stages were introduced to highlight the progressive nature of HF. While this scheme is a useful construct within which to view the stepwise development of HF, there are several features of stage A HF, as currently defined, that might warrant reevaluation in future HF guidelines.

First, stage A HF is defined by the presence of risk factors without "structural heart disease." However, "structural heart disease" does not have a uniform definition. With further proliferation of advanced cardiac imaging modalities, such as cardiac magnetic resonance and strain imaging (Carerj et al., 2010), this definition will be challenged. For example, would we consider a patient with hypertension and abnormal longitudinal strain imaging (which is associated with incident HF (Cheng et al., 2015)) to have stage A HF if the traditional echocardiographic measures are all within normal limits? Furthermore, it is probable that many individuals currently considered to have stage A HF at rest would have cardiac functional abnormalities with exercise, which might unmask occult organ dysfunction. As cardiovascular diagnostic modalities advance, therefore, the boundaries between stage A and stage B HF are likely to become blurred. For now, we would suggest that patients be characterized as having stage A HF if they have HF risk factors in the absence of cardiac structural or functional abnormalities that are readily available to the clinician.

Second, the ACC/AHA HF stages depict HF as a single condition with various risk factors. However, accumulating data suggest that HF is a heterogenous condition with different subtypes representing distinct pathophysiological pathways and treatment responses (Borlaug and Redfield, 2011). These are currently represented by HF with reduced versus preserved ejection fractions, but it is likely that further subphenotyping will allow for more personalized treatment plans and preventive measures. Therefore, we could envision a novel formulation of stage A HF in the future that considers risk factors within the context of HF subphenotypes.

Summary

Stage A HF is characterized by the presence of conditions and risk factors that are associated with an increased risk for HF in the absence of overt cardiac abnormalities. The conditions comprising stage A HF include traditional cardiovascular risk factors that are highly prevalent in the general population. CHD, hypertension, obesity, smoking, and diabetes are responsible for the highest population attributable risk of HF, while an increasing number of other conditions have been identified to be associated with the development of HF. Early identification and treatment of HF risk factors both at the population and individual level is necessary to reduce the considerable burden of HF.

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Stage B Heart Failure

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Acronyms

ACC	American College of Cardiology
AHA	American Heart Association
ARB	Angiotensin receptor blocker
CAD	Coronary art disease
CMR	Cardiac magnetic resonance imaging
CRP	C-reactive protein
CVD	Cardiovascular disease
ERNA	Equilibrium radionuclide angiography
HF	Heart failure
ICD	Implantable cardioverter defibrillator
LVD	Left ventricular dysfunction
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
MI	Myocardial infarction
MMP	Matrix metalloproteinase
MPI	Myocardial perfusion imaging
MPO	Myeloperoxidase
PET	Position emission tomography
RAAS	Renin–angiotensin–aldosterone system
ROS	Reactive oxygen species
SPECT	Single-photon emission computed tomography
TIMP	Tissue inhibitor of metalloproteinase

Introduction

Around 5.7 million Americans suffer from heart failure (HF), and nearly half of those diagnosed will die within 5 years (Mozaffarian et al., 2015). The economic weight of treating HF is as high as $30.7 billion annually (Heidenreich et al., 2011). Given the monumental health care burden, management and treatment of HF is an active area of clinical research. The American College of Cardiology and the American Heart Association (ACCF/AHA), to better manage the disease, have identified four stages to HF: stages A, B, C, and D. Briefly, stage A defines patients at risk for HF, such as those with hypertension or family history of cardiomyopathies, but have yet to develop cardiac structural changes. In stage B, patients have structural heart diseases associated with HF but have not developed symptoms, whereas in stage C patients developed HF symptoms such as dyspnea or fatigue. Lastly, patients with stage

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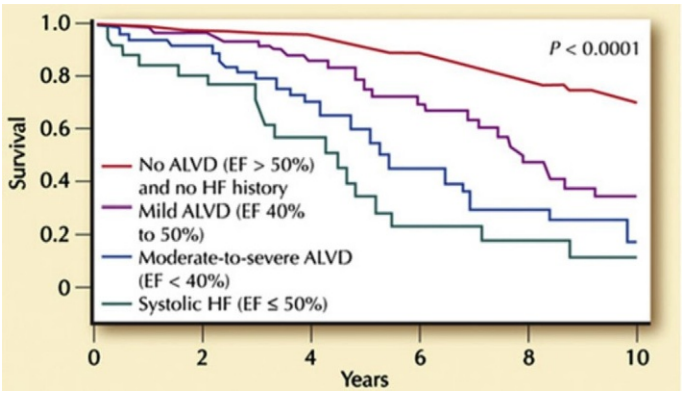

Fig. 1 Kaplan–Meier curves for survival. Referent group consists of subjects with normal left ventricular systolic function (EF >50%) and no history of congestive heart failure. Mild ALVD indicates mild asymptomatic left ventricular systolic dysfunction (EF 40%–50%); moderate-to-severe AVLD (EV <40%); systolic HF indicates congestive heart failure with EF ≤50%. From Wang, T. J., Evans, J. C., Benjamin, E. J., et al. (2003). Natural history of asymptomatic left ventricular systolic dysfunction in the community. *Circulation* **108** (8), 977–182; with permission.

D HF still demonstrate structural heart disease despite optimal medical treatment and are indicated for advanced intervention such as cardiac transplantation. Stage B HF is the culmination of a progressive disease and is a useful designation for patients at high-risk for HF. Developing measures to identify this subset of patient population, before the manifestation of signs or symptoms of HF, can dramatically improve patient outcomes. Understanding the structural basis of stage B HF, its etiology and progression, and the available clinical tools to track its clinical course will prove vital in providing the best care to HF patients.

The characteristic and functional abnormalities that fall into the category of stage B HF are diverse. In many cases of HF, cardiac risk factors that compromise the vascular endothelium and/or hemodynamics are present. Moreover, they contribute to structural changes in cardiomyocytes and the interstitium that lead to gross cardiac remodeling indicative of progressive HF. There are several etiologies that promote cardiac remodeling in progressive HF. Neurohormonal alterations of the renin–angiotensin–aldosterone axis and the adrenergic nervous system are compensatory mechanisms that exacerbate myocyte dysfunction in the long term. Additionally, pathologic states of chronic inflammation and ischemia mediated by cardiotoxins or cardiac structural diseases further play a role in ventricular hypertrophy and advancement to later stages of HF. Understanding of these disease processes and risk factors is crucial in determining the optimal treatment course and screening procedures for patients at jeopardy for HF.

Prevalence of stage B HF is rising because of an aging population and an increase in a number of patients with comorbidities. More patients are developing diabetes, left ventricular (LV) hypertrophy from hypertension, and coronary artery disease (CAD) that can result in myocardial infarction (MI) that impairs heart function. Angiotensin-converting enzyme (ACE) inhibitors and beta blockers in the case of post-MI are proven therapies that can improve survival and even reduce cardiac remodeling and prevent HF stage progression. Consequently, it is crucial to identify patients with stage B HF, as these patients have a fivefold increase in developing overt HF and manage accordingly (**Fig. 1**; Wang et al., 2003). Thus waiting for asymptomatic patients to develop symptomatic HF is a missed opportunity to positively influence the clinical course of the disease. Unfortunately, clinicians tend to focus on stage C and D HF due their symptomatic nature and ease in identification (Ditewig et al., 2010). Identifying and managing patients at earlier stages in HF can prevent progression to overt HF and improve mortality.

Pathogenesis

Cardiac Remodeling Leading to Stage B HF

Cardiac remodeling is a major feature of HF and when not present with clinical symptoms is pathognomonic for stage B HF (Savinova and Gerdes, 2012). Changes in venous return and vascular tone are the primary drivers of gross structural remodeling. Cardiomyocytes can adapt to environmental changes by altering their morphology in a myriad of pathologic states. This is accomplished through the unique positioning of cardiac cells within the heart wall. Here, cardiac cell length and diameter contribute to the heart's chamber circumference and wall thickness, respectively. How the cardiomyocyte's shape alters will determine the nature of cardiac remodeling.

Myocytes are striated cells measuring 25 μm in diameter and 100 μm in length. Each cell contains segments of sarcomeres, termed myofibrils, and the extent to which the myosin and actin filaments of the sarcomere overlap determines the force of muscle contraction. It has been widely accepted that hypertrophy (increase in cell size) rather than hyperplasia (increase in cell number) largely defines cardiac remodeling. Cardiomyocyte shape can strongly influence ventricular wall stress, a major determinant of myocardial oxygen demand. Laplace's Law reveals that wall stress (T) is proportional to chamber or vessel radius (R) and internal pressure (P) and inversely proportional to wall thickness (H):

$$T = PR/2H$$

This equation will be useful in understanding how the different etiologies of cardiac remodeling affect cardiomyocyte changes and risk for HF.

Pressure overload is one of many forms of stress that can lead to cardiac remodeling and is caused by states that increase the pressure gradient that the ventricle must overcome, such as hypertension and valvular stenosis. In addition, mechanical stress can be exacerbated by neurohormonal influences. As mentioned above, wall stress is directly proportional to internal pressure and inversely proportional to wall thickness. To normalize wall stress, the ventricle hypertrophies and increases wall thickness. Pressure overload leads to concentric hypertrophy, where there is an increase in muscle mass with either no change or a small change in chamber volume. In concentric hypertrophy, sarcomeres are added in parallel to increase the cell diameter. These changes ultimately lead to better-adapted muscle. Although left ventricular hypertrophy (LVH) at stage B HF is thought to be beneficial, it has long been found to be an independent risk factor for HF and other cardiovascular diseases (CVD) (Frohlich et al., 2011). The structural and functional changes in cardiomyocytes during hypertrophy, although beneficial at first, confer some increased risk for several cardiovascular disorders.

Volume overload also is another form of stress that can lead to cardiac remodeling. Structural cardiac volume overload has multiple etiologies and different effects on loading conditions for the four different heart chambers. For example, aortic insufficiency leads to volume overload when there is an abnormal regurgitation of blood from the aorta into the left ventricle during diastole. As with pressure overload, remodeling leads to both adaptive and maladaptive consequences. Volume overload causes eccentric hypertrophy, where increased muscle mass develops along with chamber dilation. In certain disease states both volume and pressure overload may be present, which are predisposing factors to stage B HF.

In addition, chronic ischemia of cardiomyocytes may result in remodeling. HF is a common complication of MI (Minicucci et al., 2011). The cardiac dysfunction that follows MI, such as systolic dysfunction or restrictive mitral filling patterns, can lead to compensatory cardiac remodeling. The degree of ventricular remodeling post-MI depends on infarct size and time to reperfusion. Though reperfusion has benefits, it can also cause reperfusion damage and further cardiac remodeling and ventricular dysfunction (Bolognese et al., 2002). Therefore, patients who experienced an MI or have recurrent ischemic issues should be screened for early stages of HF to improve clinical outcome.

Beyond cardiomyocytes, cardiac remodeling also occurs in myocardial interstitium, which consists of fibrillar collagen, non-collagen matrix proteins, proteoglycans, matrix metalloproteinases (MMPs), and a complex milieu of bioactive molecules. The myocardial interstitium is indispensable to cardiac function and orchestrates cardiomyocyte arrangement and contraction (Burlew and Weber, 2000). The myocardial interstitium is altered in disease states such as pressure and volume overload, valvular stenosis, and MI. These alterations are implicated in HF progression and include tissue inhibitors of MMPs (TIMPs), which leave MMPs unencumbered in their myocardial remodeling function. There are also changes in matrix composition such as an increase in galactin-3, which has been shown to be a marker in increased hospitalizations post-MI (Shah et al., 2010). Thus, understanding changes in the myocardial interstitium can offer insights to clinical outcomes and future therapeutic outlets.

Contributors to Heart Failure Progression

In response to abnormal conditions, such as pressure or volume overload, the heart can compensate by altering cardiomyocyte morphology. However, cardiac remodeling is not the only major contributor to HF progression. Studies have shown that neurohormonal stimulation, oxidative stress, inflammation, changes in arterial elasticity, and age-related cardiovascular factors all underwrite the development of HF.

Neurohormonal activation is a cornerstone to the compensatory mechanism during the development of HF. The sympathetic nervous system and renin–angiotensin–aldosterone system (RAAS) coordinate to preserve cardiac output and function. Falls in cardiac output during HF are sensed by baroreceptors in the aortic arch and carotid sinus. As blood pressure falls so does the firing rate of the glossopharyngeal and vagus nerves to the cardiovascular center in the medulla. This leads to an increase in sympathetic nervous activity to the heart and peripheral vascular system causing an increase in heart contractility, heart rate, and vasoconstriction. During HF, decreased renal perfusion, secondary to a lower cardiac output, results in the stimulation of RAAS. This promotes the release of renin, an enzyme that cleaves angiotensinogen to angiotensin I, from juxtaglomerular cells in the afferent renal arterioles. Angiotensin I is converted to angiotensin II by angiotensin-converting enzyme (ACE) in the lung and kidney to from angiotensin II. Angiotensin II is a potent vasoconstrictor that helps raise the blood pressure by increasing total peripheral resistance and leads to increases in intravascular volume by stimulating thirst in the hypothalamus. Angiotensin II also acts on the adrenal cortex to stimulate the release of aldosterone, a mineralocorticoid that promotes sodium reabsorption to augment blood volume. Though these changes are adaptive, they also lead to complications such as peripheral edema and pulmonary congestion, making neurohormonal mediators targets for therapy.

Oxidative stress is an imbalance between the generation of reactive oxygen species (ROS) and neutralizing antioxidants. During homeostasis, ROS play an important role in regulating numerous physiological processes by acting as a growth factor and cytokine. However, excess ROS can lead to tissue damage and has been implicated in countless diseases such as diabetes, atherosclerosis, hypertension, and even HF (Kojda and Harrison, 1999). Most studies have investigated the link between ROS and symptomatic HF and evidence shows that ROS play a role in pathogenesis in stage B HF (Bhimaraj and Tang, 2012). In ischemic states, secondary to structural heart disease, ROS are generated due to lack of aerobic respiration and result in a myriad of downstream consequences such as cardio-inhibition and MMP activation. Moreover, diseases that increase the risk for HF, including valvular disease, type 2 diabetes mellitus, or hypertensive disease, also lead to the generation of excess ROS in cardiomyocytes. The role of ROS in dysregulating normal cardiomyocyte function is just being appreciated, and manipulating oxidative stress in the heart could prove beneficial in the treatment of HF.

The inflammatory system is an integral to maintaining homeostasis but pathophysiological inflammatory states can alter myocardial function (Cieslik et al., 2011). Increased levels of proinflammatory medications such as C-reactive protein (CRP) and interleukin-6 (IL-6) have been linked to increased risk for symptomatic HF (Kalogeropoulos et al., 2010). Recent research has also elucidated the link between inflammation and progression from stage A to stage B HF (Kalogeropoulos et al., 2012). A proinflammatory milieu can lead to several mechanisms that compromise cardiac function such as decreasing myocardial contractility, promoting cardiac remodeling, and negatively impacting vasculature. Modulating proinflammatory factors at earlier stages of HF may prevent precipitation of symptoms and improve clinical outcome.

Arterial stiffness, or loss of arterial plasticity, is another marker with utility in identifying those at risk for HF. Reduction in arterial elasticity can impair cardiac output and put patients at increased risk for developing HF. Arterial elasticity determines the buffering capacity of arteries and pulse wave velocity coming from the heart (Laskey and Kussmaul, 1987; Mitchell, 2009). The Windkessel effect is a function of arterial elasticity and helps diminish the fluctuation in blood pressure during the cardiac cycle such that organ perfusion can be maintained during diastole. Assessment of atrial stiffness, along with other risk factors, may offer prognostic clues to the development of HF, especially in older patients who lose arterial elasticity over time.

As the population in developed countries grow older so does the prevalence of HF. This is because as more patients are successfully treated for symptomatic heart disease, the prevalence of individuals with early stages of HF increases (Mozaffarian et al., 2015). With age comes cardiovascular structural and functional changes such as arterial stiffening, increased LV wall thickness, and decreased myocyte contractility that contributes to systolic dysfunction. Additionally, the cardioprotective and repair processes are impaired and can lead to maladaptive remodeling. This leads to a higher incidence of cardiovascular diseases such as coronary artery disease and diabetic cardiomyopathy that can predicate HF. As such, it is imperative to consider aging a risk factor and screening of older patients can prevent the advent of HF.

Diagnosis and Monitoring of Stage B HF

Stage B HF is difficult to diagnose since symptoms have yet to manifest, but advanced imaging techniques and identification of biomarkers have made it possible. Patients in stage B HF stand to greatly benefit from early interventions. Advancements in no imaging modalities such as echocardiography and cardiac magnetic resonance imaging (CMR) have made it possible to detect structural changes in stage B HF. Moreover, radionuclide imaging can offer unique prognostic insights that other imaging techniques cannot. Beyond imaging, assessing biomarkers can provide information on both etiology of HF and risk of progression. In examining patients, it is advantageous to understand some of the limitations of each method and integrate the information offered by these illuminating techniques.

Echocardiography and Stage B Heart Failure

Echocardiography is relatively inexpensive and has a spectrum of applications making it a powerful tool to diagnose stage B HF. Transthoracic echocardiography is regularly used to assess measures of structure and function indicative of stage B HF. The best-studied measure is a reduction in left ventricular ejection fraction (LVEF) (Atherton, 2010). Asymptomatic left ventricular dysfunction (LVD) is typically detected by echocardiography and can be assessed in both diastole and systole to estimate the severity of stage B HF. Normally, LVEF should be between 55% and 70%. Patients with an LVEF less than 50% were found to be at an increased risk for developing symptomatic HF (Wang, 2003). In fact, the lower a patient's LVEF, the higher the risk of overt HF.

Since stage B HF is a detectable structural disease with no symptoms, LVSD, previous MI, LVD, and valve disease can all be designated as stage B HF. An advantage of echocardiography is that it can also be used to dissect structural changes by differentiating concentric and eccentric hypertrophy, valvular disease, and pericardial disease. Additionally, echocardiography can parse systolic and diastolic function by several parameters to offer greater depth of stage B HF severity. It is important to detect diastolic dysfunction since half of the patients presenting with symptomatic HF have preserved ejection fraction (Owan et al., 2006).

The echocardiogram features many settings used to screening for stage B HF (Boyd et al., 2015). In the 2-dimensional setting (2D) and M-mode, echocardiography can be used to measure LV wall thickness and chamber dimensions. Tissue Doppler echocardiography can assess both diastolic and systolic function with high temporal resolution by calculating the contractile velocity of cardiomyocytes. For example, in terms of diastolic function, echocardiography can measure useful parameters to assessing stage B HF severity such as isovolumetric relaxation time and ventricular filling pressure. Stress echocardiography can also be used in asymptomatic patients to illustrate diastolic function. Three-dimensional (3D) echocardiography offers, even more, information about cardiac structure but is diminished by poor spatial resolution. The spectrum of applications for echocardiography makes it an attractive tool for diagnosing and assessing stage B HF.

Cardiac Magnetic Resonance Imaging and Stage B Heart Failure

Cardiac magnetic resonance (CMR) is another imaging tool that can elicit both diagnostic and prognostic information for stage B HF (Partington et al., 2012). To date, this method remains the best noninvasive imaging modality for assessing heart function because it offers superior spatial and visual resolution to echocardiography. CMR, like echocardiography, can assess systolic and diastolic function while providing comprehensive myocardial tissue characterization. Moreover, it can detect inflammation, fibrosis, ischemia, and viability of cardiomyocytes. Distinguishing the cause of asymptomatic cardiac remodeling has significant

implications on risk stratification, prognosis, and treatment of the disease. Unlike echocardiography, CMR can measure cardiac structure, function, and tissue characterization in a single study. These advantages allow for more precise readings of LVED and make CMR the ideal tool for diagnosing and monitoring HF patients. There are some limitations including contraindication in patients who are at risk for radiocontrast-induced nephrotoxicity and those with pacemakers or implantable cardioverter defibrillator (ICD). Even with its limitations, this method of imaging remains a powerful tool for stage B HF.

Radionuclide Imaging

Radionuclide imaging is another tool that has proven valuable in diagnosing and monitoring stage B HF (Janardhanan and Beller, 2012). Equilibrium radionuclide angiography (ERNA) is widely used to assess left ventricular dysfunction. In ERNA, red blood cells (RBCs) are labeled with a radioactive material, namely Technetium-99 m. These RBCs can be seen flowing through the heart chambers and be used to measure systolic and diastolic function. ERNA, like CMR, has the advantage of high precision making it suitable for serial measurements of LVEF.

ERNA can be complimented with single-photon emission computed tomography (SPECT) to assess myocardial perfusion. Myocardial perfusion imaging (MPI) can be used to diagnose CAD in patients with HF and assess myocardial viability, adding another advantage to using radionuclide imaging. But unlike CMR, ERNA cannot accurately distinguish ischemic from nonischemic heart disease. Another radionuclide technique called positron emission tomography (PET) can offer insight to myocardial metabolism. In this modality, biologically active molecules, such as fludeoxyglucose, are labeled and tracked to assess tissue metabolism. This offers another degree to the characterizations of myocardial tissue.

Radionuclide imaging has great utility in investigating HF. Its precision and in-depth myocardial tissue characterization makes it ideal for detecting asymptomatic HF. It can delineate myocardial perfusion, viability, metabolism, interstitial abnormalities, and even neurohormonal receptor function. SPECT imaging can sense sympathetic stimulation, a compensatory mechanism in HF, and identify patients in early stages of HF who can benefit from intervention. Nuclear cardiology yields promising techniques to the detection and management of HF.

Biomarkers in Stage B Heart Failure

Biomarkers are measurable biological substances that indicate a disease process or risks for disease. Biomarkers in the context of HF are used to illuminate the etiology of HF, the presence of HF, and the severity of HF and risks for disease progression (Eleuteri and Di Stefano, 2012). Biomarkers for HF indicate neurohormonal activation, myocyte injury, inflammation, interstitial dysregulation, renal dysfunction, oxidative stress, and other parameters. Biomarkers have been useful in confirming or excluding HF and assessing long-term risks of disease progression.

Perhaps the most useful biomarker in inspecting for HF is the brain natriuretic peptide (BNP) and its cometabolite NT-proBNP, a prohormone. BNP is secreted by the ventricles of the heart in the response to excessive stretching (as would be seen in volume overload in stage B HF). BNP is secreted attached to NT-proBNP. BNP counteracts some of the maladaptive consequences from compensatory changes during HF by lowering systemic vascular resistance and central venous pressure, increasing natriuresis, and suppresses RAAS. The long half-life of NT-proBNP makes it a strong biomarker. In stage A and B HF, elevated NT-proBNP was associated with increased risk for MI, HF progression, and death (Luchner et al., 2000).

Biomarkers of myocyte injury and interstitial remodeling are also useful in assessing risk of HF. Troponins are proteins involved in muscle contraction and are leaked during myocyte injury. Many disease processes can elicit troponin release including HF. The release of troponin during HF may be due to a culmination oxidative stress, inflammation, wall stress, neurohormonal activation, and less perfusion due to an increase in myocardial oxygen demand (Logeart et al., 2001). The efficacy of measuring troponin levels in patients with any stage of HF has yet to be established. Volume and pressure-overload causes changes in the extracellular matrix that can be tracked by detecting changes in MMPs and TIMPs composition. For example, elevated levels of MMP-9 are associated with left ventricular remodeling and TIMP-1 with reduced systolic function (Sundström et al., 2004; Kelly et al., 2007). Despite the insights TIMP and MMP configuration have on cardiac remodeling and function, more proteomic studies need to be conducted before they can be used as reliable biomarkers for HF.

Inflammation and oxidative stress are contributors to HF progression and can be measured using biomarkers. Several proinflammatory cytokines such as IL-1, IL-6, CRP, and TNF-α are elevated in patients with HF (Anker and Von Haehling, 2004). Higher levels of these proinflammatory cytokines have been associated with HF progression and could be predictive of clinical outcome (Levine et al., 1990; Deswal et al., 2001). CRP has been shown to complement BNP screening for HF but even though that inflammatory mediators are contributors are HF, measurements are not typically made to inform therapy (Ng et al., 2006).

Similarly, ROS is observed in HF (Hafstad et al., 2013). A proinflammatory state, myocardial apoptosis, and diminished antioxidative activity are thought to be behind oxidative stress in HF. Several biomarkers have been implicated in presence of HF and HF severity. Higher plasma levels of myeloperoxidase (MPO) and isoprostane, an inflammatory mediator and marker of oxidative stress, correlate with severity of HF, degree of left ventricular remodeling, and mortality (Tang et al., 2006; Kameda et al., 2003). Oxidative stress also correlates with markers of neurohormonal activation and inflammation (Wykretowicz et al., 2004; Nonaka-Sarukawa et al., 2003). Despite the role ROS in advancing HF and these strong correlations, antioxidant therapy has had mixed clinical success in treating HF (Pacher et al., 2005; Hafstad et al., 2013). As with inflammatory markers, biomarkers of oxidative stress are not routinely used to inform therapeutic decisions for HF but are still useful in describing presence and severity of disease.

New biomarkers are emerging as the pathophysiology of HF becomes clearer. Osteoprotegerin is a glycoprotein that regulates bone resorption, inflammation, and other cellular processes and is correlated with heart diseases (Venuraju et al., 2010). Interestingly, osteoprotegerin is involved in asymptomatic left ventricular remodeling (stage B HF) (Omland et al., 2007). Other proteins have been implicated in the early stages of HF as well. Galectin-3, a protein secreted by macrophages, promotes cardiac remodeling and has amplified myocardial expression in stages A and B of HF (Sharma et al., 2004; Shah et al., 2010). As proteomics continues to evolve so will the practice of biomarkers in identifying and characterizing patients with stage B HF.

Prevention

Though the mechanism behind progression of stage A to stage B HF remains largely misunderstood some measures can still be taken to prevent advancement to stage B HF. Several antecedents to symptomatic HF have been identified such as obesity, chronic kidney disease, anemia, atrial fibrillation, smoking, diabetes, sedentary lifestyle, less education, and others. Reduction of these risk factors is the mainstay of prevention of progressing from stage A to stage B HF. Lifestyle changes such as exercising regularly, adapting healthier eating habits and lowering blood cholesterol, avoiding alcohol, and managing stress are all practical strategies for reducing risk for stage B HF. Treating hypertension, dyslipidemia, and diabetes is also beneficial in preventing progression to stage B HF. A summary of risk factors and clinical course of HF is illustrated in **Fig. 2**.

Prevention of HF progression also includes addressing dysfunction of noncardiac organs. Renal, pulmonary and hematopoietic impairment have all been associated with a 30% increased risk of overall HF, with an additive effect if more organs are involved (Lam et al., 2011). The interaction between chronic HF, chronic kidney disease, and anemia, known as cardio-renal-anemia syndrome, serves as a vicious cycle that deteriorates all three organ systems (Von Haehling and Anker, 2011). Additionally, debilitated lung function effects both systolic and diastolic cardiac performance (Barr et al., 2010; Guazzi, 2008). Therefore, improving prevention of HF progression must include attention to both cardiac risk factors and noncardiac organ impairment.

Despite knowledge of risk factors associated with HF, community-based studies suggest little progress has been made in prevention (Owan et al., 2006; Gomez-Soto et al., 2011; Levy et al., 2002). Diagnostic testing has no direct effect on clinical outcome but can improve outcome through initiating and optimizing therapeutic intervention during a critical period of the natural history of HF. Analysis from the SOLVD-Prevention and Survival and Ventricular Enlargement trials provide strong support for ACE inhibitor and β-blocker therapy during stage B HF (Vantrimpont et al., 1997; Exner et al., 1999). Although there is a clear benefit to screening patients for stage B HF and starting therapy, there is no decisive method to identify patients with stage B HF (though the use of biomarkers is promising). The following research designs will help in formulating strategies to screen and best treat patients with stage B HF (Box 1).

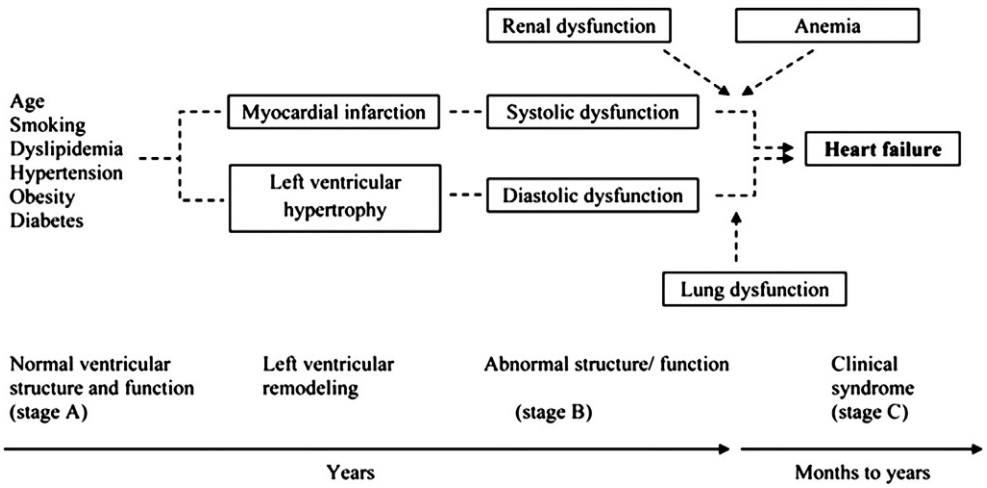

Fig. 2 Interaction of cardiac risk factors along with cardiac and noncardiac dysfunctions that contribute to heart failure progression. From Klein, L. (2011). Omnes Viae Romam Ducunt: Asymptomatic cardiac and noncardiac organ system dysfunction leads to heart failure. *Circulation* **124**, 4–6; with permission.

Box 1 Summary of proposed research designs

1. Determine the incidence of asymptomatic LVSD in community-based cohorts.
2. Characterize the natural history of asymptomatic LVSD in the community:
   a. Natural history in individuals with different degrees of LVSD
   b. Natural history in individuals with LVSD without previous MI
3. Examine the natural history of subclinical left ventricular diastolic dysfunction.
4. Examine the cost-effectiveness of various screening strategies for identifying left ventricular dysfunction and develop better screening tools.
5. Address the role of medications other than ACE inhibitors in managing patients with asymptomatic LVSD.

(*Continued*)

Box 1 (Continued)

6. Increase representation of the following types of patients in clinical trials: patients with mild LVSD, those with LVSD and no history of MI, and those with isolated left ventricular diastolic dysfunction.

ACE, angiotensin-converting enzyme; *LVSD*, left ventricular systolic dysfunction; *MI*, myocardial infarction.

From Wang, T. J., Levy, D., Benjamin, E. J., et al. (2003). The epidemiology of "asymptomatic" left ventricular systolic dysfunction: Implications for screening. *Annals of Internal Medicine* **138** (11), 907–916; with permission.

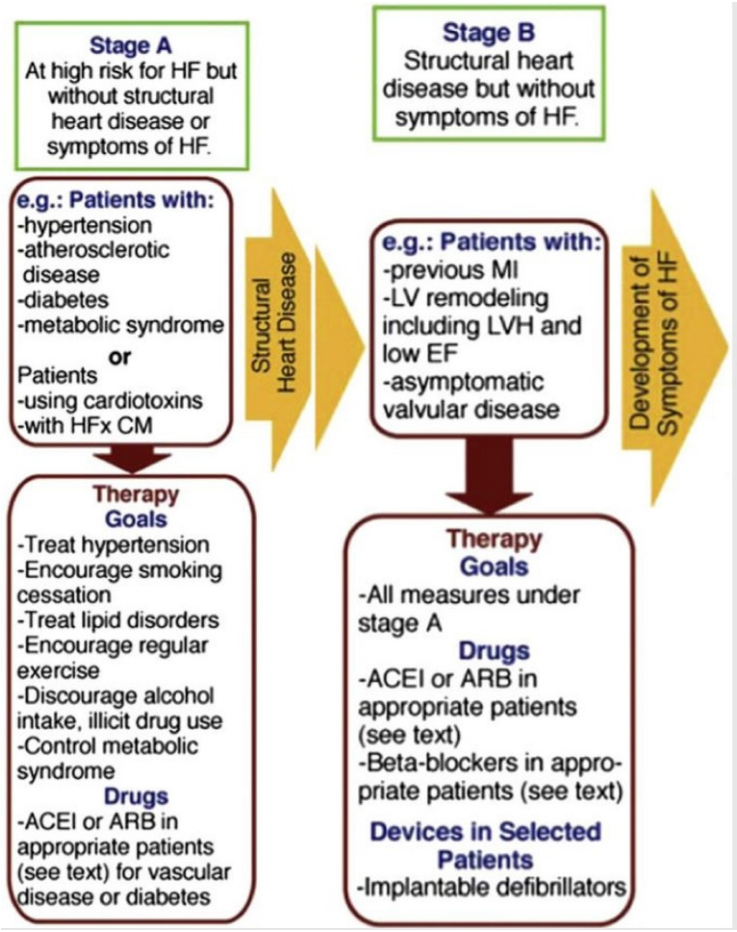

Fig. 3 Summary of asymptomatic HF characteristics and interventions. Adapted from ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult. Figure courtesy of Dr. Ragavendra Baliga.

Treatment

For patients with stage B HF, the goal of treatment is to prevent overt disease. Pharmacologic intervention has been proven to alter the natural history of HF. Currents drugs can address the underlying pathophysiology HF and target left ventricular remodeling, the RAAS, neurohormonal activation, and heart arrhythmias. This section describes asymptomatic HF interventions in detail and is summarized by Fig. 3.

Renin–Angiotensin–Aldosterone System Intervention and Stage B Heart Failure

RAAS is critical to cardiac hormonal signaling and is activated as a compensatory mechanism early on in HF to maintain adequate cardiac output and hemodynamic balance. Though adaptive at first, excessive RAAS activation can perpetuate disease mechanisms underlying HF. Pharmacologic manipulation of RAAS has proven beneficial for treating patients with a variety of cardiomyopathies, previous MIs, and LVH. Stage B HF encompasses a spectrum of pathologies exacerbated by RAAS, making RAAS a potent target for therapy.

As discussed, excess angiotensin activity during compensation ultimately leads to complications that aggravate HF. Targeting angiotensin is a pillar to the treatment of stage B HF and other cardiac disorders. ACE inhibitors can prevent the conversion of

angiotensin I to angiotensin II and lower both plasma and tissue levels of angiotensin II. ACE inhibitors facilitate vasodilation by counteracting angiotensin's vasoconstrictor effects and limiting degradation of the vasodilator bradykinin. Preservation of bradykinin levels is known to stimulate nitric oxide production and perhaps explain the antihypertensive, antifibrotic, and antithrombic effects of ACE inhibitors (Hornig et al., 1997; Pretorius et al., 2003). The preservation of bradykinin also precipitates cough, which may contraindicate the use of ACE inhibitors. ACE inhibitors are also contraindicated in patients with edema and hypersensitivity and should be used with caution in patients with renal dysfunction and cardiac outflow obstruction. For patients who are intolerable to ACE inhibitors, angiotensin receptor blockers (ARBs) also can block angiotensin activity and are an alternative. ACE inhibitors are cardioprotective in stage B postinfarct patients and reduce progression to severe HF (Pfeffer et al., 1992; Torp-Pedersen and Køber, 1999). ARB therapy has shown similar efficacy in the same clinical setting (Pfeffer et al., 2003). RAAS therapy is very effective at modulating natural history of asymptomatic heart dysfunction and can alter the natural history of HF.

β-Blocker Utility in Stage B Heart Failure

Another cause to HF as a progressive clinical syndrome is over activity of the neurohormonal system. The sympathetic nervous system modulates heart rate and activity via β1-receptors through the release of catecholamines. Overstimulation of the sympathetic nervous system is thought to contribute to progressive HF and blocking β-adrenergic has proven therapeutically beneficial. β-adrenergic blockers, or β-blockers, also have potent antiremodeling properties, though the reasons why remain unclear. Overall, β-blockers share similar efficacy and safety to RAAS inhibitors and are even associated with the most consistent improvements in LVEF (Campen et al., 1998). These properties make β-blockers an indispensable treatment for stage B HF patients.

LV dysfunction is a hallmark of HF and activates RAAS, increases catecholamine release (namely norepinephrine), and may lead to structural remodeling. Increased levels of norepinephrine (NE) exhibit cardiotoxic effects and paradoxically impair sympathetic stimulation over time (Keys and Koch, 2004). In a protective measure, the heart downregulates β1-receptor expression to reduce the deleterious effects of chronic adrenergic stimulation and counteract the cardiotoxicity of excess NE. β-blockers block NE binding and mitigate its cardiotoxic effects. Also by improving LVEF and systolic function, β-blockers can decrease adrenergic activity. β-blockers such as metoprolol succinate and metoprolol tartrate have been found helpful in treating stage B HF patients by reducing remodeling, improving systolic function, and curtailing mortality (Colucci et al., 2007). β-blockers can be contraindicated in patients with symptoms of HF, such as hypotension, but are favorable in treating stage B HF patients. Regardless of the etiology of stage B HF, patients who suffered from a previous MI should be treated with both RAAS inhibitors and β-blockers.

Implantable Cardioverter Defibrillators for Stage B Heart Failure

Implantable cardioverter defibrillators (ICDs) are devices capable of performing cardioversion, defibrillation, and in some models heart pacing. ICDs are commonly used in patients who are at risk of dying from abnormal heart rhythms and sudden cardiac death. When an abnormal heart rhythm is detected, the ICD delivers a shock to reestablish a normal rhythm. Patients with HF and/or previous MIs are at risk for sudden cardiac death and can benefit greatly from an ICD. For this reason, patients with stage B HF precipitated by an MI are often outfitted with an ICD.

Stage B Heart Failure Management with Comorbidities

Stage B HF is often present with other comorbidities. Stage B HF patients with hypertension, chronic kidney disease (CKD), or diabetes need special management. Since ACE-inhibitors are efficacious in controlling blood pressure, patients with concomitant hypertension can benefit from RAAS inhibitor treatment. Use of ACE-inhibitors and ARBs should be monitored closely in patients with CKD. While the antihypertensive effects of these have renal protective effects, they can cause hyperkalemia and other complications in CKD patients. The renal protective effects of RAAS inhibitors confer a benefit to diabetes patients. Attention should be given to what β-blockers to prescribe to diabetic patients. Carvedilol has been shown to be less detrimental to glucose control in diabetic patients than other β-blockers(Fardoun, 2006; DiNicolantonio et al., 2015). Patients who are hyperkalemic or pregnant should not be prescribed ACE-inhibitors or ARBs.

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STEMI: Diagnosis

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Introduction

ST-segment elevation myocardial infarction (STEMI) (O'Gara et al., 2013) and non-ST-segment elevation myocardial infarction (NSTEMI) (Roffi et al., 2016) are the most frequent among acute coronary syndromes (ACS) whereas unstable angina (referred to in the past as intermediate coronary syndrome (Anon., 1979)) is a less severe condition. Based on (1) chest pain presentation with typical characteristics, (2) standard electrocardiogram (ECG) with adequate modifications in time, and (3) biomarkers' evolutions, it is now highly probable to correctly diagnose to which clinical form a given patient behaves, which has immediate therapeutic and outcome-related consequences to the extent that diagnosis is a gating for short- and long-term survival which is obtained by speeding up the salvage of myocardium at risk, the real limiting factor of medical, and surgical procedures developed in recent years (Reed et al., 2017; Anderson and Morrow, 2017).

The universal definition of myocardial infarction (MI) recognizes acute myocardial ischemia as the pathophysiological mechanisms whereby cardiomyocytes undergo irreversible damage leading to necrosis in a clinical setting (Reed et al., 2017; Anderson and Morrow, 2017). However, to meet the diagnosis of MI, a combination of criteria is needed. It is, in fact, crucial the presence in the circulating blood of a biomarker normally present in intact cardiac cells only: troponin (T) (Twerenbold et al., 2012; Reiter et al., 2012; Thygesen et al., 2012a). The values of this biomarker are expected to be higher above the 99th percentile of the upper reference limit, which introduces sometimes an element of uncertainty if gender-, age-, and detection-related methods were not tested (and known) in the concerned laboratory hospital. Apart from this golden reference, the most frequently related to the high-sensitivity T-troponin (hs-TnT) (Twerenbold et al., 2012) although hs-I troponin (hs-TnI) (Shah et al., 2015) may also serve as a standard for signaling an irreversible intracellular damage due to acute ischemia, it is necessary that at least one of the following elements is present: (1) symptoms of myocardial ischemia either as chest pain or as systodiastolic dysfunctions of cardiac performance; (2) new or presumably new significant ST-T wave changes (with evolution toward Q waves) or the development of left bundle branch block (LBBB) on 12-lead ECG; (3) imaging evidence of new or presumably new loss of viability at the level of contracting myocardium, which is evidenced by regional wall motion abnormality including loss of systolic increment of wall thickness; and (4) detection of intracoronary thrombi either at selective coronary angiography or autopsy.

When ST-T wave changes persist (in obliged conjunction with critical changes of hs-TnT) more than 20 min and chest pain is typical, the patient is facing an ACS of the STEMI type which later will present with significant (>40 ms in duration: pathological) Q waves at ECG. The most probable cause of STEMI is an acute total coronary occlusion, the occurrence of which might follow to either intraluminal dislocation of a parietal subocclusive coronary atheroma due to intraplaque hemorrhage or to luminal occlusion due to thrombus formation after plaque rupture, erosion or fissuration (Falk et al., 2013; Ylä-Herttuala et al., 2013). Recent studies using optical coherence tomography (OCT) have shown that plaque rupture represents the most common cause of thrombotic coronary occlusions accounting for over 65% plaque morphologies in STEMI. Plaque erosion is recognized in at least 25%–27% of patients with STEMI. Thrombosis of a calcified stenotic lesion is recognized in the reaming 8% of STEMI patients (Higuma et al., 2015; Guagliumi et al., 2014; Burke et al., 1997; Prati et al., 2013; Manfrini et al., 2006). As a consequence of total coronary occlusion, coronary flow ceases and the dependent tissue, so frequently not irrigated by collaterals, may not survive unless reperfused by mechanical restitution of orthogonal coronary flow which is obtained by fibrinolytic dissolution of coronary thrombi and/or coronary dilation by primary (since in the acute context) coronary angioplasty and potential position of a stent to assure maintenance of adequate coronary caliper after intracoronary balloon inflation.

If atherosclerotic involvement is diffuse and all three main coronary vessels (or their major branches) are involved, it might be difficult to detect the culprit lesion actually responsible for the MI and how coronary flow reserve behaves might be useful to

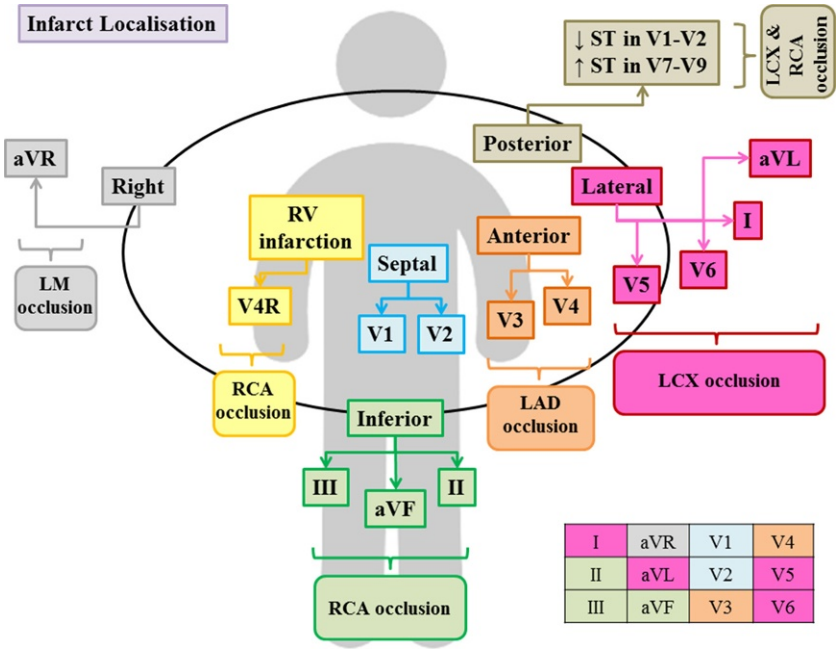

Fig. 1 Overview of ECG leads and criteria for the diagnosis of MI. For STEMI diagnosis ST-segment elevation measured at the level of the J point, should be detected in at least two adjacent leads and be of $\geq$0.20 mV in man and $\geq$0.15 mV in women in leads V_2–V_3 or $\geq$0.10 mV in the remaining leads. These cut-off values have also an age dependency and thus in younger than 40-year men, the criterion is slightly higher ($\geq$0.25 mV). Presence of ST-segment elevation alone indicates myocardial injury and impending infarction; the presence of Q-waves indicates progression to infarction. For NSTEMI, ST-segment depression and/or T-wave inversion indicate ischemic changes and might not be specific to a given coronary artery distribution. There is a certain level of colocalization between ECG exploring leads and the distribution of coronary circulation: right (RCA), left anterior descending (LAD), circumflex (LCX) of left main (LM) coronary arteries (Reed et al., 2017). Therefore, the localized diagnosis of STEMI might be performed based on ECG leads involvement and grouping: lateral (might see reciprocal changes in aVR); inferior, septal (if anteroseptal, might extend to V_3, V_4), anterior (might also see changes in V_2, V_5), right (might see reciprocal ST-depressions laterally; obtain dedicated right-sided electrocardiogram), true posterior that might see ST-depressions in V_1–V_4 (obtain dedicated posterior electrocardiogram) and the risky LM occlusion.

provide functional elements to increase the probability of detecting the actual cause of MI (Pijls et al., 1996). Nevertheless, a certain relation exists between coronary arteries' distribution and ECG leads involvement in detecting ST segment shifts, to the extent that standard 12-lead ECG might be used to localize the potentially culprit artery acting on which may prove of importance when the evolution is toward recovery of localized electromechanical viability (Fig. 1).

Clinical Presentation of STEMI

Patients stacked by STEMI present with an unremarkable physical examination although they refer quite always symptoms that may include chest pain or discomfort, shortness of breath dizziness or light-headedness, nausea or vomiting, diaphoresis unexplained by ambient temperature, palpitations, and anxiety or a feeling of impending doom. Signs of heart failure, hemodynamic or electrical instability mandate quick diagnosis to form the secure base for prompt appropriate treatments. Whereas diagnosis is based on chest pain, ECG and cardiac biomarkers, the recommendations for initial diagnosis, based on the classes and levels of evidence, point to a few actions that should be provided to all patients.

STEMI: A Clinical Overview

Coronary heart disease (CHD) is the single most frequent cause of death worldwide, both in developing than developed countries, accounting for over seven million deaths each year and >12.5% of all deaths (Ezzati et al., 2006; Puddu et al., 2011; Menotti and Puddu, 2013). Although STEMI incidence has continuously declined in the last 25 years and death rate has dramatically decreased (from 30% to << 5%) after coronary care units developed in late 1960s and very effective treatments were introduced such as acute reperfusion following thrombolysis or primary percutaneous interventions along with secondary prevention treatments, both prehospital mortality and long-term mortality following STEMI still remain substantial, leaving room to primary prevention as the most effective means of reducing the global burden of CHD. In fact, when acute myocardial ischemia develops, a particularly threatening consequence is represented by arrhythmias occurring at the border of the jeopardized zone where coronary flow stops and oxygen supply is profoundly compromised. Sudden death (SD) may occur in less than 60 min from the onset of symptoms so

that only prompt hospitalization may be life-saving. In-hospital recognition (by continuous monitoring or telemetry during transport (Puddu et al., 2013)) of the potential deterioration of apparently innocent premature ventricular contractions into serious arrhythmias such as ventricular tachycardia or, after repetitive R on T phenomena, into ventricular fibrillation, may prompt electric defibrillation as the sole treatment capable to restore the heart essential pumping function.

Initial Evaluation

The prehospital phase of STEMI has been repeatedly underlined as a particularly critical issue and might be sketched as a double-edged condition: (1) efforts to increase the probability of life-saving maneuvers by bystanders (Rea et al., 2003), pointing to the need of increasing in the public the capabilities for life support and cardiac massage before delivering heart defibrillation by appropriate electric shocks, provided these defibrillators are at hand or might be rapidly put in action from ambulances; (2) need to convince patients to seek immediate medical advice thus reducing to a minimum the delay-phase from symptoms' onset, so frequently related to negation or disease' fear. Both issues might be resumed by the quote "*time is muscle*" and underline the concept whereby "the sooner the better." As orthogonal coronary circulation is terminal and irrigates a myocardial area that, in general, does not receive transverse or parallel coronary flow from collaterals, myocytes undergo irreversible damage after approximately 20 min of ischemia (Kalogeris et al., 2012). Then the irreversibly damaged area expands (from the center to the periphery in three dimensions) and having symptoms start as time zero, different time lags have been defined (from <60 to <120 min) (Reimer et al., 1977) whereby therapeutic procedures have or have not the probability of obtaining an acceptable recovery of contractile function and might be therefore considered successful. In recent years, the American Heart Association has encouraged a new quality measure called "first medical contact-to-balloon." The goal is to have the procedure completed within 90 min of emergency medical staff (EMS) arriving at the patient's side.

The 12-lead ECG is the most important diagnostic test that is used to screen patients who present with signs and symptoms of a possible STEMI (Voci et al., 2002; Manfrini et al., 2016a). In many health care systems, the ECG will be transmitted to the hospital so that physicians can read the ECG while the patient is still out of the hospital. Regionalized systems of care for STEMI have been developing all over the United States and Europe. Organizations such as the American College of Cardiology's "Door-to-Balloon Alliance" and the American Heart Association's "Mission: Lifeline" have been helping to promote best practices in STEMI care which have resulted in improved outcomes for patients experiencing this life-threatening clinical condition.

Chest Pain

A certain interindividual degree of variability may exist between the beginning of acute myocardial ischemia and symptoms onset. Animal experiments of acute coronary artery ligature induce ST segments shifts (**Fig. 2A**) in a time window of 5 sec to 5 min (Jouve et al., 1986). The clinical presentation of STEMI might be equated with the development of typical symptoms (**Fig. 2B**). All clinical complications (including SD) and delays should refer to chest pain onset as time zero (**Fig. 3**). One of the quality measures for STEMI care is the "door-to-balloon" (D2B) time or the amount of time it takes to successfully reopen the culprit artery. The clock starts when the patient arrives at the hospital and stops when the balloon is inflated in the coronary arteries. There are certain categories of patients (**Fig. 4**), like women, elderly and diabetics in whom the precise time zero might be defined with larger uncertainty (Ricci et al., 2016; Puddu et al., 2016; Bugiardini et al., 2015; Menotti and Puddu, 2016; Mehta et al., 2016). Some of these patients experience denial and dismiss their symptoms as heartburn or indigestion. When this happens they may delay seeking care for hours and angioplasty and stenting may be unsuccessful. Other conditions presenting with ST-segment elevation should be considered for differential diagnosis versus STEMI (**Fig. 5**).

Typical chest pain as disclosed after the interview is said to be characterized by a retrosternal sensation of pressure or heaviness radiating the most frequently to the left arm (more rarely to both arms or the right arm), neck and jaw, which may be intermittent with duration of several minutes and apparent disappearance or persistent. It may have developed independently of any efforts and may be related to some oxygen-increasing demand such as excitement, passion, arousal, or fear, which, in turn, may be accompanied by signs of enhanced sympathetic activation including cold sweating and higher systolic blood pressure due to increased peripheral resistances. Sometimes chest pain may be accompanied by signs of vagal activation including nausea and vomiting or sinus bradycardia. Sympathetic activation is more probable in patients with anterior STEMI whereas vagal activation accompanies more frequently patients with infero-posterior STEMI, which may imply a difference in outcome (Pocock et al., 2015).

Relief of pain by titrated intravenous opioids is recommended (Class I, Level C) once the diagnosis of STEMI is done which will be based on ECG and cardiac biomarkers abnormalities. This may also help relieving anxiety. If pain is accompanied by breathlessness, signs of hypoxemia ($SaO_2 < 95\%$) or of acute heart failure, oxygen administration is indicated (Class I, Level C).

ECG Signs

ECG should be initiated as soon as possible in all patients with suspected STEMI, as early as within 10 min of first medical contact. Although ECG may be normal, characteristic abnormalities include ST segment elevation, but sometimes patient at first presentation may show ST segment depression, and T wave changes. It is wise in these conditions to keep leads on the patient in order to repeat the 12-lead ECG at very short delay since evolution is particularly important to be documented whereas some electrical diagnostic criteria should be met in conjunction with the occurrence of typical chest pain. Code "STEMI" is an expression used in

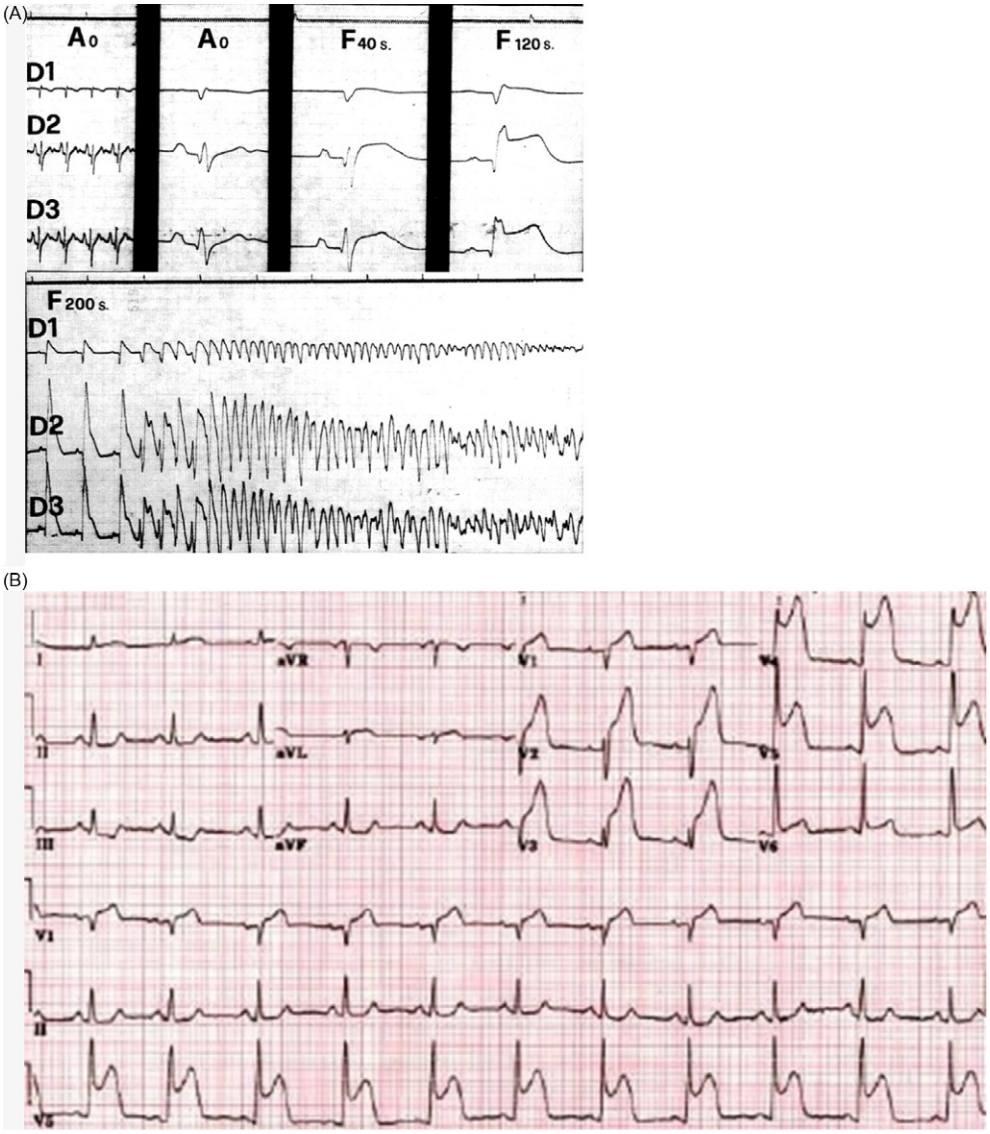

Fig. 2 ECG in experimental and clinical STEMI. Standard ECG may show immediate (earlier than 200 s) ST-segment elevation in latero-inferior leads after left circumflex coronary artery occlusion (due to ligation) in dogs (*panel A*), a critical risk factor for early postischemic ventricular fibrillation and sudden death (Jouve et al., 1986). In human, STEMI may have a similar evolution (*panel B*: here antero-septal localization) although the timing might request a longer evolution (see **Fig. 3**).

many EMS systems and emergency departments that essentially implies that physicians on first medical contact are formally requesting that a life-saving team be assembled immediately. This often means calling in the interventional cardiologist and cath-lab personnel in from home or more simply bypassing the closest hospital for the percutaneous coronary intervention (PCI)-capable hospital.

Localization of STEMI

Localization of STEMI, based on diagnostic ECG leads is important to drive the search of the potentially culprit coronary lesion (**Fig. 1**), which may give also a rough estimation of short- and long-term risks. These latter are related to the extent of the myocardial muscle loss and, thus, in part to the type of coronary artery involved. Indeed, the site of occlusion of the left anterior descending coronary artery is important in acute anterior MI because proximal occlusion is associated with less favorable outcome and prognosis. Of course, left main involvement is important as well. In that case, both left anterior descending and left circumflex coronary arteries are compromised and three quarters of the left ventricular muscle mass is at risk of acute ischemia.

Typically, ST-segment elevation in acute MI, measured at the level of the J point, should be detected in at least two adjacent leads and be of ≥ 0.20 mV in man and ≥ 0.15 mV in women in leads V_2–V_3 or ≥ 0.10 mV in the remaining leads. These cut-off values have

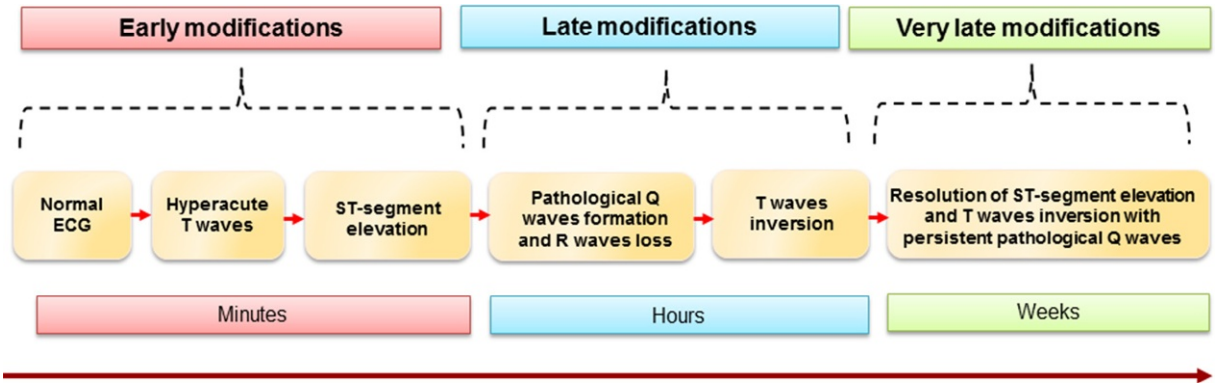

Fig. 3 Evolution of ECG in STEMI. Early to very late modifications might take weeks to occur in clinical acute myocardial infarction and are slower that what occurs after experimental coronary ligation (Goldman, 1986; Nable and Brady, 2009; Morris et al., 2008).

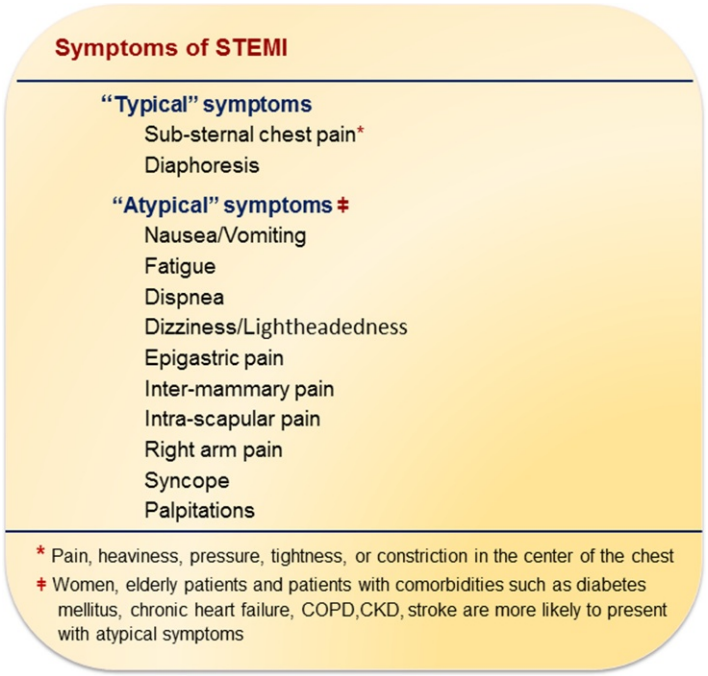

Fig. 4 Symptoms of STEMI. Typical versus atypical symptoms should be recognized in STEMI patients and might help to make the correct diagnosis in peculiar patients' groups (Vaccarino et al., 2013; Ricci et al., 2016; Milner et al., 2004; Manfrini et al.,2016b).

also an age dependency and, thus, in men younger than 40 years, the criterion is slightly higher (≥ 0.25 mV). In peculiar localizations, such as isolated right ventricular infarction with the nondominant right coronary artery, specific criteria were developed (Iannetta et al., 2013). In general, additional recording of nonstandard leads such as V_7–V_8 or V_{2R}–V_{3R} may be helpful to make the diagnosis in selected cases of infero-basal infarction where the circumflex coronary occlusion might be implicated. A quite large literature exists on standard ECG of STEMI (Nable and Brady, 2009; Ayer and Terkelsen, 2014; Deshpande and Birnbaum, 2014). Electrical abnormalities that occur at the onset of acute ischemia are easily recognized by successive ECG records where the classic evolution may be discovered, first at the level of the lesion (or Pardee's) wave consisting of the ST-T shifts and later in the development of the pathological (>40 ms in duration) Q wave. ST segment elevation in lead aVR, ST segment depression in lead V_5 and ST segment elevation in $V_1 > 2.5$ mm may strongly predict left anterior descending occlusion proximal to first septal, whereas abnormal Q wave in V_4–V_6 is often associated with occlusion distal to first septal.

Left bundle branch block

The diagnosis of STEMI in the presence of LBBB has been problematic for years. Until recently, the guidelines for management of acute MI recommended cath lab activation for patients with chest pain and new LBBB. The 2013 guidelines recommended the use of the Sgarbossa criteria, which are well known to be specific, but not sensitive enough. Still, the majority of physicians believe that

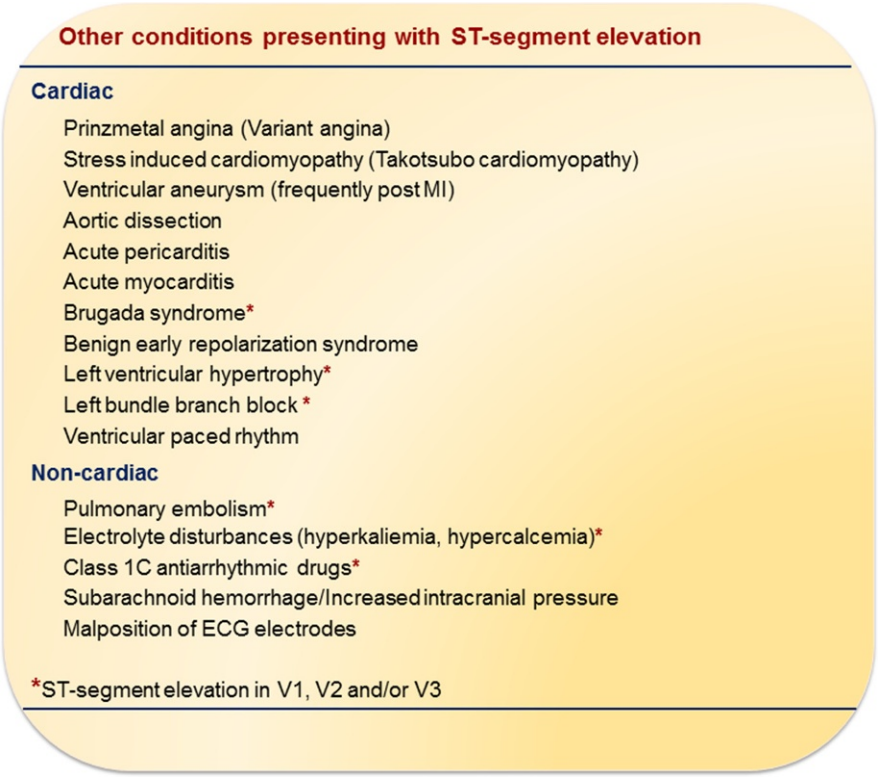

Fig. 5 Other conditions presenting with ST-segment elevation. Differential diagnosis of cardiac and noncardiac conditions should be frequently exercised and considered (Morris et al., 2008; Bonow et al., 2012; Thygesen et al., 2012b).

STEMI cannot be diagnosed in the setting of LBBB. A particularly difficult diagnosis of STEMI is that occurring in the presence of LBBB. In the presence of LBBB and typical symptoms, it is mandatory to repeat blood samples for serum marker determinations without waiting for the results before initiating reperfusion therapy (Class I, Level C).

Atypical ECG presentations

There are conditions such as ventricular paced rhythms, no ST-segment elevation but with persistent ischemic symptoms such as pain or dyspnea, isolated posterior MI, or ST-segment elevation in lead aVR that deserve prompt management and for whom appropriate differential diagnosis should be developed or the evolution of STEMI confirmed. Also among these patients, an essential target is represented by minimizing delays, not only as an objective means of indexing the quality of care but also as a practical tool for improving outcomes.

Prior myocardial infarction

STEMI resulting from total coronary occlusion leads to more homogeneous tissue damage and are usually reflected by a Q-wave MI pattern on the ECG. Pathologic Q waves are not an early sign of MI, but generally, take several hours to days to develop. If STEMI is reperfused early (e.g., as a result of PCI) stunned myocardial tissue can recover and pathologic Q waves may disappear. Definition of a pathologic Q wave can be summarized as follows: any Q-wave in leads V_2–$V_3 \geq 0.02$ s or QS complex in leads V_2 and V_3; Q-wave ≥ 0.03 s and >0.1 mV deep or QS complex in leads I, II, aVL, aVF, or V_4–V_6 in any two leads of a contiguous lead grouping (I, aVL, V_6; V_4–V_6; II, III, and aVF); R-wave ≥ 0.04 s in V_1–V_2 and $R/S \geq 1$ with a concordant positive T-wave in the absence of a conduction defect. It should be noted that the absence of pathologic Q waves does not exclude a MI.

Cardiac Biomarkers

Cardiac markers are used in the diagnosis and risk stratification of patients with chest pain and suspected STEMI (Reed et al., 2017; Anderson and Morrow, 2017). The cardiac troponins, in particular, have become the cardiac markers of choice (cardiac troponin I or T (Twerenbold et al., 2012; Reiter et al., 2012; Thygesen et al., 2012a; Shah et al., 2015)).

In 2012, the Nucleus of the Working Group of the European Society of Cardiology on Acute Cardiac Care prepared a document summarizing the use of high-sensitivity cardiac troponin in clinical routine whereby six points were defined (Thygesen et al., 2012a): (1) There is a need to use the 99th percentile concentration of the reference population as the specific cardiac troponin upper reference limit. (2) A significant change with serial testing is required for diagnosing MI. At low cardiac troponin baseline

concentrations (around the 99th percentile), the change in serial testing in order to be clinically significant requires to be marked, in case of markedly elevated baseline, a minimum change of 20% in follow-up testing is required. (3) An additional testing of other early markers of acute myocardial necrosis, such as myoglobin or creatine kinase-MB (CK-MB) is no longer needed. (4) Blood sampling in patients with suspicion of MI should be performed on admission and 3 h later. Measurement of high-sensitivity cardiac troponin should be repeated 6 h after admission in patients of whom the 3 h values are unchanged but in whom the clinical suspicion of MI is still high. (5) Cardiac troponin is a marker of myocardial necrosis and not a specific marker of MI. The latter may be only diagnosed with a rise and/or fall of cardiac troponin together with characteristic symptoms, and/or ECG changes indicative of ischemia and/or imaging evidence of acute myocardial ischemia, as stated above. In the presence of abnormal changes of cardiac troponin, therefore, also other causes of myocardial necrosis (e.g., acute heart failure or myocarditis) should be considered differentially. (6) Stable or inconsistently variable cardiac troponin values without significant dynamic changes are likely the markers of chronic structural heart disease. Thus, although elevations in the serum levels of TnI, TnT, and CK-MB indicate the presence of injury-associated necrosis of myocardial cells, such elevations do not point to the underlying mechanism of the necrosis.

There are several critical clinical concepts regarding the analytical characteristics of hs-cTn assays (Thygesen et al., 2012a). The most important criticism is that the hs-cTn assays have high precision at lower concentration ranges with an analytical coefficient of variation <10% at the 99th percentile concentration of the reference population. Thus, most TnI assays are imprecise at the 99th percentile reference limit. However, the advantage of ultrasensitive troponins is based on the premise that lower cutoff levels achieve a higher sensitivity that will allow earlier diagnosis, often within 90 min of presentation. hs-cTn is now used for confirming of ruling out MI based on an absolute value of 14 pg/mL (or 0.014 μg/L) (**Fig. 6**) as a differentiating cut-off value (Twerenbold et al., 2012).

Recently, Shah and colleagues (Shah et al., 2015; Cullen et al., 2015) reported results of a prospective observational cohort study of 6304 patients presenting at emergency departments with suspected MI. They identified a threshold for a high-sensitivity cardiac troponin I assay below which patients were at low risk of MI and potentially suitable for early discharge from the hospital. The investigators derived 5 pg/mL as the optimum cutoff for cardiac troponin I concentration, using a predefined negative predictive value of 99.5% as an acceptable threshold of safety. This threshold enabled a large proportion of the total patients presenting to emergency departments (2311 [47%] of 4870) to be identified on presentation as at low risk for events at presentation and at 30 days follow-up. This investigation represents a huge advance in the assessment of patients with possible MI in emergency departments and prompts further collaboration between clinicians and their laboratory colleagues to identify optimum assessment strategies on troponin assay.

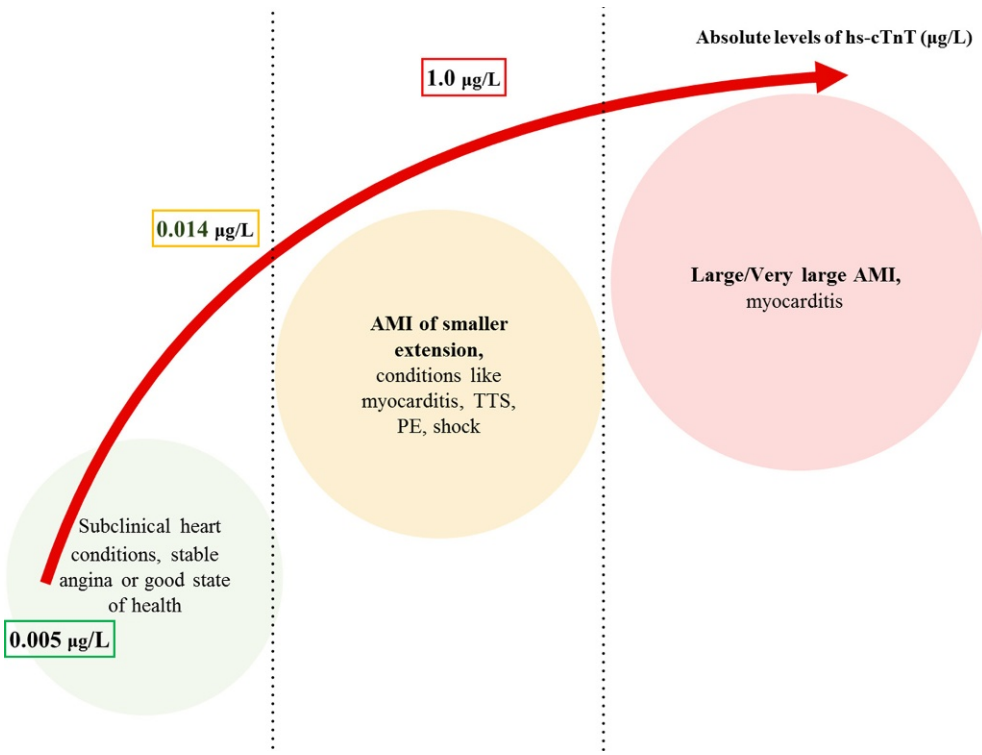

Fig. 6 Differential diagnosis based on high-sensitive troponin T. Absolute levels of hs-cTnT are directly related to the STEMI condition with a cut-off value (14 pg/mL) that might be defined with great accuracy (Twerenbold et al., 2012; Thygesen et al., 2012a).

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STEMI: Management

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Introduction

In 1971, Doctor Eugene Braunwald stated that in the setting of acute myocardial infarction "time is muscle" (Maroko et al., 1971). Subsequent numerous studies demonstrated that early reperfusion of the occluded coronary artery significantly improves in-hospital and long-term outcome, and also that benefits of reperfusion therapy are the greatest when it is performed as soon as possible from the onset of symptoms.

Consequently, the key point for a successful management of ST-elevation myocardial infarction (STEMI) patients is the timely diagnosis and the optimal revascularization of the ischemic myocardial tissue; physicians should be able to rapidly identify and treat the majority of STEMI patients, which is the most important advancement in the treatment of these patients and may positively impact morbidity and mortality (Anderson and Morrow, 2017).

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The objectives of STEMI management are:

1. Minimizing delay to reperfusion.
2. Reducing area of myocardial ongoing necrosis by selecting the timely suitable reperfusion.
3. Preventing and treating possible complications of the acute phase.
4. Preventing major adverse cardiovascular events during long-term follow-up.

Delay to Reperfusion

Definitions of Time Delays

The first medical contact (FMC) is the moment at which the patient is either initially assessed by a paramedic or a physician in the prehospital (i.e., emergency medical service (EMS)) or hospital setting (Steg et al., 2012).

The ideal time from "*FMC to diagnosis*" should be equal or inferior to 10 min. Therefore, at the point of FMC, a 12-lead electrocardiogram (ECG) should be obtained immediately with an acceptable delay $\leq$10 min.

The time between FMC and reperfusion therapy is called "*system delay*." It is an indicator of the quality of care and a predictor of outcomes. If patients undergo primary percutaneous coronary intervention (PCI), the time lag between FMC and wire passage/balloon inflation through the culprit lesion is called "*time to balloon*" and should be $\leq$120 min (preferably $\leq$90 min), whereas if the reperfusion choice is fibrinolysis (because primary PCI cannot be performed within 120 min), the time lag between FMC and starting of thrombolysis infusion is called "*time to needle*" and should be $\leq$30 min (Steg et al., 2012).

The overall time from symptom onset to reperfusion is called "*time to reperfusion therapy*" (Steg et al., 2012). It is the sum of "*system delay*" and "*patient delay*" (that is the time interval from symptom onset to FMC).

The ability of patients to recognize symptoms possibly related to myocardial ischemia and seek for medical attention is an important aspect of a successful early management of STEMI. To minimize *patient delay*, the public should be made aware of how to recognize possible symptoms of acute myocardial ischemia and about the relevance of calling immediately the EMS.

Role of the EMS

The ambulance service has a critical role in the management of STEMI because it is not only a mode of transport but also a place for initial diagnosis, triage, and treatment.

The goals of the EMS are (1) to make a working diagnosis of STEMI within 10 min, (2) to deploy emergency cardiac care, and (3) to transport the patient, in an acceptable time period, to hospital capable of advanced coronary care for reperfusion therapy (primary PCI or fibrinolysis). The availability of an EMS (i.e., ambulance), capable of recording, interpreting and/or transmitting ECGs to the emergency department (ED) or cardiac catheterization laboratory is the other fundamental factor for a successful early management of STEMI patients. The quality of the care given by EMS depends on the training of the staff. All ambulance personnel should be trained to recognize the symptoms of an acute coronary syndrome, administer oxygen, relieve pain, and provide *basic life support*. All emergency ambulances should be equipped with defibrillators and at least one person on board trained in *advanced life support*.

The initial medications to be given are aspirin (162–325 mg, chewed), sublingual nitroglycerin (0.3–0.4 mg, two times at 5 min interval) for the relief of pain, and oxygen supplementation in the case of hypoxia ($SaO_2 < 90\%–95\%$) or respiratory distress.

Candidates for primary PCI should be transferred by the EMS straight to the catheterization laboratory, bypassing the ED and intensive coronary care unit, while patient candidates for fibrinolysis can be treated in the ED, in the coronary care unit or directly in the prehospital setting (Ting et al., 2008; Amit et al., 2007). Indeed, the delay from symptom onset to treatment can be safely shortened by administration of prehospital fibrinolytic and other antithrombotic therapy, by a trained EMS unit either with a physician on board or with a hospital-based physician in direct contact, when transit time to PCI-capable hospital is longer than 120 min (O'Gara et al., 2013).

In case of cardiac arrest, advanced cardiac life support, which includes a prompt cardiopulmonary resuscitation and early defibrillation, is indicated. Therapeutic hypothermia is indicated in patients who are comatose after resuscitation, at hospital arrival, whereas infusion of cold liquid in the prehospital setting does not provide beneficial effects on outcome. Moreover, immediate angiography with a view to primary PCI is recommended in patients with resuscitated cardiac arrest whose ECG shows STEMI (Steg et al., 2012).

Early Hospital Assessment

The initial evaluation and management of patients with STEMI involves several steps which can be categorized by the following interventions, without any gender, race, and age differences.

1. Put patients at bed rest with the availability of bedside resuscitation equipment to rapidly treat sustained ventricular arrhythmias, if needed; indeed, the very early phase of an acute myocardial infarction is the most crucial phase, in which patients are at higher risk of fatal arrhythmias and cardiac arrest.

2. Get 12-lead ECG and set ECG monitoring to detect life-threatening arrhythmias and ST-segment changes (Steg et al., 2012).
3. Collect blood samples for evaluating markers of myocardial necrosis (preferably cardiac troponin).
4. Place intravenous access.
5. Take patients anamnesis and physical exam, in order to (1) recognize contraindications to recommended treatments, (2) assess signs of hemodynamic instability or left heart failure, and (3) determine baseline neurologic function (particularly if fibrinolytic therapy is to be given).

Adjunctive Medical Therapy

Most patients arrive at the hospital without the assistance of the EMS. At hospital arrival, patients who have not yet received aspirin or nitrates should receive them, immediately after the working diagnosis of STEMI is made. Soon after, morphine (if necessary) and oxygen supplementation (if necessary), other antiplatelet and anticoagulant agents (accordingly to the reperfusion strategy), as well as statin, beta-blocker, and angiotensin converting enzyme (ACE)-inhibitors should be given otherwise contraindicated (**Tables 1–4**).

The objective of the adjunctive therapy is to slow down the progression of ongoing myocardial ischemic necrosis, by means of:

– improving the oxygen myocardial demand–supply (oxygen supplementation, nitrates, ACE-inhibitors, and beta-blockers);
– antagonizing some of the pathophysiological mechanisms involved in the progression of the intracoronary thrombus: (1) platelet aggregation and activation (antiplatelet and anticoagulant agents), (2) vasoconstriction (nitrates and beta-blockers), (3) sympathetic activation (morphine, ACE-inhibitors, and beta-blockers), and (4) plaque instability (statins).

Aspirin

The great benefit of platelet aggregation inhibition therapy in patients with acute myocardial ischemia has been largely demonstrated. The use of aspirin (**Table 1**) versus placebo is associated with a decrease (>50%) in death or myocardial reinfarction (RISC Group, 1990; Cairns et al., 1985). Both American and European guidelines (Steg et al., 2012; O'Gara et al., 2013) recommend a loading dose of aspirin (162–325 mg) as soon as possible, irrespective of the reperfusion strategy (primary PCI, fibrinolysis) and even in late-comers (patients arriving at medical attention too late for reperfusion or patients who did not receive reperfusion for other reason). Moreover, indefinite low-dose aspirin (75–100 mg) is beneficial for secondary prevention (low-dose aspirin still has a protective role in ischemic events, but causes less bleeding than higher doses) (Steg et al., 2012; O'Gara et al., 2013).

Nitrates

Nitroglycerin (**Table 1**) has an endothelium-independent vasodilatory effect on the coronary and peripheral vascular beds. The administration of sublingual nitroglycerin followed by i.v. nitrates may be useful in order to relieve anginal symptoms and decrease cardiac workload in patients with uncontrolled hypertension or congestive heart failure. Hypotension, right ventricular infarction,

Table 1 Initial adjunctive therapy (Steg et al., 2012; O'Gara et al., 2013)

Drug	*Suggested dose*	*Caution/contraindication*
Aspirin	Primary PCI: loading dose 150–300 mg orally (or 80–150 mg i.v.) Fibrinolysis: starting dose 150–500 mg orally (or 250 mg i.v.) Not reperfusion: starting dose 150–500 mg orally Maintenance dose 75–100 mg/day	Active bleeding at admission. Severe chronic. Kidney disease. Allergy
Nitroglycerin	● 0.4 mg sublingual every 5 min up to 3 doses as BP allows ● i.v. dosing to begin at 10 mcg/min; titrate to desired BP effect	Suspected RV infarction. SBP <90 mmHg or if SBP >30 mmHg below the baseline. 5′-phosphodiesterase inhibitors (<24–48 h).
Morphine	● 4–8 mg i.v. initially, with lower doses in elderly ● 2–8 mg i.v. every 5–15 min if necessary	Hypotension. Bradycardia. Known hypersensitivity
Oxygen	● 2–4 L/min via nasal cannula ● Increase rate or change to face mask as needed	Caution with chronic obstructive pulmonary disease

i.v., Intravenous; *BP*, blood pressure; *RV*, right ventricular; *SBP*, systolic blood pressure; *PCI*, percutaneous coronary intervention.

Table 2 Other antiplatelet therapy (Steg et al., 2012)

Drug	*Setting*	*Suggested dose*	*Caution/contraindication*
$P2Y_{12}$ inhibitors			
Clopidogrel	Primary PCI	Loading dose 600 mg orally	Active bleeding. Use of repaglinide
	Fibrinolysis	Loading dose:300 mg orally (<=75 years old)	
	Not reperfusion	75 mg/day orally	
	Maintenance dose	75 mg/day orally	
Prasugrel[a]	Primary PCI	Loading dose 60 mg orally	Prior stroke. Transient ischemic attack. Liver disease (moderate-to-severe)
	Maintenance dose	10 mg/day (5 mg/day in pts < 60 Kg)	
Ticagrelor	Primary PCI	Loading dose: 180 mg orally	
	Maintenance dose	90 mg twice a day	
GP IIb/IIIa-inhibitors			
Abciximab	Primary PCI	Bolus of 0.25 mg/kg i.v. and 0.125 μg/kg/min infusion (max. 10 μg/min) for 12 h.	Active bleeding. Coagulation disorders. Severe hypertension
Eptifibatide	Primary PCI	Double bolus of 180 μg/kg i.v. (given at a 10-min interval), followed by an infusion of 2.0 μg/kg/min for 18 h.	
Tirofiban	Primary PCI	25 μg/kg over 3 min i.v., followed by a maintenance infusion of 0.15 μg/kg/min for 18 h.	

PCI, percutaneous coronary intervention; *i.v.*, intravenous; *GP IIb/IIIa*, glycoprotein IIb/IIIa.
[a]Prasugrel is not recommended in patients ≥ 75 years or in body weight < 60 kg.

Table 3 Anticoagulant medication (Steg et al., 2012)

Drug	*Setting*	*Suggested dose*	*Caution/contraindication*
UFH	Primary PCI	• 70–100 U/kg i.v. bolus without GPIIb/IIIa inhibitors • 50–60 U/kg i.v. bolus with GPIIb/IIIa inhibitors	Active bleeding. Thrombocytopenia with antiplatelet antibody in the presence of enoxaparin or heparin. Severe renal impairment. Severe liver disease
	Fibrinolysis[a]	60 U/kg i.v. bolus (max. 4000 U) followed by an i.v. infusion of 12 U/kg (max. 1000 U/h) for 24–48 h.	
	Not reperfusion	As in fibrinolysis	
Enoxaparin	Primary PCI	0.5 mg/kg i.v. bolus	
	Fibrinolysis	- *In pts. <75 years*: 30 mg i.v. bolus followed 15 min later by 1 mg/kg s.c. every 12 h until hospital discharge for a maximum of 8 days The first two doses should not exceed 100 mg. - *In pts. >75 years*: no i.v. bolus; start with a first subcutaneous dose of 0.75 mg/kg with a maximum of 75 mg for the first two subcutaneous doses. - *In pts. with creatinine clearance of <30 mL/min*, the subcutaneous doses are given once every 24 h.	
	Not reperfusion	As in fibrinolytic Tp.	
Bivalirudin	Primary PCI	0.75 mg/kg i.v. bolus followed by i.v infusion of 1.75 mg/kg/h for up to 4 h after the procedure as clinically warranted. Later, 0.25 mg/kg/h may be continued for 4–12 h as clinically necessary	
Fondaparinux	Fibrinolysis	2.5 mg i.v. bolus followed by a subcutaneous dose of 2.5 mg once daily up to 8 days or hospital discharge	

PCI, percutaneous coronary intervention; *UFH*, unfractionated heparin; *i.v.*, intravenous; *GP*, glycoprotein IIb/IIIa.
[a]*Target aPTT*: 50–70 s or 1.5–2.0 times that of control to be monitored at 3, 6, 12, and 24 h.

Table 4 Other adjunctive therapy (O'Gara et al., 2013)

Therapy	*Drug*	*Suggested dose*	*Caution/contraindication*
Beta-blocker	Metoprolol	• Tartrate 25–50 mg every 6–12 h orally, then transition over next 2–3 days to twice-daily dosing of metoprolol tartrate or to daily metoprolol succinate; titrate to daily dose of 200 mg as tolerated • Tartrate i.v 5 mg every 5 min as tolerated up to 3 doses; titrate to heart rate and BP	Signs of heart failure. Low output state. Increased risk of cardiogenic shock. Prolonged first-degree or high-grade. AV block. Reactive airways disease
	Carvedilol	6.25 mg twice daily, titrate to 25 mg twice daily as tolerated	
ACE-inhibitors	Lisinopril	2.5–5 mg/day to start; titrate to 10 mg/day or higher as tolerated	Hypotension. Renal failure. Hyperkalemia
	Captopril	6.25–12.5 mg 3 times/day to start; titrate to 25–50 mg 3 times/day as tolerated	
	Ramipril	2.5 mg twice daily to start; titrate to 5 mg twice daily as tolerated	
	Trandolapril	Test dose 0.5 mg; titrate up to 4 mg daily as tolerated	
ARB	Valsartan	20 mg twice daily to start; titrate to 160 mg twice daily as tolerated	Hypotension. Renal failure. Hyperkalemia
Statins	Atorvastatin	80 mg daily	Caution with drugs metabolized via CYP3A4, fibrates. Monitor for myopathy, hepatic toxicity. Combine with diet and lifestyle therapies. Adjust dose as dictated by targets for LDL cholesterol and non–HDL cholesterol reduction

ARB, Angiotensin receptor blockers; *i.v.*, intravenous; *AV*, atrioventricular; *ACE*, angiotensin converting enzyme.

or patients who have taken phosphodiesterase type 5 inhibitors (for erectile dysfunction or pulmonary hypertension) in the previous 48 h should be excluded from the administration of nitrates (Steg et al., 2012).

Morphine

Morphine (**Table 1**) is the most common analgesic used in STEMI patients, in order to relieve pain. Relief of pain is important because severe pain is associated with sympathetic activation that causes vasoconstriction and increases the workload of the heart and the risk of ventricular arrhythmias. The use of morphine may have some side effects, such as nausea and vomiting, hypotension with bradycardia, and respiratory depression (Steg et al., 2012). The hypotension and bradycardia will usually respond to atropine and the respiratory depression to naloxone (0.1–0.2 mg i.v.) (Steg et al., 2012). On the routine use of morphine in the prehospital setting, some controversies exist. Indeed, there is evidence of no benefit from prehospital morphine use, in terms of in-hospital complications and 1-year mortality (Puymirat et al., 2016).

Oxygen

Supplemental oxygen (**Table 1**), administered by mask or nasal prongs, is recommended in patients with an arterial saturation (SaO_2) < 90–95%, respiratory distress, and acute heart failure. The effects of systematic administration of oxygen in normoxic patients with STEMI, or in patients without heart failure or dyspnea, remain unclear (Cabello et al., 2016; Stub et al., 2015).

Other Antiplatelet Agents

Given that platelet activity plays a critical role in the formation and progression of intracoronary thrombus, efforts to inhibit platelet function are paramount to the management of STEMI patients. Inhibition of platelet aggregation can be achieved by different mechanisms of action, either by blockade of membrane receptors or by interaction with intracellular signaling pathways (Gresele et al., 2011). Available agents are *aspirin* (acetylsalicylic acid), which targets cyclooxygenase-1, inhibiting thromboxane A2 formation and inducing a functional permanent inhibition in platelets; *P2Y12 antagonists*, which attenuates the secondary responses to adenosine diphosphate (ADP) released by activated platelets, inhibiting activation of the glycoprotein IIb/IIIa complex and platelet aggregation; and *glycoprotein IIb/IIIa inhibitors* (abciximab, tirofiban, and eptifibatide), which target the final common pathway of platelet aggregation.

$P2Y_{12}$ Inhibitors

The $P2Y_{12}$ inhibitors are useful for further inhibiting platelet activation and aggregation. The choice of the $P2Y_{12}$ receptor blocker (*Clopidogrel*, *Prasugrel*, and *Ticagrelor*) depends on the reperfusion strategy.

Clopidogrel (**Table 2**) is a second-generation thienopyridine that irreversibly antagonizes the platelet $P2Y_{12}$ ADP receptor. Clopidogrel added to aspirin reduces the risk of death and other cardiovascular events in who will be treated with fibrinolysis (Steg et al., 2012; Chen et al., 2005a; Sabatine et al., 2005). However, in the setting of primary PCI, current guidelines prefer ticagrelor or prasugrel, because they have been proven to be superior to clopidogrel in large outcome trials in the risk of death and other major cardiovascular events at 1-year follow-up (Wiviott et al., 2007; Wallentin et al., 2009; O'Gara et al., 2013; Steg et al., 2012).

Prasugrel (**Table 2**) is a third-generation thienopyridine $P2Y_{12}$ inhibitor, which is metabolized to its active form more quickly and fully than clopidogrel, and thus has a more potent and consistent effect (Gurbel et al., 2012). The TRITON trial compared the use of prasugrel versus clopidogrel in patients with acute coronary syndromes receiving aspirin. The study showed that the use of prasugrel was associated with a reduction in risk of cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke, but an increased risk of major bleeding (Wiviott et al., 2007). Therefore, prasugrel is contraindicated in patients with prior stroke/transient ischemic attack or active pathological bleeding. Lower body weight (<60 kg) and age ≥ 75 years are relative contraindications (Steg et al., 2012).

Ticagrelor (**Table 2**) is a new $P2Y_{12}$ receptor blocker. Unlike clopidogrel or prasugrel, ticagrelor is not a thienopyridine. It is a direct-acting agent with a reversible effect, which does not require biotransformation and binds reversibly to the $P2Y_{12}$ receptors, antagonizing ADP signaling and platelet activation. The PLATO trial compared ticagrelor to clopidogrel in patients with acute coronary syndromes treated with aspirin and other standard therapy. Results showed that ticagrelor reduces the composite of cardiovascular death, myocardial infarction, or stroke at 12 months, without an increase in the rate of overall major bleeding, but with an increase in the rate of non-procedure-related bleeding (Wallentin et al., 2009).

Dual Antiplatelet Therapy

Dual antiplatelet therapy (DAPT) is the combination of aspirin and one ADP-receptor blocker (clopidogrel, prasugrel, or ticagrelor). The duration of DAPT is a matter of discussion. After coronary intervention and stent placement, struts are triggered for acute intracoronary thrombosis until a complete reendothelialization of the coronary artery. To reduce the risk of acute stent thrombosis after primary PCI, the period of DAPT, preferably with prasugrel or ticagrelor, should be up to 12 months in all STEMI patients, with a strict minimum of 1 and 6 months for patients receiving bare-metal stent and drug-eluting stent, respectively (Wiviott et al., 2007; Wallentin et al., 2009; Steg et al., 2012). DAPT with clopidogrel is indicated in addition to aspirin in adjunct to fibrinolysis (while prasugrel and ticagrelor have not been studied and should not be given) for a minimum of 14 days if coronary angiography and subsequent PCI do not increase the recommended duration of DAPT (O'Gara et al., 2013; Anderson and Morrow, 2017).

Glycoprotein IIb/IIIa (GPIIb/IIIa) Inhibitors

Abciximab, tirofiban, and eptifibatide inhibit the final common pathway of platelet activation and are the most potent antiplatelet agents (**Table 2**). Their role in the management of acute myocardial ischemia has changed through the past years. Once they were widely used. With the advent of DAPT, GPIIb/IIIa inhibitors have slowly lost their importance in routine low-risk PCI (Subban and Sarat Chandra 2013), but GPIIb/IIIa inhibitors should be considered for bailout therapy if there is angiographic evidence of massive thrombus, slow or no-reflow or a thrombotic complication (Steg et al., 2012).

Anticoagulant Agents

Benefits of the use of anticoagulant agents (**Table 3**) in STEMI patients are strongly supported by several pieces of evidence, either in patients managed invasively (primary PCI) or conservatively (fibrinolysis), and even in those STEMI patients not undergoing reperfusion. The management of acute coronary syndromes with anticoagulants can be achieved by three classes of drugs: (1) *unfractionated heparin* (UFH) and the *low molecular weight heparins* (LMWH), which are indirect thrombin inhibitors; (2) *bivalirudin*, which is a direct thrombin inhibitor (Lefkovits and Topol, 1994); and (3) *fondaparinux*, which is a synthetic heparin and an indirect inhibitor of factor Xa. The choice of the anticoagulant agent depends on the reperfusion strategy.

UFH and LMWH. It is widely demonstrated that the use of UFH in association with aspirin in the acute phase on an acute coronary syndrome reduces the incidence of myocardial infarction and death (Oler et al., 1996; RISC Group, 1990). UFH is recommended both in patients undergoing primary PCI and in those receiving fibrinolysis (Steg et al., 2012). *Enoxaparin* can be used instead of UFH both in patients receiving primary PCI or fibrinolysis. UFH is preferred in patients undergoing primary PCI, whereas enoxaparin is preferred in patients receiving fibrinolysis (Steg et al., 2012; Murphy et al., 2007).

Bivalirudin. The use of bivalirudin over UFH and GPIIb/IIIa inhibitors is recommended in patients undergoing primary PCI (Steg et al., 2012). The superiority of bivalirudin over the combination of UFH + GP IIb/IIIa inhibitor is driven by a reduction in bleeding, associated with an initial increase in stent thrombosis, which disappears after 30 days (Steg et al., 2012; Mehran et al., 2009; Stone et al., 2008).

Fondaparinux. Fondaparinux is not recommended in patients undergoing primary PCI because fondaparinux increases catheter-related thrombosis during PCI (Yusuf et al. 2006a,b).

Triple Antithrombotic Therapy

The triple antithrombotic therapy is the combination of aspirin, an ADP-receptor antagonist, and an anticoagulant agent. In STEMI patients who are not undergoing reperfusion therapy, the association between aspirin, UFH, and clopidogrel reduces in-hospital mortality and does not significantly increase the risk of major bleeding (Bugiardini et al., 2015).

Beta-Blockers

The beneficial role of beta-blockers (**Table 4**) in the very early phase of STEMI is well known (O'Gara et al., 2013; Steg et al., 2012; Bugiardini et al., 2016; Chen et al., 2005b). They exert these benefits by improving left ventricular function and in-hospital survival rate through reducing infarct size. Current guidelines recommend that *oral beta-blockers* should be initiated in the first 24 h in all STEMI patients without contraindications, such as signs of heart failure, evidence of a low-output state, and increased risk for cardiogenic shock (O'Gara et al., 2013; Steg et al., 2012). The use of *i.v. beta-blockers* should be considered in STEMI patients with ongoing ischemia or high blood pressure or tachycardia, without signs of heart failure or other contraindications (O'Gara et al., 2013; Steg et al., 2012).

Angiotensin Converting Enzyme Inhibitors

In STEMI patients with evidence of heart failure, left ventricular systolic dysfunction (ejection fraction <40%), diabetes, or an anterior infarct the use of ACE-I (**Table 4**) is indicated within the first 24 h (unless contraindicated). In patients intolerant to ACE-I, an angiotensin receptor blocker, preferably valsartan, is an alternative (O'Gara et al., 2013; Steg et al., 2012).

Statins

The beneficial effect of statins (**Table 4**) has been widely demonstrated not only for secondary prevention (Baigent et al., 2005), but also in the early phase of myocardial infarction (Schwartz et al., 2001; Cannon et al., 2004). Therefore, early high-intensity statin therapy should be administered in all STEMI patients, regardless of lipid profile (O'Gara et al., 2013; Steg et al., 2012). The PROVE-IT trial compared standard statin therapy (40 mg of pravastatin daily) with intensive statin therapy (80 mg of atorvastatin daily), showing that an intensive lipid-lowering regimen provides greater protection against death or major cardiovascular events than does a standard regimen (Cannon et al., 2004).

Reperfusion Therapy

Reperfusion therapy is the primary therapeutic goal. Reperfusion therapy is indicated in all patients with persistent ST-segment elevation (or presumed new left bundle branch block) and symptoms of ongoing ischemia.

Dissolution or removal of the intracoronary thrombus provides the best chance for mortality reduction. The atherosclerotic coronary artery plaque rupture causes intracoronary platelet aggregation, platelet activation, fibrin deposition, and downstream myocardial ischemia and necrosis. The latter is time dependent: every minute wasted increases the risk of heart failure, patient morbidity, and death. For every 30 min of ischemia, mortality increases 8–10% (Pinto et al., 2006; O'Gara et al., 2013).

Reperfusion therapy (**Fig. 1**) can be performed by pharmacological (fibrinolysis) or mechanical catheter-based (primary PCI) approaches (O'Gara et al., 2013; Steg et al., 2012).

Percutaneous Coronary Intervention

Primary PCI is defined as an emergent percutaneous catheter intervention in the setting of STEMI, without previous fibrinolytic treatment. Primary PCI is the recommended reperfusion therapy over fibrinolysis if performed by an experienced team within 120 min from FMC (O'Gara et al., 2013; Steg et al., 2012). Primary PCI should be done within 12 h from symptom onset, but even later if symptoms and ST-segment elevation are still there or have been stuttering. When primary PCI cannot be performed within 120 min from FMC, fibrinolysis should be considered, particularly if it can be given in a prehospital setting (e.g., in the ambulance) and within the first 30 min of symptom onset (Bonnefoy et al., 2002, 2009; Morrison et al., 2000; Pinto et al., 2011; Steg et al., 2012). The superiority of primary PCI has been well established by several randomized clinical trials that compared primary PCI with in-hospital fibrinolytic therapy (Zijlstra et al., 1999; Keeley et al., 2003; Andersen et al., 2003; Steg et al., 2012). Primary PCI is effective in securing and maintaining coronary artery patency and avoids some of the bleeding risks of fibrinolysis. The advantages of primary PCI over fibrinolysis include lower rates of early death, reinfarction, and intracranial hemorrhage.

According to current international guidelines, during primary PCI, in patients with significant multivessel disease, only the infarct-related artery (culprit lesion) should be treated, unless the patient is in cardiogenic shock or has persistent ischemia after PCI of the supposed culprit lesion (Steg et al., 2012). However, more recently, three randomized trials showed reductions in ischemia-driven revascularization and variable effects on the risks of recurrent myocardial infarction and death with PCI of nonculprit stenosis (Wald et al., 2013; Engstrøm et al., 2015; Gershlick et al., 2015).

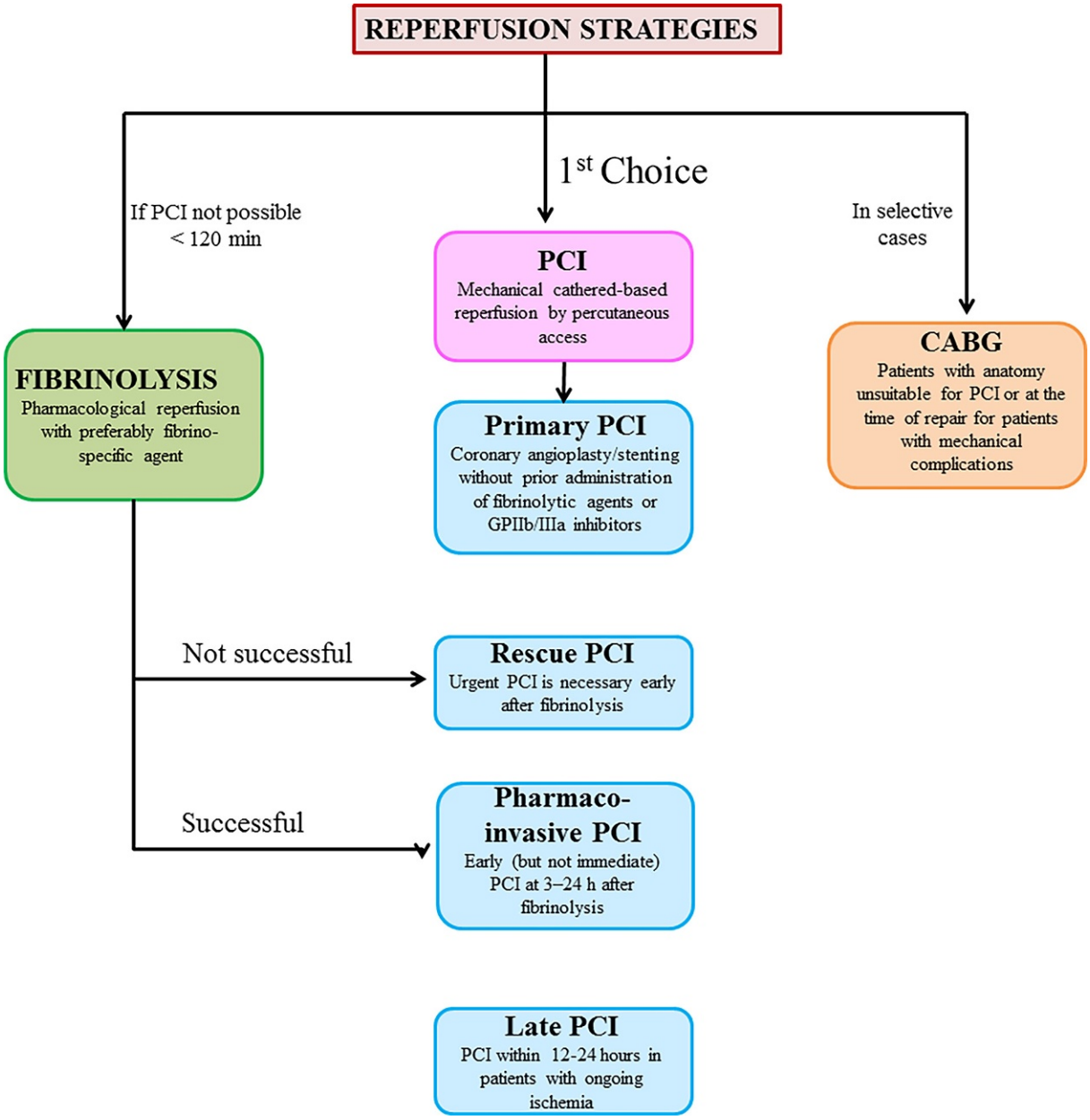

Fig. 1 Reperfusion strategies in STEMI patients (Steg et al., 2012; O'Gara et al., 2013). *PCI*, percutaneous coronary intervention; *CABG*, coronary artery bypass surgery; *GP IIb/IIIa*, glycoprotein IIb/IIIa inhibitors.

The use of stent over balloon angioplasty alone (Pinto et al., 2011; Steg et al., 2012) should be preferred because it reduces the incidence of restenosis and the rate of reintervention. For the same reason, stents coated with medication (drug-eluting stents) should be preferred to bare-metal stents. However, since drug-eluting stents carry a high risk of intracoronary acute thrombosis, prolonged DAPT is mandatory in all patients receiving drug-eluting stents. Thus, drug-eluting stents should be preferred over bare-metal stents if the patient has no contraindications to prolonged DAPT (indication for oral anticoagulation or estimated high long-term bleeding risk) and is likely to be compliant.

If primary PCI is performed by an experienced radial operator, the radial access over the femoral access should be preferred, given the lower rate of accessible bleeding (Steg et al., 2012).

During PCI, it can be reasonable to perform aspiration thrombectomy; but routine thrombectomy does not reduce mortality, recurrent myocardial infarction, heart failure, or cardiogenic shock, and might increase the risk of stroke within 30 days (Jolly et al., 2015; Fröbert et al., 2013).

Fibrinolysis

More than 30 years ago, multiple large randomized trials began to show the benefit of fibrinolytic therapy (FTT Collaborative Group, 1994) as one of the major advances in the treatment of acute STEMI. With the following arrival of primary PCI, fibrinolysis is nowadays reserved for STEMI patients presenting within 12 h from symptom onset and without contraindication to fibrinolysis, when primary PCI cannot be performed by an experienced team within 120 min (preferably 90 min) from FMC (Pinto et al., 2011; Steg et al., 2012). The greatest benefits are observed when fibrinolysis is performed within 2 h from symptom onset. The recommended absolute and relative contraindications to fibrinolysis (Steg et al., 2012) are given in **Fig. 2**.

Fibrinolytic agents are divided into two categories:

1. Nonfibrin-specific agents: streptokinase (antigenic).
2. Fibrin-specific agents: alteplase (tPA), reteplase (rPA), and tenecteplase (TNK-tPA).

Fibrin-specific agents (tenecteplase, alteplase, and reteplase) should be preferred over nonfibrin specific agents (Steg et al., 2012). Streptokinase is generally a less costly option than other fibrinolytics, but related outcomes are inferior. In the GUSTO-1 trial, alteplase was shown to reduce 30-day mortality when compared with streptokinase (GUSTO investigators, 1993). Both reteplase and tenecteplase have a similar beneficial effect as alteplase, but they are easier to administer (GUSTO III Investigators, 1997; Van De Werf et al., 1999). Moreover, tenecteplase is associated with a lower rate of noncerebral bleedings and less need for blood transfusion. **Table 5** shows the recommended doses of fibrinolysis agents.

The major complication after fibrinolysis is stroke (FTT Collaborative Group, 1994), especially during the first day after treatment. Cerebral hemorrhages are responsible for early strokes, whereas later strokes are more frequently thrombotic or embolic. The rate of intracranial bleeding ranges from 0.9% to 1.0%, whereas the rate of major noncerebral bleeds (bleeding complications requiring blood transfusion or that are life-threatening) ranges from 4% to 13% (GUSTO III Investigators, 1997; Van De Werf et al., 1999; Berkowitz et al., 1997). Patients receiving streptokinase may present hypotension and rare severe allergic reactions. The readministration of streptokinase should be avoided.

PCI After Fibrinolysis

PCI can be performed after fibrinolysis in three timely different manners.

Rescue PCI. Patients who continue to show signs of persistent ischemia after fibrinolysis, such as worsening or sustained chest discomfort, hemodynamic instability, or lack of resolution of ST elevation (by at least 50% in the worst lead at 60–90 min), should be promptly considered for an immediate coronary angiography with a window to PCI (O'Gara et al., 2013).

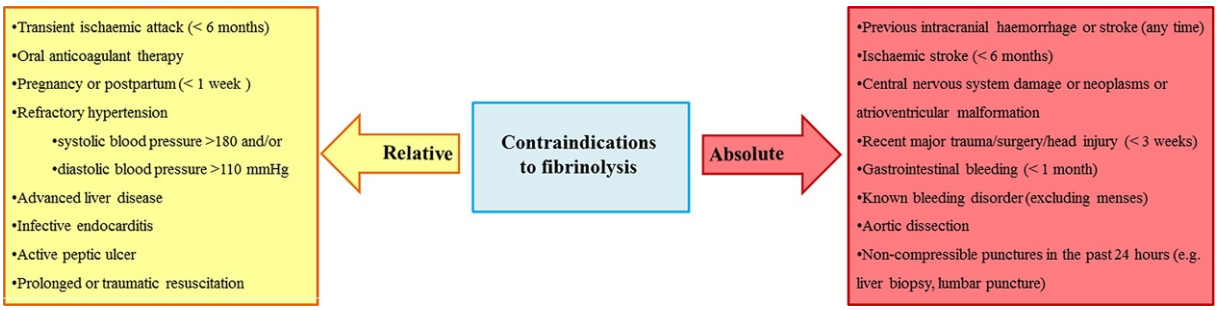

Fig. 2 Absolute and relative contraindications to fibrinolysis (Steg et al., 2012; O'Gara et al., 2013).

Table 5 Fibrinolytic agent (Steg et al., 2012)

Fibrinolytic agents	*Dose*
Streptokinase (SK)[a]	1.5 million units over 30–60 min i.v.
Alteplase (tPA)	15 mg i.v. bolus
	0.75 mg/kg (up to 50 mg) over 30 min i.v.
	0.5 mg/kg (up to 35 mg) over 60 min i.v.
Reteplase (r-PA)	10 units + 10 units i.v. bolus, given 30 min apart
Tenecteplase (TNK-tPA)	Single i.v. bolus (30 mg, if <60 kg; 35 mg, if 60–70 kg; 40 mg, if 70–80 kg; 45 mg, if 80–90 kg; 50 mg, if ≥90 kg)

[a]Contraindication: prior SK or anistreplase.

Early PCI (pharmacoinvasive strategy). The pharmacoinvasive strategy consists in early (but not immediate) PCI after fibrinolysis, that is at 3–24 h post thrombolysis. In contrast with facilitated PCI, in which PCI is performed very early after fibrinolytic therapy and has shown no benefit, recent studies have underlined the potential beneficial effect of the pharmacoinvasive strategy (Cantor et al., 2009; Di Mario et al., 2008).

Facilitated PCI. In this approach, PCI is performed very early after fibrinolytic therapy (within an hour of the initial pharmacotherapy). After early promising results, numerous studies have shown no benefits while suggesting potentially harmful complications and outcomes (increased risk of ischemic events, bleeding, and mortality) (ASSENT-4 PCI investigators, 2006; Zalewski et al., 2011; Ellis et al., 2008; Armstrong et al., 2013).

Coronary Artery Bypass Surgery

Coronary artery bypass surgery (CABG) nowadays has been substituted in most cases by primary PCI and is reserved for those STEMI patients with a coronary anatomy not suitable for PCI and ongoing ischemia or cardiogenic shock, or severe HF. In addition, CABG is performed during open heart surgery aimed at repairing STEMI mechanical complications (Pinto et al., 2011; Steg et al., 2012).

Patients Without Reperfusion Therapy

Patients who do not reach the hospital within 12 h from symptoms onset, or who have contraindications to pharmacological or mechanical reperfusion strategies, should be treated as soon as possible with an optimal medical therapy, including antiplatelet agents and anticoagulant, beta-blocker, ACE-I, and statins, unless otherwise contraindicated (O'Gara et al., 2013; Steg et al., 2012).

Management of STEMI with Normal Coronary Arteries

A patient having "normal" coronary angiography is defined as a patient in which no visible disease, or nonobstructive atherosclerotic coronary disease (epicardial artery stenosis <50%) are detectable at coronary angiography (Bugiardini and Bairey Merz, 2005; Hochman et al., 1999). In this setting, STEMI is diagnosed by clinical and electrocardiographical signs, but when the patients undergo coronary angiography, no critical lesions are detectable. Approximately 7% of STEMI patients do not show a culprit coronary lesion. Many of these patients are young women (frequently under 50 years old). In fact, 10% of women with STEMI show no culprit lesion, compared with 6% of men with the same disease (Tun and Khan, 2000; Hochman et al., 1999; Bugiardini and Bairey Merz, 2005). Furthermore, patients with normal angiograms and acute coronary syndrome seldom have cardiovascular risk factors or prior history of angina or myocardial infarction (Khan and Ansari, 1998). The etiology of this condition can be related to ischemic cardiac causes, such as coronary artery vasospasm (Ong et al., 2008), endothelial erosion (Yahagi et al., 2015), thrombophilic states (Mansourati et al., 2000; Da Costa et al., 1998, 2001; Lande et al., 1998; Larsen et al., 2005), microvascular dysfunction (Diver et al., 1994), and in situ thrombosis or embolization from a distal source with spontaneous lysis (Sharifi et al., 1995). Nonischemic causes include viral myocarditis, cardiomyopathies, pulmonary embolism (Gerbaud et al., 2012; Agewall et al., 2011), psychological factors (Ho et al., 1998), as well as the use of cocaine and methamphetamines (Minor et al., 1991; Chen, 2007.). Diagnosis requires both cardiac and noncardiac investigations and adequate drug screening. These patients present fewer complications, such as malignant arrhythmias, heart failure, and hypotension, and have been thought to have a better prognosis than patients with obstructive disease (Raymond et al., 1988; Sharifi et al., 1995.). However, recent evidence has shown that, contrary to this common belief, prognosis is not so benign, especially in women (Bugiardini and Bairey Merz, 2005; Larsen et al., 2005; Gehrie et al., 2009; Kang et al., 2011). Indeed, these patients have 2% event rate of death and myocardial infarction (Bugiardini et al., 2006). Therefore, they need to be treated with optimal medical therapy, which includes aspirin, ADP receptor antagonist, anticoagulant agent, statins, ACE-I, and calcium channel blockers, all of which have been shown to either increase survival or quality of life. Beta-blockers should be avoided when cocaine abuse is suspected (Chandrasekaran and Kurbaan, 2002).

Management of Right Ventricular Infarction

Approximately one in three patients with an inferior STEMI has a right ventricular infarction. Right ventricular involvement should be investigated in all patients with inferior STEMI. Hypotension, clear lung fields, and raised jugular venous pressure are typical of right ventricular infarction (O'Gara et al., 2013; Steg et al., 2012). The treatment of right ventricular infarction is the same as the left ventricular infarction, with the exception of diuretics and vasodilators (nitrates), which should not be included as they may cause a worsening of hypotension. Moreover, fluid loading that maintains filling pressure is essential in the treatment of this type of infarction. Atrial fibrillation or atrioventricular (AV) block, more frequently affecting these patients, should be treated as soon as possible (O'Gara et al., 2013; Steg et al., 2012).

Management of Arrhythmias

Sinus Bradycardia

Sinus bradycardia does not need any treatment, except when accompanied by severe hypotension. In this case, patients should be treated with i.v. atropine. Temporary pacing is indicated for symptomatic bradyarrhythmias unresponsive to medical treatment (O'Gara et al., 2013; Steg et al., 2012).

Supraventricular Arrhythmias

Atrial fibrillation is the most common arrhythmia complicating STEMI (incidence ranging from 6% to 28%), and is often accompanied by severe left ventricular failure (Thomsen et al., 2010; Schmitt et al., 2009; Steg et al., 2012). The administration of beta-blockers or possibly nondihydropyridine calcium antagonists (e.g., diltiazem and verapamil), if there are no clinical signs of acute heart failure, is recommended to guarantee an adequate rate control and reduce myocardial oxygen demand (Steg et al., 2012). In patients with acute heart failure or hypotension and concomitant atrial fibrillation with rapid ventricular response, amiodarone or i.v. digitalis are indicated (Steg et al., 2012). When pharmacological agents are not able to guarantee an adequate rate control in patients with atrial fibrillation and ongoing ischemia, immediate electrical cardioversion is recommended (Steg et al., 2012).

Atrioventricular Heart Block

Bradyarrhythmias and heart block are typically secondary to inferior myocardial infarction and are often self-limiting (Reed et al., 2017). First-degree heart blocks do not require treatment. Patients with second-degree Type I AV block (Mobitz I or Wenckeback) rarely develop adverse hemodynamic effects; when these patients develop adverse hemodynamic effects atropine should be the first therapeutic option, if it fails a pacing electrode should be implanted. Second-degree Type II (Mobitz II) AV block and third-degree AV block should be always considered for the insertion of a pacing electrode, certainly in the cases where bradycardia causes hypotension or heart failure (Steg et al., 2012). If the hemodynamic disturbance is severe, consideration should be given to AV sequential pacing (Steg et al., 2012). After the acute phase of STEMI, permanent pacing is indicated in patients with (1) persistent third-degree AV block, (2) persistent second degree AV block associated with bundle branch block, and (3) transient Mobitz II or third-degree AV associated with new-onset bundle branch block (Steg et al., 2012).

Ventricular Arrhythmias

Ventricular arrhythmias are the most frequent arrhythmias in the acute phase of STEMI. Ventricular arrhythmias can be classified based on time of onset: early arrhythmias arise in the first 48 h and are caused by ischemia, while secondary arrhythmias occur after 48 h and are usually due to the formation of scar tissue and the process of remodeling. In a recent study, the incidence of nonsustained ventricular tachycardia was 13%, while both sustained ventricular tachycardia and ventricular fibrillation had an incidence of 3%.

Sustained and/or hemodynamically unstable ventricular tachycardia and ventricular fibrillation require immediate and direct current cardioversion. Hemodynamically stable patients with sustained ventricular tachycardia may be treated with i.v. amiodarone, but conversion rates are low (Steg et al., 2012).

In patients with left ventricular ejection fraction <40% (assessed by echocardiography at least 40 days after the acute event), an evaluation of the risk of sudden cardiac death should be performed to consider the necessity of an implantable cardioverter-defibrillator (Steg et al., 2012).

Long-Term Management of STEMI Patients

Secondary prevention strategies are necessary to prevent recurring cardiovascular events and subsequent hospital readmissions and premature death. Patients who suffered from STEMI are considered to have a chronic coronary heart disease and are therefore at higher risk of subsequent cardiovascular events, including myocardial infarction, stroke, and death. In order to successfully prevent recurring major cardiovascular events, it is important to educate patients and their families on the importance of changes in lifestyle habits and compliance in medical therapy in terms of increasing survival and quality of life. This educational process, along with an ideal therapeutic regimen, should be instated before dehospitalization (O'Gara et al., 2013). International guidelines recommend a long-term management of STEMI patients which includes both lifestyle changes and medical therapy, and are briefly summarized in **Fig. 3**.

Lifestyle, Risk Factors, and Comorbidities

Smoking cessation is considered the most important factor of all secondary prevention measures (Thomson and Rigotti, 2003; Steg et al., 2012). When smoking cessation is successful and the prothrombotic effect of smoking is removed, the rate of death

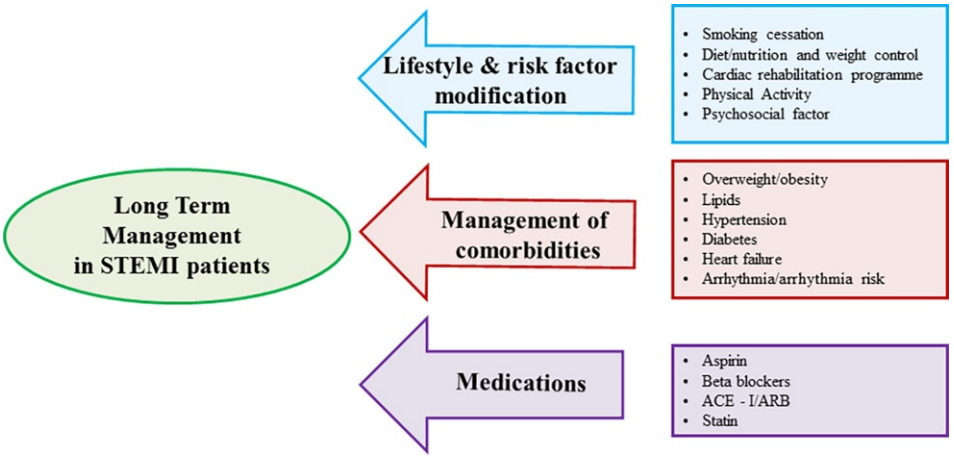

Fig. 3 Long-term management in STEMI patients (Steg et al., 2012; O'Gara et al., 2013). *STEMI*, ST-segment elevation myocardial infarction; *ACE-I*, angiotensin converting enzyme inhibitors; *ARB*, angiotensin receptor blockers.

decreases significantly when compared with patients who continued smoking. Likewise, obesity, especially central or abdominal obesity, is another major risk factor for cardiovascular disease. Even though there is little evidence of a correlation between loss of weight and reduction in mortality, guidelines recommend an ideal body mass index (BMI) of 25 kg/m^2 (Steg et al., 2012). Healthy eating habits, such as the Mediterranean diet and regular physical activity (30 min of moderate intensity aerobic exercise at least five times per week) are very important (Knoops et al., 2004; Steg et al., 2012). Following an acute myocardial infarction, systolic blood pressure should be kept <140 mmHg, but not <110 mmHg (Bangalore et al., 2010). In this complex context, the role of cardiac rehabilitation programs becomes of great utility for the reduction of risk related to chronic coronary heart disease. Cardiac rehabilitation includes exercise training, education on heart-healthy living, and counseling to reduce stress and help patients return to an active life (Cardiac Rehabilitation, 2017, www.nhlbi.nih.gov; Ades, 2001; Smith et al., 2011; Kwan and Balady, 2012).

Optimal Medical Therapy

Aspirin and other antiplatelet therapy

The protective role of aspirin on the risk of subsequent myocardial infarction, stroke, and vascular death in secondary prevention is undisputed (Hennekens et al., 1997). A maintenance dose 75–100 mg/daily should be taken indefinitely after STEMI. Clopidogrel (75 mg/day) is an alternative in patients who are intolerant to aspirin (Steg et al., 2012).

Dual antiplatelet therapy

The combination of aspirin and an ADP-receptor blocker (clopidogrel, prasugrel, or ticagrelor) is recommended to reduce the risk of stent thrombosis after PCI (Wiviott et al., 2007; Wallentin et al., 2009). DAPT must be continued in all STEMI patients undergoing primary PCI or fibrinolysis for up to 12 months (Steg et al., 2012).

Triple antithrombotic therapy

In patients with a clear indication for oral anticoagulation, the optimal duration of triple therapy (aspirin, ADP receptor antagonist, and oral anticoagulant) is still unknown. In each patient, the relative risks of bleeding and stent thrombosis should be considered (O'Gara et al., 2013; Steg et al., 2012).

Beta-blockers

The administration of beta-blockers should begin in the hospital and be continued after dehospitalization in all STEMI patients without contraindications (O'Gara et al., 2013; Steg et al., 2012).

ACE-I

Long-term use of ACE-I should be considered in all STEMI patients without a contraindication, especially in those with evidence of heart failure, left ventricular systolic dysfunction, diabetes, or an anterior infarct (Steg et al., 2012). However, some studies have shown that ACE-I may reduce the risk of death, recurrent myocardial infarction and heart failure in all patients with coronary artery disease regardless of left ventricular function (Yusuf et al., 2000; Flather et al., 2000).

Statins

The beneficial effects of statin therapy in secondary prevention are unequivocal (Baigent et al., 2005). The goal of lipid-lowering therapy after STEMI is the achievement of LDL-cholesterol serum levels $\leq$1.8 mmol/L (70 mg/dL) within 4–6 weeks (Steg et al., 2012). In addition, intensive statin therapy incrementally reduces LDL cholesterol levels and rates of nonfatal cardiovascular events. The most frequent complication associated with statins is myalgia. More severe yet rare complications include rhabdomyolysis and occasionally liver dysfunction (Golomb and Evans, 2008). Recently, two new agents (*ezetimibe* and *evolocumab*) in combination with a statin molecule (specifically simvastatin) have shown very promising results. *Ezetimibe* is a nonstatin agent, which reduces the absorption of cholesterol from the gastrointestinal tract through the action on Niemann–Pick C1-like 1 (NPC1L1) protein (Sudhop et al., 2002). The IMPROVE-IT trial showed that ezetimibe in addition to statin therapy compared to simvastatin alone lowered LDL-cholesterol and the risk of cardiovascular events (Cannon et al., 2015). *Evolocumab* is a new class of drug which is a monoclonal antibody that inhibits proprotein convertase subtilisin–kexin type 9 (PCSK9) and has recently emerged for effectively reducing LDL cholesterol levels (Giugliano and Sabatine, 2015). The FOURIER trial showed that inhibition of PCSK9 with evolocumab added to statin therapy lowers LDL-cholesterol levels and reduces the risk of cardiovascular events when compared with placebo (Sabatine et al., 2017).

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STEMI: Prognosis

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Introduction

Despite the reduction in the mortality rates, myocardial infarction (MI) remains a major cause of morbidity and mortality worldwide. Mortality rates after ST-segment elevation myocardial infarction (STEMI) are high, especially in the prehospital setting (50% of the deaths occur prior to hospital arrival) (Dudas et al., 2011). Moreover, survivors of STEMI face a substantial risk of short- and long-term cardiovascular adverse events, such as recurrent MI, stable angina, arrhythmias, heart failure (HF), stroke, and increased mortality risk. Acute atherothrombotic therapy, thrombolysis, and revascularization followed by long-term antiplatelet therapy reduce the adverse events but put these patients at higher bleeding risk. The prognosis of the individual patient depends mainly on the patient risk profile, clinical presentation, complications, and acute phase management, such as reperfusion strategies. Patient education and adherence to secondary prevention therapy have a crucial role in long-term prognosis. Therefore, knowledge concerning prognosis after STEMI is necessary to improve patient care and outcome.

Short-Term Mortality

Short-term (in-hospital or 30 day) mortality from STEMI has declined in the past two decades, which can be seen as a demonstration of the increasing use of reperfusion strategies, shortening of the total ischemic time, and increasing use of adjunctive therapies (Rogers et al., 2000; Roe et al., 2010; Schiele et al., 2010; Jernberg et al., 2011; Nallamothu et al., 2015). Currently, the short-term mortality rate of STEMI is estimated to range between 5% and 6% (O'Gara et al., 2013). Yet, in-hospital and 30-day mortality rates of patients with STEMI vary substantially across reports ranging from 3.0% to 13.3% (Gibson et al., 2008; Tatu-Chitoiu et al., 2009; Chung et al., 2014, 2015; Bugiardini et al., 2014, 2016a,b; Smith et al., 2015; Cenko et al., 2016; Kristensen et al., 2014). Outcomes are likely to be influenced by several clinical factors, such as age, sex, systolic blood pressure on presentation, heart rate, Killip class, ejection fraction, comorbidities, anatomic location of the STEMI, number of diseased vessels, flow, time, and adherence to reperfusion and preventive therapies (Steg et al., 2012; O'Gara et al., 2013). Among the factors with greater impact on mortality is the optimal use of reperfusion strategy. In patients, not undergoing any reperfusion therapy, the mortality remains high with rates ranging from 13% to 16%. Reperfusion using thrombolysis is associated with approximately 7% mortality rate, while the lowest mortality rates are reported for patients undergoing primary percutaneous coronary intervention (PCI) (3%–5%) supporting its key role in the decline of mortality rates seen in STEMI patients over these last two decades (Cenko et al, 2016; Bugiardini et al., 2014; Tatu-Chitoiu et al., 2009; Fassa et al., 2005). Greater implementations of STEMI networks and rapid reperfusion therapies, higher uptake of effective secondary preventive therapies, the availability of resources, policy initiatives, and variations in population cover may explain some of the differences in the reported mortality rates between registries and different geographical regions (Chung et al., 2014; Kristensen et al., 2014; Bugiardini et al., 2016a,b).

Long-term Mortality

Long-term mortality in patients with STEMI follows similar patterns of those seen in short-term outcomes. In the prereperfusion era, long-term mortality rates in patients with STEMI were reported nearly 20% at 1 year, rising to 30% at 5 years, with an annual mortality rate of 5% per year, most likely due to recurrent MI (Law et al., 2002). In the reperfusion era, the reported mortality rates

range from 4.2% at 1-year to 23.3% at 5 years (Haim et al., 2000; Kaul et al., 2004; Barbagelata et al., 1997; Pedersen et al., 2014; Weintraub et al., 2012; Stone et al., 2011). However, recent studies have shown that noncardiac death accounts in over 60% of long-term mortality cases in patients with STEMI. Accordingly, in contemporary STEMI patients treated with primary PCI, cardiac death is responsible for over 8% and 14% of mortality cases at 1 year and 5 years, respectively. The risk of annual cardiac mortality is reported to be less than 1.5% in these patients (Pedersen et al., 2014). A meta-analysis of 23 randomized clinical trials and over 7000 patients with STEMI, evaluated the outcomes of primary PCI as compared to thrombolysis. Overall, long-term (6–18 months) mortality was higher in patients undergoing thrombolysis as compared to primary PCI (approximately 13% vs. 9%) (Keeley et al., 2003). Long-term mortality declined substantially when data from the Should We Emergently Revascularize Occluded Coronaries for Cardiogenic Shock (SHOCK) trial, which enrolled high-risk patients in cardiogenic shock, were excluded. Excluding from the analysis patients with cardiogenic shock gives the following estimates on long-term mortality: 7% in the thrombolysis group versus 5% in the primary PCI group (Keeley et al., 2003). Similarly to short-term mortality, long-term outcomes are influenced by many prognostic factors. Among them, the strongest are age, comorbidities such as diabetes mellitus and HF, clinical presentation, time to reperfusion, the outcome of reperfusion therapy, i.e., complete restoration of coronary blood flow, and adherence to long-term secondary prevention therapies.

Sudden Cardiac Death

The reputed incidence of sudden cardiac death (SCD), after STEMI, ranges from 2% to 4% per year (Berger et al., 1992; Solomon et al., 2005). The incidence of SCD declines exponentially over time from MI. The higher incidence is reported in the first-month post-MI ranging from 1.2% to 1.4% per month as compared to 0.14% per month after 2 years (Adabag et al., 2008; Solomon et al, 2005; Zaman and Kovoor, 2014). In a recent report, the overall SCD occurred in over 15% of patients with STEMI treated with primary PCI. The median time to SCD occurrence was 2.7 years (range 1.3–5 years) (Pedersen et al., 2014). Left ventricular (LV) dysfunction is the strongest predictor of SCD. In the Valsartan in Acute Myocardial Infarction (VALIANT) trial, the incidence of SCD was markedly higher in patients with left ventricular ejection fractions (LVEF) $\leq$30% (2.3% in the first-month post-MI). Yet over half of the SCD occurred in patients with an LVEF of 31%–40% (incidence 0.90% in the first-month post-MI) (Solomon et al., 2005). According to the above-mentioned concept, prophylactic implantation of a defibrillator improves survival and should be considered as a recommended therapy as shown by the The Multicenter Automatic Defibrillator Implantation Trial II (MADIT-II). The MADIT-II trial included patients with prior MI and LVEF <30% and showed a 30% reduction in the adjusted hazard of SCD with implantable cardioverter-defibrillator (ICD) therapy at nearly 2 years follow-up (Moss et al., 2002)

Nonfatal Adverse Events

Nonfatal recurrent MI, recurrent ischemia, stroke, and bleeding are important complications after STEMI that strongly influence short- and long-term patient outcome. In the Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) trial, 30-day rates of nonfatal recurrent MI, recurrent ischemia, stroke, and major life-threatening bleeding in patients treated with thrombolysis were 4.0%, 19%, 1.4%, and 0.4%, respectively (GUSTO Investigators, 1993). In a meta-analysis of clinical randomized trials, 30-day rates of nonfatal recurrent MI, recurrent ischemia, stroke, and major bleeding in patients treated with thrombolysis were 7%, 21%, 2%, and 4%, respectively, whereas in patient rated with primary PCI were 3%, 7%, 1%, and 8%, respectively (Keeley et al., 2003). Long-term (6–18 months) rates of nonfatal recurrent MI and recurrent ischemia were 9% and 39%, respectively, in patients treated with thrombolysis and compared to 5% and 23% in patients who underwent primary PCI (Keeley et al., 2003). Contemporary trials and registry studies report rates of reinfarction, stroke, and major bleeding around 2%, 1%, and 2.8% respectively (Tobbia et al., 2013; Spencer et al., 2007). A recent registry study that assessed long-term outcomes (median follow-up 5 years) of STEMI patients treated with primary PCI reported an incidence of reinfarction, and composite of stroke, and bleedings around 6.3% and 5.6%, respectively. The median time to the occurrence of reinfarction was 8 months (range 6 days to 4.9 years) and occurrence of stroke and bleedings was 2 years (range 5 months to 4.5 years) (Pedersen et al., 2014). A longitudinal study (5.6 years of follow-up) evaluated the rate and timing of post-MI stroke found that the majority of stroke events occurred in the first month after the MI (22.6 per 1000 persons/month), most of which were ischemic. Similar to the other nonfatal adverse outcomes, the rates of post-MI strokes showed a gradual decline with an incidence of 0.9 per 1000 persons/month at 5-year follow-up (Witt et al., 2005). In the Thrombolysis in Myocardial Infarction Phase II (TIMI-II) trial, patients who experienced a nonfatal reinfarction had a twofold increase in the cumulative 3-year mortality rate (14.1% vs. 7.9%) (Mueller et al., 1995). In a posthoc analysis of GUSTO-I and GUSTO-IIb trials, symptomatic recurrent ischemia doubled the risk of reinfarction or mortality at 30 days, although the risk of reinfarction or mortality declined gradually at 6 months and 1 year, patients with hemodynamic instability maintained a higher the long-term risk (Betriu et al., 1998; Armstrong et al., 1998). Likewise, silent (not symptomatic) recurrent ischemia is associated with a worse prognosis in these patients (Bugiardini et al., 1991; Solimene et al., 1993). Patients who experience a stroke or major bleeding events are at higher mortality risk. Post-MI stroke events were associated with an almost threefold increase in the mortality risk irrespective of follow-up time (Witt et al., 2005). In the Global Registry of Acute Coronary Events (GRACE), major bleeding events in patients with MI accounted for over 10% of hospital mortality (Spencer et al., 2007). Risk factors for nonfatal outcomes after STEMI include older age, women, comorbidities such as diabetes, HF or shock, prior history of stroke and renal failure, anemia, use of fibrinolytic therapy, and antithrombotic therapy (O'Gara, et al., 2013).

Complications After STEMI

HF and cardiogenic shock complicate from 5% to 19% of patients with STEMI occurring in most cases during the first 24 h from hospital admission (**Figs. 1** and **2**). Patients presenting with HF or cardiogenic shock are at increased risk for mortality, reinfarction, and other adverse events. Using the Killip classification (Killip and Kimball, 1967) (**Fig. 1**), which stratified patients presenting with acute MI based on physical findings that can suggest HF, pulmonary edema or cardiogenic shock, the GRACE and National Registry of Myocardial Infarction II (NRMI-II) registries reported in-hospital mortality rates ranging from 17% to 21% in patients presenting with HF (Killip class II and III) as compared to those without HF (4%–7%) (Steg et al., 2004; Wu et al., 2002; Pedersen et al., 2014; Singh et al., 2007). Cardiogenic shock is associated with even higher mortality rates ranging from 40% to 70%, especially in the absence of aggressive medical care. The SHOCK trial showed that 6-month mortality rates were lower in patients with STEMI presenting with cardiogenic shock who underwent revascularization therapy (50% vs. 63%) (Hochman et al., 1999). A recent posthoc analysis of GUSTO-I trial showed that in this subset of STEMI patients, reperfusion with thrombolysis was associated with a 50% mortality rate at 30-day and 70% mortality rate at 11 years (Singh et al., 2007). Therefore, these patients should undergo prompt reperfusion regardless of time delay (O'Gara, et al., 2013; Steg et al., 2012).

Ventricular fibrillation (VF) and sustained ventricular tachycardia (VT), (**Fig. 2**) are common life-threatening complications after STEMI (**Fig. 2**). In the prereperfusion era, the incidence of VF/VT was high ranging from 4% to 40% (O'Doherty et al., 1983; Bigger et al., 1977). In the reperfusion era, the incidence of VF/VT has declined substantially, ranging from 3% to 10% in patients treated with thrombolysis (Volpi et al., 1998; Newby et al., 1998) and 6% in patients treated with primary PCI (Mehta et al., 2009). In the Assessment of Pexelizumab in Acute Myocardial Infarction (APEX AMI) trial, where patients with STEMI undergoing primary PCI were evaluated, 90% of the cases of VF/VT occurred in the first 48 h (primary VF/VT) from presentation (Mehta et al., 2009). The overall 3-month mortality rates were higher in patients who experienced VF/VT (23% vs. 3.6%). Late VF/VT (after the end of the primary PCI) was associated with a fivefold increase in 3-month mortality as compared with those patients who experienced early VF/VT (before primary PCI) (Mehta et al., 2009). The prognostic factors associated with the occurrence of VF/VT are reported to be higher Killip classes, hypotension, male sex, lower preprocedural (lower than 1) and postprocedural (lower than 3) TIMI blood flow grades, electrolyte disturbances, and ST-segment resolution less than 70% (Mehta et al., 2009; Volpi et al., 1998; Gheeraert et al., 2006). Administration of early (within 24 h) beta-blockers is associated with a reduction in the occurrence of VF/VT and reduced mortality rates (Mehta et al., 2009; Bugiardini et al., 2016b).

Atrial fibrillation (**Fig. 2**) is the common supraventricular tachycardia following STEMI with an incidence ranging from 6% to 22% (O'Gara et al., 2013; Schmitt et al., 2009). In the Cardiac Arrhythmias and Risk Stratification after Acute Myocardial Infarction (CARISMA) study, the long-term incidence of atrial fibrillation after MI in patients with LVEF <40% was approximately 30% at 2 years follow-up (Jons et al., 2011). Atrial fibrillation after STEMI has important prognostic implications as it is associated with

Killip classification

Killip Class	Clinical signs	Incidence	Mortality
Class I	No clinical signs of heart failure	33% to 79%	2% to 6%
Class II	Rales or crackles in the lungs, an S3 gallop, and elevated jugular venous pressure	12% to 38%	8% to 17%
Class III	Manifest acute pulmonary edema	5% to 10%	27% to 38%
Class IV	Cardiogenic shock, systolic blood pressure <90 mmHg, evidence of low cardiac output	3% to 19%	40% to 70%

Fig. 1 Killip classification for acute MI, incidence, and related mortality rates. Data from Killip, T., 3rd and Kimball, J. T. (1967). Treatment of myocardial infarction in a coronary care unit. A two year experience with 250 patients. *The American Journal of Cardiology* **20**, 457–464; El-Menyar, A., Zubaid, M., AlMahmeed, W., et al. (2012). Killip classification in patients with acute coronary syndrome: Insight from a multicenter registry. *The American Journal of Emergency Medicine* **30**, 97–103; Hochman, J. S., Sleeper, L. A., Webb, J. G., et al. (1999). Early revascularization in acute myocardial infarction complicated by cardiogenic shock. SHOCK Investigators. Should we emergently revascularize occluded coronaries for cardiogenic shock. *The New England Journal of Medicine* **341**, 625–634; Singh, M., White, J., Hasdai, D., et al. (2007). Long-term outcome and its predictors among patients with ST-segment elevation myocardial infarction complicated by shock: Insights from the GUSTO-I trial. *Journal of the American College of Cardiology* **50**, 1752–1758; Steg, P. G., James, S. K., Atar, D., et al. (2012). ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *The European Heart Journal* **33**, 2569–2619; O'Gara, P. T., Kushner, F. G., Ascheim, D. D., et al. American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. (2013). 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation* **127**, e362–e425. Nguyen, H. L., Yarzebski, J., Lessard, D., Gore, J. M., McManus, D. D., Goldberg, R. J. (2017) Ten-Year (2001-2011). Trends in the Incidence Rates and Short-Term Outcomes of Early Versus Late Onset Cardiogenic Shock After Hospitalization for Acute Myocardial Infarction. *Journal of the American Heart Association* **6**, e005566. Babaev, A., Frederick, P. D., Pasta, D. J., Every, N., Sichrovsky, T., Hochman, J. S., NRMI Investigators. (2005). Trends in management and outcomes of patients with acute myocardial infarction complicated by cardiogenic shock. *Journal of the American Medical Association* **294**, 448–454.

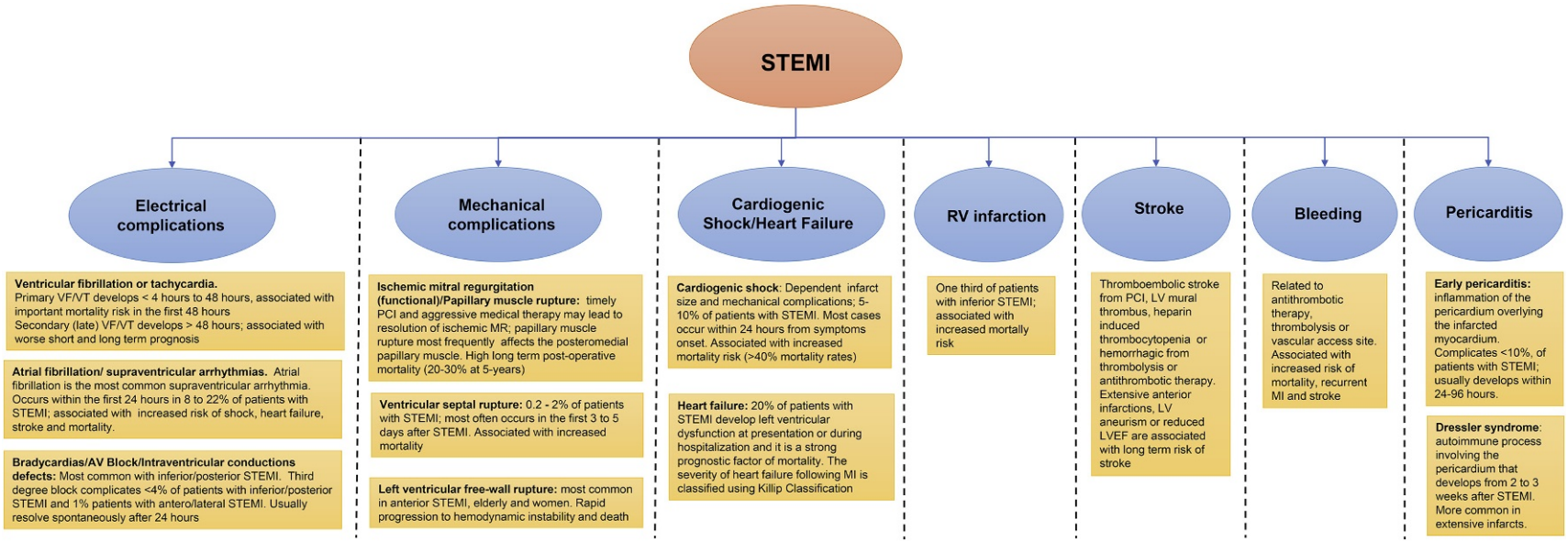


Fig. 2 Complications of STEMI. Data from Steg, P. G., James, S. K., Atar, D., et al. (2012). ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *The European Heart Journal* **33**, 2569–2619; O'Gara, P. T., Kushner, F. G., Ascheim, D. D., et al. American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. (2013). 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation* **127**, e362–e425; Reed, G. W., Rossi, J. E. and Cannon, C. P. (2017). Acute myocardial infarction. *Lancet* **389**, 197–210.

higher mortality, reinfarction, stroke, cardiogenic shock, and HF (Jons et al., 2011; Crenshaw et al., 1997). Atrial fibrillation requires oral anticoagulant therapy exposing these patients to increased risk for future bleeding events. The strongest clinical factors associated with the incidence of atrial fibrillation were found to be HF, Killip Class IV, and older age (Schmitt et al., 2009).

Bradycardias (**Fig. 2**) such as atrioventricular blocks and interventricular conductions defects can develop in approximately 4% of patients, especially in inferior/posterior STEMI (Hreybe and Saba, 2009).

Mechanical complications (**Fig. 2**) such as mitral regurgitation, ventricular septal rupture, left ventricular wall rupture, and ventricular aneurysm occur in the first 24 h (the majority) to 1 week after the clinical presentation. These conditions are associated with high mortality rates approaching 20%–60%, especially in the setting of ventricular septal rupture, and left ventricular wall rupture (O'Gara et al., 2013).

Acute pericarditis (**Fig. 2**) after STEMI has shown a decline in the incidence following reperfusion strategies with either fibrinolysis or primary PCI. The incidence of early (periinfarction) pericarditis is reported in around 12% of the nonreperfused patients, 5% in those patients treated with fibrinolysis (Correale et al., 1993; Wall et al., 1990), and 4% in those patients who underwent primary PCI (Imazio et al., 2009). The presence of a pericardial friction rub in the first 48–72 h after STEMI is associated with larger infarct size, higher incidence of anterior wall MI, and three-vessel disease, LV dysfunction and, consequently, increased short- and long-term mortality (Wall et al., 1990; Correale et al., 1993). The incidence of Dressler syndrome (late pericarditis or post-cardiac injury syndrome) was reported to be as low as 0.1% in contemporary patients treated by primary PCI. Dressler syndrome is usually self-limited and carries a relatively good prognosis, although some patients will experience recurrences (O'Gara, et al., 2013; Imazio et al., 2009).

Readmissions Rates Following STEMI

The overall rate of unplanned 30-day readmissions following STEMI is reported to range from 10% to 30%. Complication of revascularization procedures such as coronary angiography or PCI during index hospitalization, higher Killip classes, women (especially if less than 65 years old), elderly patients, those with diabetes, chronic pulmonary disease, anemia, longer index hospitalization stay, baseline quality of life, and depression are among the strongest factors associated with increased 30-day readmission rate after STEMI (Dreyer et al., 2015; Hess et al., 2016; Dunlay et al., 2012; Tisminetzky et al., 2015; Joynt et al., 2011; Curtis et al., 2009; National Quality Measures Clearinghouse (NQMC), 2015). Unplanned readmission rate is an indicator of the quality of care, therefore early risk stratification and collaboration between physicians and patients could prevent part of early readmissions.

Risk Stratification After STEMI

Given the increasing number of therapeutic options and the wide spectrum of risk in STEMI patients, risk stratification is not simply a hospital admission or predischarge commission, but it requires attention all throughout the continuum of postdischarge care. So, the current report will distinguish early prehospital or in-hospital risk stratification that should be done within the first four to six hours of hospitalization and late hospital risk stratification that is performed before or sometimes immediately after discharge.

Early Prehospital or In-Hospital Risk Stratification Using Prognostic Scores

The three major determinants of early prognosis in STEMI include (1) the extent of myocardial injury, (2) the extent of coronary artery disease, and (3) the instability of the disease and its refractoriness to management. Scores represent a simple, convenient method of risk stratification, in which a number of independent risk factors on presentation are shown to have prognostic significance. The Global Registry of Acute Cardiac Events (GRACE) risk score is a prominent example of the scoring system. It was calculated for 6-month mortality and was subsequently refined for 30-day outcomes (Eagle et al., 2004; Huang et al., 2016). The GRACE risk score measures different aspects of the extent of myocardial damage such as Killip Class, heart rate, systolic blood pressure, ST-segment deviation, raised cardiac enzymes, and resuscitation from cardiac arrest. It combines this information with other possible related variables such as age and serum creatinine concentration (**Fig. 3**). The Thrombolysis in Myocardial Infarction (TIMI) risk score focuses on 30-day as well as 1-year mortality (Morrow et al., 2000, 2001). The TIMI risk score seems to look more at the extent of coronary artery disease and its instability including predisposing factors such as diabetes mellitus, hypertension, or the prior occurrence of angina pectoris (**Fig. 4**). The Controlled Abciximab and Device Investigation to Lower Late Angioplasty Complications (CADILLAC) risk score was used to predict the 30-day and 1-year mortality and was based on patients treated with primary PCI (Halkin et al., 2005). As so, it gives primarily an overview of the extent of coronary artery disease and the pathophysiology of coronary blood flow. The advantage of these scoring systems is that they summarize important prognostic information of the disease in a single number, confidence limits can be easily calculated, and survival rates can be easily compared between given treatments.

Estimation of Long-Term Prognosis

A major issue in the management of STEMI is the risk stratification of those individuals who have survived the initial hospital admission, and are being prepared for discharge. Despite a substantial improvement in intrahospital assessment and care, patients

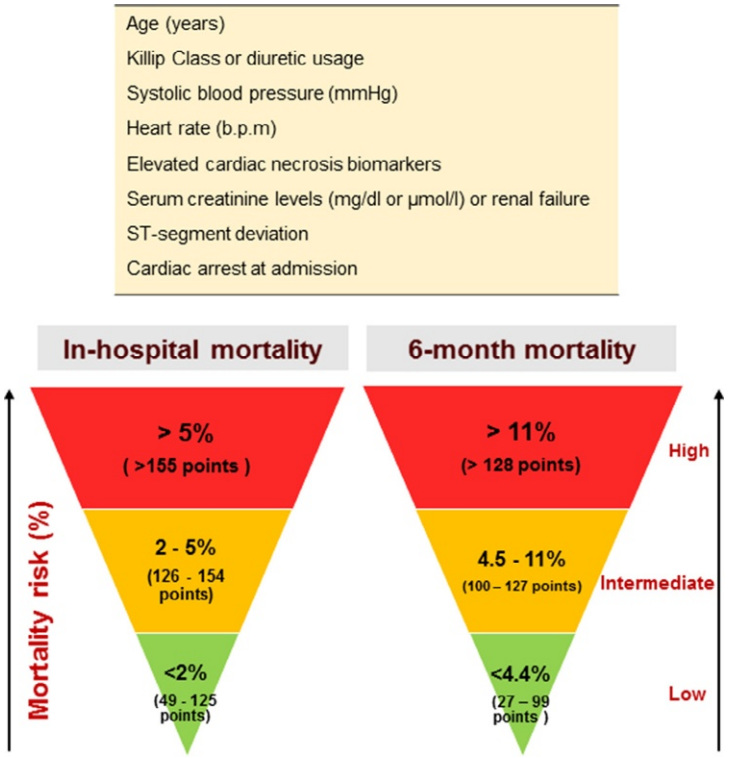
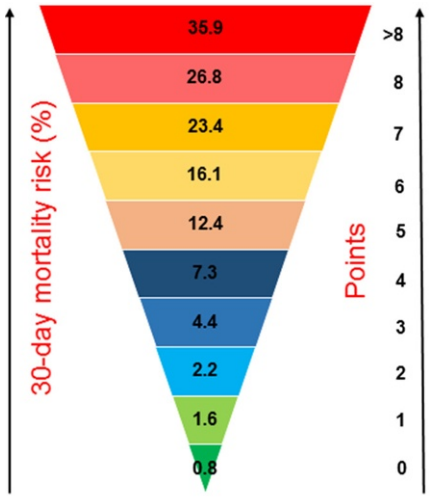


Fig. 3 GRACE risk score for in-hospital and 6-month mortality risk following ST-segment elevation myocardial infarction. Data from Eagle, K. A., Lim, M. J., Dabbous, O. H., et al. (2004). A validated prediction model for all forms of acute coronary syndrome: Estimating the risk of 6-month postdischarge death in an international registry. *JAMA* **291**, 2727–2733; Huang, W., FitzGerald, G., Goldberg, R. J., et al. (2016). Performance of the GRACE risk score 2.0 simplified algorithm for predicting 1-year death after hospitalization for an acute coronary syndrome in a contemporary multiracial cohort. *The American Journal of Cardiology* **18**, 1105–1110; https://gracescore.org/).



Age	
> 65 years old	0 points
65 – 74 years old	1 point
≥ 75 years old	2 points
Risk factors	
Diabetes mellitus, Hypertension or Angina pectoris	1 point
Clinical presentation	
Systolic blood pressure <100 mmHg	3 points
Heart rate over 100 b.p.m	2 points
Killip Class > 1	
Body weight < 67 kg (150 lb)	1 point
ECG	
Anterior ST –segment elevation or left bundle branch block	1 point
Treatment	
Time treatment over 4 hours	1 point

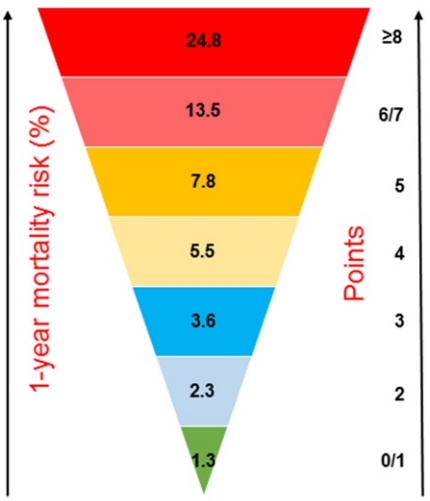
Fig. 4 TIMI risk score for 30-day mortality risk following ST-segment elevation myocardial infarction. Data from Morrow, D. A., Antman, E. M., Charlesworth, A., et al. (2000). TIMI risk score for ST-elevation myocardial infarction: A convenient, bedside, clinical score for risk assessment at presentation: An intravenous nPA for treatment of infarcting myocardium early II trial substudy. *Circulation* **102**, 2031–2037; Morrow, D. A., Antman, E. M., Parsons, L. et al. (2001). Application of the TIMI risk score for ST-elevation MI in the National Registry of Myocardial Infarction 3. *JAMA* **86**, 1356–1359; https://www.timi.org/index.php?page=calculators).

still have a considerably high incidence of medium to long-term adverse outcomes. Remarkably, approximately one-quarter of the patients in the NRMI-III database (Morrow et al., 2001) were found to be at high risk, using the TIMI risk score. At 3 years follow-up, the benefit of primary PCI was restricted to those at high risk, whereas a trend toward high mortality was seen in the majority of patients at low risk (Thune et al., 2005). Hence, once the issues of early risk assessment and the establishment of the response to reperfusion have occurred, a profile of risk should be established by characterizing the presence of inducible ischemia, the extent of

LV functional impairment, and the occurrence of malignant ventricular arrhythmias. Simply acquired clinical electrocardiographic (ECG) variables contain important prognostic information. Old and new prospective cohort studies have identified that ST shifts at 24-h ECG recordings are factors independently predictive of postdischarge outcome (Bugiardini et al., 1995; Manfrini et al., 2003; Richardson et al., 2009). These data imply plausible pathogenetic mechanisms that fit with the widely accepted notion of the disease. ST-segment shifts could reflect instability where persistence of acute stimuli may cause waxing and waning of intracoronary thrombosis and related transient myocardial ischemia. ST-segment shifts could trigger fatal arrhythmias (Pozzati et al., 1996). Signal-averaged ECG studies may be useful to identify those patients at risk for malignant ventricular arrhythmias (Steg et al., 2012; O'Gara et al., 2013). In addition, fundamental to risk stratification is the recognition that LV function is the most powerful predictor of survival. All patients should have an assessment of their LVEF in order to enhance profiling of their risk and guide medical therapy. If the LVEF is $\leq$40%, once medical therapy is optimized and after at least 1-month has intervened, then an automatic implantable defibrillator should be implanted (Steg et al., 2012; O'Gara et al., 2013). If coronary anatomy is not established as part of the initial reperfusion strategy, then a functional noninvasive test should be undertaken in order to establish the need for angiography. Profound early ischemia with standard stress tests identifies patients at relatively high risk of a subsequent cardiac event and coronary arteriography is indicated. Postinfarction residual ischemia carries a poor prognosis but is readily amenable to treatment, usually by coronary revascularization. In summary, long-term risk stratification depends critically on the underlying mechanism of disease. A better understanding of the significance of each prognostic factor is needed to quantify its strength and assess its relative importance in different subsets of patients.

Using Prognostic Scores for Late In-Hospital Risk Stratification

Risk stratification "*over time*" *is* a dynamic process, altered by a patient's changing clinical conditions. There is, therefore, a need for a risk stratification method that can be easily quantifiable at the bedside, and used as an additional component of an existing early risk stratification scoring system. Current areas of research try to address this issue. The dynamic TIMI risk score is an updated stratification model of the classic TIMI risk score (Amin et al., 2013) and includes hospital events. It also includes the concurrence of diabetes, hypertension body weight, and time delay to treatment. It is designed for the prognosis of all-cause mortality at 1 year. The baseline TIMI risk score has 0–14 potential points (**Fig. 4**), the dynamic TIMI risk score has a total of 0–29 potential points, with 0–15 points assigned based on the above mentioned in-hospital events. Scores 0–2 and all scores $\geq$8 are collapsed into a single category (**Fig. 5**). There is an approximate 25% absolute increase in 1-year mortality after discharge between a low score (0/1) and a high score ($\geq$8). These data are rational, and the underlying concept even more. Over time, a patient's condition can change, altering estimated mortality. The strength of a prognostic factor may be greater during one period of time than another.



Age	
65 – 74 years old	2 points
> 75 years old	3 points
Risk factors	
Diabetes mellitus, Hypertension or Angina pectoris	1 point
Clinical presentation	
Systolic blood pressure <100 mmHg	3 points
Heart rate over 100 b.p.m	2 points
Killip Class > 1	2 points
Body weight < 67 kg (150 lb)	1 point
ECG	
Anterior ST –segment elevation or left bundle branch block	1 point
Treatment	
Time treatment over 4 hours	1 point
Index hospital events	
Recurrent myocardial infarction	1 point
Stroke	5 points
Major bleed	1 point
Congestive heart failure or shock	3 points
Arrhythmia	2 points
Renal failure	3 points

Fig. 5 DYNAMIC TIMI risk score for 1-year mortality risk following ST-segment elevation myocardial infarction. Data from Amin, S. T., Morrow, D. A., Braunwald, E., et al. (2013). Dynamic TIMI risk score for STEMI. *Journal of the American Heart Association* **2**, e003269.

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Structure and Function of the Adult Vertebrate Cardiovascular System

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Anatomy of the Vasculature

Our overall goal is to provide the reader with a basic overview of cardiovascular structure with special emphasis on the human condition. Additionally, we discuss how this structure impacts cardiovascular function. We hope this overview will provide the reader the foundation upon which further exploration of cardiovascular structure/function can be elicited.

Evolution of the Cardiovascular System: A Very Brief Overview

The human cardiovascular system is a closed, endothelial cell-lined system with complex variation in vessel structure that is essential for its dynamic and diverse functions. Additionally, the human system is powered by a two-sided, four-chambered heart that moves blood directionally and circulating oxygenated and deoxygenated blood. Also, an independent lymphatic system that at its most proximate end connects with the cardiovascular system is present throughout the human body. It should be noted this configuration is rare in the animal kingdom. Indeed, no invertebrate possesses a closed, endothelial cell-lined system or a heart (Bettex et al., 2014; Monahan-Earley et al., 2013). Further, vertebrates, of which the human being is a member, compose only 3% of living animals. Two points emerge from this discussion. First, the cardiovascular system that we will describe later does not reflect the majority of circulatory systems seen in the animal kingdom. Our focus is on the complexity and nuance of the human condition. Second, it should be noted that there is incredible diversity in the systems that transport blood and fluids in organisms of the animal kingdom. Evolution of the human cardiovascular system is intertwined with the structure of systems seen in less complex animal models and reflects the diversity in pressures driving adaptive changes in cardiovascular structure over time. The reader is encouraged to explore the vast literature on diversity in cardiovascular structure and evolution of the cardiovascular system as the current structure and function of the human heart, and blood vessels are a reflection of that history.

Overall Structure and Function of the Human Cardiovascular System: An Overview

In simplest terms, the role of the cardiovascular system is to transport blood, and indeed, this is the underlying function driving all its activities. Still, there is a huge diversity of function in movement of blood in the human body. We will consider the diversity of cardiovascular function in the context of each component of the system. One can first subdivide the cardiovascular system into its heart and vascular tree. The heart is the centralized pump that receives blood into its left and right atria and expels blood from its left and right ventricles. Blood vessels of the cardiovascular tree distribute blood to and from the diverse components of the body to enable diverse interaction with those tissues and organs.

The Heart

The heart is the central organ of the cardiovascular system and is specifically constructed to generate high-pressure blood flow in a coordinated manner by conduction of an electric signal throughout the heart. Gross anatomically, the heart is housed in the pericardial sac. This sac and the heart are covered by a continuous simple squamous epithelium (mesothelium) creating a pericardial space between the outer sac and the surface of the heart. The major blood vessels entering and exiting the heart are

housed within the mesentery of the pericardial sac. Pericardial fluid is secreted by this epithelium and serves as a lubricant on which the heart can slide as it pumps blood. Inflammation of this space can cause the very painful pericarditis (Mann et al., 2014).

Four main chambers make up the functional area of the heart: the right and left atrium and the right and left ventricle. Fig. 1 gives a schematic representation of human heart structure. Blood returns to the heart via the pulmonary vein and vena cava entering the left and right atria. Blood flows from the atria to the ventricles through the atrioventricular (AV) valves, which prevent regurgitation to blood and ensure unidirectional flow. The right side of the heart is specialized for pulmonary circulation, which delivers deoxygenated blood to the lungs in order to supply oxygen to the blood. Deoxygenated blood enters the right atrium from the inferior and superior vena cava. Once in the right atrium, blood is pumped through the tricuspid valve (right AV valve) into the right ventricle. The right ventricle pumps the deoxygenated blood through the pulmonary semilunar valve into the pulmonary artery, which immediately branches into the left and right pulmonary arteries that enter the lungs. Once the blood has been oxygenated in the lungs, blood flows into the left atrium from the pulmonary vein, where it is pumped through the bicuspid valve (left AV valve) into the left ventricle. The thick myocardium of the left ventricle pumps sending the blood through the aortic semilunar valve into the aorta with high pressure to the tissues of the body (systemic circulation). The left and right ventricles are separated by the interventricular septum. The majority of this septum consists of a thickened muscular domain, the muscular interventricular septum, and a thinner and smaller membranous interventricular septum. The left and right atria are divided by the interatrial septum, which is thinner than the interventricular septum due to the smaller forces exerted by the atria as compared with the ventricles. The two AV valves (bicuspid and tricuspid valves) have fibrous cords known as the chordae tendineae that extend to muscular fibers connected to the walls of the ventricle called papillary muscles. Atria are much thinner than their ventricular counterparts and largely serve as a momentary holding place for blood returning to the heart.

The heart, much like arterial and venous systems, is arranged in three layers or laminae. The outermost domain is the epicardium, which has the aforementioned outer mesothelial cell layer. Subjacent to this mesothelium is a connective tissue layer that houses coronary blood vessels and nerves supplying the heart. Adipose cells also reside in the epicardium and may build up with age or in a diseased state. This layer is analogous to the tunica adventitia of vessels although the mesothelial layer is not seen

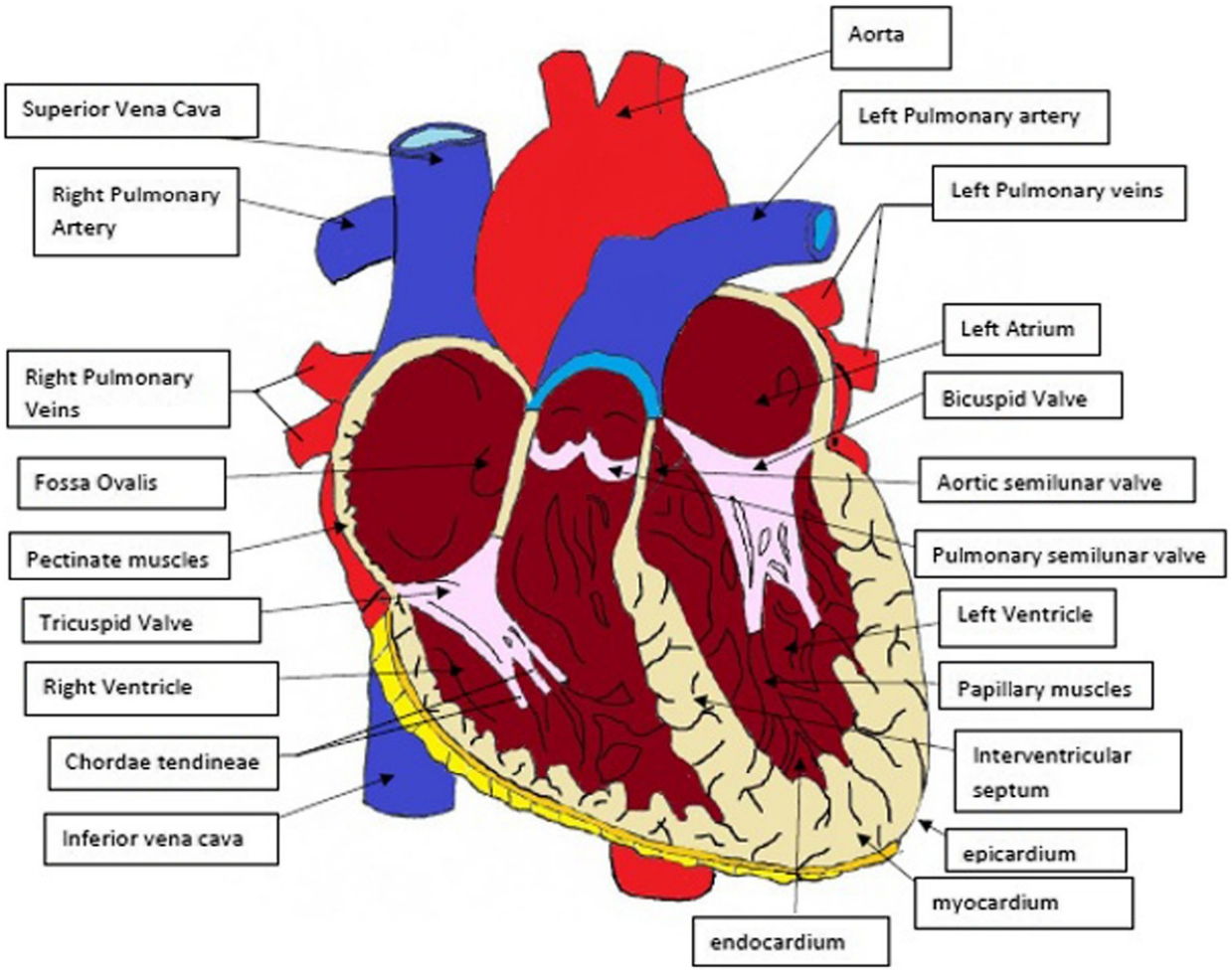

Fig. 1 Overall structure of the human heart. This schematic representation depicts the basic structure of the human heart.

in vessels (see in the succeeding text). The next layer inward is the myocardium. The major cell type occupying this layer in terms of mass is the cardiac myocyte. The myocardium is also populated by connective tissue cells and fibers that play an important role providing elasticity and, at the same time, rigidity to the heart wall. Additionally, there is an extensive capillary pool that embraces every myocyte of the myocardium. While the general structure of the myocardium is similar in the atria and the ventricles, the biggest difference between the atria and ventricles exists in this muscular layer with a much thicker layer comprising the ventricles. The third innermost layer, the endocardium, lines the heart chambers and all valvular structures housed within. This layer is made up of endothelium that faces the heart lumen and the subendothelial connective tissue space.

While the three tunics are present throughout the heart, great variation is observed in the thickness and shape of the heart walls principally due to variation in the myocardium. Most obviously, the ventricular myocardium is much thicker than its atrial counterpart (see **Fig. 1**). The ventricles are largely solid muscular structures, but the myocardium and endocardium become trabeculated in the deep region adjacent to the heart lumen. These irregularly shaped structures are called trabeculae carneae and reside in both left and right ventricles. The inner right atrium has a patterned scalloped inner surface of pectinate muscles, while the left atrium has rather smooth appearance.

Cardiac muscle is capable of spontaneous cell depolarization and is able to contract without an external stimulus from the nervous system. The steady, rhythmic heart beat is maintained by a series of cells innervated by the autonomic nervous system. The heart beat is initiated at the sinoatrial (SA) node, which is the pacemaker of the heart and is located at the intersection of the right atrium and the superior vena cava (**Fig. 2**). Modified cardiac myocytes called conduction system myocytes are present in this node and throughout the remainder of the conduction system. Myocytes of the conduction system are often larger and have fewer and more disorganized myofibrils (see **Fig. 3**). Additionally, they express differing contractile proteins (Gonzalez-Sanchez and Bader, 1985). SA node generates a muscular impulse that spreads throughout the myocardium of the right and left atria. At the right AV valve, the AV node picks up the impulse and relays the signal to the ventricles through the bundle of His (**Fig. 2**); again, specialized cardiomyocytes carry electric impulses through this portion of the conduction system. In the interventricular septum, the bundle of His further divides into left and right bundle branches serving the right and left ventricles. These bundles further divide and ultimately connect Purkinje fibers. Purkinje fibers branch out into the myocardium and make junctional contact with working myocytes. Purkinje fibers are the terminal component of the conduction system and electric conduction, and accompanying contraction continues through working cardiac myocytes. The positioning and conductivity of these modified myocytes are critical in the coordinated contraction of working cardiac myocytes, and their disruption caused by developmental or disease problems can have highly deleterious results in heart function (Milan and MacRae, 2005; Christoffels et al., 2010; Sylva et al., 2014; Neary and Breckenridge, 2013).

It is important to understand the structure of cardiac myocytes in the context of heart structure and function. Adult human cardiomyocytes have an intricate cellular structure to enable the regulation and coordination of electric flow and contraction. While both cardiac and skeletal myocytes are striated, significant differences between the two cell types exist. Most human cardiomyocytes are binucleated, the result of a lack of cell cleavage in the last round of mitosis during heart development (Ahuja et al., 2007), whereas skeletal myocytes may have hundreds of nuclei per cell resulting from the fusion of myoblasts in development. Next, cardiac myocytes are often branched with branching myofibrils, whereas skeletal myocytes are cylindrical and unbranched (**Fig. 4**). The branching nature of the cardiac myocyte and its myofibrils is essential for the multidirectional nature of their contraction in the pumping of blood. Additionally, cardiac muscle has intercalated disks, myocyte-to-myocyte junctions that allow the conduction of action potentials from one muscle cell to another via a modified gap junction. Intercalated disks also contain desmosomal and adherence junctional components that are essential in the transmission of force from myocyte to myocyte. Skeletal muscle does not

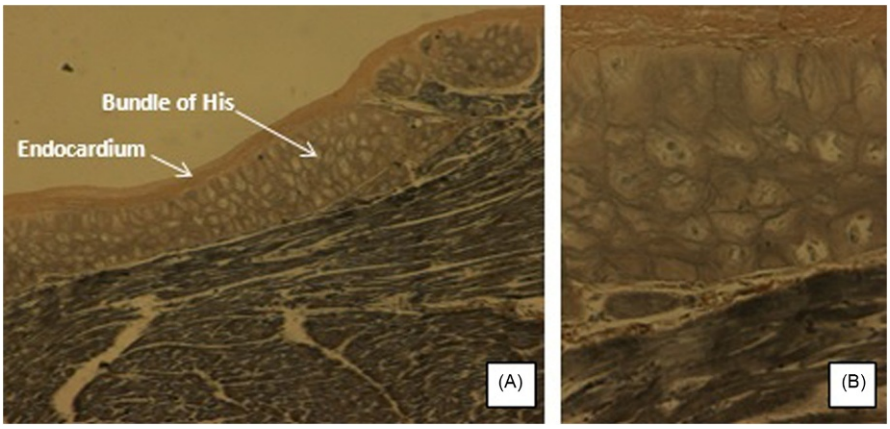

Fig. 2 Distinction between myocytes of the working myocardium and conduction system. This histological section depicts the left side of the interventricular septum with the ventricular lumen seen at the upper left of *panel A*. Myocytes of the bundle of His are seen as larger, lighter staining cells just beneath the endocardium. The higher-power view in *B* demonstrates the conduction system myocytes are larger in size and lighter stains indicating that these cells contain fewer myofibrils.

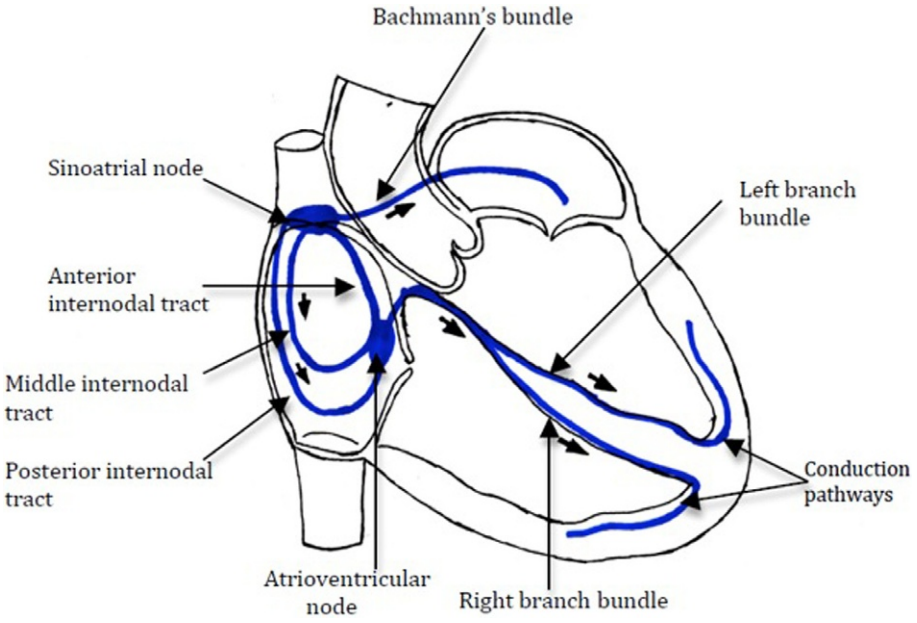

Fig. 3 Schematic diagram of the cardiac conduction system. Components of the human conduction system are given here.

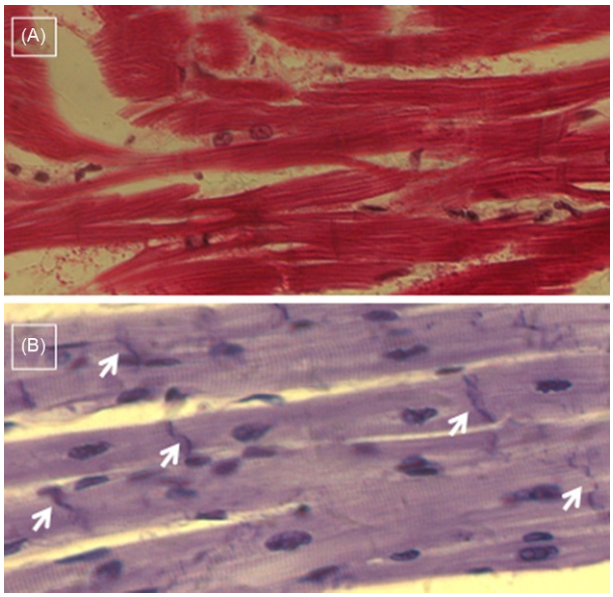

Fig. 4 Histology of working cardiac myocytes. In *panel A*, the branching nature of working cardiac myocytes can be readily seen in this trichrome stain. Darkly stained intercalated disks *(arrows)* are very apparent in toluidine blue-stained section of *panel B*. These structures contain myocyte/myocyte junctions enabling the transmission of force and electricity in the myocardium.

conduct electricity or force from muscle cell to muscle cell. Cardiac muscle cells contain transverse tubules (T tubules) and sarcoplasmic reticula similar to skeletal muscle cells but arranged somewhat differently. T tubules, which are inward extensions of the cell membrane, bring the depolarization signal to juxtaposed extensions of the sarcoplasmic reticulum. After receiving this signal, the sarcoplasm releases calcium to initiate the coordinated contraction of all sarcomeres within the myocyte. T tubule system is most prominent in myocytes of the ventricles and less in the atria. This is due to the greater number of myofibrils in the ventricular cells and the need to bring electric conduction to all of them simultaneously. In addition, the sarcoplasmic reticula do not fully encircle the myofibrils as they do in skeletal muscle but are found between them. This brief discussion of the subcellular structure of the cardiac myocyte is presented to give context in the description of the cardiovascular system. For a deeper understanding of T tubule system, see Ibrahim et al. (2011). Additionally, the reader is encouraged to explore the literature and texts for a more in-depth description of this interesting cell type.

The Blood Vessels

There are three major subdivisions of the vascular tree: arteries, capillaries, and veins. Arteries deliver blood from the heart to capillary beds positioned throughout the tissues/organs of the entire body. Capillaries serve as the major fluid/material/cell exchanger of the cardiovascular system to and from the body space. Lastly, veins return blood from the diverse and broadly positioned capillary beds of the body to the heart. It should be emphasized that there is great diversity of structure and function between each of these three categories but that diversity is also seen within each vessel type. Yet, at the same time, these vessels are a continuum of shared component parts serving the overarching function of blood transport throughout the body.

Overall structure

Arteries and veins ofttimes run in tandem throughout the body. This offers an excellent opportunity to examine the overall differences in the structure of these two vessel types. Even a quick look at Fig. 5 reveals the general differences seen in veins and their partner arteries. Note that the vein is larger than the accompanying artery. While the artery is smaller in diameter, its wall is thicker than the vein. The larger and more flaccid vein has the capacity to hold/store more blood than its arterial partner. Conversely, the smaller diameter of the artery and the constructive nature of its wall are important in regulating blood flow. The size and shape differences seen here are maintained throughout the vascular tree.

There are three distinct domains that make up the vessel walls of the cardiovascular tree. A high-power view of the mid-sized vessels seen in Fig. 5 illustrates these domains and how they differ in arteries and veins (Fig. 6). The innermost domain of a blood vessel facing the lumen is the *tunica intima*. Abutting the lumen, the tunica intima consists of an endothelial sheet and its subjacent basal lamina. In larger vessels, the intima may also contain a small connective tissue component in its structure. In smaller arteries and veins, this connective tissue component becomes very attenuated and is absent in capillaries. The middle domain in the generalized structure of vessels is termed the *tunica media*. This domain shows great variation in its size and composition ranging from scores of layers of smooth muscle cells and associated connective tissue laminae in the aorta to a complete absence in capillaries. Composition of this domain varies hugely in its resident cells and extracellular matrix. Note that the tunica media of the vein in Fig. 6 has only one or two layers of smooth muscle whereas the artery has 10–12 layers. The last and outermost layer, the *tunica adventitia*, is a connective tissue domain that links vessels to the connective tissue of the organ in which the vessel resides. Indeed, the boundary between the tunica adventitia and surrounding connective tissue space can be somewhat arbitrary. The diversity, form, and function of these domains will be discussed in the following description of the vessel types. With this background in mind, let us discuss individual components of the cardiovascular tree.

Another important consideration in understanding the overall structure and function of blood vessels is the variation in blood pressure seen throughout the system. Fig. 7 shows the systolic and diastolic blood pressures in the various vessel types discussed later. In a healthy individual, systolic and diastolic blood pressure leaving the heart is 120 over 80, and these pressures are seen in the large arteries proximate to the heart. Interestingly, blood pressures drop precipitously at the terminal arterial vessels, arterioles (discussed later), and very low pressure is observed in the capillary beds and venous return. Dealing with variant blood pressures plays a large role in the structural/functional relationships in the vascular tree.

Arteries

The overarching function of the arterial tree is to deliver/distribute blood to organs of the body. Important in the consideration is that the total volume of blood in the body is much smaller than the volume of the entire cardiovascular network; that is, there is not enough blood to fill all the blood vessels in the body at the same time if all vessels were in a relaxed or fully opened state. Also, the

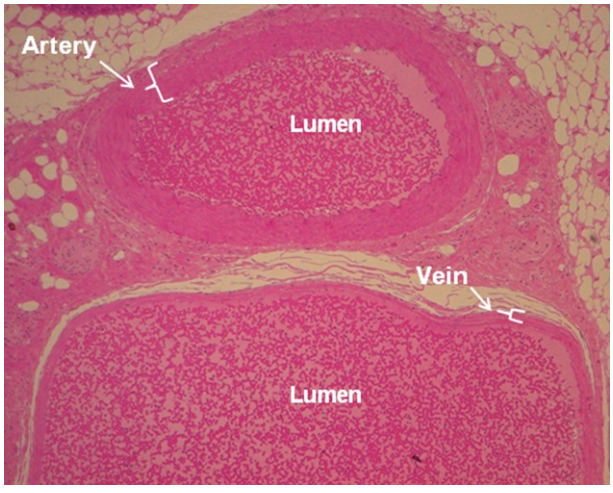

Fig. 5 Histological structure of the vascular tree. This cross section through a mid-sized artery and accompanying vein provides an example of the overall configuration of the vascular tree. Note that the luminal diameters of the vein and artery vary greatly as does the thickness of the vessel walls *(brackets)*.

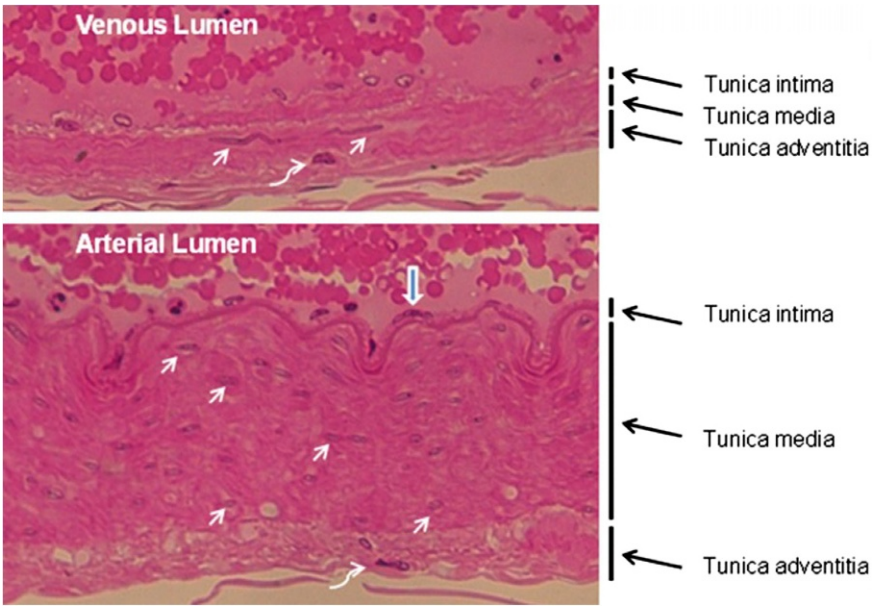

Fig. 6 Differences in venous and arterial vessel walls. This is a higher magnification of the vessels seen in **Fig. 5**. Note the overall variation in wall thickness between the two vessels and the relative thickness of the arterial tunica media compared with its venous partner. The *arrow with the blue interior* marks the nucleus of an endothelial cell in the arterial tunica intima. The *arrowheads* denote smooth muscle nuclei in both vessel types; the vein has a media consisting of only one or two smooth muscle cells, whereas the artery has 10–12 of these cells in its media. The *curved arrow* in both the venous and arterial walls marks a fibroblast of the tunica adventitia. These nuclei are typically more heterochromatic than smooth muscle counterparts giving them a darker blue color in this H and E stain.

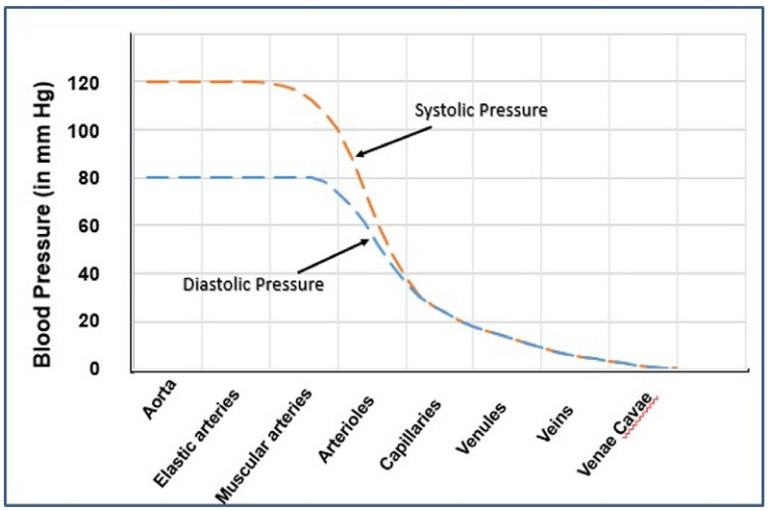

Fig. 7 Variations in systolic and diastolic blood pressure in different vessels of the cardiovascular tree. Systolic and diastolic blood pressure in the aorta and other elastic arteries is maintained at 120/80. These pressures decrease greatly in the muscular/distributing arteries and arterioles. Capillaries and veins have constant pressures less than 20 mmHg. Obviously, the structure and function of these varying vessels are intimately tied to the blood pressures observed within.

potential pool for housing blood is much greater in the venous side of circulation. Thus, the body senses where and when blood is needed, and the arterial tree responds by constricting and relaxing specific blood vessel pathways so that blood is delivered to the proper locale. The mechanism by which this is achieved is given in the succeeding text. There are three major subdivisions of the arterial tree: elastic and muscular arteries and arterioles. As one might expect, there are huge differences in the composition of the three tunicae in these vessels.

Elastic arteries

As the blood leaves the ventricles of the heart, the first type of vessel encountered is an elastic artery being either the aorta or pulmonary artery. The stroke volume of right and left ventricles is just under 100 ± 15 mL in a 150 pound man (Maceira et al.,

2006). Considering that healthy individuals have heart rates from 60 to 100 beats min^{-1}, these arteries are under tremendous strain dealing with rapid and constant volume changes. The structure of the elastic arterial wall is built to withstand this turbulence.

The tunica intima of elastic arteries is thicker than seen in other vessels. From the lumen outward, endothelial cells, oriented longitudinally along the vessel, are first seen. Next, a connective tissue space abutting the endothelial basal lamina extends to the inner boundary of the tunica media and accounts for the relatively thicker nature of the tunica intima. The thicker nature of the tunica intima in large elastic arteries is likely important in allowing distensibility of the vessel. Moving away from the vessel lumen, the tunica media is next seen. The smooth muscle cells of this layer have their longitudinal axis oriented perpendicular to the long axis of the vessel and are thus seen in longitudinal fashion in cross sections of the elastic artery. Elastic sheets and collagen fibers are situated between the layers of smooth muscle and give the entire tunica media its distinctive alternating pattern of smooth muscle cells and elastic laminae (**Fig. 8**). Elastin, collagen, and all other components of the laminae and interstitial matrix are secreted by smooth muscle cells of the media. The tunica media of elastic arteries has numerous smooth muscle cells with alternating elastic laminae; the aorta of humans may have 70 or more elastic laminae with accompanying layers of smooth muscle. By definition, the innermost elastic lamina is called the internal elastic lamina and constitutes the inner boundary of the tunica media. In turn, the external most lamina is defined as the external elastic lamina and is the external boundary of the tunica media. Interestingly, the embryonic source of smooth muscle cells in elastic arteries is diverse with specific descendants of these progenitors populating specific domains of the vessels (Pfaltzgraff and Bader, 2015). These regional differences in derivation of smooth muscle may underlie variation in vascular physiology and disease response. Exterior to the outermost elastic lamina is the tunica adventitia, which is composed primarily of connective tissue. This connective tissue blends with connective tissue of the surrounding area and serves to integrate the vessel into the specific region of the body.

In the human body, elastic arteries extend from the pulmonary trunk and the aorta and its major branches including the common carotid, brachiocephalic, vertebral, pulmonary, subclavian, and common iliac arteries. The aorta and pulmonary artery are very large vessels with a significantly large luminae and diameters, which are tapered as the cardiovascular tree becomes more distant from the heart (compare **Figs. 8** and **9**, which have been at the same magnification). Interestingly, the larger vessels and their accompanying veins need their own blood supply to survive. This capillary system is called the vasa vasorum, meaning "vessels of the vessel" and bring oxygenated blood to the outer vessel wall.

Muscular arteries

There is a gradual transition from a purely elastic artery to a purely muscular artery. A decrease in overall size and luminal diameter of the vessels accompanies this transition. Thus, the exact anatomical position when an elastic artery becomes a muscular artery is somewhat arbitrary. Examples of muscular (also termed distributing) arteries are the branchial, ulnar, and radial arteries of the arm and the femoral, popliteal, and tibial arteries of the leg. In the head and neck region, arteries distal to the brachiocephalic artery take on the characteristics of muscular arteries. The tunica intima of these vessels is thinner than that of an elastic artery but has the same characteristic features. This tunic and the internal elastic lamina are often seen in a distinctly scalloped fashion in tissue preparations (**Fig. 9**). This is likely due to the contraction of vesicular smooth muscle upon fixation. The tunica media is bounded by the prominent internal elastic lamina with its scalloped shape and at its periphery by a more fragmented external elastic lamina with the intervening space filled by smooth muscle. These vessels have elastin and collagen throughout their tunica media, but there is not the distinctive configuration into elastic laminae seen in elastic arteries. The tunica adventitia as seen in other vessels is predominantly composed by connective tissue that blends with the connective tissue proper of the surrounding organ. Ofttimes, the tunica adventitia of these arteries and the accompanying veins are conjoined (see arrows in **Fig. 9**). From a proximal to distil position in the

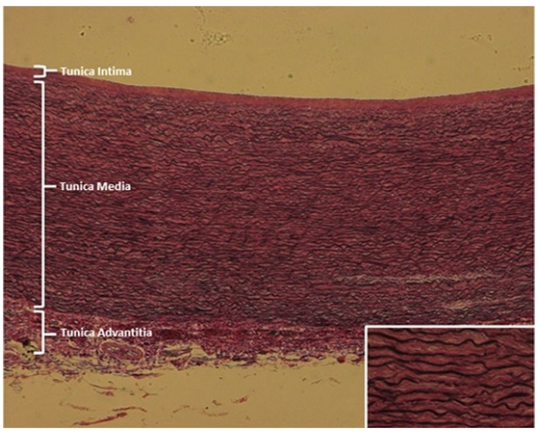

Fig. 8 Histological section of the human aorta (an elastic artery). This low-power view of the aorta stained with an elastin stain shows the three distinct tunicae of the vessel; the intima, media, and adventitia. The *purple structures* in the tunica media are the elastic laminae produced by the intervening smooth muscle of this region. The higher-power *inset* shows the "wavy" purple elastic laminae with the pink-stained smooth muscle. This arrangement allows for the rhythmic expansion and recoil of the elastic aorta.

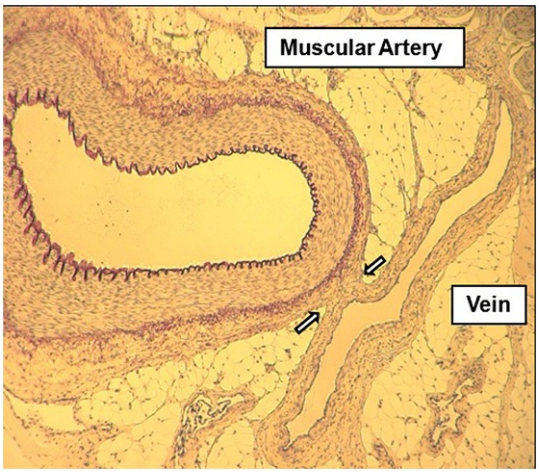

Fig. 9 Histology of muscular artery with an accompanying vein. A cross section of a muscular artery and its accompanying vein is seen in this elastin-stained specimen. The distinct internal elastic lamina is seen in its characteristic "scallop" shape. At this power, the tunica intima, lying toward the luminal side of the internal elastic lamina, is difficult to detect. The external elastic lamina is more fragmented in appearance and separates the tunica media from the tunica adventitia. The *arrows* in this figure show a region of where the adventitia of this artery and its accompanying vein merge. Note the collapsed nature of the vein versus the artery and that the venous wall is predominately made up of the tunica adventitia. Further, the magnification of this specimen is the same as seen in **Fig. 4**, the depiction of the elastic aorta. Note the difference in the relative size of the vessels and especially their tunica media.

arterial tree, muscular/distributing arteries decrease in total size and luminal diameter and have a reduced amount of elastic tissue in their tunica media. The number of smooth muscle cells in the tunica media also attenuates.

The major function of muscular arteries is regulating the "selective" distribution of blood. The muscular tunica media of muscular arteries is proportionately the largest muscular component of any blood vessel and is most active in vasoconstriction. In considering this function, think of blood flow after you have consumed a large meal. Your body senses the presence of food stuffs in your digestive tract, and the relaxation of smooth muscle in muscular arteries serving the gut provides a larger volume of blood to those organs. The same is true when running a race. Blood is preferentially distributed to skeletal muscles of the extremities and, simultaneously, diverted from the gut. This large-scale regulation of blood distribution is coupled with a more fine-scaled regulatory system, the arteriole, which is next in our discussion.

Arterioles

The terminal unit of the arterial tree is the arteriole, which is directly adjacent to the capillary bed. The lumen of these vessels tapers to just under 40 mm. The tunica intima of arterioles is highly attenuated consisting of an endothelium and accompanying basal lamina with scarcely any connective tissue components. The tunica media houses one to five layers of smooth muscle with little if any elastin. A thin tunica adventitia is present and, as always, blends with the surrounding connective tissue of the organ.

The major function of arterioles is to regulate the flow and, thus, the volume of blood entering capillary beds. When arteriolar smooth muscle is relaxed, blood flows into adjoining/distal capillaries, and the entire bed is filled in blood. Conversely, when arteriolar smooth muscle contracts, blood flow is lessened to distal capillaries and, thus, to that region of the body. Lastly, it is important to understand that blood pressure drops precipitously at the arteriole/capillary border (**Fig. 10**). Given the extremely delicate nature of the capillary wall, this decrease in pressure is essential in capillary function.

Capillaries

Capillaries are the most distal component of the cardiovascular system. Throughout the body, they are the anastomosing connection between arterial and venous vessels. Capillaries are by far the most prevalent members of the vascular tree, and approximately 60,000 miles of capillaries are present within the human body (Siddiqui, 2011). The human brain alone has 100 billion capillaries extending 400 miles (van Tellingen et al., 2015). Concerning gas, fluid, and material exchange, the capillary is the "business end" of the cardiovascular system. These beds anastomose within the connective tissue space of organs such that all cells of the supplied area are within one to three cell widths from a capillary. The luminal diameter of capillaries is approximately 9–10 μm, marginally larger than a red blood cell (RBC). White blood cells that have larger diameters reshape/redistribute their cytoplasm to pass through capillary beds. Interestingly, intermittent thoroughfare channels (also known as metarterioles and also depicted in **Fig. 10**) provide an "express route" to move blood more directly to the venous return virtually bypassing the capillary bed. These vessels do not have the robust layer of smooth muscle seen in arterioles at their proximal/arterial end. Instead, a single, discontinuous layer of smooth muscle is seen at the arterial/proximate region of the thoroughfare channel. This smooth muscle layer gradually subsides toward the venous/distal region of this channel. In this way, when arterioles in one region of the body collectively close, arterial blood is quickly shunted through these thoroughfares to the venous return making this blood available for other parts of the organism that are in greater need of blood.

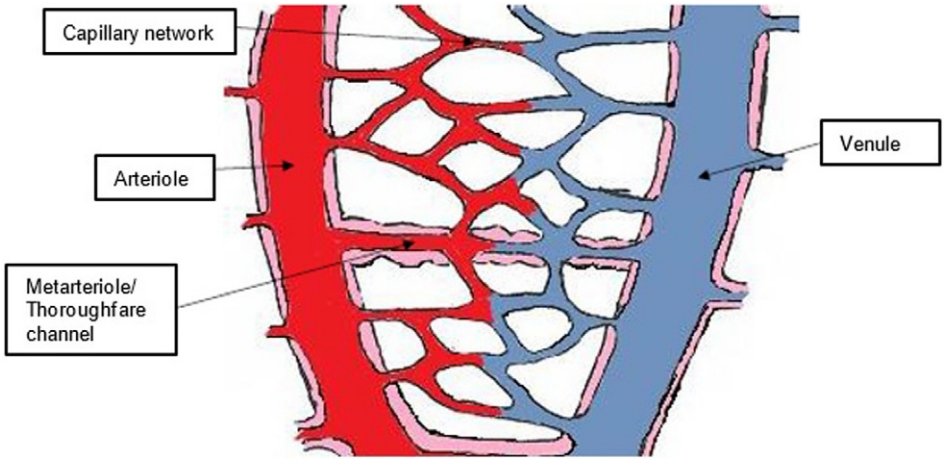

Fig. 10 Arterioles, capillaries, and venules. This schematic depicts the arteriole system leading to a capillary bed. Arterioles regulate the flow of blood to capillary beds through constriction and dilation of their terminal orifices. A metarteriole/thoroughfare channel is also shown. This vessel can provide a direct shunt from the arteriole to venous pole, thus avoiding the capillary bed. Venules, at the distal end of the capillary bed, are the first component in the venous return.

The overarching function of capillaries is to enable exchange of oxygen, fluids, nutrients, carbon dioxide, and other substances between the cardiovascular system and other body compartments. Capillaries have the same basic structure throughout the body, although critical variation in structure/function is noted later. In essence, capillaries are a continuation of the tunica intima seen in arteries and veins. Capillaries consist of an inner endothelium resting on a basal lamina, which is secreted by the endothelium. While a definitive tunica media and adventitia are absent in capillaries, specific mesenchymal cells are seen intermittently adjacent to the basal lamina of the capillary. One of these cell types is called the pericyte. This cell is connective tissue cell with contractile properties. Other connective tissue cells with phagocytic and fibroblastic properties are also scattered along the capillary network. This basic structure is modified throughout the body depending on the particular function of each capillary bed.

There are three major subcategories of these vessels: continuous, fenestrated, and discontinuous capillaries. We give an example of each capillary as a means of describing their varying function.

Continuous capillaries, as the name implies, have a continuous endothelial sheet of cells at the luminal surface with a continuous subjacent basal lamina (**Fig. 11**). Cell/cell junctions insure that the luminal blood is restricted to the vessel while the attenuated endothelial cytoplasm promotes exchange of materials from the blood supply to the surrounding tissue. Let us consider the blood supply to the lung as a means of discussing the structure and function of continuous capillaries.

Deoxygenated blood originating from the right ventricle enters the lung via the elastic pulmonary artery. This artery quickly branches into muscular distributing arteries that roughly follow the branching pulmonary tree that supplies the lungs with air. Many orders of branching and subdivision follow in both systems until the airway is reduced to a single epithelial sheet called the terminal alveolus, while the cardiovascular system ends in continuous capillaries. As seen in the accompanying figure, these two simple squamous epithelia rest on a shared basal lamina. Thus, the structure between the air of the outside world and the lumen of your cardiovascular system is reduced to two attenuated epithelial sheets and a common basal lamina. It is important to remember that the endothelial cells in the lungs form a continuous capillary system. While the cells are very attenuated and very closely associated with the epithelia of the airways, the continuous nature of these capillaries keeps the blood within the cardiovascular system preventing you from drowning in your own blood. Thus, the complex branching of the airway and bloodstream results in a simple cellular arrangement where inhaled oxygen is in close proximity to RBCs.

A similar configuration of continuous capillaries is seen in the brain and spinal cord, in smooth, skeletal and cardiac muscle, and in most of the connective tissue compartments of your body. Indeed, continuous capillaries are the most prevalent capillary seen in the human body.

The next type of capillary seen in the body is the fenestrated capillary. These vessels are present where more extensive fluid interchange between the cardiovascular system and the organ/body compartment takes place. Examples are the glomeruli of the kidneys, villi of the small intestine, and endocrine glands. Fenestrated capillaries are continuous in nature with a continuous basal lamina but have domains within the endothelial cell where very thin windows or fenestrae reside (**Fig. 11**). These windows are thinner than the diameter of a single plasma membrane and vary in diameter. Interestingly, with the great advances science has made over the past years, the definitive structure of capillary fenestrae has not been resolved although factors influencing their development have been identified (Satchell and Braet, 2009).

Fenestrae vary in number and size in different populations of capillaries, but it should be noted that transport across these structures is in the range of molecules and not at the level of intact cells. Obviously, fluid exchange is a major function in the kidney. Glomeruli of the kidney are the most distal structures of the nephron and are intimately associated capillary beds. The capillaries within the glomeruli actually have small opening or gaps within their fenestrae and have a mildly elevated hydrostatic pressure that pushes fluids out of the vessel across the associated basal lamina. Glomerular epithelial cells (called podocytes) have gaps between

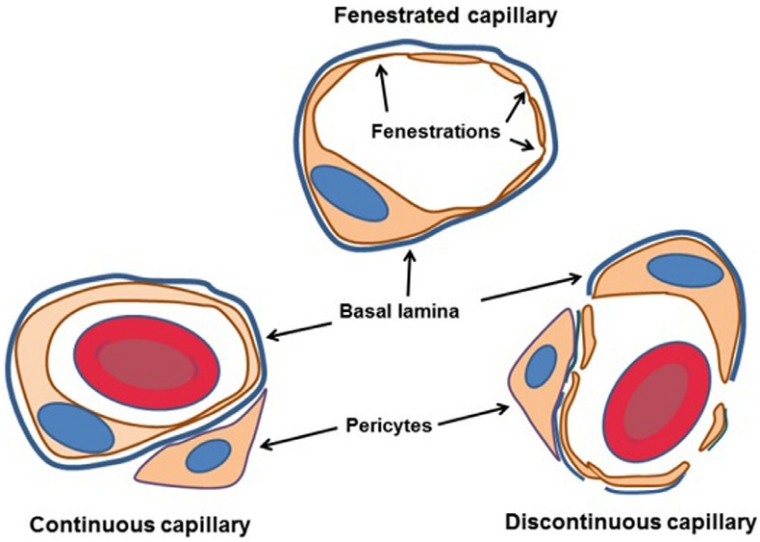

Fig. 11 Capillary structure. The three major types of capillaries are depicted. At the lower left, a continuous capillary is seen in cross section with its continuous endothelial lining, continuous basal lamina, and an associated pericyte. A fenestrated capillary with its "windowed" fenestrations is seen at the top and middle. At the lower right, a discontinuous capillary is shown with gaps in both the cellular endothelial lining and basal lamina of this vessel type. The relative size of these vessels can be discerned by comparing the diameter of the red-colored RBC (6–8 μm in the human) to the capillary lumen.

cells allowing the passage of fluids, ions, and small molecules into the initial nephric space. This is the first step in production of urine and the elimination of excise fluids from the cardiovascular system. The configuration of the capillaries within the glomerulus promotes the movement of fluids out of the blood system to the urinary tract.

The third major capillary form is the discontinuous capillary. This type of capillary is found in regions of the body where whole-cell entry and exit from the cardiovascular system is observed and includes organs such as the liver, spleen, and bone marrow. In this setting, gaps in the endothelial cell sheet occur with accompanying spaces in the subjacent basal laminae (Fig. 11). Hematopoiesis is a major function of the blood marrow, and newly matured red and white cells can freely migrate from the stroma through discontinuous endothelial cells in the marrow to enter the blood stream. Conversely, blood flow in the spleen is virtually an open system where cells, such as RBCs, exit the cardiovascular system and are surveyed by resident splenocytes. These splenocytes can determine which RBCs are ready to be eliminated due to their age (remember that human RBCs function to deliver oxygen for 120 days and then are eliminated in the spleen by those splenocytes). RBCs that have not reached 120 days of maturity are not eliminated and return to the bloodstream through the same discontinuous capillaries and exit the spleen to continue their duties in gas exchange.

It is interesting how nature and evolution have conserved the overall function of capillaries in these three different settings while, at the same time, promoting variation to meet the varying needs of a complex organism such as the human being.

Veins

From a cursory glance at veins, one sees a rather boring trip back to the heart for blood. But, a closer look reveals interesting properties in these vessels.

The venous return begins with the postcapillary venules. These vessels have the three tunics, but they are highly attenuated (see Figs. 5, 6, and 9). Endothelia of postcapillary venules can be continuous or discontinuous, basically following the pattern seen in the accompanying capillary bed. The tunica media of these vessels is very slight housing pericytes, fibroblasts, reticular cells, and smooth muscle cells. These cells are usually no more than one or two cell layers in thickness that are ofttimes seen individually. The adventitia is also very thin and blends with surrounding connective tissue. Postcapillary venules are an important component in fluid and cell exchange from the vascular tree throughout the body.

Veins can be categorized as small, medium, or large and are accompanied by their arterial partner throughout the body (Figs. 5 and 9). In histological analyses, defining one vessel as small, medium, or large vein is somewhat arbitrary as transition from one category to the next is gradual. As one might expect, the venous vessel size and luminal diameter increase the closer they are to the heart. The luminal diameter of a vein may be four or five times larger than its accompanying artery anywhere along the cardiovascular tree. All veins are composed of the three tunics. In general, the tunica adventitia is proportionately larger than seen in arteries and makes up the majority of the vessel wall. The tunica media is proportionately smaller, and the boundary between these two tunics is often difficult to define. Smooth muscle is limited in even the largest vessels. The tunica intima contains the standard cellular and extracellular components and is thinner than its arterial counterpart.

A major consideration in the analysis of the venous return is their gross anatomical position relative to the heart. The flow of blood throughout the venous tree is largely powered by the overall movement and position of the body and not by the vessels themselves; remember smooth muscle is not prominent in veins. Indeed, gravity is a major driving force in venous blood flow.

Think about sitting at your desk for long periods of time and noticing that your feet feel "puffy." That is in part due to a lack of body movement and an accumulation of fluids in your lower extremities.

Veins can be thought of as supra- or infracardiac; that is, they are located above or below the heart. Gravity is a positive factor in blood flow in vessels located above the heart, while it is a negative factor in vessels located below the heart. Infracardiac veins have valves that protrude into the vessel lumen. These valves are an extension of the tunica intima having an endothelial covering with a slight connective tissue core. These structures are essential in preventing backflow of blood in infracardiac veins.

Lymphatic Vessels

The overall function of this system is to transport fluids, materials, and cells from the tissue space of the body through a series of lymphatic tissues/nodes back to the major veins entering the heart where the lymph is again reunited with blood. The lymphatic system is highly integrated throughout the human body with the notable exceptions of cartilage, bone, thymus, teeth, and the central nervous system. Unlike the cardiovascular tree, flow in the lymphatic system is unidirectional with all flow from the periphery of the body back to the heart. Lymph, the fluid present in this system, is a filtrate of blood. Fluid that exits the cardiovascular tree moving into the tissue space throughout the body is an essential component of that domain. The lymphatic system is basically an open circulatory tract with an open connection to the tissue space of the body, and lymph can freely move between the tissue space and lymphatics. Lymphatic capillaries, the distal most component of the lymphatic system, are composed of a simple endothelium with an accompanying basal lamina. This endothelium is largely discontinuous and lacks the accompanying connective tissue components seen in blood capillaries. These lymphatic capillaries are somewhat larger than the blood capillary and often have a flattened appearance. Lymphatic vessels increase in diameter and size as they move proximally in the body. Still, the vessel wall remains very thin relative to arteries and veins in the same body regions. Valves, like those of the infracardiac venous system, are present in larger lymphatic vessels. The larger lymphatic vessels do have a limited amount of connective tissue and the occasional pericyte or smooth muscle cell. Distinguishing definitive tunics is difficult even in the largest vessels of this system.

As noted earlier, fluid from the diverse tissue compartments of the body freely enters the lymphatic tree at its distal branches. This fluid that also contains particulate matter and cells of the lymphoid system moves proximally as branches converge to form larger vessels. These larger vessels encounter a series of lymph nodes in the neck, groin, and axillary regions of the body. Lymph nodes have a connective tissue capsule that is penetrated by these vessels. Vessels that feed into the node are termed afferent vessels. Circulation within the node in essentially open lymph is surveyed by cells of the lymphatic system. Infectious microorganisms, damaged cells, and particulate debris in the lymph are eliminated or attacked by macrophages, plasma cells, and lymphocytes that are present in the node. The open sinuses of the node converge and form a single efferent lymphatic vessel that emerges from the node opposite to the region where afferent vessels enter. This vessel proceeds proximally and, ofttimes, encounters another node. In this case, the vessel would be then considered an afferent vessel. Like the cardiovascular tree, lymphatic vessels continue to join together making ever larger vessels. The two largest lymphatic vessels, the thoracic duct and the right lymphatic duct, connect with the venous return in the region of the neck, reuniting lymph with its parental blood.

To reiterate, fluids leaving the cardiovascular system are placed in diverse compartments of the body's connective tissue space. This fluid can freely enter the lymphatic system that transports it back toward the heart. Along the way, lymph is filtered and monitored by the lymph nodes until it is returned to the blood system. Thus, the two circulatory systems operate in a parallel yet connected manner.

Overall Summary

In reflecting on the structure and function of the cardiovascular system, one understands that there is great variation in its components. Yet, there are also concatenating themes that remain constant in these diversities. We hope this presentation will provide a groundwork upon which the reader can then explore this vital component in biological systems.

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Sudden Cardiac Death

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Epidemiology and Risk Factors

Sudden cardiac death (SCD) is defined as unexpected, nontraumatic death occurring within 1 hour of the onset of new or worsening symptoms (witnessed arrest) or, if unwitnessed, within 24 h of last being seen alive. By definition, a patient with SCD does not survive. When the patient survives, the event is termed aborted SCD or sudden cardiac arrest (SCA) (Priori et al., 2015; Zipes et al., 2006).

The clinical presentation of SCD generates a broad differential diagnosis. However, in both males and females over the age of 30, SCD is most commonly associated with ischemic heart disease (Priori et al., 2015). SCD may be preceded by cardiovascular symptoms such as chest pain, dyspnea, palpitations, presyncope, or syncope, but many individuals have no symptoms prior to their SCD event. The proximal cause of SCD in most instances is either ventricular fibrillation (VF) or ventricular tachycardia (VT). However, in a significant minority of cases, asystole or pulseless electrical activity is the initial documented rhythm. In these cases, the potential etiologies are more numerous and may include noncardiac causes (Priori et al., 2015; Zipes et al., 2006).

The most common causes of SCD are listed in **Table 1**. Broad categories of SCD include those related to ischemic heart disease, nonischemic structural heart disease, primary electrical disease, and noncardiac disease (**Fig. 1**).

SCD is common with an annual worldwide incidence that ranges from 1.4 per 100,000 in females to 6.8 per 100,000 person-years in males (Priori et al., 2015). Of the approximately 17 million cardiovascular deaths per year worldwide, SCD accounts for approximately 4.25 million (25%) of them. Other data suggest a lower overall incidence. Using death certificate data, it is estimated that SCD accounts for approximately 15% of all deaths in western nations (Zipes et al., 2006). In fact, death certificate data may overestimate the incidence since many deaths may be attributed to SCD but are actually unexplained. For example, extrapolated data from the Oregon Sudden Unexpected Death Study (SUDS) suggests that SCD accounts for only around 5.6% of all deaths in the United States (Zipes et al., 2006).

SCD incidence increases with age. The incidence of SCD in younger populations (<30 years) is 100-fold lower than that in older individuals (Zipes et al., 2006). Females are relatively protected from the risk of SCD until the postmenopausal years when incidence then approaches that of males. However, even in younger females, conventional coronary artery disease (CAD) risk factors are predictive of SCD events. Genetic factors play a role in SCD at multiple steps in the pathophysiologic pathway (Arking et al., 2011; Bezzina et al., 2010). At least two studies have described a familial propensity for SCD as the initial presentation of CAD. Mutations and polymorphisms modulate the risk of SCD associated with both CAD- and non-CAD-related events. Some studies have demonstrated an increased risk of SCD in African Americans compared to Caucasians while Hispanic individuals may be at lower risk (Zipes et al., 2006).

Relative to other causes of death, SCD accounts for a very significant proportion of events (Stecker et al., 2014). In males, SCD incidence exceeds that of other causes of death including individual cancers (lung, prostate, and colorectal), accidents, chronic respiratory diseases, diabetes, and cerebrovascular disease. In females, SCD incidence is similar to that of lung cancer and cerebrovascular disease and is higher than that of other causes of death including breast cancer, colorectal cancer, Alzheimer's disease, and accidents. When expressed as years of potential life lost, the impact of SCD is impressive (Kuriachan et al., 2015).

At the time of their SCA, nearly 50% of people were not known to have structural heart disease (Priori et al., 2015). Accordingly, in this patient population, risk stratification is particularly challenging. Furthermore, 40% of SCD occurs in individuals with known heart disease but with a left ventricular ejection fraction (LVEF) greater than 40% (Wellens et al., 2014). Our ability to predict SCD

Table 1 Major causes of sudden cardiac death

Ischemic heart disease
Coronary artery disease with myocardial infarction or angina
Coronary artery embolism
Nonatherogenic coronary artery disease (arteritis, dissection, congenital coronary anomalies)
Coronary artery spasm
Nonischemic heart disease
Hypertrophic cardiomyopathy
Dilated cardiomyopathy
Valvular heart disease
Congenital heart disease
Arrhythmogenic right ventricular cardiomyopathy
Myocarditis
Cardiac tamponade
Acute myocardial rupture
Aortic dissection
No structural heart disease
Idiopathic ventricular fibrillation/J-wave syndrome
Brugada syndrome
Long QT syndrome with torsades de pointes
Familial sudden cardiac death
Commotio cordis
Noncardiac disease
Pulmonary embolism
Intracranial hemorrhage
Drowning
Pickwickian syndrome
Drug overdose/toxicity
Central airway obstruction
Sudden infant death syndrome

Adapted from Kuriachan, V. P., Sumner, G. L. and Mitchell, L. B. (2015). Sudden cardiac death. *Current Problems in Cardiology* **40**, 133–200.

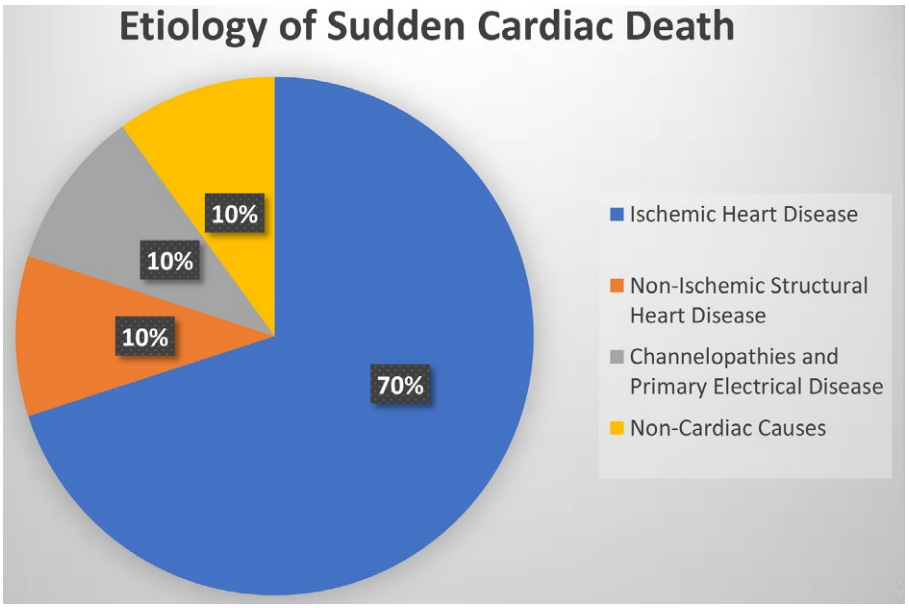

Fig. 1 Etiology of sudden cardiac death (all ages).

in this population is also limited. The remaining (approximately 10%) incidence of SCD affects individuals with known structural heart disease and an LVEF less than 40%. It is, therefore, not surprising that current risk stratification tools and therapies such as implantable cardioverter defibrillators (ICDs) have had only a modest impact on the overall problem of SCD (Stecker et al., 2014; Wellens et al., 2014). Inherited channelopathies account for only 2% of all SCD. Because the majority of SCD events occur in

individuals without known structural heart disease, efforts to prevent SCD should be directed toward the identification and modification of conventional risk factors.

The risk factors for SCD in older adults (age >35) are similar to those for ischemic heart disease and include tobacco smoking, systemic hypertension, dyslipidemia, and diabetes (Kuriachan et al., 2015). In the Framingham population, smoking conferred a 2-fold increased risk of SCD. Furthermore, in one study, people who continued to smoke after their aborted SCD had a risk of recurrent SCA of 27% over the subsequent 3 years compared to 19% in those who stopped smoking (Stecker et al., 2014). Obesity also confers an increased risk of SCD in patients with CAD (Wellens et al., 2014). There is conflicting data with respect to sedentary lifestyle as a risk factor for SCD. The risk of SCD is increased (approximately 17-fold) during vigorous physical activity especially in those who are generally sedentary (approximately 74-fold). However, the absolute risk of SCD during a single episode of vigorous exercise is very low (1 per 1.51 million episodes of exercise) (Albert et al., 2000). Furthermore, regular participation in physical exercise attenuates the overall risk of SCD both during exercise and at rest. Psychosocial stressors also increase the risk of SCD. Major disasters such as earthquakes and war increase the incidence of SCD in affected populations (Kark et al., 1995). Heavy alcohol consumption, defined as six or more drinks per day, increases the risk of SCD, while moderate alcohol consumption, defined as one to two drinks per day, may decrease the risk of SCD (Albert et al., 1999). In contrast, caffeine intake has not been shown to be a risk factor for SCD. Higher levels of long chain n-3 polyunsaturated fatty acids (eicosapentaenoic acid and docosahexaenoic acid; "fish oil") measured in plasma and in red cell membranes are associated with a lower risk for SCD (Albert et al., 2002a). Higher serum C-reactive protein (CRP) levels, a marker of generalized inflammation, have been shown to be associated with increased risk of SCD (Albert et al., 2002b). In 97 cases of SCD among apparently healthy men in the Physicians Health Study, serum CRP levels in the highest quartile were associated with a 2.78-fold increased risk compared to men in the lowest quartile (Albert et al., 2002b). Prospective studies are required to evaluate the impact of direct treatment or mitigation of these risk factors on the incidence of SCD.

In the patient population with established structural heart disease and LVEF >40% several risk stratification tools have been evaluated including tests of autonomic nervous system function (heart rate variability, heart rate turbulence, baroreceptor sensitivity, and, more recently, imaging assessment of cardiac sympathetic activity) (Clements et al., 2016; Exner et al., 2007; Huikuri et al., 2010). In addition, electrocardiographic markers of electrophysiological instability (ventricular premature beat frequency and complexity, QT interval prolongation, QT dispersion, microvolt T-wave alternans) have also been employed as potential risk stratification tools. To date, none of these markers have demonstrated independent, incremental predictive value for the future occurrence of SCD over LVEF alone. Ongoing randomized trials such as Risk Estimation Following Infarction Noninvasive Evaluation-ICD efficacy (REFINE-ICD) (clinicaltrials.gov) and AdreView™ Myocardial Imaging for Risk Evaluation-A Multicentre Trial to Guide ICD Implantation in NYHA Class II & III Heart Failure Patients With 30% ≤ LVEF ≤ 35% (ADMIRE-ICD) (clinicaltrials.gov) are directly assessing combinations of these biomarkers for their ability to select patient populations that have a mortality advantage in randomized fashion in order to clarify risk in this patient cohort.

SCD in Young Persons (Aged 35 Years and Under)

SCD in a young individual (aged 35 years and under) is an especially devastating event because of both its unexpected nature and because of the number of years of life lost. A 3-year prospective study of the general population in Australia and New Zealand performed between 2010 and 2012 identified 490 SCD events that occurred in people aged 1–35 years (Bagnall et al., 2016). The overall incidence of SCD in this age group was estimated to be 1.3 per 100,000. Of these events, the highest incidence was in the 31–35 age category. In this age group, CAD was the dominant etiology. SCD events in the youngest patient cohort (<30 years of age), where a diagnosis could be established, were predominantly due to cardiomyopathies and channelopathies. In the entire cohort, 65% of events occurred either during sleep or at rest (Bagnall et al., 2016). In the population aged 30 and under, approximately 40% of SCD events were unexplained after conventional toxicology and a complete autopsy examination. In 57% of those patients whose SCD events were classified as unexplained, molecular autopsy was performed. Genetic analysis related to candidate genes related to SCD in these 113 patients whose SCD was initially unexplained revealed an inheritable etiology of SCD in 31 patients (27%) (Bagnall et al., 2016). During clinical follow-up, a familial cardiac condition was identified in another 13% of these patients.

From these data, the authors concluded that the addition of genetic testing to the standard clinical investigation, including autopsy, significantly amplified the identification of a potential SCD etiology in this relatively young population. Such testing may have a role in the screening, risk stratification, and follow-up of immediate family members of the decedent (so-called cascade screening) (Bagnall et al., 2016)

Noncardiac Causes of SCD

Most studies have evaluated causes of death using medical records and death certificates. A small population study in Japan, in which 78% of the subjects had an autopsy, suggested that the major causes of sudden death were heart disease (49%), stroke (33%), and aortic aneurysm/dissection (12%) (Nagata et al., 2013).

Neurologic Disorders

Central nervous system disorders that can directly (or indirectly via cardiac interaction) result in sudden death include epilepsy, ischemic stroke, intracranial bleeding, and traumatic head injury (Kuriachan et al., 2015; Finsterer and Wahbi, 2016).

Uncontrolled epilepsy may lead to Sudden Unexplained Death of Epilepsy (SUDEP). Among patients with epilepsy, the incidence of SUDEP is 6/1000 patient-years. SUDEP is usually triggered by a generalized tonic–clonic seizure. It is believed that rare channelopathies may predispose to both epilepsy and SCD. Apnea, bradycardia, and asystole have also been implicated, as has the use of certain antiseizure medications. Finally, associated stress-induced cardiomyopathy (Takutsubo) may also play a role (Finsterer and Wahbi, 2016).

Intracerebral catastrophes, such as stroke and major intracranial bleeding/injury, can cause ECG changes and autonomic imbalance. The ECG changes may include QT-interval prolongation and torsade de pointes VT. Accordingly, it is possible that associated arrhythmias (severe bradycardia or VT/VF) are responsible for SCD in these instances. Patients with these neurologic disorders may also be predisposed to SCD due to underlying cardiovascular disease and an inflammatory state (Kuriachan et al., 2015; Finsterer and Wahbi, 2016).

Preventative treatments include good medical control of a seizure propensity and measures to reduce the incidence of stroke and cerebral injuries including blood pressure control and appropriate anticoagulation for patients with atrial fibrillation. Cardiac monitoring after cerebral catastrophes is important for recognition of related cardiac arrhythmias.

Acute Aortic Syndromes

The acute aortic syndromes include entities that disrupt aortic integrity with complications ranging from aortic rupture to organ ischemia and death. These syndromes include aortic dissection, aneurysm expansion, trauma, penetrating ulcer, and intramural hematoma (Kuriachan et al., 2015; Bonaca and O'Gara, 2014).

Patient risk factors for an acute aortic syndrome include age and hypertension. Loss of tensile strength due to Marfan's syndrome, Ehlers–Danlos syndrome, familial thoracic aortic aneurysm disease, and the aortopathy associated with congenital bicuspid aortic valve also increase risk. Cocaine use is a risk factor due to the increases in blood pressure and heart rate that it produces.

Treatment imperatives include rapid diagnosis, control of blood pressure and shear stress with β-blockers and vasodilators, and early surgical intervention (Kuriachan et al., 2015; Bonaca and O'Gara, 2014).

Electrolyte, Metabolic, and Endocrine Derangements

Life-threatening arrhythmias may result from electrolyte imbalances (especially hypokalemia and hypomagnesemia), metabolic derangements (including acid–base disorders), and severe endocrine disorders (especially hypothyroidism, hyperthyroidism, and hypercatecholaminergic states). Treatments are focused on the underlying disorder and acute correction of electrolyte, metabolic, and endocrine imbalances. Arrhythmias can also accompany eating disorders and aggressive treatments intended to correct them. Weight reduction in obesity and refeeding in anorexia should be controlled with attention to electrolyte fluctuations (Priori et al., 2015).

One major patient group prone to metabolic and electrolyte derangements are those on dialysis. Dialysis patients have a high mortality rate and up to 43% of their deaths are cardiac, with most being due to a cardiac arrhythmia. Risk factors for SCD in dialysis patients include electrolyte shifts, hypervolemia and hypovolemia, left ventricular hypertrophy, systolic and diastolic heart failure, CAD, hyperphosphatemia, sympathetic overactivity, and autonomic dysfunction (Kanbay et al., 2010).

The prevention of SCD in dialysis patients is challenging. During dialysis, care is taken to minimize electrolyte shifts. Traditional medical therapies for CAD, such as statins, have lesser benefits in dialysis patients, suggesting that the role of coronary plaques in SCD may be less important in dialysis patients (Ritz and Wanner, 2008). β-Blockers not only decrease ischemia but also have antiarrhythmic and sympathoinhibitory effects. Accordingly, they also decrease sudden deaths and increase the survival probability of cardiac arrest in diabetic patients on dialysis. Survival benefits from angiotensin-converting enzyme inhibitor (ACE-I) and angiotensin receptor blocker (ARB) treatments have also been demonstrated (Ritz and Wanner, 2008).

ICDs play a major role in the prevention of SCD in many predisposed patient populations. Many of the major trials demonstrating the benefits of ICDs excluded patients with end-stage renal disease. To date, no randomized trials of ICD use have been performed in patients with end-stage renal disease. Nevertheless, a patient with end-stage renal disease is considered for an ICD if survival expectation exceeds 1 year and functional status is good, especially in those patients who have survived aborted SCD (Lehrich et al., 2007). The comorbidities in this patient population generate competing nonarrhythmic causes of death and lead to higher ICD complication rates, thereby limiting the benefits of ICD therapy. Although the benefits have been difficult to document, dialysis staff should have basic life-support training and an external cardiac defibrillator should be on-site.

Medication-Related SCD

Many drugs can cause proarrhythmia that may lead to syncope and sudden death (Kuriachan et al., 2015). The most common mechanism of this proarrhythmia is suppression of the rapid component of the delayed rectifier potassium current (I_{Kr}) resulting in

prolongation of the QT interval and torsade de pointes VT (Li et al., 2010). Risk factors for this form of proarrhythmia include other factors that prolong the QT interval including hypokalemia, hypomagnesaemia, bundle branch block, bradycardia, CHF, inherited long QT abnormalities, and female sex. Accordingly, patients with a QTc greater than 460 msec should be exposed to additional QT prolonging drugs only under close observation.

The noncardiovascular drugs associated with this form of proarrhythmia include certain antibiotics, psychotropic medications, and diuretics (by magnesium and potassium depletion) (Li et al., 2010). A web-based listing of potential culprit medications is available at www.crediblemeds.org.

Cardiovascular drugs may also be proarrhythmic (Kuriachan et al., 2015). Vaughn Williams Class III antiarrhythmic drugs (amiodarone, dronedarone, dofetilide, ibutilide, and sotalol) are potassium channel blockers that prolong QT and may produce torsade de pointes VT. Such agents must be administered with close monitoring of QT intervals, maintenance of normal potassium and magnesium levels, and assurance of normal renal function for those agents that are so excreted (dofetilide, sotalol) (Li et al., 2010). Vaughn Williams Class Ia antiarrhythmic agents (disopyramide, procainamide, quinidine) are also potassium channel blockers that prolong QT and may produce torsade de pointes VT. In addition, these drugs and other Class I agents (lidocaine, mexiletine, propafenone, flecainide) are sodium channel blockers that may prolong QRS duration. Class I antiarrhythmic agents have been associated with SCD particularly in patients with underlying structural heart disease and myocardial fibrosis (Echt et al., 1991). Accordingly, Class I agents are usually reserved for patients with no structural heart disease. Vaughn Williams Class II antiarrhythmic agents (β-blockers) and Class IV agents (nondihydropyridine calcium channel blockers) may result in sinus and/or AV nodal suppression bradycardia especially if used in high doses or in combination.

Although many recreational drugs may result in SCD, the one most implicated is cocaine. Studies in Europe report that 1%–4% of young adults have used cocaine in the previous year (Kuriachan et al., 2015; Morentin et al., 2014). Cocaine is an indirect sympathomimetic agent and may increase blood pressure, increase heart rate, increase ventricular contractility, and lower VT threshold placing users at risk for myocardial infarction, major hemorrhages, and arrhythmias. Up to 9% of young adults with SCD were recent cocaine users (Kuriachan et al., 2015; Morentin et al., 2014).

Clearly, the best prevention of sudden death related to proarrhythmic drug effects is to limit the use of these agents and to carefully monitor their effects when they are used, particularly when therapy is first prescribed and shortly after dosage increments.

Other Causes of Noncardiac Sudden Death

Other causes of noncardiac sudden death are extremely rare and include pulmonary hemorrhage, massive pulmonary embolism, chronic obstructive pulmonary disease, asthma, rupture of esophageal varices, and rupture of ectopic pregnancy. Prevention of sudden death from these causes mainly involves diagnosis and treatment of the underlying disorder (Kuriachan et al., 2015).

Clinical Approach to Investigation of SCA

The approach to investigation of the survivor of SCA involves the following components (Krahn et al., 2010):

1. assessment for reversible causes
2. assessment for structural heart disease
3. assessment for primary electrical disease when there is no significant structural heart disease
4. evaluation of family members if an inheritable syndrome is suspected or identified

Table 2 lists some of the potentially reversible causes that are associated with SCA.

Assessment of the cardiac arrest survivor begins with a complete history and physical examination that includes evaluation of the presence of symptoms or signs suggestive of underlying structural heart disease (Krahn et al., 2012). The patient's ingestion history and medication list (both prescribed and nonprescribed) should be appraised for the possibility of drug- or toxin-induced arrhythmia or electrolyte disturbance. A three-generation family history should include careful review for the presence of SCA (often called a massive heart attack by patients) (Dorian and Krahn, 2016), syncope, neuromuscular disorders, unexplained drowning, sudden infant death, and sensorineural deafness. Laboratory testing should be performed immediately upon presentation to assess for electrolyte, acid–base, and metabolic abnormalities that may aid in diagnosis or that might require immediate treatment. However, the natural tendency to ascribe SCA to mild electrolyte abnormalities is to be avoided as such abnormalities may be the result of rather than the cause of the cardiac arrest (Kuriachan et al., 2015).

Serial ECG evaluation should be performed to screen for myocardial ischemia or infarction, conduction abnormalities, and repolarization or depolarization abnormalities. Continuous ECG telemetry monitoring may identify nonsustained and sustained arrhythmias or reversible myocardial ischemia.

Invasive coronary angiography should be performed in most cardiac arrest survivors to seek CAD that may require intervention. When coronary spasm is suspected, provocative testing with either ergonovine or acetylcholine may be performed. Noninvasive computed tomography coronary angiography may also be of value to rule out the presence of CAD and coronary artery anomalies when coronary angiography is not to be performed.

Table 2 Reversible causes of sudden cardiac arrest

Acidosis
Cardiac tamponade
Hypothermia
Hypovolemia, hemorrhage, anemia
Hypoxia
Hypomagnesemia
Hyperkalemia and hypokalemia
Myocardial infarction
Poisoning
Pulmonary embolism
Tension pneumothorax

Adapted from Kuriachan, V. P., Sumner, G. L. and Mitchell, L. B. (2015). Sudden cardiac death. *Current Problems in Cardiology* **40**, 133–200.

Cardiac imaging is indicated to assess cardiac structure and function, to stratify risk in terms of adverse clinical outcomes, and to guide therapeutic intervention. Clinical experience and experimental data indicate that there is often significant myocardial stunning and global impairment of left ventricular function in the first few days after a SCA event. Accordingly, in SCA survivors, formal assessment of left ventricular systolic function should be repeated approximately 1 week after the event.

2D echocardiography will identify many important etiologies of SCA including CAD (focal wall motion abnormalities), hypertrophic cardiomyopathy (asymmetric left ventricular hypertrophy), arrhythmogenic right ventricular cardiomyopathy (ARVC) (right ventricular wall motion abnormalities and aneurysms), aortic stenosis, and nonischemic dilated cardiomyopathies (global left ventricular systolic dysfunction). Nevertheless, cardiac magnetic resonance imaging (cMRI) is now considered the gold standard for assessment of cardiac volumes and function. In addition to those conditions listed above, cMRI may be useful to clarify or rule out the diagnosis of myocarditis, cardiac sarcoidosis, and cardiac amyloidosis. Furthermore, cMRI is more sensitive for the detection of right ventricular wall motion abnormalities which are of importance in the diagnosis of ARVC. Using the technique of late gadolinium enhancement, cMRI can also quantify the extent and distribution of myocardial scar. Finally, T2-weighted imaging, which identifies unbound myocardial water, characterizes myocardial edema associated with inflammatory conditions such as myocarditis, sarcoidosis, and acute ischemia (Krahn et al., 2010).

Exercise testing may be performed to provide a functional assessment and to expose symptoms and signs of exercise-related myocardial ischemia, myocardial dysfunction, or arrhythmia. Exercise testing is of particular value in the diagnosis of catecholamine-dependent arrhythmias including catecholaminergic polymorphic ventricular tachycardia (CPVT) and long-QT interval syndrome (LQTS). A failure to increase systolic blood pressure by at least 20 mmHg with exercise identifies a potentially high-risk individual with HCM.

Approximately 5%–10% of SCA survivors have neither structural heart disease nor a noncardiac etiology for their event (Krahn et al., 2010). Such individuals are considered to have a primary electrical disorder. Pharmacological challenge testing with epinephrine and with a Class Ic sodium channel blocker (procainamide, ajmaline, or flecainide) may unmask the otherwise subtle ECG abnormalities in conditions such as congenital LQTS, CPVT, and Brugada syndrome (BrS). When a graded epinephrine infusion elicits an absolute increase in the QT interval of at least 30 milliseconds then congenital LQTS-1 should be strongly considered. When the same infusion elicits polymorphic VT or bidirectional VT, the diagnosis of CPVT is supported. Similarly, when a Class IC antiarrhythmic drug infusion elicits a Type I BrS pattern ECG (>2 millimeters J-point elevation and coved ST-T segment elevation in leads V1 and V2), a diagnosis of BrS is likely (Kuriachan et al., 2015).

Transvenous catheter electrophysiologic studies (EPS) are not routinely performed in the assessment of SCA. In a patient with a structurally normal heart, lack of inducibility of VT or VF with programmed stimulation does not necessarily predict a favorable outcome, and an ICD would be indicated regardless of the results of such testing. In patients with manifest Wolff–Parkinson–White (WPW) syndrome, EPS may identify an accessory connection with a very short refractory period. Ablation of the accessory connection would then be indicated.

When an inheritable condition such as congenital LQTS, BrS, CPVT, or ARVC is identified, the patient and immediate family members may benefit from genetic testing. One study evaluated the yield of systematic advanced cardiac imaging and provocative testing in patients with unexplained SCA. In 63 patients with SCA of unknown cause, a diagnosis was obtained with advanced testing in 56% of patients. Targeted genetic testing revealed causative mutations in 47% of the 63 patients. (Krahn et al., 2010). In addition, of the 64 immediate family members screened, 24% were subsequently treated for their clinically silent and previously undiagnosed SCD risk.

Role of the Molecular Autopsy

The use of clinical forensic autopsy has declined significantly from its use in up to 50% of cases 50 years ago to less than 10% in 2008. However, over the same time frame, the development and availability of genetic testing to evaluate selected patients for

potential SCD risk has grown. Recently, published data from the Scripps Translational Science Institute (Torkamani et al., 2016) showed that molecular autopsy can provide important data to suggest a likely or possible etiology of SCD in 40% (10/25) of a series of sequential cases. The authors suggest that more comprehensive sharing of phenotypic and genetic data between databases may lead to the identification of even more SCD genotype/phenotype correlations (Torkamani et al., 2016). Doing so would provide important risk quantification information for living relatives of SCD victims.

Preventative Therapies

When an individual patient is identified at being at a higher risk of SCD, preventative therapies are considered. The most appropriate preventative therapies are a function of the underlying cause of the higher SCD risk. Specific therapies are discussed further in chapters dealing with specific etiologies. This chapter will only address the preventative therapies that are considered in patients with a demonstrated ischemic cardiomyopathy or nonischemic congestive cardiomyopathy in whom the most common pathways to SCD are VT and VF.

In patients with a demonstrated ischemic cardiomyopathy or nonischemic congestive cardiomyopathy the prevention of SCD begins with optimizing the treatment of the underlying structural heart disease. Indeed, randomized clinical trials in such patients have demonstrated that VT/VF, SCD, cardiovascular death, and all-cause mortality are reduced with beta-adrenoreceptor blocking agents; with hydroxymethylglutaryl coenzyme-A reductase inhibitors (statins) in patients with ischemic cardiomyopathy and possibly also in patients with nonischemic cardiomyopathy; and with renin–angiotensin–aldosterone system suppression by angiotensin-converting enzyme (ACE) inhibitors, by ARB, or by aldosterone blockers (Mitchell, 2008). In contrast, Vaughn Williams Class I antiarrhythmic drugs prevent VT/VF at the expense of an increase in SCD and all-cause mortality, Class III antiarrhythmic drugs prevent VT/VF with variable, drug-specific, effects on SCD and all-cause mortality, and digoxin may increase the incidence of VT/VF and SCD (Mitchell, 2008).

The ICD was conceived not to prevent VT/VF but rather to react to VT/VF so that the ventricular dysrhythmia does not result in SCD. Current ICDs reliably detect VT/VF and react with automatic antitachycardia pacing and automatic shock therapies to terminate the VT/VF with remarkable efficacy. Such devices do not obviate SCD but do dramatically reduce its probability. Of course, an ICD would be expected to provide the greatest survival benefit in patients with ischemic cardiomyopathy or nonischemic congestive cardiomyopathy that have a higher risk of future VT/VF episodes. The best predictor of a future episode of VT/VF is a past episode of VT/VF that occurred in the absence of a transient or completely reversible cause. In this regard, a first episode of VT/VF that develops in the 48 h after the onset of an acute ST-segment elevation myocardial infarction is not considered to portend a higher risk of future episodes. The risk of future episodes of VT/VF in such a patient is not demonstrably higher than that of patients with an equivalent myocardial infarction who did not develop VT/VF in the first 48 h after their infarction (Zipes et al., 2006). The vast majority of other patients who have experienced sustained VT/VF are a higher risk of future episodes and, when an ICD is used for prevention of SCD in such patients, the indication is referred to as being for the secondary prevention of SCD.

Three large, randomized clinical trials—the Antiarrhythmics Versus Implantable Defibrillator (AVID) trial (AVID Investigators, 1997), the Cardiac Arrest Study Hamburg (CASH) (Kuck et al., 2000), and the Canadian Implantable Defibrillator Study (CIDS) (Connolly et al., 2000a)—evaluated the efficacy of ICD therapy versus amiodarone therapy (1.3% of the total population of these trials received sotalol therapy rather than amiodarone therapy) for the secondary prevention of SCD. An individual patient-level meta-analysis of their results (Connolly et al., 2000b) reported that, after a mean follow-up of 2.33 years, arrhythmic deaths occurred at an annual rate of 2.7% in ICD-treated patients compared to 5.7% in amiodarone/sotalol-treated patients (Hazard ratio (HR), 95% CI, *p*-value: 0.49, 0.36–0.67, $p < 0.001$). In the absence of an effect on nonarrhythmic cardiac death or noncardiac death, this difference in arrhythmic death rates translated into a difference in annual all-cause mortality rates of 8.8% in ICD-treated patients compared to 12.3% in amiodarone/sotalol-treated patients (HR, 95% CI, *p*-value: 0.73; 0.60–0.87, $p < 0.001$) (**Fig. 2**). In this meta-analysis, the advantage of ICD therapy over amiodarone/sotalol therapy was restricted to patients with an LVEF ≤ 0.35. The secondary prevention ICD trials did not include patients with hemodynamically stable VT. Nevertheless, an analysis of the patients that were screened for participation in AVID but were not enrolled compared the outcomes patients with hemodynamically stable VT and patients with hemodynamically unstable VT and found that patients with underlying structural heart disease who presented with hemodynamically stable VT have a subsequent mortality rate that is at least as high as that of patients presenting with hemodynamically unstable VT suggesting that hemodynamically stable VT may be a marker for subsequent risk of hemodynamically unstable VT/VF and sudden death (Raitt et al., 2001). These studies have informed practice guidelines that recommend placement of an ICD in all patients with structural heart disease who have sustained VT or VF in the absence of a reversible cause. Some guidelines give an equivalent recommendation for chronic amiodarone therapy in patients with an LVEF >0.35.

Patients with structural heart disease but no previous episodes of sustained VT/VF who are considered to be at higher risk of future episodes of VT/VF are considered for an ICD for the purpose of primary prevention of SCD. Such instances are predicated on the ability to select patients at higher future risk of VT/VF. To date, the only predictor of a future first episode of VT/VF that has been demonstrated to select a patient population that was then shown to have a survival benefit from placement of an ICD in a randomized clinical trial is depressed left ventricular systolic function defined as a LVEF ≤ 0.35. A total of eight randomized clinical trials have evaluated the advantages, disadvantages, and risks of ICD therapy versus placebo therapy, antiarrhythmic drug therapy, or both in patient populations selected, at least in part, by virtue of significant depression in LVEF (Theuns et al., 2010).

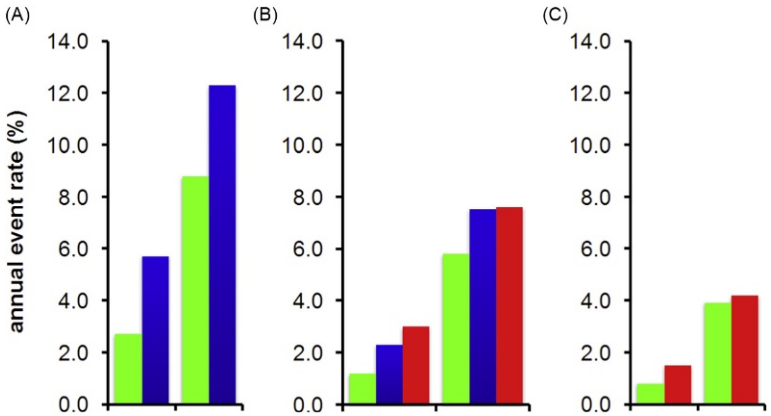

Fig. 2 Annual event rates for arrhythmic death (*left column group in each panel*) and for all-cause mortality (*right column group in each panel*) for patients treated with an ICD (*green columns*), for patients treated with antiarrhythmic drugs (almost exclusively with amiodarone) (*blue columns*), and for patients treated with neither (*red columns*). Panel A shows results of a meta-analysis of randomized trials of ICD therapy for secondary prevention of SCD. Panel B shows results of the SCD-HeFT randomized trial of ICD therapy for primary prevention of SCD. Panel C shows results of the DANISH randomized trial of ICD therapy for primary prevention of SCD in patients with well-treated nonischemic congestive cardiomyopathy. The annual event rates are higher in patient populations a prior life-threatening ventricular tachyarrhythmia (secondary prevention trials—Panel A) than they are in patient populations without a prior life-threatening ventricular tachyarrhythmia (primary prevention trials—Panels B and C). The annual event rates are higher in less contemporary patient populations that include patients with either ischemic or nonischemic congestive cardiomyopathy (Panel B) than in more contemporary patient populations that only include patients with nonischemic congestive cardiomyopathy (Panel C). Nevertheless, in each patient population the probability of arrhythmic death is halved by an ICD compared to control with or without antiarrhythmic drug therapy. Whether or not the ICD advantage with respect to arrhythmic death translates into a reduction in all-cause mortality is dependent on other factors.

No patient-level meta-analysis of the results of these trials has been reported. Accordingly, to derive annualized mortality rates in patients evaluated for a primary prevention ICD that might be compared to those of patients evaluated for a secondary prevention ICD the results of the largest of these trials, the Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT) (Bardy et al., 2005), are cited here. After a median follow-up of 3.79 years, arrhythmic deaths occurred at an annual rate of approximately 1.2% in ICD-treated patients compared to approximately 2.3% in amiodarone-treated patients (statistics for this comparison not provided) and compared to approximately 3.0% in placebo-treated patients (HR, 95% CI, *p*-value: 0.40, 0.27–0.59, $p < 0.001$). In the absence of an effect on nonarrhythmic cardiac death or noncardiac death, these differences in arrhythmic death rates translated into differences in annual all-cause mortality rates of 5.8% in ICD-treated patients compared to 7.5% in amiodarone-treated patients (statistics for this comparison not provided) and compared to 7.6% in placebo-treated patients (HR, 97.5% CI, *p*-value: 0.77, 0.62–0.96, $p < 0.001$) (Fig. 2). In SCD-HeFT, the advantage of ICD therapy over amiodarone therapy and over placebo therapy was not a function of whether the underlying structural heart disease was ischemic or nonischemic. Similarly, a meta-analysis of SCD-HeFT and the other seven randomized primary prevention ICD trials in patients with ischemic cardiomyopathy or with nonischemic congestive cardiomyopathy similarly did not demonstrate a difference in response to ICD therapy in these two patient population groups. However, more recently, the Danish Study to Assess the Efficacy of ICDs in Patients with Nonischemic Systolic Heart Failure on Mortality (DANISH) (Kober et al., 2016) randomly assigned patients with a nonischemic congestive cardiomyopathy and a LVEF $\leq$0.35 to receive or not to receive an ICD in addition to optimal medical therapy and, when indicated, cardiac resynchronization therapy (CRT) (58% of each group). This study randomized 1116 patients. After a median follow-up of 5.6 years, arrhythmic deaths occurred at an annual rate of approximately 0.8% in ICD-treated patients compared to approximately 1.5% in control patients (HR, 95% CI, *p*-value: 0.50, 0.31–0.82, $p = 0.005$). The SCD rates in DANISH were substantially lower than those of the other trials described above, an observation that may reflect advances in both medical therapy and CRT in this patient population (Fig. 2). Both nonarrhythmic cardiac death and noncardiac death occurred statistically nonsignificantly more often in the ICD-treated group. Accordingly, the difference in arrhythmic death rates did not translate into a statistically significant difference in annual all-cause mortality rates of approximately 3.9% in ICD-treated patients compared to approximately 4.2% in control patients (HR, 95% CI, *p*-value: 0.87; 0.68–1.12, $p = 0.28$). A subsequent meta-analysis of DANISH and the other five randomized controlled trials of ICD therapy for the primary prevention of SCD in 2970 patients with nonischemic congestive cardiomyopathy reported a statistically significant 23% risk reduction in all-cause mortality in favor of ICD therapy [HR 0.77, 95% CI (0.64, 0.91)] (Golwala et al., 2016).

Most patients with ischemic cardiomyopathy or nonischemic dilated cardiomyopathy who are candidates to receive a CRT pacemaker are also candidates to receive an ICD, and many patients who are candidates to receive an ICD are also candidates to receive a CRT pacemaker. The outcomes following use of the monotherapy devices (CRT or ICD) have been compared to use of a combined CRT defibrillator (CRT-D) in nine randomized controlled or two-arm prospective studies in patients with congestive heart failure. A meta-analysis of their results (Deng et al., 2015) reported that, compared to use of a CRT-D, use of a CRT pacemaker was associated with a higher follow-up incidence of all-cause mortality (HR, 95% CI, *p*-value: 1.46; 1.19–1.78, $p < 0.001$) and compared to use of a CRT-D, use of an ICD alone was associated with a higher follow-up incidence of all-cause mortality (HR, 95% CI, *p*-value:

1.27; 1.06–1.52, $p = 0.009$) in patients who are candidates for both devices. The ventricular reverse remodeling that accompanies a patient's favorable response to CRT pacing might be expected to reduce the frequency and severity of follow-up VT/VF episodes given the well-recognized association between left ventricular systolic function and ventricular arrhythmogenesis. Data supporting this contention include a systematic review of six cohort studies that compared the follow-up incidence of appropriate ICD therapies between CRT-D recipients who were responders to CRT pacing and CRT-D recipients who were nonresponders to CRT pacing (Chatterjee et al., 2015). Four trials defined responders to CRT pacing as those whose LVEF improved to ≥ 0.35. The annual rate of appropriate ICD therapies was significantly lower in patients who were so defined as modest responders to CRT pacing at 5.4% than in patients who were not responders to CRT pacing at 11.5% ($p < 0.001$). Two trials defined responders to CRT pacing as those whose LVEF improved to ≥ 0.45. The annual rate of appropriate ICD therapies was significantly lower in patients who were so defined as significant responders to CRT pacing at 2.3% than in patients who were not responders to CRT pacing at 8.2% ($p < 0.001$).

Conclusion

SCD is one of the most common single causes of death in developed nations. Although very significant advances in the understanding of SCD and in the prevention of SCD have been made over the past decade, the persistence of the epidemic of SCD indicates that there is more to be done both with respect to new knowledge creation and with respect to implementation of the knowledge already in hand.

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Surgical Management of Atrial Fibrillation

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Introduction

Atrial fibrillation (AF) is the most common arrhythmia in the world. Its detrimental effects stem from atrioventricular (AV) dyssynchrony causing hemodynamic compromise and left atrial (LA) stasis resulting in thromboembolism. AF is associated with a fivefold increased stroke risk (Kannel et al., 1998), a threefold increased risk of heart failure (Wang et al., 2003), and a twofold increased mortality risk (Wang et al., 2003). Patients may experience palpitations, fatigue, exercise intolerance, and dyspnea. Patients frequently require anticoagulation, which predisposes to bleeding complications and hemorrhagic stroke (Mitchell et al., 2013). The inadequacies of pharmacologic management and catheter intervention have justified a role for surgical ablation.

Mechanisms

Proposed electrophysiological mechanisms of AF include random ectopic depolarization of atrial tissue, rapid and irregular depolarization of a single focus, and reentrant circuits (Moss, 2003; Cox et al., 1991). Moe (1962) proposed the multiple wavelet hypothesis that AF was maintained by multiple changing wave fronts that randomly reentered the atria. Allessie et al. (1985), in an animal model of cholinergic AF, demonstrated that at any one time, multiple wavelets were present during AF. Factors contributing to the electrophysiological substrate that determines whether AF is initiated and/or sustained include a trigger, the atrial refractory period, the conduction velocity, and atrial geometry. A critical mass of atrial tissue is required to support a reentrant circuit (Byrd et al., 2005).

Haissaguerre et al. (1996) demonstrated that the trigger for AF originated in the pulmonary veins (PVs) in most patients with paroxysmal AF (PAF). However, up to a third of the triggers lie outside the PVs (Lee et al., 2005). Electrically excitable cardiac muscle extends 1–4 cm beyond the ostium of the PVs (Spach et al., 1972). Pacemaker tissue has been shown to be present in the PVs during development (Blom et al., 1999).

AF is characterized by anatomical, electrical, cellular, and structural remodeling (SRM). SRM characterized by interstitial fibrosis is well described in AF. Late gadolinium enhancement MRI (LGE-MRI) can identify SRM and stratify patients likely to benefit from ablation. McGann et al. (2014) showed that the extent of LA wall fibrosis on LGE-MRI strongly correlates with ablation outcome and histological evidence of remodeling. Arrhythmia recurrence following catheter ablation was significantly higher when $\geq$30% fibrosis was present on preprocedural MRI scans.

Indications for Surgical Ablation

The indications for surgical ablation of AF are detailed in the 2012 Expert Consensus Statement of the Heart Rhythm Society Task Force on Catheter and Surgical Ablation of AF (European Heart Rhythm Association (EHRA) et al., 2007).

Stand-alone AF

Symptomatic AF refractory to medical therapy
Failed catheter ablation
Contra-indications to anticoagulation
LA thrombus
High-risk patients with persistent AF who suffered a cerebrovascular event while anticoagulated

Concomitant AF

Symptomatic AF, regardless of whether antiarrhythmic medication has been started.

Asymptomatic AF when surgical ablation would not significantly add to operative risk (Lancaster et al., 2016a).

Contra-indications

Patients with a LA of >8 cm, significant LA fibrosis/calcification on imaging, or age >85 years are considered to have relative contraindications since recurrence rates tend to be high (Damiano et al., 2011a).

Surgical Principles

The Cox-Maze procedure consists of a series of incisions in both atria designed to theoretically interrupt the macroreentrant circuits that sustain AF, leaving a pathway for the sinus node to activate both atria and the AV node. By restoring sinus rhythm (SR) and AV synchrony while maintaining atrial transport function, the Cox-Maze procedure has the potential to reduce the risk of thromboembolism, stroke, and hemodynamic compromise associated with AF.

Pulmonary Vein Isolation or Cox-Maze Procedure?

The catheter mapping performed by Haissaguerre et al. (1996) was performed in patients with "stand-alone" AF. Approximately 60% of patients with PAF have PV triggers (Cox, 2014). Pulmonary vein isolation (PVI) works well in a small group of patients with isolated PAF and normal-sized left atria, short-term AF, and contraindications to anticoagulation. However, the late results with PVI alone have been relatively poor in the catheter ablation literature (Boersma et al., 2012) and have also had high late recurrence rates in some surgical series (Boersma et al., 2012). Data from Washington University would strongly support that PVI alone should never be performed. The minimal requirement for successful late outcomes is isolation of not only the PVs, but all of the posterior left atrium. Without this, late results have shown a success rate of less than 30% at 5 years (Henn et al., 2015).

Patients with non-PAF (NPAF) have more complex mechanisms that may involve multiple rotors, non-PV focal sources, and both macro- and microreentrant circuits. In this situation, simple PVI is no longer effective.

A retrospective review compared patients who underwent a Cox-Maze procedure ($n=100$), with those who had a limited Cox-Maze procedure ($n=7$), or PV isolation alone ($n=23$). Freedom from AF was 90%, 86%, and 59% in the in the Cox-Maze procedure group, limited Cox-Maze procedure group, and PV isolation alone group, respectively (Melby et al., 2006). Since surgeons deal primarily with concomitant AF, Haissaguerre's observation even in PAF may not be applicable in our surgical patients.

LA or Biatrial Maze?

A common dilemma is the necessity to perform right atrial lesion sets. Two metaanalyses (Barnett and Ad, 2006; Zheng et al., 2016) concluded that biatrial surgical procedures were more effective than left-sided procedures in eliminating AF. Sixty-nine studies (5885 patients) were included in the metaanalysis by Ad et al. Biatrial surgical procedures (range, 92.0%–87.1%) demonstrated superior freedom from AF at all-time points compared with LA surgical procedures (range, 86.1%–73.4%). At 3 months, 1, 2, and 3 years, the freedoms from AF were 92% versus 86%, 89% versus 76%, 86% versus 75%, and 87% versus 73%, respectively.

Since the mechanisms of AF in the individual patient are rarely well defined, we usually default to performing a biatrial lesion set, as this has had excellent long-term success rates in restoring SR. In patients with PAF, of short duration (<1 year), no LA enlargement (LA diameter <4.5 cm) and no RA enlargement or right-sided pathology (i.e., tricuspid regurgitation, pulmonary hypertension), we have performed a full left-sided Cox-Maze lesion set with good late results. However, this represents less than 10% of patients referred for surgical ablation at Washington University.

Concomitant AF

The three groups of patients with concomitant AF, who represent the vast majority of patients undergoing cardiac surgery, are those undergoing aortic, coronary, or mitral surgery (Cox, 2014).

CABG and AVR patients

PVI alone is not an adequate or appropriate approach in these patients. Patients require a Maze procedure. The surgical dilemma in this situation is the need to open the LA, which many surgeons are reluctant to do. Adding a Maze procedure does not increase the morbidity or mortality of patients undergoing coronary surgery (Ad et al., 2012a).

Ad et al. (2012a) reported 485 patients who underwent the Cox-Maze procedure of whom 95 had a Cox-Maze with an AVR or CABG (Cox-Maze/AVR = 30; Cox-Maze/CABG = 47; Cox-Maze/AVR/CABG = 18). 4255 patients with no history of AF underwent AVR or CABG without surgical ablation (AVR = 422; CABG = 3518; AVR/CABG = 315).

In unadjusted analyses, the Cox-Maze group was similar to the non-Cox-Maze group on perioperative permanent stroke (1.1% vs. 1.4%), prolonged ventilator time (>24 h) (6.3% vs. 6.1%), renal failure requiring dialysis (1.1% vs. 1.2%), readmissions within 30 days of discharge (12.6% vs. 8.3%), and operative mortality (within 30 days; 1.1% vs. 1.7%). The Cox-Maze group did have significantly more patients who developed perioperative pneumonia (5.3% vs. 1.9%) and reoperation for bleeding (5.0% vs. 2.0%) although none of the bleeding was related to the atriotomies or LA appendage (LAA) amputation.

All 95 patients who underwent the Cox-Maze procedure were propensity score matched with patients who did not undergo the Cox-Maze. Addition of the Maze procedure did not predict receipt of intraoperative blood products (OR, 0.83; CI, 0.28–2.54) or postoperative blood products (OR, 0.63; CI, 0.25–1.57), permanent stroke (0.55; 0.07–4.35), prolonged ventilation (>24 h) (0.78; 0.31–1.93), renal failure requiring dialysis (0.76; 0.09–6.20), or operative mortality (0.34; 0.04–2.72), in the propensity score-matched groups.

Mitral valve surgery patients

Patients with AF undergo a Maze procedure, as the LA has to be opened to perform mitral surgery.

Technology

Surgical ablation devices either use a unipolar energy source or bipolar clamps. Unipolar energy sources (cryothermy, unipolar radiofrequency (RF) energy) radiate either heat or cold from a single source, in an unfocused fashion and can cause collateral damage (Gillinov et al., 2001). Transmural lesions are difficult to create from the epicardial surface on the beating heart, due to the heat sink effect of circulating blood (Santiago et al., 2003).

Radiofrequency

RF energy uses an alternating current between 100 and 1000 kHz which is high enough to prevent rapid myocardial depolarization and the induction of ventricular fibrillation, yet low enough to prevent tissue vaporization and perforation. Lesions are created through thermal injury. Resistive heating occurs within a narrow rim of tissue in direct contact with the electrode. Passive conduction continues from this interface to create deeper lesions.

With bipolar RF ablation, focused energy is delivered between two closely approximated electrodes embedded in the jaws of a clamp, thereby creating discrete lesions. This reduces collateral damage. The device continuously measures tissue conductance to assess transmurality (Damiano et al., 2007). When the conductance drops to a stable minimum level, the lesion is deemed to be transmural. Bipolar ablation enables shorter ablation times but is incapable of fully ablating the right and LA isthmuses (Damiano et al., 2007). Newer unipolar RF ablation devices have been developed with suction adherence to aid in minimally invasive applications.

The COBRA Fusion Surgical Ablation System (AtriCure, Inc.) is a suction-assisted, combined bipolar/monopolar linear RF device designed to reduce the heat sink effect of circulating blood and allow ablation on the beating heart (Lancaster et al., 2016b).

Cryoablation

The two sources of cryothermal energy are nitrous oxide and argon, which at one atmosphere of pressure reach temperatures of _89.5 degrees C, and _185.7 degrees C, respectively (Lee et al., 2009). Cryoablation causes tissue injury through the freezing and rewarming process. Acutely, ice crystals disrupt cell membranes, whereas, chronically, microvascular damage leads to local tissue ischemia. Cryoablation preserves the fibrous skeleton of the heart, making it safe for use around valvular tissue, but is ineffective on the beating heart. Cryolesions take longer to create (1–3 min) (Lee et al., 2009).

The ability of bipolar clamps to create reliable transmural lesions on the beating heart has been confirmed in chronic animal models (Melby et al., 2006).

Operative Techniques

The Cox-Maze IV Procedure

Preoperative planning

A transthoracic echocardiogram is performed to determine LA size and detect the presence of LA thrombus and valve disease (Lawrance et al., 2016). A contrast chest computed tomography (CT) scan is performed to rule out PV stenosis in patients who have failed catheter ablation. Patients scheduled to have a right mini-thoracotomy (RMT), receive a CT angiogram of the aorta, iliac, and femoral arteries, if they are older than 40 years, or have peripheral vascular disease (PVD) or risk factors for PVD. Coronary angiography is performed to define coronary anatomy and stenosis (Robertson et al., 2014). Antiarrhythmic and rate-control medication should be continued until the day of the procedure. Patients on warfarin or a novel anticoagulant are transitioned to intravenous heparin (Robertson et al., 2014).

Surgical access

The Cox-Maze procedure can be performed through a median sternotomy or a RMT. A RMT approach is used for stand-alone AF ablation and in patients undergoing concomitant mitral or tricuspid valve surgery. Median sternotomy is reserved for patients with contraindications to the RMT approach (PVD that precludes femoral cannulation for cardiopulmonary bypass (CPB), prior right thoracotomy, severe LV dysfunction, or chest wall deformities) (Lawrance et al., 2016), and when the maze procedure is performed in conjunction with aortic or coronary surgery.

Ablation principles

The essential prerequisites for an effective ablation are lesion transmurality and continuity. The only guarantee of effectiveness of a procedure is the creation of a fully transmural lesion, from either the epicardial or the endocardial surface (Melby et al., 2013). Very small gaps in ablation lines have the ability to conduct impulses, thereby emphasizing the importance of creating complete and continuous lesions (Melby et al., 2008). A continuous LA isthmus lesion to the mitral annulus is critically important. Gaps in this ablation line can lead to LA flutter which is often more symptomatic than AF.

Each RF ablation line is created by performing two to three sets of firings of the bipolar RF clamp, to ensure a transmural lesion. For each set, the clamp is left in place and ablations are repeated until the ablation time falls below 10 s. All cryoablations are performed for 3 min at a temperature less than −40°C.

Sternotomy approach

The Cox-Maze procedure has been well described by Dr Damiano's group (Lawrance et al., 2016). A median sternotomy is performed and CPB is initiated. The absence of LA clot is confirmed by TEE. Cardioversion is performed after the administration of IV amiodarone, if the patient is in AF and there is no LA clot.

PV isolation

Pacing thresholds are measured from each PV. All four PVs are dissected and isolated. The intraatrial septum is completely dissected to remove the fat. A generous cuff-of atrial tissue is clamped between the jaws of bipolar RF clamp and the clamp is fired to ensure electrical isolation between the PVs and the LA. Isolation is confirmed by documenting exit block demonstrated by a failure to epicardially pace from each of the PVs.

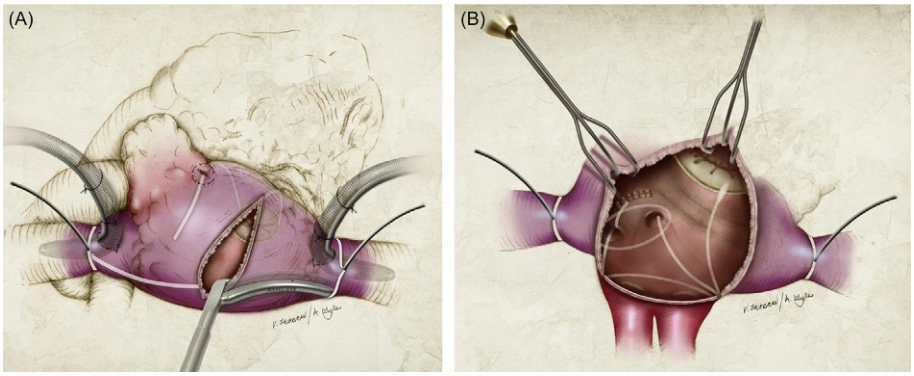

Fig. 1 CMIV lesion set as performed through a sternotomy. (A) Right atrial lesion set. RF ablations consist of SVC/IVC ablation and RA free wall ablation. Cryoablations include linear cryoablations through a purse-string suture to the 10 o'clock position of the tricuspid valve annulus and through the superior aspect of the atriotomy to the 2 o'clock position of tricuspid valve annulus. (B) Left atrial lesion set. Bipolar RF ablation lines consist of bilateral pulmonary vein isolations, ablations between the left superior pulmonary vein and amputated atrial appendage, pulmonary vein roof and floor connecting ablations, and ablation between the inferior aspect of the atriotomy to mitral valve annulus. Cryoablations include linear epicardial cryoablation over the coronary sinus and at the mitral valve annulus. Reproduced from Lawrance, C. P., Henn, M. C., Damiano, R. J., Jr. (2016). Surgery for atrial fibrillation. *Heart Failure Clinics* **12**(2), 235–243. With permission from Elsevier.

Right atrial lesion set

The RA lesion set is performed on the beating heart (Fig. 1A).

Appendage lesions: Through a purse-string suture at the base of the RA appendage, a RF lesion is created across the free wall of the RA, toward the superior vena cava (SVC), and down the aortic side of the RA appendage to avoid the SA node region. The linear cryoprobe is inserted through the purse-string suture, and an endocardial cryoablation is created down to the tricuspid valve annulus at the 10 o'clock position.

Free wall lesion: A vertical atriotomy is made from the intraatrial septum toward the AV groove. This incision should be at least 2 cm from the first free wall ablation to avoid an area of slow conduction.

Tricuspid annulus cryolesion: From the superior extent of this incision, a linear cryoprobe is used to create an endocardial cryoablation down to the tricuspid annulus at the 2 o'clock position.

Intercaval lesion: From the inferior aspect of the RA incision, RF lesions are placed along the lateral aspects of the SVC and the inferior vena cava (IVC).

LA lesion set

The aorta is cross-clamped, and the heart is arrested using cold blood cardioplegia (Fig. 1B).

LAA: The LAA is amputated. Through the amputated LAA, an RF ablation line is created into either of the left PVs. The LAA is oversewn.

Left atrium: A standard left atriotomy is performed. The bipolar RF clamp is used to create ablation lines from the inferior and superior aspects of this incision to the left inferior and superior PVs, respectively. A third bipolar RF lesion is placed from the inferior aspect of the atriotomy, across the atrial floor, toward the mitral annulus. This lesion heads toward the junction of the P2–P3 scallop of the mitral valve in patients with a right dominant circulation, and toward the posterior commissure if the patient has a left dominant circulation. This ablation should cross the coronary sinus in the space between the right coronary artery and the circumflex artery.

The AV groove containing thick tissue is located here and requires cryoablation to bridge the 1–2 cm gap from the end of the RF ablation line to the mitral annulus. To complete the LA lesion set, the coronary sinus is ablated in line with the isthmus lesion by performing cryoablation from the epicardial surface. This often requires use of a monopolar device from the epicardial surface to ensure transmurality. The mitral isthmus line is the "Achilles heel" of the LA lesion set and prevents postoperative atrial flutter. This lesion requires both bipolar and unipolar energy, due to the thickness of the AV groove and the inability of the bipolar clamp to reach the annulus. Failure to create the mitral annulus lesion is associated with a higher incidence of postoperative atrial flutter.

The left atriotomy is closed. The aorta is unclamped after dearing, and the patient is weaned from CPB. Epicardial right atrial and ventricular wires are placed. The chest is closed after securing hemostasis.

Right mini thoracotomy

Preparation

The patient is positioned supine with the right chest elevated 45° and the hips flat (Saint et al., 2014). TEE is performed to characterize the mitral regurgitation, and detect the presence of AR, a patent foramen ovale or LA clot. Significant aortic insufficiency is a contraindication to this approach. Both groins are prepped for femoral cannulation.

The right femoral artery and vein are exposed through a subinguinal incision and cannulated. Cannula position is verified by TEE. The right pleura is entered through the 4th Interspace, through a 5–6 cm RMT incision. Normothermic CPB is initiated. The right lung is deflated. If in AF, the patient is cardioverted.

PV isolation

The right PVs are ablated similar to that described in the sternotomy approach.

Right atrial lesion set

The right atrial lesion set is performed through three separate purse strings during CPB on the beating heart (Fig. 2A).

Intercaval lesion: The first purse string is placed just above the intraatrial septum between the IVC and SVC. RF lesions to the SVC and IVC are placed through the first purse string, similar to that described in the sternotomy approach.

Free wall lesion: An RF line of ablation is created through this purse string similar to that described in the sternotomy approach. This RF lesion substitutes for the vertical atriotomy in the sternotomy approach. Care is taken not to ablate over the AV fat pad to prevent right coronary artery injury.

Tricuspid annulus cryolesion: At the superior extent of this lesion, a second purse string is placed, through which an endocardial cryolesion is placed to the TV annulus, at the 2 o'clock position.

Appendage lesions: A third purse string is placed at the base of the RA atrial appendage. RF and cryolesions are created similar to that described in the sternotomy approach.

The endocardial cryoablations are performed with the heart beating. The circulating warm blood through the right coronary prevents injury from cryoablation.

LA lesion set

This lesion set is performed after aortic cross clamping and administration of cold blood cardioplegia. Through a left atriotomy the mitral valve and PVs are exposed (Fig. 2B).

LAA: The LAA is oversewn from the endocardial side.

Left atrium: The LA ablation lines are similar to that described in the sternotomy approach. To complete the isolation of the posterior LA, the two ablation lines to the left inferior and superior PVs are connected behind the PVs and along the lateral ridge by performing endocardial cryoablations.

The left atriotomy is closed. The aorta is unclamped after dearing, and the patient is weaned from CPB. Epicardial right atrial and ventricular wires are placed. The femoral vessels are decannulated. Distal femoral arterial flow is confirmed by Doppler. The groin and chest are closed after obtaining hemostasis.

Pitfalls

1. Postoperative bleeding

   Bleeding can occur from the atriotomy incisions or from the LAA, which tends to be friable.

2. Coronary artery injury

   RF energy and cryoablation can damage coronary arteries (Roberts-Thomson et al., 2009). Bipolar RF ablation acutely has been shown not to damage the coronary arteries. However, the late histological changes are unknown. Cryoablation has been associated with intimal hyperplasia and has been implicated in late coronary stenoses.

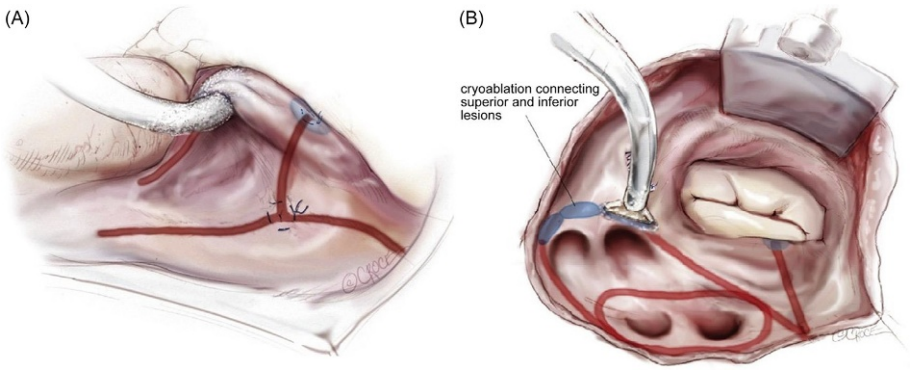

Fig. 2 CMIV lesion set as performed through a right mini sternotomy. (A) Right atrial lesion set. Three separate purse-string sutures and replacement of right atriotomy with a line of bipolar RF ablation. All other lesions are identical to the sternotomy approach. (B) Left atrial lesion set. Left pulmonary vein isolation is achieved through sequential endocardial cryoablation, and LAA is oversewn in two layers endocardially. All other lesions are identical to the sternotomy approach. Reproduced from Lawrance, C. P., Henn, M. C., Damiano, R. J., Jr. (2016). Surgery for atrial fibrillation. *Heart Failure Clinics* **12**(2), 235–243. With permission from Elsevier.

3. PV isolation
   Repeat ablations are necessary if exit block is not achieved.
4. Posterior LA isolation
   The posterior LA is an important source for the triggers and drivers that initiate and maintain AF (Ndrepepa et al., 2003). The box lesion reduces the critical mass of atrial tissue available for sustaining AF (Voeller et al., 2008). Isolating the entire posterior LA yields significantly higher freedom from AF and antiarrhythmic drugs (AADs). This finding is consistent with data from the electrophysiology laboratory, in which wide-area circumferential ablation of the posterior LA was more effective than targeting only the PVs (Oral et al., 2003).

Postoperative Management

Postoperative junctional and atrial tachyarrhythmias (ATAs) are not uncommon but usually resolve spontaneously after several days. Patients with stable ATAs are usually rate controlled pharmacologically and are DC cardioverted if ATAs persist. It is preferable to wait 1–3 weeks, before cardioversion, to allow postoperative inflammation to subside. Antiarrhythmic medication is not commenced until the return of SR, particularly in patients with bradyarrhythmias.

If heart block develops, AV sequential pacing is used. About 5% of patients will require a permanent pacemaker after a Cox-Maze procedure and the incidence increases with age (Robertson et al., 2013).

Warfarin is initiated postoperatively after the return of SR and is continued for at least 3 months. Warfarin is discontinued after 3 months, if the patient has no ATAs, off antiarrhythmic medication on prolonged monitoring (at least a 24-h Holter) and shows no atrial stasis on echocardiography.

Monitoring

Recommendations for follow-up and standardized outcomes reporting are provided in the 2012 Heart Rhythm Society consensus statement (Calkins et al., 2012). Recurrence is defined as any episode of AF, flutter, or atrial tachycardia (collectively referred to as ATAs) of at least 30 s duration that occurs after a 3-month blanking period. Success rates are reported as the percentage of patients free from ATAs, in the absence of class I and III AADs. Patients are seen at 3 months following ablation and then every 6 months for at least 2 years. Rhythm assessment at a minimum includes 24-h Holter monitoring at 6- and 12-months, and ECGs at each postoperative visit.

A prospective multicenter study, comparing conventional intermittent methods of monitoring with continuous monitoring using an implantable loop recorder (ILR), showed an increasing trend in compliance when using ILR (93%) compared to ECG (85%) and Holter monitoring (76%) (Damiano et al., 2016). Freedom from ATAs was not different between these modalities. Implantable devices are useful in patients who are unlikely to return for follow-up. The major limitations of the ILR devices are (1) a limited storage capacity and (2) a high rate of false-positive readings.

Surgical Results: Cox-Maze Procedure

The Cox-Maze procedure is the gold standard in surgical ablation and is the interventional procedure with the single highest success rate of terminating AF. Henn et al. (2015) reported a 5-year freedom from ATAs with and without AADs of 78% and 66%, respectively in a cohort of 576 patients. Ad et al. (2015) demonstrated 5-year freedom from ATAs with and without AADs of 85% and 71%, respectively in a cohort of 120 patients.

Damiano et al. (2003) examined the long-term efficacy of the Cox-Maze procedure in patients with ischemic heart disease. In 47 patients undergoing coronary grafting with a concomitant Maze procedure, 98% of patients were free of AF, at a mean follow-up of 5.7 years. 19% required a permanent pacemaker postoperatively. 78% of these patients had a preoperative diagnosis of sick sinus syndrome which is a known risk factor. Most patients were off warfarin and AADs. No patient had a cerebrovascular accident during follow-up.

When AF ablation is performed concomitant to other cardiac procedures, it is necessary to consider the incremental risk incurred by a concomitant AF ablation. In patients undergoing mitral surgery, the postoperative mortality rate was 4.6% in those not undergoing a Cox-Maze IV procedure versus 2.9% in those who did (Saint et al., 2013).

Risk factors for recurrence (Damiano et al., 2011b) at 1 year include (1) failure to perform a box lesion, (2) increasing LA size, with a probability of recurrence exceeding 50% once the LA approaches 8 cm (Byrd et al., 2005) and (3) early ATAs, which are likely a marker of more advanced pathology within the atrial substrate.

LA Appendage

Most strokes due to AF have their origin in the trabeculated portions of the LAA (Cox, 2013). Several percutaneous and surgical methods for closing the LAA have been developed. The ACC/AHA Guidelines for the Management of Patients with Valvular Heart

Disease recommend amputation of the LAA at the time of mitral surgery to reduce the risk of thromboembolism (Bonow et al., 2008).

It is believed that elimination of the LAA debulks the LA, eliminates potential focal LAA triggers, and decreases macroreentrant circuits and conduction abnormalities within the LAA (Hocini et al., 2011).

Surgical techniques to isolate the LAA include (1) suture ligation, (2) clipping, (3) excision, and (4) stapling with or without excision (Ailawadi et al., 2011). These techniques are imperfect and sometimes traumatic to the appendage. Unsuccessful appendage closure is defined as the presence of: (1) a patent appendage due to dehiscence of the suture or staple line; (2) a color flow jet between the LA and the LAA, or (3) a residual pouch in the LAA <1 cm in maximal length after closure (Damiano, 2008).

Moreover, when using techniques that do not remove the appendage, studies have documented residual blood flow through the closure line into the appendage in up to one-third of patients.

Patients with persistent flow into the appendage have a greater likelihood of LA thrombus (Kanderian et al., 2008). A retrospective study from the Cleveland Clinic of 137 patients reported successful LAA closure in 73%, 23%, and 0% of patients who underwent surgical excision and closure, suture ligation, and staple exclusion, respectively (Kanderian et al., 2008).

In a study reported by Aryana et al. (2015), complete appendage ligation (cLAA) was detected in 64%, incomplete ligation (ISLL) in 24%, and a residual LAA stump in 12%. Spontaneous systemic embolization (SSE) occurred in 2% of patients with cLAA versus 24% with ISLL and 0% with LAA stump ($P=0.006$). ISLL emerged as the sole predictor of SSE in a multivariate analysis. Anticoagulation is recommended in this high-risk cohort.

The only reliable techniques appear to be surgical excision and clipping (Damiano, 2008). Evidence indicates that less-invasive procedures that suture exclude or staple the appendage are inadequate (Kanderian et al., 2008).

Minimally Invasive Surgery for AF

The development of new ablation technology has not only simplified and shortened the Cox-Maze procedure but has also spurred interest in minimally invasive procedures on the beating heart. New devices use parallel bipolar electrodes to create lesions from the epicardial surface, in an attempt to mitigate the heat sink effect, and improve the efficacy of ablation on the beating heart. However, compared to bipolar clamps, these devices are not as reliable at creating lesions that are both transmural and continuous (Watanabe et al., 2014). Other innovations include chest wall soft tissue retractors, atrial retractor systems, lighted dissectors, LAA occlusion devices, and high definition endoscopes.

The most frequently performed minimally invasive procedure on the beating heart for stand-alone AF uses the bilateral video-assisted mini-thoracotomy or thoracoscopic approach. A RF bipolar device is used for PVI with or without ganglionic plexus disruption, division of the ligament of Marshall and LAA exclusion (Wolf et al., 2005).

The longest-term outcomes reported with this technique (Wolfe technique) include 139 patients in whom freedom from ATAs off AADs was 52%, 28%, and 29% for paroxysmal, persistent, and long-standing persistent AF, at 5-year follow-up (Zheng et al., 2013). The bilateral mini-thoracotomy approach for PVI has been modified to a bilateral, completely thoracoscopic approach. La Maeir and colleagues have recently reported on a unilateral thoracoscopic approach via the left chest (La Meir, 2013).

A multicenter report on the bilateral approach showed success rates of 93% for PAF, 96% for persistent AF, and 71% for permanent AF, with 61% of these patients able to discontinue AADs and 65% discontinuing anticoagulation therapy (Beyer et al., 2009). The complication rate was 13% and included pacemaker implantation, phrenic nerve injury, hemothorax, and transient ischemic attacks. Kasirajan et al. (2012) reported a freedom from AF of 80% off antiarrhythmic medication, at a mean follow-up of 16.5 months in 118 patients. Permanent pacemakers were required in 2% of the patients.

Notwithstanding the inferior results of minimally invasive PVI compared to those of the Maze procedure, surgical PVI is superior to catheter ablation in patients with PAF and small left atria. In the FAST trial, 124 patients with short-duration AF, LA dilation (>4.0 cm), and previous failed catheter ablation were randomized to surgical PVI or catheter ablation. For surgical PVI, the 12-month freedom from ATAs off AADs was 66% versus 37% for catheter ablation ($P=0.002$) although there was a 23% surgical complication rate due to a high rate of pneumothorax (Boersma et al., 2012).

Some centers have used additional epicardial ablation lines to construct an extended LA lesion set, to address the suboptimal outcomes of stand-alone PVI in patients with NPAF. A recent report of 229 patients with persistent AF, undergoing the “5 box” lesion set demonstrated a 92% freedom from ATAs off AADs at 2 year follow-up although only 37 patients were included at this time point (Sirak, 2012).

Hybrid Procedures

The hybrid approach leverages the ability of the surgeon to perform epicardial ablations, with the electrophysiologist’s ability to map lines of block, identify breakthrough conduction, and perform endocardial catheter ablation to complete lesion sets.

Advantages of the hybrid approach include (1) the ability to confirm conduction block, (2) the ability to close identified gaps, (3) reduction of phrenic nerve and esophageal injury, (4) reduced risk of tamponade due to an open pericardium, and (5) reduced risk of embolism from catheter ablations (as fewer ablations are used) (Lawrance et al., 2015).

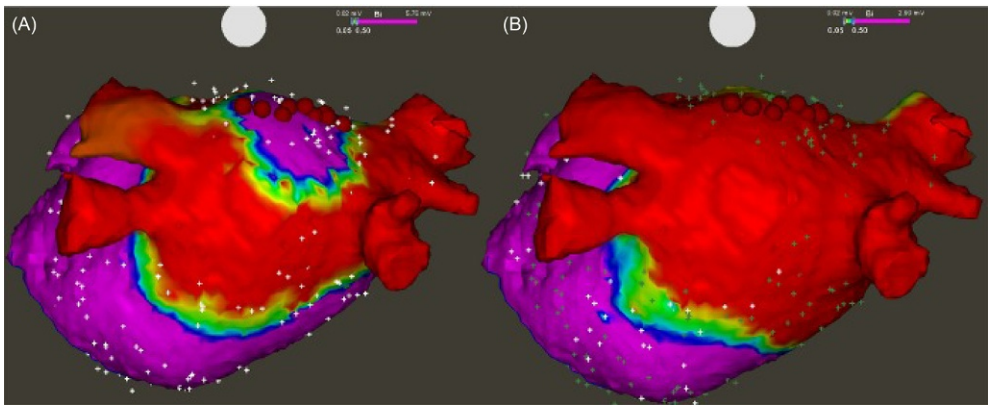

Fig. 3 Bipolar voltage map of the left atrium after epicardial radiofrequency thoracoscopic ablation, posterior view. (A) All four pulmonary veins were found isolated, while a gap was found in the middle of the roof line allowing electrical activation of the posterior wall. *Red dots* show sites that were reablated with endocardial touch-up. (B) Remapping after ablation revealed no potentials in the posterior wall, which confirmed a complete box-lesion. Reproduced from Bulava, A., Mokracek, A., Hanis, J., Kurfirst, V., Eisenberger, M. and Pesl, L. (2015). Sequential hybrid procedure for persistent atrial fibrillation. *Journal of the American Heart Association* **4**(3), e001754. With permission from Wiley Blackwell.

The disadvantages of hybrid approaches include: (1) long operative times when compared with catheter or surgical ablation alone, (2) higher complication rates in some centers, and (3) lack of good ablation technology to create the connecting lesions (Lawrance et al., 2015).

Hybrid procedures can be performed in either single-stage or two-stage fashion. A single-stage approach avoids a second procedure and hospitalization (Pison et al., 2012). A sequential two-stage procedure might be more cost-effective in identifying patients with AF recurrence after surgical ablation and treat these patients with a subsequent percutaneous endocardial ablation (Mahapatra et al., 2011).

A common hybrid procedure involves minimally invasive bilateral video-assisted thoracoscopic approach using a bipolar RF clamp and a surface bipolar linear probe to create lesions on a beating heart. This procedure is done under general anesthesia with TEE guidance and single lung ventilation. The lesions include bilateral PVI, and roof and floor lines to complete the "box lesion" and isolate the posterior LA. The ligament of Marshall is divided and the LAA is clipped. Epicardial mapping is performed to confirm PVI.

The endocardial portion is performed through a femoral vein approach. A transseptal sheath is placed for left heart access. RF ablation of the cavotricuspid isthmus is performed. Catheter mapping of the PVs, the posterior LA wall, and the mitral isthmus line is performed, to confirm isolation or electrical reconnection. If an electrical reconnection is suspected, detailed mapping is performed to determine sites of earliest potential, and these regions are ablated until all gaps are closed and electrical silence is achieved (Gelsomino et al., 2014).

Bulava et al. (2015) investigated the sequential (staged) hybrid method, which consists of a surgical thoracoscopic RF ablation procedure (PVI using an ablation clamp (Isolator Synergy; Atricure) and roof and inferior lines using a linear pen device (Bipolar linear pen; Atricure)), followed by RF catheter ablation 6–8 weeks later in 50 patients with long-standing persistent AF and a dilated LA (>45 mm) (**Fig. 3**). During catheter ablation, all four PVs were isolated in 74% of the patients; the roof line was complete in 34%, while the inferior line was complete in 60%, resulting in an overall box-lesion success rate of only 30% (**Fig. 4**).

Despite direct visualization of the epicardial RF lesions, and the use of multiple ablations, acutely observed electrical isolation was only temporary in many patients. Maturation of epicardial "burns" allows easier identification of target points during subsequent endocardial mapping. This highlights the ineffectiveness of epicardial RF ablations in creating transmural linear lesions and the potential importance of delaying the endocardial phase of the procedure (Bulava et al., 2015). Twelve months after the completed hybrid ablation, 94% were in SR. Surgical ablation was associated with a 13.7% major complication rate.

Convergent Approach

The convergent approach avoids lung deflation (Gehi et al., 2013). It employs an abdominal transdiaphragmatic approach to the pericardium to access and ablate the posterior LA. Following this, a standard left-sided endocardial ablation is performed to complete and confirm PVI. Initial results demonstrated SR with and without AADs of 80% and 52%, respectively, at 12 months in 73 patients (Geršak et al., 2014). An 11% complication rate was reported.

Monolateral Right Thoracic Approach

A staged approach consisting of the monolateral right thorascopic approach and endocardial catheter ablation has been performed. The epicardial "box" lesion is performed using a RF monopolar or combination monopolar/bipolar RF device with suction

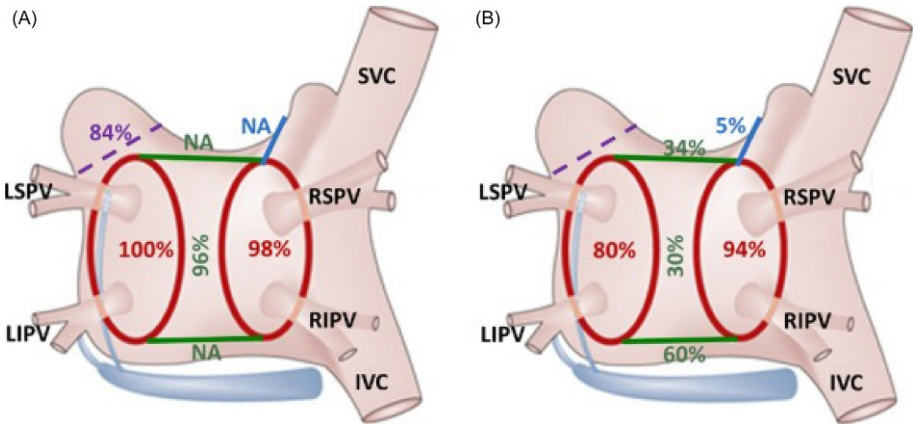

Fig. 4 Success rate of surgical thoracoscopic epicardial radiofrequency isolation of pulmonary veins, linear ablation lines connecting both superior and inferior pulmonary veins, and the trigone line connecting the right superior pulmonary vein across the left atrial roof toward the noncoronary aortic cusp as they were assessed (A) immediately after the ablation during surgery and (B) during the electrophysiological examination 6–8 weeks following the index procedure. Percentage of deployed left atrial appendage clips is also depicted (*violet color*). *IVC*, inferior vena cava; *LIPV*, left inferior pulmonary vein; *LSPV*, left superior pulmonary vein; *NA*, not applicable; *RIPV*, right inferior pulmonary vein; *RSPV*, right superior pulmonary vein; *SVC*, superior vena cava. Reproduced from Bulava, A., Mokracek, A., Hanis, J., Kurfirst, V., Eisenberger, M. and Pesl, L. (2015). Sequential hybrid procedure for persistent atrial fibrillation. *Journal of the American Heart Association* **4**(3), e001754. With permission from Wiley Blackwell.

adherence followed a month later by transcatheter endocardial ablation. At a mean follow-up of 28 months (range: 1–55 months), 87.5% of patients were in SR (Muneretto et al., 2012).

The limitation of this approach as well as the convergent approach is the inability to exclude the LAA. They have therefore been modified to include LAA ligation with the LARIAT suture delivery device (Bartus et al., 2013) (SentreHEART, Inc., Redwood City, CA).

In a systematic review of nine observational studies that each included more than 10 patients and at least 3-month follow-up, freedom from ATAs off AADs at 12 months in 335 patients ranged from 86% to 92% when bipolar RF energy was used, and 37%–89% with monopolar RF energy. Complications occurred in 4% of patients, including three conversions to sternotomy (Gelsomino et al., 2014).

An additional report detailed outcomes in 78 patients who underwent simultaneous thoracoscopic PVI and endocardial catheter ablation with at least 1-year follow-up (median follow-up of 24 months). Freedom from ATAs off AADs at last follow-up was 87% overall and 88% in NPAF although this includes the 13% of patients who underwent repeat catheter ablation for recurrent ATAs (Pison et al., 2014). Complications occurred in 8% of patients.

A recent systematic review of 1877 patients undergoing stand-alone minimally invasive surgical procedures for AF ablation found the Cox-Maze procedure to be superior to both beating heart epicardial ablation and hybrid procedures in terms of major complications and freedom from ATAs at 12 months (Je et al., 2015).

Conclusion

The greatest potential to impact the overall AF burden is in patients with concomitant cardiac disease. A review of the Society of Thoracic Surgeons database from 2005 to 2010 demonstrated that 62,152 concomitant ablations were performed compared to 4893 stand-alone ablations (Ad et al., 2012b).

The RMT Maze procedure is the only minimally invasive ablation technique that fully replicates the complete lesion set of the Cox-Maze procedure. For stand-alone AF ablation, minimally invasive PVI has demonstrated inferior results to those of the Maze procedure in NPAF and should therefore be considered only for patients with PAF and small LAs. The techniques for minimally invasive PVI and extended LA lesion sets, in contrast, are a closer approximation of a beating-heart Maze procedure. Hybrid procedures represent an alternative strategy for extending the atrial lesion set.

The goal of an entirely epicardial, minimally invasive, off-pump ablation procedure with high efficacy has remained elusive. The ideal surgical treatment for AF would consist of a patient-tailored lesion set using devices that consistently achieve transmurality and continuity through a minimally invasive approach.

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T

Takotsubo Syndrome

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Takotsubo syndrome (TTS), also known as Takotsubo cardiomyopathy, stress cardiomyopathy, and broken heart syndrome, is an acute and typically reversible heart failure often precipitated by a period of intense emotional or physical stress (Lyon et al., 2016; Templin et al., 2015). Its name is derived from the ampulla-shaped fishing pots used by Japanese fisherman to catch octopi (Sharkey et al., 2011). Characterized by a regional hypocontractility in one or more left ventricular segment, with a relative hypercontractile spared segment, TTS patients present as an acute coronary syndrome, yet no culprit coronary pathology can be found on angiography (Tranter et al., 2013). The most common apical TTS variant occurs when the apical myocardium is hypocontractile and the basal myocardium is hypercontractile; the mid-LV may or may not be involved (Tranter et al., 2013). Atypical variants include solely mid-LV hypocontractility, or basal hypocontractility, with or without mid-LV involvement and biventricular TTS. Since its initial description in 1990 after an earthquake in Japan (Satoh et al., 1990), recognition of the disease has increased, particularly due to the use of early coronary intervention in cases of seemingly cardiac chest pain (Shao et al., 2012).

Causes of TTS are numerous and include emotional stress, physical stress, sepsis, hyper-catecholamine endocrine disorders (e.g., pheochromocytoma, thyrotoxicosis), electroconvulsive therapy, sepsis, anesthetic induction, acute exacerbations of respiratory disease, and epilepsy (Lyon et al., 2016).

The pathophysiology is complex and incompletely understood. Preclinical animal models of TTS have been created by a number of research groups (Izumi et al., 2009; Shao et al., 2013; Paur et al., 2012; Ueyama et al., 2002; Cao et al., 2015), whereas studies of the pathophysiology of the human disease are mostly limited to endomyocardial biopsy samples (Nef et al., 2010) and epidemiological data (Schneider et al., 2013). There have been no randomized controlled trials assessing different therapeutic strategies for TTS, and therefore current clinical management is based on a *primum non nocere* principle, with supportive therapies favored for low-risk (stable) TTS patients and intensive care-based strategies (e.g., extracorporeal circulatory support) for high-risk (unstable) TTS patients who develop profound cardiogenic shock (Lyon et al., 2016). General advice is to avoid catecholamine-based positive inotropes and β2-agonist bronchodilators and these drugs can trigger TTS and may worsen a preexisting case.

Epidemiology

It is estimated that 1%–2% of acute coronary syndrome patients will be diagnosed with TTS (Gianni et al., 2006), although the actual figure may be higher as the size of a potentially large cohort of patients that either suffer an out-of-hospital cardiac arrest or do not suffer a high enough degree of LV dysfunction to attend hospital (subclinical TTS). In a study of 33,506,402 patients carried out in the United Stated, TTS accounted for 0.02% of hospitalizations, with risk factors including smoking, hyperlipidemia, anxiety, and alcohol abuse (Deshmukh et al., 2012).

In the German TTS registry, over 90% of TTS patients are female, with a mean age of 66 years, although disease characteristics were similar between males and females. TTS is also more common in elderly people, with less than 10% of cases occurring in those under 50 years of age (Deshmukh et al., 2012; Pilgrim and Wyss, 2008). Furthermore, TTS patients over the age of 75 years have an increased mortality rate (Citro et al., 2012). There has often been the assertion that TTS is a disease primarily affecting the

postmenopausal woman (Schneider et al., 2013), and there is limited evidence to show that patients previously taking hormone-replacement therapy may have reduced complications from an episode of TTS (Patel et al., 2013).

Diagnosis

A TTS patient will typically present with cardiac chest pain and dyspnea, mimicking an acute coronary syndrome (Tranter et al., 2013). ECG abnormalities are present in the acute phase (<12 h from symptom onset) in almost all cases of TTS (Lyon et al., 2016), namely ST-segment elevation and/or depression, left bundle branch block, T-wave inversion, and QT prolongation. T-wave inversion in aVr with no negative T-wave in V1 is particularly sensitive for TTS (Kosuge et al., 2012). QT prolongation can be profound, and daily ECG monitoring is suggested to risk stratify for *torsade de pointes* arrhythmia, a potentially fatal form of ventricular tachycardia (Ahn et al., 2011).

TTS is a diagnosis made after the exclusion of culprit coronary pathology. All patients, even when there is a high suspicion of TTS over an acute coronary syndrome, should undergo urgent coronary imaging, usually diagnostic angiography. This can be followed by left ventriculography which will confirm TTS with the Takotsubo shape and myocardial dysfunction affecting >1 coronary artery territories.

Echocardiography is also a valuable imaging modality in both diagnosis and monitoring, particularly to detect the presence of acute complications including LV apical thrombus, mitral valve regurgitation, left ventricular outflow tract obstruction, and decreasing cardiac function.

To date, a clinically validated specific biomarker for TTS has not been reported. There is limited evidence to suggest that NT-proBNP could be used to risk-stratify patients with TTS, as lower levels of NT-proBNP are associated with a more favorable prognosis (Lyon et al., 2016).

Cardiac magnetic resonance can provide further information about the contractile deficit present in the heart, acute myocardial inflammation of the affected segments, and right ventricular involvement where present (Eitel et al., 2011). Furthermore, myocardial edema consistent with the anatomical region of hypocontractility can help to rule-in TTS as a diagnosis (Joshi et al., 2010; Iacucci et al., 2013).

Pathophysiology

There have been a number of proposed mechanisms for the hypocontractility seen in TTS: with the recent reclassification of Takotsubo cardiomyopathy to TTS (i.e., a clinical diagnosis based on a collection of symptoms and signs), a single mechanistic pathway is not required, and a number of pathological processes could lead to regional hypocontractility.

The frequently described precipitating stressful event or catecholamine administration (e.g., dobutamine stress echocardiography, Epi-Pen injection) suggests catecholamine excess to be central to the development of TTS, with perhaps a predisposition for increased "gain" through the sympathetic axis (i.e., an increased catecholamine release for the same emotional/physical stimulus). An increased incidence of TTS in people with a Type D personality has been reported consistent with higher susceptibility to a given stressful trigger (Compare et al., 2013). Serum catecholamine levels have been shown to be increased at the time of presentation and remain acutely elevated (Wittstein et al., 2005).

Proposed hypotheses for TTS include multivessel vasospasm, left ventricular outflow tract obstruction, direct myocardial catecholamine stunning, and a cardio-circulatory interaction; it is unlikely that a single coronary vessel pathology could cause the whole apex to dysfunction as this is perfused by more than one artery, and the frequency of the "wrap-around" left anterior descending artery is not higher in TTS patients compared to MI controls (Hoyt et al., 2010). These hypotheses are covered in depth in a number of reviews (Tranter et al., 2013; Omerovic, 2011; Redfors et al., 2013). Acute effects of high dose epinephrine on β2-adrenergic receptor signaling to a cardioprotective but negative inotropic secondary messenger signaling pathway in the ventricular myocardium may play a role (Lyon et al., 2008). Endomyocardial biopsy samples have shown a number of changes that show a metabolic dysfunction and cardioprotective phenotype in the setting of a high catecholamine load, and myocardial edema appears to correlate well with the area of dysfunction, although this may be merely epiphenomenal (Nef et al., 2008, 2009; Shao et al., 2013; Perazzolo Marra et al., 2013). There is conflicting evidence of a genetic susceptibility to TTS, with some studies showing a GRK5 polymorphism that acts as an endogenous beta-blocker could be a risk factor (Spinelli et al., 2010; Novo et al., 2015; Figtree et al., 2013).

Management

During the acute phase many TTS patients have a high risk of complications and require close monitoring before recovery occurs. Risk stratification may be helpful at admission to identify those at highest risk (Lyon et al., 2016). Acquired QT prolongation in the acute phase predisposes to *torsade de pointes* and ventricular arrhythmias, and therefore continuous ECG monitoring in a coronary care unit or high dependency unit is advised which access to resuscitation and electrical cardioversion. Monitoring of cardiac function through echocardiography will help the clinician to risk stratify TTS patients, with the most severe cases with cardiogenic

shock requiring interventions such as extracorporeal membrane oxygenation or a temporary left ventricular assist device. If pharmacological inotropic support is required, a low-dose levosimendan infusion may be of benefit (Paur et al., 2012; Santoro et al., 2013); administration of sympathomimetics such as isoprenaline, dobutamine, and epinephrine is generally contraindicated (Lyon et al., 2016) and was associated with an increased mortality in the InterTak registry (Templin et al., 2015).

Long-Term Prognosis for TTS Patients

There are conflicting reports for the long-term morbidity and morality for patients after a single episode of TTS, but in a recent study using the SWEDEHEART registry, mortality at 3 years post-TTS episode was similar to patients who had a STEMI (Redfors et al., 2015). However, other studies have found that TTS did not increase mortality compared to controls matched for age and gender (Molina et al., 2014; Elesber et al., 2007).

Conclusion

TTS is a form of acute heart failure that is typically triggered by a stressful stimulus or stimuli, typically presenting with acute cardiac chest pain and ECG changes similar to an acute coronary syndrome. The diagnosis requires culprit coronary pathology to be excluded, and TTS is characterized by a hypocontractile apical myocardium coupled to a hypercontractile basal myocardium, although anatomical variants do exist. Due to its relatively recent discovery (1990), its pathophysiology is poorly understood, but is likely to be multifactorial. Epidemiological data showed an increased prevalence among postmenopausal females, although it can be observed in younger women and ~10% of cases are male. As the disease is typically self-limiting, treatment should be supportive, with mechanical support in intensive care settings to treat cardiogenic shock and other rare but severe complications. Further work is required, both in basic and clinical science, to understand the mechanisms and optimal treatment strategies for TTS.

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Tobacco and Cardiovascular Diseases

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Cigarette smoking remains the number one preventable cause of death worldwide, and it contributes significantly to cardiovascular disease (CVD) (World Health Organization, 2016). The extraordinary burden of CVD and related diseases caused by tobacco products is even more remarkable in that the pandemic is entirely manmade. A combination of commercial marketing and physical addiction sustains the CVD epidemic, and tobacco is the major human risk factor. Tobacco marketing creates the illusion that smoking is desirable and thus encourages nonsmokers to experiment with tobacco usage. Physical addiction to nicotine then forces continued usage, making it extremely difficult for many smokers to quit. Smokers need the chronic exposure to the numerous risk factors in the cigarette and other toxic substances due to continue smoking to maintain some concentration of nicotine, and then they have the hazards of smoking to the health.

Over the past decades, a large body of evidence has clearly indicated that exposure to smoking is associated with increased risks of CVD and accelerated atherosclerosis. The CVD risk attributable to smoking increases sharply at low levels of tobacco consumption and with exposure to secondhand smoke. The harmful effects of smoking on CVD and atherosclerosis are attenuated by smoking cessation, however. This article focuses on the relationship between smoking and CVD, the effects of smoking on the atherosclerotic process, the benefits of smoking cessation, the progress in smoke-free legislation, and the prevention of CVD.

Smoking and CVD

Smoking is an independent major risk factor for all types of CVD, including coronary heart disease (CHD), stroke (Jee et al., 1999), sudden cardiac death (SCD) (Kannel et al., 1975), aortic aneurysm (Lee et al., 1997), and peripheral artery disease (PAD) (Murabito et al., 2002). The incidence of myocardial infarction (MI) is sixfold higher in women and threefold higher in men who smoke 20 or more cigarettes daily compared to never smokers (Njolstad et al., 1996). According to the INTERHEART study conducted across 52 countries, smoking was responsible for 36% of the population attributable risk (PAR) of a first MI (Yusuf et al., 2004). In a meta-analysis of data from 32 studies, the overall relative risk (RR) for stroke related to smoking was 1.5 (95% confidence interval (CI), 1.4–1.6) (Shinton and Beevers, 1989). The RRs altered across the subtypes of stroke: 1.9 for ischemic stroke, 0.7 for hemorrhage stroke, and 2.9 for subarachnoid hemorrhage (Shinton and Beevers, 1989).

Most SCDs are due to CVD. In multivariate analyses of the combined data from the Framingham Heart Study and the Albany Study, which followed men aged 45–64 years, smoking was the SCD risk factor with the highest statistical significance (Kannel et al., 1975). Current and recent former smokers had an increased risk of aortic aneurysm compared to long-time former smokers and never smokers (odds ratio (OR), 2.63; 95% CI, 1.53–6.21) (Lee et al., 1997). The Framingham Offspring Study demonstrated an increased risk of PAD among current smokers (OR, 2.0; 95% CI, 1.1–3.4) compared to noncurrent smokers (Murabito et al., 2002). Moreover, smoking increases all-cause mortality and CVD mortality. The adjusted hazard ratios (HRs) during a 35-year follow-up period for current smokers compared with never smokers were 1.62 (95% CI, 1.40–1.88) and 1.63 (95% CI, 1.24–2.13), respectively, and those who persisted in smoking cessation had no increased risk of death compared with never smokers (Qiao et al., 2000).

Patients with established CHD who were active smokers had increased risk of recurrent coronary events compared to those who were nonsmokers (RR, 1.51; 95% CI, 1.10–2.07) (Rea et al., 2002) and for SCD (HR, 2.47; 95% CI 1.46–4.19) (Goldenberg et al., 2003). Among patients who successfully underwent percutaneous coronary intervention for CHD, those who continued to smoke had higher risks of Q-wave infarction (RR, 2.08; 95% CI, 1.16–3.72) and death (RR, 1.44; 95% CI, 1.04–2.04) than nonsmokers (Hasdai et al., 1997). After coronary artery bypass graft (CABG), persistent smokers had elevated risks of cardiac death (RR, 1.75; 95% CI, 1.30–2.37) and revascularization (RR, 1.41; 95% CI, 1.02–1.94) compared to the CABG patients who gave up smoking for at least 1 year after surgery (van Domburg et al., 2000).

Dose and Duration of Smoking Exposure and CVD

A clear dose-response relationship exists between the number of cigarettes smoked daily and the risk of CVD; another clear dose-response relationship exists between an individual's smoking duration and the risk of CVD. In the INTERHEART study, compared with never smokers, even a very small number of daily cigarettes (i.e., <5) was associated with a higher risk of CHD; the risk increased by 5.6% for every additional cigarette smoked (OR, 1.056; 95% CI, 1.05–1.06) (**Fig. 1**) (Teo et al., 2006). In addition, the odds of an MI were ninefold higher among those who smoked ≥40 cigarettes daily compared to never smokers (Teo et al., 2006). Similarly, the risk of stroke related to smoking increased with the number of cigarettes smoked daily, and this risk was present for even a small number of cigarettes; those who smoked <10 cigarettes daily still had an increased risk of stroke compared to never smokers (RR, 1.37; 95% CI, 1.24–1.52) (Shinton and Beevers, 1989).

An increased risk of CHD was also observed with a longer duration of smoking regardless of the number of cigarettes smoked daily (Burns, 2003). In the Nurses' Health Study, the number of cigarettes smoked daily and the smoking duration were linearly associated with the SCD risk among current smokers (Sandhu et al., 2012). Even small to moderate cigarette consumption (1–14 per day) was associated with a significant 1.84-fold (95% CI, 1.16–2.92) increase in SCD risk, and every 5 years of continued smoking was associated with an 8% increase in SCD risk (HR, 1.08; 95% CI, 1.05–1.12) (Sandhu et al., 2012).

Type of Tobacco Used

Available evidence suggests that all types of smoking increase the risk of developing CVD. A cohort study of 17,774 men followed for 14 years showed that, compared to never smokers, the men who smoked cigars were at a higher risk of incident CHD (RR, 1.27; 95% CI, 1.12–1.45) in a dose-dependent manner: the RRs were 1.20 (95% CI, 1.03–1.40) for the men who smoked <5 cigars per day and 1.56 (1.21–2.01) for those who smoked >5 cigars daily (Iribarren et al., 1999). A longitudinal cohort of 16,932 Norwegian men exhibited a significantly increased risk of CVD, CHD, and stroke deaths from pipe smoking (Tverdal and Bjartveit, 2011). The RRs for those who smoked 14 g of tobacco or more daily compared to never smokers were 3.35 (2.28–4.91) for death from CVD, 3.34 (2.02–5.51) for death from CHD, and 3.76 (1.61–8.78) for death from stroke, in a dose-dependent manner (Tverdal and

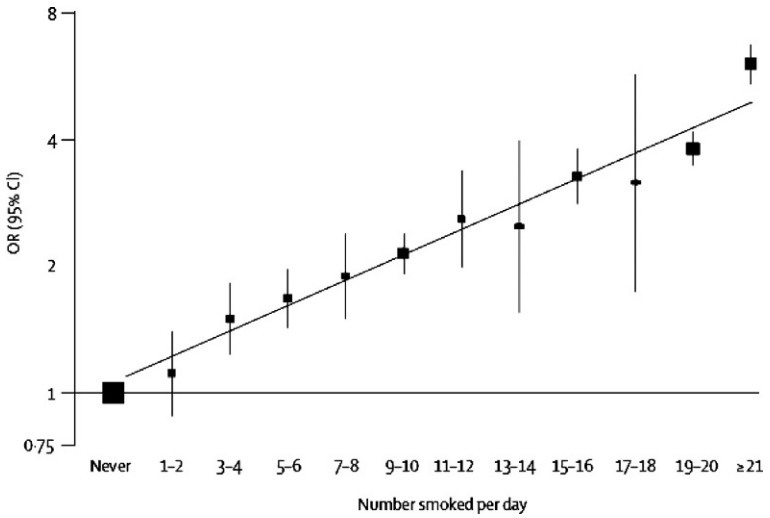

Fig. 1 Risks of myocardial infarction (MI) with increasing numbers of cigarettes smoked compared with never smokers, from the INTERHEART study conducted across 52 countries. The risk increased by 5.6% for every additional cigarette smoked (OR, 1.056; 95% CI, 1.05–1.06). At least 21 cigarettes smoked per day represent approximately 1.5 cigarette packs, associated with an OR of 6.00–7.00. Reprinted from Teo, K. K., Ounpuu, S., Hawken, S., Pandey, M. R., Valentin, V., Hunt, D., Diaz, R., Rashed, W., Freeman, R., Jiang, L., Zhang, X., Yusuf, S. and Investigators IS. (2006). Tobacco use and risk of myocardial infarction in 52 countries in the INTERHEART study: a case-control study. *Lancet* **368**, 647–658 with permission from Elsevier.

Bjartveit, 2011). No or only minor differences were found between pipe smokers and cigarette smokers in mortality from any cause and the specified CVD diseases (Tverdal and Bjartveit, 2011).

In the INTERHEART study of a case–control investigation in 52 countries, the use of other forms of tobacco was largely confined to specific regions (Teo et al., 2006). Smoking beedies (or bidis; a thin hand-rolled tobacco cigarette (i.e., indigenous to South Asia)) alone was associated with an increased risk of acute MI (OR, 2.89; 95% CI, 2.11–3.96), similar to the OR associated with cigarette smoking (Teo et al., 2006). Chewing tobacco alone was associated with an OR for acute MI of 2.23 (95% CI, 1.41–3.52), and smokers who also chewed tobacco had the highest increase in risk (OR, 4.09; 95% CI, 2.98–5.61) (Teo et al., 2006). The INTERHEART study found that the risk of MI increased with all forms of tobacco use, including different types of smoking and chewing tobacco. The use of snuff and other types of smokeless tobacco is increasing, particularly among adolescents and young adults. However, the CVD risks related to smokeless tobacco are not well-established and deserve further investigation (Gupta et al., 2004).

Women

Sex differences exist in the adverse effects of smoking. A systematic review and meta-analysis of 75 prospective cohort studies demonstrated that female smokers were 25% more likely to develop CHD than male smokers (female-to-male RR ratio, 1.25; 95% CI, 1.12–1.39) (Huxley and Woodward, 2011). Female smokers are more likely to experience adverse events after acute coronary syndrome (ACS) than male smokers (Howe et al., 2011). These findings suggest that tobacco-control programs should consider women, particularly in those countries where smoking among young women is increasing in prevalence.

Race and Ethnicity

The risk of CVD associated with cigarette smoking persists across all races. Smoking was identified as a significant risk factor for CVD among Hispanic populations (Mendelson et al., 1998) and Asian populations (Kiyohara et al., 1990; Ueshima et al., 2004). In the INTERHEART case–control study, the PAR for acute MI in current smokers ranged from a low of 33.1% (95% CI, 29.7–36.6) in China and Hong Kong to a high of 53.3% (46.2–60.3) in Australia and New Zealand (Teo et al., 2006). The PAR for other world regions fell within this narrow range, showing that the PAR for current tobacco smoking is consistent and high across the globe (Teo et al., 2006).

Secondhand Smoke Exposure and CVD

Exposure to secondhand smoke increases the risk of CVD in never smokers. Large epidemiologic studies have estimated a 20% higher risk of CHD among never smokers exposed to secondhand smoke (Steenland et al., 1996; Wells, 1994). A meta-analysis of 18 epidemiologic (10 cohort and 8 case–control) studies also found a 25% increased risk of CHD (95% CI, 1.17–1.32) related to a dose-dependent exposure to secondhand smoke (He et al., 1999). In the INTERHEART study, secondhand smoke was associated with an incremental increase in the risk of acute MI related to exposure; the OR was 1.24 (1.17–1.32) in individuals who were least exposed (1–7 h/week) and 1.62 (1.45–1.81) in individuals who were most exposed (>21 h/week) (Teo et al., 2006). Findings from the Nurses' Health Study indicated that compared with women not exposed to secondhand smoke, the RR of CHD was 1.58 (95% CI, 0.93–2.68) among women who reported occasional exposure and 1.91 (95% CI, 1.11–3.28) among those reported regular exposure (Kawachi et al., 1997).

Secondhand smoke may also be associated with extracoronary vascular disease. A large cross-sectional study of 60,377 women in China showed an association between stroke in the women and smoking by their husbands (Zhang et al., 2005). The prevalence of stroke increased with the duration of smoking and with the number of cigarettes smoked daily (Zhang et al., 2005). Secondhand smoke exposure among never smokers is potentially a modifiable risk factor for CVD. Before–after studies have indicated that laws that limit public and workplace smoking may result in a rapid decrease in the risk of ACS in both smokers and nonsmokers (Callinan et al., 2010). One meta-analysis estimated that the rate of hospitalization for acute MI decreased 12 months after the implementation of smoking restriction laws (0.83, 95% CI, 0.80–0.87) (Lightwood and Glantz, 2009).

Smoking and Atherosclerosis

Various clinical imaging techniques have been used to confirm the association of smoking and atherosclerosis directly. The Multi-Ethnic Study of Atherosclerosis (MESA) evaluated the relationship between smoking and the severity of modality-determined subclinical atherosclerosis (McEvoy et al., 2015). Current and former smoking were associated with subclinical atherosclerosis at three anatomically distinct sites: coronary artery calcification (CAC), carotid intima-media thickness (IMT), and ankle-brachial index (ABI) (McEvoy et al., 2015). A Japanese population-based study also demonstrated strong associations between current and former smoking and subclinical atherosclerosis in multiple vascular beds such as CAC, carotid IMT, ABI, and aortic artery

calcification (AoAC), with a dose-dependent relationship with cumulative pack-years exposure or smoking intensity (Hisamatsu et al., 2016). A significant relationship between current or former smoking and CAC was also reported in other cohorts (Jockel et al., 2009; LaMonte et al., 2005; Loria et al., 2007; Rasmussen et al., 2013). Similarly, carotid IMT (Richey Sharrett et al., 2004; Raitakari et al., 2003; Baldassarre et al., 2009; Howard et al., 1994) and AoAC (El-Saed et al., 2013) findings associated with smoking have been confirmed.

Endothelial dysfunction is a well-established response to CVD risk factors, and it precedes the development of atherosclerosis. Smoking was associated with endothelial dysfunction in a dose-dependent manner, as assessed by flow-mediated dilatation in asymptomatic young adults (Celermajer et al., 1993). Several large autopsy studies have found an association between cigarette smoking and atherosclerosis in the major coronary arteries (Strong and Richards, 1976; Berenson et al., 1998; Zieske et al., 1999). The Bogalusa Heart Study examined autopsy data from 93 children and young adults (age range 2–39 years) who had died principally from trauma and for whom data on risk factors were available (Berenson et al., 1998). Investigators observed that cigarette smoking increased the percentage of the intimal surface involved with fibrous plaques in the aorta (1.22% in smokers vs. 0.12% in nonsmokers, $p = 0.02$) and fatty streaks in the coronary vessels (8.27% vs. 2.89%, $p = 0.04$) (Berenson et al., 1998). Similar associations between smoking and early stage atherosclerosis were observed in the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study (Zieske et al., 1999). These autopsy studies indicate that even among young smokers, atherosclerotic plaque progresses faster and to a greater degree than among nonsmokers.

Mechanisms Underlying the Links Between Smoking and CVD

The evidence linking smoking exposure to various CVDs is clear, although the mechanisms underlying these associations have not been fully elucidated (Ambrose and Barua, 2004). Multiple factors are likely involved, since smoking exerts a variety of effects that may contribute to atherogenesis (Ambrose and Barua, 2004). Atherosclerosis is considered a critical player in the pathophysiology of smoking-induced CVD (Ambrose and Barua, 2004), and the adverse effects of atherosclerosis can be reduced by smoking cessation (Johnson et al., 2010).

Smoking is associated with an adverse effect on serum lipids (i.e., elevated low-density lipoproteins (LDL) and triglycerides and reduced high-density lipoproteins (HDL)) and insulin resistance (Craig et al., 1989; Reaven and Tsao, 2003). Free radicals generated by smoking also damage lipids, resulting in the formation of proatherogenic oxidized particles, specifically oxidized low-density lipoprotein cholesterol (Miller et al., 1997; Heitzer et al., 1996). In addition, smoking enhances the prothrombotic state through an elevation of blood fibrinogen concentrations, an increased platelet activity, an increased expression of tissue factors, and the inhibition of tissue plasminogen activator release from the endothelium (Newby et al., 1999; Matetzky et al., 2000). Smoking also activates the sympathetic nervous system, elevating heart rate and blood pressure, and constricting peripheral and coronary arteries (Winniford et al., 1986; Narkiewicz et al., 1998).

Smoking and passive smoking can impair the functionality of endothelium-dependent vasodilation in coronary arteries and reduce coronary flow reserve (Sumida et al., 1998; Barua et al., 2001; Kaufmann et al., 2000). The decline in endothelial function is caused by oxidative stress from oxidized LDL and reduced nitric oxide (Barua et al., 2001; Kaufmann et al., 2000; Kugiyama et al., 1996; Mazzone et al., 2001). Moreover, smoking has been related to higher levels of C-reactive protein, fibrinogen, homocysteine (Bazzano et al., 2003), soluble intercellular cell adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1) (Mazzone et al., 2001). These findings indicate that inflammation and hyperhomocysteinemia could be important factors by which smoking promotes the pathogenesis of atherosclerosis.

Smoking Cessation

Smoking cessation is a key element in both the primary and secondary prevention of CVD. Among individuals without prior CHD, the reduction in cardiac events after quitting smoking ranged from 7% to 47% (Multiple Risk Factor Intervention Trial Research Group, 1982; Hjermann et al., 1981; Hurt et al., 2012). The risks of CHD associated with cigarette smoking decrease relatively soon after smoking cessation is initiated, and the risks continue to decrease over time (U.S. Department of Health and Human Services, 1990).

Smoking cessation improves outcomes in patients with known CHD. In a meta-analysis of 20 prospective cohort studies with a follow-up period of ≥ 2 years among 12,603 patients with known CHD, of whom 5659 gave up smoking and 6944 continued to smoke, the RR of mortality in the patients who gave up smoking was 0.64 (95% CI, 0.58–0.71) compared with those who continued to smoke (Critchley and Capewell, 2003). A retrospective cohort study of 2619 patients discharged after a first MI, with never smokers as the reference, found a multivariable RR for recurrent CHD of 1.17 (95% CI, 0.93–1.43) for former smokers and 1.51 (95% CI, 1.10–2.07) for current smokers (Rea et al., 2002). Compared with never smokers, the RR among former smokers decreased progressively with time since smoking cessation (RR, 1.62 (95% CI, 1.02–2.61) for the duration <6 months; 1.60 (0.97–2.60) for 6–18 months; 1.48 (0.76–2.51) for 18–36 months; and 1.02 (0.54–1.86) for 36 months or more; P for trend $= 0.01$) (Rea et al., 2002).

This finding indicates that patients who gave up smoking after having suffered an MI had reductions in the risk for recurrent CHD over time; within 18–36 months, the risk was almost similar to that of never smokers. In addition, the relative benefits of smoking cessation were shown to be similar in young and old patients with CHD (Hermanson et al., 1988).

Smoking cessation was also associated with a reduction in the risk of SCD primarily in patients with CHD (Goldenberg et al., 2003; Peters et al., 1995; Vlietstra et al., 1986; Hallstrom et al., 1986). In a prospective study with a mean follow-up period of 8.2 years, patients with known CHD who gave up smoking experienced a significant reduction in SCD risk (HR, 1.06; 95% CI, 0.70–1.62) compared to those who had never smoked (Goldenberg et al., 2003). However, there are limited data on the impact of smoking cessation on SCD risk among individuals without known CHD (Sandhu et al., 2012; Gordon et al., 1974). In the Framingham study, male smokers aged <65 years at study entry who subsequently stopped smoking cut their rate of SCD by almost 50% compared to those who continued to smoke over 18 years of follow-up (Gordon et al., 1974). The Nurses' Health Study among women without known CVD at baseline found that the risk of SCD decreased linearly over time after smoking cessation and was equivalent to that of never smokers after 20 years of cessation (Sandhu et al., 2012). The risk of stroke also decreases over time after smoking cessation. For total and ischemic stroke, excess risks among women who stopped smoking largely disappeared 2–4 years after cessation, independent of age at cessation and the number of cigarettes smoked per day (Kawachi et al., 1993).

Some community-based studies of participants without known CVD have reported an association between smoking cessation and measures of atherosclerosis in multiple vascular beds (McEvoy et al., 2015; Hisamatsu et al., 2016; Jockel et al., 2009). In the MESA multiethnic cohort, the associations of smoking with CAC, carotid IMT, and ABI decreased over time in former smokers (McEvoy et al., 2015). Similarly, in a general Japanese population, time since cessation was linearly associated with lower burdens of coronary, carotid, aortic, and peripheral atherosclerosis (Hisamatsu et al., 2016). A 1-year prospective investigation of the impact of smoking cessation on endothelial function in over 1500 smokers revealed that endothelial function improved significantly in smokers who had successfully given up smoking compared to the baseline function (although these subjects gained an average of 5 kg), but it did not improve in those who continued to smoke (Johnson et al., 2010).

Smoke-Free Legislation and CVD

Most of the available studies conducted worldwide regarding smoke-free legislation and CVD observed that legal smoking regulations and the implementation of fully tobacco-free areas in public spaces or workplaces decreased the local rate of hospitalization due to heart attacks (such as acute MI) soon after the legislation was implemented (Sargent et al., 2004; Bartecchi et al., 2006; Barone-Adesi et al., 2006; Khuder et al., 2007; Juster et al., 2007; Tan and Glantz, 2012). Here, we describe several examples from the United States and Italy.

Helena, Montana, United States

The city of Helena, Montana, is a geographically isolated area that had a population of 68,140 people in 1998. The city's regulations to prohibit smoking in public places and workplaces were implemented from Jun. to Dec. 2002. There was only one hospital in this area, and all individuals with an acute MI were conveyed to this hospital. This research (Sargent et al., 2004) was carried out using the medical records of all inpatients admitted to the hospital from Dec. 1997 to Nov. 2003, by the Poisson analysis. The number of hospitalizations with acute MI within the 6 months after the smoking prohibition regulations were started was 24 cases, which was 16 cases (95% CI, −31.7 to −0.3) less, that is, 40% lower compared to the mean number of cases (approximately 40 cases per month) before the regulations. In the area surrounding Helena during the same period, the number of heart attacks increased by 5.6 cases, from 12.4 cases to 18 cases per month. This difference showed a statistically significant decrease between inside and outside Helena.

These results suggested that the legal regulations to make fully tobacco-free areas in public places and workplaces decreased the number of heart attacks soon after the regulation in this population. Moreover, the rate of heart attacks in this area increased again after the regulations were canceled, to 38 cases over the 6 months after the cessation of the regulations.

Pueblo, Colorado, United States

Based on the results obtained in the earlier-mentioned study, this study (Bartecchi et al., 2006) examining the implementation of smoke-free areas in public places and hospitalization rates was carried out in Pueblo, Colorado, which is geographically similar to Helena, Montana. The hospitalization rates for AMI in Pueblo were assessed each 1.5 years before and after the implementation of a smoke-free ordinance. These AMI rates were also compared with those of another geographically isolated but proximal community, El Paso, Colorado, that did not have a smoke-free ordinance. Between Jan. 1, 2002 and Dec. 31, 2004, a total of 855 patients were hospitalized with AMI in Pueblo. A reduction in AMI hospitalization was observed in the 1.5-year period after the ordinance's implementation among residents living within Pueblo's city limits (RR, 0.73; 95% CI, 0.63–0.85). No significant changes in the AMI rate were observed among the residents outside the Pueblo city limits (RR, 0.85; 95% CI, 0.63–1.16) or in El Paso (which no longer had smoke-free public places and workplaces) during the same period (RR, 0.97; 95% CI, 0.89–1.06). The reduction in AMI rate within Pueblo differed significantly from changes in the control group (El Paso).

Piedmont, Northern Italy

In Jan. 2005, the Italian Government banned smoking in all indoor public places, including cafés, bars, restaurants, and discos. This study (Barone-Adesi et al., 2006) evaluated whether the national law banning smoking in public resulted in a short-term reduction in hospital admissions for AMI, by analyzing the hospital discharge records of the Piedmont region in northern Italy. The rates of admission for AMI before the ban (Oct.–Dec. 2004) and after the ban's introduction (Feb.–Jun. 2005) were analyzed. Each period was compared with the corresponding period 12 months before. Among persons aged under 60, the number of admissions for AMI decreased significantly after the introduction of the ban: from 922 cases in Feb.–Jun. 2004 to 832 cases in Feb.–Jun. 2005 (sex- and age-adjusted rate ratio, 0.89; 95% CI, 0.81–0.98). No effect was found among persons aged $\geq$60. This study was estimated that the observed reduction in active smoking after the introduction of the ban could account for a 0.7% decrease in admissions for AMI during the study period, suggesting that most of the observed effect (11%) might be due to the reduction of passive smoking.

Bowling Green, Ohio, United States

In Mar. 2002, the city of Bowling Green, Ohio, implemented a clean indoor air ordinance banning smoking in workplaces and public places. Compared with a matched control city (Kent, Ohio) with no clean indoor air ordinance, the largest reduction in hospital admissions was for CHD, for which the rate in Bowling Green decreased significantly by 39% after 1 year and by 47% after 3 years following the implementation of the ordinance (Khuder et al., 2007). The findings of that study suggest that clean indoor air ordinances lead to a reduction in hospital admissions for CHD.

The State of New York, United States

Another study (Juster et al., 2007) examined whether the 2003 implementation of a comprehensive smoking ban in New York State was associated with reduced hospital admissions for CVDs. In 2004, there were 3813 fewer hospital admissions for AMI than would have been expected in the absence of the comprehensive smoking ban. Direct healthcare cost savings of $56 million were realized in 2004. There was no reduction in the number of admissions for stroke.

A Meta-Analysis of Hospitalizations for Cardiac Events and Cerebrovascular Diseases

A random-effect meta-analysis (Tan and Glantz, 2012) was conducted to determine the relationship between smoke-free legislation and hospital admissions or death from cardiac events and cerebrovascular diseases. The studies were identified by using a systematic search for relevant studies published before Nov. 30, 2011. Changes in hospital admissions (or deaths) in the presence of a smoke-free law, the duration of follow-up, and the smoking prohibition laws' comprehensiveness (i.e., workplaces only; workplaces and restaurants; or workplaces, restaurants, and bars) were recorded. Forty-five studies of 33 smoke-free laws with the median follow-up of 24 months were included. Comprehensive smoke-free legislation was associated with significantly lower rates of hospital admissions (or deaths) for three of the following diagnostic groups: coronary events (RR, 0.848; 95% CI, 0.816–0.881), other heart disease (RR, 0.610; 95% CI, 0.440–0.847), and cerebrovascular accidents (RR, 0.840; 95% CI, 0.753–0.936). Thus, smoke-free legislation was associated with a lower risk of smoking-related cardiac events and cerebrovascular diseases, with more comprehensive laws associated with greater reductions in the risks.

Prevention of CVD

Two complementary approaches are needed to reduce the devastating effects of tobacco on health. In the long term, progress depends on the systematic application of primary prevention measures that can reduce the initiation of tobacco use by young people and end the smoking pandemic during the second half of the 21st century. In the near term, substantial reductions in smoking-attributable CVDs can be achieved by providing counseling and treatment to facilitate smoking cessation among the millions of people around the world who currently smoke.

A variety of community-based interventions have proven effective in reducing tobacco consumption and decreasing smoking initiation when these measures are applied as part of comprehensive tobacco control (Centers for Disease Control and Prevention, 1999). They include regulatory approaches (restrictions on tobacco marketing, laws ensuring clean indoor air, and the enforcement of laws restricting minors' access to tobacco), economic approaches (e.g., increasing the price of cigarettes through excise taxes, thereby decreasing demand), and counter marketing campaigns to redefine social norms regarding tobacco use. It is possible to prevent tobacco use by young people if measures that have been proven to be effective are applied.

Most current smokers want to quit smoking. Approved therapies include nicotine replacement (gum, patch, inhaler, nasal spray), sustained-release bupropion hydrochloride, and behavioral treatment. The latter methods are most effective when they include practical counseling (problem solving/skills training), social support as part of treatment, and help with securing social support outside of treatment. The combination of pharmacologic and behavioral treatment enables 20%–25% of persons attempting to quit smoking to remain abstinent 1 year after treatment (U.S. Department of Health and Human Services, 2000). However, tobacco dependence frequently involves relapse, and it is best characterized as a chronic disease that may require periodic treatment.

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Total Artificial Heart

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Introduction

The total artificial heart (TAH) offers complete mechanical circulatory support (MCS) for patients with biventricular heart failure as a bridge-to-transplantation. By replacing the two native ventricles and four native valves, this device is very invasive and also unique in that it removes the risks associated with dysfunctional native myocardium. Understandably, utilization of TAHs is limited to very sick patients, a fact due to its associated risks and also the widespread implantation of ventricular assist devices. We believe that the TAH provides the best option for an important and growing subset of patients, and that technological advances with the device may promote increased utilization in the future. This article provides a comprehensive overview of the TAH as it relates to its history, current indications, technique of surgical implantation, postoperative management, outcomes to date, and future developments.

Historical Perspective

The TAH is founded in concepts of both MCS and cardiac transplantation, and thus its development has been touched by many pioneers of cardiac surgery.

The discovery of heparin by Jay McLean in 1916 was foundational to all subsequent work (McLean, 1959). In the 1920s and 1930s, Alexis Carrel teamed with Charles Lindbergh to develop a perfusion system to preserve the explanted organs of fowls and cats (Carrel, 1935). In 1935, John Gibbon successfully bypassed the heart and lungs of a cat, a concept Gibbon conceived while famously witnessing a patient expire from a massive pulmonary embolus (Gibbon, 1937).

A more modern version of the TAH began to appear in 1937, when Russian surgeon Vladimir Demikhov developed a device to aid in transplantation of dog lungs: its design featured two adjacent pumps driven by an external motor and a transcutaneous drive shaft (Kwann-Gett et al., 1969). Additional strides were made in 1948, when William Sewell developed a pump system which was pneumatically powered, thereby creating the pressurized system upon which many current pumps depend (Eli Whitey Museum, www.eliwhitney.org). In 1952, Forest Dodrill bypassed the left ventricle of a 41-year-old male for 50 min to perform a mitral valve repair through a left anterolateral thoracotomy in what is considered to be the first successful machine-supported open heart operation in a human (Dodrill et al, 1952). In 1953, John Gibbon again made his mark, utilizing a heart–lung machine to close an atrial septal defect in an 18-year-old woman (Gibbon, 1954).

Initial efforts at creating a complete cardiac replacement artificial organ in the United States began as one of the several scientific initiatives advocated during the Kennedy administration in the 1960s. In 1964, the National Institute of Health launched an artificial heart program, fostering the development of partial and full cardiac replacement devices. Parallel efforts began in Texas and Cleveland on circulatory replacement systems. In 1963, William DeBakey implanted the first left ventricular assist device (LVAD) in a human at Baylor University in Houston, Texas, though the patient expired 4 days later (Debakey, 1971).

In 1967, Christiaan Barnard completed the first human-to-human cardiac transplantation in South Africa (Barnard, 1967), followed shortly thereafter by U.S. transplants overseen by Kantrowitz (1998) and Norman Shumway (Haller and Cerruti, 1968). Motivated by these events, in 1969 Denton Cooley performed the first human implantation of a TAH (Cooley et al., 1969). It occurred on April 4, 1969 after a failed ventricular resection procedure for the enlarged heart of Mr. Haskell Karp, who could not be weaned from heart–lung machine, resulting in Denton Cooley implanting a Liotta-TAH in this 47-year-old man. The patient regained consciousness, was extubated, and then bridged to transplant after 64 h of TAH support but died after 32 h posttransplant due to pneumonia. 12 years later, Cooley implanted his second artificial heart (Cooley et al., 1981), this time with a pneumatic dual-chambered Akutsu TAH, in a patient who survived 39 h postimplant.

In 1982, the Jarvik-7-100 TAH, a pneumatically powered, biventricular, pulsatile pump, was implanted by William DeVries (DeVries et al., 1984). The patient Dr. Barney Clark survived 112 days postoperatively, and due to this impressive survival, many consider this to be the first successful implantation of a TAH. The second implantation of this device occurred in November 1984 and extended the life of William Schroeder by 620 days postoperatively (Altman, 1986).

The rising enthusiasm over heart transplantation in the early years of 1980, along with the development of new immunosuppressive drugs and the influence of increased regulatory guidance, evolved the initial indication for TAH from destination therapy to bridge to heart transplantation (BTT).

In recent years only the SynCardia CardioWest TAH has been continuously used as a TAH, achieving worldwide reliable results as BTT, with more than 1300 implants at the time of writing (SynCardia, www.syncardia.com). Ownership of the device changed hands to CardioWest in 1992, and the device was renamed as the CardioWest TAH, with subsequent CE Mark in Europe in 1999 and Food and Drug Administration (FDA) approval in 2004 as a bridge-to-transplantation. A second renaming in 2010 resulted in the device currently known as the SynCardia TAH (**Figs. 1** and **2**). New TAHs have recently been adopted or entered in the commerce, though with very limited available clinical data, paving the way for the next generation of TAH technology.



Fig. 1 SynCardia total artificial heart, connected to the freedom portable driver console. Courtesy of syncardia.com.

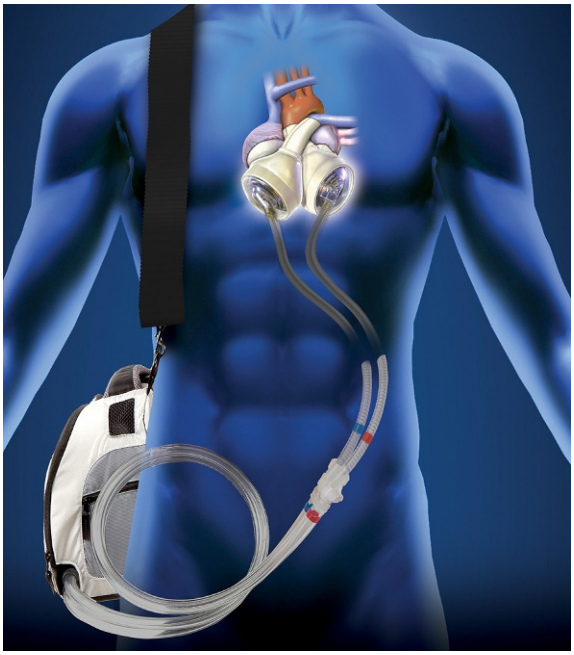

Fig. 2 Orthotopic positioning of an implanted SynCardia total artificial heart. Courtesy of syncardia.com.

Current Role of Total Artificial Hearts

Approved Devices

The SynCardia TAH (SynCardia Systems LLC, Tuscon, Arizona) is currently the only FDA-approved TAH, and it has gained CE Mark (Europe) and Health Canada approval as well. The SynCardiaTAH is a pulsatile biventricular device which completely replaces the native ventricles and all four cardiac valves orthotopically. It weighs 160 kg and attaches to the native atria via synthetic cuffs. A four-layered polyurethane diaphragm within each polyurethane ventricle is pneumatically driven, with a with a maximum stroke volume of 70 mL and maximum cardiac output of 9.5 L per minute. Four Medtronic-Hall mechanical tilting disc valves maintain unidirectional flow. Air pressure as well as device monitoring and backup power supplies are provided by an external console which is connected to the device by drivelines, which pierce the skin. The console is a large unit on wheels which requires patients to remain admitted within the hospital. More recently, a Freedom portable driver console has allowed patients to be discharged from the hospital.

Indications

The SynCardia TAH is indicated for use as abridge-to-transplantation for transplant-eligible patients at imminent risk of death from biventricular failure (Parker et al., 2014; Feldman et al., 2013; Thanavaro et al., 2014). This necessitates that patients meet criteria for transplantation (Mehra et al., 2016) and are actively listed for transplant (**Table 1**).

Several additional criteria specific to the TAHs include New York Heart Association (NYHA) class IV, adequate patient size (body surface area (BSA) 1.7–2.5 m^2), and adequate patient chest size (a distance of $\geq$10 cm from inner table of the sternum to the anterior vertebral body of the 10th thoracic vertebra).

Contraindications

Absolute contraindications for TAH implantation include patient inability to receive anticoagulation (**Table 1**). A relative contraindication is patient size less than 1.7 m^2, as this has been found to be associated with worse survival following TAH implantation (Kawaguchi et al., 1990). A second relative contraindication is a small chest size, defined as a distance of $<$10 cm between inner table of the sternum to the anterior vertebral body of the 10th thoracic vertebra, for fear that the device may compress the inferior vena cava (IVC) or pulmonary veins. Finally, despite the fact TAH is now approved as destination therapy for a very limited subset patients based on FDA humanitarian use device (HUD) designation, the large majority of TAHs are implanted as a BTT and therefore contraindications to heart transplantation (**Table 1**) apply for TAHs.

Table 1 Indications and contraindications to total artificial hearts and cardiac transplantation

Indications and contraindications to TAH (Mehra et al., 2016; Parker et al., 2014; Feldman et al., 2013; Thanavaro et al., 2014)	*Indications and Contraindications to Cardiac Transplantation (Mehra et al., 2016)*
Indications: ● Approved for transplant and on transplant list, with compatible heart not currently available ● Irreversible biventricular dysfunction refractory to optimal medical therapy Absolute contraindications: ● Inability to take anticoagulation Relative contraindications: ● BSA <1.7 m^2 ● Distance of <10 cm between inner table of the sternum to the anterior vertebral body of the 10th thoracic vertebra ● Pregnancy	Indications: ● NYHA class III or IV heart failure refractory to optimal medical therapy, including intravenous inotropes ● Debilitating ischemia not amenable to revascularization ● Recurrent, symptomatic ventricular arrhythmias refractory to medical therapy, implantable cardioverter defibrillator therapy, and surgical treatment Absolute contraindications: ● Pulmonary hypertension (PVR >6 Woods units despite maximal therapy) ● Significant reversible hepatic dysfunction (e.g., bilirubin >3.0 mg/dL) ● Severe cerebrovascular disease ● Active substance users (including alcohol) ● Poor compliance with drug regimens Relative contraindications: ● Age >70 years ● Obesity >35 kg/m^2 ● Diabetes with end-organ damage (other than nonproliferative retinopathy) or persistent poor glycemic control (glycosylated hemoglocin [HbA1c] $>7.5\%$) ● Significant irreversible renal dysfunction (e.g., eGFR <30 mg/mL/1.73 m^2) ● Severe lung disease ● Active peptic ulcer disease ● Uncorrected abdominal aortic aneurysm greater than 4 to 6 cm ● Peripheral vascular disease not amenable to surgical or percutaneous therapy ● Active infection (except in the setting of severe device complication, which is a status 1A criterion) ● Systemic infection with immune suppression risk (ehuman immunodeficiency virus, hepatitis B virus, cytomegalovirus) ● Active malignancy ● Osteoporosis ● Tobacco use ● Patient with insufficient social support system

TAH indicates total artificial heart; *NYHA*, New York Heart Association; *BSA*, body surface area; *PVR*, pulmonary vascular resistance; *eGFR*, estimated glomerular filtration rate.

Evidence for Total Artificial Hearts

TAHs vs. Optimal Medical Therapy

The evidence supporting use of TAHs in patients with advanced heart failure was proven by a landmark study published in 2004 (Copeland et al., 2004). This nonrandomized, prospective trial followed 95 transplant-eligible patients at risk for imminent death due to irreversible biventricular cardiac function despite maximum medical therapy, who were implanted with a CardioWestTAH. 81 patients within this cohort met all study criteria and were compared to 35 control patients who met identical criteria but in whom a device was not implanted. The TAH was associated with significantly improved near- and long-term outcomes. At 30 days posttransplant, the percentage of patients deemed to have been treated successfully (defined as alive, in NYHA class I/II, not bedridden, off-ventilator, and not requiring dialysis) was 69% in the device group and 37% in the control group ($P=0.002$). The group with implanted TAHs also had greater survival to transplantation (79% vs. 46%, $P<0.001$) and 1-year survival (70% vs. 30%, $P<0.001$) (Fig. 3). The authors concluded the CardioWest TAH "prevents death in critically ill patients who have irreversible biventricular failure and are candidates for cardiac transplantation." Among this very sick cohort of patients, frequent adverse events with the TAH within 30-days postimplant included infection (77% of patients), bleeding (62%), hepatic dysfunction (37%), respiratory dysfunction (36%), and renal dysfunction (31%). Several recent case series have shown similar rates of treatment success and complications, and an increasing number of patients surviving beyond 1-year postimplant (Torregrossa et al., 2014a,b; Copeland et al., 2012; Kirklin et al., 2015).

TAHs vs. Ventricular Assist Devices

Ventricular assist devices (VADs) are the predominant form of implanted MCS. Beginning June 2006, the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) has compiled data on MCS device utilization in >15,000 patients from 158 participating hospitals and has regularly published reports on trends regarding relative use and outcomes. During this time period, LVADs without right ventricular support have represented 92% of implanted MCS devices, while TAHs have comprised 2% (Kirklin et al., 2015).

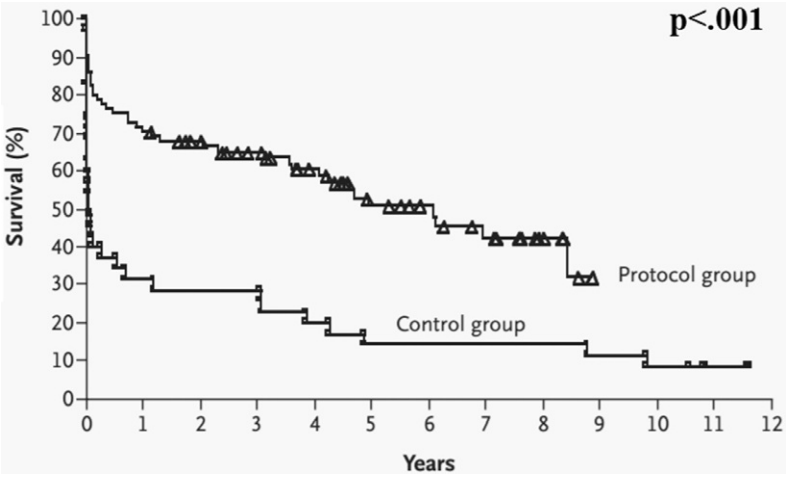

Fig. 3 Overall survival rate among patients implanted with a total artificial heart (protocol group) and patients with similar baseline status who did not receive an artificial heart. Data from Copeland, J. G., Smith, R. G., Arabia, F. A., et al. (2004). Cardiac replacement with a total artificial heart as a bridge to transplantation. *New England Journal of Medicine* **351**, 859–867.

The dominance of LVADs can be directly traced to a randomized trial published in 2001, which found improved 2-year survival with LVADs compared to medical therapy in heart-failure patients ineligible for transplantation (Rose et al., 2001). Since then, LVAD practice patterns have been continuously refined, with two additional randomized trials being particularly influential: a first finding continuous-flow devices to provide improved survival compared to pulsatile-flow device (Slaughter et al., 2009), and a second finding improved 6-month outcomes with magnetically levitated centrifugal-flow pumps versus axial-flow pumps (Mehra et al., 2017). These data have kept the LVADs front of mind for providers as a successful option for MCS, and the combination of this familiarity and LVAD approval as both bridge-to-transplant and destination therapy has helped maintain LVAD market dominance. Patients with borderline biventricular failure are often considered candidates for LVADs, based on the logic that improved left heart flow rates will improve right heart function. Additionally, INTERMACS data have consistently found patients implanted with LVADs have superior survival to patients implanted with biventricular support devices (Kirklin et al., 2015): while this is undoubtedly due in large part to patient selection of sicker patients receiving biventricular support, the association remains. No randomized data exist directly comparing these LVADs and TAHs.

For patients requiring unequivocal biventricular support, an alternative option to TAHs is the biventricular assist device (BiVAD), which augments the native ventricles and valves as opposed to replacing them. In the most recent INTERMACS report, BiVADs comprise approximately 5% of implanted MCS devices (Kirklin et al., 2015). Recent data have shown comparable or improved outcomes with TAHs as compared to BiVADs. A retrospective study of patients across 16 French centers who required biventricular support and were implanted with a paracorporeal BiVAD ($n=255$, 67%), TAH ($n=90$, 24%), or implanted BiVAD ($n=38$, 10%) between 2000 and 2010 found similar actuarial survival with each device but significantly decreased stroke rates with TAHs as well as a trend toward improved survival with TAHs among patients who required support greater than 90 days (Kirsch et al., 2012). In the most recent INTERMACS publication (Kirklin et al., 2015), patients with a TAH implanted between 2012 and 2014 had a 3-year survival rate of 60% which was superior to cohorts of patients implanted with BiVAD between 2008–11 and 2012–14 (Fig. 4). This trend toward improved outcomes with the TAH relative to BiVADs in the modern era bears watching, and the authors of the study note "early and midterm survival with a TAH is currently somewhat better than BiVAD support, but the patient groups may not be comparable." Patient survival for the cohort with TAHs implanted between 2012 and 2014 was constant after 1 year, representing durable life support with modern TAHs and device management. It is our opinion that the TAH is superior to the BiVAD due to its larger and more linear cannulas, thereby allowing flow rates and decreasing turbulent flow and associated thromboembolism.

Surgical Technique

Device Preparation

Arterial outflow conduits are prepared by first preclotting three times with the patient's own blood, approximately 30 cc for each graft each time, streteched, and then left to dry for 5 min before preclotting again. The exterior of the grafts are then coated with a mixture of cryoprecipitate, calcium, and topical thrombotic to serve as an adhesive. They are once again stretched and left to dry. This should be very early in the operation so as to allow time for preclotting. If patients have been heparinized, such as scenarios when there is a late decision to implant a TAH, the arterial conduits are preclotted with a combination of heparinized blood, protamine, and thrombin.

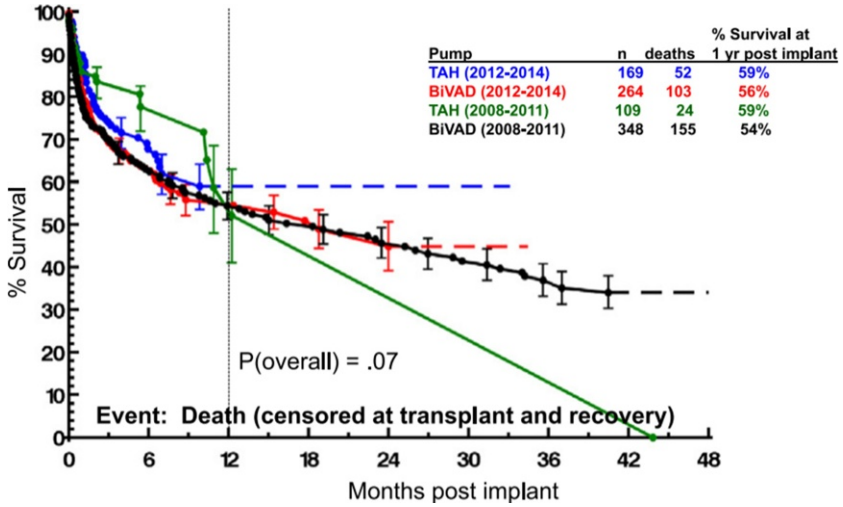

Fig. 4 Actuarial survival curves stratified by biventricular assist device (BiVAD) vs. total artifical heart and by Era. Data from Kirklin, J. K., Naftel, D. C., Pagani, F. D., et al. (2015). Seventh INTERMACS annual report: 15,000 patients and counting. *Journal of Heart and Lung Transplantation* **34**, 1495–1504.

The atrial cuff, or "quick connect," edges are trimmed in a circular fashion so that 5–7 mm of the cuff remains. These cuff edges will be sewed to the native atria and serve as connection points for the artificial ventricles.

Patient Preparation

The patient is positioned supine and general anesthesia is induced. Previous surgical scars are noted and marked. We then mark the skin at future exit sites for drive lines. The exit site of the left drive line is the left midclavicular line, 7–10 cm below the costal margin, and the exit site of the right drive line is 5 cm medial to the left's. Note that a distance of 5 cm is required to prevent the development of skin necrosis between the two sites. Central venous access is gained and a transesophageal echocardiogram probe inserted (TEE). A Swan-Ganz catheter is not required. Peripheral vessels should be exposed for planned or emergency cannulation, including for peripheral cardiopulmonary bypass institution.

Sternotomy and Access

The procedure is performed through a median sternotomy. Exposure of the heart and great vessels is similar to that of heart transplantation, with dissection of the aorta and pulmonary artery minimized in anticipation of planned future surgery. Heparin is administered. Aortic and bicaval cannulation are performed in a standard fashion, with both venous cannula through the right atrium to preserve the cava for subsequent transplant. Caval tapes may be utilized for both venous cannula, though we find this unnecessary with vacuum-assisted bypass. Cardiopulmonary bypass is instituted.

Cardiac Excision

The aorta is clamped. The heart may be arrested or fibrillated.

The excision differs from that of transplantation in that it preserves the two atria, the two annulus of the tricuspid and mitral valves, and 2 cm of ventricular muscle. The heart should be freed posteriorly down to the level of the pulmonary veins. We begin with an excision in the acute margin of the right ventricle with a #10 scalpel or other large blade, parallel and 1 cm anterior to the right atrioventricular groove. The incision is then extended with a scalpel or knife, anteriorly and clockwise along across the right ventricular outflow tract and proximal to the pulmonary valve while preserving as much pulmonary artery as possible. We again return to the acute margin of the right ventricle and extend the initial incision posteriorly and anticlockwise toward the interventricular septum. Pacing wires are transected if present.

Next we focus on the left ventricle, incising the inferior wall with care to preserve the mitral valve and annulus as well as 2 cm of left ventricular muscle. The incision is continued counterclockwise around the left ventricle through the lateral and then anterior walls. We transect the ascending aorta at the sinotubular junction. The aortic root is dissected from the roof of the left atrium. The aortic root and valve are excised while preserving the atrio-mitral curtain; aortic root tissue close to the curtain is preserved, as this tissue supports later anastomoses. The bilateral ventriculectomy is completed, and the excised ventricles are removed from the operative field.

Atrial Cuffs

Preparation for the anastomses of the atrial cuffs begins with trimming the remaining ventricular muscle such that a 1-cm cuff remains attached to the atrioventricular valves. Pacing leads are identified and divided at the entrance of the SVC, and intracardiac

portions are dissected from the endocardium; the extracardiac portions will be removed lateral after heparin reversal but before chest closure. Central venous catheters within the right atrium should be trimmed if they lie close to the tricuspid valve. The mitral and tricuspid valves are hallowed, removing leaflet tissue, chordae, and papillary muscles but preserving the annuli. We oversew the coronary sinus from within the atrium to prevent bleeding from the transected sinus and oversew the left atrial appendage in preference to external ligation to prevent adhesion formation which may complicate future transplant. The atrial septum should be inspected for defects and these should be closed if present.

The cuff of the left atrial quick connect device is sutured to the left atrial tissue using continuous 3-0 prolene suture. In the area of the aortic-mitral curtain, sutures will pass through the right fibrous trigone and aortic root. A second running 3-0 prolene suture is performed to complete a double suture line and ensure total hemostasis. The same process is repeated on the right. Care should be taken with the depth of the sutures into the left atrium, especially near the pulmonary veins, as the amount of scarring around these sutures will affect the availability of nonfibrosed tissue for future transplant anastomoses. We do not regularly test the anastomosis with pressurized saline injections. Felt strips may be utilized to support the atrial suture line, though we avoid this technique because it may result in excessive scarring and thus complicate future transplantation.

Arterial Conduits

The distal ends of the arterial outflow conduits and the transected ends of the great arteries are anastomosed. The conduits are trimmed to size, with the aortic conduit generally being 3–4 cm and the pulmonary conduit being 5–6 cm. Anastomoses are performed with 4-0 prolene sutures in a continuous end-to-end fashion, again layered twice.

Ventricle Implantation

We begin by suturing two large Gore-Tex sheets to the atrioventricular groove so that the implanted ventricles and atrial suture lines will not communicate with the posterior, inferior, and lateral pericardium. This will prevent adhesion formation and facilitate future device explantation prior to transplantation. The ventricle drivelines are then tunneled through the skin at the points marked previously, in a manner similar to chest tube placement: a skin incision is made with a scalpel, followed by enlargement with a clamp, passing of a 40 French chest tube through the pericardium into the clamp, and pulling of the chest tube through. Driveline tubing is passed through the tubes on the left, then the right.

The left ventricle is connected first (Fig. 5). Two Mayo clamps are used to grasp one end of the left atrial quick connect, gasping at two positions separated by 1/4th of the circumference of the cuff, and traction is provided to facilitate insertion of the ventricle

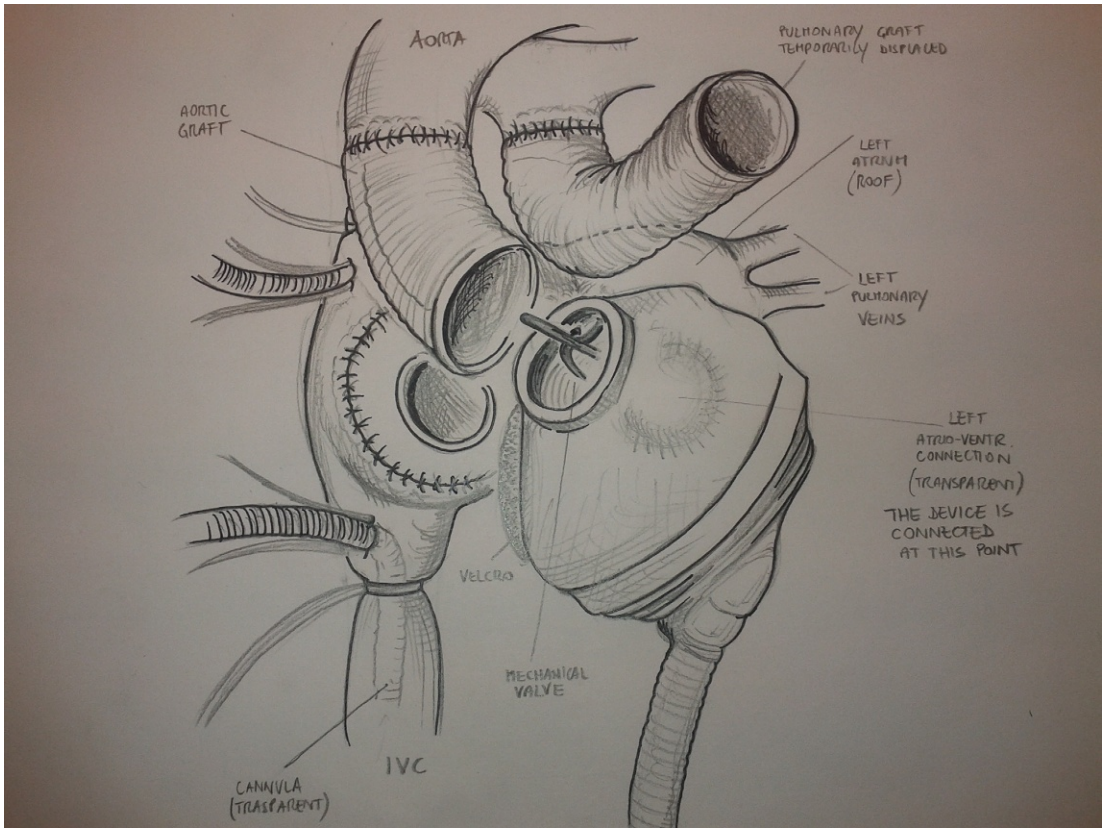

Fig. 5 Implantation of the total artificial heart. Courtesy of Z Fabio.

connect. The ventricle is rotated in place to properly align the outflow of the ventricle with the arterial conduit. The aortic cross-clamp is removed briefly under low flow to allow filling of the aortic conduit, and aortic quick connect is inserted into the ventricle in a manner which prevents kinking or tension on the arterial conduit. If kinking exists, the arterial quick connect should be disconnected via a spatula/flat instrument placement between the quick connect and the ventricular connection. Once the ventricular–arterial connection is sufficient, the patient is placed in steep Trendelenburg and the aortic graft is vented with a needle.

The right ventricle is connected in a similar fashion. The atrial connect is sutured first. The distal cava and right atrium should be deaired by briefly occluding the venous bypass cannula until blood flows out of the atrium. The right ventricle is rotated and the pulmonary conduit is connected, again ensuring satisfactory lie. The pulmonary conduit and aorta are deaired with a needle. The cross-clamp is removed. The patient is again placed in Trendelenburg and the lung ventilated. Pump flow rates are begun with low-flow initially, with deairing through the aortic vent. Further deairing may be performed with assistance of TEE and gentle manual compression of the heart, with this complete process taking 10–15 min. This time will allow the TAH pump, the heart to regain its normal rate, and the patient to be weaned from bypass.

Closure

Protamine is administered, and hemostasis is ensured by meticulous inspection of all anastomotic sites with the patient at physiologic pressures. Bypass cannula are removed in a normal fashion. The Gore-Tex membrane is wrapped around the anterior and lateral surfaces of both ventricles and the drivelines. A saline breast implant or tissue expander may be inserted inferior to the ventricles within the pericardium to maintain apical pericardial space, as contraction of the pericardial cavity is a rare but avoidable occurrence with artificial hearts which complicates future transplantation. The chest is closed in a standard fashion with particular care during sternal closure, as mediastinitis and sternal dehiscence are catastrophic events for TAH patients. For large patients, closure devices other than simple wires may be considered.

Challenging Implantation Scenarios

Small Patients

A patient BSA of less than 1.7 m^2, or a distance between the sternum and the anterior vertebral body of less than 10 cm, is traditionally considered relatives contraindication to the SynCardia TAH (**Table 1**). Small BSAs have been associated with worse outcomes post-TAH implantation, though some centers have reported successful implantation in many patients of BSA less than 1.7 m^2 with similar or superior outcomes to patients of large size (Leprince et al., 2005). A small chest size predisposes patients to a risk of compression of the IVC and left-sided pulmonary veins by the device.

In this settings we utilized several techniques, which in totality cause the ventricles to lie mostly to the left of the sternum and the left ventricle to lie in the left pleural cavity (as opposed to the usual placement where the right ventricle is immediately posterior to the sternum).

The diaphragmatic attachment of the pericardium must be mobilized and the left pleura is opened to allow the device to sit more leftward and posterior in the chest. A Gore-Tex membrane should be wrapped around the left ventricle to prevent lung adherence to the device. The device is then anchored by its ventricles to a rib on the left chest wall, which laterally displaces the TAH away from the IVC and left pulmonary veins. This anchoring is achieved with umbilical tape, heavy silk, or polyester suture, which is wrapped three times around the main body of the left artificial ventricle. The two ends of the tape or suture are then tunneled outside the chest through an intercostal space and tied around one of the ribs. Position of the ventricle is finetuned by echocardiographic and hemodynamic evaluation combined with observation of the TAH fill-volumes. Once the ideal position is identified, the suture or tape is tied. Further adjustments to device positioning may be required when the sternum is closed.

It is critical to confirm via echocardiogram that there is laminar flow into all left-sided veins and the IVC prior to chest closure, and additionally that ventricular filling is maintained after closure. If attempts to close the chest result in severe venous obstruction despite corrective maneuvers, the chest is left open and the patient returned to intensive care, and chest closure can be reattempted at a later date. Rarely, if chest closure cannot be accomplished, then musculocutaneous flap closure may be required.

Complex Congenital Heart Disease

Patients with complex congenital heart disease and end-stage cardiomyopathy are strong candidates for TAH implantation, as most intracardiac anomalies will be improved by excision of the native ventricles. For these patients, the TAH is implanted in a similar way to orthotopic heart transplantation, though there are some situations where congenital cardiac disease poses unique surgical challenges for TAH implantation: in the setting of single ventricular physiology or transposition of the great vessels.

In case of failed "Fontan" operations, the TAH has been implanted after constructing a "neo right atrium" (neo RA) in order to fit the right atrial quick connector (Rossano et al., 2014). The extracardiac conduit and IVC are both separated from the right pulmonary artery, which is reconstructed with a bovine pericardial patch or autologous tissue explanted from the failing heart. A 24 or 26 mm Gore-Tex graft is then inserted between the SVC and IVC to serve as the neo RA. The left atrial cuff is prepared in a standard fashion and any interatrial communication is addressed. The neo RA tubing graft is opened in a generous elliptic shape in order to accommodate the right atrial quick connector. The outflow grafts are then sutured in an end-to-end fashion on the aorta

and end-to-side fashion at the pulmonary artery bifurcation. The length of the pulmonary graft is generally longer than the usual six to seven cm and should be addressed before proceeding with the distal anastomosis.

The SynCardia TAH has also been used in cases of congenital corrected transposition of the great arteries (Adachi and Morales, 2014). In this situation, implantation is made difficult by the position of the great arteries (aorta and pulmonary artery): with the aorta anterior and leftward to the pulmonary artery, the right and left pumps must be implanted in a parallel rather than a normal crisscross arrangement.

Extracorporeal Membrane Oxygenation

Pulmonary edema is often present in cardiogenic shock patients requiring biventricular support, and patients may be on extracorporeal membrane oxygenation (ECMO) to maintain adequate oxygenation either at the time of TAH implantation or afterward.

Veno-arterial (VA) ECMO may be undertaken but will result in two parallel circuits. This causes reduced flow through the TAH, which may predispose patients to thrombosis of the mechanical valves. Therefore, VA ECMO it is not ideal if prolonged ECMO is anticipated.

Veno-venous (VV) ECMO is preferable, as it allows the ECMO and TAH circuits to run in series with maximal flows on both devices. For patients who require ECMO to wean from bypass, our preferred approach is to draw blood from the infrahepatic IVC and return it to the right atrium (Hosseinian et al., 2014). This is done by placing a femoral venous cannula and advancing it such that the tip lies in the intrahepatic portion of the inferior IVC. The ECMO outflow cannula is a separate venous cannula placed in the other femoral vein or a subclavian vein, which is advanced a few centimeters into the right atrium under sonographic guidance. Blood returning via the SVC is not oxygenated. If ECMO is anticipated preoperatively, then cannule are placed before instituting cardiopulmonary bypass, with final adjustment of outflow cannula position made under direct vision after excision of the native heart to ensure adequate distance of the cannula tip from the right-sided atrioventricular valve. This allows the explantation of VV ECMO at the bedside of the patient. Adequate distance between the tips of both cannulas should be ensured to avoid short-circuit phenomenon.

For patients who develop respiratory failure postimplant, a single dual-lumen catheter may be placed via the internal jugular vein. Care must be taken when introducing the cannula with the Seldinger technique, so as not to entrap the wire or the cannula into the mechanical right atrioventricular valve of the TAH.

Postoperative Management

Early Postoperative Management

Antibiotics are administered preoperatively as prophylaxis against infection and should be continued for 24 h after implantation. Lifelong anticoagulation is mandatory to prevent device thrombosis. This should be achieved with warfarin and the target international normalized ratio (INR) is 2.5–3.5 (Feldman et al., 2013). Patients are prescribed aspirin and potentially another oral anticoagulant, especially if thromboembolic events occur. Expected hemodynamic changes include increased diastolic pressure, increased diastolic flow, and decreased pulse pressure. Arterial lines are required for hemodynamic monitoring postoperatively and should be compared against console readings. Standard blood measurements must be gathered regularly to ensure sufficient end-organ perfusion and to identify metabolic changes.

Late Postoperative Management

Late management includes rehabilitation and education. The wheeled console may be cumbersome and prevents hospital discharge but is intended to allow ambulation; a newer portable device console is available and permits discharge. Note that complications are common in the postoperative period including bleeding, infections, and thrombosis: judicious monitoring is required.

Complications

The most frequent complications following TAH implantation are bleeding and infection. Bleeding may be secondary to surgery or diffuse coagulopathy. This can contribute to infection and transfusion-related complications. Diffuse coagulopathy may result from changes such as dilutional thrombocytopenia and exposure to antithrombicand antiplatelet agents, and patients may be treated with platelets, fresh frozen plasma, or cryoprecipitate. Significant refractory bleeding may require a return to the operating room.

Device-specific infections may occur within the implanted device, at the sternal wound or the drivelines. Antibiotic prophylaxis preoperatively is required. Should infections arise, judicious treatment with antibiotics should be undertaken, and may be followed with surgical drainage, vacuum-assisted wound closure, and device exchange. Endocarditis may only be definitively treated by device removal. Infection is generally not a contraindication to heart transplant. In the landmark study of 95 patients implanted with TAHs, the most common infections immediately posttransplantation were respiratory and genitourinary (Copeland et al., 2004). Note that in long-term follow-up, mortality and bleeding events were increased among patients with BSA $<1.8\ m^2$ (Torregrossa et al., 2014a,b).

Additional complications with the device are rare but may have significant consequences. The most significant device malfunctions reported was a perforation of one of the four layers of the device diaphragm, which occurred once in 12,000 patient-days of device support of the landmark study (Copeland et al., 2004), and three times in a study of TAH patients who survived beyond 1 year (Torregrossa et al., 2014a,b); this was the cause of three deaths. Issues with device fit is more common and may be corrected with simple repositioning or surgical treatment, though the risk of improper device fit is low if the device is meticulously implanted. Hypotension or hypertension should be managed carefully with the console, inotropic agents, vasopressors, and vasodilators. End-organ perfusion must be maintained to minimize the risk of secondary complications such as renal dysfunction, hepatic dysfunction, respiratory dysfunction, and neurologic events. Proper anticoagulation is additionally required to reduce the risk of thromboembolic events.

Long-Term Outcomes

Several case series have reported the rates of successful bridge-to-transplantation to be approximately 75%, and 1 year-survival on the device to be approximately 65% (Copeland et al., 2004, 2012; Kirklin et al., 2015). In a study of 47 patients supported by the SynCardia TAH beyond 1 year, 72% of patients were successfully bridged to transplantation (Torregrossa et al., 2014a,b).

1-year and 5-year postimplant survival rates of patients who successfully bridged to transplant with the TAH are70%–80% and 60%–65%, respectively (Copeland et al., 2004). At the time of writing, the longest patient survival on SynCardia TAH was in a Turkist man who was actively being supported beyond 4.6 years (SynCardia, www.syncardia.com).

The Future of Total Artificial Hearts

The TAH has several distinct and achievable frontiers for improvement. Among these are approval as destination therapy, continuous flow systems, methods of powering the device which do not break skin and therefore decrease associated infections, components which reduce the rate of thrombosis, hemolysis, and inflammation, and the ability to support small patients.

The SynCardia TAH is currently enrolling patients to investigate it suitability as a destination therapy. The company is also developing a 50 mL device, intended to better support small patients than the current 70 mL device.

The AbioCor TAH (Abiomed, Danvers, MA) was approved by the FDA for humanitarian device exemption (HDE) status in 2006 but is no longer being utilized or developed by the company due to unproven efficacy. However, several of its features may be utilized in future artificial hearts. This device was fully implantable and utilized a battery system which transferred energy across the skin, thereby obviating the need for skin incisions. It also weighed 1 kg and thus allowed impressive patient mobility.

Devices Under Development

The continuous flow total artificial heart (CFTAH) is being developed by Cleveland Clinic and features a continuous flow pump (Fukamachi et al., 2010). It measures six cm by 10 cm, is comprised of biocompatible titanium and plastic, and is without mechanical valves, flexing membranes, sensors, or actuators (**Fig. 6**). Conceptualized differently, this pump is a combined right and LVAD connected to a single axial rotor. This system senses inlet systemic and pulmonic pressures and self-modulates speed of rotor to generate separate and appropriate pressures within the left and right heart (Shiose et al., 2010). This device has proven viability in calves, with durability and extended battery life being two remaining issues.

The Carmat TAH (CARMAT, Velizy-Villacoublay, France) features a unique design which is part prosthesis and part bovine (Mohasci and Leprince, 2014). It consists of two chambers, each with a flexible biomembrane-separating hydraulic fluid from blood (**Fig. 7**). Force is created through the hydraulic fluid to display the blood. The aspect of the membrane interfacing with the blood is comprised of bovine pericardium to improve biocompability: while it has been shown to reduce platelet adhesion and blood cell deposition on the membrane, it raises concerns of low mechanical durability. The device also self-modulates cardiac output by measuring the patient's level of physical activity and responding accordingly. Clinical trials of the device began in Europe in December 2013, with the first patient living 75 days. Development of this device is being lead by Dr. Alain Carpentier.

Finally, the BiVACOR TAH (BiVACOR, Inc., Houston, Tx, USA) is a device which utilizes third-generation magnetic suspension technology (Timms et al., 2008) (**Fig. 8**). Two centrifugal impellers are mounted to a single rotating hub, with flow balancing achieved with rotor movement along the shared axis (Greatrex et al., 2010). The expected benefit is that this design reduces device thrombus formation, with this benefit seen in clinical trials of the magnetically levitated HeartMate III LVAD versus the HeartMate II (Mehra et al., 2017).

Areas of Future Growth

Between 2012 and 2014, destination therapy was the device strategy in 44% of patients implanted with a continuous flow LVAD or BiVAD (Kirklin et al., 2015). However, the SynCardia TAH is only approved as a bridge-to-transplantation. An ongoing trial is

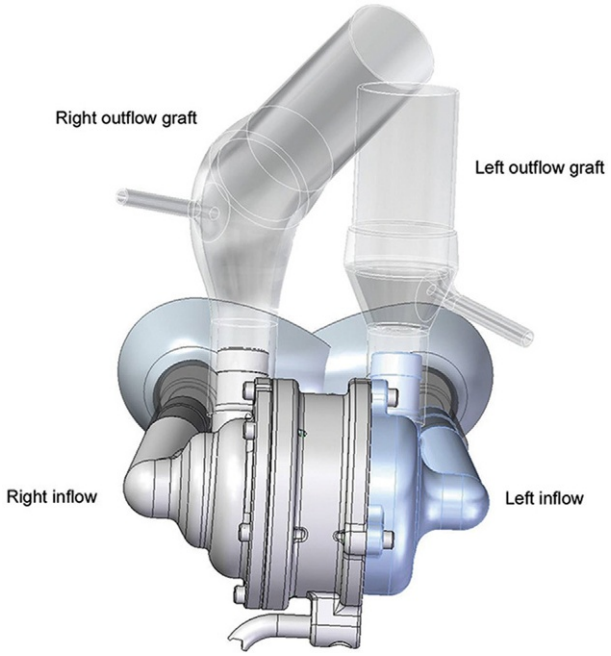

Fig. 6 Continuous flow total artificial heart. Karimov et al. First report of 90-day support of 2 calves with a continuous-flow total artificial heart. *The Journal of Thoracic and Cardiovascular Surgery* 2015. J Thorac Cardiovasc Surg. 2015 Sep;150(3):687-93.e1. https://doi.org/10.1016/j.jtcvs.2015.06.023. Epub 2015 Jun 18. First report of 90-day support of 2 calves with a continuous-flow total artificial heart. Karimov JH, Moazami N, Kobayashi M, Sale S, Such K, Byram N, Sunagawa G, Horvath D, Gao S, Kuban B, Golding LA, Fukamachi K.

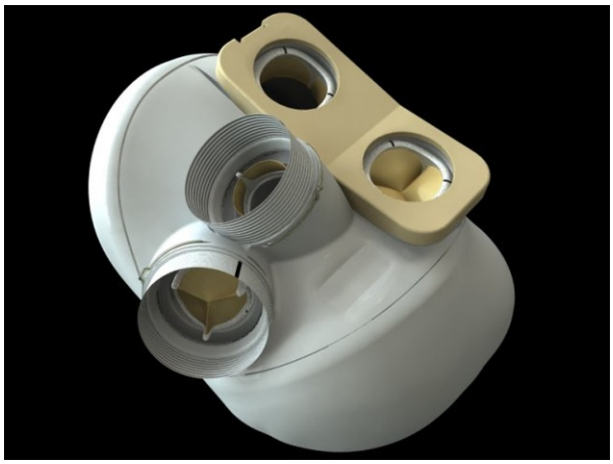

Fig. 7 Carmat total artifical heart. Reproduced with permission from CARMAT.

currently enrolling patients to investigate TAH outcomes for patients requiring MCS for destination therapy, an important area of potential growth for this technology.

Truly increasing the utilization of TAHs will likely require more comparative data showing increased benefit for TAHs against the BiVAD and potentially the LVAD. This is not unreasonable given that in the INTERMACS registry, the cause of mortality for continuous flow VAD patients was due to causes potentially avoided with TAHs: multisystem organ failure (19.5% of deaths), right ventricular failure (5.5%), device malfunctions (4.6%), and congestive heart failure (4.1%) (Kirklin et al., 2015).

Conclusions

The TAH provides durable MCS as a bridge-to-transplantation for patients at an imminent risk of death secondary to biventricular failure. The predominant device is the SynCardia TAH, which has been approved and utilized for more than a decade. Indications and surgical techniques are well established, with the result being consistent outcomes and high rates of successful transplant.



Fig. 8 BiVACOR total artifical heart. Reproduced with permission from BiVACOR, Inc.

Utilization is limited by its indication as a bridge-to-transplantation, the invasiveness and complexity of devices, and the prevalence of VADs. However, future utilization may increase as the population ages, the technology advances, its indications broaden, and data convince the medical community of its benefit.

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Transcriptomics in Cardiovascular Medicine

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Transcriptomics:An Overview

The remarkable progress achieved in the "omics" field has successfully improved the understanding of cardiovascular disease (CVD) pathophysiology by comprehensively interrogating disease states at the molecular level (Ozsolak and Milos, 2011). Transcriptomics, the simultaneous study of ribonucleic acid (RNA) transcripts and their expression patterns at a genome-wide level, is a particularly promising tool. The transcriptome is the sum of RNA transcripts that comprehend messenger RNAs, ribosomal and transfer RNA, and regulatory noncoding RNAs (Ozsolak and Milos, 2011).

Transcriptomic approaches include microarray-based methods where tens of thousands of transcripts are simultaneously analyzed by chemically labeling RNA molecules and subsequent hybridization to probes on the microarray. With the novel RNA sequencing technologies, a population of RNA is converted to a cDNA library which is subsequently sequenced in a high-throughput base-by-base manner to obtain short sequences, providing a much better and deeper coverage for detection of low abundance transcripts.

This chapter describes the methodological underpinnings in the transcriptomics field and provides an overview about recent advances of transcriptomics in the cardiovascular field.

RNA, Gene Expression, and Transcriptomics

RNA has long been considered as the messenger molecule between genes and proteins, where RNA is transcribed from DNA to messenger RNA (mRNA) and subsequently translated into protein (Siemelink and Zeller, 2014).

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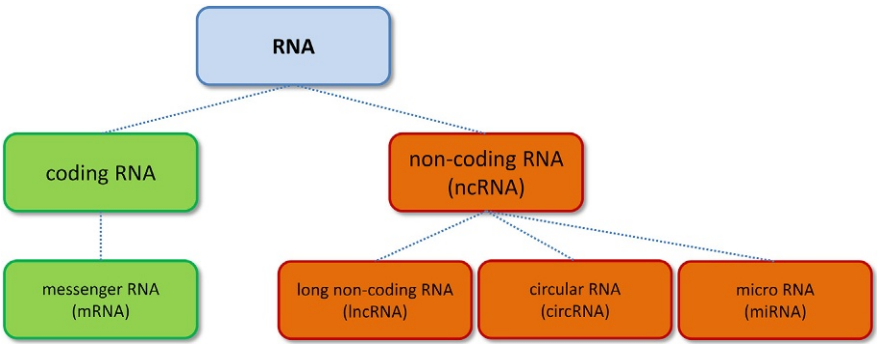

Fig. 1 Overview of RNA molecules. Ribonucleic acids can be categorized into protein-coding and noncoding RNAs; mRNAs are translated into proteins, while several noncoding RNAs, including lncRNAs, circRNAs, and miRNA, are not translated into proteins (Devaux, 2016).

In recent years, noncoding RNA species have been characterized including micro-RNAs (miRNAs), long noncoding RNAs (lncRNAs) and circular RNA (**Fig. 1**). miRNAs are endogenous, noncoding small RNAs of about 22 nucleotides regulating gene expression at a posttranscriptional level (Fichtlscherer et al., 2011). miRNAs are the best investigated ncRNAs so far. Since their discovery in 1993 (Lee et al., 1993) approximately 1800 miRNAs have been annotated (www.mirbase.org). miRNAs are produced by all cell types and interact mainly with the 3′ untranslated region (of protein-coding genes thereby inhibiting the translational process). They are involved in a broad range of biological processes and their dysregulation impacts disease development (Small and Olson, 2011).

lncRNAs cover RNA molecules over 200 nucleotides and are observed in a wide range of tissues. lncRNAs are transcribed from either intergenic regions, from introns of protein-coding sequences or from antisense strand of genes (Boon et al., 2016). In contrast to miRNA sequences, the primary sequence of lncRNAs is only poorly conserved. The number of lncRNAs within cells is believed to be around 9000 (Pennisi, 2012). They exert a broad repertoire of functions and have been linked to differentiation and developmental processes and disease (Mercer et al., 2009; Ponting et al., 2009).

Circular RNAs have been known for some time, however, only recently this class of ncRNAs gained interest. Due to their circular state these RNA molecules are highly stable, and show an evolutionary conservation. circRNAs can be reproducibly detected at high levels in peripheral blood and other body fluids.

The number of RNA transcripts represents the amount at which the corresponding gene is expressed ("gene expression") and reflects the cell function and gene regulation. Measuring and analyzing gene expression can give an insight into regulation and effects of the corresponding gene, while a transcriptomics analysis investigates all RNA transcripts of the cell simultaneously.

Technology for RNA Transcript Detection

Recent advances in technology enabled the detection and measurements of RNA transcripts, by using microarrays or sequencing as high-throughput techniques, or polymerase chain reactions as low-throughput technology. qPCR is mainly used for verification of results obtained by microarrays and/or sequencing technologies. In general, such experiments involve the following steps: (1) RNA sample collection, (2) sample purification, (3) sample processing, (4) detection of transcripts, (5) data analysis and visualization, and (6) validation. These steps are complemented by additional technology-specific processes as detailed below.

Microarray technology

Transcriptomics analyses often rely on microarray technology, assessing thousands of transcripts (mRNA, miRNA, ncRNA) on a genome-wide scale in parallel. It allows to rapidly screen the entire transcriptome by chemically labeling and hybridizing RNA molecules to probes on the microarray. The main advantages of the microarray technology include low costs, high-throughput applicability, large coverage, and straightforward data analysis, while it is limited by the large amount of RNA required, limited dynamic range for quantification, the semiquantitative approach and the detection of predefined transcripts (Siemelink and Zeller, 2014). Generally, the main steps involved in a microarray experiment are (1) RNA sample collection, (2) sample purification, (3) reverse transcription (cDNA synthesis), (4) sample labeling, (5) hybridization and washing, (6) scanning of the probe array, and (7) visualization and data analysis (**Table 1**, **Fig. 2**).

Most microarrays are manufactured as spotted microarrays, self-assembled arrays, or in situ synthesized arrays (Bumgarner, 2013). In spotted microarrays oligonucleotides are synthesized and deposited onto a glass slide, while in situ synthesized arrays can be produced by inkjet oligo synthesis methods or photolithography methods (e.g., Affymetrix GeneChip, Agilent), and self-assembled arrays are manufactured using the BeadArray technology (e.g., Illumina Bead Chip). The Affymetrix GeneChip arrays rely on standardized assays and reagents, and instrumentation is provided within the platform. The GeneChip probe arrays are oligonucleotide microarrays being produced in a highly standardized manner using photolithography applying predefined masks and producing 25-mer probes of known sequence. Performing a GeneChip experiment includes different steps: (1) obtaining RNA samples, (2) target amplification and sample labeling, (3) injection of the labeled sample into the probe and hybridizing, (4) probe

Table 1 Microarrays as a high-throughput transcriptomics technology. Using microarray technology involves different analytical and computational steps which are specific for this technology, including wet lab procedures (sample labeling, hybridization and washing, and probe scanning) and dry lab procedures (data preprocessing and data analysis)

Microarrays	
Wet lab	• User-specific dyes can be chosen • Platform-specific protocol exists • Straightforward visualization of the experiment • Technical batch effects can occur • External hardware and software required, which may be platform-specific
Dry lab	• Identification and removal of batch effects • Various approaches available, no gold standard • Analysis can be complex

Here, several exemplary technology-specific steps are categorized into wet lab and dry lab procedures, along with their advantages and challenges.

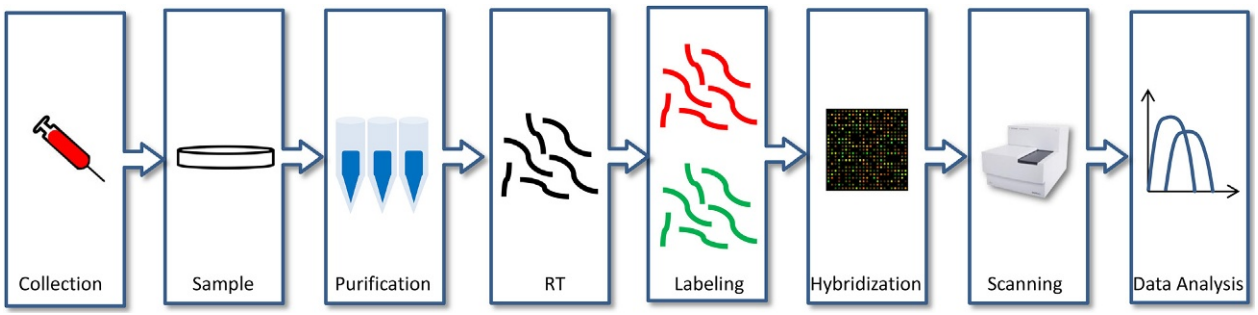

Fig. 2 Overview of the main steps involved in a microarray experiment. Generally, the following steps are involved: Sample collection, RNA sample collection, sample purification, reverse transcription (RT), sample labeling, hybridization and washing, scanning of the probe array, and visualization and data analysis.

array washing and staining, and (5) scanning of the probe array in the Affymetrix GeneChip scanner (Dalma-Weiszhausz et al., 2006). In contrast, the Bead Chips from Illumina use the BeadArray technology, with gene-specific probes attached to beads and assembled into arrays. Basically, it is based on a high-density silica bead-based microarray technology, covering each bead with hundreds of thousands of copies of a specific oligonucleotide acting as the capture sequences in a given Illumina assay.

Next-generation RNA-sequencing

Along with evolving deep-sequencing technology, next-generation RNA-sequencing (RNA-Seq) has become a novel and powerful method for quantifying RNA transcripts at a high-throughput, high-resolution, and high-quality genome-wide transcriptome scan (Wang et al., 2009). A typical RNA-Seq uses deep-sequencing technologies involving the following steps: (1) RNA fragmentation to convert long RNAs into a library of cDNA fragments, (2) addition of sequencing adaptors to each cDNA fragment, (3) high-throughput sequencing of short cDNA fragments, (4) alignment of sequence reads with reference transcriptome, and (5) generation of a base-resolution expression profile for each gene (Wang et al., 2009) (**Table 2**).

In an RNA-Seq experiment, first, RNAs are converted into a library of cDNA fragments through RNA fragmentation or DNA fragmentation. Subsequently, sequencing adaptors are added to each cDNA fragment and a short sequence is obtained from each cDNA using high-throughput sequencing technology (NGS). The resulting sequence reads are aligned with the reference genome or transcriptome, and classified as exonic reads, junction reads or poly(A) end-reads, which are subsequently used to generate a base-resolution expression profile for each gene (Wang et al., 2009).

RNA-Seq clearly outperforms microarray technology in terms of throughput, resolution, and dynamic range, while it includes splice variants, unknown RNAs, and RNAs too short to be captured by microarrays. However, RNA-Seq is more expensive than microarray technology, as it requires large data storage, computation resources, and advanced bioinformatics resources for subsequent data analysis (Wang et al., 2009; Siemelink and Zeller, 2014; Wu et al., 2014) (**Table 3**).

Real-time quantitative polymerase chain reaction

Many applications require verification of individual transcripts as obtained from microarrays or RNA-Seq experiments. Real-time quantitative polymerase chain reaction (RT qPCR) is a well-established, but low-throughput technology for monitoring the amplification of a single cDNA molecule serving as an independent validation of preperformed experiments. The quantification is performed in comparison to a housekeeper gene (relative) or to the standard curve (absolute). PCR is a low-throughput technology for measuring the expression of individual or a small amount of transcripts, and, thus, is often applied for validation or verification.

Table 2 RNA-Seq as a high-throughput transcriptomics technology. Using RNA-Seq technology involves different analytical and computational steps which are specific for this technology, including wet lab procedures (sequencing, mapping, annotation, and assembly) and dry lab procedures (data analysis)

RNA-Seq	
Wet lab	• High-throughput • High resolution • High dynamic range • RNA library construction can be biased • Expensive • Large data storage required • Polymorphisms, which occur when sequence reads align to multiple locations of the genome
Dry lab	• Advanced and complex bioinformatics approaches required • Bioinformatics tools available, but various approaches available, no gold standard • Computational expensive • Can be difficult to reconstruct and tease apart all splicing isoforms

Here, several exemplary technology-specific steps are summarized, along with their advantages and challenges.

Table 3 Comparison of transcriptomics technologies (microarrays, RNA-Seq, qPCR)

	Microarray technology	*RNA-Seq technology*	*qPCR*
Throughput	High	High	Low
Resolution	Medium	High	Low
Dynamic range	Medium	High	Low
Expenses/costs	Medium	High	Low
Coverage	High	High	Low
Data storage	Medium	High	Low
Data analysis	Easy	Complex	Easy
Batch effects	Possible	Possible	Possible as plate effects
Aim of analysis	Discovery	Discovery	Mainly validation/verification

The three technologies mainly differ on the level of experiment costs and time, amount of transcripts that can be analyzed, coverage, resolution, data storage, and data analysis.

Table 4 qPCR as a low-throughput transcriptomics technology for target verification. Using qPRC technology involves different analytical and computational steps which are specific for this technology, including wet lab procedures (primer design and PCR reaction) and dry lab procedures (data analysis)

qPCR	
Wet lab	• Low-throughput • Transcript-specific • Time-consuming for a large number of transcripts • Transcript for normalization required
Dry lab	• For validation or verification rather than for transcriptomics analysis • Easy to interpret and analyze • Software available • No gold standard

Here, several exemplary technology-specific steps are summarized, along with their advantages and challenges.

A single RT qPCR reaction is characterized by the PCR cycle at which fluorescence first rises above threshold background levels (threshold cycle, Ct), and consequently, results in a low Ct value if the RNA levels are large (Jozefczuk and Adjaye, 2011). A general workflow of RTqPCR involves multiple steps: (1) RNA isolation, (2) primer design, (3) reverse transcription to obtain cDNA from RNA, (4) cDNA amplification, (5) PCR reaction, and (6) data analysis (Thermofisher) (**Table 4**). As a result, the nucleic acids are quantified either relatively (fold-difference in expression using a reference transcript) or absolutely (exact number of DNA molecules). Due to its low throughput, RT qPCR is nowadays mainly used for validation of individual RNA transcripts.

Detection and measuring of noncoding RNA

Recent advances allow the detection and measurement of noncoding RNA along with coding RNA transcripts. Using microarray technology, different platforms exist that cover the whole-transcriptome (e.g., Affymetrix GeneChip Human Gene ST Array, Affymetrix Gene Chip miRNA Array, Agilent SurePrint Human Gene Expression Microarray, Illumina BeadArray), detecting both

coding and noncoding transcripts. However, due to the large amount of unknown ncRNAs, the annotation of the corresponding probes is still challenging (Zhang et al., 2015a). Using sequencing technology to measure ncRNAs allows detecting the exact transcript on a single nucleotide resolution (Zhou et al., 2011), independent on the availability of a priori transcript information or genome sequence (Veneziano et al., 2015). However, for annotating transcript sequences, reference transcriptomes are needed. Again, unknown ncRNA cannot be annotated without corresponding a priori information.

Computational Methods for Transcriptome Analyses

High-throughput technology (microarray and RNA-Seq experiments) detects and measures the whole transcriptome. Subsequently, technology-specific approaches are applied for quantifying expression levels, including preprocessing and normalization in microarray experiments, while RNA-Seq experiments involve sequencing, mapping, and assembly (**Tables 1** and **2**). Subsequently, based on such processed data, gene expression levels can be directly quantified, or, in more complex analyses, combined with other data types to handle more advanced scientific questions [e.g., TWAS (gene expression and phenotype data), eQTL (gene expression and genomic data)]. Alternatively, large gene expression data sets can be combined and meta-analyzed, or, in a follow-up study, analyzed in a longitudinal expression analysis (**Table 5**).

Preprocessing and normalization of data from microarray experiments

Transcriptome data derived from microarray experiments can suffer from technical variation, that is, batch effects. A recent study analyzing Illumina gene expression microarray data identified technical factors to substantially influence signal intensity values, including the chip assignment, RNA amplification batch, RNA isolation batch, and the sample storage time, and the authors concluded that adjusting for such batch effects had improved the reliability of subsequent data analyses (Schurmann et al., 2012).

Often, microarray experiments are performed to compare expression levels between different conditions (e.g., disease vs. healthy, different medication, different time points) on a transcript level. However, before such analysis can be done, the microarray data must be adjusted for technical effects, and several data transformations are needed to identify and exclude invalid measurements, to adjust chip intensities, and to select transcripts that are significantly differentially expressed between two conditions. Such transformations include data normalization which adjusts the individual hybridization intensities (e.g., unequal quantities of input RNA, differences in labeling, systematic biases in measured expression levels) to balance them appropriately so that meaningful biological comparisons can be made (Quackenbush, 2002). Multiple computational methods for gene expression data normalization have been proposed, among which loess normalization (Bolstad et al., 2003), RMA (Irizarry et al., 2003), MAS (Affymetrix), GCRMA (Wu and Irizarry, 2005), and dChip (Li and Wong, 2001) are well-established methods. Loess normalization is a locally weighted regression approach, which models the intensity-dependent trends by a regression curve. RMA (robust multiarray average) uses a convolution background correction, quantile normalization, and summarization using median polish, while GCRMA is a similar approach which computes the background in a different manner. In contrast to RMA, MAS5 normalizes each array independently and sequentially, while RMA uses a multichip model. Additionally, MAS5 uses data from mismatch probes to calculate a robust average, while RMA does not use mismatch probes. Different benchmark studies on the Golden Spike data has intensively analyzed and benchmarked these methods for normalizing Affymetrix gene expression data (Choe et al., 2005; Pearson, 2008).

Sequencing, mapping, and assembly of data from RNA-Seq experiments

RNA-Seq uses deep-sequencing technology, such as Illumina IG (18516045, 18451266), Applied Biosystems SOLiD (Cloonan et al., 2008) or Roche 454 Life Science (Emrich et al., 2007, Barbazuk et al., 2007), revealing short reads that have to be mapped to a reference genome (e.g., SOAP (Li et al., 2008b), MAQ (Li et al., 2008a), and RMAP (Smith et al., 2008)). For low-copy repetitive sequences, multimatch short reads can be proportionally assigned based on the number of reads mapped to their neighboring unique sequences (Mortazavi et al., 2008), while for longer sequence reads paired-end sequencing strategy can be applied that determine short sequences from both ends of DNA fragment (Campbell et al., 2008). For a detailed overview refer to Wang et al. (2009). Alternatively, reads can be assembled into contigs, followed by an alignment into the final genomic sequence. Multiple de novo RNA-Seq assemblies have been developed, for example, DETONATE (RSEM-EVAL and REF-EVAL) (Li et al., 2014), Trans-ABySS (Robertson et al., 2010), or Trinity (Grabherr et al., 2011).

Table 5 Computational methods for transcriptome analyses

Computational method	*Description*
Transcriptome-wide association study (TWAS)	Investigates the association between gene expression and trait or phenotype
Expression quantitative trait loci (eQTL)	Represents genomic loci that contribute to variation in gene expression levels
Meta-analysis	Statistical study that systematically assesses and combines multiple studies quantitatively by statistically pooling estimates from individual studies into one meta-analytical estimate
Longitudinal expression analysis	Measures and analyzes gene expression at different time points

Quantification of expression levels

To quantify the differences in gene expression levels, an expression ratio can be computed. This ratio can be either a simple ratio (e.g., disease/healthy), or a log-transformed ratio [e.g., log (disease/healthy)]. The advantage of the simple ratio is their intuitive understanding, although they treat up- and down-regulated transcripts differently (e.g., upregulation by a factor of 2: $2/1=2$; downregulation by a factor of 2: $1/2=0.5$). This limitation can be solved by log transforming the data (e.g., upregulation by a factor of 2: $\log_2(2/1)=1$; downregulation by a factor of 2: $\log_2(1/2)=-1$), providing an intuitive understanding of up- and down-regulation of transcripts.

To assess a transcript's statistical significance level, *P*-values are computed, corrected for multiple testing (e.g., Bonferroni, Benjamini-Hochberg) if more than one transcript is assessed. P-values can be computed by comparing their differential expression level to a random distribution (Nitsch et al., 2009), by computing a simple *t*-test or a regularized *t*-test [e.g., Cyber-T (Baldi and Long, 2001)]. Alternatively, a standard score (*z*-score) can be computed which is the signed number of standard deviations a transcript is above the mean; here, a positive *z*-score indicates an upregulation, while a negative *z*-score indicates a downregulation of a transcript. Often, a *z*-score threshold of -1.96 and $+1.96$ is used to assess a statistical significance of a transcript, corresponding to a *P*-value of 0.05 and a 95% confidence interval.

Transcriptome-wide association studies

In relation to a genome-wide association study (GWAS), assessing the association between genetic variation and trait or phenotype, a transcriptome-wide association study (TWAS) investigates the association between gene expression and trait or phenotype. A recent TWAS leveraged imputed gene expression to identify significant expression-trait associations. For imputing gene expression, an integrative approach was suggested that combines gene expression measurements with summary association statistics from large-scale GWAS to identify genes whose cis-regulated expression is associated with complex traits. For examples, this approach was applied to gene expression data from blood and adipose tissue measured in ~3000 individuals, revealing 69 new genes significantly associated with obesity-related traits (BMI, lipids, and height) (Gusev et al., 2016).

Expression quantitative trait loci

Genomic loci that contribute to variation in gene expression levels are represented by expression quantitative trait loci (eQTLs). Studies could show that cis-eQTLs, that is, those that map to the nearby location of their corresponding gene, outnumber trans eQTLs, that is, those that map far from the location of their corresponding gene (Dixon et al., 2007; Goring et al., 2007). While *cis*-eQTLs directly influence gene expression, trans-eQTLs are not directly involved in regulating gene expression.

eQTLs are identified by studying a population of genetically different individuals. To detect those variants [single nucleotide polymorphisms (SNPs)] that influence gene expression, the genotype and transcriptome for each individual has to be collected and quantified. Next, eQTLs are identified by comparing the genotypes with the expression levels using association or linkage analysis. Subsequently, statistical analyses determine whether a given variant has an effect on the expression of a given gene: those genes that have significantly higher expression levels for one allele than for the other alleles influence the expression of the corresponding gene (Albert and Kruglyak, 2015).

A recent study performed a genome-wide eQTL analysis in a European population, revealing three regions of the genome reproducibly associated with specific patterns of gene expression. Two of these regions overlap genetic variants previously known to be involved in the susceptibility to type 1 diabetes, celiac disease, and hypertension. Genes whose expression is modulated by these genetic variants may act as mediators in the causal relationship linking the variability of the genome to complex disease. These findings illustrate how integration of genetic and transcriptomic data at an epidemiological scale can help decipher the genetic basis of complex diseases (Rotival et al., 2011).

Meta-analysis for combining multiple gene expression data sets

Sometimes there is a need to systematically review or assess multiple gene expression studies, whether it is to increase statistical power or to generalize conclusions based on studies from different origin. A meta-analysis is a statistical study that systematically assesses and combines multiple studies quantitatively by statistically pooling estimates from individual studies into one meta-analytical estimate, while its outcome can include a more precise estimate of the effect under study that the individual studies would reveal (Haidich, 2010). As a precondition of a meta-analysis and to ensure a harmonization of the heterogeneity of the data and phenotypes, the individual gene expression data sets to be pooled should be harmonized in terms of gene identifiers, gene expression values (e.g., fold change, log fold change, odds ratio, risk ratio), and—if applicable—applying consistent preprocessing and normalization, or mapping and assembling methods. Recently, a tool called ARepA has been developed for standardized acquisition and analysis of high-throughput and heterogeneous biological data, facilitating the retrieval and standardization of microarray gene expression data from different sources (Börnigen et al., 2015). The Expression Atlas represents a valuable integrated database of gene and protein expressions in different organisms, providing information on gene expression patterns under different biological conditions (e.g., knock-out), and including microarray and RNA-Seq data. In a large meta-analysis, all underlying expression data sets of the Expression Atlas were reanalyzed to assure consistency and, subsequently, to detect promising expression patterns under the condition of the underlying experimental setup (Kapushesky et al., 2012).

After harmonizing the individual gene expression data sets, the effect sizes of the individual studies are pooled into a weighted meta-analytical effect size. Here, the concept of weighting reflects the value of the evidence of the individual study (Haidich, 2010). There are different statistical models to pool the effects of the studies, the most prominent being an unsupervised data integration

model (Börnigen et al., 2015), supervised data integration model [e.g., Bayesian model (Huttenhower et al., 2009; Börnigen et al., 2016)], fixed-effects model (Schmidt et al., 2009), or random-effects model (Schmidt et al., 2009; Börnigen et al., 2015). An unsupervised data integration model can be applied for homogeneous data sets without training data, while a supervised model (e.g., Bayesian model) requires prior knowledge for training. Additionally, a random-effects model allows heterogeneity of the individual study results, while a fixed-effects model requires homogeneity across all individual studies (Haidich, 2010).

A recent meta-analysis incorporated three population-based cohorts from different tissues to identify novel associations between cardiovascular risk factors and the transcriptome (Schurmann et al., 2012). Detailed statistical analyses revealed technical factors substantially influencing signal intensity values, especially the Illumina chip assignment, the RNA amplification batch, the RNA isolation batch, the sample storage time, and RNA integrity number (RIN), while biological factors effecting signal intensity values included the tissue, gender, and age. To overcome these technical and biological effects, a harmonized workflow was established to ensure consistent and harmonized sample processing in each cohort, including sample storage, RNA isolation, RNA amplification, hybridization, scanning, and data preprocessing. Additionally, appropriate covariate adjustment, that is, for technical covariates was implemented to reduce the unexplained variance.

Longitudinal expression analysis

Measuring and analyzing gene expression at different time points (longitudinal gene expression) is a well-established approach to study the effects of drug treatment or disease progression on gene expression. As longitudinal expression analyses require a direct comparison or integration of measurements from different time points, similar challenges apply to what was observed during meta-analyses (see above). A recent study investigated such batch effects occurring in longitudinal gene expression, in particular the time at which the RNA was collected, assay and scanning differences if applicable, and storage time (Müller et al., 2016). To minimize the influence of such batch effects, a harmonized workflow was suggested, involving a harmonized protocol for sample collection, handling, and storage, RNA isolation and processing, hybridization, and scanning. This workflow was applied at all time points in the same way to reveal harmonized measurements. Again, this procedure is concordant with a meta-analysis (Schurmann et al., 2012).

Application of Transcriptomics in CVDs: Examples

Due to technical and methodological advances, in particular NGS methods, gene expression analyses have been widely established in the field of CVD to identify novel disease-related genes, genes responding to treatment, transcripts as diagnostic markers of gene expression signatures, and to study molecular biomarkers aiming at improving our understanding of the molecular mechanisms of CVD, facilitating the development of personalized medicine in CVD, and reducing the mortality rates in CVD (Wu et al., 2014). Biomarkers play an important role in CVD, serving either as diagnostic indicators, or as predictors of prognosis. In CVD, most of the biomarkers are proteins, while protein coding RNAs and ncRNAs (e.g., miRNAs) in blood cells might also be useful cardiovascular biomarkers in the future (Devaux, 2016). Consequently, the study of the transcriptome to identify novel biomarkers in CVD is an emerging field. Examples of studies using transcriptomics analyses for biomarker identification as presented in **Table 6**.

Large Scale Transcriptomics Analysis

A meta-analysis of large-scale transcriptomics data sets

The MetaXpress (Meta-Analysis of Gene Expression) Consortium published a recent meta-analysis investigating the influence of body-mass-index (BMI) as a cardiovascular risk factor on gene expression patterns in whole blood (Homuth et al., 2015). Here, the authors analyzed microarray gene expression profiles from almost 2000 individuals in two large independent population-based cohort studies, namely the Study of Health in Pomerania (SHIP-TREND) (Volzke et al., 2011) and the Cooperative Health Research in the Region of Augsburg (KORA F4) (Holle et al., 2005). Whole-blood mRNA levels were measured on Illumina HumanHT-12 v3 BeadChips and scanned using the Illumina Bead Array Reader, while preprocessing and normalization included quantile normalization and log transformation. The subsequent meta-analysis of gene expression included linear regression models to assess the association between BMI with gene expression patterns in both cohorts individually before combined. To combine the gene expression data from both cohorts, the authors computed z-scores based on the sample sizes of the cohorts. This meta-analysis revealed in a set of 3762 genes significantly associated with BMI after adjusting for multiple testing. Subsequent analyses identified extensive BMI-associated alterations of the whole-blood transcriptome, three gene expression signatures associated with increased BMI, as well as a BMI-associated genes involved in insulin signaling and protection against oxidative stress. These results demonstrate the power of meta-analyses in gaining novel insight into the pathomechanisms of metabolic syndrome and type 2 diabetes.

Identification of gene expression signatures by transcriptomics analysis

Grouping several mRNAs into a combined set can reflect a broader picture of the pathophysiological mechanisms and pathways, and thus for the understanding of disease pathobiology, and might be useful for prioritizing novel therapeutic targets for treating the disease. Consequently, the combination of different mRNAs to a gene expression signature might prove to be a powerful biomarker for CVD. To date, a number of studies have been published examining whole blood gene expression profiling to identify subjects at risk for CVD.

Table 6 Examples of biomarkers discovered by transcriptomics

Biomarker	*Cardiovascular phenotype*	*Reference*
Genes		
ST2	MI	Weinberg et al. (2002)
GDF-15	MI	Kempf et al. (2011)
RNASE1, FMN1, JDP2	HF	Maciejak et al. (2015)
IL6	MI	Anderson et al. (2013)
IL18	Atherosclerotic plaque	Mallat et al. (2001)
	HF	Mallat et al. (2004)
	Peripheral artery disease (PAD)	Deser et al. (2016)
FOX	HF	Hannenhalli et al. (2006)
miRNAs		
miR-499, miR-133a, miR-126	Coronary artery disease (CAD)	De Rosa et al. (2011)
miR-140-3p, miR-182, miR-92a, miR-92b	CAD	Taurino et al. (2010)
miR-191-3p, miR-455-3p, miR-1281	Abdominal aortic aneurysm (AAA)	Zhang et al. (2015b)
let-7e, miR-15a, miR-196b, miR-411	Abdominal aortic aneurysm (AAA)	Stather et al. (2015)
miR-126, miR-223, miR-197	CHD	Zampetaki et al. (2012)
miR-1, miR-122, miR-133, miR-208a/b, miR-375, miR-499	AMI	Cheng and Zhang (2010), D'Alessandra et al. (2010), Gidlof et al. (2011), Kuwabara et al. (2011), Creemers et al. (2012)
miR-21, mir-29a, miR-208a	Post-MI	Zile et al. (2011)
miR-1, miR-133a, miR-499, miR-208	CAD	De Rosa et al. (2011); Widera et al. (2011)
lncRNAs		
LIPCAR	HF	Archer et al. (2015)
CoroMarker	CAD	Yang et al. (2015)
LncPPARδδ	CAD	Cai et al. (2016)

Transcriptomics has been an emerging field for identifying novel biomarkers in CVD. Such studies revealed novel genes, miRNAs, and lncRNAs as novel biomarkers in different cardiovascular phenotypes.

To study the cardiovascular risk factors blood pressure and hypertension, a recent meta-analysis of genome-wide gene expression profiles was performed using six independent cohort studies (Huan et al., 2015). First, differentially expressed genes in blood pressure were determined, which were subsequently replicated. Next, a meta-analysis was performed, identifying 34 differentially expressed genes associated with blood pressure traits. Such genes defined the gene expression signature for blood pressure, and were attributable for 5%–9% of blood pressure variability. Subsequently, these signature genes were further integrated with eQTLs and GWAS results in an effort to differentiate downstream transcriptomic changes due to blood pressure from putatively causal pathways involved in blood pressure regulation.

Another recent study investigated whether gene expression patterns in peripheral blood correlate with the severity of coronary artery disease and whether these patterns correlate with the extent of atherosclerosis in the vascular wall (Sinnaeve et al., 2009). Here, blood from 120 patients with CAD was selected, revealing a gene expression signature 160 genes to be correlated with the severity of CAD. Molecular pathways covered by these 160 genes included angiogenesis, inflammatory response, apoptosis, cell adhesion, cell growth, arrest and cell-cell communication, lipid homeostasis as well as immune response. This study showed that a large-scale expression analysis of peripheral blood cells can identify gene expression patterns or signatures whose expression correlates with the severity of angiographically documented coronary artery atherosclerosis.

In the Personalized Risk Evaluation and Diagnosis in the Coronary Tree (PREDICT) study, a whole blood 23 gene expression score was developed and validated for the assessment of obstructive CAD in nondiabetic patients (Elashoff et al., 2011). This score was further evaluated in the multicenter COMPASS study with regard to the diagnostic accuracy in symptomatic patients referred for myocardial perfusion (Thomas et al., 2013). This gene expression score was a significant predictor of CAD and resulted, at a predefined threshold, in a high sensitivity and high negative predictive value making this score an extremely promising and one of the best examples of the value of transcriptomics-based biomarkers in the cardiovascular field today.

An integrative network analysis of transcriptomics data reveals deeper insights into the molecular mechanisms

Along with large-scale meta-analyses, integrative approaches play an emerging role. In general, integrative approaches involve different types of experimental data (e.g., transcriptomics, genomics, and interactomics) that will be first harmonized (i.e., standardization of gene identifier and data format), and subsequently, computationally integrated into one data set. This integrated data set then includes the combination of all original information from the experimental data sources.

A recent study integrated blood pressure GWAS with mRNA expression profiles as a coexpression network and applied network biology approaches, revealing transcriptomic signature for blood pressure at the single-gene and coexpression network module (Ding et al., 2015). This study identified gene subnetworks entailing molecular interactions between key drivers and genes related to blood pressure. Consequently, one of the top driver, *SH2B3*, was validated in a mouse model, revealing a significant number of genes predicted to be regulated by *SH2B3* in gene networks were perturbed in $Sh2b3^{-/-}$ mice, demonstrating an exaggerated pressor response to angiotensin II infusion. This finding may help to identify novel targets for the prevention or treatment of hypertension.

A large-scale eQTL analysis reveals susceptibility to CVD

An eQTL analysis associates gene expression measurements with genetic variants. A study investigating the monocyte transcriptome as a potent integrator of genetic and nongenetic influences of relevance for disease pathophysiology and risk assessment performed an eQTL analysis for CVD risk factors in a population-based cohort study (Zeller et al., 2010). The results identified expression traits significantly associated with at least one risk factor, and genome-wide interaction analyses suggested that genetic variability and risk factors mostly acted additively on gene expression.

From the Transcriptome to Biomarkers

ST2 in myocardial infarction: From discovery toward an early marker of CVD

After being discovered as an interleukin-1 receptor family member (Kakkar and Lee, 2008; Ciccone et al., 2013), the ST2 mRNA was identified as being exceptionally upregulated in cardiac myocytes subjected to mechanical strain (4.7 ± 0.9-fold) (Weinberg et al., 2002). ST2 was identified as the major inducible transcript by mechanical strain. Additionally, ST2 was induced by interleukin-1β, while additive and synergistic effects of strain and strain plus interleukin-1β were not observed, suggesting common pathways for induction of ST2 by stimuli. Additional experiments showed that neither treatment with angiotensin II, nor Interleukin-4, nor lipopolysaccharides induced the biomechanical induction of ST2, suggesting that oxidant signaling pathways do not mediate induction of ST2, and that the induction of ST2 is not indiscriminate for multiple pathways. An in vivo experimental setting in mice showed significantly increased serum levels of ST2 after MI compared with sham operated mice, revealing that ST2 is induced after myocardial infarction (MI). Translating these results into human, ST2 protein levels were measured in serum, resulting in increased levels in MI patients, and suggesting an association between the extent of myocardial injury or biomechanical load and ST2 in the circulation (Weinberg et al., 2002).

From the Transcriptome to Molecular Diagnostic Tools

Novel myocardial gene expression signatures of heart failure by RNA-Seq

To identify gene signatures for heart failure (HF) RNA-Seq was applied in resolution case control study. The transcriptomes of the left ventricle tissue were sequenced, and the sequenced short reads were aligned to the hg19 reference genome using Tophat (Trapnell et al., 2009), and log ratios for all transcripts were computed to assess their differential expression using Cufflinks (Roberts et al., 2011). Such obtained differentially expressed genes were used to classify heart failure status in a set of 313 individuals using Affymetrix Human Exon ST1.1 arrays, which were subsequently preprocessed and normalized using ComBat (Johnson et al., 2007). The results of this study showed that using the gene signatures identified from this small RNA-Seq data set enabled to classify individuals with failing or nonfailing hearts, and the misclassification rates of individuals with different heart failure status were one to three times lower than those obtained from microarray data. These results are likely due to the highly accurate gene expression measurements obtained from RNA-Seq and careful selection of feature vectors in classification (Liu et al., 2015).

The Noncoding Transcriptome

Noncoding transcriptome analyses in CVD: miRNAs

In most noncoding transcriptome analyses in CVD, miRNAs have been investigated, as they are stable, easily detectable and because of their influence on of a wide variety of target genes (Lee et al., 2007). In most cases, miRNAs have been analyzed as noninvasive biomarkers (singly or as signature) in CVD (Meder et al., 2008; Keller et al., 2011).

As an examples, a genome-wide study reported alterations of all of the expressed miRNAs analyzed in the peripheral blood of AMI (acute myocardial infarction) patients (Meder et al., 2011), revealing a set of 121 miRNAs to be significantly dysregulated. As these results were solely based on single point measurements and blood samples, obtained at different times after the onset of symptoms, the authors performed a proof-of-concept study to investigate the kinetic changes of the whole blood miRNome in the early stage of suspected myocardial infarction at five predefined time points using microfluidic primer extension arrays and to replicate the results in an independent cohort using qPCR (Vogel et al., 2013). Here, they identified a signature of seven consistently dysregulated miRNAs as an early biomarker of AMI, including miR-566, -1291, -7-1*, -1254, -455-3p, -380*, and -636. Additionally, two novel biomarkers (miR-1915 and miR-181c*) were identified to be associated with early myocardial infarction.

lncRNAs and circular RNAs

In the last years, the knowledge of lncRNA has expanded widely. However, the cellular origin of circulating lncRNAs is often unclear (Skroblin and Mayr, 2014) and little knowledge on the causal involvement in the underlying disease is currently available.

A recent transcriptomics analysis of heart failure patients showed the potential of lncRNAs as biomarkers in CAD (Archer et al., 2015). Higher levels of LIPCAR (long intergenic noncoding RNA predicting cardiac remodeling) were associated with a higher risk of cardiovascular mortality in HF patients in addition to traditional risk factors. The lncRNA CARMEN (Ounzain et al., 2015) was identified by a genome wide profiling of the cardiac transcriptome and was found to be a crucial regulator of cardiac cell differentiation and homeostasis. Most recently, lncRNA CoroMarker (Yang et al., 2015) and LncPPARδδ (Cai et al., 2016) were identified as predictive biomarkers for CAD.

Even though the existence of circRNAs has been known for some time, only recently this class of ncRNAs gained interest. Due to their circular state these RNA molecules are highly stable recent study revealed over 9000 candidate circRNAs detected in heart tissue (Werfel et al., 2016) and further studies are ongoing to dissect the role and potential of circular RNAs in CVD.

Summary and Future Direction

Due to technological and methodological advances, large-scale transcriptomics analyses can be performed to gain deeper insight into underlying molecular mechanisms, but also to identify novel disease genes or cardiovascular biomarkers to serve as risk factors, diagnostic indicators, or predictors of prognosis. An exemplary workflow is shown in **Fig. 3**.

In the future, single-cell transcriptomics based on single-cell RNA-Seq (scRNA-Seq) technology may play a promising role in understanding diseases and associated biological processes at a high resolution. It is designed to investigate expression profiles at the cell level and, thus, increases the discovery power as compared to the population-averaged RNA-Seq technology (Khera and Mehta, 2014; Poirion et al., 2016). Despite the large amount of bioinformatical approaches that have been developed throughout the years, there is still a need for novel methods as the amount and complexity of high-throughput and heterogeneous data sets and types are increasing. Additionally, novel approaches will be needed to improve the translation into the clinical application.

Systems medicine, a novel field using interdisciplinary approaches, emerged as a powerful tool to study complex and multifactorial diseases, including CVD, and in particular risk assessment, prevention, and treatment as well as biomarker and drug development need to be improved in the future. Due to the interdisciplinary nature, many challenges have to be faced, such as establishing interdisciplinary and an appropriate infrastructure (wet lab, dry lab, study center), setting up large size study cohorts, processing samples and phenotypes, analyzing the data, and translation into the clinical application (Haase et al., 2016; **Fig. 4**).

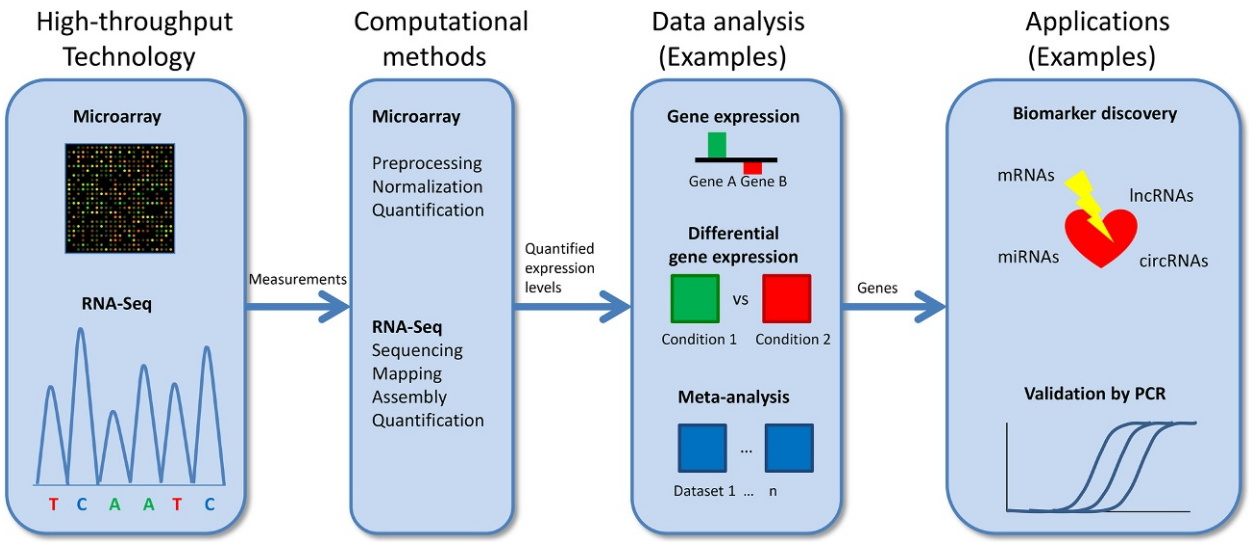

Fig. 3 Workflow of an exemplary transcriptomics analysis including detection and computational technology. High-throughput technology (microarrays and RNA-Seq) detects and measures gene expression, which is subsequently processed by technology-specific computational approaches, resulting in quantified gene expression levels. These expression levels can be analyzed in different manners, for example, a simple gene expression analysis, a differential gene expression analysis, or a multi-experiment meta-analysis. Finally, such retrieved genes can be subsequently validated by PCR and applied as novel biomarkers, for example.

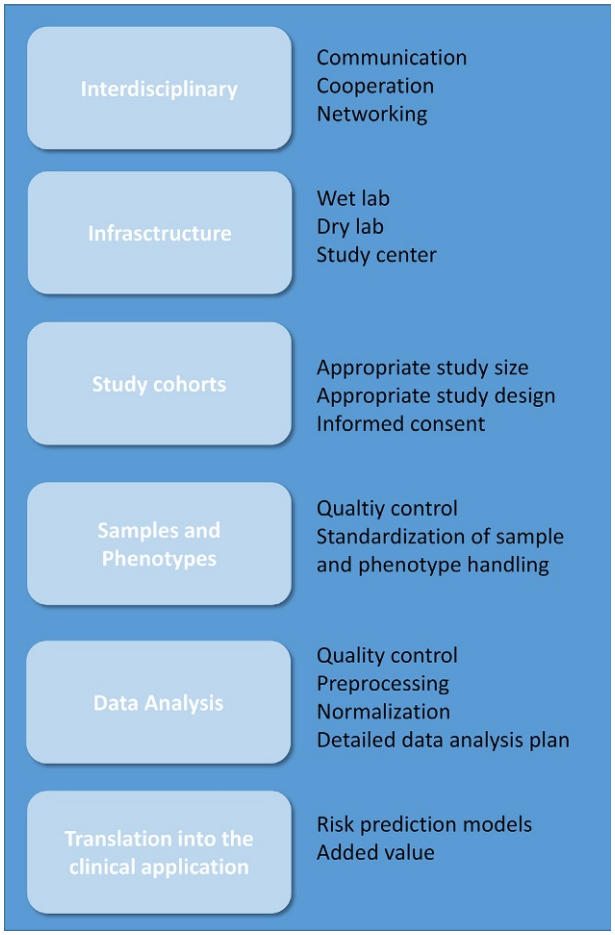

Fig. 4 Challenges for systems medicine. The major challenges that can be observed in the systems medicine field include working in interdisciplinary teams, installing appropriate infrastructure, initiating study cohorts, performing quality control of samples, phenotypes, and data, appropriate handling of sample and phenotype, performing data analysis, and subsequently, translating novel findings into clinical applications.

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Transcriptome and Epigenome Applications for Coronary Heart Disease Research

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Introduction

Having been the leading cause of death for many decades (CDC, 2009), coronary heart disease (CHD) has been one of the focus areas of research efforts. However, CHD is a complex disease involving the interplay of multiple genetic and environmental risk factors (Schwartz et al., 2012) and thereby dissecting such complexities has been a formidable challenge. On top of such challenge also lies the difference of CHD risks between sexes (Jousilahti et al., 1999) or among ethnicities (Finkelstein et al., 2004), along with the associated behavioral difference (Ram and Trivedi, 2012), which blurs the line between genetic and environmental factors.

Studies on risk factors to CHD have grown from the traditional set, such as hypertension, smoking status, age, and diabetes mellitus (O'Donnell and Elosua, 2008), to a more sophisticated set, such as C-reactive protein, coronary artery calcium (CAC) score, and lipoprotein (a) (Akhabue et al., 2014), in the hope for early prognosis and to ultimately reduce the public health burden of CHD. On the other front, numerous genome-wide association studies (GWAS) have been performed to discover novel genetic variants that may predispose individuals to CHD (O'Donnell and Nabel, 2011). Recent advances in such studies include multiple discoveries of rare variants that elevate risks to CHD (Zanoni et al., 2016). Nonetheless, none of such studies addresses the complex interplay between genetic and environmental factors, which may explain the disease pathophysiology at molecular level.

Advances in transcriptomic and epigenomic technologies have enabled the whole-genome profiling beyond genetics. These advances present the opportunity to examine the impact of gene regulation in relation to CHD, which has the potential to dissect the interaction of genetic factors, disease activity, metabolism, and environmental factors (Aziz et al., 2007; Huan et al., 2012). This article highlights several studies in these areas. The goal is to help the readers understand how each technology has been used in cardiovascular disease research in synthesizing evidence for research and clinical applications.

Brief Overvies of CHD GWAS

Technological advances of high-throughput DNA microarray technology has opened many opportunities in investigating the genetic predisposition of various diseases, including CHD. In general, the association was carried out using variations of regression model, one genetic variant, or single nucleotide polymorphism (SNP), at a time. As the microarray technology advances, more and more SNPs can be fit into one microarray, and thus bring concern about controlling the Type I errors. Several attempts have been performed, but variants declared significant typically at $p < 5 \times 10^{-8}$ Bonferroni threshold. This threshold was taken as the standard because it was postulated that there are about 1 million haplotype blocks exist in human genome. Such p-value threshold is close to the result of a simulation study (Dudbridge and Gusnanto, 2008).

Among the first genetic locus found significantly associated with CHD using this technology is the 9p21.3 locus (McPherson et al., 2007; Helgadottir et al., 2007; Samani et al., 2007). As genotyping technologies became cheaper and more accessible, many more studies were able to genotype more participants and therefore increasing the number of significantly associated SNPs. Some landmark studies include the Framingham 100 K study (Larson et al., 2007), the Myocardial Infarction Genetic Consortium (Myocardial Infarction Genetics Consortium et al., 2009), the CARDIoGRAM Consortium (Schunkert et al., 2011), the Coronary Artery Disease (C4D) Genetics Consortium (Coronary Artery Disease Genetics consortium, 2011), CARDIoGRAMPlusC4D Consortium (CARDIoGRAM Consortium et al., 2013), and the 1000 Genomes-based (1000 Genomes Consortium et al., 2012) Coronary Artery Disease (Nikpay et al., 2015). The last of these studies encompasses over 60,000 cases and 123,000 controls, investigating over 9.5 million variants (Nikpay et al., 2015). Such massive undertaking validated 55 previously known loci and discovered 10 new ones. Among the findings of the 55 known loci reside in lipid genes, such as *PCSK9*, *LIPA*, *LPL*, *LDLR*, *APOB*, and

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ABCG5, or in inflammation genes, such as *IL6R* and *CXCL12*. Among the novel loci are in genes with unknown effect on CHD, except genes such as nitric oxide synthase 3 (*NOS3*).

Such studies have been on populations of European ancestry, which have different haplotype structure from populations of other ethnicities (Lonjou et al., 2003). Thus, studies in non-European population may lead to the discovery of novel CHD loci. For example, CHD association study done on a multiethnic cohort has failed to replicate many of the findings in European ancestry (Franceschini et al., 2011). Another study in cohorts of African ancestry also failed to replicate such CHD findings, but do replicate findings from European-ancestry cohorts for CHD risk factors (Lettre et al., 2011). On the other hand, many of such findings do replicate in East Asian (Marigorta and Navarro, 2013) and South Asian Consortium (CARDIoGRAM Consortium et al., 2013) populations.

The subsequent technology, the whole genome sequencing (WGS), has afforded much higher resolution than that of microarray and the examination of rarer variants (Hurd and Nelson, 2009). Such technology is presently very costly, thus hampering the adoption. Nonetheless, there are some pilot studies (Chambers et al., 2012), but these studies have been underpowered due to the low number of samples. The upcoming Trans-Omics for Precision Medicine (TOPMed) of the National Heart, Lung, and Blood Institute (NHLBI) will bring the total number of WGS samples to over 120,000 (https://www.nhlbiwgs.org/). Such precision medicine initiative will realize the advantages of the WGS technology.

Transcriptome

RNA is the product of DNA transcription with various biological roles. The level of RNA indicates the level of expression of the underlying gene, giving rise to the term "gene expression." RNA generally functions as a messenger between DNA and protein. The regulation of RNA, and thereby its expression levels, is known to be tissue-specific (Ong and Corces, 2011). There are multiple types of RNA, with the most prominently discussed being the messenger RNA (mRNA). Prior to its translation to protein, mRNA may be spliced, edited, excised, transported, or even degraded. These processes may be dynamically influenced by environmental factors (Pleiss et al., 2007; Whangbo and Hunter, 2008). Due to this nature, RNA may capture some of the interaction between genetic and environmental factors that contribute to diseases.

Early RNA profiling was performed using polymerase chain reaction (PCR)-based technologies, which allows examination of a few genes. Such genes were usually selected a priori based on evidence in literature. Hence, studies using this technology are confirmatory in nature. It was successfully used in several experiments (Nickenig et al., 1998; Dieterich et al., 2000). Using this technology, Nickenig et al. validated the role of estrogen on AT_1 receptor (now gene *AGTR1*, angiotensin II receptor type 1), which has been implicated in the pathogenesis of hypertension and atherosclerosis (Nickenig et al., 1998). Dieterich et al. validated the role of genes MnSOD (now *SOD2*, superoxide dismutase 2), CuZnSOD (now *SOD1*, superoxide dismutase 1), and GPX (now *GPX3*, glutathione peroxidase 3) on oxidative stress that eventually result in cardiomyopathy (Dieterich et al., 2000).

Subsequent development uses custom-built microarray-based technologies, which allows for investigation and more precise quantification of thousands of genes. Such technology involves labor-intensive library preparation and microarray building. Yet it was successfully used to query "global" gene expression at the time. Barrans et al. (2002) were able to query 10,848 transcripts in human genome across 12 samples and identified several genes that were differentially expressed, such as atrial natriuretic peptide (now gene *NPPA*, natriuretic peptide A) and TGF-Beta (now gene *TGFB1*, transforming growth factor beta 1). Another study by van den Bosch et al. (2006) queried up to 6881 gene transcripts in mice in relation to cardiac hypertrophy and found gene Anf (homolog to gene *NPPA* in human), Myhc-b (now Myh7, homolog to *MYH7*, myosin heavy chain 7 in human), Pln (homolog to *PLN*, phospholamban in human), among others that are differentially expressed. The number of significantly differentially expressed genes increases proportional to the number of queried genes. Thus, studies also group such genes into "pathways" or "processes" to explain the roles of such genes. For example, van den Bosch et al. (2006) identified acetyl-CoA metabolism, fatty acid metabolisms, and other processes represented by the significant genes they have found.

These early technologies also had several challenges. They are labor intensive and costly, limiting the number of samples to assay. There was lack of standardized protocol, standardized quality control, or standardized gene name or location (Brazma et al., 2001; Pruitt et al., 2002). In addition, as the number of queried genes increases and statistical methods at the time being underdeveloped to cope with such technological development, the number of false positives or false negatives could be high. Nonetheless, there was some coherence in the results, such as gene *NPPA*.

The next development of the technology was the prefabricated microarray technology, which allows the examination of whole genome transcriptome with better precision. Combined with better standardization of protocols (Shi et al., 2006), quality controls (Shi et al., 2010), gene name and location (Pruitt et al., 2005, 2011, 2014), and much reduced labor requirement (Auburn et al., 2005), this technology brought down the cost significantly. This allowed the assay of many more samples, increasing the power of detection of significantly differentially expressed genes. Statistical methods also progressed (Benjamini and Hochberg, 1995; Storey and Tibshirani, 2003; Johnson et al., 2007; Leek and Storey, 2007) to reduce the number of false positives or false negatives.

A flurry of studies took advantage of this newer technology. Taurino et al. (2010) used the Illumina Sentrix humanref-6 to assay 24 samples in relation to coronary artery disease (CAD) and found 365 differentially expressed genes. In addition, Taurino et al. used quantitative real-time PCR (qRT-PCR) technology (Freeman et al., 1999), an improvement of the PCR technology, to assay

microRNAs (Bartel, 2004), a short noncoding RNA (ncRNA) of 16 to 26 nucleotides, in relation to CAD. Sinnaeve et al. (2009) used the Affymetrix microarray HGU133A technology to assay 241 samples in relation to CAD and found 160 differentially expressed genes, which in aggregate yield 0.776 partial least square. Szmit et al. (2010) used ScanArray technology to assay 12 patients in relation to heart failure and found 275 differentially expressed genes. Wingrove et al. (2008) used Agilent 41 K technology to assay 41 samples in relation to CAD and found 526 differentially expressed genes. There are many more studies using such technology in relation to CAD (Calverley et al., 2010; Rosenberg et al., 2010; Healy et al., 2006; Kiliszek et al., 2012). Lin et al. (2014) associated gene *PBX1*, among others, with atrial fibrillation on 2126 participants of Framingham Heart Study. It is worth noting that despite the increasing number of significantly differentially expressed genes, very few number of genes are in common across multiple studies. Nonetheless, these results all seem to point to processes commonly associated with CAD, such as oxidative stress, apoptosis, immune response, and inflammatory response.

The lack of replicability across studies highlights several important points: the necessity of better experiment design, heterogeneity of disease definition (Joehanes et al., 2013), variability across different expression platforms, tissue specificity of gene expression (Joehanes et al., 2012), and the importance of quality control procedures. Joehanes et al. (2013) to some degree resolved some of these issues but were unable to completely overcome the lack of replicability across study results. Chen and Stewart recently summarized replicable results (Chen and Stewart, 2016) across gene expression studies. Nonetheless, the pathways pointed by disparate sets of genes seem to be in common, as noted earlier.

Another avenue afforded by the new technology is to examine the coexpression patterns across genes in relation to CAD. Huan et al. (2012) investigated such patterns and confirmed the multiple pathways involved in CAD. Huan et al. also integrated findings from GWASes to reveal "key drivers," genes that are coexpressed with many other genes of CAD. These key drivers include *TNFRSF13C*, *SPIB*, *TSPAN13*, *CD79B*, *PLEKHG1*, and *CELSR1*, some of which were also identified in previous study (Joehanes et al., 2013).

Several studies also combine multiple high-throughput datasets to paint a more coherent picture of CAD pathophysiology. In addition to the study of Huan et al. (2012), several other studies integrate genetic findings with gene expression findings (Zhang et al., 2014a; Yao et al., 2015; Huan et al., 2015a) in relation to CAD. Such studies highlight two important points. First, genetic variance associated with CAD may manifest in gene expression changes. Second, there are similarities of differential expression patterns across several conventional CAD risk factors, such as low density lipoprotein, blood pressure, diabetes mellitus, and smoking.

Integration of multiple high-throughput evidence has the potential to point to the pathophysiology or etiology of CAD or its risk factors. In fact, the support of multiple human genetic evidence significantly improves the rate of clinical trial success of a drug targeted to a certain gene (Nelson et al., 2015). A study of Sen et al. (2014). integrated evidence from DNA, RNA, and protein that implicates gene *TREML4* in CAC. Burkhardt et al. (2015) and Yao et al. (2015) highlighted apparent pleiotropic genetic variants across multiple CAD risk factors and found some concordance in gene expression. Smith et al. (2016) used gene expression and DNA methylation evidence to follow up findings of GWAS and implicated single nucleotide polymorphism (SNP) rs9885413 to heart failure. The SNP variation eventually leads to lower expression of a chemokine gene *TSLP*, which is a key gene in inflammation response (Isaksen et al., 1999; Ziegler et al., 2013). Joehanes et al. (2017) were able to follow up GWAS results from CARDIoGRAMplusC4D consortium (CARDIoGRAMplusC4D Consortium et al., 2013) using expression quantitative trait locus dataset and identified potential causal variants to those results.

The primary limitation of probe-based microarray technology is the inability to detect novel isoforms of transcripts. Isoforms of known genes are sometimes vital to the pathophysiology of heart disease. For example, δ isoform of gene *CAMKII* (now *CAMK2B*, calcium/calmodulin dependent protein kinase 2B) has been implicated in failure of human myocardium (Hoch et al., 1999). Another classic example is the isoform of α_1 subunit of the voltage-dependent calcium channel (now gene *CACNA1C*) (Slish et al., 1989). There are some microarray technologies that partially overcome such limitation by incorporating known or putative isoforms in the array, such as Affymetrix Human Exon Array ST 1.0. Such platform has been used to detect putative isoform in relation to myocardial infarction (MI) in gene *PTGS1*, which may be modulated by genetic variance (Zhang et al., 2015a). Using the same platform, Joehanes et al. (2013) also detected potential alternative splicing of genes *GUK1* and *TMEM56* that may be associated with MI. There is also a growing evidence that alternative isoforms may play an important role in CAD etiology (Park et al., 2011).

The second important limitation of probe-based microarray technology is the relative inability to detect short transcripts. Microarray probes are usually 25 nucleotides or longer and rely on a few nucleotides of mismatch in the transcript sequence for their measurement precision (Draghici et al., 2006). However, multiple studies show the increasing importance of such short transcripts in the regulation of genome organization and gene expression (Morris and Mattick, 2014). They also have been implicated in cardiovascular development and diseases (Ono et al., 2011). Some microarray technologies (Liu et al., 2004; Okoniewski and Miller, 2008) also incorporated assays of short transcripts. However, for measuring the expression of such transcripts, microarray platform generally suffers from lower intensity, limiting its usefulness on less commonly expressed transcripts (Git et al., 2010). During this time, for assaying short transcripts, ncRNA, which includes a broad range of short molecules such as microRNA, piwi-interacting RNA, or short-interfering RNA, qRT-PCR was a technology of choice. Huan et al. implicated 15 microRNA in relation to CAD (Huan et al., 2015a). The significantly differentially expressed microRNAs are involved in intracellular transports, cell cycles,

protein localization, and gene silencing. MicroRNAs, such as mir-208a and mir-1, also have the potential for diagnosing acute MI (Wang et al., 2010).

Prefabricated microarray technologies were then superseded by sequence-based RNA profiling, called the RNA-seq technology (Wang et al., 2009), which is the newest to date. In this technology, the RNAs are sequenced directly and then quantified (Conesa et al., 2016). In general, RNA-seq has been shown to be superior to microarray technology, having a much better resolution (Black et al., 2014; Zhao et al., 2014; Zhang et al., 2015b). However, results from microarray are highly correlated with those of RNA-seq for a subset of genes for which microarray has little to no limitation of detecting (Nookaew et al., 2012; Xiong et al., 2012). Currently, RNA-seq technology is costlier than microarray technology and thus current RNA-seq experiments have low sample size. Yet, as the cost of sequencing declines, RNA-seq experiments with higher number of samples are expected to rise (Sboner et al., 2011; Muir et al., 2016).

A study of Liu et al. (2015) identified up to 492 genes differentially expressed in relation to heart failure ($n=6$). The findings were then followed up using a microarray experiment on a larger cohort ($n=313$) and could be used to classify disease status. A bigger case-control study ($n=90$) (Muehlschlegel et al., 2015) identified 1241 genes in relation to myocardial ischemia. None of the featured genes are in common to previous microarray-based results (Huan et al., 2012; Joehanes et al., 2013), however. As in microarray results, these genes point to a common set of processes, such as immune response and B-cell immunity (Huan et al., 2012).

RNA-seq technology has also been exploited to identify short RNA sequences in relation to heart diseases. ncRNA also plays important molecular regulatory roles, such as epigenetic imprinting, guide for transcription factors, and activation of enhancers (Mattick, 2006). Several ncRNAs have been implicated in heart diseases, such as *Novlnc6* (Ounzain et al., 2014) or *Mhrt* (Han et al., 2014). An excellent review of the role of ncRNA in CVD can be found in Uchida and Dimmeler (2015).

Bottom Line: Can Transcriptomic Biomarkers Be Used for Clinical Applications?

The lack of a common set of significantly differentially expressed genes in current findings highlights the need to overcome many challenges, such as appropriate experimental design, heterogeneity of disease definition, and, most importantly, sample size. On the other side, the common set of pathways pointed by the disparate sets of results mean that results of gene expression, regardless of the underlying technology, are in some way coherent. However, due to the lack of proper longitudinal study design, it is indeed not clear whether gene expression captures signals that may be causal to heart disease or signals that are consequence of heart disease.

Transcriptome-based prognostic biomarkers may still be achievable by incorporating biomarkers from CAD risk factors, such as diabetes (Patti, 2004), blood pressure (Huan et al., 2015b), cigarette smoking (Huan et al., 2016), age (Peters et al., 2015), or body mass index (BMI) (Mendelson et al., 2017). Indeed, transcripts of several genes are associated with multiple of these risk factors. For example McManus et al. (2017) showed that four genes, *FAM13A*, *CSF2RB*, *HIST1H2AC*, and *WNK1*, are significantly differentially expressed with six cardiometabolic factors, triglyceride, BMI, systolic and diastolic blood pressure, high density lipoprotein, and fasting glucose and four microRNAs, miR-505-5p, miR-197-3p, miR-145-5p, and miR-328, are significantly differentially expressed with the first four factors. These findings were then followed up with the integration of coexpression network to reveal transcriptomic regulatory architecture underlying CHD. Also, in many cases, dose–response relationship between the risk factor and the biomarkers is available (Huan et al., 2016), which may be useful to monitor disease progression even prior to symptoms manifestation. Combining these biomarkers with genetic biomarkers, which may help identify individuals predisposed to the disease, may be the future for clinical application of transcriptome.

Epigenome

Epigenome is a set of modifications to the DNA that are, in some cases, heritable (Jaenisch and Bird, 2003). Epigenetic modifications may affect gene expression (Romanoski et al., 2015; Attwood et al., 2002) by modulating the accessibility of surrounding promoter or enhancer of the gene (Greco and Condorelli, 2015). Such modifications include DNA methylation and histone modification (Allis and Jenuwein, 2016). Epigenetics modification has been documented to reflect the effects of environmental and dietary influences (Feil and Fraga, 2012; Aguilera et al., 2010). For example, air pollution has been documented to affect the methylation of certain genes (Bind et al., 2014). Gut microbiota have also been implicated as epigenetic regulator in the body of the host (Kumar et al., 2014). In addition, epigenetic modifications have also been associated with cardiac development (Gilsbach et al., 2014). Therefore, epigenetics hold an excellent potential to serve as biomarkers to CHD (Ordovás and Smith, 2010; Baccarelli et al., 2010).

DNA methylation (Moore et al., 2013), being the discovered first (Avery et al., 1994), is perhaps the most studied form of epigenome (Weinhold, 2006). It is a process where a methyl group (CH3) is added to the fifth position of cytosine, resulting in 5-methylcytosine. In mammals, such methylation occurs predominantly in cytosine-phosphate-guanine (CpG) sequence

(Jaenisch and Bird, 2003). The most common effect of such methylation is long-term gene expression silencing (Jaenisch and Bird, 2003). DNA methylation is also shown to be tissue specific (Gutierrez-Arcelus et al., 2015).

Early technology for DNA methylation detection is also based on PCR using bisulfite treatment of DNA, which selectively converts cytosine to uracil, but not 5-methylcytosine (Clark et al., 1994). Dong et al. (2002) reviewed studies using such technology and highlighted several genes in relation to atherosclerosis, such as estrogen receptor alpha (*ESR1*) and tumor protein 53 (*TP53*). Kim et al. (1772) showed that epigenetic changes in estrogen receptor beta (*ESR2*) are associated with atherosclerosis. Although initially the technology was gene-specific, a later breakthrough enabled global DNA methylation profiling (Yang et al., 2004), which will be the basis of later technologies.

After PCR-based technology, microarray is used to assay DNA methylation profile. A review by Muka et al. (2016) combined the results of early microarray technology and the PCR technology, and highlighted a few genes in relation to cardiovascular disease, such as *LINE1*, *ABCA1*, *KCNQ1*, and *C1QL4*. Haas et al. (2013) highlighted the role of genes *LY75* and *ADORA2A* in relation to dilated cardiomyopathy. Rask-Andersen et al. (2016) highlighted the methylation of a few genes in relation to MI, such as *IL6R*, *ABCG5-ABCG8*, and *HDAC9*. A preliminary result of a study of ten cohorts spanning 9725 individuals (Agha et al., 2016) identified 16 CpG sites differentially methylated in relation to both MI and incident heart disease (IHD), such as *IGF1R*, *GARNL3*, *ATP6V1H*, *FAM113B*, and *AHRR*. If examined separately, the study found 72 CpG sites significantly associated with MI and 149 CpG sites significantly associated with IHD. In addition, Kim et al. (2010) explored associations of DNA methylation with cardiometabolic risk factors across 286 individuals in an attempt to create a set of biomarkers for CHD.

The current technology to assay the DNA methylation profile is based on next generation sequencing (Masser et al., 2015). There are several techniques associated with such technology, such as whole-genome bisulfite sequencing, MeDIP-seq, Methyl Cap-seq, reduced representation bisulfite sequencing, and SureSelect Methyl-seq (Soto et al., 2016). Some of these sequencing technologies, such as MeDIP-seq and Methyl Cap-seq, overcome the primary limitation of bisulfite sequencing technology: inability to distinguish 5-hydroxymethylcytosine from 5-methylcytosine. The former promotes gene expression, while the latter represses it (Branco et al., 2011). However, as of this writing, no experiments on cardiovascular disease have been performed on the latest technology.

Histone modification is a class of covalent posttranslation modification to histones that may affect chromatin structure, which in turn affect transcription regulation (Bannister and Kouzarides, 2011). Tingare et al. (2013) discusses a role of histone modification in relation to cardiac remodeling and highlighted how the methylation of H3K9 governed cardiac biology. Montgomery et al. (2007) showed that histone deacetylases regulate cardiac morphogenesis, growth, and contractility, which leads to arrhythmias and dilated cardiomyopathy. Histone acetylation and deacetylation affect multiple pathways that lead to cardiac remodeling, such as inflammation, apoptosis, angiogenesis, oxidative stress, and fibrosis, which in turn affect multiple cardiovascular diseases (Wang et al., 2014). Kaneda et al. (2009) reported that tri-methylated histones H3H4 and H3K9 are associated with heart failure. Multiple histone modifications can be a mechanism that explains renal failure connection to cardiomyopathy in type 2 diabetes (Gaikwad et al., 2010). Mahmoud and Poizat summarized multiple epigenetic modifications in relation to cardiomyopathy (Mahmoud and Poizat, 2013).

Bottom Line: Can Epigenomic Biomarkers Be Used for Clinical Applications?

Although there are several epigenomic marks that are in common across several studies, most notably *LINE1*, *ABCA1*, *KCNQ1*, *C1QL4*, and *IGF1R*, more studies are needed (Aslibekyan et al., 2015). As the majority of such replicable signals came from microarray-based technology, at least replication based on a different technology is needed to avoid false positives. As in transcriptome, integration of biomarkers across multiple CHD risk factors is needed. Kim et al. (2010) has a proof of concept that it is possible. However, larger study is needed to bring in more statistical power.

Discussion and Future Direction

Despite the enormous progress in transcriptome and epigenome research on biomarkers for CHD, few biomarkers are replicable across multiple studies. Nevertheless, as indicated earlier, the findings so far seem to point to common set of pathways. Further research is needed to understand the variability in such findings. In addition, better, more precise technology is certainly needed to partially overcome this challenge.

The lack of replicability may be caused by several confounding factors. One of such factors is the heterogeneity of phenotypic definition of heart diseases. Such heterogeneity substantially affects GWAS results (Kitsios et al., 2011) and manifests in gene expression levels (Joehanes et al., 2013). Another contributing factor is the technical variability in transcriptome and epigenome that typically outweighs biological variability (Bryant et al., 2011). Such technical variability exists even in the latest technology (McIntyre et al., 2011; Meyer and Liu, 2014). This underscores the importance of standardized laboratory protocol, technical and biological replication, experimental design, and techniques to identify, account, and mitigate such variability. The next factor is the specificity of gene expression and epigenomic modifications (Maruyama et al., 2011), which underscores the importance of tissue

selection in CHD research. The question of tissue selection is dependent on the scientific question of the research. For purposes of finding biomarkers, blood-based biomarkers would be most desirable because of affordability, expediency, and relative noninvasiveness. However, caution must still be exercised since variability in expression levels (Joehanes et al., 2012) and DNA methylation levels (Thompson et al., 2013) does exist among different blood-derived samples.

Once these confounding factors have been overcome, the obvious next question is whether transcriptome or epigenome biomarkers provide better prognostic power for heart diseases, whether independent from or in conjunction to known risk factors (van Holten et al., 2013). For transcriptome-based biomarkers, caution needs to be exercised since the expression of thousands of genes tends to fluctuate due to circadian and ultradian rhythm (Zhang et al., 2014b; Ono et al., 2015; van der Veen and Gerkema, 2017), which diminishes their utility as stable biomarkers (Ramachandran, 2006). On the other hand, epigenome-based biomarkers tend to be more stable (Sarkies and Sale, 2012). Given that CHD is a multifactorial disease and preliminary evidence points to multiple biomarkers with low effects, if such biomarkers were found and validated, there will be dozens of them combined into a score. Wingrove et al. (2008) and Rosenberg et al. (2010) gave a proof of concept that it could be done. Although models using multiple biomarkers to predict cardiovascular events or cardiovascular death have been developed (Wang et al., 2006; Zethelius et al., 2008), using multiple biomarkers may lead to conflicting results and thereby confusion (Wang, 2010).

Another next question is whether these biomarkers can help dissect the genetic and genomic architecture of CHD. Given there are cross-talks (Ritchie et al., 2015) between genetic variants and both gene expression (Joehanes et al., 2017; Westra et al., 2013; Liang et al., 2013) and epigenomic modifications (Gaunt et al., 2016; Banovich et al., 2014; Gutierrez-Arcelus et al., 2013), biomarkers from disparate molecular datasets assembled together in a network can help dissect the intricacies of the architecture. Ritchie et al. (2015) described methods to dissect such architecture. Another method to uncover such architecture is to find common sets of genes and pathways across multiple CHD risk factors using one or more molecular datasets. An example of this is the study of McManus et al. that has been discussed in the previous section.

In summary, this article chronicles transcriptome and epigenome technologies. It surveys their findings in relation to cardiovascular diseases. It discusses its challenges of lack of replicability and potential solutions. Although transcriptomic and epigenomic technologies present enormous potential for clinical applications for CHD, further studies are needed.

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Transplant Arteriosclerosis

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Introduction

Organ transplantation is a life-prolonging procedure for many people with end-stage organ failure but almost all grafts eventually fail. Transplant arteriosclerosis (TA; also called cardiac allograft vasculopathy, transplant vasculopathy, or transplant vascular disease) is a vascular condition characterized by concentric intimal thickening and vasomotor dysfunction of arteries that develops in all solid organ transplants (Mitchell and Libby, 2007; Radio et al., 1996). It is widely established to be a main cause of heart transplant failure after the first postoperative year. The development of TA has diminished only minimally over the past 30 years despite advances in immunosuppressive drug formulation and delivery that have considerably reduced acute rejection (Stehlik et al., 2010). This differential effect of immunosuppressive drugs on TA and acute rejection highlights the therapeutic challenge of TA and may reflect uniqueness in its pathology. In this article, we will discuss the immunologic and vascular processes that cause TA.

Immune Mechanisms in Vascular Rejection and TA

T cells recognize peptides bound in the context of major histocompatibility complex (MHC) proteins, also called human leukocyte antigens (HLAs) in humans, through the T cell receptor. In addition to recognition of peptide-MHC, activation of T cells also requires the expression of costimulatory molecules and inflammatory cytokines that bind to partner molecules and receptors on T cells. These three signals act together to drive T cell activation and differentiation into specific effector lineages (Janeway and Medzhitov, 2002). The ability to activate resting T cells through the provision of these signals is restricted to certain cell types, collectively termed antigen-presenting cells. Dendritic cells, macrophages, and B cells are the main antigen-presenting cells that potently induce T cell activation. Dendritic cells and macrophages reside within or circulate through peripheral tissues where they acquire antigen and then interact with T cells in lymphoid organs and sometimes directly within nonlymphoid tissues. In addition to these cells, human vascular endothelial cells are also capable of presenting antigen and activating resting memory T cells (Pober and Tellides, 2012).

Depending on the strength of antigen stimulation and type of costimulation and inflammatory cytokines present, activated T cells differentiate into distinct effector cell types that migrate to peripheral tissues. CD8 T cells mostly differentiate into cytotoxic effectors that kill target cells and secrete interferon-γ (IFNγ). Killing occurs mainly through the secretion of granzymes, a family of serine proteases, and perforin, a pore-forming protein needed to deliver granzymes into target cells, that are stored in the granules of activated CD8 T cells (Choy, 2010). The expression of FasL on the surface of CD8 T cells can also induce cell death in some cell types by activating the death receptor Fas (Guicciardi and Gores, 2009). CD4 T cells differentiate into Th1, Th2, or Th17 cells that migrate to sites of inflammation. Th1 cells secrete IFNγ; Th2 cells secrete IL-4, 5, and 13; Th17 cells secrete IL-17. Each CD4 effector T cell type has distinct roles in protective immunity against pathogens and the cytokine profile secreted by each has specific immunopathological effects (Sallusto, 2016).

Immunologic responses toward organ grafts are a result of genetic differences between the donor and recipient. This is called alloreactivity. T cells recognize and are activated by foreign MHC-peptides on donor cells and by foreign peptides that are acquired from the graft and that are presented in the context of self-MHC molecules on antigen-presenting cells of the graft recipient. The majority of T cells that infiltrate grafts are cytotoxic CD8 T cells and Th1 cells, although experimental models have implicated Th2

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and Th17 cells in some forms of graft rejection (van Besouw et al., 1995; Chan et al., 1995; Yuan et al., 2008). B cells are also activated to produce antibodies that bind foreign MHC and some non-MHC proteins (Djamali et al., 2014).

Immune-mediated damage to arteries causes the development of TA because this vascular condition does not occur in arteries outside the graft and it does not develop in experiments where grafts are performed between genetically identical animals or into recipients that lack adaptive immune cells (Shi et al., 1996). CD4 and CD8 T cells are present in the intima and adventitia of arteries with TA, and are normally absent from the media (Salomon et al., 1991). The T cell population is predominated by Th1 cells that secrete IFNγ and vascular cells in these arteries respond to this cytokine as is evident by their expression MHC class II, an IFNγ-induced gene (Burns et al., 2005; van Loosdregt et al., 2006; Salomon et al., 1991). Experimental models have established that T cells play a causative role in TA. Specifically, this vascular condition does not develop in grafts transplanted into animals that lack T cells (Shi et al., 1996). Depending on the model utilized, both CD4 and CD8 T cells have been shown to be important (Shi et al., 1996; Vessie et al., 2005). Further, the activation of Th1 cells is needed for the development of TA because intimal thickening is reduced in graft recipients that lack STAT4, a transcription factor needed to induce the development of Th1 cells (Koglin et al., 2000). In addition to Th1 cells, IL-17 expression is observed to a varying degree in TA and animal models have determined that Th17 cells can contribute to the development of this vascular condition, although these experiments were performed using graft recipients that cannot mount Th1 responses (Liu et al., 2012; Yuan et al., 2008). Amplification of Th2 responses in graft recipients does not affect intimal thickening but results in expansive medial damage and rupture that is reminiscent of aneurysms, a feature not observed in clinical cases of TA (Shimizu et al., 2004).

Antigens presented by dendritic cells, macrophages, B cells, and endothelial cells initiate immune responses toward allograft arteries. Arteries normally house populations of dendritic cells that reside primarily in the adventitia and intima (Ma-Krupa et al., 2004; Pryshchep et al., 2008; Millonig et al., 2001). Under physiological conditions, artery-associated dendritic cells impose tolerance toward arterial antigens and disruption of these dendritic cells can increase immunopathological responses in atherosclerosis (Choi et al., 2011). Activation of artery-associated dendritic cells with inflammatory toll-like receptor signals upregulates their expression of costimulatory signals and results in T cell activation that can drive immune activation in the arterial wall (Ma-Krupa et al., 2004). It is clear that antigen-presenting cells from the graft migrate into lymph nodes and spleen of graft recipients within days after transplantation and activate T cells that migrate back to the graft and cause rejection (Larsen et al., 1990). Dendritic cells that reside within arteries are also capable of activating T cells locally in these blood vessels (Ma-Krupa et al., 2004). Human vascular endothelial cells also express HLA class I and II and can activate resting memory CD8 and CD4 T cells (Pober et al., 1983; Page et al., 1994; Epperson and Pober, 1994). The pattern of costimulatory molecules expressed by human endothelial cells is partially distinct than that expressed by dendritic cells because these vascular cells do not express CD80/86 but instead express primarily LFA-3, 4-1BB ligand, ICOS ligand, and OX40 ligand (Hughes et al., 1990; Shiao et al., 2005). This may result in differences in the activation of certain T cell subsets by endothelial cells as compared to dendritic cells. For instance, human endothelial cells stimulate alloreactive memory T cells and poorly stimulate naïve T cells (Dengler and Pober, 2000; Ma and Pober, 1998). The phenotype of T cells activated by human endothelial cells is also different from those activated by professional antigen-presenting cells, and this may define a chronic and smoldering immune response in arteries that is a feature of TA (Lorber et al., 1999). Specifically, endothelial cells activate CD8 T cells to differentiate into a relatively immature phenotype (characterized by sustained expression of CD69, CD25, and CD62L) as compared to other antigen-presenting cells (Dengler and Pober, 2000). This effect on T cell activation is dominant over other antigen-presenting cells and may define the unique quality of local T cell activation in TA. Activation of T cells by allogeneic endothelial cells is sufficient to cause vascular rejection and intimal thickening reflective of TA in humanized mouse models (Murray et al., 1994; Lorber et al., 1999). Endothelial cells also have immune regulatory properties. They can induce the differentiation of T regs when IL-6 activity is prevented, support the differentiation of suppressive CD8 T cells, and can be induced to express the immune-inhibitory cell surface protein PD-L1 that opposes the development of TA (Fogal et al., 2011; Manavalan et al., 2004; Bolinger et al., 2010).

B cells can serve as effectors of immune responses by producing antibodies and can also activate immune responses by acting as antigen-presenting cells that stimulate T cells. Antibody-mediated rejection of grafts is vascular in nature, with endothelial changes observed in biopsies being a foundation for its clinical diagnosis (Berry et al., 2011). The development of antidonor antibodies and the incidence of antibody-mediated rejection are associated with the development of TA (Petrossian et al., 1989). The transfer of antidonor antibodies into graft recipients that lack T and B cells is sufficient to induce intimal thickening of allograft arteries in mice, indicating that antidonor antibodies are capable of inducing TA (Galvani et al., 2009). Due to their polymorphic nature, MHC molecules are main targets of antidonor antibodies. Studies have shown that antibody binding to MHC class I triggers the activation of Rho kinase, ERK, and mechanistic target of rapamycin (mTOR) signaling pathways in vascular cells (Ziegler et al., 2012; Jindra et al., 2008). This causes phenotypic alterations in endothelial cells including increased proliferation, survival, and migration (Jin et al., 2005). Antibody binding to MHC class I also results in inflammatory activation of the endothelium by increasing P-selectin and increasing secretion of von Willebrand factor (Valenzuela et al., 2013). These effects increase leukocyte transendothelial migration. Antibodies toward non-MHC molecules are also detected in patients with TA, correlate with poor graft outcome, and are sufficient to induce the development of TA in animal models. These antigens include vimentin, a fragment of the extracellular matrix protein perlecan, and angiotensin type II receptor (Jurcevic et al., 2001; Mahesh et al., 2007, 2010; Soulez et al., 2012; Dragun et al., 2005). These antibodies likely develop due to immune activation in the context of large-scale inflammation, which overrides tolerance mechanisms that are usually dominant in preventing activation against these antigens. Although the production of antibodies usually occurs in B cells activated within secondary lymphoid tissues, recent studies suggest that some antidonor antibodies can be generated within tertiary lymphoid structures that develop locally in allograft arteries (Huibers et al., 2015,

2016). This may reflect chronic immune activation in the setting of TA. In addition to producing antibodies, B cells also cause TA through antibody-independent mechanisms that involve the activation of alloreactive T cells through antigen presentation. TA and associated T cell responses are not affected in mice that contain B cells but cannot produce antibodies, but both are reduced in mice containing B cells that cannot present antigens to T cells (Zeng et al., 2014).

The role of NK cells in rejection of organ transplants is multifactorial and complex. Activation of these cells is determined by the combinatorial strength of inhibitory and activating signals presented by target cells. Self-MHC class I proteins are inhibitory NK cell ligands and the absence of self-MHC class I on target cells triggers NK cell activation (Sun and Lanier, 2011). As such, the absence of self-MHC class I on organ transplants may lead to NK cell activation. Experimental studies have shown a role for these innate immune cells not only in causing acute heart transplant rejection in immunosuppressed recipients but also in opposing rejection by killing donor antigen-presenting cells and competing with effector T cells for cytokines (McNerney et al., 2006; Laffont et al., 2008; Yu et al., 2006; Zecher et al., 2010). NK cells may contribute to the development of TA. Transplantation of hearts into recipients that express MHC class I proteins not present in the donor results in the development of TA that is dependent on NK cell activation (Uehara et al., 2005). Interestingly, T cells are needed for NK cell-mediated development of TA although the mechanism is currently not clear. NK cells may also contribute to vascular inflammatory changes and resultant development of TA induced by antidonor antibodies (Hirohashi et al., 2012).

Vascular Changes That Cause Arteriosclerotic Thickening in TA

Immunological effects on vascular cells cause structural and functional changes in TA. Structurally, arteriosclerotic thickening of the intima results from immune-mediated death of endothelial cells that line the arterial lumen as well as from cytokine-induced phenotypic alterations in endothelial and smooth muscle cells. Blood vessels also control blood flow by dilating or constricting. This vasomotor function is abnormal in TA, which further compromises blood flow through affected arteries and contributes to ischemic damage of grafts.

Immune-Mediated Vascular Cell Injury

The integrity of vascular endothelial and smooth muscle cells is essential for maintenance of arterial structure. Studies in the 1970s and 1980s that examined the effects of mechanical injury on arteries determined that endothelial injury resulted in a "response-to-injury" process in the artery wall that culminated in intimal thickening (Ross et al., 1977; Lindner et al., 1990; Ferns et al., 1991; Fingerle et al., 1989). Lesions consisted mostly of smooth muscle cells that migrated from the media into the intima and proliferated locally as well as of macrophages that infiltrated from the circulation. Intimal thickening was caused by the action of growth factors, such as fibroblast growth factor and platelet-derived growth factor, that were produced by injured vascular cells and infiltrating leukocytes (Ross et al., 1977; Lindner and Reidy, 1991). Although this early work focused mainly on mechanical injury to the arterial wall, the findings suggested that other pathological processes that lead to disruption of the luminal endothelium of arteries could have a similar effect.

Cytotoxic CD8 T cells infiltrate and kill target cells within transplanted organs. Cultured endothelial cells are sensitive to killing by alloreactive CD8 T cells mainly through the granzyme B/perforin pathway and may normally be resistant to FasL-mediated killing (Zheng et al., 2002; Krupnick et al., 2002; Choy et al., 2005; Sata et al., 2000). However, endothelial cells become sensitized to FasL-mediated killing by exposure to IFNγ or modified forms of low-density lipoproteins that are present in TA lesions (Stefanescu et al., 2008; Sata and Walsh, 1998). In TA, CD8 T cells that express perforin are observed to accumulate underneath the luminal endothelium (Fox et al., 1993). This is associated with histological features of endothelial injury, suggesting that endothelial killing through this mechanism could be a triggering event for the development of intimal thickening (Hruban et al., 1990). Indeed, increased apoptosis of luminal endothelial cells is observed in allograft arteries in early stages of TA development but not at later stages, suggesting that endothelial regeneration occurs after early endothelial injury (Dong et al., 1996). The expression of granzyme B is abundant in the intima of arteries with TA and is spatially correlated with cells undergoing apoptosis (Choy et al., 2003). Also, endothelial cell apoptosis in TA is spatially correlated with expression of the death receptor Fas, implicating a role of this cytotoxic mechanism in early endothelial injury (Dong et al., 1996) (**Fig. 1**).

Consistent with clinical observations that early endothelial cell death in allograft arteries may be a triggering event in the development of TA, early loss of luminal endothelial cells is observed in animal models of this vascular condition and this is reduced by administration of immunosuppressive drugs (Lai et al., 2003). The immune response toward transplanted organs is multifactorial, with several processes potentially having overlapping effects on the general outcome of graft failure or arteriosclerotic thickening in the case of TA. In animal models of TA in which grafts are transplanted across a complete MHC mismatch, intimal thickening is not affected by elimination of CD8 T cells. CD4 T cells and B cells play nonredundant roles in this model (Shi et al., 1996). However, T cell-mediated rejection in the absence of antibodies is prevalent in clinical organ transplantation. In models of TA that do not involve appreciable antibody responses, TA develops as a result of CD8 T cell killing of luminal endothelial cells through a perforin and granzyme B mechanism (Schnickel et al., 2004; Choy et al., 2004b, 2005).

Medial smooth muscle cell injury may also trigger the development of arteriosclerotic thickening of arteries (Fingerle et al., 1990; Yu et al., 2011a). Although medial damage and immune infiltration is normally minimal in clinical specimens of TA, this analysis of arterial changes at late stages of disease may not entirely reflect early transient events that are important in the pathogenic process.

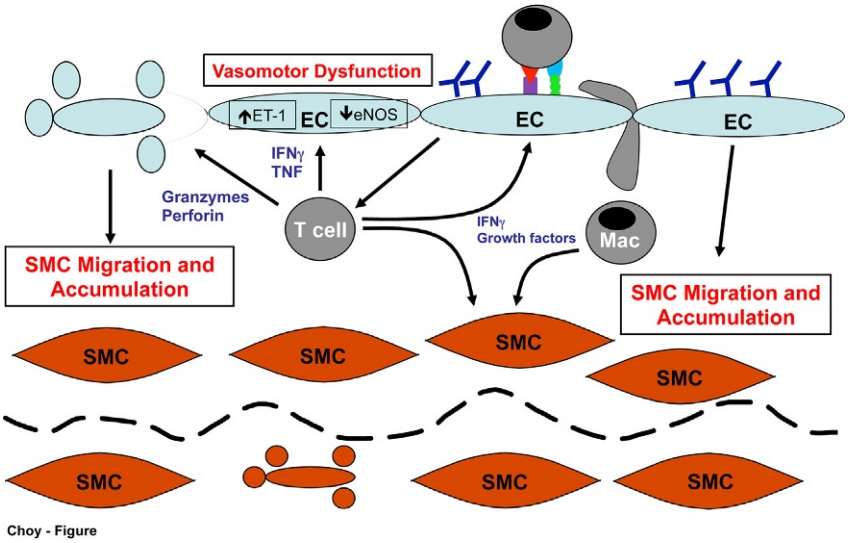

Fig. 1 Immunological causes of TA. Effector T cells within allograft arteries injure and cause dysfunction of vascular cells. Cytotoxic T cells induce endothelial cell death through granzyme B/perforin and potentially FasL pathways, which triggers a response to injury process in which smooth muscle cells (SMCs) migrate into the intima and proliferate. Effector T cells also secrete cytokines that increase inflammatory activation of the endothelium to amplify T cell and macrophage infiltration into the intima. Cytokines and growth factors produced by T cells and macrophages stimulate the intimal migration and proliferation of SMCs. IFNγ and TNF secreted by effector T cells also cause vasomotor dysfunction, characterized by reduced vasodilation and increased vasoconstriction, by reducing eNOS and increasing ET-1 expression. Antidonor antibodies contribute to TA by binding foreign MHC and non-MHC antigens on the surface of endothelial cells.

Medial smooth muscle cell death occurs in animal models of vascular rejection and TA, and injury of this vascular cell type depends on CD8 T cells and FasL (Legare et al., 2000; Hart-Matyas et al., 2010). Smooth muscle cells are normally resistant to FasL-mediated cell death but IFNγ can sensitize this vascular cell type to FasL by relocating Fas to the cell surface (Chan et al., 2000). Vascular smooth muscle cells can also be killed by granzyme B and this can occur through the extracellular proteolysis of matrix proteins (Choy et al., 2004a). In addition to CD8 T cells, neutrophils infiltrate allograft arteries early after transplantation and may also contribute to smooth muscle cell death (King et al., 2010; So et al., 2013). One mechanism by which smooth muscle cell death causes intimal thickening in TA involves the release of the chemokine CXCL12, also called stromal cell-derived factor-1, from injured cells, which triggers the migration and proliferation of mesenchymal stem cells into the intima (Li et al., 2012).

Cytokine-Mediated Vascular Cell Dysfunction

Activated T cells infiltrate allograft arteries and secrete effector cytokines. Most CD4 T cells in TA are Th1 cells that secrete IFNγ and vascular cells express IFNγ-induced proteins, indicating that they are responding to this cytokine (Burns et al., 2005). One of the main effects of IFNγ is the induction of MHC class I and II. This enables endothelial cells to activate resting memory CD8 and CD4 T cells as well as to trigger effector responses from infiltrating T cells that have been previously activated in lymphoid organs (Collins et al., 1984). The secretion of IFNγ from T cells causes intimal thickening through effects on smooth muscle cells (see later). IFNγ also induces the expression of proteins that support leukocyte infiltration into allograft grafts. This includes the chemokine IFN-inducible protein-10 and the upregulation of cell adhesion molecules, the latter occurring in cooperation with TNF (Burns et al., 2005; Pober et al., 1987). These effects contribute to arteriosclerotic thickening by exacerbating inflammation in the arterial wall.

Effector T cells also secrete TNF, which has potent inflammatory effects on the endothelium. TNF upregulates the surface expression of cell adhesion molecules, such as Intercellular adhesion molecule (ICAM) and Vascular cell adhesion molecule (VCAM), which are required for infiltration of all leukocytes into transplanted organs (Pober et al., 1987). In allograft arteries, TNF secretion by T cells likely acts as a positive feedback mechanism to amplify the infiltration of effector T cells and monocytes. Monocytes differentiate into macrophages, which can contribute to intimal thickening by secreting growth factors. Th17 cells are also present in TA and secrete IL-17. This cytokine induces similar signaling pathways as TNF. However, the level of inflammatory activation in endothelial cells induced by IL-17 alone is minimal although this cytokine can act cooperatively with TNF to synergistically upregulate cell adhesion molecules and leukocyte recruitment (Griffin et al., 2012). A main effect of IL-17 may be on the induction of cytokine production by pericytes that supports neutrophil infiltration and activation within inflamed tissues (Liu et al., 2016). The role of vascular effects of IL-17 in TA remains poorly understood because neutralization of its activity in a humanized mouse model of vascular rejection does not affect intimal thickening although a mouse model of TA using graft recipients that cannot mount Th1 responses showed that it contributes to the development of this vascular condition (Rao et al., 2008; Yuan et al., 2008).

T cell effector cytokines also change the phenotype of vascular smooth muscle cells. IFNγ upregulates the expression of MHC class II in these cells (Jonasson et al., 1988). Unlike endothelial cells, antigen presentation by smooth muscle cells does not lead to

activation of resting T cells (Murray et al., 1995). This is a result of a relative paucity of costimulatory molecules, expression of PD-L1, expression of the immune-inhibitory enzyme indoleamine 2,3-dioxygenase that depletes tryptophan and synthesizes the inhibitory metabolite kynurenine, and the secretion of the antiinflammatory molecule TGFβ (Zhang et al., 2010; Cuffy et al., 2007; Lebastchi et al., 2011; Koga et al., 2004). However, recognition of peptide-MHC on intimal smooth muscle cells by activated T cells elicits effector cytokine secretion. IFNγ and IL-17 induce expression of inflammatory cytokines and chemokines by vascular smooth muscle cells and, in this way, increases leukocyte infiltration into the arterial intima (Eid et al., 2009). Finally, IFNγ induces proliferation of vascular smooth muscle cells and this is sufficient for driving intimal proliferation in a humanized mouse model of arteriosclerosis (Tellides et al., 2000). The mitogenic effects of IFNγ are mediated through the activation of PI3K and mTOR signaling pathways, and can be inhibited by the RAS GTPase-activating protein ASK-1 interacting protein 1 (Yu et al., 2011b).

Antibody-Mediated Alterations in Vascular Cells

The development of antidonor antibodies and incidence of antibody-mediated rejection episodes correlate with the development of TA, suggesting a pathogenic role for antibodies (Petrossian et al., 1989; Cherry et al., 1992). Animal models have also established that anti-MHC antibodies can induce the development of TA (Galvani et al., 2009). Most antidonor antibodies bind to MHC molecules and cause cellular changes in endothelial cells that include increased proliferation, migration, and expression of inflammatory molecules (Jindra et al., 2008; Valenzuela et al., 2013). Increased endothelial proliferation and migration may disrupt the homeostatic properties of the luminal endothelium. This could increase vascular permeability that results from morphological changes associated with these cellular processes, which may contribute to intimal thickening (Choy et al., 2001). Antibodies also induce the expression of cell adhesion molecules that support leukocyte transendothelial migration and inflammation within the arterial wall (Valenzuela et al., 2013). In addition to having direct effects on endothelial cells, antibodies activate complement and this has immune activating properties. Complement activation by antidonor antibodies leads to the formation of membrane attack complexes, which is the terminal outcome of the complement cascade, on the surface of endothelial cells. Cell lysis does not occur but the membrane attack complex induces activation of inflammatory signaling pathways in endothelial cells that increases T cell activation (Jane-Wit et al., 2013). Overall, antibodies are likely to contribute to the development of TA by directly activating inflammatory signaling pathways in endothelial cells that pathologically alter endothelial cell phenotype and inflammatory gene expression as well as by indirectly stimulating endothelial cells through complement activation, which increases the activation of T cells by the graft endothelium.

Vasomotor Dysfunction in TA

There is also defective vasodilation and increased vasoconstriction of allograft arteries in TA, which further compromises blood flow to transplanted organs and contributes to ischemic graft damage. The endothelium is essential in regulating vasomotor function of arteries by producing vasodilatory and vasoconstrictive factors (Vanhoutte et al., 2016; Vanhoutte, 2011). Reduced agonist-induced vasodilation is observed early after heart transplantation in some patients and this precedes the development of intimal thickening (Pethig et al., 1998; Hollenberg et al., 1998, 2001).

Dysregulation of Vasodilation

One of the main mechanisms that controls the dilation of arteries is the production of the bioactive gas, nitric oxide (NO), by the endothelium (Palmer et al., 1987). NO is synthesized in endothelial cells by endothelial NO synthase (eNOS) where it diffuses into the arterial media and relaxes smooth muscle cells through the activation of soluble guanylate cyclase and downstream dephosphorylation of the cellular contractile apparatus (Janssens et al., 1992; Pollock et al., 1991; Rapoport and Murad, 1983). eNOS is basally expressed in the endothelium, and its expression and activity can be modified during immune responses to affect NO output. Agonist-induced vasodilation mediated by eNOS-derived NO is compromised in allograft arteries (Pethig et al., 1998; Hollenberg et al., 1998, 2001). Inflammatory cytokines alter the expression and function of eNOS. IFNγ and TNF secreted by effector T cells inhibit eNOS expression in endothelial cells and this contributes to pathological vasoconstriction in allograft arteries (Koh et al., 2004). Further, prolonged exposure to abnormally high levels of NO can desensitize blood vessels to NO-mediated vasodilation. In TA, NO is also produced by inducible NOS (iNOS) in infiltrating macrophages and T cells, and iNOS-derived NO can compromise vasodilation by desensitizing smooth muscle cells to this vasodilatory stimulus in allograft arteries (Koh et al., 2004; Choy et al., 2008). The regulation of eNOS by TNF and IFNγ also extends to effects on enzyme activity. Interestingly, both these cytokines increase eNOS activity and NO output by inducing the synthesis of cofactors needed for eNOS activity (Rosenkranz-Weiss et al., 1994; Katusic et al., 1998). Activated T cells also synthesize similar cofactors (Chen et al., 2011). Increased eNOS activity caused by cytokines may support acute vasodilation during inflammatory responses while decreased eNOS expression has chronic effects that are important in TA.

IL-17 also regulates eNOS expression and activity. This cytokine increases expression of eNOS, and resultant NO output, in cultured human endothelial cells through NF-κB, MAPK, and JNK signaling pathways. The level of IL-17 in human arteries with TA is positively correlated with increased eNOS protein levels and greater lumen size, suggesting that this cytokine may support increased blood flow into heart transplants by promoting outward expansion of arteries (Liu et al., 2012). However, IL-17 has been shown to inhibit eNOS activity in rat endothelial cells and contribute to hypertension in mouse models (Nguyen et al., 2013;

Madhur et al., 2010). The mechanisms controlling the context-specific effects of IL-17 on vasodilatory properties of endothelial cells are not clear and require further study.

Dysregulation of Vasoconstriction

The endothelium also produces vasoconstrictive molecules, with endothelin-1 (ET-1) being the most potent and having clear pathological indications in humans (Virdis et al., 2010). ET-1 levels are increased in TA and this factor increases pathological constriction inherent in allograft arteries (Ravalli et al., 1996; Weis et al., 1999; Larose et al., 2009). Expression of ET-1 is induced in endothelial cells by TNF, IL-1, IFNγ, and IL-6 (Lamas et al., 1992; Kahaleh and Fan, 1997). Also, the immunosuppressive drug cyclosporine may have the unintended consequence of increasing arterial vasoconstriction by increasing ET-1 levels while tacrolimus does not (Petrakopoulou et al., 2006). In addition to endothelial-dependent effects on vasoconstriction, vascular smooth muscle cells in the media have inherent myogenic constriction in response to outward flow-mediated pressure on the arterial wall. This is needed to maintain proper arterial diameter and blood flow. Myogenic constriction may be compromised by immune-mediated smooth muscle cell death during vascular rejection, which could pathologically increase intravascular hydrostatic pressure and concomitant fluid leakage into the interstitial space (Moien-Afshari et al., 2004). The resultant edema could lead to loss of ventricular compliance and heart transplant failure. Immunosuppressive drugs prevent medial damage and preserve myogenic constriction (Moien-Afshari et al., 2004).

Nonimmune Factors in TA

In addition to the immunological foundation that drives the development of TA, some nonimmune factors also contribute. Given the vascular nature of TA, risk factors associated with atherosclerotic coronary artery disease are also associated with TA. This includes age, male sex, hyperlipidemia, obesity, hypertension, hyperglycemia, and hyperhomocysteinemia (Caforio et al., 2004; Escobar et al., 1994; Valantine et al., 2001; Mehra et al., 1995; Gupta et al., 1998). These factors likely act in a similar manner as they do in coronary artery disease to increase arteriosclerotic thickening in TA. Increased and altered lipid deposition is observed in human TA (McManus et al., 1995; Lin et al., 1996). Hyperlipidemia, glucose, and homocysteine are proinflammatory and increase arterial inflammation (Gauthier et al., 1995; Kim et al., 1994; Postea et al., 2006). All of the mentioned factors activate vascular endothelial cells to increase expression of cell adhesion molecules that support leukocyte infiltration. They also promote inflammatory cytokine and chemokine secretion from vascular cells and leukocytes. This may amplify the immunologic response toward allograft arteries and/or increase endothelial dysfunction. Finally, viral infection with cytomegalovirus (CMV) is a risk factor for development of TA and prophylactic prevention of this infection reduces development of TA (Valantine et al., 1999). Most individuals exist in a state of chronic infection with CMV that is maintained in a latent state. Reactivation of the virus can occur as a result of immunosuppression and this likely contributes to the development of TA by increasing inflammation in the arterial wall, as a result of increased systemic inflammation and/or direct infection of vascular cells (La Rosa and Diamond, 2012).

Therapeutic Management of TA

The rate of late graft loss associated with TA has diminished only minimally over the past 30 years despite improvements in the formulation and delivery of calcineurin inhibitors that prevent acute rejection, thereby highlighting the therapeutic challenge of this vascular condition (Stehlik et al., 2010). Prevention of TA likely requires approaches that are able to inhibit both the chronic immune response and vascular reactions that cause arterial remodeling. Repurposing of existing drugs, such as mTOR inhibitors and statins, is promising. Experimental approaches that specifically inhibit immune responses, such as T reg therapy and B cell depletion, are being investigated in clinical trials.

Calcineurin inhibitors block calcium signaling pathways that are essential for T and B cell activation. Cyclosporine and tacrolimus are the main members of this drug class and act through a similar mechanism, although with different direct binding partners (Azzi et al., 2013). The majority of heart transplant recipients are given tacrolimus, which may have a slightly better effect at preventing the development of TA as compared to cyclosporine (Stehlik et al., 2010). The mechanism is not clear, although could be due to greater freedom from acute rejection or reduced adverse effects on lipid levels.

Proliferation signal inhibitors include everolimus and sirolimus. Both inhibit the signaling protein mTOR, a kinase that induces cellular processes needed to support cell growth and division (Fine and Kushwaha, 2016). Everolimus and sirolimus effectively inhibit lymphocyte proliferation so, in this way, are immunosuppressive. They also prevent proliferation of vascular smooth muscle cells and were initially used to prevent arteriosclerotic thickening caused by implantation of coronary artery stents (Grube and Buellesfeld, 2004). Seminal trials examining proliferation signal inhibitors in heart transplantation showed that administration of everolimus or sirolimus beginning early after transplantation reduces the development of TA (Eisen et al., 2003, 2013; Keogh et al., 2004; Andreassen et al., 2014). However, most reports do not show a clear survival benefit potentially because of complications associated with mTOR inhibition. Importantly, the therapeutic effect of these drugs on TA depends on early administration within the first year after transplant, suggesting that the pathogenic nature of TA involves targetable processes that occur early during development.

Statins have been a mainstay drug for the treatment of cardiovascular disease for several decades. This class of drugs reduces circulating cholesterol levels by inhibiting HMG CoA reductase. This effect on circulating cholesterol levels is a main mechanism of

its therapeutic effect in cardiovascular disease. Increased circulating lipid levels are a nonimmune risk factor for TA and TA lesions contain aberrant lipid accumulation. Treatment of adult heart transplant recipients with statins reduces the progression of TA and mortality (Kobashigawa et al., 1995). Potential effects of statins on TA in pediatric heart transplant recipients are less clear (Mahle et al., 2005; Greenway et al., 2016). The therapeutic effect of statins appears to be minimally related to reduction of circulating lipid levels, suggesting that other effects of these drugs are beneficial. Indeed, use of statins is related to less acute rejection and these drugs have antiinflammatory effects. Statins inhibit activation of proinflammatory signaling pathways in endothelial cells, which reduces the expression of cell adhesion molecules and prevents leukocyte transendothelial migration. These drugs may also inhibit T cell and macrophage activation (Li et al., 2002; Seljeflot et al., 2002; Forrester and Libby, 2007). The mentioned effects are established to contribute to the therapeutic effect of statins in cardiovascular disease and likely contribute to their effects in TA.

The drugs above have immune and/or vascular effects that oppose the development of TA. However, all lack specificity for immune reactions toward the graft and have undesirable side effects. Specific modulation of the immune response to prevent rejection while maintaining protective immunity, termed tolerance, is the "holy-grail" of transplantation. T regs are an essential tolerance component of the immune system that prevents immunopathological responses by inhibiting the activation and function of effector T cells (Josefowicz et al., 2012). Many experimental models have established a role for endogenous T regs in opposing immunologic rejection of many types of organ grafts as well as the potential effectiveness of administering large numbers of T regs to prevent rejection in the absence of immunosuppressive drugs (Lee et al., 2005; Joffre et al., 2008). Given the promise of these preclinical studies, phase 1/2 clinical trials are being conducted to examine the utility of this cell therapy in humans (Juvet et al., 2014). Although the majority of studies have focused on the prevention of acute transplant rejection, injection of T regs prevents the development of TA in preclinical studies using humanized mice (Nadig et al., 2010). Considerations that need to be addressed for the clinical application of T regs include optimization of protocols for expanding antigen-specific cells, precision of bulk-activated cells that are not specific for transplantation antigens, and the phenotypic stability of the injected T regs (MacDonald et al., 2012; Ezzelarab et al., 2016).

B cell depletion with rituximab has shown remarkable efficacy for the management of many immunopathological conditions. The beneficial effects of rituximab appear related to effects on antibody production and also to inhibition of T cell activation that is dependent on antigen presentation by B cells. Early stage clinical trials are underway to examine B cell depletion as an induction therapy in kidney transplant recipients (van den Hoogen et al., 2015; Tyden et al., 2009). Experimental studies have established that B cells contribute to the development of TA through antibody-dependent and independent mechanisms (Colvin et al., 2015; Zeng et al., 2014). Moreover, B cell depletion significantly reduces the severity of TA in nonhuman primate studies of heart transplantation when done in combination with costimulatory blockade for immunosuppression (Azimzadeh et al., 2016).

Conclusions

The pathogenesis of TA involves immunological processes that have distinct effects on vascular cells. This involves injury of vascular cells by T cells and antibodies that lead to altered reparative responses that drive arteriosclerotic thickening. Alterations in the phenotype of vascular cells by cytokines and antibodies also contribute to arteriosclerotic thickening by promoting inflammation and proliferation of smooth muscle cells. Vascular cells also have immunomodulatory properties that may influence the nature of immune activation locally within arteries and, in this way, define the unique pathogenesis of TA. Understanding these processes will be necessary for advancements that are able to specifically prevent immune activation toward allograft blood vessels and the arteriosclerotic response of vascular cells in order to prevent solid organ transplant failure. Similar processes may also be involved in other immune-mediated vascular diseases, such as giant cell arteritis and atherosclerosis, so knowledge of the pathogenesis of TA may contribute to further understanding of these diseases.

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Further Reading

Wenzel U, Turner JE, Krebs C, Kurts C, Harrison DG, and Ehmke H (2016) Immune mechanisms in arterial hypertension. *Journal of the American Society of Nephrology* 27: 677–686.

Trends in the Incidence and Mortality of Cardiovascular Disease

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Cardiovascular disease (CVD), including coronary heart disease (CHD) and stroke, is one of the leading causes of death in developed countries. Advances in medical technology and changes in lifestyle during the past several decades have likely affected the prevalence of cardiovascular risk factors and thereby the incidence and mortality of CVD.

Fig. 1 shows the secular trends in mortality rates of CHD and stroke based on the data from vital statistics in three major developed countries in the world. Because the age distributions have differed among the countries and have changed with time, the mortality rates shown in these graphs were adjusted for age using the World Standard Population. In 1968, age-standardized mortality rates of CHD per 100,000 were 295 in the United States, 195 in the United Kingdom (England and Wales), and 43 in Japan. The corresponding rates in 1997 were 107, 119, and 30, respectively. During this period of three decades, the mortality rate of CHD in Japan had been much lower than in Western countries. While the mortality rates of CHD decreased greatly with time in the United States and in the United Kingdom, the decreasing trend in the CHD mortality in Japan was much smaller. On the other hand, age-standardized mortality rates of stroke per 100,000 in 1966 were 93 in the United States, 113 in the United Kingdom, and 223 in Japan, and the corresponding rates in 1997 were 35, 50, and 57, respectively. The mortality rate of stroke in Japan was much higher than in Western countries in the 1960s and decreased greatly with time during the next three decades.

Secular trends in CVD mortality during the period from 1979 to 2013 were recently published using the data from national statistics in the United Kingdom (Bhatnagar et al., 2016). Age-standardized mortality rate of total CVD in the United Kingdom declined by 70% during this period. Similar decreases were seen for both CHD and stroke (72% and 71%, respectively).

These statistics indicate that the mortality risks of CVD in developed countries have decreased with time in general, probably as a result of the improvement in medical technology and the management of cardiovascular risk factors. However, the patterns in the secular trends in CVD mortality in Japan have been completely different from those in Western countries, probably because of the different genetic and lifestyle backgrounds.

Vital statistics based on death certificates cannot determine whether the changes in mortality rates reflected a change in the incidence of CVD or the prevalence of its risk factors, or an improvement in case fatality. In addition, the diagnoses on death certificates are not always accurate and are not generally based on standardized diagnostic criteria. Accordingly, to examine accurate trends in the incidence and mortality rates of CVD and in the prevalence of risk factors, population-based studies with standardized criteria of CVD diagnosis are more useful. In the first two sections of this article, two population-based prospective studies that examined the secular trends in the incidence and mortality rates of CVD and the prevalence of its risk factors, the Framingham Heart Study (FHS) in the United States and the Hisayama Study in Japan, are introduced. The meta-analysis data from the Global Burden of Disease (GBD) studies are also introduced briefly in the last section.

The Framingham Heart Study

The FHS is a prospective epidemiological study of CVD following a two-third population sample of the residents of Framingham, Massachusetts, the United States (Sytkowski et al., 1996). The original cohort of this study consisting of 5209 participants aged 28–62 years was established in 1948. Information on newly developed CVD and its risk factors was collected through biennial health examinations including a medical interview, physical examination, and laboratory tests. Information on CVD events and deaths was collected through daily hospital and death surveillance. In the FHS, CHD was defined as myocardial infarction, angina pectoris, sudden and nonsudden coronary death, and coronary insufficiency. Stroke included both ischemic and hemorrhagic symptomatic stroke. All CVD events were diagnosed and adjudicated using standard criteria at regular meetings of a panel of physicians.

Using the prospective follow-up data from the FHS original cohort, three successive cohorts of residents aged 50–59 years without a history of CVD were established in 1950 ($n=1375$), 1960 ($n=1402$), and 1970 ($n=1432$) (Sytkowski et al., 1996).

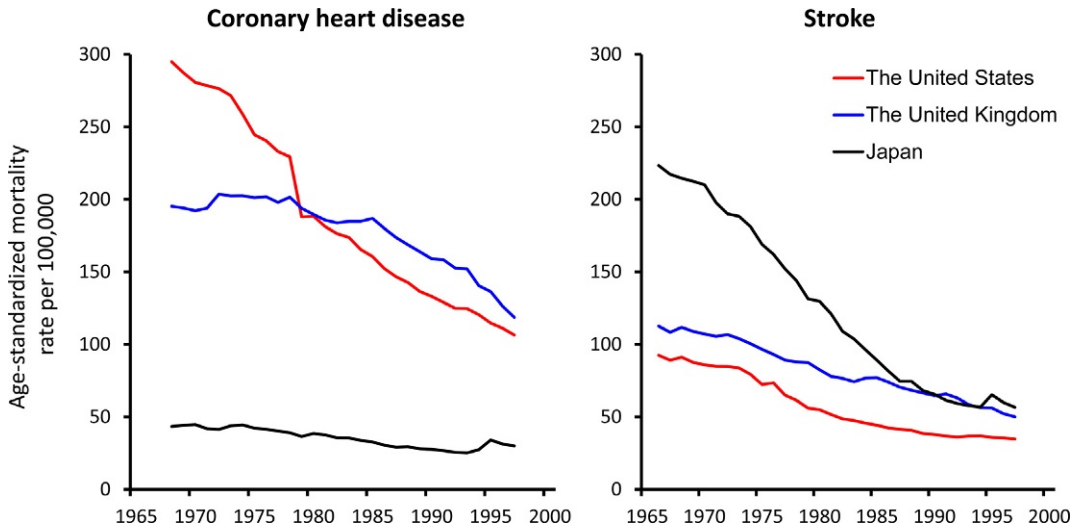

Fig. 1 Age-standardized mortality rates of coronary heart disease and stroke per 100,000 in the United States, the United Kingdom (England and Wales), and Japan, from the vital statistics (World Health Statistics Annual).

Then, the prevalence of risk factors at baseline and the incidence and mortality of CVD during the next 20 years were compared among the three cohorts established at different time points.

Prevalence of Risk Factors in Framingham

Prevalence or mean values of risk factors at the baseline examinations in 1950, 1960, and 1970 in the FHS are summarized in Table 1 (Sytkowski et al., 1996). In both men and women, mean levels of systolic and diastolic blood pressure decreased significantly with time as a result of the spread of antihypertensive medication over the years from 1950 to 1970. During this 20-year period, the prevalence of diabetes increased significantly with time in both sexes. The mean values of total cholesterol decreased significantly with time in women, but did not show a clear change in men. The mean values of body mass index increased significantly and the smoking rates decreased significantly in men. In contrast, the mean values of body mass index decreased significantly and the smoking rates increased significantly in women.

CVD Incidence and Mortality in Framingham

For each cohort, the participants were followed up for 20 years from the baseline examination (Sytkowski et al., 1996). Fig. 2 shows the secular trends in the 20-year cumulative incidence and mortality of CHD and stroke in the FHS cohorts. In both men and women, the risks of CHD incidence were much higher than those of stroke incidence. In men, the cumulative incidence of CHD and stroke showed no clear change, probably because the benefits from the improved blood pressure control and decreased smoking rates were negated by the increased prevalence of diabetes and obesity. In women, the cumulative incidence of CHD and stroke decreased significantly with time, probably as a result of the decreased levels of blood pressure, total cholesterol, and body mass

Table 1 Prevalence or mean values of risk factors at baseline of each cohort, the Framingham Heart Study

	Men			*Women*		
	1950 cohort	*1960 cohort*	*1970 cohort*	*1950 cohort*	*1960 cohort*	*1970 cohort*
Blood pressure (mmHg)	143/88	135/85	135*/83*	153/91	138/84	131*/80*
Antihypertensive medication (%)	0	4	7*	0	11	11*
Diabetes mellitus (%)	3.7	3.4	6.1*	2.9	2.2	5.9*
Total cholesterol (mg dL^{-1})[a]	226	240	227	252	258	241*
Body mass index (kg m^{-2})	26.0	26.0	26.7*	27.1	25.6	25.5*
Smoking (%)	56	53	44*	25	36	54*

*$P < 0.05$ for trend.

[a]To convert cholesterol from mg dL^{-1} to mmol L^{-1}, multiply by 0.02586.

Source: Sytkowski, P. A., D'Agostino, R. B., Belanger, A. and Kannel, W. B. (1996). Sex and time trends in cardiovascular disease incidence and mortality: the Framingham Heart Study, 1950–1989. *American Journal of Epidemiology* **143**, 338–350.

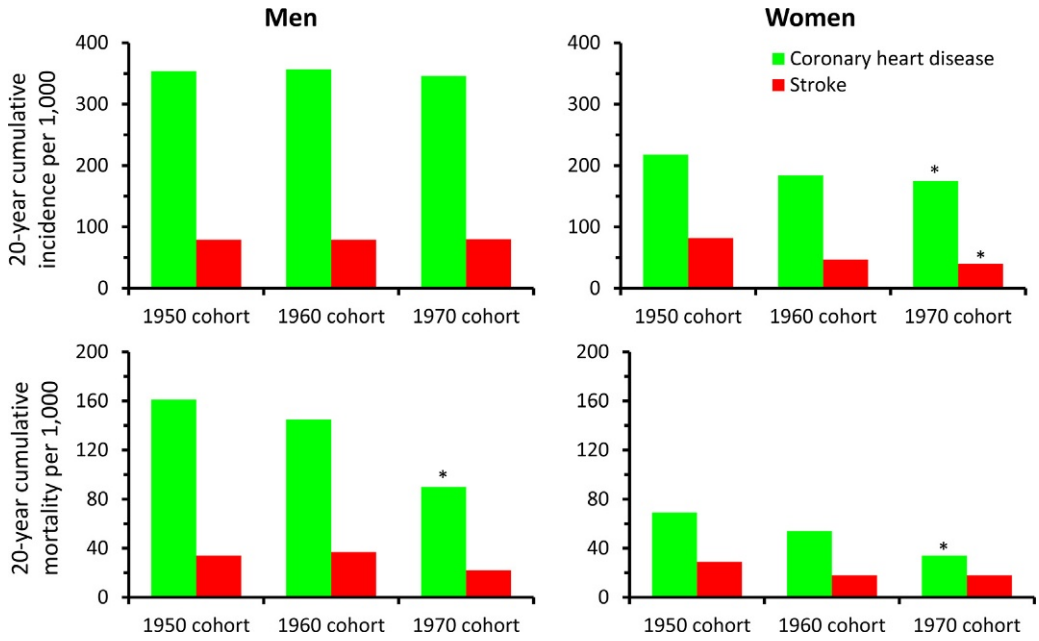

Fig. 2 Cumulative incidence (upper graphs) and mortality (lower graphs) of coronary heart disease and stroke per 1000 during 20 years of follow-up of each cohort, the Framingham Heart Study. $^{*}P$ for trend <0.05. Adapted from Sytkowski, P. A., D'Agostino, R. B., Belanger, A. and Kannel, W. B. (1996). Sex and time trends in cardiovascular disease incidence and mortality: the Framingham Heart Study, 1950–1989. *American Journal of Epidemiology* **143**, 338–350.

index, although the increased prevalence of diabetes and smoking rates might have influenced the CVD risk to some degree. The mortality risks of CHD in both sexes decreased significantly with time probably as a result of the improvement in medical management and reduction in case fatality after CHD, as well as a decreased incidence of CHD in women.

The Hisayama Study

The Hisayama Study is a population-based prospective study for CVD in Japan. The town of Hisayama is located in the eastern suburb of the Fukuoka metropolitan area on Kyushu Island, Japan. According to the national census, the age and occupational distributions in the town were very similar to those in the country of Japan as a whole (Hata et al., 2013). Since 1961, health examinations for residents of Hisayama aged ≥ 40 years have been repeated annually. To compare the incidence and mortality rates of CVD and the prevalence of its risk factors, five cohorts, consisting of residents aged ≥ 40 years without a history of CVD, established in 1961 ($n=1618$), 1974 ($n=2038$), 1983 ($n=2459$), 1993 ($n=1983$), and 2002 ($n=3108$), were used (Hata et al., 2013). Then, the prevalence of risk factors and the incidence and mortality of CVD during the next 7 years were compared among the cohorts. To compare the proportion of ischemic stroke subtypes or the location of intracerebral hematoma, three cohorts consisting of CVD-free residents aged ≥ 40 years established in 1961 ($n=1618$), 1974 ($n=2038$), and 1988 ($n=2637$) were followed up prospectively over the next 13 years, respectively (Kubo et al., 2008; Gotoh et al., 2014). Information on newly developed CVD was collected through annual health examinations and mail or telephone interviews, and a daily monitoring system organized by the study team, local physicians, and town government. All CVD events were diagnosed, classified, and adjudicated by a panel discussion of study physicians including cardiologists and stroke neurologists according to the standardized diagnostic criteria.

Prevalence of Risk Factors in Hisayama

Table 2 summarizes the age-adjusted prevalence rates or mean values of cardiovascular risk factors at baseline examination in the five Hisayama cohorts (Hata et al., 2013). The prevalence of hypertension increased slightly during the earlier period from 1961 to 1983 and then decreased slightly during the more recent period from 1983 to 2002, but these changes were not drastic. However, the mean values of systolic blood pressure among participants with hypertension decreased significantly as a result of the spread of antihypertensive treatment over the study period. In contrast, the prevalence of metabolic risk factors, namely, glucose intolerance (including diabetes mellitus and prediabetes), hypercholesterolemia, and obesity, increased significantly with time in both sexes, probably due to the westernization of dietary habits and physical inactivity as a result of motorization. The smoking rates in men and women decreased significantly from 1961 to 1993.

Table 2 Age-adjusted prevalence or mean values of risk factors at baseline of each cohort, the Hisayama Study

	1961	*1974*	*1983*	*1993*	*2002*
Men					
Hypertension (%)	38.4	43.1	47.7	43.7	41.3
Antihypertensive agents (%)	2.0	8.4	10.9	14.7	17.5*
Blood pressure in hypertensive individuals (mmHg)	161/91	157/90	152/92	152/88	148*/89*
Glucose intolerance (%)	11.6	14.1	14.3	29.9	54.0*
Hypercholesterolemia (%)	2.8	12.2	23.0	25.2	22.2*
Obesity (%)	7.0	11.6	20.2	26.7	29.2*
Current smoker (%)	75.0	73.3	57.2	47.0	47.4*
Women					
Hypertension (%)	35.9	40.1	41.2	34.6	30.8*
Antihypertensive agents (%)	2.1	7.4	11.5	15.2	16.2*
Blood pressure in hypertensive individuals (mmHg)	163/88	161/87	155/87	155/84	149*/86*
Glucose intolerance (%)	4.8	7.9	7.0	21.0	35.1*
Hypercholesterolemia (%)	6.6	19.9	33.5	35.7	35.3*
Obesity (%)	12.9	21.5	23.5	26.2	23.8*
Current smoker (%)	16.6	10.2	7.4	4.6	8.5*

*$P < 0.05$ for trend.

Hypertension was defined as blood pressure $\geq$140/90 mmHg or the use of antihypertensive agents. Glucose intolerance included both diabetes mellitus and prediabetes. Hypercholesterolemia was defined as total cholesterol $\geq$5.7 mmol L^{-1} (220 mg dL^{-1}). Obesity was defined as body mass index $\geq$25 kg m^{-2}.

Source: Hata, J., Ninomiya, T., Hirakawa, Y., et al. (2013). Secular trends in cardiovascular disease and its risk factors in Japanese: half-century data from the Hisayama Study (1961–2009). *Circulation* **128**, 1198–1205.

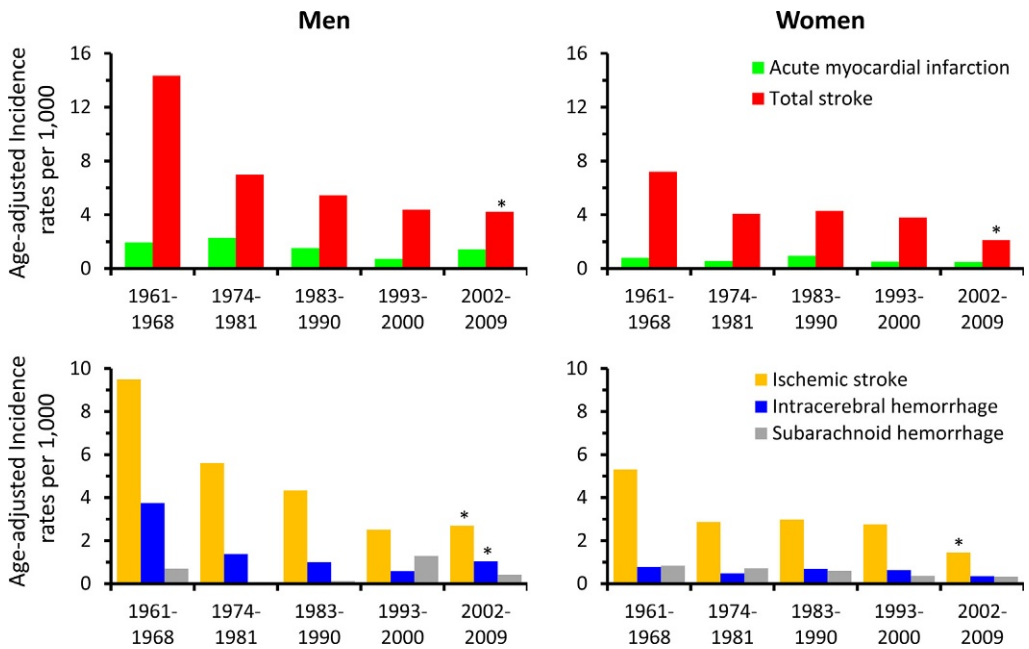

Fig. 3 Age-adjusted incidence rates of acute myocardial infarction, total stroke (upper graphs), and stroke subtypes (lower graphs) per 1000 person-years during 7 years of follow-up of each cohort, the Hisayama Study. *P for trend <0.05. Adapted from Hata, J., Ninomiya, T., Hirakawa, Y., et al. (2013). Secular trends in cardiovascular disease and its risk factors in Japanese: half-century data from the Hisayama Study (1961–2009). *Circulation* **128**, 1198–1205.

CVD Incidence and Mortality in Hisayama

For each of the five cohorts, the participants were followed up for 7 years from the baseline examination. Fig. 3 shows the secular trends in the age-adjusted incidence rates of acute myocardial infarction (AMI), total stroke, and each stroke subtype in the Hisayama cohorts (Hata et al., 2013). In both men and women, the incidence rates of AMI were much lower than those of stroke over the past half century. The incidence rates of AMI did not show a clear change during the study period. In contrast, the incidence rates of total stroke and ischemic stroke decreased greatly in the earlier period from the 1960s to the 1970s, but these decreasing trends slowed down in the subsequent period in both sexes. While the incidence rate of intracerebral hemorrhage in men decreased

consistently from the 1960s to the 1990s, the incidence of intracerebral hemorrhage in women did not show a clear change over the study period.

In this study, the incidence rates of total and ischemic stroke decreased mainly in the earlier period, probably because of the improvement in hypertension management resulting from the increased use of antihypertensive agents, reduction in salt consumption, and decreasing trends in smoking rates. However, the decreasing trends in the incidence of total and ischemic stroke slowed down in recent years, and there was no clear change in the incidence of AMI over the study period, probably because the benefits of hypertension control and smoking cessation were negated by the increasing prevalence of metabolic risk factors such as glucose intolerance, hypercholesterolemia, and obesity. While the decreased incidence of intracerebral hemorrhage in men was likely attributable to the improvement in hypertension management, a comparable trend of decrease was not seen in women, probably because of the small number of events. In addition, the Hisayama Study previously reported that alcohol consumption and hypertension synergistically increased the risk of intracerebral hemorrhage (Kiyohara et al., 1995). Thus the impact of hypertension on the development of intracerebral hemorrhage may have been smaller in women because the drinking rate in women was much lower than that in men.

Then, the participants who developed stroke or AMI during each 7-year period were further followed up for the subsequent 5 years. **Fig. 4** shows the age- and sex-adjusted survival curves after the onset of stroke or AMI in each cohort (Hata et al., 2013). The estimated 5-year survival rates after stroke and AMI improved greatly from the 1960s cohort to the 2000s cohort as a result of an improvement in medical management in the acute and chronic phases of CVD.

Fig. 5 shows the secular trends in the mortality rates from AMI and stroke in the same Hisayama cohorts during each 7-year period (Hata et al., 2013). The mortality rates from stroke in both sexes decreased significantly as a result of the decreased incidence and improved survival rates.

Ischemic Stroke Subtypes in Hisayama

Ischemic stroke was further classified into subtypes according to the size of affected arteries and the mechanisms of ischemia, namely, lacunar infarction, atherothrombotic infarction, and cardioembolic stroke. In the Hisayama Study, three cohorts without a history of CVD were established in 1961, 1974, and 1988 and followed up for 13 years each. During the follow-up periods, ischemic stroke events were observed in 134 subjects in the 1961 cohort, 142 subjects in the 1974 cohort, and 154 subjects in the 1988 cohort (Kubo et al., 2008). **Fig. 6** shows the secular trends in the proportion of ischemic stroke subtypes in each cohort. The proportion of lacunar infarction, the most common subtype of ischemic stroke in Japan, decreased with time from 62% in the 1961 cohort to 48% in the 1988 cohort. In contrast, the proportion of the other two subtypes, namely, atherothrombotic and cardioembolic infarction, increased with time. Lacunar infarction develops by the occlusion of small perforating arteries in deep parts of the brain, such as the basal ganglia, thalamus, and brainstem, as a result of lipohyalinosis or microatheroma mainly caused by hypertension. The decreased proportion of lacunar infarction in the Hisayama Study may be explained by an improvement in hypertension control during the study period. Atherothrombotic infarction is developed by atherosclerosis of large intracerebral or extracerebral arteries and its risk factors include metabolic disorders such as hypercholesterolemia and diabetes. Cardioembolic infarction is developed by thromboembolism from the heart in patients with atrial fibrillation or other cardiac diseases. The proportions of

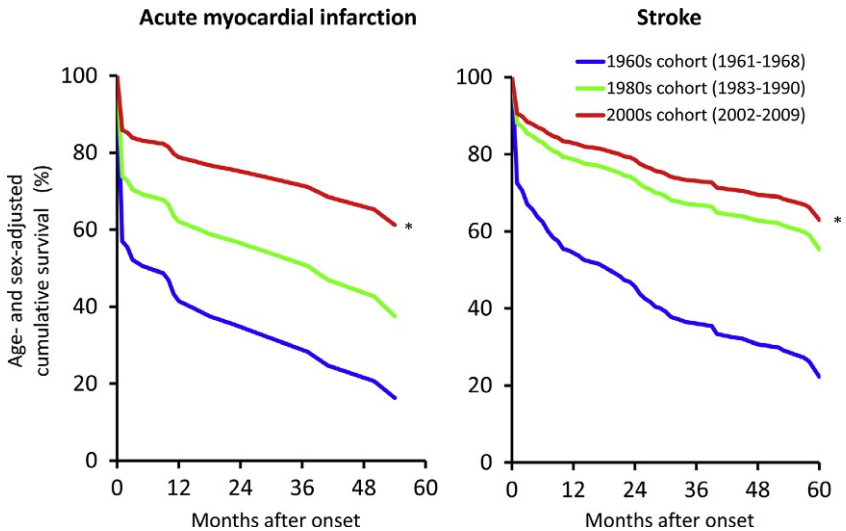

Fig. 4 Age- and sex-adjusted 5-year survival curves after the onset of acute myocardial infarction and stroke, the Hisayama Study. *P for trend <0.05. Adapted from Hata, J., Ninomiya, T., Hirakawa, Y., et al. (2013). Secular trends in cardiovascular disease and its risk factors in Japanese: half-century data from the Hisayama Study (1961–2009). *Circulation* **128**, 1198–1205.

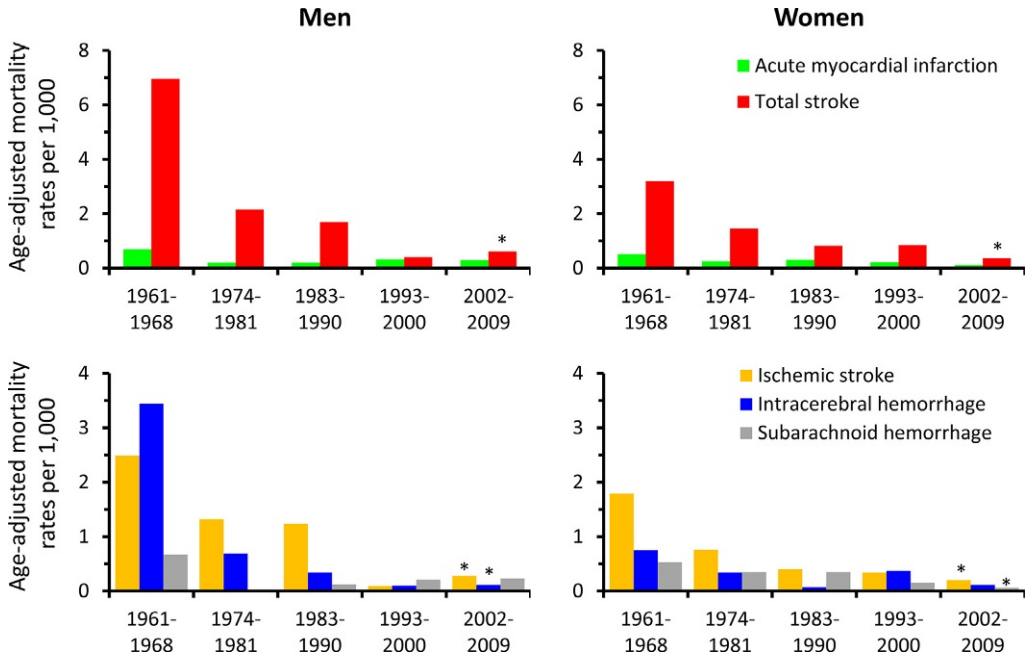

Fig. 5 Age-adjusted mortality rates of acute myocardial infarction, total stroke (upper graphs), and stroke subtypes (lower graphs) per 1000 person-years during 7 years of follow-up of each cohort, the Hisayama Study. *P for trend <0.05. Adapted from Hata, J., Ninomiya, T., Hirakawa, Y., et al. (2013). Secular trends in cardiovascular disease and its risk factors in Japanese: half-century data from the Hisayama Study (1961–2009). *Circulation* **128**, 1198–1205.

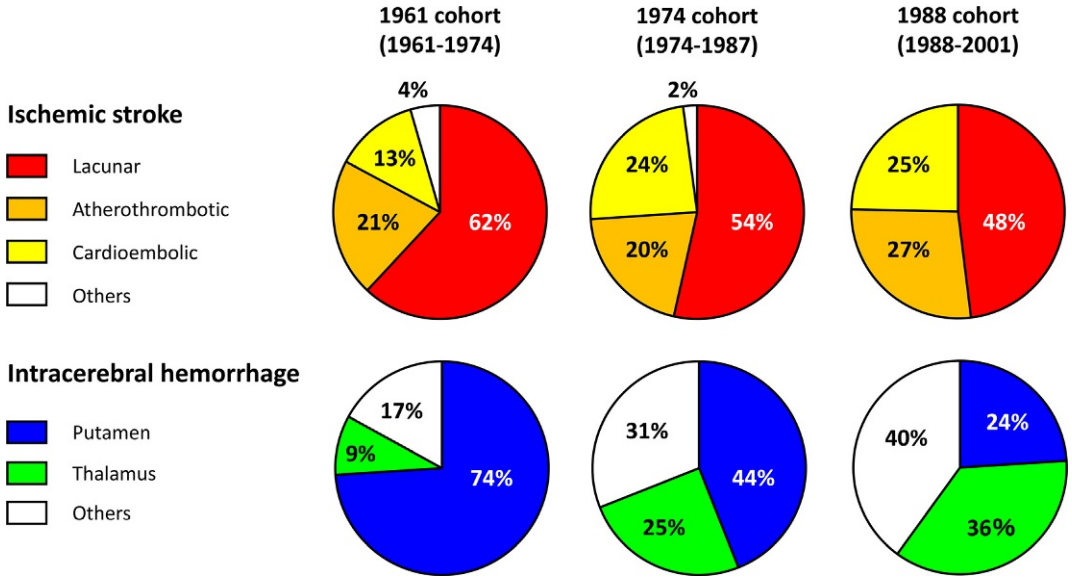

Fig. 6 Proportion of ischemic stroke subtype among patients with ischemic stroke (upper graphs) and proportion of hematoma location among patients with intracerebral hemorrhage (lower graphs) during 7 years of follow-up of each cohort, the Hisayama Study. Adapted from Kubo, M., Hata, J., Doi, Y., et al. (2008). Secular trends in the incidence of and risk factors for ischemic stroke and its subtypes in Japanese population. *Circulation* **118**, 2672–2678; Gotoh, S., Hata, J., Ninomiya, T., et al. (2014). Trends in the incidence and survival of intracerebral hemorrhage by its location in a Japanese community. *Circulation Journal* **78**, 403–409.

atherothrombotic and cardioembolic infarction increased with time probably as a result of increased prevalence of metabolic risk factors and increased number of patients with atrial fibrillation as a result of the super-aged society in Japan (Ohsawa et al., 2005).

Location of Intracerebral Hemorrhage in Hisayama

In the same 13-year cohorts started in 1961, 1974, and 1988, intracerebral hemorrhage events were observed in 43, 33, and 53 subjects, respectively (Gotoh et al., 2014). Fig. 6 shows the secular trends in the distribution of hematoma location among

patients with intracerebral hemorrhage. In the 1961 cohort, the most common location of hematoma was the putamen. While the proportion of putaminal hemorrhage decreased steadily, the proportion of thalamic hemorrhage increased with time. In this study, the incidence of putaminal hemorrhage decreased with time mainly in the relatively young age group (60–69 years) and the incidence of thalamic hemorrhage increased especially in the very old subjects ($\geq$80 years) (Gotoh et al., 2014). The precise reasons for these findings are not clear, but the influence of aging and hypertension, the most powerful risk factors for hemorrhagic stroke, may differ among the locations studied.

The GBD Studies

The GBD studies (GBD 2010 and GBD 2013) have estimated country-specific and global incidence and mortality of stroke using the meta-analysis data from published studies in the world. The detailed country-specific data from the GBD 2010, which examined the secular trends in stroke burden during 1990–2010, are available elsewhere (Krishnamurthi et al., 2013). In this two decades, incidence rates of ischemic and hemorrhagic stroke decreased in high-income countries (13% for ischemic stroke and 19% for hemorrhagic stroke), probably due to improved prevention strategies and better health care. In contrast, in low- and middle-income countries, incidence rates of ischemic and hemorrhagic stroke increased in the same period (6% for ischemic stroke and 22% for hemorrhagic stroke). In these countries, diseases related to infection and undernutrition have been replaced with more chronic diseases such as stroke and other CVD as a result of changes in lifestyles, industrialization, and urbanization. During the same period, mortality rates of ischemic and hemorrhagic stroke showed decreasing trends in high-income countries (37% for ischemic stroke and 38% for hemorrhagic stroke) and in low- and middle-income countries (14% for ischemic stroke and 23% for hemorrhagic stroke) as a result of improved acute and chronic treatment of stroke.

Sex difference in stroke incidence is also an important issue. In the GBD 2013 study, incidence rates of ischemic and hemorrhagic stroke in 1990 and 2013 were compared between men and women (Barker-Collo et al., 2015). Age-adjusted incidence rates of ischemic stroke per 100,000 showed a nonsignificant decrease in men (147.40 in 1990 and 132.77 in 2013) and a significant decrease in women (113.31 in 1990 and 98.85 in 2013). For hemorrhagic stroke, there was little change detected in incidence over time (65.31 in 1990 and 64.89 in 2013 for men; 44.25 in 1990 and 45.48 in 2013 for women). Men had a higher incidence of ischemic stroke than women probably because cardiovascular risk factors are more frequent and severe in men than in women.

The risk of stroke in young adults is generally lower than that of older adults, but the societal impact is high due to the greater number of years of life lost and the resulting loss in productivity. The GBD 2013 study examined the mortality rates of stroke in young adults aged 20–64 years in 1990 and 2013 (Krishnamurthi et al., 2015). During this period, mortality rates of stroke per 100,000 young adults declined in both developing countries (47 in 1990 and 39 in 2013) and developed countries (33.3 in 1990 and 23.5 in 2013) as a result of improved treatment of stroke. However, mortality rate of stroke in developing countries was higher than in developed countries, suggesting that a more aggressive approach toward primary prevention and adequate healthcare services is required to narrow these disparities.

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Ultrafiltration for the Treatment of Acute Decompensated Heart Failure

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Introduction

Heart failure (HF) is a syndrome that affects over 5 million people in the United States and countless additional people across the world (Mozaffarian et al., 2016). A significant feature of HF is the episodic worsening of congestive symptoms, often referred to as acutely decompensated HF (ADHF), which often leads to a hospitalization. The major hallmark of most ADHF hospitalizations is retention of fluid leading, which is the cause of characteristics HF symptoms such dyspnea, orthopnea, and edema (Ambrosy et al., 2014). As such, the main focus of most HF hospitalizations is delivery of therapies aimed at adequately removing excess volume in order to improve symptoms of congestion.

Despite this seemingly straightforward strategy, historical data suggests inadequate volume removal during many ADHF hospitalizations (Fonarow and Horwich, 2003). Incomplete or inadequate therapies can lead to subsequent worsening outcomes including recurrent hospitalizations and decline in overall functional status (Setoguchi et al., 2007; Crespo-Leiro et al., 2016). Therefore, it is paramount to understand methods aimed to facilitate volume removal during an ADHF hospitalization. One such method is extracorporeal removal of fluid, otherwise known as ultrafiltration (UF) or aquapheresis.

Historical Background

The utilization of an extracorporeal circuit to manipulate physiologic volume status can trace its roots back to early attempts to treat acute renal failure. These early attempts were focused on the removal of unwanted metabolic by-products due to inadequate filtration by failing kidneys using a dialysis membrane as part of an "artificial kidney" (Kolff et al., 1944). Subsequent evolution led to the use of intermittent hemodialysis to treat renal failure (Scribner et al., 1960). The modern experience with hemodialysis focuses on the appropriate removal of metabolic by-products unable to be filtered by the failing kidney as well as control of volume status, which is also controlled by the hemodialysis circuit (Flythe and Brookhart, 2014; Weiner et al., 2014).

In the setting of ADHF, however, filtration of metabolic by-products is not necessarily required. Although renal function is known to be impaired in many HF scenarios, there is typically not a complete loss of native renal clearance (Patel et al., 2010). In many instances, appropriate removal of excess volume can result in restoration of renal function (Mullens et al., 2009). Therefore, specifically targeting the excess fluid retention that occurs in ADHF by using extracorporeal means could be an effective strategy. Indeed, early exploratory studies utilized variations of traditional dialysis circuits to treat HF with some degree of efficacy. These early results led to the development of specific UF filters, designed to remove plasma water alone, to be used in HF patients (Costanzo et al., 2017).

Alternative Therapies

The major accepted alternate therapy for volume removal during an ADHF event has been intravenous loop diuretics such as furosemide. Robust clinical data supporting the use of diuretic therapies in the settings as well as data guiding dosing strategies is somewhat sparse. Diuretic therapies earned their place in the treatment of HF prior to the advent of clinical testing, and as such have always been considered "gold standard" against which other therapies have been compared.

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Generally, intravenous loop diuretics are recommended for patients presenting with worsening signs of congestion at a dose equivalent to or greater than their baseline dosing (Yancy et al., 2013). Although typically effective in improving symptoms and volume overload, the use of intravenous loops diuretics may incur some physiologic cost. First, the mechanism of action of loop diuretics results in hypotonic urine, implying less total sodium loss per volume of fluid removed compared to the plasma sodium concentration of the patient. This effect may counteract the intended goal of removing both sodium and water in volume overloaded patients (Costanzo et al., 2017). Additionally, the use of loop diuretics activates neurohormonal pathways that are considered to be detrimental in the setting of HF. One consequence of such activation is diminished response to the same loop diuretics being utilized for therapy (Braam et al., 2012). Historically, utilization of diuretic-based strategies has not resulted in significant improvement in the overall treatment of ADHF (Gheorghiade and Filippatos, 2005).

Some of the difficulties in demonstrating improved outcomes with the use of diuretic therapies may lie in the lack of clarity currently available guiding dosing strategies. This issue was addressed in the Diuretic Optimization Strategies Evaluation (DOSE) Trial. In this trial, 308 patients presenting with ADHF for randomized and a 2×2 factorial designed to receive either low- or high-dose furosemide as either a continuous infusion or via bolus dosing. Patients were followed for the primary endpoint of a change in their own global assessment of symptoms and changes in renal function within the initial 72 h of therapy. Secondary endpoints measured included worsening kidney function. Ultimately, no difference was demonstrated between bolus and continuous infusions, and no statistical difference was found between low- and high-dose strategies (Felker et al., 2011). At present, the prompt administration of diuretic therapy for ADHF remains a Class I recommendation; however adjustments in therapy due to inadequate response, including increasing diuretic dosing or adding a second diuretic agent, carry Class IIa recommendations (Yancy et al., 2013). Specific recommendations on dosing strategies are still lacking.

Tolvaptan, an arginine vasopressin antagonist, can be used to facilitate volume removal. By utilizing a mechanism unique compared to loop diuretics, tolvaptan aims to facilitate free water excretion without activating the neurohormonal pathways seen with loop diuretics. When used in the treatment of an acute decompensation event, tolvaptan was initially shown to improve markers of dyspnea and fluid loss, along with serum sodium levels. However, the effect of tolvaptan was no better than placebo when added to standard HF therapies when assessing long-term outcomes (Konstam et al., 2007). In a follow-up investigation, the Targeting Acute Congestion with Tolvaptan in Congestive Heart Failure (TACTICS-HF) trial, tolvaptan was not associated with improvements in dyspnea but did show greater weight loss and fluid loss (Felker et al., 2017). Currently approved uses for tolvaptan are limited to those patients with hyponatremia and ADHF, which is a Class IIb recommendation (Yancy et al., 2013). The routine use of tolvaptan for ADHF, though, is not supported (Starling and Young, 2017).

Description of UF

UF is an extracorporeal circuit that passes blood through a semipermeable membrane. The creation of a pressure gradient across the membrane allows passage of an iso-osmotic and isonatremic filtrate that is removed from circulation. Hematologic components and other larger molecules, namely potassium, do not pass through the membrane and are returned to the patient. The resultant loss of fluid is achieved without the need to administer loop diuretics and hence can be considered a "diuretic holiday" during therapy (Ronco et al., 2001; Costanzo et al., 2017).

Selection of the membrane to determine which molecules are filtered distinguishes UF from other forms of hemofiltration. Both goals can be achieved through the use of traditional hemodialysis machines if adjusted to the desired effect (Sharma et al., 2001). For situations where only UF is required, such as volume overload in ADHF, two smaller, specialized UF machines exist. These devices allow the use of temporary peripheral, and not central, vascular access to connect a patient with the circuit (Jaski et al., 2003). Depictions of the circuits are shown in **Fig. 1** (Costanzo et al., 2017).

During a treatment course, the rate of fluid removal occurs at a rate set by the user. This rate is adjustable and should be set at a level to allow adequate volume removal without excessively depleting vascular volume, which can lead to hypotension or hypoperfusion (Costanzo, 2008). Theoretically, the removal rate should be set a level equal to the plasma refill rate to allow refilling of the vascular volume by "third spaced" fluid (Marenzi et al., 2001). Although conceptually relatively simple, matching this rate is clinically challenging since it is currently immeasurable in clinical situations. Once the patient's volume status has reached an optimal level, the circuit is discontinued and standard therapies can be resumed.

Clinical Evidence Regarding UF

Several clinical trials have been conducted to examine the efficacy of UF in the treatment of ADHF. Selected studies are described below:

1. Early Ultrafiltration in Patients With Decompensated Heart Failure and Observed Resistance to Intervention with Diuretic Agents (EUPHORIA) trial

   Nonrandomized trial enrolling 20 patients, all of which were treated with UF early in the presentation of ADHF (prior to initiation of IV diuretics). UF resulted in short length of stays with adequate volume removal. Rehospitalizations rates were low following therapy (Costanzo et al., 2005).

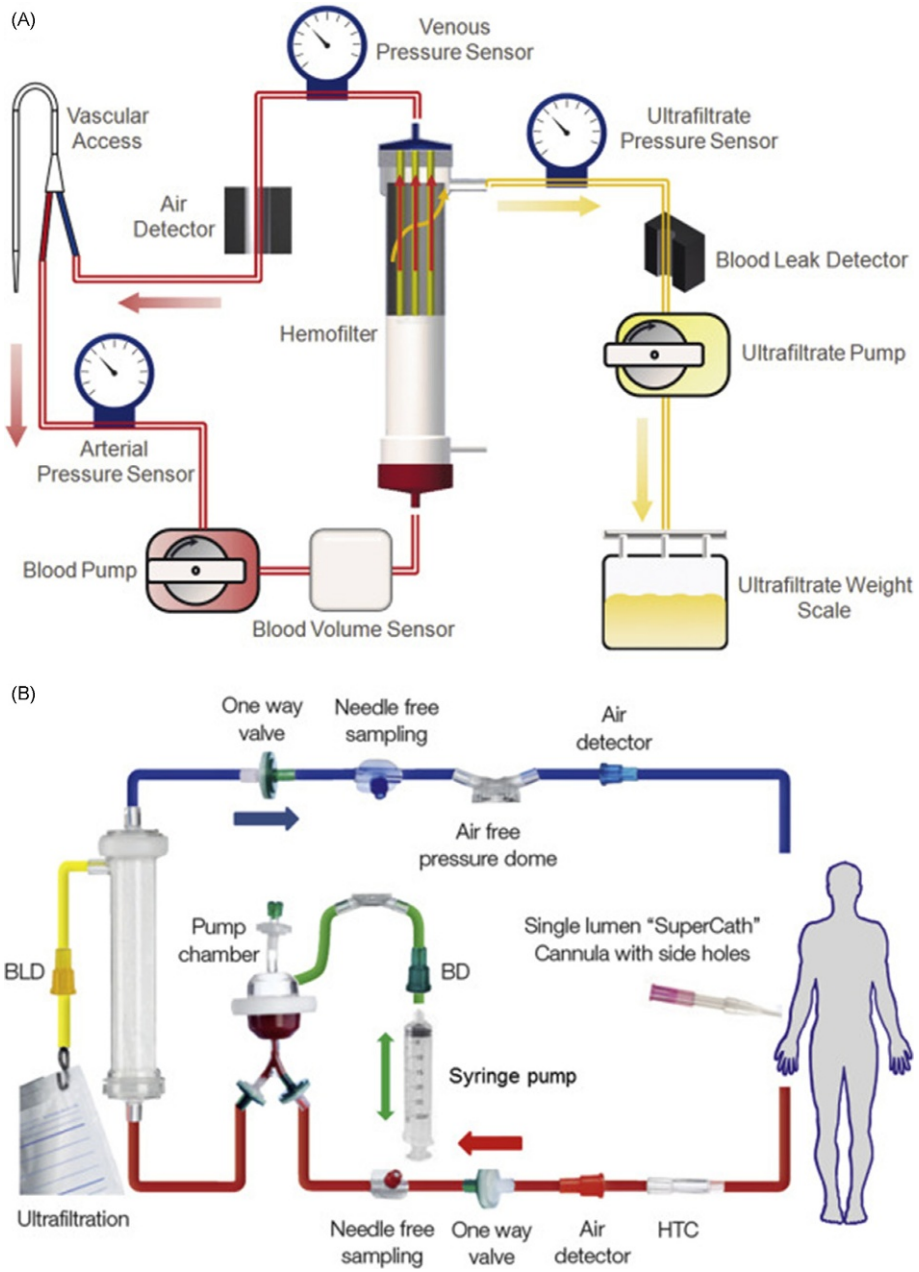

Fig. 1 (A) UF circuit using a multi-lumen venous access catheter allowing blood to pass through a hemofilter to extract ultrafiltrate prior to return into the patient. (B) UF circuit using a single-lumen, multihole peripheral access point. Two one-way valves guide blood flow through a syringe pump-controlled chamber and across a filter from which the ultrafiltrate is removed (Costanzo et al., 2017).

2. Ultrafiltration Versus IV Diuretics for Patients Hospitalized for Acute Decompensated Congestive Heart Failure (UNLOAD) trial
   Randomized controlled trial enrolling 200 patients, treated with either early UF or standard diuretic therapy when presenting with ADHF. Greater weight loss and fluid loss was seen in the UF arm, with suggested lasting benefits after discharge. UF patients had lower diuretic regimens postdischarged compared to IV diuretic patients (Costanzo et al., 2007).
3. Cardiorenal Rescue Study in Acute Decompensated Heart Failure (CARRESS-HF) trial
   Randomized controlled trial intended to enroll 200 patients but halted after 188 enrollments due to worsening renal function in the UF arm. UF was compared to stepped pharmacologic therapy (IV diuretics) for the bivariate endpoint of weight change and kidney function change. In the endpoint analysis, UF fared worse than IV therapies (Bart et al., 2012).
4. Continuous Ultrafiltration for Congestive Heart Failure (CUORE) trial
   Randomized controlled trial comparing adjustable-rate ultrafiltration (AUF) to standard IV therapies for ADHF. A total of 64 patients were enrolled, with those treated by AUF were less likely to be rehospitalized for ADHF within the first year (Marenzi et al., 2014).

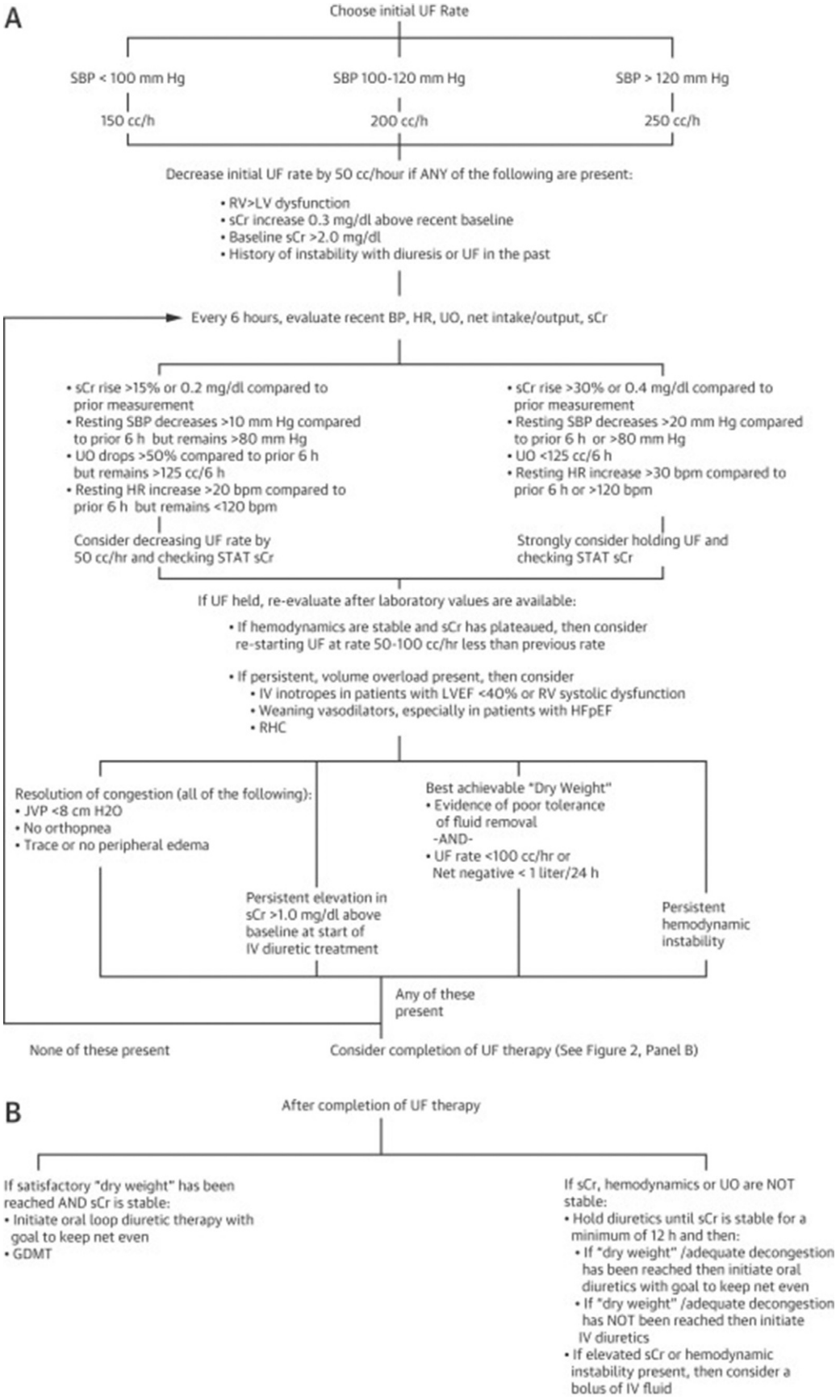

Fig. 2 Suggested algorithm for UF management in treating ADHF. (Costanzo MR, et al, 2017)

5. Aquapharesis Versus Intravenous Diuretics and Hospitalizations for Heart Failure (AVOID-HF) trial

   Randomized controlled trial intended to compare AUF to stepped pharmacologic therapy for the treatment of ADHF. The trial designed called for 810 patients to be enrolled in order to detect a difference in 90-day readmissions for HF. The trial was discontinued after 224 patients following withdrawal of sponsor support. No safety or efficacy concerns were identified, but the trial was underpowered to demonstrate the primary endpoint. Improved outcomes with AUF were suggested but did not meet prespecified criteria for significance (Costanzo et al., 2016).

With the exception of CARRESS-HF, the remaining trials suggest efficacy of UF in the treatment of ADHF. Although enrollment was small in most trials, there exists a fairly consistent signal of improved outcomes beyond the hospitalization when using UF for fluid removal compared to IV diuretics. Nonetheless, the safety concerns and worsening renal function noted in CARRESS-HF must be considered. In evaluating the result of CARRESS-HF further, two specific considerations are noteworthy. First, the signal for worsening renal function in the trial was noted to be an increase of serum creatinine by 0.23 mg/dL when using UF compared to IV diuretics (Bart et al., 2012). This small incremental increase was statistically significant, but the clinical relevance of this change is debatable. A more widely used definition of worsening renal function is a change in serum creatinine of $\geq$0.3 mg/dL (Mehta et al., 2007). If this more standardized definition of kidney injury were to be applied, the true magnitude of worsening kidney function seen in CARRESS-HF may be diminished.

Second, the protocol-specified use of UF was universally set at 200 mL/h of fluid removal (Bart et al., 2012). The use of the same rate for all patients goes against the need to match the needed volume removal rate, either by estimating the plasma refill rate or by individualizing for blood pressure and total excess volume present. The efficacy as well as the safety profile of UF may have been affected the chosen approach. These concerns would have been better addressed by the AVOID-HF trial, which was designed to compare adjustable-rate UF to adjusted medical therapy. The aforementioned early termination of the trial, unfortunately, leaves the question of AUF versus adjustable diuretic therapy unanswered.

Current Status of UF

At present, UF carries a Class IIb recommendation for use in ADHF for both patients in whom response to standard therapy is suboptimal and in patients who have significant signs and symptoms of volume overload (Yancy et al., 2013). UF can be considered as an alternative to intravenous diuretics to treat ADHF, especially when high doses of diuretics may be anticipated. Specific guidelines on the management of UF have not yet been conclusively been proven, but suggested algorithms exist to guide clinicians (**Fig. 2**). UF can be delivered at the bedside using compact machines and may provide lasting benefits beyond the acute treatment phase. Ongoing investigations will help determine the validity of the proposed benefits, as well as better inform the patient selection and management portions of the knowledge base.

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Unstable Angina: Presentation, Diagnosis, and Management

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Introduction

Coronary artery disease (CAD) is characterized by stable or dynamic atherosclerotic obstructions of the coronary vasculature that may result in myocardial ischemia or infarction. CAD itself is a broad descriptor that encompasses several syndromes that may be broadly divided into two categories: the acute coronary syndromes (ACS) and stable ischemic heart disease (SIHD).

The acute coronary syndromes may be further divided into three subcategories: ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI), and unstable angina (UA). In patients with STEMI (also referred to as STE-ACS), plaque instability occurs, typically with components of ulceration, plaque rupture, vasospasm and thrombosis. This results in complete occlusion of the affected coronary artery and necrosis of the subtended myocardium if coronary revascularization is not rapidly achieved. In contrast, NSTEMI and UA are typically the result of subtotal, non-occlusive coronary obstruction resulting in either reversible ischemia (UA) or myocardial necrosis (NSTEMI).

As such, NSTEMI and UA represent different points on the same pathophysiologic continuum of subtotal dynamic coronary artery occlusion, with the distinguishing feature being the presence of elevated cardiac biomarkers in patients with NSTEMI. Furthermore, at presentation these two entities may be indistinguishable owing to initially normal cardiac biomarkers in both settings, and, thus, the initial management of NSTEMI and UA is similar. Given their pathophysiologic similarities, the most recent ACC/AHA guidelines and others have combined these entities into a single group termed the non-ST elevation acute coronary syndromes (NSTE-ACS) (Amsterdam et al., 2014; Roffi et al., 2015). With the advent of ultra-high-sensitive cardiac biomarker assays, very low levels of biomarkers can now be detected, thereby making a biomarker-negative category of ACS increasingly less common (Braunwald and Morrow, 2013). However, for the present time the traditional framework continues to have clinical utility. This chapter will review the current clinical understanding of unstable angina, focusing on its presentation, diagnosis, and management.

Presentation and Diagnosis of Unstable Angina

Presentation

The clinical hallmark of coronary artery disease is that of anginal chest pain, which was originally described by Heberden in 1772. This description has stood the test of time, and is worth revisiting: "They who are afflicted with it, are seized while they are walking (more especially if it be up hill, and soon after eating), with a painful and most disagreeable sensation in the breast, which seems as if it would extinguish life, if it were to increase or continue; but the moment they stand still, all this uneasiness vanishes" (Heberden, 1772). The contemporary description of classic angina is that of a pressure-like discomfort that is deep substernal in location but not precisely localized, that builds over minutes rather than achieving maximum intensity at its onset, that is provoked by exertion or by

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Table 1 Performance of selected clinical findings in ACS

Clinical finding	*LR*	*CI*	*Sens. (%)*	*Spec. (%)*
Chest pain radiating to both arms	2.6	(1.8–3.7)	11	96
Pain similar to prior ischemia	2.2	(2.0–2.6)	47	79
Change in pattern over 24 h	2.0	(1.6–2.5)	27	86
Typical chest pain	1.9	(0.9–2.9)	66	66
Radiation to neck/jaw	1.5	(1.3–1.8)	24	84

Modified from Fanaroff, A. C., Rymer, J. A., Goldstein, S. A., Simel, D. L., Newby, L. K. (2015). Does this patient with chest pain have acute coronary syndrome?: The rational clinical examination systematic review. *JAMA* **314** (18), 1955–1965. https://doi.org/10.1001/jama.2015.12735.

emotional stress, and is relieved with rest or the administration of nitroglycerin. When added to age and gender, the extent to which a patient's symptoms are typical of angina is predictive of the presence of coronary artery disease at angiography (Diamond and Forrester, 1979; Fihn et al., 2012).

While this description of typical angina remains useful, atypical symptoms are present in many patients, particularly in the elderly, diabetics, and females. A review of national myocardial infarction databases demonstrated heterogeneity in symptoms at presentation, and warned against the expectation of typical symptoms in all patients suspected of having ACS (Canto et al., 2000). Dyspnea in isolation is the most commonly noted atypical anginal symptom; other less frequent "angina equivalents" include nausea, epigastric pain, and fatigue.

The pattern of anginal symptoms defines the stability of the coronary syndrome. Chronic stable angina is that which occurs on exertion, usually after a predictable or reproducible amount of effort. In contrast, the symptoms of unstable angina/ACS may occur in several patterns including the presence of symptoms at rest, symptoms that occur with less exertion than previously required, or symptoms occurring for a longer duration (generally greater than 20 min) or with more intensity than usual. The new appearance of angina is also compatible with the presentation of an acute coronary syndrome. It should be noted that the symptoms of UA may be indistinguishable from those of MI, although the later tends to be more intense and prolonged. A classification scheme of different subtypes of UA based on the clinical phenotype has been proposed in the past, but is not routinely used in clinical practice (Braunwald, 1989; Hamm and Braunwald, 2000).

The variety of potential symptoms makes the clinical evaluation of patients suspected of having unstable angina challenging. A recent systematic review and meta-analysis examined the test performance of symptoms in patients presenting with suspected acute coronary syndromes (**Table 1**) (Fanaroff et al., 2015). Pooling from multiple studies, the investigators calculated the test performance characteristics of various presenting symptoms in terms of their sensitivity, specificity, and positive and negative likelihood ratios (LR). From this data, symptoms that were strong predictors of acute coronary syndrome included pain that radiated to both arms (LR 2.6), pain similar to prior episodes of ischemia (LR 2.2), a change in symptom pattern over the preceding 24 h (LR 2.0), pain that is worse with exertion (LR 1.5–1.8) and pain that radiates to the jaw or neck (LR 1.5). "Typical" chest pain had modest sensitivity and specificity (0.66 for both) and while a positive likelihood ratio was noted (LR 1.9) the confidence interval crossed 1 and did not meet statistical significance. Other symptoms that were associated with a low likelihood ratio included pleuritic pain (LR 0.35–0.36) and associated syncope (LR 0.55).

Physical Examination

The physical examination in acute coronary syndrome may be unremarkable; however, important clinical features may be present and may be prognostically important and useful in determining the timing of revascularization and in triage of the patient to the appropriate level of care. A general inspection provides information about the overall comfort of the patient, and may identify associated findings such as diaphoresis, dyspnea, or postures such as Levine's sign (fist clutched over the left chest) suggesting ischemic chest discomfort. Cardiac auscultation may reveal a new murmur suggestive of ischemic mitral regurgitation, a third heart sound suggestive of ventricular volume overload, or a fourth heart sound consistent with impaired diastolic filling. The physical examination may also identify evidence of an alternative diagnosis including musculoskeletal pain (often reproducible on examination), aortic dissection (asymmetric pulses and discrepant BP in upper extremities), pericarditis (friction rub, pulsus paradoxus), pneumothorax (unilateral diminished breath sounds), and pneumonia (rhonchi or pleural rub).

The evidence base for the utility of these physical findings in the setting of UA is rather sparse. A recent systematic review found only one high quality study on physical examination findings in 700 patients with suspected ACS. None of the physical signs studied had a positive likelihood ratio. The physical exam finding with the highest positive LR was hypotension with an LR of 3.9 (95% CI 0.98–15). The sign with the lowest LR was chest pain that was reproduced with palpation, with an LR of 0.28 (95% CI 0.14–0.54) (Fanaroff et al., 2015).

A particular focus at the time of presentation should be the identification of heart failure, as this portends a worse prognosis in all forms of ACS. The Killip classification scheme was originally developed in the 1960s, and provides a prognostic framework for the patient with ACS based entirely on clinical examination findings compatible with heart failure (see **Table 2**). While this classification was originally developed in patients with acute MI, its prognostic value in unstable angina has been validated. In the GRACE

Table 2 Killip classification

	Defining clinical features
Class I	Absence of evidence of heart failure on examination
Class II	Mild to moderate heart failure, evidenced by rales less than halfway up the posterior lung fields, presence of S3, or JVP elevation
Class III	Overt pulmonary edema
Class IV	Cardiogenic shock

Modified from Killip, T., Kimball, J. T. (1967). Treatment of myocardial infarction in a coronary care unit. A two year experience with 250 patients. *American Journal of Cardiology* **20**, 457–464.

registry, patients with unstable angina who developed heart failure during admission had a 15.9% case fatality rate, whereas those who did not develop signs of heart failure had a 1% case fatality rate (Steg et al., 2004).

Diagnostic Evaluation

The clinical diagnosis of unstable angina depends on the presence of ischemic symptoms, which may or may not be accompanied by changes in the electrocardiogram (ECG), and which occur without a detectable increase in cardiac enzymes. As such, UA is the subset of acute coronary syndrome whose diagnosis depends nearly entirely upon clinical history. The discussion above regarding the limited test performance of chest pain characteristics speaks to the difficulty in making the diagnosis of UA, especially at the initial presentation. Without the ability to rely on laboratory testing for diagnosis, unstable angina may be difficult to distinguish from noncardiac, or nonischemic, causes of chest pain, or from myocardial infarction.

The ECG may provide supportive evidence of the diagnosis of UA but is frequently normal (Lev et al., 2003; Slater et al., 1987). Common findings on ECG during an episode of UA that support the diagnosis include ST depression, T wave inversions, or transient ST segment elevations. These abnormal ECG findings in a patient with anginal symptoms have relatively low sensitivity for diagnosing UA, but are fairly specific. A contemporary systematic review of 7 studies examining the performance of the ECG in the diagnosis of any acute coronary syndrome found a positive LR of 5.2 (95% CI 2.8–8.6) for ST depressions, and positive LR of 1.8 (95% CI 1.3–2.7) for T wave inversions (Fanaroff et al., 2015). Serial EKGs should be checked in the initial stages of suspected UA to monitor for dynamic or evolutionary changes suggestive of ACS.

The third universal definition for myocardial infarction is useful in providing a framework by exclusion for distinguishing NSTEMI and STEMI from unstable angina, driven largely by the presence or absence of elevations in cardiac biomarkers (Thygesen et al., 2012). The diagnosis of myocardial infarction requires the typical rise and fall of cardiac biomarkers with at least one value above the 99th percentile upper reference limit, along with at least one of the following: symptoms of ischemia, changes in electrocardiogram (Q waves, ST segment elevation or depression, new left bundle branch block), imaging evidence of loss of viable myocardium, or demonstration of coronary thrombus during angiography or at autopsy. At initial presentation with ACS, it may be unclear if a patient has MI or UA; thus, serial assessment of cardiac biomarkers is necessary. Biomarkers should be checked at presentation and 3–6 h later. The vast majority of patients with MI will develop elevated biomarkers within 6 h of symptom onset.

The development of high sensitivity troponin assays has increased the ability to detect very low-level elevations of troponin in plasma. This increased sensitivity of contemporary troponin assays to detect myocardial necrosis far exceeds that of other cardiac biomarkers. As a result, current guidelines state that CKMB and myoglobin are not useful in the diagnosis of ACS (Amsterdam et al., 2014). The increased sensitivity in laboratory testing may reduce the frequency of the diagnosis of unstable angina, as troponin positivity at lower levels could recategorize many patients with unstable angina as having NSTEMI (Braunwald and Morrow, 2013). Furthermore, while false negatives will be decreased substantially by the use of high-sensitivity troponin assays, detectable elevations in troponin may be found in a sizeable portion of the population not clinically suspected of having an acute coronary syndrome (Deckers, 2013). Some single centers have already reported declines in the rates of diagnosis of unstable angina with current troponin assays (D'Souza et al., 2015), and other studies have demonstrated that detectable troponin levels in asymptomatic individuals correlate with the risk of future coronary events (Ford et al., 2016).

Management of Unstable Angina

Risk Stratification

After the initial evaluation and diagnosis, a critical step in the management of all patients diagnosed with unstable angina is the performance of risk stratification. The identification of patients who are at higher risk provides important prognostic information and allows for triage decisions to be made, especially as related to the decision to pursue an invasive approach with early coronary angiography. While contemporary practices have favored cardiac catheterization for most patients with ACS, risk stratification tools continue to provide a useful framework by which to guide therapeutic decisions.

There are several validated risk stratification tools for use in patients with NSTEMI/UA; two of the most commonly used are the TIMI (Thrombolysis In Myocardial Infarction) and GRACE (Global Registry of Acute Coronary Events) scores. The TIMI risk score was derived from the pooled data of two randomized, controlled trials (RCTs) examining anticoagulation in selected patients

Table 3 TIMI risk score and prognosis

TIMI risk factor	*Comment*	*Points*
Age >65		1
≥3 traditional CAD risk factors	Family history CAD, HTN, HLD, DM, current smoking	1
Known prior coronary stenosis	≥1 known stenosis of >50% on coronary angiography	1
ST segment deviation on EKG		1
Severe anginal episodes	≥2 episodes in 24 h	1
Use of aspirin in last 7 days		1
Elevated cardiac enzymes	Not applicable in unstable angina	1

TIMI Score	*Risk of 14d all-cause mortality, new or recurrent MI, or severe recurrent ischemia requiring revascularization*
≤1	4.7%
2	8.3%
3	13.2%
4	19.9%
5	26.2%
≥6	40.9%

Adapted from Antman, E., Cohen, M., Bernink, P. J. L. M. et al. (2000). The TIMI risk score for unstable angina/non—ST elevation MI. *JAMA* **284** (7) 835–842. https://doi.org/10.1001/jama.284.7.835.

presenting with chest pain, including those with unstable angina (**Table 3**) (Antman et al., 2000). From a regression analysis, seven variables were identified that were predictors of the primary outcome, a composite of 14-day all-cause mortality, MI, or urgent revascularization. The TIMI scoring system has been validated in additional cohorts of patients, and higher TIMI scores correlate with a higher risk of coronary events (Mega et al., 2005). Patients with elevated TIMI scores (e.g., ≥3) derive greater benefit from aggressive antiplatelet therapies and early invasive therapy than do their lower risk counterparts.

The GRACE score was similarly devised to provide prognostic information about patients presenting with ACS, but incorporates more data points than does the TIMI score (Granger et al., 2003). The GRACE score was derived from the international registry of the same name, and the scoring system was subsequently validated among separate patient subsets. The original model estimated the risk of in-hospital mortality with high predictive accuracy (c-statistic value: 0.84). Studies have since demonstrated that the GRACE score not only provides prognostic data during the acute hospitalization for ACS but also predicts outcomes at 6 months, 12 months, and 3 years after ACS (Fox et al., 2014). The GRACE scoring system is not reproduced in this chapter owing to the numerous variables that it takes into consideration; however, several on-line calculators and smartphone applications are available for the calculation of GRACE scores.

Regardless of the exact scoring system used, the prognostic information provided helps guide decision-making in regard to the use of further diagnostic or therapeutic measures. This decision-making process is discussed in more detail below, under invasive therapies.

Medical Therapies

Overview

The medical management of unstable angina involves therapies aimed at the relief of angina, and the inhibition of platelet aggregation (**Table 4**) and of thrombosis (**Table 5**), and consideration of catheterization and revascularization of culprit vessel stenosis.

Antiplatelet therapies

Aspirin

The use of aspirin as a cornerstone of platelet inhibition in ACS has been well established. Aspirin is an irreversible inhibitor of the COX-1 enzyme resulting in a reduction in the synthesis of thromboxane A2, a potent stimulus for platelet aggregation (Brunton, 2006). The data supporting the use of aspirin in unstable angina comes from early trials that were performed prior to the widespread use of early interventional approaches to ACS, and showed a substantial 46% relative risk reduction in major vascular events compared to placebo (Antithrombotic Trialists' Collaboration, 2002). Aspirin has since been a routine part of standard medical therapy in ACS trials, and should be administered to all patients who are suspected of having unstable angina. In aspirin-naïve patients, it should be given as an initial loading dose of 162–325 mg (Amsterdam et al., 2014). The optimal long-term aspirin dosing has been studied in several trials in the NSTEMI and STEMI population, and may be extrapolated to UA. The CURRENT-OASIS trial was designed as a 2×2 factorial trial examining optimal dosing of clopidogrel and aspirin, and the primary endpoint was a composite of death, MI, or stroke at 30 days. When the aspirin dosing subgroups were examined, there were no significant

Table 4 Selected guideline recommendations on antiplatelet therapy for NSTE-ACS

Therapeutic agent	*ACC/AHA*[a]	*ESC*[b]
Aspirin	COR:I, LOE:A	COR:I, LOE:A
Clopidogrel in addition to ASA for 12 months (invasive or ischemia-driven strategy)	COR:I, LOE:B	COR:I, LOE:B
Prasugrel in addition to ASA for at least 12 mo in post–PCI patients treated with coronary stents	COR:I, LOE:B	COR:I, LOE:B
Ticagrelor in addition to ASA for 12 months (invasive or ischemia-driven strategy)	COR:I, LOE:B	COR:I, LOE:B
GpIIb/IIIa Inhibitors (eptifibatide, tirofiban) in early invasive strategy and DAPT with intermediate/high-risk features	COR:II, LOE:B	NR

COR, class of recommendation; *LOE*, level of evidence; *NR*, no recommendation.

[a]Adapted from Amsterdam et al. (2014). AHA/ACC guideline for the management of patients with Non-ST-elevation acute coronary syndromes: A report of the American College of Cardiology/American Heart Association task force on practice guidelines. *Journal of the American College of Cardiology* **64** (24), e139–e228. https://doi.org/10.1016/j.jacc.2014.09.017.

[b]Adapted from Roffi, M., Valgimigli, M., Bax, J. J. et al. (2015) ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *European Heart Journal* **32**, 2999–3054. https://doi.org/10.1093/eurheartj/ehv320.

Table 5 Selected guideline recommendations on antithrombotic therapy for NSTE-ACS

	ACC/AHA[b]	*ESC*[c]
UFH (for 48 h or until PCI performed)	COR:I, LOE:B	COR:I, LOE:B
Enoxaparin (for 48 h or until PCI performed)	COR: I, LOE:A	COR:I, LOE:B
Fondaparinux (for duration of hospitalization or until PCI performed)[a]	COR:I, LOE:B	COR:I, LOE:B
Bivalirudin (until diagnostic angiography or PCI is performed in patients with early invasive strategy only)	COR:I, LOE:B	NR

NR, no recommendation; *COR*, class of recommendation; *LOE*, level of evidence.

[a]Recommended as first-line therapy by the ESC guidelines.

[b]Adapted from Amsterdam et al. (2014). AHA/ACC guideline for the management of patients with Non-ST-elevation acute coronary syndromes: A report of the American College of Cardiology/American Heart Association task force on practice guidelines. *Journal of the American College of Cardiology* **64** (24), e139–e228. https://doi.org/10.1016/j.jacc.2014.09.017.

[c]Adapted from Roffi, M., Valgimigli, M., Bax, J. J. et al. (2015) ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *European Heart Journal* **32**, 2999–3054. https://doi.org/10.1093/eurheartj/ehv320.

differences in ischemic outcomes between low dose (75–100 mg) and high dose (300–325 mg) groups (Mehta et al., 2010). A similar result was found in the TRANSLATE-ACS study, which examined a registry of more than 10,000 patients with STEMI or NSTEMI who underwent percutaneous coronary intervention (PCI) and found no difference in cardiovascular events between high (325 mg) and low dose (81 mg) aspirin (Xian et al., 2015).

Based on these data, aspirin is recommended for all patients with suspected unstable angina without contraindications at the time of presentation. It should be administered as an initial loading dose of 162–325 mg and a maintenance dose of 75–100 mg daily thereafter (Amsterdam et al., 2014).

P2Y12 Inhibitors

The diversity of stimuli that lead to platelet aggregation and contribute to thrombosis has resulted in considerable interest in the development of additional platelet inhibitors for use in patients with ACS. The P2Y12 receptor is a platelet surface receptor that, when activated, results in activation and aggregation of platelets. Inhibitors of this receptor currently available and in use include clopidogrel, prasugrel, and ticagrelor. Older agents, such as ticlodipine, have fallen out of use as a result of their side effect profile.

Clopidogrel

The efficacy of clopidogrel was established in the landmark CURE trial, examining the benefit of clopidogrel when added to aspirin therapy in >12,000 patients with NSTEMI-ACS, approximately 75% of whom were diagnosed with unstable angina at study entry (Yusuf, 2001). This study found a significant reduction in the composite cardiovascular endpoint of death from cardiovascular causes, nonfatal myocardial infarction, or stroke at both 30 days and 1 year. Clopidogrel is absorbed as a prodrug and requires activation to an active form in the liver. Polymorphisms in the CYP system required to metabolize the prodrug have been identified, resulting in variations in the amount of active drug in circulation. Some of these polymorphisms have been associated with higher rates of death from any cause, nonfatal stroke, or myocardial infarction during 1 year of follow-up in those taking clopidogrel (Simon et al., 2009). High rates of these polymorphisms have been identified in patients of Asian heritage and warrant consideration when administering this medication to this patient group (Jang et al., 2012). Despite these considerations, the routine genetic testing for these polymorphisms in patients taking clopidogrel continues to be debated and is not routinely recommended (Aradi et al., 2014; Levine et al., 2011).

Prasugrel

Prasugrel is another P2Y12 inhibitor and is also absorbed as a prodrug. However, it is more rapidly and reliably converted to the active metabolite than is clopidogrel and, therefore, also produces more rapid inhibition of platelet function (Wiviott et al., 2007a). The use of prasugrel was studied in the TRITON-TIMI 38 trial randomizing over 13,000 patients with ACS (of whom 10,000 had either NSTEMI or unstable angina) and who were scheduled for percutaneous coronary revascularization, to treatment with prasugrel or clopidogrel (Wiviott et al., 2007b). The results of the trial showed a statistically significant reduction in the primary composite endpoint of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke in those patients treated with prasugrel. A retrospective analysis of the NSTEMI-ACS patients enrolled in TRITON was performed and confirmed the benefit of prasugrel over clopidogrel in this population (De Servi et al., 2014). However, prasugrel was also associated with an increased risk of major bleeding that was particularly problematic in patients who were elderly (age >75 years), had low body weight (<60 kg), or had a prior stroke. As a result, the use of prasugrel should be avoided in these subgroups. Whereas prasugrel has benefit in patients with ACS who are undergoing PCI, a large RCT revealed that it was not superior to clopidogrel in patients who did not undergo revascularization (Roe et al., 2012). As such, in the setting of UA, prasugrel is currently only indicated for those patients planned to undergo PCI (Amsterdam et al., 2014).

Ticagrelor

Ticagrelor is among the newest of the P2Y12 inhibitors. Unlike clopidogrel and prasugrel, it is neither a prodrug nor a thienopyridine, and it functions as a reversible P2Y12 inhibitor. Its efficacy was evaluated in the PLATO trial, which studied over 18,000 patients with ACS (17% of patients with UA) who were randomized to receive either clopidogrel or ticagrelor in addition to aspirin (Wallentin et al., 2009). The trial showed a 16% reduction in the composite primary endpoint of cardiovascular death, myocardial infarction, or stroke in patients treated with ticagrelor. While there were no differences in overall major bleeding between the ticagrelor and clopidogrel groups, there was an increase in nonprocedure-related major bleeding including fatal intracranial bleeding in the ticagrelor treated group.

Current guidelines suggest the addition of clopidogrel or ticagrelor to aspirin for patients with NSTEMI-ACS; however, there is no compelling data to favor one over the other. The upstream use of P2Y12 inhibitors in patients with unstable angina who ultimately undergo CABG for revascularization is potentially problematic due to the persistence of the medication, and a higher risk of operative bleeding. Thus, the possibility of the need for surgical revascularization should be considered prior to starting dual antiplatelet therapy in patients with UA. It is currently recommended to discontinue clopidogrel or ticagrelor for 5 days prior to surgery and prasugrel for 7 days prior to surgery (Amsterdam et al., 2014; Roffi et al., 2015).

GpIIb/IIIa Inhibitors

Inhibition of the GpIIb/IIIa platelet surface complex produces significant inhibition of platelet function, and may be useful in the treatment of unstable angina. The currently available GpIIb/IIIa inhibitors are tirofiban and eptifibatide (both small molecule inhibitors), and abciximab (a monoclonal antibody). Previously, the use of GpIIb/IIIa inhibitors was common in the setting of ACS; however, since the advent of oral P2Y12 inhibitors the use of GpIIb/IIIa inhibitors has declined and their routine use in the management of ACS is no longer recommended (Amsterdam et al., 2014; Roffi et al., 2015).

Antithrombotic therapies

Unfractionated heparin

Unfractionated heparin (UFH) is a polymeric inhibitor of the coagulation cascade. Its pharmacologic effect relates to the inactivation of thrombin and factor Xa in a manner dependent on interaction with the molecule antithrombin (Halperin, 2001). The original efficacy of heparin was established in addition to aspirin, with reduction in cardiovascular events in patients with unstable angina (Theroux et al., 1988). In the setting of UA, the administration of UFH for 48 h decreases the risk of recurrent ischemic events. There is, however, wide variability in pharmacokinetics and patients require weight-based dosing and frequent dose adjustments to ensure that the anticoagulant effect remains within a relatively narrow therapeutic window. Despite these limitations, UFH has the advantage of being able to turn on or off a continuous infusion and to rapidly reverse its effects with protamine, rather than await the longer half-lives of alternative antithrombotic agents. It is in this regard that heparin may have an advantage for those imminently planned for catheterization. Given its practicality, UFH persists as a reasonable antithrombotic choice albeit with justifiable concerns about its narrow therapeutic window and variability of pharmacokinetics.

Low-molecular-weight heparin

The low-molecular-weight heparins (LMWHs), of which enoxaparin is the most commonly used, are glycosaminoglycan chains similar to unfractionated heparin but synthesized at a much lower and more uniform weight (mean molecular weight of 5000) (Weitz, 1997). As a result, LMWH has greater specificity for the inhibition of Factor Xa than does UFH, and has been found to have a more reliable dose response and more stable pharmacokinetics. LMHW is dosed in a weight-based fashion and requires adjustments for reduced GFR due to its renal clearance.

Several studies have established the efficacy of LMWH in acute coronary syndromes. The ESSENCE trial was performed in patients with NSTEMI or unstable angina, who were randomized to receive either UFH or LMWH (Cohen et al., 1997). The results of

the trial showed a reduction in the composite endpoint of death, myocardial infarction, or recurrent angina at 30 days in the LMWH group (16.6% vs 19.8%). There was no difference in major bleeding noted between the agents; however, LMWH was associated with an overall increased risk of bleeding compared with UFH, primarily resulting from ecchymoses at injection sites. Importantly, this trial was performed in patients being treated primarily with medical therapy, and was performed prior to the widespread use of P2Y12 inhibitors. Examination of the difference between LMWH and UFH in more contemporary cohorts with higher rates of P2Y12 inhibitor use, and higher rates of coronary angiography and interventions, has yielded more equivocal results with noninferiority seen in some trials and meta-analyses (SYNERGY Investigators, 2004; Silvain et al., 2012).

The use of enoxaparin as an initial therapy in patients with unstable angina should continue until PCI, or for a period of at least 48 h for those managed noninvasively. In the patient who is given enoxaparin as initial therapy and is then taken for percutaneous intervention, enoxaparin should be continued as the antithrombotic agent of choice during the procedure, given an observed increase in significant bleeding in patients who cross over to UFH at the time of catheterization (Petersen et al., 2004).

Fondaparinux

Fondaparinux is a pentasaccharide molecule that results in selective inhibition of factor Xa, has high bioavailability and a reliable dose response. The medication is administered subcutaneously, and can be given once a day. Its efficacy in the management of acute coronary syndromes was established in the OASIS trial, which compared the use of fondaparinux to enoxaparin in more than 20,000 patients, 45% of whom had a diagnosis of unstable angina. The overall results of the trial demonstrated noninferiority of fondaparinux with regard to the primary composite endpoint of death, myocardial infarction, or refractory ischemia (Anon, 2006). There was a strong trend toward reduced bleeding events, including procedural bleeding, in the group treated with fondaparinux. Importantly, patients undergoing catheterization were noted to have increased rates of catheter thrombosis. Subsequent analyses have demonstrated that this effect was minimized with the use of a bolus of UFH at the time of catheterization.

Bivalirudin

Bivalirudin is an intravenous direct thrombin inhibitor that is a congener of hirudin, an anticoagulant first identified in leeches. It is a reversible inhibitor of thrombin and requires dose adjustment to maintain anticoagulation parameters within an acceptable therapeutic window. The use of bivalirudin in ACS including unstable angina has been studied extensively in comparison to other antithrombotic regimens. The early studies were somewhat confounded by the co-administration of GpIIb/IIIa inhibitors with heparin and a variable use of P2Y12 inhibitors (Stone et al., 2006). A meta-analysis of these early trials included a subgroup analysis of the use of bivalirudin compared to heparin alone (not in combination with GpIIb/IIIa inhibitors) and demonstrated similar rates of mortality and MI, and reduced bleeding with bivalirudin (Navarese et al., 2015). There was notably a significant increase in acute stent thrombosis appreciated when bivalirudin was compared to heparin alone in this meta-analysis. However, a recent randomized control study comparing heparin and bivalirudin in patients with NSTE-ACS being managed with a planned invasive strategy demonstrated similar rates of cardiovascular events (Valgimigli et al., 2015). Bivalirudin is a reasonable option for antithrombotic therapy in patients with UA who are planned for coronary angiography and intervention.

Antianginal therapies

An important goal in the early management of unstable angina is the relief of ischemic symptoms, which is usually achieved by correcting the oxygen supply–demand mismatch that is present in ischemic myocardium. Given that oxygen extraction in resting myocardium is already near maximal, restoring oxygen balance to the myocardium can only be accomplished in one of two ways: reduction in myocardial demand or increase in coronary oxygen delivery. The therapies used to achieve these goals are described below.

Oxygen

Supplemental oxygen may increase the oxygen content of blood and thereby deliver more oxygen to ischemic myocardium. However, given the sigmoidal curve of the oxygen–hemoglobin dissociation curve, the oxygen-carrying capacity of hemoglobin is generally saturated at ambient levels of oxygen unless an additional process is ongoing (Hall and Guyton, 2011). The use of oxygen by nasal cannula had been a routine measure taken for patients presenting with unstable angina; however, an increased awareness of oxygen toxicity, including adverse effects of hyperoxia on coronary hemodynamics, has led to a reduction in the routine use of supplemental O2. The potential for adverse effects is supported by the results of randomized studies in patients with STEMI, in which no benefit, and some markers of harm, were reported in patients without hypoxemia who were treated with supplemental oxygen (Stub et al., 2015). In unstable angina, the use of supplemental oxygen should be restricted to the hypoxemic patient.

Beta-blockers

Beta-blockers work to slow heart rate, decrease myocardial contractility, lower blood pressure (and ventricular wall stress), and thereby reduce the oxygen consumption of myocardial cells in unstable angina. While there is no defined goal for heart rate reduction to guide titration, initiation of beta-blockers at a low dose to ensure tolerance is recommended (Amsterdam et al., 2014). The evidence for the efficacy of beta-blockers in unstable angina is extrapolated from studies in acute coronary syndrome in general, but a trend toward reduced cardiovascular events (reinfarction, ventricular arrhythmias, and long-term mortality) has been seen in several meta-analyses (Kontos et al., 2011; Chatterjee et al., 2013). However, beta-blockers must be used with caution in patients at risk for cardiogenic shock including those >70 years of age, and those with hemodynamic abnormalities including a heart rate

greater than 110 beats/min, and systolic blood pressure below 120 mmHg. Similarly, the use of beta-blockers in patients with suspected or known left ventricular dysfunction must be judiciously considered. Nonetheless, beta-blockers should be administered to all patients with ACS who do not have a contraindication.

Calcium channel blockers

Similar to beta-blockers, the non-dihydropyridine calcium channel blocking agents produce a decrease in heart rate, contractility, and wall stress, thereby reducing the myocardial oxygen demand. They are generally considered as second line to beta-blockers, as the evidence supporting their use is generally less robust. Early trials in unstable angina showed reduction in the rates of recurrent infarction with the use of diltiazem or verapamil; however, these agents must be used with caution in patients with depressed left ventricular systolic function or with overt heart failure (Smith et al., 1998).

Nitrates

Nitroglycerin preparations produce endothelium-dependent veno- and vasodilation resulting in decreases in preload and afterload as well as coronary vasodilation. These effects help to decrease myocardial oxygen demand and increase coronary blood flow, thus reducing myocardial ischemia. The primary utility of nitrates has been for the relief of ischemic symptoms, as the use of these agents has not translated into a reduction in hard clinical events in the limited trials that have addressed this question in the setting of NSTE-ACS. Sublingual nitroglycerin should be administered acutely to patients with angina symptoms and can be repeated every 5 min until relief of symptoms, or the medication is no longer tolerated. Patients with persistent angina should be started on an intravenous infusion of nitroglycerin, which can be up-titrated to achieve angina relief. Intravenous nitroglycerin may also be useful for the management of hypertension or heart failure during NSTE-ACS. Nitrates may produce hypotension in patients with aortic stenosis, right ventricular dysfunction/infarct, or volume depletion, and should be avoided in these settings. In addition, nitrates are contraindicated in patients who have recently used phosphodiesterase inhibitors such as sildenafil and tadalafil, owing to the risk of hemodynamic compromise.

Morphine

Morphine has several potential beneficial effects in patients with unstable angina, including the relief of pain and anxiety, factors which may increase myocardial oxygen demand through increased heart rate and blood pressure. There have been no definitive trials to define the role of morphine in UA; rather, its use is supported by clinical experience. Nonetheless, caution should be used when administering morphine to ACS patients with acute heart failure as observational studies suggest an increase in adverse events in this setting. There is some data to suggest that morphine decreases absorption of P2Y12 receptor inhibitors such as clopidogrel resulting in decreased circulating active metabolites; however, there is currently no data to suggest an increase in ischemic events in patients receiving both agents (Hobl et al., 2014). Thus, at present, morphine remains the analgesic of choice in patients with UA and ongoing chest pain.

Cholesterol Lowering Therapies

Cholesterol lowering therapy, primarily achieved with statin medications, is well established in the secondary prevention of coronary artery disease. Meta-analyses of statin therapy in patients with CAD have demonstrated a reduction in the risk of mortality on the order of 16% (Wilt et al., 2004). A complete discussion of statin therapy is beyond the scope of this chapter; however, given the beneficial effects of these agents in the setting of ACS and stable CAD, all patients presenting with unstable angina should receive high-intensity statin therapy in the absence of contraindications (Amsterdam et al., 2014; Roffi et al., 2015). A high-intensity statin therapy has been defined by the ACC/AHA as therapy known to reduce LDL cholesterol by 50% or more, and includes atorvastatin 40–80 mg daily and rosuvastatin 20–40 mg daily (Stone et al., 2014). The use of statin therapy in patients with established CAD and age >75 is not well studied, and a moderate intensity strategy may be reasonable in this older population.

Invasive Strategies for Unstable Angina

As an important early step in the management of unstable angina, all patients should undergo risk stratification to estimate their short- and medium-term risk of adverse events. The TIMI and GRACE scoring systems are described above, and help to identify patients who are at increased risk for complications such as death, recurrent MI, and the need for repeat revascularization.

An understanding of this risk is necessary to inform decision-making in regard to the timing and necessity of coronary angiography and PCI. There have been multiple studies comparing a conservative approach of optimized medical therapy with an invasive strategy in patients with NSTE-ACS, including in patients with unstable angina. These studies have taken place over several decades, during which medical therapies and invasive techniques have changed significantly, and the outcomes of the studies have varied. Nonetheless, several meta-analyses of routine invasive strategies for patients with NSTE-ACS have shown a reduction in future adverse cardiovascular events when an invasive approach is pursued. One such meta-analysis revealed a relative risk reduction in cardiovascular death or nonfatal MI of 19% at 5 years of follow-up in patients receiving invasive therapies compared with conservative management (Fox et al., 2010).

In general, patients with high-risk features derive more benefit from an early invasive approach than do low-risk patients. Patients with persistent or recurrent angina despite optimal medical therapy should undergo urgent catheterization with the goal of revascularization if feasible, as should patients in whom UA is accompanied by heart failure, hemodynamic instability, or sustained ventricular arrhythmias. An invasive approach should also be considered for patients with UA who have elevated risk scores (e.g., TIMI score >2, GRACE score >109), depressed LVEF, diabetes, prior coronary revascularization, or evolutionary ischemic changes on serial electrocardiograms. Patients without high-risk clinical markers and with low-risk scores (e.g., TIMI score 0–1, GRACE score <109) can often be managed with an ischemia-guided strategy. With this strategy, optimal medical therapy is the primary therapeutic approach with invasive evaluation reserved for those patients who have refractory angina despite medical therapy or have objective evidence of ischemia on predischarge noninvasive stress testing.

A discussion of invasive procedural considerations and decisions regarding revascularization strategies (i.e., PCI versus CABG) is beyond the scope of this chapter, and is described elsewhere. Patient comorbidities and preference should be taken into consideration when making decisions regarding the invasive evaluation of patients with unstable angina and their subsequent revascularization.

Conclusions

The acute coronary syndromes represent a critical instability in coronary atherosclerotic disease. The diagnosis of unstable angina remains a clinical challenge but is supported by historical features and findings on physical examination. Unstable angina reflects one end of the spectrum of ACS, and is distinguished from myocardial infarction by the presence of elevated cardiac biomarkers in the latter setting. The prevalence of UA may decrease in the future as emerging biomarkers with greater sensitivity are able to identify minimal elevation in cardiac enzymes and, thereby, reclassify patients previously diagnosed with UA as having NSTEMI. Aggressive medical therapy should be initiated early in patients with UA with a combination of antiplatelet, antithrombotic, and antianginal agents, as well as cholesterol lowering therapies. Early risk stratification should be performed to both inform prognosis and guide the consideration of invasive evaluation with catheterization and subsequent coronary revascularization. With the advances in therapies described above, there has been substantial improvement in the natural history of this ominous condition.

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Further Reading

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V

Vascular Guidance Cues

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The functioning of essentially all organs and tissues of vertebrates relies on the circulation of blood, which provides the necessary nutrients and removes harmful waste products, and on the conveyance of biochemical and electrical signals that control and coordinate the execution of tissue and organ tasks (Standring, 2016). These complex functions are, respectively, under the control of two pervasive and ubiquitous anatomic systems, namely, the vascular and the nervous system, which physically interacts and ramifies in architecturally similar patterns (Standring, 2016). Altogether, these observations would imply some degree of functional interdependence, such as the requirement for oxygen and nutrients by nerves, as well as the responsiveness to the same guidance cue (s) by the two systems during embryonic development, when vascular endothelial cells (ECs), neurons, and neuronal growth cones travel over long distances to achieve their final destinations.

In 1989, Paul Martin and Julian Lewis (Martin and Lewis, 1989) provided the first experimental evidences supporting the hypothesis that nerves and blood vessels frequently navigate along the same route because they likely respond to the same steering cues whose molecular nature and signaling mechanisms were, at that time, still unknown. Over the last two decades, several laboratories contributed in developing a wide and detailed knowledge about the different guidance cues and signaling pathways that control both nervous and vascular guidance emerged (Blockus and Chédotal, 2016; Kania and Klein, 2016; Mehlen et al., 2011; Worzfeld and Offermanns, 2014). It emerges that ECs and neuron growth cones are guided in their journey throughout by the same families of plasma membrane-associated or secreted proteins that elicits either attractive or repulsive cytosolic pathways that impact on cytoskeletal, trafficking, and adhesive dynamics. Here, we will review the literature describing how the major vascular guidance cues were first identified and their signaling mechanisms and function were then thoroughly characterized.

Ephrins and Eph Receptors

The first Eph receptor was isolated in a screening aimed at identifying new tyrosine kinases endowed with tumorigenic function (Hirai et al., 1987). The name Eph derives from erythropoietin-producing hepatocellular carcinoma cell line, from which its cDNA was initially cloned. Since then, additional Eph receptors were discovered and divided in categories based on their sequence homology (Kania and Klein, 2016). Fourteen Eph receptors members exist and are classified into two subfamilies: type A (EphA1-8 and EphA10), which bind to their cognate ligand type A ephrins (ephrin-A1-5) and type B (EphB1-4 and EphB6), which bind to type B ephrins (ephrin-B1-3) (Kania and Klein, 2016). However, this sort division is somehow arbitrary since some EphB receptors can bind ephrin-A ligands and EphA receptors can bind ephrin-B ligands (Taylor et al., 2017).

EphA and EphB receptors share a similar structure (Kania and Klein, 2016). They have a ligand-binding domain and a cysteine-rich region (Cys domain) followed by two FNIII domains in the extracellular portion, a single transmembrane domain and an intracellular juxtamembrane domain (important for receptor activation), a kinase domain, a sterile alpha motif (required for receptor oligomerization), and a PDZ domain-binding motif (Kania and Klein, 2016). Ephrins are ligands tethered to the cell

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surface, and the two subfamilies are very different from each other (Kania and Klein, 2016). Class B ephrins have an extracellular receptor-binding domain, a transmembrane domain, and a short cytoplasmic tail, containing conserved tyrosine residues and a PDZ-binding site. On the contrary, class A ephrins are smaller, being composed only by a receptor-binding domain tethered to the membrane through a glycosylphosphatidylinositol (GPI) anchor. The fact that Eph ligands are linked to cell surface implies that the repulsive or attractive signaling through these receptors depends on cell-to-cell contacts. Moreover, the ephrin-Eph signaling can occur bidirectionally, and the interaction takes place between ligand and receptor on two neighboring cells or on the same cell (Kania and Klein, 2016). Another order of complexity is achieved thanks to the fact that, to be efficiently activated, both ephrins and Eph receptors aggregate into homotypic or heterotypic clusters (Taylor et al., 2017).

The development of the vascular system largely relays on the specific ephrin-B2/EphB4 ligand-receptor pair although some findings highlighted functional redundancies involving other ephrins and Eph receptors (Adams et al., 1999). From the onset of embryonic angiogenesis, Ephrin-B2 and its cognate receptor EphB4 are expressed by arterial and venous ECs, respectively, and serve to establish the identities of each vascular compartment (Wang et al., 1998). Moreover, ephrin-B2 and EphB4 reciprocal interaction promotes the structural and functional cooperation between arteries and veins that is essential for their effective angiogenic remodeling. Indeed, due to severe embryonic and yolk sack defects in both arterial and venous vessels growth and remodeling, ephrin-B2 or EphB4 knockdown causes the death of mouse embryos at midgestation (Gerety et al., 1999; Wang et al., 1998). Ephrin-B2 and EphB4 participate in the formation of arteries and veins acting downstream of the transcription factors Notch and chicken ovalbumin upstream promoter–transcription factor II (Coup-TFII). While Notch signaling promotes arterial and suppresses venous fates by upregulating ephrin-B2 and downregulating EphB4, Coup-TFII represses Notch signaling in venous ECs (You et al., 2005). Ephrin-B2 and EphB4 repulsive signaling is required for the formation of the two major embryonic blood vessels, the dorsal aorta and the cardinal vein. First in the zebrafish and more recently in mouse models, it has been demonstrated that the dorsal aorta assembles first and contains venous precursors. This mixed population of ECs in dorsal aorta is characterized by the presence of two different signatures: arterial precursors expressing ephrin-B2 and venous ones expressing EphB4. Ephrin-B2–EphB4 repulsive signaling promotes the relocation of venous ECs and the ensuing assembly into cardinal vein (Herbert et al., 2009; Lindskog et al., 2014).

The Ephrin-B2–EphB4 interaction is also an important regulator of sprouting angiogenesis, as extensively studied in postnatal mouse retina and in pathological tumor vascularization mouse models, wherein it cooperates with and controls VEGF signaling. VEGF gradients promote the migration of tip cells of vascular sprouts, promoting internalization of their receptors, which is necessary to foster downstream signaling and induce filopodia extension (Sawamiphak et al., 2010; Wang et al., 2010). In this context, ephrin-B2 and its intracellular interactors, the clathrin-associated protein disabled 2 (Dab2) and the cell polarity regulator PAR-3, enable clathrin-dependent VEGFR2 internalization and downstream signaling, being both negatively regulated by atypical protein kinase C (Nakayama et al., 2013b; Sawamiphak et al., 2010; Wang et al., 2010).

Upon EphB binding, ephrin-B reverse signaling is activated thanks to the phosphorylation of its cytoplasmic domain by Src family kinases. Once phosphorylated, ephrin-B recruits the PDZ containing phosphatase PTP-BL that switches off the signaling of ephrin-B itself (Palmer et al., 2002). Recently, ephrin-B2 was shown to control EC survival and death, which in turn regulate hyaloid and retinal vessel pruning in the eye, in a VEGF-independent manner (Salvucci et al., 2015). Ephrin-B2 is phosphorylated in the remodeling vasculature during eye development, wound healing, and tumor growth (Salvucci et al., 2015). At sites of blood vessel pruning, signals that induce ephrin-B2 dephosphorylation promote JAK2/STAT1 activation and the ensuing c-Jun N-terminal kinase 3-mediated EC apoptosis and vascular degeneration (Salvucci et al., 2015). Interestingly, mature quiescent blood vessels do not depend on ephrin-B2 phosphorylation for their integrity, suggesting that other mechanisms are involved in the stabilization and maintenance of the nonangiogenic vasculature. Ephrin-B2 was also found in venous valves, and the induction of its expression in venous ECs is one of the first events during valve development (Bazigou et al., 2011). Continuous ephrin-B2 signaling is also required for valve maintenance, albeit the precise underlying mechanism of action remains to be clarified (Bazigou et al., 2011).

Ephrin-B2 is also expressed in pericytes and vascular smooth muscle cells (VSMCs) and regulates their migration and adhesion during the assembly of the blood vessel wall. A conditional gene targeting approach, aimed to selectively disrupt the expression of ephrin-B2 in mural cells, revealed that knockout pericytes and VSMCs failed to develop mature contacts with ECs, causing alterations in blood vessel architecture and hemorrhages. A complex formed by the adaptor proteins Crk and p130Cas is involved in the ephrin-B2 signaling that promotes mural cell spreading and motility (Foo et al., 2006). In addition, a more recent study revealed that in mural cells ephrin-B2 interacts with PDGF receptor β (PDGFRβ) to control its localization, internalization, and signaling (Nakayama et al., 2013a). In the absence of ephrin-B2, PDGFRβ internalization and the ensuing Tiam1–Rac1 activation is impaired, while mitogen-activated protein kinase and JNK signalings are instead increased (Nakayama et al., 2013a). As a result, mural cells migrate and proliferate less, finally resulting into blood vessel wall defects and aortic aneurysms (Nakayama et al., 2013a).

Semaphorins, Neuropilins, and Plexins

Semaphorins (Semas) are a large family of secreted or membrane-associated glycoproteins that elicits repulsive or attractive signals on migrating cells. Originally identified as axon guidance molecules in the developing nervous system (Kolodkin et al., 1993; Luo et al., 1993), Semas were later involved in many physiological and pathological processes, such as vascular EC motility, cardiovascular development, lymphocyte activation, bone and lung morphogenesis, cancer angiogenesis, and metastatic dissemination

(Epstein et al., 2015; Kruger et al., 2005; Neufeld et al., 2012; Serini et al., 2013). The Sema family consists of a large number of phylogenetically conserved proteins characterized by an extracellular amino-terminal homodimeric "sema" domain consisting of two β-propellers that bind and elicit the dimerization of Sema receptors (Siebold and Jones, 2013). Based on the carboxyl-terminal domains and phylogenetic analyses, eight Sema classes have been identified: class 1 and 2 are encoded by invertebrates, classes 3–7 are from vertebrates, and class V Sema are found in viruses. The seven vertebrate class 3 Semas (Sema3A–G) are produced as secreted proteins by cells of multiple lineages, including ECs and epithelial cells, neurons, and tumor cells. From the N-terminus to the C-terminus, Sema3 contains a sema domain followed by a plexin–semaphorin–integrin (PSI) domain, an immunoglobulin (Ig)-like domain, and a basic domain. Sema3 holoreceptor complexes belong to the families of plexins and neuropilins (Nrps).

Plexins are a family of nine transmembrane proteins subdivided into four (A to D) classes. Their intracellular region contains a guanosine triphosphatase (GTPase)-activating protein (GAP) domain (Wang et al., 2012, 2013; Worzfeld and Offermanns, 2014; Worzfeld et al., 2014), whereas their N-terminal Sema-PSI domain combination mediates the binding to Sema homodimers (Janssen et al., 2010, 2012; Liu et al., 2010; Nogi et al., 2010; Olsson et al., 2006; Siebold and Jones, 2013). Compared to membrane-associated Semas, secreted Sema3 proteins display a less hydrophobic dimeric interface that need to be stabilized by disulphide bonds between Ig domains, which are negatively regulated by the proteolytic activity of furins (Adams et al., 1997; Koppel and Raper, 1998). Electrostatic interactions mediate a head-to-head interface between each sema domain of a Sema dimer and the sema domain of a monomeric plexin, giving rise to a 2:2 Sema–plexin heterotetramer (Janssen et al., 2010; Liu et al., 2010; Nogi et al., 2010; Siebold and Jones, 2013). The same head-to-head interface is probably employed by Sema3A to bind to and signal through plexin receptors (Nogi et al., 2010). Nevertheless, since no high-affinity binding has been revealed so far (Janssen et al., 2012), the interaction between the sema domains of Sema3A and plexins must be extremely weak and the involvement of coreceptors, such as Nrp1 (Janssen et al., 2012) or proteoglycans (Casazza et al., 2013; Pang et al., 2015), is likely needed. The intracellular region of plexins is highly conserved and contains two large portions that are highly homologous to Ras GAP domains (Hu et al., 2001; Rohm et al., 2000). These domains were reported to exert their enzymatic activity on two Ras-related small GTPase proteins: R-Ras (Oinuma et al., 2004) and M-Ras (Saito et al., 2009). However, subsequent studies did not confirm that Plexins exert GAP activity toward R-Ras or M-Ras (Sakurai et al., 2010; Tong et al., 2009; Wang et al., 2012). Similarly, analyses of knock-in mice carrying inactivating mutations in the GAP domain of Plexin D1 or B2 unveiled an R-Ras and M-Ras independent function for those domains in the control of embryonic development (Worzfeld et al., 2014). Wang and colleagues provided instead evidence that purified cytoplasmic regions of different Plexins exert their GAP activity on the small GTPase Rap1 and that this function was required for Plexin-mediated neuronal growth cone collapse (Delcommenne et al., 1998). The same laboratory also described the crystal structures of zebrafish Plexin C1 cytoplasmic region in complex with Rap1 (Wang et al., 2013). Of note, the small GTPase Rap1 effectively controls vascular morphogenesis (Chrzanowska-Wodnicka, 2013) and promotes the conformational activation of integrins via talin and the adhesion of different cell types to the extracellular matrix (Calderwood et al., 2013; Chrzanowska-Wodnicka, 2013).

In vertebrates, Nrp1 and Nrp2 act as Sema3 coreceptors (Guo and Vander Kooi, 2015). The extracellular parts of both Nrps contain two complement binding domains (a1/a2), two coagulation factor V/VIII homology domains (b1/b2), and a MAM domain (c); while the short cytoplasmic domain is about 40 amino acids long and contains a C-terminal PDZ-binding SEA motif. In addition to Sema3, Nrp1 and Nrp2 are coreceptors also for vascular endothelial growth factor-A (VEGF-A) and -C (VEGF-C) family members, respectively (Guo and Vander Kooi, 2015; Parker Matthew et al., 2015; Soker et al., 1998). The b1 domain of Nrp1 mediates the high-affinity binding to the basic domain of VEGF-A and Sema3 proteins (Giger et al., 1998; Nakamura et al., 1998; Parker et al., 2012b,c; Renzi et al., 1999; Siebold and Jones, 2013), in which a C-terminal arginine is naturally present or exposed upon furin-dependent cleavage, respectively (Adams et al., 1997; Guo et al., 2013; Guo and Vander Kooi, 2015; Parker et al., 2010, 2012b,c). The a1 domain of Nrp1 does not directly bind with high affinity the sema domain of Sema3A (Janssen et al., 2012) but favors its attachment to the sema domain of type A plexins, such as Plexin A2 (Janssen et al., 2012; Parker et al., 2012a; Siebold and Jones, 2013).

Sema3A

Sema3A is required for the appropriate pattern establishment of trunk intersegmental blood vessels in the developing zebrafish embryo (Torres-Vázquez et al., 2004; Zygmunt et al., 2011). Sema3A1 and Sema3A2 proteins keep the vascular sprouts budding from trunk large blood vessels within the intersomitic boundaries (Torres-Vázquez et al., 2004). Indeed, *sema3a1*/*sema3a2* and *plxnd1* morphants, along with the *plxnd1* knock-out mutant *out-of-bounds* (*obd*) present patterning anomalies of intersegmental blood vessels that invade somites (Torres-Vázquez et al., 2004). In addition, in quiescent aortic ECs adjacent to somites, Sema3A/PlexinD1 signaling was found to promote the autocrine secretion of soluble VEGFR1 that, sequestering VEGF, decreases blood vessel sprouting in somite boundaries (Zygmunt et al., 2011). Moreover, the implantation of anti-Sema3A antibody-soaked beads substantially alters the vascular pattern in the developing forelimb of chick embryos (Neufeld and Kessler, 2008). Similarly, retrovirus-mediated delivery of dominant negative constructs of Sema3A holoreceptor impairs vascular remodeling in the developing chick embryo (Serini et al., 2003). *Sema3a* knockout embryos present severe abnormalities in the remodeling of both cephalic plexus and dorsal longitudinal anastomotical vessel (Serini et al., 2003). Furthermore, *Sema3a*$^{-/-}$ pups that survive until the adulthood exhibit an aberrantly increased number of glomerular ECs together with defects of renal blood vessels (Reidy et al., 2009). The reported lack of vascular abnormalities in one study on *Sema3a* null mice (Vieira et al., 2007) may be due to the comparison between wild-type and *Sema3a* null embryos by using an age-and-stage matching strategy that inherently misses growth

retardation phenotypes (Hamada et al., 2005), such as that of *Sema3a* null mice (Serini et al., 2003). Endothelial tip cells of murine retinal vascular spouts were also found to express much more *Sema3a* mRNA than stalk ECs (Strasser et al., 2010) and EC-specific *Sema3a* knockout mice exhibit a significantly increased number and length of endothelial tip cell filopodia in retinal vascular sprouts (Ochsenbein et al., 2016).

The role of Nrp1 in Sema3A signaling in ECs incompletely understood. A $Nrp1^{Sema-}$ mouse strain carrying mutations in a1 domain of Nrp1 that impair Sema3 signaling was previously generated (Foo et al., 2006). Differently from *Nrp1* null mice, which die by E12.5, 60% of $Nrp1^{Sema-}$ mouse were found to survive until P7 and to exhibit cardiac, but not vascular abnormalities (Foo et al., 2006). However, two subsequent reports (Joza et al., 2012, 2013) showed that only 18% of $Nrp1^{Sema-}$ mice survive until P4 and exhibit lung alveolar capillary dysplasia. The lack of angiogenic remodeling defects in major head and trunk blood vessels in $Nrp1^{Sema-}$ mice (Gu et al., 2003) and the severity of the vascular phenotype in both Sema3A (Serini et al., 2003) and Nrp1 (Kawasaki et al., 1999) knockout mice support the possibility that the reduced responsivity of ECs to Sema3A in mutant $Nrp1^{Sema-}$ may be due to additional Sema3A coreceptors, such as proteoglycans (Casazza et al., 2013; Pang et al., 2015). Indeed, *Plxna4* null mice display several misprojected axon bundles that are not present in *Nrp1* (Kitsukawa et al., 1997) or $Nrp1^{Sema-}$ (Gu et al., 2003) mutant animals, implying that Plexin A4 may elicit Nrp1-independent Sema3A signals (Yaron et al., 2005). Hence, similar to membrane-associated Semas, Sema3A might bind at very low affinity and signal *via* plexins (Nogi et al., 2010).

Sema3A can signal through Plexin A1 (Takahashi et al., 1999), Plexin A2 (Janssen et al., 2012; Takahashi and Strittmatter, 2001), Plexin A4 (Kigel et al., 2011; Yaron et al., 2005), and Plexin D1 (Zhang et al., 2009), the latter being significantly more efficient that type A plexins in forming high-affinity Nrp-dependent holoreceptor complexes for Sema3A and Sema3C (Gitler et al., 2004). Sema3A-elicited EC collapse depends on both Plexin A1 and Plexin A4 (Kigel et al., 2011), and Sema3A inhibits the sprouting of aortic blood vessels or the migration of primary ECs isolated from $Plxnd1^{-/-}$ less efficiently than wild-type animals (Zhang et al., 2009). Therefore, Sema3A may control in vivo vascular morphogenesis by binding with high affinity to coreceptors, such as Nrp1 or proteoglycans, and signal through low-affinity receptors, for example, Plexin A1, Plexin A2, Plexin A4, and Plexin D1.

Sema3E

Independently from Nrp1, Sema3E binds to Plexin D1 with high affinity (Nakayama et al., 2013a). Both in *Sema3e* and *Plxnd1* knockout embryos, blood vessels invade somites resulting in the loss of the typical stereotyped intersomitic vascular pattern (Nakayama et al., 2013a). However, while *Plxnd1* knockout pups become cyanotic sudden after birth and die within 24 h (Gitler et al., 2004), $Sema3e^{-/-}$ mice are alive, fertile, and survive throughout adulthood although displaying initially severe vascular defects (Meadows et al., 2012; Nakayama et al., 2013a). This suggests that Plexin D1 likely transduces signals of Sema3 proteins other than Sema3E, such as Sema3A (Zhang et al., 2009) and Sema3C (Gitler et al., 2004). Interestingly, both Sema3A (Behar et al., 1996) and Plexin-D1 (Kanda et al., 2007) null mice share common axial skeletal defects. Moreover, endothelial *Plxnd1* gene inactivation in mice induces myocardial defects and skeletal malformations, associated witho a strong reduction of the bone microvasculature (Zhang et al., 2009).

Sema3E protein produced by the lateral plate mesoderm is required for dorsal aortae patterning and to generate the avascular zones laterally to the dorsal aortae and along the midline (Meadows et al., 2012, 2013). In this regard, $Sema3e^{-/-}$ embryos develop highly branched plexiform vessels that convert into single, unbranched dorsal aortae between E8.25 and E8.75 (Meadows et al., 2013), due to unidentified repulsive cues originating from the lateral plate mesoderm (Nakayama et al., 2013a). Furthermore, differently from zebrafish *sema3a1*/*sema3a2* and *plexind1* morphants as well as *obd* mutants (Torres-Vázquez et al., 2004) (Lamont et, al., 2009), *sema3e* zebrafish morphants do not display any overshooting of intersomitic sprouts.

Other Sema3 Proteins

Sema3B inhibits angiogenesis through its binding to Nrp1 (Varshavsky et al., 2008). Sema3B knockout mice are alive and fertile (Falk et al., 2005). An unbiased transcriptomic analysis revealed that SEMA3B is upregulated in severe forms of human preeclampsia and inhibits the differentiation of placental cytotrophoblasts; SEMA3B derived from the cytotrophoblast acts in a paracrine way to impair uterine microvascular EC functions (Zhou et al., 2013).

Sema3C protein binds with high affinity to Nrp1–Plexin D1 and to Nrp2–PlexinD1 complexes with lower affinity (Gitler et al., 2004). Accordingly, Sema3C was reported to inhibit angiogenesis by signaling *via* Nrp1 and Plexin D1 (Yang et al., 2015a). Deletion of *Sema3c* (Feiner et al., 2001), *Nrp1* (Kawasaki et al., 1999), or *Plxnd1* (Gitler et al., 2004) genes causes postnatal lethality due to cardiovascular defects, for example, the improper septation of the cardiac outflow tract, resembling the persistent truncus arteriosus observed in humans (Crupi et al., 1977). A recent study proposed that neural crest cell-derived Sema3C elicits both the Nrp1-dependent endothelial-to-mesenchymal transition in the endocardial cushions and the myocardialization of septum (Plein et al., 2015).

Sema3D inhibits EC spreading and migration through a Nrp1 and PI3K/Akt-dependent pathway (Aghajanian et al., 2014). In the developing embryo, Sema3D is expressed in a subpopulation of proepicardial cells, which will form the sinus venosus and the coronary endothelium (Dunah et al., 2005), and in the mesocardial reflections located between the splanchnic mesoderm and the venous pole of the heart, where Sema3D exerts a Nrp1-dependent repulsive effect to direct pulmonary venous EC migration toward the left atrium (Degenhardt et al., 2013). Consistently, *Sema3d* null mice exhibit anomalous pulmonary venous connection (Degenhardt et al., 2013). In venous ECs, Sema3D inhibitory signals are also conveyed through a Nrp1–ErbB2 signaling complex (Aghajanian et al., 2016).

Unc5, Netrins, and FLRTs

Netrin 1 (Ntn1) was first identified as an axon guidance molecule secreted by floor plate cells and capable of promoting the growth of commissural axons in the embryonic spinal cord (Kennedy ,et al., 1994; Serafini e,t al., 1994). It belongs to a family of laminin-related factors, which includes other two secreted Netrins (Ntn3 and Ntn4) and two GPI-anchored membrane proteins, netrin G1 and netrin G2 (Mehlen and Guenebeaud, 2010). Ntn1 and Ntn3 are structurally similar to the short arms of laminin-γ chains and contain a laminin VI domain, three epidermal growth factor (EGF)-like repeats related to the laminin V domain (V-1, V-2, and V-3), and a positively charged heparin-binding COOH-terminal motif termed domain C. In contrast, Ntn4 is more related to laminin-β chains and shares little homology with Ntn1 and Ntn3. Ntn4 regulates the assembly of the basement membrane in the vasculature and other organs, affecting branching morphogenesis (Koch et al., 2000; Schneiders et al., 2007).

Through its laminin VI domain, Ntn1 interacts with and activates several receptors, including Down's syndrome cell adhesion molecule, neogenin, deleted in colorectal cancer (DCC), adenosine A2b receptor, and uncoordinated-5 (UNC5) homologue family members (UNC5A, UNC5B, UNC5C, and UNC5D in humans, and Unc5h1, Unc5h2, Unc5h3, and Unc5h4 in rodents) (Mehlen et al., 2011). Unc5b is so far retained the only Netrin 1 receptor expressed in murine embryonic ECs (Lu et al., 2004), whereas putative expression of the other receptor remains unclear. However, adult aortic ECs express DCC and the receptor is required for Netrin 1-induced ERK1/2 activation and the ensuing nitric oxide production (Nguyen and Cai, 2006).

Strong genetic evidences indicate that Netrin 1 and its receptor play a critical role in angiogenesis; however, conflicting data about their function during vascular development appear to reveal the complexity of the mechanisms of action. Lu and colleagues demonstrated that inactivation of Unc5b gene in mice and targeting by means of morpholinos *unc5b* or *ntn1a* in zebrafish leaded to aberrant blood vessels navigation and branching, hence suggesting a possible antiangiogenic role for Netrin 1. Mice lacking *Unc5b* died of heart failure at embryonic day E10.5 or E12, depending on the genetic background, and the vascular branching defects were ascribed to the loss of Ntn1 inhibition of EC filopodia extension and migration (Lu et al., 2004). In a follow-up study, authors demonstrated that Unc5b is downregulated in adult quiescent blood vessels and expressed in sprouting vasculature of tumor implants, thus defining an inhibitory role for Ntn1 in sprouting angiogenesis (Larrivée et al., 2007). On the contrary, a series of data from other *in vivo* and *in vitro* studies reported a proangiogenic function for Ntn1 (Epting et al., 2010; Navankasattusas et al., 2008; Park et al., 2004; Wilson et al., 2006). In 2004, Park et al. found that Ntn1 promotes EC migration and proliferation (Park et al., 2004), while a follow-up study revealed that *ntn1a* silencing in zebrafish embryos impair the formation of parachordal vessels, that are precursors of lymphatic vessels, and that Netrins promote the revascularization of ischemic tissues in murine models (Wilson et al., 2006). In 2008, Navankasattusas and colleagues generated endothelial-specific *Unc5b* knock-out mice in which they observed a decrease in the density in placental labyrinth arterioles, finally resulting in embryonic death between E12 and E13.5 (Navankasattusas et al., 2008). Recently, Netrin 1a and its receptor Unc5b were found to be upstream activators of a signaling cascade involving the ELMO1–DOCK180-dependent activation of Rac1 in cultured ECs and blood vessel formation in the zebrafish embryo (Epting et al., 2010). Moreover, adeno-associated viral overexpression of Ntn1 induced in vitro EC proliferation and migration and elicited the formation of new artery-like blood vessels in the adult rodent brain (Fan et al., 2008).

In an alternative model, it was proposed that Ntn1 may function as prosurvival factor, blocking cell death induced by its unbound Unc5b that in ECs also functions as a dependence receptor (Castets et al., 2009). Authors proposed that, in the absence of Netrin 1, Unc5b activates the serine/threonine death-associated protein kinase (DAPK), which in turn leads to cell apoptosis, through the action of caspases (Castets et al., 2009). On the contrary, in the presence of Ntn1 the proapoptotic caspase signaling cascade is blocked, thus promoting EC survival and proliferation (Castets et al., 2009).

More recently, Yung and colleagues generated a *Ntn1* null mouse, by inserting *loxP* sites around the second exon of *Ntn1* and crossing animals with a germline-specific *Cre* line. In these mice, in which no Ntn1 protein was expressed, phenotypic analysis confirmed a Ntn1 primary function during midline guidance of commissural axons, but not in other Unc5b-mediated effects such as repulsion, neuronal survival, or blood vessel branching. The authors proposed that Ntn1 may not be the primary ligand for Unc5 receptors *in vivo*, thus suggesting that other Unc5 ligands might play key roles in the control of EC survival and angiogenesis (Yung et al., 2015). In this regard, the fibronectin leucine-rich transmembrane proteins (FLRTs) have been found to interact with Unc5 receptors and act as repulsive guidance cues (Yamagishi et al., 2011). FLRT1–3 are the three members of the FLRT family of ligands that mediate both homophilic and heterophilic cell-to-cell adhesive interactions (Seiradake et al., 2014). Moreover, transmembrane FLRT proteins can be shed and act as soluble Unc5 ligands (Seiradake et al., 2016). FLRTs are regulators of early embryonic cardiovascular and neural development and are characterized by the presence of an N-terminal LRR domain followed by a linker region, a type 3 fibronectin domain (FN) and a juxtamembrane linker, containing a metalloprotease cleavage site (Seiradake et al., 2016). During development, upon proteolytic cleavage and shedding from the cell surface, FLRT2 and FLRT3 extracellular domains may function as repulsive guidance cues for Unc5-positive neurons (Yamagishi et al., 2011). Yet, the homophilic interaction among FLRT molecules on neighbor cells may promote instead cell-to-cell adhesion, thus indicating that FLRT can trigger both adhesive and repulsive signals in the same cell (Karaulanov et al., 2006). Recently, FLRT3 was found to be expressed by primary human artery ECs together with its receptor UNC5B and to control retinal vascularization in mouse models. In both cases FLRT–UNC5B interaction exerts a repulsive function toward ECs (Seiradake et al., 2014).

Ntn4, the most divergent member of the Ntn family, is a secreted protein which functions in axon guidance and regulates both mammary and lung morphogenesis in addition to blood vascular development. Some reports showed an inhibitory role for Ntn4 specifically in pathological angiogenesis (Lejmi et al., 2008; Nacht et al., 2009). Ntn4 was found to be upregulated by VEGF

stimulation and to inhibit EC migration and angiogenesis in laser-induced choroidal neovascularization and tumor xenograft models in mice (Lejmi et al., 2008). Ntn4 exerts its function by binding to its receptor neogenin and promoting its association with Unc5b, both receptors being necessary for downstream signaling (Lejmi et al., 2008). Hypoxia was found to increase Ntn4 expression in the retina (Lange et al., 2012) and in an oxygen-induced model of retinopathy neovascularization was significantly increased in *Ntn4*$^{-/-}$ mice (Kociok et al., 2016). Ntn4 was also found to promote in vivo lymphangiogenesis and to contribute to tumor metastatic dissemination (Larrieu-Lahargue et al., 2010). Indeed, the high-affinity binding of Ntn4 to α6β1 integrin, which is expressed on lymphatic ECs, leads to the activation of Src signaling and to the formation of cell-to-matrix contacts (Larrieu-Lahargue et al., 2011). The role of Ntn4i n the control of developmental angiogenesis is instead less clearly understood. Despite a low *Ntn4* gene transcription during embryonic zebrafish development, Lambert and colleagues showed that Ntn4 plays a crucial role for *in vivo* blood vessel formation (Park et al., 2005). Authors showed that Ntn4 induced EC survival, proliferation, and migration through the activation of different kinase pathways, that is, ERK1/2, Akt, JNK, and FAK, and that these signaling cascades finally lead to sprouting angiogenesis in zebrafish embryos (Lambert et al., 2012).

A recent structural study revealed that Ntn4 inhibits axonal growth, angiogenesis, and tumor progression by blocking laminin polymerization and disrupting preexisting laminin network in a nonenzymatic manner, independently from its binding to canonical Ntn receptors (Reuten et al., 2016). In particular the high-affinity binding of Ntn4 to laminin γ1 would cause the disruption of the basement membrane lying between ECs and pericytes, thus inhibiting the stabilization of capillaries and finally resulting in a collapse of the whole vascular network (Reuten et al., 2016).

Slits and Robos

Signaling by the Slit–Roundabout (Robo) ligand/receptor pair was first discovered to be implicated in the control of axon guidance (Carmeliet and Tessier-Lavigne, 2005), and more recently this interaction has been shown to be involved in vascular injury (Yuen and Robinson, 2013) as well as in physiological (Yadav and Narayan, 2014) and tumor angiogenesis (Wang et al., 2003). The Robo receptor family is composed of four known members, named Robo 1–4. While Robo2 and 3 are mainly neuronal, Robo1 is expressed in both nervous and vascular systems. Robo1–3 transmembrane receptors share a common molecular structure, consisting of five Ig-like and three fibronectin type III (FNIII) domains in the extracellular portion and four cytoplasmic conserved motifs (CC0, CC1, CC2, and CC3) (Kidd et al., 1998). The most newly discovered member of the family Robo4, also known as magic Roundabout, is an endothelial-specific protein that was identified independently by using bioinformatics data mining (Huminiecki et al., 2002) and in a study aimed at finding genes perturbed in mice with altered developmental angiogenesis (Park et al., 2003). Robo4 displays significant structural differences form the other family members, since it contains in the extracellular portion only two Ig and two FN repeats and in the intracellular domain only is variants of CC0 and CC2 motifs.

Slit proteins are the cognate ligand for the Robo receptors. Slits are a family of secreted glycoproteins composed of three members in vertebrates (Slit 1–3), *D. Melanogaster* and *C. Elegans* expressing instead only one single Slit protein. All mammalian Slits are expressed in the nervous system, but Slit2 and 3 can also be found in other organs. Slits are large proteins containing a stretch of four LRR, seven to nine EGF-like domains, a domain named Agrin, Laminin, Perlecan, and SLIT (ALPS) or laminin G-like module, and a C-terminal cysteine knot (Brose et al., 1999). Structural studies conducted in *Drosophila* showed that a site located in the second LRR domain of slit binds to the Ig region of robo1-3 and transduces cellular signaling (Howitt et al., 2004). Moreover, *Drosophila* slit binding to robo is enhanced by the high-affinity interaction of the C-terminal portion of slit with the heparan–sulfate (HS) proteoglycan syndecan, which probably acts by concentrating slit on the cell surface (Hohenester et al., 2006). Later, a corresponding HS/heparin-binding site was identified in the Ig1 and Ig2 domains of robo1, suggesting the importance of ternary complex formation to foster the stabilization of the signaling unit (Fukuhara et al., 2008). Of note, mammalian Robo3 and Robo4 lack the key amino acid residues required for Slit and heparin binding (Morlot et al., 2007; Zelina et al., 2014), and cell-based assays failed so far to demonstrate a direct Slit2–Robo4 interaction (Koch et al., 2011).

Despite the importance of Slit and Robo in the regulation of angiogenesis, the exact effect of Slit2 on ECs remains to be investigated due to the controversial reports on its attractive or repulsive signaling. Wang and colleagues were the first to investigate the role of Slit2 and Robo1 in vascular guidance. In particular, they demonstrated that Slit2 produced by tumor cells was able to attract Robo1-expressing ECs and promote angiogenic sprouting in a PI3K-dependent manner (Wang et al., 2003). Once the endothelial-specific Robo4 was discovered, different groups supported the hypothesis of an inhibitory effect of Slit2 on EC migration (Jones et al., 2008; Kaur et al., 2008; Park et al., 2003; Seth et al., 2005). In particular, *Robo4*-null mice are viable and fertile and have normal vessel patterning in multiple organs but exhibit increased basal and VEGF-induced retinal vascular permeability and show an increased experimentally induced pathological neovascularization. Robo4 is expressed by the endothelium of various vascular beds of embryonic and adult mice, but it is absent from tip ECs of retinal blood vessels, suggesting a possible role of Robo4 in the maintenance of vascular stability. Accordingly, Slit2 exerts a Robo4-dependent inhibitory function respect to VEGF-A165-induced migration and permeability by blocking the activation of Src kinase family proteins (Jones et al., 2008). This study was confirmed by a genetic dissection analysis, which revealed that Slit2 and Slit3 produced by mural cells inhibit VEGF-induced blood vessel growth in mammary gland, in a Robo4-dependent manner (Marlow et al., 2010). The clinical implication of these findings was further highlighted in multiple murine models of inflammation-induced endothelial permeability, wherein administration of the exogenous active N-terminal fragment of Slit2 (Slit2N) enhances the endothelial barrier function

and reduces cytokine storm-dependent vascular leakage by acting through Robo4 (London et al., 2010). The molecular mechanisms underlying the maintenance of vascular stability by Robo4 were dissected and a yeast two-hybrid screening identified paxillin and its paralog Hic-5 as Robo4 interactors. Accordingly, the formation of the Robo4/paxillin/Hic-5 complex was enhanced by Slit2. Moreover, upon Robo4 binding, paxillin recruits the ADP-ribosylation factor GTPase-activating proteins (Arf-GAP) GIT1 and inhibits VEGF-induced Arf6 GTP loading and the downstream activation of Rac (Jones et al., 2009). Since it has been demonstrated that Slit2 enhances vascular barrier function by stabilizing VE-cadherin at the EC surface (Jones et al., 2009), this may depend on the inhibition of the Arf6-induced internalization of VE-cadherin.

In a protein–protein interaction screening, the extracellular portion of Robo4 was found to interact with Ntn receptor UNC5B, another vascular specific axon-guidance receptor. In the model conceived by Koch and colleagues, Robo4 acts as a ligand that binds and signals through Unc5B receptor. This hypothesis is corroborated by the finding that soluble Robo4 treatment rescued vessel barrier function in Robo4$^{-/-}$ mice and blocked VEGF-induced vascular permeability in wild-type animals, but not in mice treated with an Unc5B inhibitory antibody. The suggested mechanism of action is that Robo4-activated Unc5B could associate with phosphorylated Src and prevent it from binding to VEGFR2 upon VEGF stimulation. Blocking Robo4–Unc5B interaction increases Src association with VEGFR2 and VE-cadherin phosphorylation. Moreover, since Robo4 knockdown leaves developing retinal vessels unaffected, it has been hypothesized that the absence of Robo4 may be compensated by alternative Unc5B ligands, such as netrins or FLRTs (Koch et al., 2011).

A possible explanation for ROBO4 responsiveness to SLIT2 in absence of direct binding comes from a study conducted on HUVECs by Sheldon and colleagues. Authors found that ROBO1 and ROBO4 can form heterodimers and, upon SLIT2 binding to ROBO1, ROBO4 acts as a signaling unit that recruits Wiskott–Aldrich syndrome protein (WASP), neural WASP (NWASP), and their regulatory proteins WASP-interacting protein (WIP) and syndapin. The formation of this signaling complex would promote local actin polymerization, filopodia formation, and directional EC migration toward the source of SLIT2 (Sheldon et al., 2009).

Other studies described a promigratory role for Slit2 in postnatal retina and in mouse models of pathological ocular neovascularization. In blood vessels of the mouse retina, Slit2, signaling *via* Robo1 and Robo2, cooperates with VEGF to induce the establishment of EC front-to-rear polarity and motility. Slit2 binding to Robo leads to the recruitment of the scaffolding protein Nck1/2 and the ensuing activation of Cdc42 and Pak2, which in turn promote cytoskeletal remodeling and directional migration (Dubrac et al., 2016; Rama et al., 2015). On note, compared to its wild-type counterpart, the Slit2-ΔE15 alternative splicing variant, which lacks the exon 15-encoded C-terminus of the second LLR responsible for the direct binding to the first and second Ig domains of Robo1, exhibits a stronger and Robo-independent ability of inhibiting EC motility and stabilizing blood vessel integrity (Yang et al., 2015b). Notably, Slit2-ΔE15 expression is lost in many cancers (Yang et al., 2015b).

Slit–Robo can also signal to influence the behavior of other cell types, which contribute to the formation of the blood vessels wall. Two recent studies described the expression of Slit2, Robo1, and Robo4 in VSMCs and pericytes that surround medium/large diameter blood vessels and capillaries, respectively. In both studies, Slit2 inhibited platelet-derived growth factor-induced in vitro cell migration (Guijarro-Muñoz et al., 2012; Liu et al., 2006). These results suggest the existence of autocrine and paracrine circuits among cells localized within different layers of blood vessel walls, which may be involved in vascular guidance and remodeling.

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Vascular Repair at the Interface of the Endothelium: The Roles of Protease-Activated Receptors and Neuregulin-1

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Introduction

Vascular repair in the cardiovascular system is a complex biological process involving orchestrated responses among and between the endothelium, smooth muscle cells, cardiac fibroblasts, and myocytes. The endothelium serves as a nexus for molecular signaling events that contribute to postinjury tissue remodeling. While cardiac tissue remodeling plays an initially protective role in response to injury, excessive activation of fibroblasts leads to adverse remodeling and fibrosis that exacerbates heart failure. Endothelial cell-derived factors can both facilitate and inhibit vascular tissue repair, the balance of which ultimately determines the extent of fibrosis and cardiac dysfunction.

One of the first endothelium-mediated responses to myocardial infarction (MI) is the generation of thrombin that promotes a cascade of cellular effects to facilitate vascular repair. Thrombin-mediated cellular signaling occurs via classical G protein-coupled protease-activated receptors (PARs), which initially facilitate vascular repair but also contribute significantly to the pathological consequences of cardiac remodeling. PARs are the most abundant GPCR expressed on fibroblasts, which upon ligand-binding promote detrimental vascular remodeling and fibrosis.

Vascular injury also induces endothelial cell-mediated production of cardioprotective factors, such as neuregulin-1 (NRG-1), a growth and survival factor critical for heart development and cardiac repair. Similar to PARs, NRG-1 receptors are expressed throughout the cardiovascular system, but in contrast to PARs elicit mainly positive signaling events that contribute to attenuation of inflammation and fibrosis.

A detailed description of all the known molecular factors of vascular injury and repair is beyond the scope of this article. Rather, a novel perspective is provided in which the roles of PARs and NRG-1 in vascular repair are compared and contrasted, which is especially relevant given that both PARs and NRGs are promising therapeutic targets.

Role of Fibrosis in Vascular Repair After MI

MI is a major cause of heart failure, in part due to the limited capacity of the adult heart to regenerate after injury. Following an MI, cardiomyocyte death and loss of architectural integrity, together with specific hormones, growth factors, and cytokines induces infiltration of circulating immune and stromal cells to the injured area, proliferation of resident fibroblasts, and transdifferentiation of fibroblasts into a profibrotic phenotype (i.e., myofibroblasts). In concert with a multiphase inflammatory reaction, MI prompts myofibroblast-mediated replacement of the affected tissue with a fibrotic scar (van den Borne et al., 2010). This replacement fibrosis (i.e., scar formation) is a pivotal process in preventing ventricular wall rupture after an ischemic insult (van den Borne et al., 2010; Shinde and Frangogiannis, 2014). Impairment of cardiac function with subsequent heart failure may result from remodeling of the uninjured myocardium and in the infarct border zone due to exaggerated fibrotic response and reactive fibrosis (Talman and Ruskoaho, 2016; Sutton and Sharpe, 2000). This eventually leads to increased ventricular thickening (cardiomyocyte hypertrophy), ventricular stiffness, and altered chamber compliance with subsequent heart failure (Weber et al., 2013). Therefore, therapies for post-MI treatment that reduce pathological cardiac remodeling and fibrosis represent an attractive target for therapeutic intervention.

The Role of PARs in Vascular Repair

PARs are classical G protein-coupled receptors, but they are uniquely activated by proteases, resulting in release of a tethered ligand (Coughlin, 2000; Patterson et al., 2001). The PAR family, which consists of PAR-1, PAR-2, PAR-3, and PAR-4, can be activated by numerous serine proteases, including trypsin, plasmin, activated protein C, cathespsin G, kallikreins, tryptase, and matriptase. Most notably, all PAR family members, with the exception of PAR-2, can also be activated by the potent hemostatic protein thrombin. PARs reside in vascular cells, including vascular smooth muscle cells (VSMCs), endothelial cells, platelets, fibroblasts, and cardiac myocytes, and are intimately involved in vascular repair (Fig. 1). PAR-1 activation in endothelial cells leads to endothelial cell proliferation, attachment of polymorphonuclear cells, and increased permeability, steps that are important in the inflammatory cascade and hence intimately associated with vascular repair (Coughlin, 2000; Cleator and Vaughan, 2008).

PAR Activation by Thrombin

Thrombin stimulates PAR-1 and PAR-4 on human platelets and is the most potent activator of platelets. Moreover, thrombin-mediated PAR-1 stimulation on VSMCs causes contraction (Alberelli and De Candia, 2014). In addition, PAR-1 mediates the proliferation, migration, and hypertrophy of VSMCs in the setting of endothelial dysfunction, which contributes to the pathophysiology of atherosclerosis. Counteracting these deleterious effects, thrombin mediates activation of endothelial nitric oxide synthase (eNOS) to generate nitric oxide, which causes vasodilation in VSMCs and inhibits platelet activation (Alberelli and De Candia, 2014) which has been referred to as the thrombin paradox.

Thrombin induces an increase in thrombosponsdin-1 (TSP-1) gene expression in human microvascular endothelial cells as demonstrated by microarray analysis, which also corresponds to an increase in TSP-1 protein expression (McLaughlin et al., 2005). TSP-1 is an "adhesion-modulating" component of the extracellular matrix (ECM) (Mosher and Adams, 2012) that contributes to wound healing, in part via activation of latent transforming growth factor-β (TGF-β) (Murphy-Ullrich and Poczatek, 2000), a potent profibrotic cytokine important for fibroblast activation and secretion of ECM components, such as collagen. Thrombin-induced

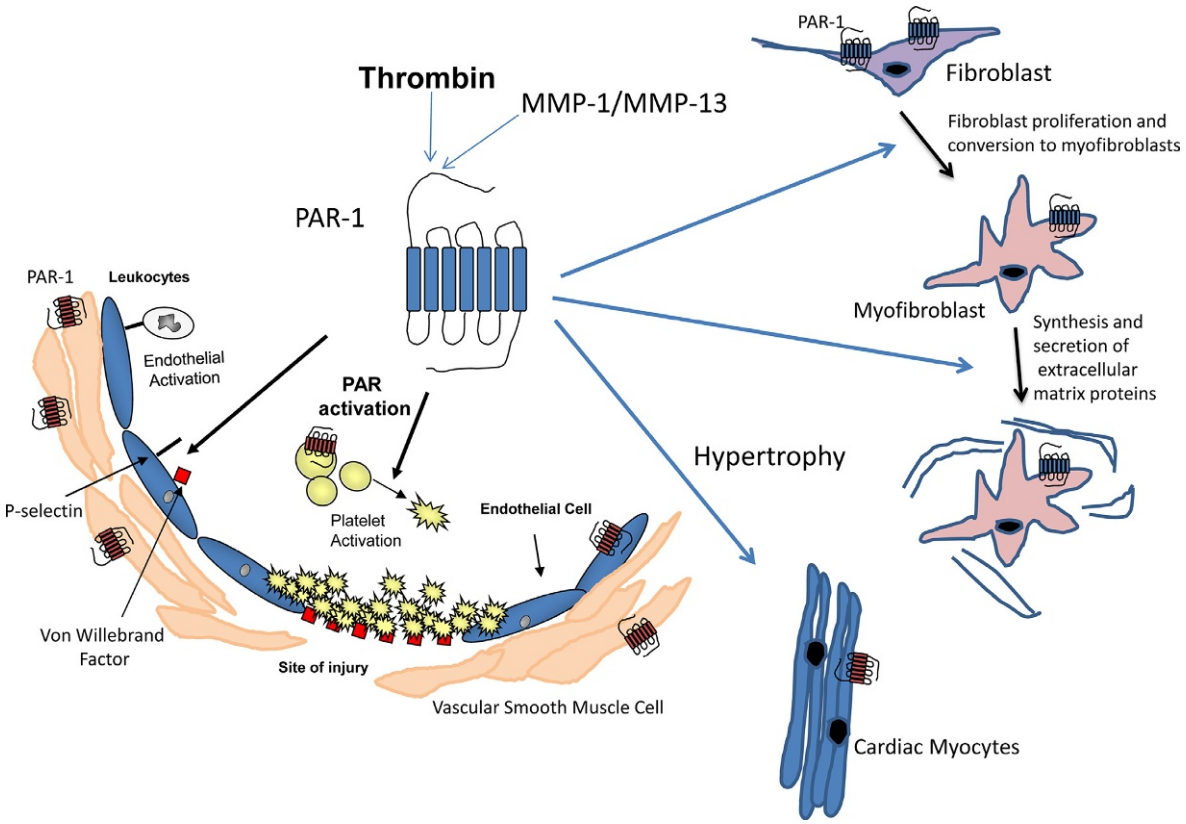

Fig. 1 PAR-1 mediation of vascular effects that influence vascular repair. Depicted centrally is the main thrombin receptor, PAR-1 which is activated when thrombin cleaves the N-terminal domain, unmasking a tethered ligand. PAR-1 activation leads to platelet activation and endothelial cell activation with exocytosis of Von Willebrand factor and P-selectin, which are critical steps in thrombosis and inflammation. PAR-1 activation also leads to fibroblast proliferation and conversion to myofibroblasts, partly through increased expression of TGF-β. In addition, PAR-1 is also expressed on cardiac myocytes, and PAR-1 activation leads to cardiac hypertrophy.

cleavage of PAR-1 also causes the proliferation and migration of VSMCs and inhibits angiogenesis (Krishna and Golledge, 2013). TSP-1 has also been implicated in wound-healing "gone awry," enhancing profibrotic signaling, matrix synthesis, and endothelial dysfunction (Krishna and Golledge, 2013). TSP-1 was shown to localize to calcified atherosclerotic lesions (Hansen et al., 2009) and to promote the formation of platelet–monocyte rosettes in patients with severe coronary artery stenosis and atherothrombotic cerebral ischemia (Jurk et al., 2010).

PAR Activation by Matrix Metalloproteinases

In addition to thrombin, PAR-1 can be activated by matrix metalloprotease 1 (MMP-1) and MMP-13, which results in biased activation, as opposed to classical canonical G protein signaling mediated by thrombin. MMP-1 and MMP-13 cleave the N-terminal domain of PAR-1 at a different site than thrombin resulting in distinct signaling that may be leveraged for therapeutic gain in vascular repair (Austin et al., 2013). MMPs are zinc-dependent endopeptidases, which are interstitial collagenases that play a pivotal role in vascular repair and development (Shoulders and Raines, 2009). Within the vasculature, MMPs affect the proliferation, migration, and apoptosis of VSMCs, endothelial cells, and inflammatory cells. Targeting of MMPs has long been investigated in treating vascular disease ranging from inhibition of MMP-1 in treating tissue accumulation after balloon injury (i.e., restenosis) to attenuating vascular remodeling after MI.

PAR Activation in Cardiac Cells

PAR-1 is also expressed on cardiomyocytes, as well as on fibroblasts that mediate cardiac remodeling (Sabri et al., 2000, 2002). In fact, PAR-1 is the most highly expressed GPCR present on cardiac fibroblasts and when stimulated leads to increased proliferation, conversion to the profibrinogenic myofibroblast phenotype, and increased production of TGF-β (Sabri et al., 2002; Snead and Insel, 2012). PAR-1 activation in cardiac fibroblasts also causes a significant increase in the expressions of MMP-1 and MMP-13 (Sonin et al., 2013). At the level of the cardiomyocyte, PAR-1 activation leads to hypertrophy, as demonstrated in a rat neonatal model (Glembotski et al., 1993). Furthermore, PAR-1 expression is increased in the hearts of patients with cardiomyopathy (CM) (both ischemic and nonischemic) (Moshal et al., 2005a), which correlates with basic science studies demonstrating increased PAR-1 expression in mice with experimentally induced chronic heart failure (Moshal et al., 2005b). Multiple groups have reported that inhibition of PAR-1 decreases cardiac remodeling and also limits myocardial ischemia reperfusion injury (**Table 1**).

Table 1 Studies of effects of PARs on vascular repair

Author	*Vascular model*	*Intervention*	*Outcome*
Rajput et al. (2014)	Rat middle cerebral artery occlusion	Knockdown of PAR-1	Decreased infarction and vascular disruption
Mao et al. (2010)	Mouse transient middle cerebral artery occlusion	PAR-4 knockout	Decreased infarction with attenuation of microvascular inflammation and inhibition of platelet activation
Xie et al. (2015)	Rat middle cerebral artery occlusion	Ginsenoside-Rg administration correlated with downregulation of PAR-1 expression	Reduced infarct volume, permeability, and neurobehavioral function score
Olson et al. (2004)	Mouse combined cerebral hypoxia/ischemia model	PAR-1 knockout and PAR-1 antagonist studies	Reduced infarct volume, cellular death, and motor behavior impairment
Junge et al. (2003)	Transient focal middle cerebral artery occlusion	PAR-1 knockout and PAR-1 antagonist studies	Reduced infarct volume
Strande et al. (2007)	Rat left coronary artery occlusion	Selective PAR1 antagonist SCH79797	Reduced infract size and increased ventricular recovery
Strande et al. (2008)	Rat left coronary artery occlusion	PAR4 antagonists (tc-y-NH2 and P2pal10)	Reduced infarct size and increased ventricular recovery
Kolpakov et al. (2016)	Mouse LAD artery occlusion	PAR-4 knockout	Reduced infarct size and cardiomyocyte apoptosis
Pawlinski et al. (2007)	Mouse LAD artery occlusion	PAR-1 knockout	Reduced remodeling but not infarction. Cardiomyocyte overexpression of PAR-1 induced adverse remodeling
Gu et al. (2015)	Rat left coronary artery ligation	Hirudin (a direct thrombin inhibitor)	Increased cardiac function which was reversed by PAR-1 agonist peptides
Baker et al. (2009)	Total body irradiation	Bone marrow transplantation	Increased PAR-1 expression correlated with adverse cardiac remodeling

Also, knockdown of either PAR-1 or PAR-4 confers a protective effect, with less cerebral infarction after stroke (middle cerebral artery infarct model, **Table 1**). This expands the potential therapeutic role of PARs in vascular repair to the cerebral vasculature, which shares a postischemic thromboinflammatory injury pattern with the cardiac vasculature (Bai and Lyden, 2015; De Meyer et al., 2016). Interestingly, it has become increasingly evident that paradoxically low levels of thrombin might by cytoprotective in the central nervous system (Krenzlin et al., 2016).

Therapeutic Applications of PAR Antagonists

Recently, multiple large randomized trials have investigated the effects of vorapaxar, a PAR-1 antagonist, in preventing adverse major cardiovascular events in patients with coronary artery disease. In patients presenting with acute coronary syndrome undergoing percutaneous coronary intervention, administration of vorapaxar failed to reduce the primary endpoint of ischemic events and increased the rates of severe bleeding (Tricoci et al., 2012; Morrow et al., 2009). However, a larger secondary prevention trial utilizing vorapaxar in patients with either a history of MI, history of ischemic stroke, or peripheral artery disease (TRA2 TIMI 50) demonstrated favorable results (Morrow, 2012). In a subgroup analysis, patients with a history of MI benefitted from addition of vorapaxar with a decrease in recurrent ischemic events (Scirica et al., 2012). It has been opined that off-target effects of PAR-1 antagonism on nonplatelet vascular cells may attenuate adverse remodeling contributing to beneficial effects.

Unintended deleterious side effects of vorapaxar, such as intracranial bleeding, have led to the development of alternative antithrombotic inhibitors that might block platelet aggregation while retaining beneficial PAR-mediated effects on cardiovascular endothelial cells. A small anatagonist peptide was recently reported to successfully elicit these cellular effects, including inhibition of thrombin-triggered clot formation in rodents (Ramachandran et al., 2017). Antibody blockage of PAR-4 in an arterial thrombosis monkey model was similarly efficacious, with minimal bleeding and the added advantage of oral administration (Wong et al., 2017).

NRG-1 Signaling in the Cardiovascular System

NRG-1 is encoded by one of four NRG genes belonging to the epidermal growth factor (EGF) superfamily of potent growth factors, which are potent modulators of cellular development, differentiation, and survival in multiple cell types and organ systems (Buonanno and Fischbach, 2001; Falls et al., 1993; Talmage and Role, 2004). NRG transduces signaling via erythroblastic leukemia viral oncogene homolog (ErbB) receptors (ErbB2, ErbB3, and ErbB4) (Yarden and Sliwkowski, 2001), which are tyrosine kinases that function similarly to the founding member (ErbB1, also called EGFR).

NRG-1 signaling is initiated upon engagement of its EGF-like domain to the extracellular binding region of either ErbB3 or ErbB4, which leads to receptor auto-phosphorylation of its cytosolic kinase region, dimerization with a second subsequently phosphorylated ErbB receptor, and recruitment and phosphorylation of one of several cytosolic adaptor proteins. ErbB3 is virtually kinase-dead, and thus NRG-1/ErbB3-mediated signaling requires recruitment and activation of ErbB2, which exhibits strong tyrosine kinase activity but lacks an extracellular ligand-binding domain. As is the case for ErbB1/EGFR, ErbB4 possesses both the ligand-binding and kinase domains and can therefore transduce growth factor-mediated signaling via homo- or hetero-dimerization.

Some of the specificity of NRG-1 signaling is achieved by variable expression and surface presentation cycling of ErbB receptors, which is highly dependent upon spatial and temporal context. NRG-1 and its receptors are widely expressed throughout the body, but the most prevalent source of NRG-1 in the cardiovascular system is the microvascular endothelium (Galindo et al., 2014a; Rupert and Coulombe, 2015). Endothelial cell-derived NRG-1β induces both autocrine and paracrine signaling within the myocardium (Kuramochi et al., 2004a; Noireaud and Andriantsitohaina, 2014; Lemmens et al., 2006; Kalinowski et al., 2010; Iivanainen et al., 2007). NRG-1 also acts distally to modulate adult heart homeostasis, most notably the control of heart rate and blood pressure via sympathetic vasomotor neurons in the rostral ventrolateral medulla (**Fig. 2**) (Matsukawa et al., 2011).

The Indispensability of NRG-1 in Cardiovascular Development and Repair

It is widely appreciated that NRG-1/ErbB signaling is critical for embryological development of the heart. Genetically altered mice with disruption in NRG-1, ErbB2, ErbB3, or ErbB4 die in utero due to defects in development of cardiac trabeculation and/or endocardial cushion formation (Meyer and Birchmeier, 1995; Gassmann et al., 1995; Lee et al., 1995). NRG-1/ErbB signaling also plays a crucial role in adult cardiovascular homeostasis and repair (**Fig. 2**), as evidenced by conditional knockout studies in mice and in human breast cancer patients. In mice, disrupted expression of ErbB2 or ErbB4 in the postnatal heart results in the development of dilated CM. More importantly, a higher incidence of heart failure was observed in women who received anthracyclines in combination with trastuzamab treatment (Slamon et al., 2001). Trastuzamab is a monoclonal antibody that was developed to specifically target ErbB2 (more often called HER2 in the arena of tumor biology), which is inappropriately activated (in the absence of ligand-bound dimerization partners) in some highly invasive and metastatic breast cancers.

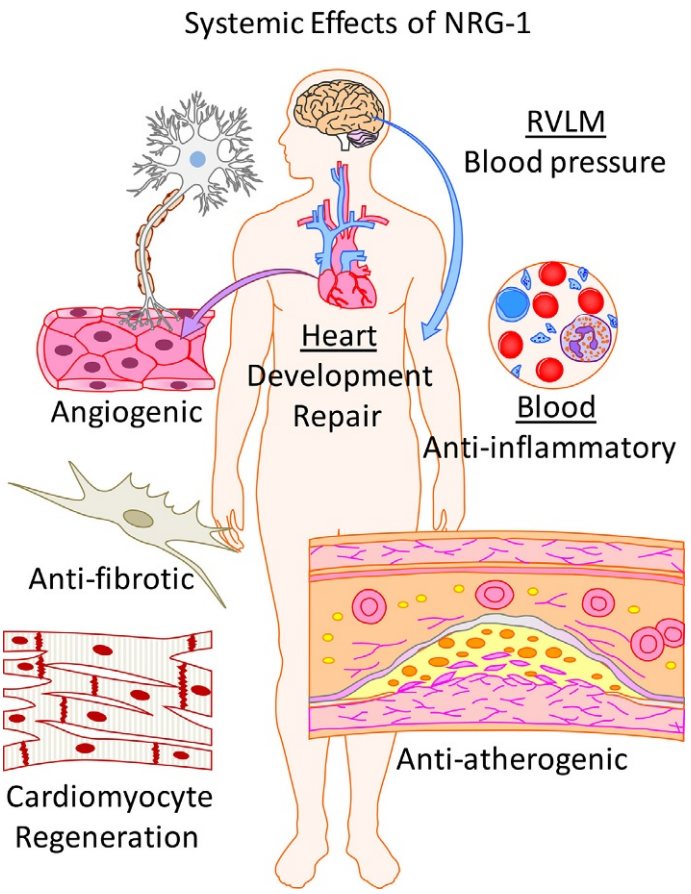

Fig. 2 Systemic effects of neuregulin-1 (NRG-1). NRG-1 plays a role in the development of the heart and in postnatal maintenance of the cardiovascular system, including regulation of heart rate and blood pressure via sympathetic vasomotor neurons in the rostral ventrolateral medulla (RVLM). NRG-1 is regenerative in response to cardiac injury, with direct effects on the endothelium, fibroblasts, and myocytes. NRG-1 might also inhibit atherosclerosis, as depicted.

In a reciprocal fashion, ErbB2 inhibition is detrimental for cardiovascular and nervous systems that require this receptor for homeostatic and stress-induced responses to NRG-1.

Ischemia/reperfusion injury is a potent activator of NRG-1/ErbB signaling in the intact heart, and selective disruption of endothelial NRG-1 expression impairs recovery of cardiac contractile function after an ischemic insult (Hedhli et al., 2011). Endothelial-derived NRG-1 is important for adaptive cardiomyocyte hypertrophy and cell lengthening, as indicated by genetically altered mice with conditional knockout of ErbB2 or ErbB4, which develop severe dilated CM characterized by chamber dilation, wall thinning, and decreased contractility (Ozcelik et al., 2002; Crone et al., 2002; Garcia-Rivello et al., 2005). ErbB2 expression is significantly decreased in a dog model of ischemic heart disease, as well as in human ischemic CM (Gordon et al., 2009). With ErbB2 blockade, however, in animals with chronic hypertrophy secondary to aortic stenosis, expressions of ErbB2 and ErbB4 are maintained during the compensatory stage of the disease and decline during the transition to failure (Rohrbach et al., 1999). A similar situation is observed in humans. Although ErbB2 is upregulated following ventricular unloading in heart failure patients (Uray et al., 2002), ErbB2 and ErbB4 expressions and activities are reduced in patients with advanced heart failure (Rohrbach et al., 2005). NRG-1/ErbB signaling might also play a role in the normal physiological adaptation of the heart to pregnancy (Lemmens et al., 2011), and its perturbation might therefore contribute to postpartum CM.

Cellular Effects of NRG-1/ErbB Signaling in the Heart

In vascular endothelial cells, NRG-1 induces proliferation and angiogenesis (Kalinowski et al., 2010; Russell et al., 1999; Bagheri-Yarmand et al., 2000), prevents endothelial dysfunction (Wang et al., 2017; Hedhli et al., 2012), and contributes to eNOS-dependent cardioprotection (Matsukawa et al., 2011; Ebner et al., 2013). NRG-1/ErbB signaling in the vasculature also prevents intimal thickening (Clement et al., 2007; Hintsanen et al., 2007), smooth muscle hyperplasia (Clement et al., 2007), and rarefaction of capillaries (Russell et al., 1999; Hedhli et al., 2012; Safa et al., 2011; Lok et al., 2009). In addition to autocrine signaling in microvascular endothelial cells (Kalinowski et al., 2010), NRG-1 induces paracrine signaling in cardiomyocytes (Pentassuglia et al., 2007, 2009; Baliga et al., 1999; Giraud et al., 1985; Sawyer et al., 2002; Matsui et al., 1999; Miao et al., 2000; Fujio et al., 2000;

Timolati et al., 2006; Cote et al., 2005; Fukazawa et al., 2003; Zhao et al., 1998; Kuramochi et al., 2004b, 2006; Okoshi et al., 2004; Jamnicki-Abegg et al., 2005; Lemmens et al., 2004; Mansour et al., 2004; Boateng et al., 2005; Kuppuswamy, 2002; Pfister et al., 2007; Peng et al., 2006, 2008), aortic smooth muscle cells (Yoshizumi et al., 2001), cardiac fibroblasts (Galindo et al., 2014b; Kirabo et al., 2017), resident and circulating stem cells (Liang et al., 2015; Polizzotti et al., 2015; Sun et al., 2011; Wang et al., 2008, 2009; Zhu et al., 2010; Hao et al., 2014), and monocytes (Ryzhov et al., 2017) (**Fig. 2**). NRG-1/ErbB signaling is strongly induced in myocytes in response to stress or injury, resulting in cellular proliferation (Polizzotti et al., 2015; Bersell et al., 2009; Ganapathy et al., 2016; Wadugu and Kuhn, 2012; Parodi and Kuhn, 2014; D'Uva et al., 2015) and survival (Kuramochi et al., 2004a; Rohrbach et al., 2005; Pentassuglia et al., 2007; Giraud et al., 1985; Matsui et al., 1999; Fujio et al., 2000; Fukazawa et al., 2003; Zhao et al., 1998; Belmonte et al., 2015; Liu et al., 2006; Jie et al., 2012; Li et al., 2007a; Fang et al., 2017), preserved excitation-coupling (Timolati et al., 2006), reduced oxidative stress (Timolati et al., 2006; Belmonte et al., 2015), reduced endoplasmic reticulum stress (Fang et al., 2017), and mitochondrial dysfunction (Guo et al., 2012), increased glucose uptake and protein synthesis (Baliga et al., 1999; Cote et al., 2005), and enhancement of sarcomere organization (Baliga et al., 1999; Sawyer et al., 2002; Cote et al., 2005; Mansour et al., 2004; Liu et al., 2006).

In addition to its mitogenic and protective effects on vascular cells and cardiomyocytes, NRG-1 alters the cardiac interstitium and associated cell populations (Galindo et al., 2014a). NRG-1 inhibits cardiac fibrosis (Gui et al., 2012; Li et al., 2011), presumably via decreased production of ECM structural and regulatory proteins (Galindo et al., 2014b; Li et al., 2011), alterations in MMPs (Yuan et al., 2008a,b; Li et al., 2009; Momeny et al., 2015; Asrani et al., 2013; Kim et al., 2012; Sigala et al., 2006), and fewer ventricular myofibroblasts (Galindo et al., 2014b). Paradoxically, NRG-1 enhances proliferation and survival of normal human cardiac ventricular fibroblasts when cultured in vitro (Kirabo et al., 2017), which could point to a pro-wound-healing effect if these functional results are recapitulated in the intact heart in conjunction with cardiovascular injury.

Circulating monocytes express ErbBs and respond to NRG-1, which reduces production of inflammatory mediators (Ryzhov et al., 2017; Marballi et al., 2010; Zhou et al., 2017). In animal models of vascular disease, NRG-1 inhibits stress and ischemia-induced accumulation of leukocytes (Hedhli et al., 2011; Lok et al., 2009; Liu et al., 2006; Li et al., 2009; Marballi et al., 2010; Xu et al., 2005; Panutsopulos et al., 2005). In monocytes acquired from heart failure subjects, higher surface expression of ErbB3 was found to be correlated with lower levels of the inflammatory cytokine tumor necrosis factor α (TNFα) (Ryzhov et al., 2017). Surprisingly, an inverse relationship between TNFα and ErbB3 was not observed in this same subpopulation of monocytes obtained from healthy individuals (Ryzhov et al., 2017), which underscores the highly contextual nature of NRG-1/ErbB signaling and the need for further investigation of this phenomenon. Recent evidence also supports a role for NRG-1 in the alteration of macrophages from a proinflammatory to a regenerative phenotype in the postinfarct setting (Diaz-Herraez et al., 2017).

Distant NRG-1 signaling can also impact cardiovascular function and homeostasis (**Fig. 2**). Recent evidence suggests that NRG-1 stimulation of resident cardiac fibroblasts might contribute to a multicellular, intersystemic signaling network (Kirabo et al., 2017). It is possible that NRG-1-induced production of cytokines modulates the inflammatory response during wound healing and pathological cardiac remodeling. Additionally, NRG-1-induced secretions from activated cardiac fibroblasts might stimulate influx of circulating monocytes, pericytes, endothelial progenitors, resident stem cells, bone marrow-derived stem cells, or pluripotent mesenchymal cells. Consistent with this theory, NRG-1 enhances embryonic stem cell cardiomyogenesis (Sun et al., 2011; Wang et al., 2008, 2009; Hao et al., 2014; Chen et al., 2013; Kim et al., 2007), regulates cardiac subtype specification (Zhu et al., 2010; Ruhparwar et al., 2007), and may also stimulate new cardiomyocyte production from endogenous cardiac progenitor cells in response to workload (Polizzotti et al., 2015; Waring et al., 2014). Given the importance of NRG-1 in normal physiological and pathophysiological responses to stress and tangential stem cell studies for cardiac regeneration, these intersecting research areas are ripe for investigation.

NRG-1 as a Potential Therapy for Cardiovascular Disease and Injury

Preclinical data support the efficacy of NRG-1 as a therapeutic candidate in multiple areas of cardiovascular medicine (**Fig. 2**), including vasculopathy. Endogenous NRG-1 is required for angiogenesis and arteriogenesis following ischemic injury, and administration of exogenous NRG-1 enhances this process (Hedhli et al., 2012). Plasma levels of NRG-1 were shown to be significantly lower in patients with coronary artery syndrome and angina, compared to those with mild hypertension and normal individuals (Wang et al., 2009). NRG-1 expression is amplified in atherosclerotic lesions in coronary artery disease (Sigala et al., 2006; Panutsopulos et al., 2005). Administration of exogenous NRG-1 suppressed macrophage foam cell formation in primary human monocyte cultures (Wang et al., 2009). Further, in apolipoprotein E-deficient mice, NRG-1 was shown to inhibit atherogenesis (Wang et al., 2009). In a rat model of carotid vascular injury, NRG-1 injection reduced lesion size by 50% compared with vehicle-treated control animals and prevented mitogen-stimulated VSMC proliferation and migration (Clement et al., 2007). These findings suggest that NRG-1 may be a novel therapeutic candidate for the treatment of atherosclerosis. Local administration of NRG-1 could also mitigate restenosis following endovascular therapy, which might possibly reduce re-intervention and increase vessel preservation for future treatment options.

Multiple in vivo studies have established the therapeutic potential for NRG-1 for the treatment of heart failure (**Tables 2** and **3**). The EGF-domain fragment of recombinant human NRG-1β2a (rhNRG) was used in a series of small and large animal models of systolic heart failure to demonstrate a positive effect on heart function and survival (**Table 2**). For example, rhNRG administration to myocardial-infarcted rodents improved LV remodeling and cardiac function, induced angiogenesis, activated the cell cycle in

Table 2 Studies of effects of recombinant NRG-1 on heart failure (animal studies)

Animal model	*Species*	*NRG-1 formulation*	*Treatment*	*Outcome*
Carotid vascular injury (Clement et al., 2007)	Rat	rhNRG	2.5 ng/kg, before balloon injury then every 2 days, for 14 days	Attenuated neointima formation, 50% reduction in lesion size
MI (Liu et al., 2006)	Rat	rhNRG	10 μg/kg i.v. daily, for 5 days, 10 days, 1 week, or 2 months after LAD	Attenuated dysfunction and improvements in cardiac performance. Increased capillary abundance in the fibrotic peri-infarct area (angiogenesis)
MI (Bersell et al., 2009)	Mouse	rhNRG	2.5 μg/mouse i.p. daily for 12 weeks, 1 week after LAD	Induced cell cycle in cardiac myocytes and improved cardiac function and structure
MI (Gu et al., 2010)	Rat	rhNRG	5 μg/kg/h at 10 μL/h osmotic pump, for 7 days, 8 weeks after LAD	Upregulated cardiac myosin light chain kinase associated with improved cardiac function
MI (Guo et al., 2012)	Rat	rhNRG	10 μg/kg i.v. daily, for 5 days, 10 days, 1 week, or 2 months after LAD	Improved LV remodeling and cardiac function, reduced mitochondrial dysfunction, myocyte apoptosis, and oxidative stress
MI (Xiao et al., 2012)	Rat	rhNRG	rhNRG-carrying lentivirus injected in infarcted area	Improved LV function, decreased apoptosis, promotion of neovascularization and angiogenesis
MI (Hill et al., 2013)	Rat	rhGGF2	0.625 or 3.25 mg/kg, with or without high fat diet, twice weekly for 4 weeks, 7 days post-MI	Improved LV contractile function, corrected gene expression alterations, beneficial effects independent of dietary fat content
MI (Galindo et al., 2014b)	Swine	rhGGF2	0.67 mg/kg or 2 mg/mL i.v. twice weekly, for 4 weeks, 1 week post-MI	Improved LV contractive function, decreased fibrosis
MI (Hill et al., 2013)	Rat	rhGGF2	0.65, 2.6, or 3.25 mg/kg at variable freq. and durations	Sustained improvement of LV function and morphology
I/R	Rat	rhNRG	1, 2, 4, or 8 μg/kg i.v. for 20 min prior to I/R	Improved LV structure, reduced infarct size, decreased apoptosis
I/R (Fang et al., 2017)	Rat	rhNRG	4 μg/kg i.v. for 20 min prior to I/R	Decreased myocardial infarct size, inhibited ER stress
Viral myocarditis (Liu et al., 2006)	Mouse	rhNRG	30 μg/kg i.v. daily, for 5 days	Improved echocardiographic parameters and survival
Dox-induced CM (Liu et al., 2006)	Mouse	rhNRG	20 μg/kg i.v. daily, for 5 days, 4 weeks post-Dox treatment	Prevented cardiac dysfunction and improved survival
Dox-induced CM (Bian et al., 2009)	Mouse	rhGGF2	0.75 mg/kg *sub.q* 1 day prior to Dox, daily thereafter, 5 days	Improved survival and cardiac function
Dox-induced CM (Jay et al., 2013)	Mouse	NN	20 μg/mouse i.p. daily for 1 week, 16 weeks post-Dox	Improved LV function, increased posterior wall thickness, decreased end-systolic diameter
Diabetic CM (Li et al., 2011)	Rat	rhNRG	10 μg/kg i.v. daily, every 2 days for 2 weeks, 12 weeks post-STZ injection	Improved LV structure and dysfunction, decreased apoptosis, decreased fibrosis
Diabetic CM + atherosclerosis (Vandekerckhove et al., 2016)	Mouse	rhNRG	20 μg/kg i.p. 5 days/week for 14 weeks	Prevented LV dilatation, improved LV contractile function, reduced atherosclerotic plaque size, attenuated nephropathy
Cardiac cryoinjury (Polizzotti et al., 2015)	Mouse	rhNRG	100 ng/g sub-q. daily, for the first 34 days of life	Improved myocardial function, reduced prevalence of transmural scars
Cardiac cryoinjury (Ganapathy et al., 2016)	Mouse	rhNRG	100 ng/g *sub-q.* daily, for first month of life	Cardiac regeneration in absence of neoplasia
Pacing-induced CM (Liu et al., 2006)	Dog	rhNRG	5 μg/kg *i.v.* daily, 5 days with continuous pacing, 3 weeks after start of pacing	Improved LVEDP and LVESP, improved cardiac contractility and relaxation
Pacing-induced CM (Li et al., 2007b; Li and Qiang, 2007)	Rhesus monkey	rhNRG	3 μg/kg *i.v.* daily, for 10 days	Improved LV function, increased MHCα

LV, left ventricular; *LVEDP*, left ventricular end-diastolic pressure; *LVESP*, left ventricular end-systolic pressure; *MI*, myocardial infarction; *I/R*, ischemia/reperfusion; *NRG*, neuregulin-1; *rhNRG*, recombinant human neuregulin-1; *rhGGF2*, recombinant human glial growth factor 2; *NN*, bivalent NRG-1.

cardiomyocytes, and reduced mitochondrial dysfunction, myocyte apoptosis, and oxidative stress I (Liu et al., 2006; Guo et al., 2012; Gu et al., 2010; Xiao et al., 2012). In rat models of ischemia reperfusion, pretreatment with rhNRG improved LV structure, reduced infarct size, and inhibited endoplasmic reticulum stress and apoptosis (Fang et al., 2010, 2017). Treatment with rhNRG similarly increased cell survival and myocardial function in a mouse model of viral myocarditis (Liu et al., 2006), in mice with doxorubicin-induced CM (Liu et al., 2006; Bian et al., 2009), in Type I diabetic CM rodent models with and without atherosclerosis

Table 3 Human studies of effects of recombinant NRG-1 on heart failure

Trial	*Description*	*Formulation of NRG-1*	*Dose*	*Effect*
ACTRN12607000330448[a] Completed (Gao et al., 2010)	Phase II, randomized, double-blind, multicenter, background-therapy-based, placebo-controlled, parallel group study	rhNRG	0.3, 0.6, or 1.2 μg/kg 10 h intravenous infusion, daily for 10 days	Improved and sustained LVEF % and decreased LVEDV and LVESV at 30 and 90 days after treatment
ACTRN12607000330448[a] Completed (Jabbour et al., 2011)	Single-center, prospective, nonrandomized, open label study	rhNRG	Initial dose of 1.2 μg/kg for 6 h; followed by 0.6, 1.2, or 2.4 μg/kg for 12 h intravenous infusion, daily for 10 days	Acute increase in CO and improvement in LVEF
NCT01258387[b] Completed (Lenihan et al., 2016)	Phase I, single ascending dose study of cimaglermin alfa (neuregulin 1β3) in patients with systolic dysfunction and heart failure	rhGGF2	0.007, 0.021, 0.063, 0.189, 0.378, 0.756, or 1.512 mg/kg for 30 m, single intravenous infusion	Persistent (90 day) increase in LVEF, with minimal side effects for 0.189 mg/kg and higher doses

LVEF %, % left ventricle ejection fraction; *LVEDV*, left ventricle end-diastolic volume; *LVESV*, left ventricle end-systolic volume; *CO*, cardiac output; *rhNRG*, recombinant human neuregulin-1; *rhGGF2*, recombinant human glial growth factor 2.

[a]Registered with the Australian New Zealand Clinical Trials Registry, anzctr.org.au.

[b]Registered with U.S. National Institutes of Health, ClinicalTrials.gov.

(Li et al., 2011; Vandekerckhove et al., 2016), cryo-injured mice (Polizzotti et al., 2015; Ganapathy et al., 2016), and in two large animal models of pacing-induced CM (Liu et al., 2006; Li et al., 2007b; Li and Qiang, 2007).

Another version of NRG-1, representing the full-length NRG-1β3 isoform (rhGGF2), was also developed and likewise shown to be efficacious in the failing myocardium (**Table 2**). Most interestingly, rhGGF2 has been demonstrated to prevent adverse remodeling after injury, in part via antifibrotic effects in the heart. In post-MI swine, rhGGF2-treated pigs exhibited less fibrosis, improved ECM structure, and reduced myofibroblast percentages in myocardial tissue remote from the infarct (Galindo et al., 2014b). More recently, Parry et al (Parry et al., 2017). directly compared the kinetics and pharmacological effects of the two different recombinant NRG-1 formulations in vitro and in post-MI rats and concluded that (1) rhGGF2 has higher ErbB4 receptor binding affinity than rhNRG, (2) better protects doxorubicin-challenged cardiomyocytes, and (3) provides sustained improvements in post-MI cardiac function with relatively infrequent dosing compared to a daily dosage regimen with rhNRG.

These promising preclinical animal studies have led to ongoing clinical trials examining whether these NRG-1βs have similar effects in humans (**Table 3**).

Developing Novel Methods for Target-Specific NRG-1 Therapies

Cooperative intercellular signaling via NRG-1/ErbB has been demonstrated for cardiomyocytes and endothelial cells (Lemmens et al., 2006) and likely occurs among other cell types. NRG-1-mediated activation of other EGFR family members provides further complexity, enhancing feed-forward signaling cascades that tend to confound disease-specific therapeutic applications. Off-target effects are of prime concern, given the strong associations of both ErbB2 and ErbB3 in tumor development and progression. Two recent advances provide potential solutions to these issues: bivalent ErbB receptor agonists (Jay et al., 2011, 2013), and conjunctive therapies using microparticles or stem cells.

Diaz-Herraez et al. (2017) recently demonstrated synergistic, vasculogenic, and cardioreparative effects of NRG-1 combined with adipose-derived stem cells in post-MI rats, when administered in microparticles coated with collagen and poly-D-lysine. This combinatorial treatment approach induced significant heart regeneration, including a reduction of ~36% in infarct size, stimulation of cardiomyocyte proliferation, and formation of both arterioles and capillaries (Diaz-Herraez et al., 2017). Similarly, controlled delivery of a combination of NRG-1 and fibroblast growth factor, encapsulated in biodegradable microparticles, promoted cardiac regeneration in post-MI rats (Formiga et al., 2014). Transplantation of ErbB4-overexpressing mesenchymal stem cells was also efficacious in post-MI mice, which exhibited a greater reduction in infarct size and better preserved cardiac function than in mice treated with the corresponding wild-type stem cells (Liang et al., 2015). This therapeutic strategy was also superior to post-MI mice treated with recombinant NRG-1 alone (Liang et al., 2015), implying endogenously produced NRG-1 was sufficient to activate the cells once administered, or possibly that other ErbB4 ligands contributed to the observed improvement in cardiac regeneration and performance.

A Possible Link Between PAR-1 and NRG-1

A simplified view of thrombin/PAR-1 and NRG-1/ErbB signaling is that they respectively act negatively and positively on the vasculature. This "inverse functional relationship" is not entirely unrealistic, because there is some evidence to support

NRG-1-mediated inhibition of thrombin activities via increased production of inhibitory factors in the postinfarct setting (Galindo et al., 2014b). Most notably, NRG-1 administration to pigs, beginning 1 week after MI and continuing twice weekly for 4 weeks, resulted in a significant increase in thrombomodulin (THBD) mRNA in myocardium remote from the infarct (Galindo et al., 2014b).

THBD is the other major cofactor/receptor for thrombin. Although the level of thrombin is a result of the net balance between its synthesis and degradation, its physiological effects are determined primarily by availability of PAR-1 and THBD. THBD alters vascular remodeling and decreases neointima formation in animal models of vascular injury. In a mouse carotid ligation model, binding of THBD to thrombin inhibited thrombin-induced PAR-1 activity, which resulted in significantly attenuated atherosclerosis and neointima formation (Wei et al., 2011). In a separate study, THBD was likewise shown to decrease the severity of neointima formation and reduce arterial remodeling via thrombin-mediated PKC activation. In this same study, THBD inhibited leukocyte adhesion to the vessel wall after carotid ligation (Li et al., 2006). NRG-1-mediated alterations in the coagulation cascade via upregulation in THBD transcription might therefore contribute to its proreparative effects in response to vascular injury.

Summary and Conclusions

PARs and NRG-1 are indispensable in the response of the vasculature to ischemia and other injurious insults. The complex balance between vascular repair and maladaptive remodeling leading to fibrosis, atherosclerotic plaque formation, or thrombosis has complicated efforts to harness NRG or inhibit PAR. Nonetheless, clinically translatable progress has provided insights into the underlying mechanisms associated with these two inducible signaling cascades. Novel, selective therapies continue to evolve as we improve our basic understanding of vascular injury and repair, in keeping with the general trend of modern medicine toward more personalized, context-driven solutions.

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Vasculogenesis in Development

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Introduction

The vascular system is one of the earliest functional organ systems to develop in all vertebrate embryos. Comprising a highly complex and intricate network of arteries, veins, capillaries, and lymph vessels, the circulatory system transports gases, nutrients, and metabolites throughout the body and plays important physiological roles in regulating homeostasis in the developing embryo as well as in the adult. The vascular system retains morphogenetic plasticity in the adult, which is important in normal physiological processes like the oestrous cycle and wound healing. Furthermore, vascular anomalies have been implicated in the pathology of several diseases including cancer (Carmeliet, 2003). Thus, the establishment of a proper vascular network is imperative for the normal development and functioning of all organ systems in the body.

The development of the vascular system involves two distinct processes known as vasculogenesis and angiogenesis (Poole and Coffin, 1989; Risau et al., 1988). Vasculogenesis, which is the de novo differentiation and assembly of endothelial cells into primary blood vessels, is the process by which the first major axial vessels, the dorsal aorta (DA) and cardinal vein (CV), are formed in most vertebrates (Coffin and Poole, 1988; Poole and Coffin, 1989). Following the formation of a primitive vascular plexus and the major axial vessels, elaboration of the primary vasculature by coordinated sprouting of blood vessels occurs by angiogenesis (Poole and Coffin, 1989). While these two processes are equally important in the establishment of the vascular network, this review will focus on the mechanisms underlying vasculogenesis. Vasculogenesis is initiated during late gastrulation when a subset of cells in the lateral plate mesoderm (LPM) is specified as vascular endothelial progenitor cells (EPCs), also known as angioblasts. Angioblasts migrate toward the midline, differentiate into vascular endothelial cells, and coalesce to form solid tubes (Kimmel et al., 1995; Stainier et al., 1995). In response to extracellular cues, the endothelial cells acquire either an arterial or venous identity. The final step in vasculogenesis is the process of lumenization of the vessels into hollow tubes, which allows blood cells to circulate through the primitive vessels. The current review aims to summarize the key events during vasculogenesis together with models used to describe these events.

Sites of Vasculogenesis

In amniotes, the earliest vasculogenesis occurs in the blood islands of the yolk sac which give rise to the extraembryonic primitive vascular plexus. In mouse, yolk sac vasculature forms at around E7.5. This is followed by the intraembryonic vasculogenesis which takes place within the LPM and gives rise to the major axial vessels, such as the DA and the CV (Drake and Fleming, 2000). Early studies have suggested that the vasculature in many organs is derived from vascular endothelial progenitors which originate de novo and are not derived from the existing vascular network (Gouysse et al., 2002). However, in most cases, lineage tracing has not been performed to confirm these observations. More recent studies showed that many organ-specific vessels are derived from existing vasculature through a combination of vasculogenesis and angiogenesis. For example, intestinal vasculature in zebrafish is formed from vascular endothelial progenitors that originate from the posterior CV (PCV) and migrate as individual cells and subsequently coalesce into the subintestinal artery and subintestinal vein (Goi and Childs, 2016; Hen et al., 2015; Koenig et al., 2016). Genetic lineage tracing studies have demonstrated that the murine hepatic plexus is derived from the endocardium and shares a common developmental origin with coronary arteries (Zhang et al., 2016). A substantial portion of murine coronary vasculature is formed by de novo vasculogenesis from endocardial progenitors (Tian et al., 2014), which themselves originate from the LPM. In addition to

the LPM, cell-tracking experiments have demonstrated that some vascular endothelial cells are derived from somites in chick embryos (Ambler et al., 2001; Wilting et al., 1995). It is somewhat controversial whether any endothelial cells are derived from somites in nonamniote embryos although recent experiments in zebrafish have suggested this possibility (Nguyen et al., 2014).

Hemangioblast—The Common Precursor of Vascular Endothelial and Blood Cells

Based on multiple observations including the spatial proximity of endothelial and hematopoietic cell development in avian and mammalian embryos, it has been speculated that a common precursor cell known as the hemangioblast gives rise to both blood cells and endothelial cells (Detrich et al., 1995; Haar and Ackerman, 1971; Moore and Owen, 1965). The concept of a common progenitor of both cell types arose from initial observations made nearly a century ago by Sabin and Murray, who described the close proximity of hematopoietic and endothelial cells in the developing chick embryo (Murray, 1932; Sabin, 1920). The support for the existence of such a common progenitor comes from studies of in vitro-differentiated embryonic stem (ES) cells or mouse posterior primitive streak regions, which were shown to harbor a vascular endothelial growth factor (VEGF)-responsive progenitor, which expresses VEGFR2/FLK1 and has a potential of generating both blood and endothelial cells in culture at a single cell level (Choi et al., 1998; Faloon et al., 2000; Huber et al., 2004). Studies on human ES cell differentiation in vitro have identified a progenitor which has the potential to give rise to both endothelial and hematopoietic cells (Choi et al., 1998; Kennedy et al., 1997; Zambidis et al., 2005). Moreover, cell lineage tracing studies using *Flk1-Cre* and Rosa-stop–EYFP or Rosa-stop–LacZ reporter mice also supported the notion that blood and endothelial cells originate from the Flk1-expressing progenitors (Lugus et al., 2009; Motoike et al., 2003). Single-cell fate mapping experiments and retrospective clonal analysis in zebrafish embryos have shown that while the majority of cells at the blastula and gastrula stages give rise to either endothelial or blood cells and that these two types of cells differentiate through different paths, a small fraction of cells gave rise to both endothelial and blood cells, suggesting that only a subset of mesodermal cells could give rise to hemangioblasts (Vogeli et al., 2006).

The recent identification of the zebrafish *cloche* mutant gene *npas4l* will likely add to our understanding of hemangioblast as *cloche* mutants lack nearly all hematopoietic and endothelial cells (Reischauer et al., 2016; Stainier et al., 1995). While it is tempting to postulate that the earliest cells which express *npas4l* could be the presumptive hemangioblasts which give rise to both endothelial and primitive erythroid cells, accumulating evidence points to other possibilities. Some endothelial progenitors which express *npas4l* could form a specialized intermediate hemogenic endothelium which gives rise to primitive erythroid cells in addition to giving rise to vascular endothelial cells (Lancrin et al., 2009). Another possible explanation is that *npas4l* is expressed only in endothelial progenitors which go on to form endothelial cells (Parker and Stainier, 1999). These endothelial cells then provide essential signals and the appropriate microenvironment for the differentiation of hematopoietic progenitors into primitive erythroid cells. In both scenarios, the loss of *npas4l* leads to loss of both endothelial cells and primitive blood cells.

The Role of Etv2 in Vascular Endothelial Specification

One of the earliest transcription factors expressed in vascular endothelial progenitors and/or hemangioblast cells is an ETS transcription factor Etv2/ER71/Etsrp which functions (Lee et al., 2008; Sumanas and Lin, 2006). In the developing mouse embryo, *Etv2* is expressed in the primitive streak, embryonic mesoderm, amnion, allantois, and yolk sac blood islands at around E7.5 (Lee et al., 2008). Its expression becomes rapidly downregulated as the embryo develops and becomes restricted to major vessels, including the DA and branchial arches, at around E8.25–E9.5 (Lee et al., 2008). *Etv2* expression in the adult is mainly observed in the testis (Brown and McKnight, 1992). Similarly, *Etv2* expression in differentiating ES cells, embryoid bodies (EBs), is initiated around the same time as *Brachyury* expression. *Etv2* expression within EBs is also transient and becomes undetectable once hemangiogenic progenitors have been generated (Lee et al., 2008). Consistently, transgenic reporter expression driven by the *Etv2* promoter/enhancer region or the endogenous *Etv2* locus can be detected in the extraembryonic mesoderm, lateral mesoderm, and vascular structures in the embryo proper and yolk sac. However, the *Etv2*-reporter is no longer expressed at later stages of development (Kataoka et al., 2011; Rasmussen et al., 2011; Wareing et al., 2012).

Similarly, in zebrafish embryos, *etv2* expression is initiated in the anterior LPM (ALPM) as early as the 1-somite stage. *npas4l* function is required for *etv2* expression (Reischauer et al., 2016). *etv2*-expressing cells in the ALPM give rise to the endocardium, cranial vasculature, and myeloid progenitors (Proulx et al., 2010). Shortly afterward, at the 2–3 somite stage, *etv2* expression is observed in endothelial progenitors in the posterior LPM (PLPM). Similarly, in *Xenopus* embryos, *etv2* expression is observed in the dorsal LPM which gives rise to the DA and hematopoietic stem cells, in the ventral blood island where primitive hematopoietic cells originate, and in the vascular endothelial progenitors that correspond to the vascular plexus and PCV (Ciau-Uitz et al., 2013; Salanga et al., 2010).

Transient *Etv2* expression pattern is manifested by its transient requirement in hematopoietic and vascular development. In particular, *Etv2*-deficient mice display a complete block in blood, endothelial cell and endocardial formation, which indicates that ETV2 performs a nonredundant and indispensable function in hematopoietic and vessel development (Ferdous et al., 2009; Lee et al., 2008). Similarly, *Etv2* functional knockdown in zebrafish using morpholinos, as well as genetic mutant data demonstrate the critical role of *Etv2* during embryonic vasculogenesis and hematopoiesis. Specifically, vascular endothelial cells fail to differentiate, and the expression of multiple vascular endothelial markers is lost or downregulated in *etv2* morpholino (MO) knockdown

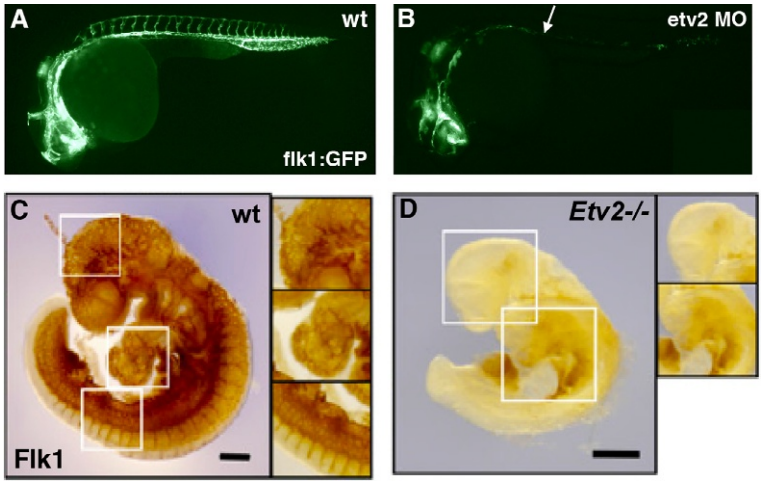

Fig. 1 Etv2 is required for specification of vascular endothelial cells in vertebrates. (A and B) Vascular endothelial specific *flk1:GFP* (*kdrl:GFP*) expression is greatly reduced in Etv2 MO-injected embryos at 24 hpf. (C and D) Flk1 immunostaining is absent in mouse *Etv2* knockout embryos at E9.5.

zebrafish embryos (morphants) and *etv2* mutants during early and mid-somitogenesis stages (**Fig. 1**) (Pham et al., 2007; Sumanas and Lin, 2006). Etv2 directly activates the transcription of many vascular endothelial specific genes by binding to vascular enhancers in combination with other transcription activators such as FOXC family members (De Val et al., 2008; Liu et al., 2015). *etv2*-expressing EPCs in Etv2 translation blocking MO-injected zebrafish embryos fail to migrate to the midline, remain positioned bilaterally, and do not coalesce into blood vessels due to their failure to form junctions between adjacent cells (Sumanas and Lin, 2006). In striking contrast to a complete block in hematopoietic and vascular formation seen in *Etv2* knockout mouse embryos, *Etv2* deletion after the hemangiogenic program commitment leads to normal development. Specifically, *Etv2* deletion using *Tie2-Cre* (hematopoietic and endothelial cell specific), *Vav-Cre* (hematopoietic specific), or *VEcadherin-Cre* (endothelial cell specific) systems do not lead to any defects in hematopoietic or endothelial cell development (Park et al., 2016; Xu et al., 2017). However, in zebrafish Etv2 plays a redundant role with other ETS factors such as Fli1b during later vascular development (Craig et al., 2015). It is also important for vascular regeneration during mouse hindlimb ischemia and during tumor angiogenesis in the zebrafish model (Baltrunaite et al., 2017; Park et al., 2016). This suggests that while *Etv2* is not essential in homeostatic hematopoietic and vascular functions, it plays an important role during vascular regeneration and pathological angiogenesis.

VEGF Signaling During Vasculogenesis

VEGF-VEGFR2/Flk1 signaling plays a critical role in vasculogenesis. In primitive streak stage mouse embryos, VEGF receptor tyrosine kinase *Flk1* is first detected in the extraembryonic mesoderm and the LPM (Dumont et al., 1995; Ema et al., 2006; Yamaguchi et al., 1993). Subsequently, its expression becomes downregulated in hematopoietic cells (Kabrun et al., 1997) and confined to endothelial cells of the yolk sac and embryo proper, and the developing endocardial tube. *Flk1*-deficient mice display similar defects to *Etv2*-deficient mice in the formation of yolk sac blood islands, blood vessels, and endocardium (Shalaby et al., 1995, 1997). VEGFA has been identified as an exclusive ligand for FLK1 in the formation of hematopoietic and vascular systems, as *Vegfa* deficiency results in similar defects observed in *Flk1* knockout mice. Importantly, $Vegfa^{+/-}$ embryos display similar blood and vessel defects that are observed in $Flk1^{-/-}$ embryos (Carmeliet et al., 1996; Ferrara et al., 1996). This indicates that VEGFA dosage is critical for properly forming hematopoietic and vascular systems. Consistent with this idea, mice homozygous for the *Vegfa* hypomorphic allele, *Vegfa*(*lo*), die around E9.0 with severe yolk sac vasculature abnormalities and dorsal aortae defects (Damert et al., 2002). Conversely, overexpression of *Vegfa* also leads to an embryonic lethality (Miquerol et al., 2000). These studies clearly demonstrate that VEGFA levels and the optimal FLK1 signaling are critical for proper hematopoietic and vascular development and embryogenesis.

Cell lineage tracing studies have revealed that Flk1-expressing progenitors contribute to blood, endothelial cells, cardiomyocytes, and skeletal muscle cells (Lugus et al., 2009; Motoike et al., 2003). By examining *LacZ* expression from the *Flk1-LacZ* knock-in targeted allele, hemangiogenic progenitors were shown to have high expression levels of *Flk1*, while cardiac and somite progenitors have low *Flk1* expression levels. Thus, it was suggested that while FLK1 mesoderm can contribute to blood, endothelial cell, skeletal muscle cells and cardiomyocytes, FLK1 expression levels, presumably reflecting VEGF signaling strength, might determine the cell lineage outcome of the $FLK1^{+}$ cells in the embryo (Ema et al., 2006). As such, while blood and endothelial cells are generated from hemangiogenic progenitors expressing high levels of FLK1 ($FLK1^{high}$), cardiac or muscle cells would be generated from $FLK1^{low}$ progenitors (Ema et al., 2006). Consistent with this idea, $FLK1^{+}$ hemangiogenic progenitors can be readily detected around embryonic day (E) 7.5, which progressively expand during E7.5–E8.5. Similar to the *LacZ* reporter expression pattern in *Flk1-LacZ* knock-in mice, the mean fluorescence intensity of FLK1 expression within a subset of the $FLK1^{+}$ cell population becomes

greater as the embryo develops, suggesting an elevated FLK1 signaling activity within a subset of FLK1^{+} cells. Remarkably, the FLK1^{high} cell population is exclusively missing in *Etv2*$^{-/-}$ embryos. This cell population represents a small fraction of the embryo, suggesting that hematopoietic and endothelial cells develop from a small pool of FLK1^{high} progenitors (Liu et al., 2015). Thus, ETV2 function is to generate FLK1^{high} hemangiogenic progenitors to ensure proper hematopoietic and vascular development.

Recently, a reporter ES cell line expressing tdTomato and GFP from the *Etv2* and *Scl* loci, respectively (named as SGET cells), has been generated to track molecular and cellular events leading to hemangiogenic cell lineage specification (Zhao and Choi, 2017). *Scl* is a direct target of ETV2 and is expressed shortly after *Etv2* (Liu et al., 2015). Flow cytometry analysis of tdTomato and GFP expression confirmed that the onset of Scl-GFP expression was later than that of Etv2-tdTomato. Importantly, emerging Scl-GFP expressing cells were mainly observed within cells expressing high levels of Etv2-tdTomato (Etv2^{high}). RNA-seq analysis of Etv2^{high} and Etv2$^{low/intermediate}$ cells has revealed that previously identified ETV2 targets (Liu et al., 2015) were activated within Etv2^{high} cells, which suggests that the hemangiogenic lineage program becomes activated after the threshold of *Etv2* expression has been achieved. Thus, it appears that *Etv2* directly specifies the hemangiogenic fate in a threshold-dependent manner. Importantly, VEGF is required for generating Etv2^{high} cells. *Flk1*$^{-/-}$ ES cells fail to generate Etv2^{high}, not Etv2^{low}, cells. As such, VEGF plays an instructive role in the hemangiogenic cell lineage specification (**Fig. 2**). Consistent with this idea, *Etv2* inactivation using the *Flk1-Cre* system results in embryonic lethality, apparently showing decreased generation of the FLK1^{high} cell population (Liu et al., 2015). This suggests that any perturbation of *Etv2*, VEGF, and FLK1 balance causing insufficient generation of Etv2^{high} and FLK1^{high} cells might lead to defects in hematopoietic and endothelial cell development.

In zebrafish, two homologues of VEGFA are present—*vegfaa* and *vegfab* (Bahary et al., 2007). While fish heterozygous for *vegfaa* do not exhibit a phenotype, homozygous *vegfaa*$^{-/-}$ mutants display defects in vasculogenesis and angiogenesis (Casie Chetty et al., 2017; Rossi et al., 2016). Although *flk1/kdrl*$^{+}$ endothelial cells are present in *vegfaa* mutants, the embryos form only a single vessel instead of a separate DA and PCV (Casie Chetty et al., 2017; Rossi et al., 2016). Previous studies have demonstrated that chemical inhibitors blocking Vegf signaling leads to a reduction in the expression levels of endothelial genes, suggesting an impairment in endothelial cell differentiation (Chan et al., 2002). In contrast, a study using Vegfaa morpholinos reported an increase in venous gene expression but a decrease in arterial gene expression in the morphants (Lawson et al., 2002). These data point to separate functional roles of Vegfa in promoting endothelial differentiation and inducing arteriovenous specification. In an attempt to elucidate these different functional roles of Vegfa, a recent study analyzing endothelial gene expression in both *vegfaa* mutants and Vegf signaling-inhibited embryos (by chemical treatment) pointed to a dose- and time-dependent role of Vegfa in promoting endothelial cell differentiation (Casie Chetty et al., 2017). Similar to mice, Vegfa in zebrafish during early vasculogenesis appears to promote endothelial differentiation and survival by enhancing the expression of the ETS transcription factor *etv2*, which in turn activates the expression of multiple endothelial genes (Casie Chetty et al., 2017; Sumanas and Lin, 2006).

Signaling Pathways That Induce Specification of Vascular Endothelial Progenitors and Hemangioblasts

While FLK1 has served as an excellent marker for hemangiogenic progenitors, FLK1^{+} progenitors are heterogeneous and include cardiac progenitors (Kattman et al., 2006; Moretti et al., 2006). Recently, PDGFRα expression was used to further segregate FLK1^{+} progenitors. As such, FLK1+PDGFRα-progenitors are enriched for hemangiogenic progenitors (Kattman et al., 2011; Liu et al., 2012). Additional markers including Brachyury (T), a pan-mesoderm marker, and Scl, an ETV2 direct target, were used to track hemangiogenic lineage development. These studies revealed that hemangiogenic progenitors develop through a distinct developmental route (Zhao and Choi, submitted). In particular, as previously reported, both FLK1^{+} and PDGFRα^{+} cells were found mainly within T-GFP^{+} cells in early EBs, confirming their primitive streak origin. Initially arising FLK1^{+} cells were also positive for PDGFRα, suggesting that PDGFRα expression precedes that of FLK1. Importantly, Etv2-tdTomato^{high} cells were found mainly within FLK1^{+} cells. Comparison of RNA-seq data of T-GFP^{+}PDGFRα^{-}FLK1^{-}, T-GFP^{+}PDGFRα^{+}FLK1^{-}, T-GFP^{+}PDGFRα^{+}FLK1^{+}, and Etv2-tdTomato^{high} as well as Etv2-tdTomato^{int}, Etv2-tdTomato^{high}Scl-GFP^{-} Etv2-tdTomato^{high}Scl-GFP^{int}, and Scl-GFP^{high} cell populations to those published RNA-Seq of undifferentiated ES cells, ES cell-derived epiblast-derived stem cells (EpiSCs), endoderm, and cardiac progenitors (Choi et al., 2012; Factor et al., 2014; Wamstad et al., 2012) has revealed that while genes expressed in the primitive streak/early mesoderm such as *T*, *Mesp1*, *Gsc*, *Pdgfra*, *Tbx6*, and *Mesp2* were enriched in T-GFP^{+}PDGFRα^{+}FLK1^{-} cells, genes enriched in hemangiogenic or cardiac progenitors still remained low in these cells. This suggests that T-GFP^{+}PDGFRα^{+}FLK1^{-} cells represent primitive streak/nascent/early mesoderm. Consistently, T-GFP^{+}PDGFRα^{+}FLK1^{-} transcriptome was more similar to the proximal regions of the primitive streak of the E7.0 gastrulating embryo (Zhao and Choi, submitted), where nascent mesoderm emerges, than to the distal regions, which form definitive endoderm (Peng et al., 2016). From these studies, hemangiogenic progenitors (ETV2^{high}SCL^{+}) develop from the pluripotency stage through the following developmental path: gastrulating mesendoderm (T-GFP^{+}), nascent/early mesoderm (PDGFRα^{+}), and LPM (FLK1^{+}) (**Fig. 2**).

Previous in vitro and in vivo experiments have shown the involvement of bone morphogenetic protein (BMP), fibroblast growth factor (FGF), Wnt and Indian hedgehog (IHH) signaling pathways in the generation of hemangioblast, and/or vascular EPCs (Marcelo et al., 2013). BMP4 has been shown to be sufficient for the induction of mesoderm in vitro, and its ablation results in embryonic lethality in mice due to a failure in the generation of mesoderm (Park et al., 2004; Pearson et al., 2008; Winnier et al., 1995). Similarly, loss of the downstream effector of BMP4, *Smad5*, leads to a severe disruption of yolk sac vasculature, similar to *Bmp4*$^{-/-}$ mutant mice (Chang et al., 1999). Along the same lines, genetic deletion studies have shown that FGF2 signaling via the FGF receptor type I is a key player in the induction of the mesoderm (Flamme and Risau, 1992; Saxton and Pawson, 1999;

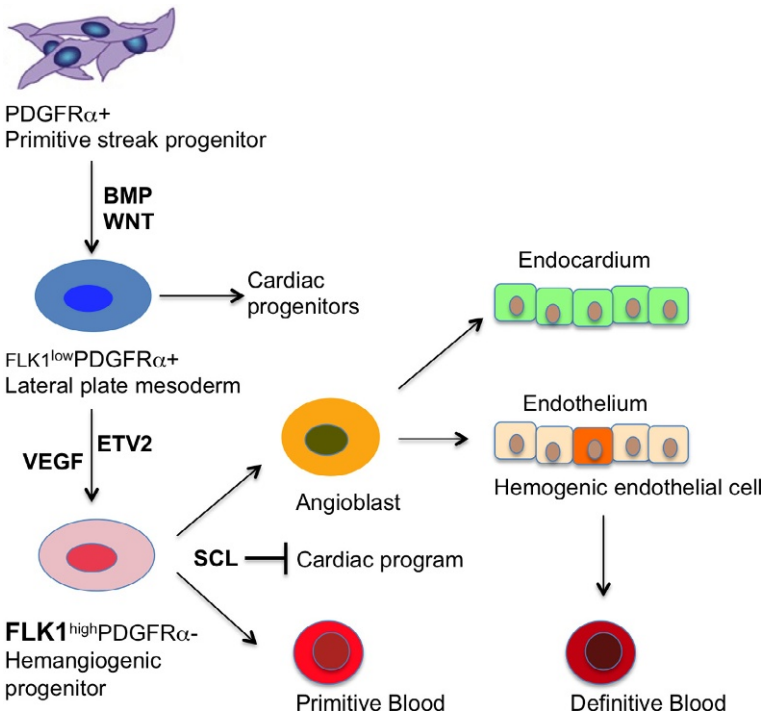

Fig. 2 A model showing the developmental route and factors involved in the generation of hematopoietic and endothelial cells.

Yamaguchi et al., 1994). The disruption of these pathways generally causes a defect in mesoderm formation, which in turn could result in the failure of endothelial and hematopoietic cell fate specification. Additionally, the IHH signaling pathway has been demonstrated to play a critical role in the differentiation of endothelial cells during development in mice. IHH, which is secreted by the yolk sac visceral endoderm, binds to its receptors expressed in the underlying mesoderm in developing mouse embryos. Experiments from mouse embryo explant cultures that lack endoderm have revealed that IHH is sufficient to promote the formation of both endothelial and hematopoietic cells (Kelly and Hirschi, 2009). However, loss of IHH or its receptor Smoothened does not completely inhibit endothelial cell differentiation, indicating the contribution of multiple signaling pathways in this process (Byrd et al., 2002). More recent in vitro studies have demonstrated the combined roles of the BMP, Notch, and Wnt pathways in generating FLK1^{+} mesoderm (Lee et al., 2008). Although the hierarchy of these signaling pathways in specifying hemangioblasts in the mesoderm is still not well understood, BMP4 and Wnt are likely to be involved in the generation of FLK1^{low}PDGFRα^{+} LPM, while VEGF further plays an instructive role in the generation of the FLK1^{high}PDGFRα^{-} hemangioblast by promoting Etv2 threshold expression (Fig. 2, Zhao and Choi, submitted). In the future, further in-depth experiments looking at the direct signaling pathways governing hemangioblast formation would be necessary to better understand the mechanisms of hemangioblast specification in the mesoderm.

Assembly of the Major Embryonic Vessels

Following the specification of angioblasts in the PLPM, the angioblasts coalesce to form the major axial vessels—the DA and CV—in the trunk. In many vertebrates, including zebrafish and frog Xenopus laevis, this involves migration of angioblast progenitors toward the midline. In zebrafish embryos, the first bilateral lines of angioblasts can be observed by *npas4l* expression as early as the tailbud stage and by both *etv2* and *npas4l* expression at the 2-somite stage (Reischauer et al., 2016). At the 10-somite stage, the second set of bilateral lines of angioblasts located laterally to the medial angioblasts can be observed by *etv2* expression. The two medial lines of angioblasts begin to migrate between the somites to the midline starting at around the 9–10 somite stage and continue to migrate till around the 15-somite stage when all the medial angioblasts congregate at the midline (Kohli et al., 2013). The angioblasts migrate as individual cells and subsequently coalesce after they have reached the midline (Jin et al., 2005). It is interesting to note here that this migration occurs in a wave-like manner, beginning with the more anteriorly located angioblasts and then progressing posteriorly. It is unclear what spatiotemporal cues coordinate this pattern of migration of angioblasts. Starting at around the 16-somite stage, the lateral lines of angioblasts begin to migrate toward the midline and largely complete their migration by the 22-somite stage although individual migrating angioblasts can be seen even at later stages. Lineage tracing and cell labeling experiments have demonstrated that the first wave of medial angioblasts gives rise to the DA and the second wave of lateral angioblasts contributes to the PCV (Kohli et al., 2013). Thus, DA and PCV in zebrafish originate at distinct locations and different time points; arterial progenitors originate earlier and closer to the midline than venous progenitors (Fig. 3A and B).

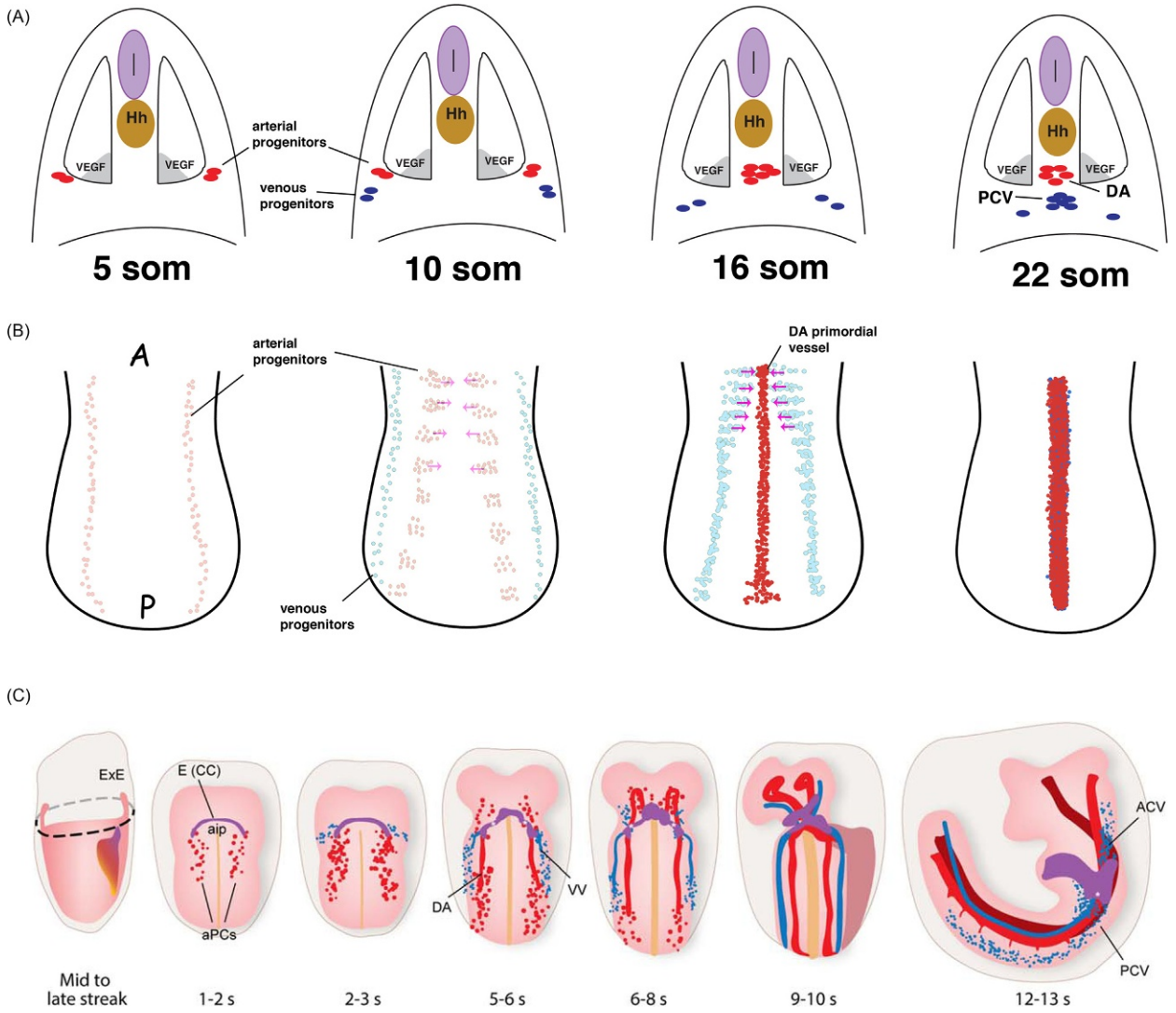

Fig. 3 Assembly of the major axial vessels in zebrafish (A and B) and mouse (C) embryos. (A and B) Transverse cross-sectional (A) and dorsal views of vascular endothelial progenitors in the trunk region. The first group of endothelial progenitors appears as a pair of bilateral lines (*light-red colored cells*) at around the 5 somite stage. At approximately the 10 somite stage, a second pair of bilateral lines are observed (*light-blue cells*), located laterally to the initial lines of endothelial progenitors. At around the same time, the more medially located lines of angioblasts begin to migrate toward the midline (indicated by *pink arrows*) between the somites (at the somite boundaries), beginning at the anterior end of the embryo. This initial migration is completed at approximately the 16ss, and at this point the second population of angioblasts commences their migration toward the midline. Angioblasts in this second wave of migration congregate at a position more ventral to the first group of cells. By around the 22ss, nearly all the angioblasts have completed their migration to the midline. Hedgehog (Hh) secreted by the notochord induces the secretion of VegfA from the somites. The first group of endothelial cells has a more dorsal location and is thus closer in proximity to the source of Vegf. In response to the high concentration of Vegf ligand, these cells differentiate into arterial cells (*red*) and form the DA. The more ventrally located group of endothelial cells experience a lower concentration of Vegf and are specified as venous cells (*blue*) which form the PCV. Adapted from Kohli, V., Schumacher, J. A., Desai, S. P., Rehn, K. and Sumanas, S. (2013). Arterial and venous progenitors of the major axial vessels originate at distinct locations. *Developmental Cell* **25**, 196–206. (C) In mouse embryo, Flk1 + angioblasts are apparent in the LPM starting at the 1–2-somite stage, posterior to the endocardial cells underlying the cardiac crescent (E(CC), *purple*). Aortic progenitor cells (aPCs) coalesce to form paired dorsal aortae (DA). Venous progenitors coalesce into viteline veins (VV) by 5–8-somite stage. The embryo turns between 9–12-somites. Anterior (ACV) and posterior cardinal veins (PCV) are apparent by the 12–13-somites. Reprinted from Fish, J. E. and Wythe, J. D. (2015). The molecular regulation of arteriovenous specification and maintenance. *Developmental Dynamics* **244**, 391–409, with permission from John Wiley and Sons.

In contrast, a different mechanism of DA and PCV formation, termed ventral sprouting has been proposed by Herbert and colleagues. According to it, both the medial and lateral lines of angioblasts migrate to the same location at the midline, to form a common precursor vessel (Herbert et al., 2009). Subsequently, venous progenitors begin to migrate ventrally out of the precursor vessel to give rise to the PCV. Both models can be reconciled if the majority of the cells form the PCV by direct migration while some venous progenitors contribute to PCV through ventral sprouting. Intriguingly, studies in mouse embryos also showed that some

venous progenitors are initially present within the DA. Ephrin B2/EphB4 signaling was implicated in clearing venous-fated ECs from the DA (Lindskog et al., 2014). Additional studies will need to resolve the remaining discrepancies and clarify the details of DA and PCV assembly.

Similar to zebrafish, arterial cells which give rise to the paired dorsal aortae in mammalian embryos originate earlier and closer to the midline compared to the venous progenitors. As early as the 1–2-somite stage Flk1 + positive angioblasts align bilaterally and start coalescing to the paired aortae at the 5–6-somite stage (Chong et al., 2011). Because aortae are initially paired in mouse embryos, differently from fish or frogs, angioblasts do not need to migrate to the midline. Arterial progenitors are thought to spread in the anterior and posterior direction as they coalesce into the dorsal aortae. Scattered venous progenitors appear first near the endocardium and sinus venosus at the 2–3-somite stage. They migrate laterally and posteriorly and coalesce into the vitelline veins. After the embryo completes turning at 13-somites, anterior and PCVs begin to appear (**Fig. 3C**) (Chong et al., 2011). It is currently not clear if the mechanisms involved in the midline migration of angioblasts in zebrafish and frog embryos are equivalent to guiding migration in mammalian embryos.

Several guidance cues have been implicated in the guiding the migration of angioblasts from the LPM to the midline. In *Xenopus*, VegfA was demonstrated to play an important role in guiding the migrating angioblasts to the midline (Cleaver and Krieg, 1998). It was suggested that a similar function of VegfA as an angioblast chemoattractant would be present in zebrafish as well (Coultas et al., 2005; Gore et al., 2012; Verma et al., 2010). However, since then, many studies have revealed that VegfA signaling is not required for this process in zebrafish. The knock down of Vegf or its downstream signaling molecule *plcγ1* did not affect angioblast migration (Helker et al., 2015). Additionally, the *vegfaa* mutant does not appear to have a clear defect in migration (Rossi et al., 2016).

Upstream of Vegf signaling, Sonic Hedgehog (Shh) signaling from the notochord is known to be important for proper angioblast migration in zebrafish (Lawson et al., 2002). Previous studies have shown that either chemical inhibition of Hh signaling (Gering and Patient, 2005) or a lack of a notochord (*noto/floating head* mutants) or defective notochord (*ta/no tail* mutants) in zebrafish leads to an impairment in angioblast migration (Fouquet et al., 1997; Sumoy et al., 1997). As mentioned earlier, it has been well established that there are two waves of angioblasts that migrate to the midline and that the DA forms before the PCV in both mouse and zebrafish (Herbert et al., 2009; Kohli et al., 2013; Lindskog et al., 2014; Torres-Vazquez et al., 2003). In embryos lacking Hedgehog signaling, the first wave of angioblast migration appears to be absent and all the angioblasts migrate in the second wave, giving rise to the PCV (Williams et al., 2010). Despite the lack of Vegf signaling in these Hedgehog-deficient zebrafish embryos, the angioblasts are still able to migrate to the midline, suggesting that neither Vegf nor Hedgehog signaling is required for angioblast migration (Helker et al., 2015). Intriguingly, studies in chick embryos have shown that the notochord produces BMP inhibitors Noggin and Chordin which prevent endothelial cells from entering the midline regions resulting in the bilateral position of dorsal aortae (Bressan et al., 2009; Reese et al., 2004).

Relatively recent studies have identified a novel signaling mechanism which guides angioblast migration in zebrafish (Helker et al., 2015). Apelin, which is a short peptide encoded by the *apln* gene, is secreted by the notochord and has been shown to be involved in regulating heart field development (Zeng et al., 2007). Apelin is secreted by the notochord at around the same time that its corresponding receptors *apelin receptor a* (*aplnra*) and *apelin receptor b* (*aplnrb*) are expressed by angioblasts, prior to their migration. While knocking down either receptor caused a reduction in the efficiency of migration of angioblasts to the midline, migration was completely abolished in double knock-down embryos. This was confirmed in double-mutant embryos for the *aplnra* and *aplnrb* genes, emphasizing the necessity of apln receptor signaling during the migration of angioblasts to the midline during early vasculogenesis (Helker et al., 2015). Surprisingly, the depletion of the Apln ligand by MO and genetic knockout revealed no defects in migration and vasculogenesis, suggesting that another ligand might be involved in the process. The recently identified novel peptide hormone, Elabela, has been shown to bind and activate apln receptors (Chng et al., 2013; Pauli et al., 2014). Homozygous ela mutants exhibited a strong defect in angioblast migration although it was not as severe as that seen in the apln receptor double mutants. However, homozygous double mutants for *apln* and ela ($apln^{-/-};ela^{-/-}$) have a severe migration defect, similar to that seen in the apln receptor double mutants. These results suggest that Ela, which is also secreted by the notochord, plays a central role angioblast migration to the midline, while Apln plays a minor role in the process. Understanding the downstream signaling cascades of Apln signaling will provide essential insight into one of the earliest and most important steps in vasculogenesis.

Although defects are observed in the axial vasculature in *vegfaa* and *shh* mutants, the anterior vasculature remains unaffected, suggesting that different mechanisms regulate the formation of vessels from the angioblasts in the ALPM. Time-lapse videos using *etv2:GFP* reporter showed that the majority of cranial vascular endothelial progenitors come from two distinct regions. The most anterior region, the rostral organizing center (ROC) gives rise to the most rostral cranial vasculature while the rest of the cranial vasculature comes from the MOC region, which is located within the LPM ventral from the midbrain (Proulx et al., 2010). While the ROC and MOC angioblast clusters are derived by vasculogenesis, the majority of the cranial vasculature including the lateral DA (LDA) forms by angiogenesis when angioblasts coalesce into cord-like structures and extend angiogenic sprouts. In addition to endothelial progenitors of the cranial vasculature, MOC also gives rise to the primitive macrophages as well as the endocardial progenitors (Proulx et al., 2010). Interestingly, all myeloid progenitors originate in the MOC region and not the more rostral region. Therefore clearly not all EPCs go through the hemangioblast stage. Similar to the formation of the LDA, at around 17hpf a group of more laterally located angioblasts in the ALPM and in the anterior region of the PLPM begin to migrate toward each other and eventually form the posterior hindbrain channel which is the largest vein in the head region (Bussmann et al., 2011; Siekmann et al., 2009). This migration event has been shown to be mediated by the chemokine Cxcl12b, expressed in the endoderm underlying the

lateral aortae and its receptor Cxcr4a (Siekmann et al., 2009) which is restricted to endothelial cells. The knockdown of *cxcr4a* or Cxcl12b leads to impaired fusion of the angioblasts and results in the disrupted formation of the LDA.

Several studies have pointed to a critical requirement of the endoderm during vasculogenesis (Bielinska et al., 1996; Palis et al., 1995; Vokes and Krieg, 2002). Experiments on *Xenopus* embryos have indicated a requirement of the endoderm for proper angioblast migration and vessel formation during vasculogenesis (Vokes and Krieg, 2002). Loss of the endoderm by surgical removal resulted in an impairment in the formation of functional vascular tubes in avian and frog embryos (Vokes and Krieg, 2002). Similarly, angioblasts were shown to be in close association with the endoderm during their migration in mouse and quail embryos, further underscoring the significance of the endoderm during early stages of vascular development (Vokes et al., 2004). However, a more recent study on zebrafish embryos indicated that the endoderm, while necessary for the proper temporal regulation of angioblast migration, was not essential for the formation of functional vascular tubes. In zebrafish mutant embryos showing either a complete lack of endoderm (*casanova*) or a strong reduction in endoderm (*bonnie and clyde*), migration of the angioblasts to the midline was severely delayed and the angioblasts appeared to migrate in aggregates by forming cell–cell junctions with each other, as opposed to migrating as individual cells (Jin et al., 2005). Despite their abnormal migration, the angioblasts appeared to form two normal axial vessels at the midline. Thus, though the endoderm plays an important role in the temporal regulation of angioblast migration, it does not affect the overall formation of the axial vessels.

Arteriovenous Specification

A functional vascular system consists of arteries and veins, which are both formed by angioblasts, but have distinct molecular and structural properties. Classical models of vasculogenesis have attributed these differences between arteries and veins to hemodynamic forces exerted on the walls of the vessels during circulation (Gonzalez-Crussi, 1971). Indeed, blood flow can regulate arterial-venous identity in some cases (le Noble et al., 2004). However, growing evidence over the past twenty years has demonstrated that cells acquire distinct arteriovenous fates at early stages prior to the initiation of circulation (Wang et al., 1998; Zhong et al., 2000, 2001). During the early stages of vascular development, endothelial cells acquire either an arterial or venous fate, and this step is critical for the correct architecture of the vascular network. Early cell labeling experiments demonstrated that a single endothelial progenitor can give rise to either arterial or venous cells but not to both lineages (Zhong et al., 2001). However, this does not necessarily demonstrate that each progenitor is committed to a specific lineage. Although it is unclear as to exactly when an endothelial cell makes the decision to become either arterial or venous, the signaling pathways and downstream effectors that govern this decision-making process have been fairly well described.

Several initial studies in zebrafish delineated a Shh–Vegf–Notch signaling axis for promoting arterial specification and repressing venous identity (Lawson et al., 2001, 2002, 2003). Sonic hedgehog, which is secreted by the notochord, induces the secretion of VegfA from the somites (Lawson et al., 2001, 2002). VegfA then binds to Vegfr2/*kdrl* present on the surface of endothelial progenitors and activates notch signaling which, in turn, promotes arterial fate (Lawson et al., 2001, 2002). However, it has been recently shown that Shh can also act directly on endothelial cells and promote arterial fate in a Vegf-independent manner via a calcitonin receptor-like receptor (Wilkinson et al., 2012). Vegf role in arterial specification appears evolutionarily conserved. High concentrations of Vegf promote arterial differentiation ES cell culture (Lanner et al., 2007). Mouse VegfA mutants display loss of arterial development (Carmeliet et al., 1996; Ferrara et al., 1996). Vegf signaling through VegfR2 has been shown to promote arterial fate by inducing the expression of several arterial-specific genes. In zebrafish it has been proposed that the first population of angioblasts that arise in a more medial location in the LPM and migrate to the midline to form the DA experience a higher concentration of VegfA ligand secreted from the somites than the second wave of lateral angioblasts that form the PCV, which is located ventrally to the DA (Kohli et al., 2013). This higher concentration of VegfA experienced by the more dorsally located endothelial cells results in the suppression of venous genes and the induction of arterial genes (**Fig. 3A**) (Kohli et al., 2013). A recent study demonstrates separate roles of Vegfa signaling during initial endothelial cell differentiation and subsequent arteriovenous specification. While low levels of Vegfa signaling at early stages appears to promote endothelial cell differentiation through the induction of *etv2* expression, high levels of Vegf signaling at subsequent stages appear to repress venous gene expression and promote the expression of arterial genes (Casie Chetty et al., 2017). High level of Vegf results in the higher expression of *etv2* in the arterial progenitors, which in turn upregulates *dll4* and *notch* expression. Subsequently, Dll4–Notch signaling promotes arterial and inhibits venous differentiation. While several studies have shown that VegfA signaling promotes arteriogenesis, there is some controversy surrounding the downstream effectors that are activated in this process. The identification of a mutant with defective artery formation revealed a critical role of phospholipase C gamma-1 (plcg1) downstream of Vegf signaling, in regulating arterial specification (Lawson et al., 2003). Several studies have shown that the activation of extracellular signal-regulated kinase (ERK) drives arterial gene expression downstream of plcg1, through a complex cross talk with PI3K (Deng et al., 2013; Hong et al., 2006, 2008; Ren et al., 2010). However, a more recent study demonstrated that VegfA signaling via ERK does not promote arterial specification but instead is responsible for the initiation of angiogenesis (Shin et al., 2016).

According to the current model of arteriovenous specification, which is based on the Shh–Vegf–Notch axis, Notch signaling is activated downstream of Vegf signaling (Gore et al., 2012). In zebrafish, similar to the mammalian embryos, both the receptors (Notch1b, Notch5) and ligands (Delta homolog Dll4, Jagged) of Notch signaling are expressed exclusively in arterial endothelial cells. The upregulation of the Notch ligand Dll4 in response to Vegf signaling results in high Notch signaling activity within the developing aorta (Quillien et al., 2014). This increase in Notch activity appears to be a critical event in the repression of venous

genes, which allows cells to take on an arterial identity (Quillien et al., 2014). A deficiency in Notch signaling resulted in the expansion of venous markers into arterial domains and the loss of arterial marker expression, while overactivation of Notch leads to enhanced arterial gene expression and a suppression of venous genes in the PCV (Lawson et al., 2001, 2002, 2003). Similar to zebrafish, Dll4–Notch signaling is required for arterial specification in mouse embryos (Duarte et al., 2004; Gale et al., 2004; Kim et al., 2008). With regard to the regulation of Notch activity, multiple ETS transcription factors including Etv2 have been shown to bind to the promoters of several endothelial genes, including that of *dll4* (De Val et al., 2008; Lee et al., 2008; Liu et al., 2015; Wythe et al., 2013). It has been proposed that Vegf-MAPK-dependent induction of ETS factors including Erg and Fli1 regulates the expression of *Dll4* and *Notch4* and thus promotes arterial fate in mouse embryos (Wythe et al., 2013).

Apart from the well-established Shh–Vegf–Notch signaling pathway, additional signals and transcription factors are important for arteriovenous specification. COUP-TFII/Nr2f2 transcription factors are required in mouse embryos to establish venous identity by suppressing Notch signaling (You et al., 2005). The SRY-related HMG box (Sox) group of transcription factors has also been implicated in the regulation of arteriovenous specification. Loss-of-function studies have demonstrated that Sox7 and Sox18 (which are expressed in the artery and vein) are important in ensuring proper arteriovenous morphogenesis (Cermenati et al., 2008; Herpers et al., 2008; Pendeville et al., 2008). Sox factors promote arterial differentiation by directly binding together with RBPJ/Notch Intracellular domain to an enhancer that regulates Dll4 expression (Sacilotto et al., 2013). Murine Sox17 is selectively expressed in arteries, and endothelial-specific inactivation of Sox17 in the mouse embryo results in the absence of arterial differentiation (Corada et al., 2013). In addition, Wnt/beta-catenin pathway has been implicated in murine vascular development and arterial differentiation by upregulating Dll4/Notch signaling (Corada et al., 2010). In vitro experiments also support a combined role of Notch and beta-catenin in arterial differentiation (Yamamizu et al., 2010).

Vessel Lumenization

The formation of a hollow tube is one of the final steps in vasculogenesis and is critical for ensuring proper circulation in the developing embryo. Tubulogenesis can occur by several mechanisms and varies between different types of tubes (Baer et al., 2009; Lubarsky and Krasnow, 2003). During the formation of the vascular network, the two waves of endothelial progenitors which migrate to the midline as individual cells form aggregates which represent a vascular cord-like structure (Jin et al., 2005). The now-differentiated endothelial cells then undergo different morphogenetic processes to form a tubular structure. This can occur by several mechanisms including (a) cell hollowing—vacuoles form within cells and then fuse between cells to create a contiguous intracellular luminal space; (b) cord hollowing—cells within the cord acquire apicobasal polarity resulting in membrane separation and the formation of a lumen between cells; (c) wrapping—cells become polarized and migrate to form a lumen; (d) cavitation—cells within the center of the cord undergo apoptosis and leave a luminal space (Hogan and Kolodziej, 2002; Kucera et al., 2007; Lubarsky and Krasnow, 2003). In zebrafish embryos, the DA and PCV are fully lumenized by around the 28–30hpf, while mouse aortae are lumenized at 3–5 s (around E8.0) (Chong et al., 2011; Isogai et al., 2001).

When endothelial cells aggregate at the midline to form a vascular cord in zebrafish, they do not appear to form cell–cell junctions and instead remain as clusters of cells (Jin et al., 2005). At about 17hpf, the cells begin to form tight junctions between each other as seen by zona occluding-1 staining. Beginning at ~18hpf, the DA begins to lumenize by a "hollowing out" mechanism (Jin et al., 2005). Similar observations were reported by Parker et al. who identified an EC-derived secreted factor EGF-like domain 7 (*EGFL7*) which appears to regulate vessel lumenization (Parker et al., 2004). Following the formation of the solid vascular cord, angioblasts undergo spatial separation and rearrangement to form a lumen within the center of the nascent vessel. This involves the breakdown of tight junctions between angioblasts within the center of the tube—a process controlled by EGFL7. Loss of EGFL7 function results in a failure of the angioblasts to separate and form a lumen in the center of the vascular cord, indicating that EGFL7 is required for the downregulation of tight junctions during tubulogenesis (Parker et al., 2004). It has been proposed that the DA and PCV lumenize by different mechanisms: while the DA appears to lumenize by a cord hollowing-out mechanism, the PCV is formed by ventrally sprouting venous cells that coalesce around blood cells (Herbert et al., 2009). Subsequently, intersegmental vessels lumenize by the fusion of intracellular vacuoles (Kamei et al., 2006).

Similar to zebrafish, aortic lumenization in the mouse also appears to occur by a cord-hollowing mechanism. Studies examining the molecular mechanisms of lumen formation in the paired aortae of mouse embryos revealed the formation of intercellular adherens junctions by VE-cadherin between endothelial cells followed by junctional remodeling and the polarization of endothelial cells (Dejana et al., 2009; Strilic et al., 2010). The initial establishment of apical–basal polarity in endothelial cells appears to rely on the presence of VE-cadherin without which the accumulation of antiadhesive molecules such as CD34-sialomucins and podocalyxin at cell–cell contact sites does not occur (Strilic et al., 2010). Both these molecules which contain negative charges accumulate at the future luminal surface of the ECs and cause repulsion between the cells, thus initiating the formation of a lumen. The establishment of EC polarity is also dependent upon proper GTPase signaling, which regulates structural changes in the cytoskeleton and overall cell shape and polarity. Mice which lack an endothelial-restricted Ras-interacting protein Rasip1 fail to form lumenized vessels due to dysregulation of GTPase signaling which leads to a defect in the establishment of cell polarity (Xu et al., 2011). Following polarization of ECs, Vegf induces the localization of myosin II to the apical surface of the cells, causing constriction of the apical membrane. Further cell shape changes occur by the recruitment of F-actin to the apical surface, a process dependent on CD-34 sialomucins and Moesin (Strilic et al., 2010; Wang et al., 2010). Together, these processes lead to the formation of a mature lumen (**Fig. 4**).

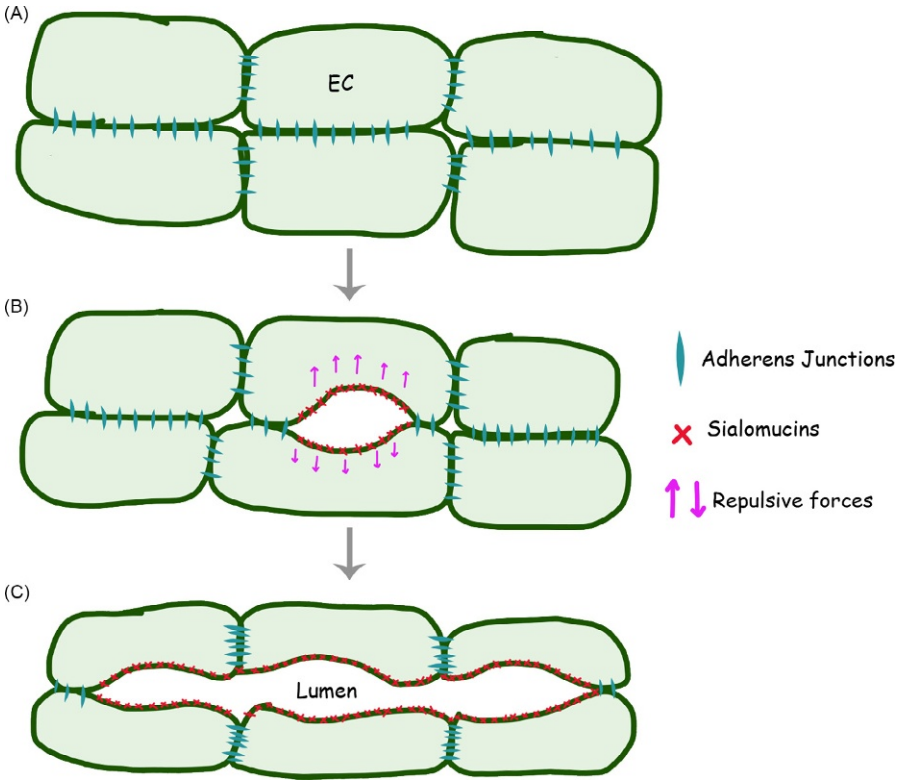

Fig. 4 Following the migration of endothelial progenitors, the cells form a vascular cord-like structure which consists of an aggregation of cells. The formation of adherens junctions by VE-cadherin between neighboring cells is important for the establishment of these aggregates (A) and also for the establishment of apicobasal polarity of the ECs. Negatively charged sialomucins become localized at the future-luminal surface of the ECs and cause repulsion between cells at the luminal surface (B). This is accompanied by a reorganization of adherens junctions and a change in cell shape (C). Repulsion between multiple cells along the length of the vascular cord results in the formation of a continuous lumen (C). Note that the lumen in smaller vessels forms by vacuole fusion (not shown).

Concluding Remarks

While this review focuses on the mechanisms of vasculogenesis during embryonic development, many of the described steps can be affected in vascular diseases. For example, mutations in Notch affect arteriovenous specification and can result in arterial–venous malformations (AVMs) (Delev et al., 2016). Furthermore, vasculature defects in AVMs can be alleviated in the mouse model by activating Notch (Murphy et al., 2012), which points to the potential future clinical therapies. Another example of potential therapeutic intervention is the ETS transcription factor Etv2, which is critical for embryonic endothelial specification. Overexpression of Etv2 can transdifferentiate fibroblasts into vascular endothelial progenitors and promotes recovery in the mouse hindlimb ischemia model (Lee et al., 2017; Morita et al., 2015; Park et al., 2016). Therefore, research on the molecular mechanisms related to embryonic vasculogenesis will ultimately greatly contribute toward the development of novel therapeutic approaches for numerous vascular disorders, wound healing, ischemia, and cancer.

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Ventricular Arrhythmias and Sudden Cardiac Death in Hypertrophic Cardiomyopathy

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Introduction

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease caused by mutations in genes coding for cardiac sarcomeres. There are multiple genetic variants, but all phenotypic expressions display pathology characterized by myocardial hypertrophy and disarray. HCM is the most common inherited heart disease with a prevalence of 0.2% in the general population (Maron et al., 1995). Patients with HCM are at an increased risk of potentially lethal ventricular arrhythmias (Elliott et al., 2006a; Maron et al., 2000). Prevention of sudden cardiac death (SCD) is best accomplished by implantation of a cardioverter-defibrillator (ICD) in patients who are at risk of SCD due to ventricular arrhythmias (Maron et al., 2000; Russo et al., 2013). Selection of patients in whom ICD implantation is appropriate is guided by risk stratification using the presence or absence of specific clinical risk factors (Russo et al., 2013).

Ventricular Arrhythmias and SCD

Conventional Risk Factors

Patients with HCM are at an increased risk of the development of ventricular dysrhythmias, including ventricular tachycardia (VT) and ventricular fibrillation (VF). SCD is the most serious consequence of the development of ventricular dysrhythmias. The young are at particular risk for SCD, while those over 60 are less affected by SCD (Russo et al., 2013). Five risk factors have been found to be associated with SCD; these include family history of sudden death, personal history of unexplained syncope, massive left ventricular wall thickness, nonsustained VT, and an abnormal blood pressure response to exercise (Dimitrow et al., 2010; Maron et al., 2013a; Gersh et al., 2011). While these risk factors were derived from tertiary referred patients, the same risk factors were found to be important even in a community-based cohort (Anastasakis et al., 2013).

LV hypertrophy

The degree of left ventricular hypertrophy (LVH) directly correlates to the risk of SCD, increasing progressively in relation to the maximal wall thickness (Spirito et al., 2000). While the highest risk was observed in those with maximal wall thicknesses $\geq$30 mm, there was not a sudden increase in risk with this measurement but rather a linear increase in risk as wall thickness increased. The burden of VT also directly correlates with increasing LVH; in one study of patients with HCM in whom VT had been documented,

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the extent of LV hypertrophy was compared with a control group of HCM patients without documented VT. The study found significantly more LV hypertrophy in patients with documented VT (53%) versus those without VT (21%) (Spirito et al., 1987).

A more recent study including 157 HCM patients evaluated the ventricular arrhythmia events over a median of 3.7 years (Puntmann et al., 2010a). Patients were evaluated with 2-D echocardiography for LV wall measurements at baseline and followed clinically thereafter. Maximal ventricular wall thickness and basal anterior thickness above 15 mm were associated with a 4.5 fold increase in relative risk of ventricular arrhythmias. Additionally, equatorial inferior thicknesses above 19 mm had a 5.9 fold increase in relative risk. The two regional measures (basal and equatorial) were independently associated with risk of ventricular arrhythmias.

Syncope

Syncope is present in approximately 14%–35% of patients with HCM. Unexplained syncope has been repeatedly identified as a predictor of SCD (Spirito et al., 2009; Fananapazir et al., 1992). A large cohort study of 1511 patients with HCM found 205 patients to have unexplained or neurally mediated syncope. Over a mean follow-up of 5.6 ± 5.2 years, 74 of the 1511 patients experienced sudden death (Spirito et al., 2009). The relative risk of sudden death was 1.78 (95% CI: 0.00–3.51, $P = 0.08$) in patients with unexplained syncope and 0.91 (95% CI 0.00–3.83, $P = 1.0$) in those with neurally mediated syncope compared with HCM patients without syncope. Additionally, the timing of unexplained syncope was independently associated with SCD; patients who had an episode of unexplained syncope within 6 months before initial evaluation had a 5-fold increase in risk compared with patients without syncope (HR 4.89%, 95% CI 2.19–10.94). A smaller but similar study of 174 consecutive patients with HCM and with a follow-up of 74 ± 22 months reported eight cardiovascular deaths. A history of syncope was noted in all eight patients (Kyriakidis et al., 1998).

Family history of sudden death

A family history of sudden death due to HCM has been identified as a significant risk factor for sudden death in patients with HCM. Alone, a positive family history has a 1.9 (95% CI 0.8–4.5, $P = 0.15$) risk ratio for sudden death. However, when combined with history of syncope, the risk ratio increases to 5.3 (95% CI 1.9–14.9, $P = 0.002$) (Elliott et al., 2000). In the 2011 ACC/AHA Guidelines a single family member with SCD qualifies as a risk factor, while in the 2006 European Guidelines it took two family members dying suddenly from HCM. Additionally, it is not clear how proximate the family member must be; whether 2nd degree relatives, who presumably carry the same gene confer as much risk as 1st degree relatives is not completely understood.

Nonsustained ventricular tachycardia

Nonsustained VT (NSVT) in patients with HCM has been independently associated with sudden death in multiple studies. In one study examining 1380 HCM patients during exercise testing, 24 had NSVT and 3 had VF (Gimeno et al., 2009). Eight of these 27 patients died or had a cardiac event requiring ICD implant or ICD firing, compared with 150 patients without exercise-induced NSVT/VF ($P = 0.008$). Patients with NSVT or VF had a 3.73 fold increase in risk of sudden death or ICD firing (HR 95% CI: 1.61–8.63, $P = 0.002$). Beyond arrhythmias in the setting of exercise, another study used Holter monitoring for arrhythmia detection. In this study, the estimated rate for sudden death was 1.8% per year in patients with NSVT compared with 0.8% in those without NSVT (Adabag et al., 2005). However, the presence of NSVT has some conflicting evidence as to its contribution to SCD. In one study of an unselected patient population with HCM, a history of NSVT was not associated with adverse prognosis (Cecchi et al., 1998). Evidence is accumulating that not all NSVT portends an equivalent risk. NSVT in the patient less than 30 years is more highly predictive of SCD than NSVT in individuals >30 years (Monserrat et al., 2003). Additionally, faster and longer NSVT is more predictive of SCD than slower and shorter NSVT (**Figs. 1** and **2**) (Wang et al., 2017).

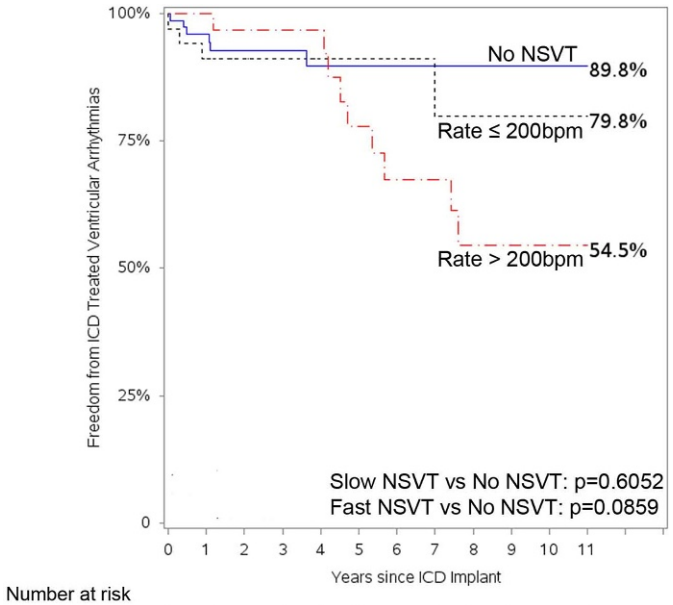
Abnormal blood pressure response to exercise

A hypotensive or normotensive response to exercise was initially proposed as a marker for increased disease-related mortality in a study of HCM patients by Olivotto. Of 126 patients, 19 had failure of blood pressure to rise and 9 had a hypotensive response (Olivotto et al., 1999). On follow-up of 4.7 ± 3.7 years, 9 patients had HCM-related deaths and 4 of them had abnormal blood pressure response to exercise. Additionally, survival analysis found that patients aged ≤50 years with abnormal blood pressure response had an increased risk for cardiovascular mortality. Although the positive predictive accuracy was low, the negative predictive accuracy was high, allowing for possible consideration of this test to be used in screening fashion to identify low-risk patients.

Emerging Risk Factors

Fibrosis

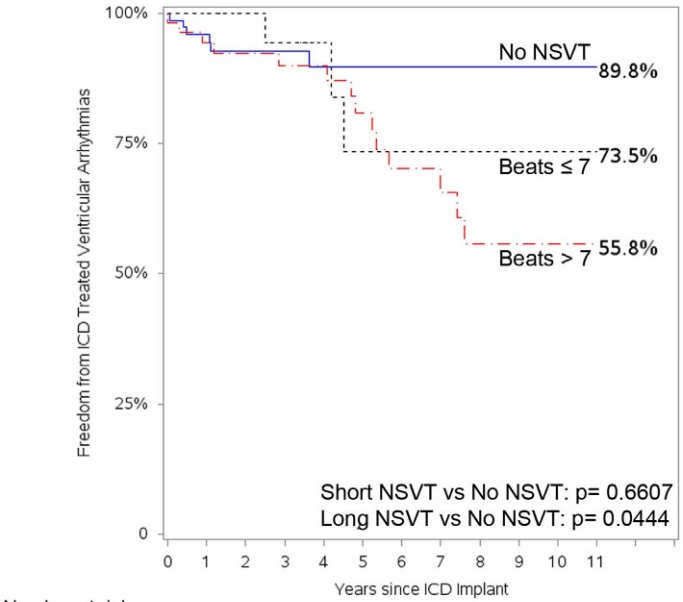
Myocardial fibrosis is a common finding in HCM patients. Late gadolinium enhancement (LGE) on cardiac magnetic resonance imaging (CMR) assesses for fibrosis. Multiple studies have found associations between the percentage of myocardial scar due to fibrosis, as measured by LGE on CMR, and ventricular tachyarrhythmias (Oka et al., 2008; Kwon et al., 2008; Adabag et al., 2008; Leonardi et al., 2009; Chan et al., 2014). The largest of these studies examined 1293 patients undergoing CMR, and fibrosis as



Number at risk

No NSVT	74	61	47	37	26	22	18	15	9	6	3	3
Rate ≤ 200bpm	34	30	24	20	15	13	10	6	5	4	1	1
Rate > 200bpm	32	31	28	23	22	16	13	11	6	5	3	1

Fig. 1 Survival probability for freedom from first ICD treated ventricular tachyarrhythmia stratified by the rate of nonsustained ventricular tachycardia (NSVT) ($\leq$200 bpm vs. $>$200 bpm) in 66 patients. Survival was estimated using Kaplan–Meier curves and compared using log-rank test.



Number at risk

No NSVT	74	61	47	37	26	22	18	15	9	6	3	3
Beats ≤ 7	25	24	19	15	11	7	7	5	2	2	1	0
Beats > 7	54	48	43	34	33	25	18	14	9	7	4	2

Fig. 2 Survival probability for freedom from first ICD-treated ventricular tachyarrhythmia stratified by length of nonsustained ventricular tachycardia (NSVT) ($\leq$7 vs. $>$7 beats) in 79 patients. Survival was estimated using Kaplan–Meier curves and compared using log-rank test.

quantified by LGE correlated linearly with risk of ventricular arrhythmias (Chan et al., 2014). A total burden of scar by LGE $\geq$15% of LV mass connoted a two-fold increase in SCD event risk. Additionally, certain locations of scarring may be at an increased likelihood of ventricular arrhythmias. The frequency of VT was several-fold higher in patients with basal septal scarring on CMR as compared to those patients without (27% vs. 5%, $P=0.03$) (Kwon et al., 2009). In a separate study, a higher number of scarred segments was shown to be a significant arrhythmogenic risk factor in patients with basal septal hypertrophy (Amano et al., 2014).

Additionally, signal intensity may be a useful tool to prognosticate arrhythmic risk. One study examining signal intensities of LGE found that intermediate signal intensity was a better predictor of ventricular tachyarrhythmias including NSVT, ventricular couplets, and PVCs, as compared to high intensity LGE (Appelbaum et al., 2012).

Left ventricular apical aneurysm

LV apical aneurysm has an estimated prevalence of approximately 2% in HCM patients (Maron et al., 2008). In a large cohort study, these patients experienced an adverse event rate of 10.5% per year, events including sudden death, aborted cardiac arrest, and appropriate ICD interventions for SCD. In this study, >40% of those with LV aneurysm who experienced cardiac arrest, SCD, or appropriate ICD interventions had no otherwise conventional risk factors (Maron et al., 2008). Apical aneurysms are typically ringed with scar and associated with additional transmural myocardial scarring and fibrosis. This degree and location of scarring likely serves as the arrhythmogenic substrate (Maron et al., 2008; Paul et al., 2006).

End-stage phase

The end-stage phase of HCM, characterized by LV systolic dysfunction and often dilatation, is associated with poor outcomes from clinical heart failure, and an increased risk for sudden death (Harris et al., 2006). In a large multicenter cohort of HCM patients, end-stage disease had a SCD prevalence of 3.5%, appropriate ICD interventions in 10% of patients annually, and an overall annual mortality rate of 11% per year (Harris et al., 2006). Although often occurring concomitantly with other traditional risk factors, given the high rate of severe and mortal events at least somewhat associated with ventricular arrhythmias, it is not unreasonable to consider end-stage HCM as an independent risk factor for SCD. Clinically, patients with end-stage HCM risk being overlooked, as they may not fulfill traditional criteria for ICD implantation in the nonischemic population (ejection fraction $\leq$35%). However, an ejection fraction below 50% in a patient with HCM, when systolic function is usually hyperdynamic, indicates significant myocardial dysfunction and elevated risk (Harris et al., 2006).

Left ventricular outflow tract obstruction

About 1/3 of HCM patients have left ventricular outflow tract obstruction at rest and another 1/3 only with exertion (Maron et al., 2003a; Elliott et al., 2006b; Autore et al., 2005). Obstructive patients are at a significantly higher risk of SCD than those without obstruction (Maron et al., 2003a; Elliott et al., 2006b; Maki et al., 1998). In one large cohort study, an LVOT basal gradient $\geq$30 mmHg was an independent predictor of sudden death or appropriate ICD discharge, and the severity of outflow tract obstruction positively correlated with a higher occurrence of sudden death and appropriate ICD discharges (Elliott et al., 2006b). Due to its high prevalence in patients with HCM, when considered as a lone risk factor, outflow tract obstruction has a high negative predictive value (95%–95.9%) but a low positive predictive value (7%–9.7%) (Maron et al., 2003a; Elliott et al., 2006b).

High risk genetic mutations

Early studies reported that patients with troponin T mutations were at a high risk of SCD at a young age (Fiset and Giles, 2008; Baudenbacher et al., 2008; Watkins et al., 1995; Tsoutsman et al., 2006). Additionally, recent studies have shown that double or compound sarcomere mutations may predispose patients to advanced disease progression and higher risk of sudden cardiac risk absent conventional risk factors. However, with over 1000 different mutations currently known in HCM, including many involving troponin T, and significant phenotypical heterogeneity, clinical risk stratification based on genetic testing has a very limited role in risk stratification (Alcalai et al., 2008; Maron, 2010; Maron et al., 2012). Further prospective observations of large genetic databases with clinical correlations will be required to further elucidate whether individual mutations or gene defects correlate with elevated risk, and to what degree.

Myocardial ischemia

Atherosclerotic coronary artery disease (CAD) compounds and elevates risk of SCD in patients with HCM. Patients with HCM and concomitant CAD have reduced survival free of SCD compared with HCM patients with no CAD (Sorajja et al., 2003). The rate of SCD in these patients is 2% annually and overall mortality is 6.6% per year, far exceeding historical rates seen otherwise in patients with CAD with normal left ventricular function. Independent of atherosclerosis, patients with HCM have poor coronary flow reserve, of which the resulting ischemia severity correlates negatively with long-term outcome (Maron et al., 2009). This microvascular dysfunction also may cause abnormal stress findings, and in some studies was an independent predictor of death from cardiovascular causes (Cecchi et al., 2003; Petersen et al., 2007).

EKG parameters

Heart rate

A recent study of 106 HCM patients evaluated cardiovascular event end points including arrhythmias (as measured by Holter monitoring) over a mean 10.1 year follow-up (Kawasaki et al., 2012). The study found no evidence of differences in heart rate variability and turbulence on Holter monitoring between patients with or without cardiovascular events; however, average heart rates were significantly lower in patients with cardiovascular events (64.7 ± 11.2 beats/min) versus patients without cardiovascular events (73.7 ± 10.2 beats/min). Further studies are needed to confirm the significance of heart rate and prognosis in HCM.

Fragmented QRS

Surface electrocardiogram (ECG) findings have been evaluated to assess risk for future arrhythmic events. Using appropriate ICD therapies as a surrogate, fragmented QRS (fQRS) as seen on surface ECG at ICD implant was found to predict arrhythmic events in HCM patients along with history of VT/VF. Lateral lead fQRS significantly increased the risk of appropriate ICD therapy ($P < 0.0001$) (Femenia et al., 2013).

QT intervals/dispersions

QT variables have also been evaluated as a part of noninvasive risk stratification (Uchiyama et al., 2009). A study of 479 HCM patients found the mean QTc to be 440 ± 28 ms and 13% of patients exceeded 480 ms (Johnson et al., 2011). Patients with QTc >480 ms were found to have greater obstructive, thicker ventricles, and be more symptomatic. The QT interval should be assessed as it may reflect the degree of hypertrophy and obstruction in addition to its pro-arrhythmic potential.

T-wave alternans

In one study from 2002, 27 of 53 evaluated patients with HCM were noted to have T-wave alternans (TWA). These 27 patients were found to have an increased maximal number of successive ventricular ectopic beats, NSVT, as well as histopathological disarray score (Kuroda et al., 2002). A similar study conducted years later found that TWA is not useful for predicting SCD in HCM; however, this study measured TWA while the patients were maintained on anti-arrhythmic medications, as opposed to the aforementioned study (Fuchs and Torjman, 2009). A recent third study performing TWA-exercise testing and follow-up found TWA to be associated with greater maximal and regional left ventricular wall thickness, an otherwise independent risk factor (Puntmann et al., 2010b).

Methods of Prevention of SCD

Medical Management

Medical management of HCM patients for the prevention of SCD has never been proven to be effective. Anti-arrhythmic agents (including amiodarone and Ia antiarrhythmic drugs), β-blockers, and calcium antagonists have been proposed as potentially useful for prevention of SCD (Maron et al., 2003b). While early reports suggested amiodarone as potentially protective (Cecchi et al., 1998; Maron et al., 2003b; McKenna et al., 1984, 1985), it is now clear that amiodarone does not provide absolute protection from sudden death in HCM patients (Maron et al., 2000; Spirito et al., 2000; Melacini et al., 2007). In addition, amiodarone is associated with significant cumulative toxicity, making it unsuitable for long-term use in young patients (Maron et al., 2003b).

Implantable Cardioverter Defibrillators

Defibrillator use for prevention of sudden cardiac death

Defibrillators are the only proven therapy for prevention of SCD in patients with HCM (**Figs. 3** and **4**) (Maron et al., 2003b, 2007, 2013a; Epstein et al., 2008; Zipes et al., 2006). Despite questions of whether ICDs could reliably terminate ventricular arrhythmia due to increased LV mass, ICDs have been shown to reliably terminate VT and VF (Maron et al., 2000; Alsheikh-Ali et al., 2013). High-risk patients with ICDs have annual appropriate discharge rates of 4%–7% per year, with appropriate treatment occurring more frequently when placed for secondary prevention (7%–11% per year) compared to primary prevention (3%–5% per year) (**Figs. 1–3**) (Maron et al., 2000, 2007, 2013a; Woo et al., 2007). Subcutaneous defibrillators also appear to be effective in patients with HCM, but the experience is more limited (Weinstock et al., 2016; Maurizi et al., 2017).

Patient selection and guideline recommendations

2011 ACC/AHA consensus guidelines

The most recent AHA/ACC/HRS consensus guidelines for the implantation of ICDs in patients with HCM was published in 2011 (**Fig. 5**) (Gersh et al., 2011). Recommendations for management are graded in this as well as the AHA/ACC/ESC guidelines into class I: recommended; class IIA: reasonable; class IIB: may be considered; and class III: not advisable or harmful. In addition the strength of evidence supporting the recommendations is graded into levels of evidence (LOE). In general, LOE A is supported by several randomized trials, LOE B is supported by prospective or retrospective data, and LOE C is supported by expert opinion. In HCM there are very few randomized trials; thus most recommendations are based on level B or C evidence.

According to these guidelines, patients with resuscitated SCD or sustained VT should have an ICD implanted (class I, LOE B). For primary prophylaxis, ICD implantation is recommended if any one of the following three risk factors is present: massively thick myocardium, family history of SCD, or personal history of syncope (class IIA, LOE C). For patients with NSVT or hypotensive response to exercise, the presence of additional risk modifiers (LVOT Obstruction, LGE on CMR, LV Apical Aneurysm, Genetic Mutations (especially multiple)) may be useful when making the decision for implantation of an ICD. The presence of one or more of these risk modifiers yields a recommendation for ICD implantation (class IIA, LOE C). However, the absence of these additional risk factors drops the recommendation class (class IIB, LOE C) (Gersh et al., 2011). More recent data on NSVT finds that NSVT at a younger age, and longer and faster NSVT are more predictive of SCD risk (Wang et al., 2017). An additional IIB recommendation (LOE C) is made for those individuals with class III or IV heart failure with an LVEF less than or equal to 50%. Implantation of an ICD is not recommended (class III, LOE C) solely to permit athletic participation, or solely

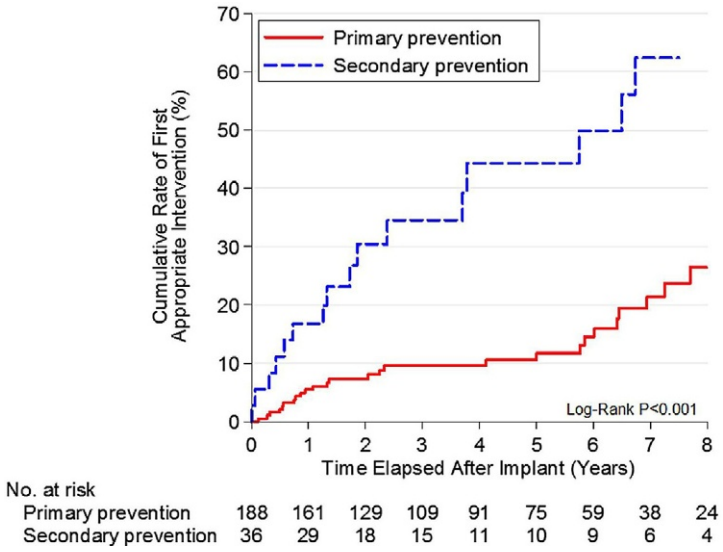


Fig. 3 ICD intervention rates. Cumulative rates for first appropriate implantable cardioverter-defibrillator (ICD) intervention, shown separately for patients who underwent implantation for primary ($n=188$) or secondary ($n=36$) prevention. The rate of first appropriate ICD shock for secondary prevention exceeded that for primary prevention by fourfold. From Maron BJ, Spirito P, Ackerman MJ, Casey SA, Semsarian C, Estes NA 3rd, Shannon KM, Ashley EA, Day SM, Pacileo G, Formisano F, Devoto E, Anastasakis A, Bos JM, Woo A, Autore C, Pass RH, Boriani G, Garberich RF, Almquist AK, Russell MW, Boni L, Berger S, Maron MS, and Link MS (2013a) Prevention of sudden cardiac death with implantable cardioverter-defibrillators in children and adolescents with hypertrophic cardiomyopathy. *Journal of the American College of Cardiology* **61**, 1527–1535, with permission.

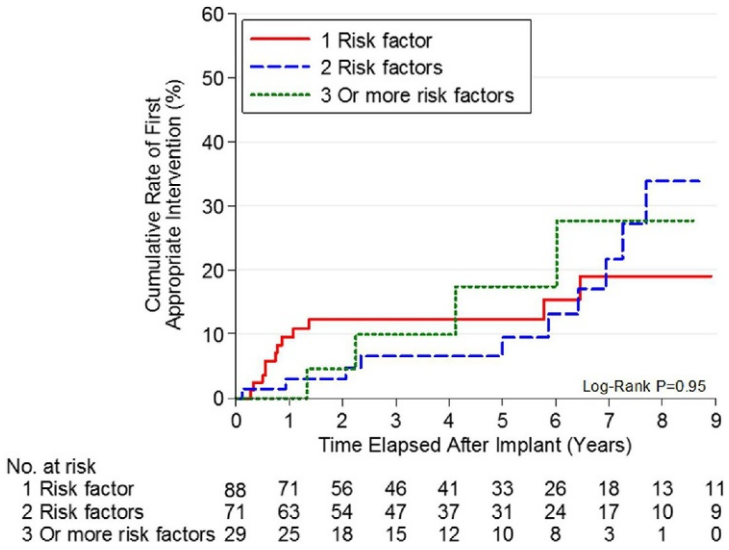


Fig. 4 Number of sudden death risk factors and ICD interventions. Cumulative rates for first appropriate implantable cardioverter-defibrillator (ICD) intervention in 188 patients who underwent implantation for primary prevention and 1, 2, or ≥ 3 risk markers. Event distribution was nonuniform during the follow-up period, with early interventions disproportionate ($P=0.023$). From Maron BJ, Spirito P, Ackerman MJ, Casey SA, Semsarian C, Estes NA 3rd, Shannon KM, Ashley EA, Day SM, Pacileo G, Formisano F, Devoto E, Anastasakis A, Bos JM, Woo A, Autore C, Pass RH, Boriani G, Garberich RF, Almquist AK, Russell MW, Boni L, Berger S, Maron MS, and Link MS (2013a) Prevention of sudden cardiac death with implantable cardioverter-defibrillators in children and adolescents with hypertrophic cardiomyopathy. *Journal of the American College of Cardiology* **61**, 1527–1535, with permission.

on the basis of prior alcohol septal ablation or need for permanent pacemaker. There is some data that these risk factors play less of a role for individuals greater than 60 years of age. In a study of 428 patients older than 60 years, only 16 (3.7%) died of HCM-related events (Maron et al., 2013b).

In addition, guidelines offer guidance on the type of ICD to be implanted. Single-chamber ICDs (class IIA) are recommended unless the need atrial pacing is anticipated (LOE C). Additionally, a dual chamber device is recommended in patients with sinus bradycardia or who have a history of atrial fibrillation (AF) (LOE C) or those with an LV outflow gradient >50 mmHg and clinical heart failure who may potentially benefit from RV pacing (LOE B) (Gersh et al., 2011).

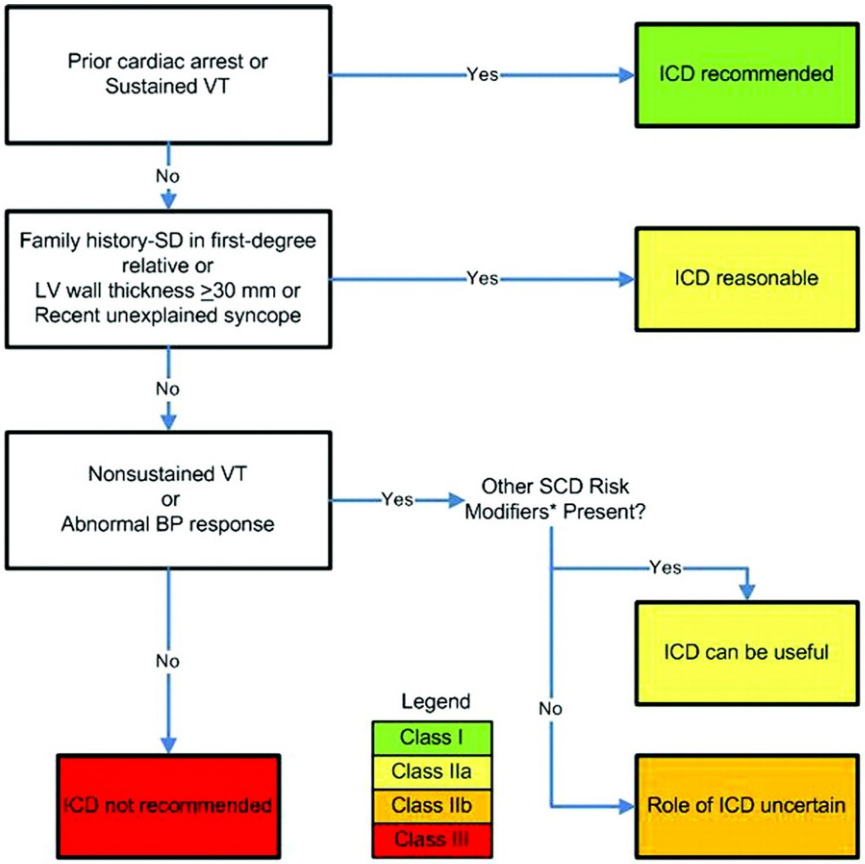


Fig. 5 Guidelines for implantation of implantable defibrillators (ICDs) in patients with HCM. From Gersh, B. J., Maron, B. J., Bonow, R. O., Dearani, J. A., Fifer, M. A., Link, M. S., Naidu, S. S., Nishimura, R. A., Ommen, S. R., Rakowski, H., Seidman, C. E., Towbin, J. A., Udelson, J. E. and Yancy, C. W. (2011). 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation* **124**, 2761–2796, from Circulation with permission.

A balance of benefit must be weighed against potential for harm, however, when choosing to implant ICDs. Insertion of dual chamber ICDs is associated with a greater risk of lead complications, both in short-term and long-term follow-up. Due in large part to a high incidence of AF in patients with HCM, inappropriate discharges are also common in HCM patients, with up to 25% receiving inappropriate shocks (Maron et al., 2000, 2007, 2013a; Lin et al., 2009). Young age and AF have been shown to be associated with inappropriate ICD shocks (Maron et al., 2007, 2013a; Woo et al., 2007). Inappropriate discharge due to sinus tachycardia, AF, and lead malfunction are the most common ICD complications in HCM. Infection, hemorrhage, thrombosis, lead fracture, dislodgement, and T-wave oversensing are found at rates similar to the general population of patients with pacemakers and ICDs (Lin et al., 2009).

European guidelines

Data from Europe has also been published on the risk of SCD and included in the most recent European Society of Cardiology Guideline update in 2014 (Elliott et al., 2014). In a multicenter consortium 3675 patients were followed for a median of 5.7 years. Of prespecified predictors eight were associated with SCD or appropriate ICD shock, including age, maximal left ventricular wall thickness, left atrial diameter, left ventricular outflow tract gradient, family history of SCD, nonsustained VT, and unexplained syncope. A score based on these risk factors is calculated by a HCM-SCD calculator. This algorithm calculates an individual HCM patient's estimated risk of SCD at 5 years. Three tertiles of groups are delineated by 5-year risk estimations given as percentages; a low-risk estimation is denoted for patients whose risk is <4%, an intermediate-risk estimation is denoted for patients whose risk is

4%–6%, and a high-risk estimation is denoted for patients whose risk is $\geq$6%. A low-risk estimation carries a recommendation of "ICD generally not indicated," an intermediate risk recommendation is "ICD may be considered," and a high-risk recommendation is "ICD should be considered" (Elliott et al., 2014; O'Mahony et al., 2014).

Conclusion

SCD is a major cause of mortality in patients with HCM, especially in the young, but can occur at any age. The substrate of HCM is highly vulnerable to the development of ventricular arrhythmias, which are responsible for the elevated rate of SCD in this population. In patients with HCM, SCD is the predominant concern in young patients, while progressive heart failure, AF, and stroke become more significant causes of morbidity and mortality in the later years. Major risk factors for SCD include prior resuscitated SCD or sustained VT, a family history of SCD due to HCM, recent unexplained syncope, and marked cardiac hypertrophy over 3.0 cm. Other risk factors include NSVT, abnormal blood pressure response to exercise, and newer emerging risk factors including fibrosis burden on CMR, genetic mutations, and the presence of apical aneurysm. Medications may be palliative and reduce symptoms, but are ineffective at prevention of SCD. The only proven effective treatment for prevention of SCD is implantation of an ICD. Risk assessment schemes, performed at initial presentation and repeated annually or when new risk factors arise, are vital in the management of patients.

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Ventricular Assist Devices and Heart Transplantation

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VAD Introduction

A ventricular assist device (VAD) is an implantable mechanical device which can partially or fully replace the function of a ventricle of a failing heart. The growing heart failure population and limited number of donor hearts available for transplantation (for survival posttransplant, see **Fig. 1**) have resulted in rapid technological advances and increased implantation of VADs. They may be classified into devices that support the left ventricle, right ventricle, or both ventricles. The decision to implant a specific type of device is based on the patients underlying diagnosis as well as assessment of left and right ventricular function. The patients are classified into those who are given a VAD as:

- Bridge to recovery (BTR)
- Bridge to candidacy (BTC)
- Bridge to transplant (BTT)
- Destination therapy (DT)

Notwithstanding these implant definitions, in the current era VADs are used to treat refractory heart failure and the ultimate disposition of the patients depends on the response to mechanical circulatory support.

The first clinical use of a VAD was in 1966 by Michael DeBakey. He successfully supported a 37-year-old woman for 10 days after complex cardiac surgery using a pneumatically driven VAD (DeBakey, 1971). The subsequent development of these devices initially focused on bridging patients to heart transplantation. The early generation Novacor and paracorporeal pneumatic VAD were followed by the implantable pneumatic Heartmate VAD in 1992. The REMATCH trial published in 2001 confirmed the superior survival for patients with implantable left VADs as compared with optimal medical management (Rose et al., 2001). This shifted the focus to long-term support of heart failure patients.

The dramatic evolution in VADs from large bulky pulsatile devices to miniature ventricular assist devices (MVAD) weighing less than 100 g is illustrated in **Fig. 2** (Moayedi and Ross, 2017).

The registry for mechanically assisted circulatory support (INTERMACS) keeps track of the volumes related to durable mechanical cardiac support. From 2006 to 2014, there were 15,745 patients (2500/year) who received a durable device (Kirklin et al., 2015). Over 90% of these patients received a continuous flow ventricular assist device (CF-LVAD). In 2014, almost half of the devices implanted were designed for DT. Patients who underwent implant were predominantly INTERMACS profile 2–3 (65%), less than 20% of patients were INTERMACS 4–7 (**Table 1**) (Kirklin et al., 2015).

Procedure of Implantation of VAD

Before implantation of a VAD, the patient should be medically optimized. This may require inotropic support, diuresis, clearing of infection, and removal of any unnecessary central lines. Intraoperatively they will require invasive monitoring as well as transesophageal echocardiogram (Slaughter, 2011).

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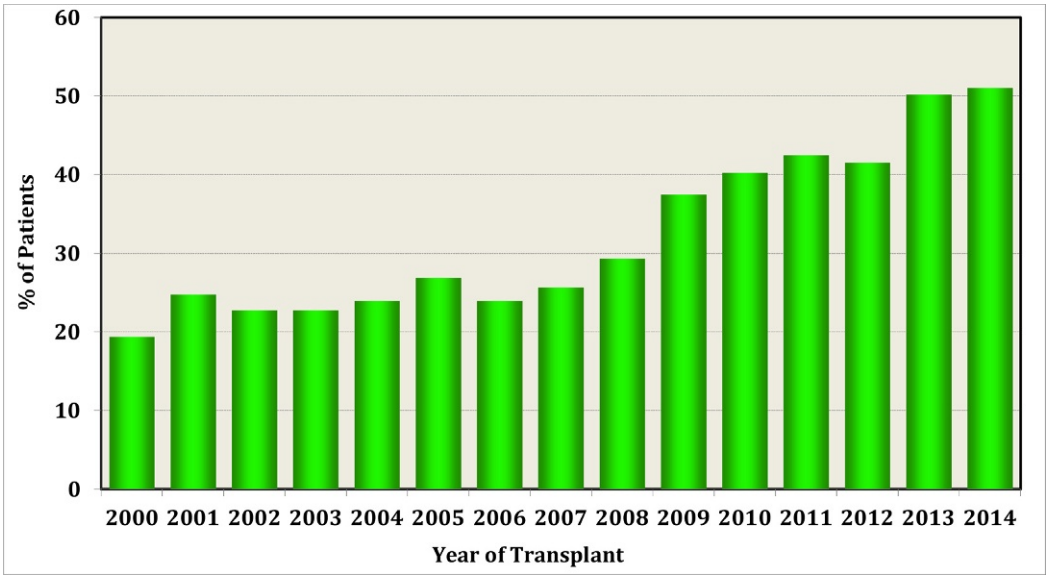

Fig. 1 Survival posttransplant.

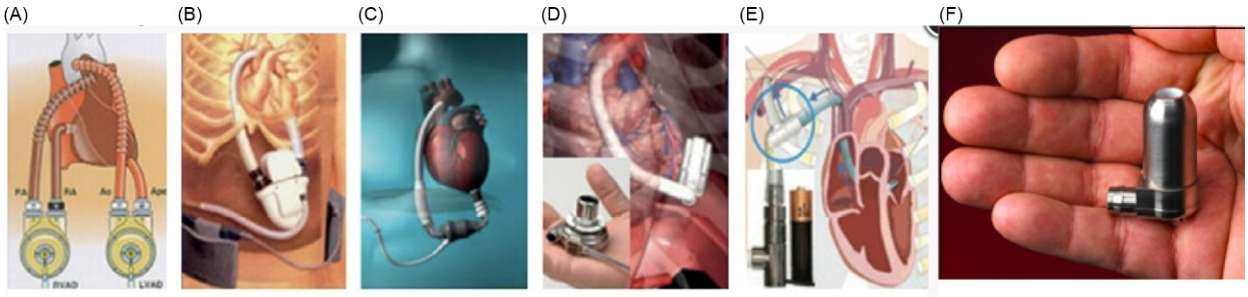

Fig. 2 Evolution of devices from external paracorporeal devices to implantable smaller sophisticated continuous flow with axial and centrifugal technology. (A) Thoratec LVAD, (B) Novocor World heart, (C) Heartmate II, (D) Heartware HVAD, (E) Circulite synergy micropump, and (F) MVAD.

Table 1 INTERMACS profiles (Kirklin et al., 2015)

Profile	*Profile description*
1	Critical cardiogenic shock (Crash and burn)
2	Progressive decline (Sliding fast on inotropes)
3	Stable but on inotrope dependent
4	Resting symptoms or oral therapy at home
5	Exertion intolerant (housebound)
6	Exertion limited (walking wounded)
7	Advanced NYHA class III

The most common method for the implantation of VADs utilizes cardiopulmonary bypass with the heart beating. They can also be inserted while stopping the heart using aortic cross clamping and cardioplegia. The inflow cannula is most commonly placed in the LV apex to ensure adequate decompression. First the LV apex is cored, 2 cm lateral to the left anterior descending artery, and then the inflow cannula is secured with either running or interrupted sutures. The use of pledgets is dependent on the surgeon's preference and the quality of the heart muscle. The inflow cannula is then attached to the pump as per the manufacturer's instructions (Whitson, 2015). It should be parallel to the septum and pointing toward the mitral valve.

The outflow cannula is attached to the ascending aorta as far distal as possible, with a beveled anastomosis, to the sinotubular junction, using a partial occlusion clamp. Commonly this is done using a 4-0 or 5-0 nonabsorbable monofilament suture. The outflow conduit should be away from the midline to ensure easier reopening should the patient be transplanted. If the conduit is too redundant there is a risk of turbulent blood flow at the aortic anastomosis which may predispose to pump thrombus.

The percutaneous driveline is tunneled through the subcutaneous tissues and exits in the midclavicular line 3–4 cm below the costal margin. This can be done before systemic heparinization to limit bleeding. Old scars should be avoided and the drive line (DL) should be in a location that is accessible to the patient for change of dressings. Consideration is given to the patient's preference for a left- or right-sided DL exit site. Adequate de-airing is confirmed using echocardiogram guidance before starting the device.

Once the VAD has been implanted and hemostasis achieved, the device should be wrapped in a pericardial patch or in Gortex fabric to reduce adhesions should the patient recover or be suitable for transplantation.

Traditionally VADs have been implanted using a full sternotomy. However, there is a move to use a left thoracotomy and hemimedian sternotomy. As the implantable devices get smaller and more surgeons are using minimally invasive approaches for their routine surgeries, this will become more common.

Indications for LVAD

The contemporary indications for VAD therapy for patients as a bridge and DT are listed in **Table 2** (Feldman et al., 2013).

Contraindications to VAD Therapy

Contraindications to VAD therapy include mechanical aortic valve without plan to replace or close, profound thrombocytopenia, coagulopathy, uncontrolled systemic infection, recent stroke, or cerebrovascular disease that increases risk for intraoperative stroke, contraindication to systemic anticoagulation or antiplatelet therapy, significant right heart failure, psychosocial instability (ongoing substance abuse, lack of care plan, noncompliance), or other medical conditions that limit survival to less than 24 months (Feldman et al., 2013). Center-specific contraindications such as body size (BSA) $<1.2\ m^2$, right ventricular (RV) failure such as an RV stroke work index (RVSWI) $<250\ mm\ Hg \times mL/m^2$, end organ renal failure with a creatinine >3 mg/dL, moderate to severe pulmonary dysfunction, BMI $>45\ kg/m^2$, restrictive cardiomyopathy, and hypertrophic cardiomyopathy (Mancini and Colombo, 2015).

Preoperative Assessment and Selection

Patients who are being investigated for a durable LVAD should also be assessed for heart transplantation eligibility (Mehra et al., 2016; Costanzo et al., 2010). To determine eligibility for LVAD, the following tests should be completed as per the most recent ISHLT guidelines (Feldman et al., 2013):

1. All patients require assessment of RV function with hemodynamic and echocardiographic measures.
2. Patients with a history of coronary bypass should have a chest computed tomography (CT) to provide the location of bypass graft(s).
3. Patients with a history of vascular and coronary artery disease should have preoperative assessment of the ascending aorta for dilatation and atherosclerotic burden.
4. All patients with a history of atherosclerotic vascular disease require screening for peripheral and carotid/vertebral vascular disease.
5. A recent echocardiography or contrast cardiac CT should rule out the presence of apical thrombus.
6. All patients should have psychosocial risk factors assessed and a screen for cognitive dysfunction.

Palliative Care Input Before VAD

Palliative care is a critical part of the multidisciplinary team management of patients with advanced heart failure. In this complex group of patients, advanced care planning focusing on goals of care and desired therapies is vital. In fact pilot studies have shown

Table 2 Indications for VAD therapy: inclusion criteria (Kirklin et al., 2015)

Indications for VAD therapy	
Bridge (BTR, BTC, BTT)	*Destination therapy*
Listed for heart transplantation Status 1A or 1B listing NYHA class IV	LVEF equal or less than 25% Peak oxygen consumption (VO_2) less than 14 mL/kg/min (50% age/sex predicted) And 1 of 3 following criteria: ● NYHA class IIIB–IV symptoms(Mehra et al., 2016) despite medical therapy for at least 45 days or the prior 60 days, or ● Dependence on IV inotropes for greater or equal to 14 days, or ● Dependence on IABP for greater than 7 days

VAD, Ventricular assist device; *LVEF*, left ventricular ejection fraction; *NYHA*, New York Heart Association; *IABP*, intraaortic balloon pump.

benefits in quality of life, depression, and symptom control (Luo et al., 2016). Patients with heart failure have episodic exacerbations requiring regular reassessment, making timing of referral for palliative care challenging. Results from the recent randomized control trial of Palliative Care in Heart Failure are awaited (Mentz et al., 2014).

The goals of palliative care input for mechanical support patients include:

- Supporting the family while deciding goals of care
- Discussing care preferences in the event of a complication or poor quality of life
- Engaging the patient, family, and their appointed decision maker
- Empowering the family to make decisions in the event of device deactivation
- Providing a better understanding of the patient's treatment plan
- Helping with caregiver stress

The Decide-Left VAD (LVAD) trial is currently in progress across six sites. This randomized controlled trial is assessing illustrated pamphlets as well as a video to aid patients and caregivers with decision-making. As more patients have mechanical circulatory support implanted as DT, it is important to plan for the long-term management of these patients.

Survival of Patients With LVAD

Research related to LVADs can be traced back to over 60 years ago; with the first case of an extracorporeal VAD described by Dr. Debakey for a 37-year-old woman with mitral stenosis and aortic regurgitation (DeBakey, 1971; Hetzer and Delmo Walter, 2017). The REMATCH trial was the first randomized trial that showed the use of durable LVAD resulted in a clinically meaningful survival benefit in patients ineligible for heart transplantation. The rate of survival at 1 year was 52% for those with the pulsatile flow device (PF-LVAD) heartmate XVE versus 25% with medical therapy (Rose et al., 2001). In the subsequent HeartMate II Bridge to transplant trial (HM BTT), was a prospective nonrandomized trial where 80% either underwent transplantation or were alive at 180 days still listed as status IA or IB (Miller et al., 2007). The HM II CF-LVAD has also been compared to the PF-LVAD HMXVE device in the HeartMate II DT trial which showed an improved survival free from debilitating stroke and need for pump repair. More recently, the centrifugal CF-LVAD Heartware (HVAD) device was shown to be noninferior to the axial CF HM2 counterpart for DT. The HVAD bridge to transplant study device also showed that the HVAD was noninferior to HMII (Aaronson et al., 2012). The third generation of CF-LVAD, HM3, a fully magnetically levitated pump, was compared to the second-generation HM2, where there were no significant differences in the rates of death or disabling stroke, but pump thrombosis was less frequent with HM3 (0.7% vs. 7.7%, $P=0.02$) (Mehra et al., 2017). The current 1- and 2-year survival of patients with continuous flow LVAD devices is 80% and 70%, respectively (Kirklin et al., 2015).

Care of the Patient With the LVAD

The care of the patient with an LVAD depends on a collaborative multidisciplinary staff involving the cardiovascular surgeon, the heart failure clinician, nursing staff, MCS coordinator, and other specialists such as gastroenterology and infectious disease when needed (Feldman et al., 2013). The frequency of visits depends on the institution and the clinical stability. For example, in our institution, patients are seen weekly for the first month post discharge and then monthly. Each clinic visit would include a clinical assessment, interrogation of the LVAD to evaluate pump parameters and alarm history and routine blood work including coagulation and hemolysis screening. In our program, we routinely complete an echocardiogram, a RAMP study in all our VAD patients up to 3 months post discharge. Additional testing such as repeat echocardiogram and/or hemodynamic assessment may be warranted in patients who are bridge to transplantation or in the presence of change in clinical parameters.

Complications of VADs

While mechanical circulatory support has revolutionized treatment of heart failure patients, they are associated with significant complications. These can be classified into:

- Short term (<3 months post implantation)
- Long term (>3 months post implantation)

The most reliable information on adverse events is published by the INTERMACS registry. **Table 3**, published in September 2016, summarizes the adverse events in patients receiving primary implants between June 23rd 2006 and September 30th 2016.

INTERMACS also reports the most common cause of death in this patient population. The table published in September 2016 summarizes the causes of death in patients receiving primary implants between June 23rd 2006 and September 30th 2016.

Figs. 3–7 demonstrate some common pathological complications of VADs (**Table 4**).

Table 3 Adverse events

Adverse event type	*Early <3 months (%)*	*Late >3 months (%)*
Arterial non-CNS thromboembolism	0.9	0.5
Bleeding	29.6	23.5
Cardiac arrhythmia	21.9	11.5
Device malfunction +/− pump thrombosis	8.1	19.1
Hepatic dysfunction	3.6	2.5
Infection	28.3	31.4
Myocardial infarction	0.3	0.4
Neurological event	10.1	14.2
Other serious adverse event	21.0	16.0
Pericardial drainage	4.5	0.2
Psychiatric episode	5.7	3.5
Rehospitalization	38.3	65.3
Renal dysfunction	9.7	5.6
Respiratory failure	16.0	6.2
Venous thromboembolism	3.7	0.9
Wound dehiscence	1.2	0.5



Fig. 3 Picture of an explanted (recipients) heart with an LVAD in place. The outflow cannula (*arrow*) which is connected to the aorta has been cut here at explantation. The white line (*white arrow*) is the drive line, comes out of the skin, and is connected to the power pack.

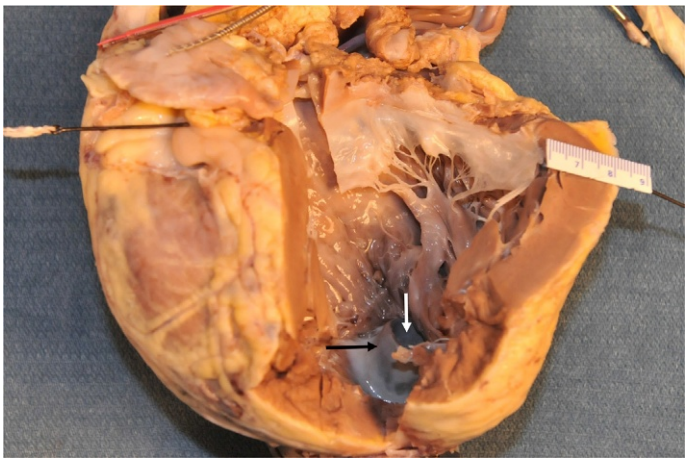

Fig. 4 This explanted (recipient) heart shows the inflow cannula (*black arrow*) and its orifice (*white arrow*). The cannula takes blood from the left ventricle to the LVAD. The cannula is covered with a layer of gray-white, shiny tissue called pannus or host tissue reaction. Among other things, pannus can help prevent thrombus deposition.

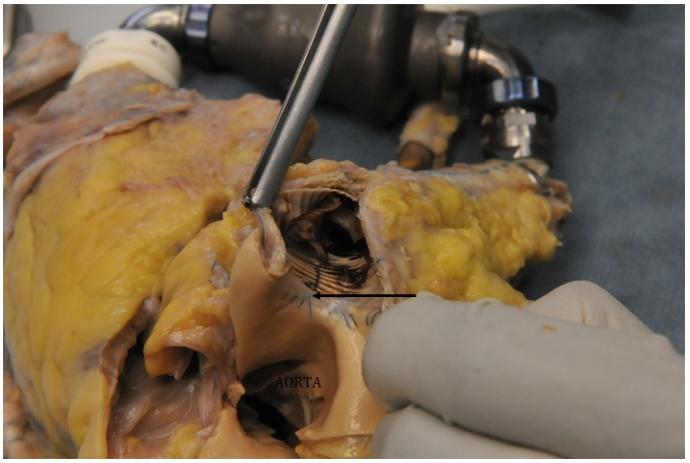

Fig. 5 This image shows the outflow graft at its junction with the aorta. At 5 years post implantation, there is a mild degree of tissue overgrowth (*arrow*) at the anastomosis. Dark gray-black, post mortem clot is seen in the synthetic (pleated dacron) graft.



Fig. 6 Explanted (recipients) heart shows a Heart Mate 2 LVAD. Device in place for over 5 years. There are extensive fibrous adhesions around the inflow cannula and the outflow cannula (*arrow*). A defibrillator pack is also seen.

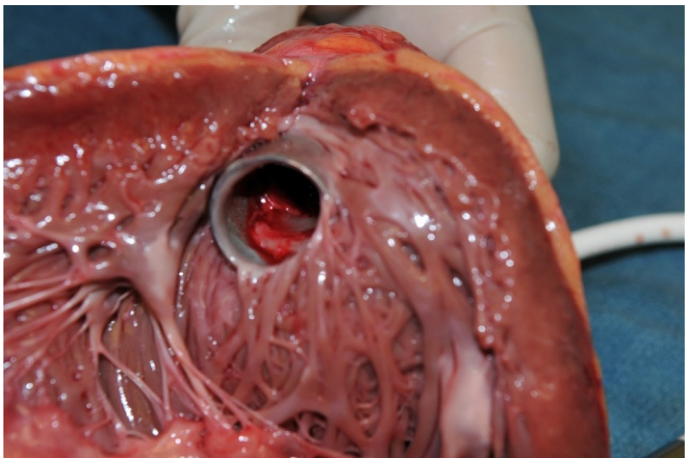

Fig. 7 This photograph of a left ventricle shows the LVAD inflow cannula in place. The cannula is adherent to the LV septal myocardium.

Table 4 Causes of death post VAD insertion

Cause of death	*Percentage (%)*
• Congestive heart failure	3.4
• Cardiac arrhythmia	2.6
• Major bleeding	2.8
• Right heart failure	4.1
• Sudden unexplained death	4.0
• Device malfunction	3.3
• Major infection	8.6
• Multisystem organ failure	16.1
• Neurological event	19.3
• Respiratory failure	5
• Withdrawal of support	9.3

The Future of VADs

It is estimated that 10 million people in the United States alone will be affected by heart failure by 2030 (Heidenreich et al., 2013). There is a significant discrepancy between donor heart availability and number of patients awaiting transplantation. VADs offer the option of bridge to heart transplantation or as DT.

Patients who had the devices implanted were in the advanced stages of heart failure and often inotrope dependent (Lima et al., 2015). The REVIVE-IT is an NIH-supported trial attempted to look outcomes of patients with less-advanced heart failure supported with durable LVAD (Baldwin and Mann, 2010; Pagani et al., 2016). The control group was noninotrope dependent, ambulating and only in moderately advanced heart failure. Unfortunately, this trial was discontinued early and failed to achieve its goal of widespread use of LVAD in less sick patients. The ROADMAP trial was a prospective trial which assessed the use of LVAD support as compared to optimized medical management in patients with INTERMACS profile 4 symptoms. In the as-treated analysis, the 12-month survival was significantly greater for LVAD than medically managed patients (Estep et al., 2015). With the evolving mechanical support technology mitigating significant adverse events, there the use of LVADs will perhaps be available to the less sick patient to further improve functional status and overall survival.

Introduction Heart Transplantation

Heart transplantation is the gold standard therapy for patients with advanced heart failure. It has resulted in an improved quality of life and survival in patients with stage D heart failure. As per the most recent ISHLT registry data, there have been an increasing number of heart transplantations since the 1980s, 4500 reported adult and pediatric heart transplantations occurred most recently in 2014; with a median recipient age of 53 years. The median survival is increasing and is currently 10.7 years with a median conditional on surviving the first year is 13.3 years (Lund et al., 2016).

Criteria for Heart Transplantation Listing

Patients with ACC/AHA Stage D heart failure comprise approximately 5% of all patients with heart failure. Such patients require several investigations to complete heart transplantation evaluation. Based on the updated 2016 ISHLT criteria for heart transplantation guidelines, **Table 3** describes some of the essential tests recommended to guide listing (Mehra et al., 2016).

Care of Patient Post Heart Transplant

The practice of care post heart transplantation is guided by clinical expertise and can be different from one institution to another (Costanzo et al., 2010). The core principles of care include monitoring for rejection, treating infection, and managing the late potential complications of heart transplantation such as chronic side effects of immunosuppression (IS) (e.g., hypertension, diabetes, renal dysfunction, cancer) and coronary allograft vasculopathy (CAV) (**Table 5**).

Patients post heart transplant require clinical assessment and surveillance endomyocardial biopsy frequently for the first 6–12 months after transplant for allograft rejection. The ISHLT guidelines recommend weekly biopsies for the first month, then bimonthly for the second month, monthly until 6 months, and then every few months until 18 months (Costanzo et al., 2010). An endomyocardial biopsy may be expedited if there are signs of rejection or a change in IS.

The standard maintenance therapy for IS includes calcineurin inhibitor, mycophenolate mofetil, and corticosteroids, which can be weaned or maintained at a low dose. Where there is clinical indication, there may be addition or a switch for a proliferative signal inhibitor such as everolimus or sirolimus.

Table 5 Criteria for heart transplant listing (Mehra et al., 2016)

Cardiopulmonary stress test	• Listing only based on peak VO_2 should not be performed • Respiratory exchange ratio (RER) >1.05 • When intolerant of beta-blocker peak VO_2 ≤14 mL/kg/min • In presence of B-blocker ≤12 mL/kg/min • In presence of submaximal stress test (RER <1.05) a ventilation equivalent of carbon dioxide slope ≥35
Role of diagnostic right heart catheterization	• Should be performed on all adult candidates • A vasodilatory challenge when pulmonary artery systolic pressure ≥50 mmHg and either transpulmonary gradient ≥15 and pulmonary vascular resistance >3 Wood units while maintaining a systolic blood pressure >85 mmHg
Age	• Carefully selected patients ≥70 years of age
Obesity	• Body mass index area <35 kg/m^2
Diabetes	• HbA1c <7.5%
Renal	• Estimated glomerular filtration rate if <30 mL/min/1.73 m^2 relative contraindication
Vascular	• Vascular Doppler and carotid Doppler to assess severity of peripheral vascular disease and cerebrovascular disease
Assessment of frailty	• Using modified frailty score where a score of 0 is nonfrail, 1–2 prefrail, and 3–5 frail (Fried et al., 2001)
Tobacco and substance use	• Cessation of smoking for 6 months • No active substance abuse including alcohol
Psychosocial evaluation	• Social supports need to be deemed sufficient

Complications of Heart Transplantation

The ISHLT registry provides information on patient profiles as well as morbidity and mortality after heart transplantation. The number of patients who are bridged to transplantation with mechanical circulatory support is steadily increasing. For causes of death posttransplant and significant risk factors for first year mortality, see **Tables 6** and **7**.

The most common morbidities post heart transplantation are listed in **Table 8**.

Among these complications, cardiac allograft vasculopathy (CAV) is the most notable as more than 50% of patients post heart transplantation have CAV by 10 years. It is a most common cause of death after the first year (Lund et al., 2016; Clerkin et al., 2017). CAV is characterized by intimal thickening and narrowing of the artery and results in flow limitation which results in restrictive remodeling (Clerkin et al., 2017). Causes of CAV can be associated with traditional cardiovascular risk factors, nonimmunological causes such as ischemic time, CMV infection, and immunological causes such as donor-specific antibodies, antibody, and cellular rejection (Clerkin et al., 2017). Patients require screening for CAV, most commonly with coronary angiography and a follow-up angiogram to assess disease progression. Although CAV does significantly limit posttransplant survival, there are limited therapies that can prevent or reverse progression. Of the immunosuppressive regimens, everolimus and sirolimus are associated with a decrease in a combined endpoint of death, CAV progression, or need for coronary intervention (Mancini et al., 2003).

Future of Heart Transplantation

Until a fully dependable artificial heart becomes available, the need for heart transplantation will continue. For monitoring graft hearts for rejection, the endomyocardial biopsy remains the gold standard, whether antibody mediated (AMR) or cell mediated (ACR). The biopsy is however associated with a 0.5%–1.5% risk of complications such as pericardial effusion, tricuspid regurgitation, and very rarely death (Crespo-Leiro et al., 2015). Based on the ISHLT guidelines, a patient will undergo 13 endomyocardial biopsies within the first year of transplantation. Gene expression profiling can be used to create a global picture of cellular function as an alternative to assessing cellular and antibody rejection. Currently, AlloMap, a proprietary algorithm based on 11 genes, is a

Table 6 Causes of death post heart transplantation

Cause of death	*0–30 days (%)*	*>31 days–1 year (%)*	*>3–5 years (%)*	*>10–15 years (%)*
Cardiac allograft vasculopathy	1.3	3.5	13.4	12.8
Acute rejection	4.4	9.3	4.9	0.9
Lymphoma	0	1.1	3.3	2.8
Malignancy, other	0	2.4	19.2	21.1
CMV	0	1.0	0.2	0.1
Infection, non-CMV	13.4	31.1	10.3	10.9
Graft failure	40.2	17.3	23.3	17.4
Technical	7.3	1.6	0.8	1.2
Other	4.4	6.8	7.6	6.6
Multiple organ failure	17.8	15.9	5.9	8.4
Renal failure	0.5	0.9	3.1	8.1
Pulmonary	2.9	3.8	4.5	4.4
Cerebrovascular	7.7	5.3	3.4	5.4

Table 7 Significant risk factors for 1-year mortality include

- Recipient age
- Donor age
- Recipient creatinine
- Ischemia time
- Donor height
- Recipient BSA
- Transplant centre volume
- Total bilirubin

Table 8 Causes of morbidity, post heart transplantation

Morbidity	*Within 1 year (%)*	*Within 5 years (%)*	*Within 10 years (%)*
Hypertension	70.8	90.9	
Renal dysfunction	25.4	50.9	68
Abnormal Cr	17.3	32.8	39.5
Cr >2.5 mg/dL	6.1	13.7	18.6
Chronic dialysis	1.7	3.0	6.2
Renal transplant	0.3	1.3	3.7
Hyperlipidemia	59.9	87.6	
Diabetes	23.3	36.6	
Cardiac graft vasculopathy	7.8	29.4	47.6
Malignancy	5.1	15.7	27.4

simple blood test being used clinically to assess for cellular rejection. The AlloMap score is associated with a negative predictive value that estimates the probability that a patient is not experiencing moderate (Grade 2R) rejection (Crespo-Leiro et al., 2015; Deng et al., 2006). It is hoped that these gene profiling scores will become an adequate substitute to endomyocardial biopsy for both cellular- and antibody-mediated rejection.

The future of heart transplantation rests on research that focuses on CAV and the need for immune tolerance to give, improve, and extend years of productive life for patients, post heart transplantation.

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Ventricular Fibrillation and Defibrillation

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Introduction

Ventricular arrhythmias are an important cause of morbidity and sudden cardiac death (SCD) in almost all forms of heart disease. In North America and Europe the annual incidence of SCD ranges between 50 and 100 per 100,000 in the general population (Fishman et al., 2010). About half of these SCDs are caused by ventricular fibrillation (VF) or ventricular tachycardia (VT). VF is defined as "turbulent, disorganized electrical activity of the heart in such a way that the recorded electrocardiographic deflections continuously change in shape, magnitude, and direction" (Robles de Medina et al., 1978) (**Figs. 1–3**).

Mechanism of VF

As mentioned earlier, VF is defined as turbulent cardiac electrical activity, which implies a large amount of irregularity in the electrical waves that underlie ventricular excitation (Mandapati et al., 1998). Electrocardiography (ECG) during VF is characterized by chaotic, variable amplitude, morphology of deflection and which suggests complex and disorganized cardiac activation (**Fig. 1**).

Despite many decades of research, the underlying mechanism of VF is not fully understood. The electrical activity of the cardiac muscle during VF has been studied using extracellular and optical recordings in either vivo, or in perfused, isolated heart. Studies suggest that VF is unlikely to be one static mechanism but rather a dynamic process of electrical derangement that changes with duration (Tabereaux et al., 2009). The two major proposed mechanisms of VF are multiple wavelets theory and mother rotors theory, and whether VF is maintained by wandering wavelets or mother rotors remains controversial.

In 1930, Wiggers first proposed four progressive phases of VF based on epicardial movement using high-speed cinematography (Wiggers, 1930). Wiggers concluded that VF could not be adequately described as an asynchronous contraction of individual myocardial fibers. His observations suggested that the apparent lack of coordination and synchrony first involved relatively large areas of the myocardium, which seemed to gradually multiply and decrease in size as fibrillation evolved through its various stages (Jalife, 2000). Relatively recently Huang et al. investigated stages of VF using a high-density epicardial array during VF (**Fig. 4**) (Huang et al., 2004). Their classification was different from that of Wiggers'. They found that VF rapidly gained complexity in the first minute, followed by a recovery of organization over the next 10 min. In conclusion, they classified VF into five stages based on observation of the electrically mapped epicardial activation patterns during VF. According to their theory, stage 1, lasting the first 11 s after the onset of VF, demonstrates many wavefronts, a rapid frequency of activation, reentry, and a rapid propagation velocity. During stage 2 (12–62 s) these measures decrease, albeit with rapid dynamic fluctuations. During a brief stage 3 (63–86 s), many of these descriptors again increase consistent with a higher degree of organization, perhaps caused by a temporary decrease in extracellular potassium. Stage 4 (87–310 s) shows a steady decrease in most quantitative measures of VF organization, whereas during stage 5 (311–600 s), these measures all gradually toward zero, indicative of cell death and cessation of electrical activity.

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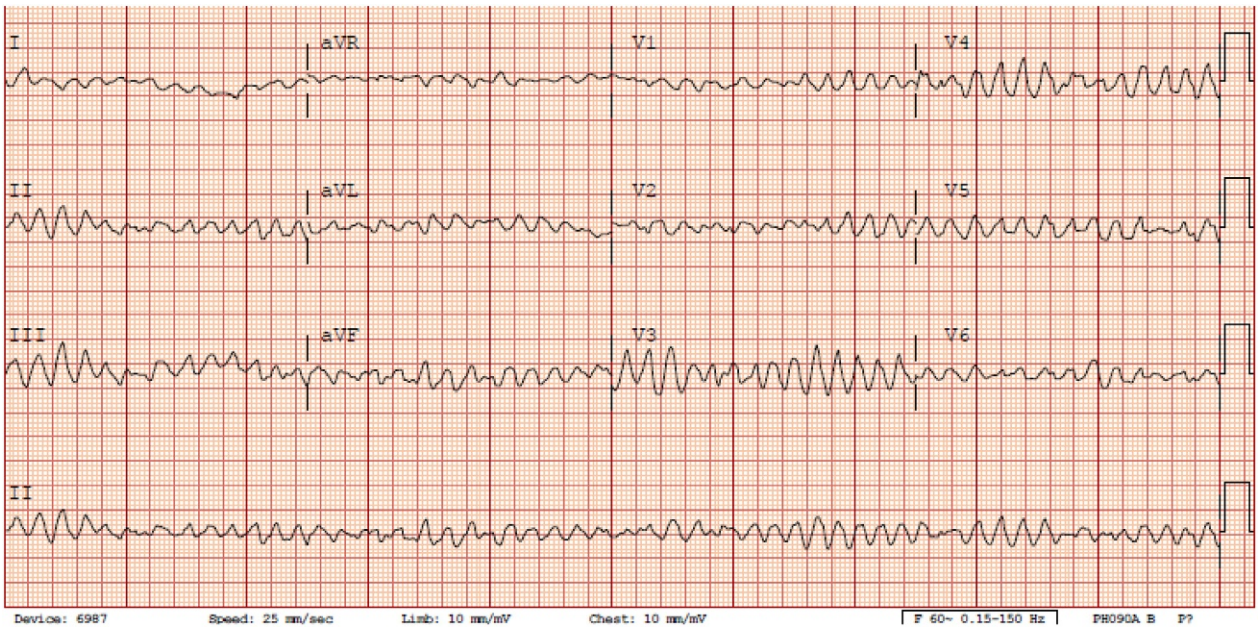

Fig. 1 Twelve lead ECG during ventricular fibrillation. Irregular deflections of varying amplitude and contour were seen in all 12 leads.

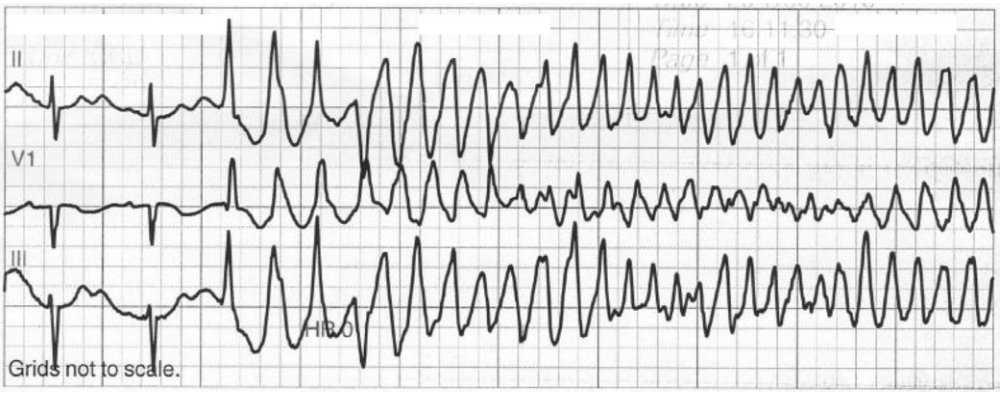

Fig. 2 Initiation of ventricular fibrillation on telemetry, VF was initiated by PVC on T wave.

Huizar et al. (2007) found three distinct phases of local VF dynamics during the first 10 min of VF: (1) a distinct increase in wavefront conduction velocity and organization during the brief third stage beginning ~1 min after VF induction and lasting ~30 s, (2) a high variation for most descriptors from second to second during VF, especially during the second stage, and (3) an increased incidence of reentry beginning ~40 s after VF induction and lasting for ~2 min. Although there are some differences among these theories, it seems that VF continues to change through different stages as it progresses. These different stages raise the possibility that the relative importance of different arrhythmogenic mechanisms changes as VF is initiated and then progresses through different stages (Wiggers, 1930; Huang et al., 2004; Huizar et al., 2007).

Initiation

Similar to the mechanisms of atrial fibrillation (AF), the mechanisms of VF can be divided into initiation by triggers and maintenance by substrate. The Purkinje system and premature ventricular contractions (PVCs) have been shown to be a trigger for VF in patients with no structural heart disease (Bänsch et al., 2003; Haïssaguerre et al., 2003; Marrouche et al., 2004). If a PVC occurs during the vulnerable period of the T wave, the impulse can propagate chaotically through the variably refractory myocardial cells and establish reentrant patterns that result in turbulent cardiac electrical activity. Computerized mapping techniques showed that reentrant wave fronts underlie the onset of VF in intact canine ventricles (Lee et al., 1996; Chen et al., 1986; Cha et al., 1994).

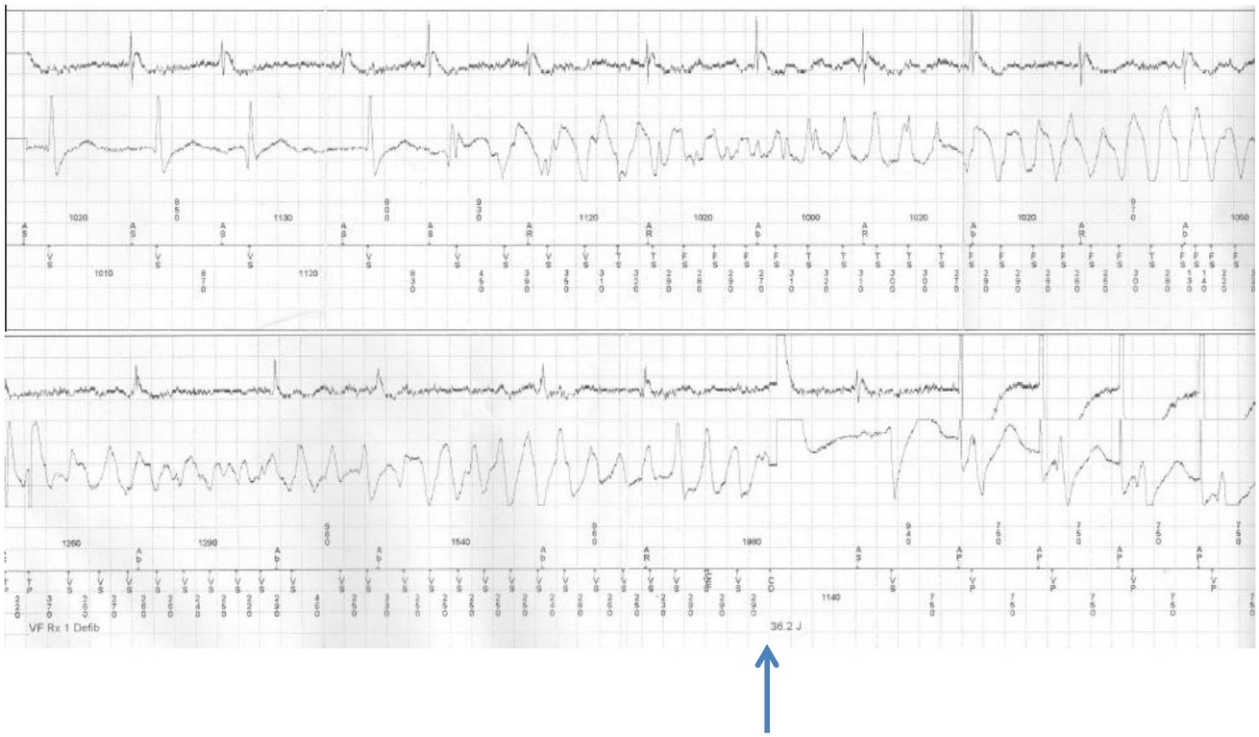

Fig. 3 Intracardiac ECG recorded on ICD. Ventricular fibrillation was detected and terminated by electrical shock of 36.2 J (*arrow*).

Clinical observations also indicate that VF is almost always preceded by VT, the duration of which can be a few beats to many beats (Kempf and Josephson, 1984).

Maintenance of VF "Wandering Wavelets" or "Mother Rotors"

Wandering Wavelets Theory

On the basis of a computer model of AF by Moe developed in 1962, fibrillation was hypothesized to be maintained by multiple small, wandering wavelets, in which the activation pattern is constantly changing (Moe, 1962). According to the wandering wavelet hypothesis, wavelets continue to change pathways and are short lived. Wandering wavelets terminate in two different mechanisms: (1) they are extinguished when they collide with another wavelet, strike a nonconducting boundary, or completely block; (2) they partially block and fractionate into two or more daughter wavelets. If the amount of tissue exceeds a critical mass, enough daughter wavelets that block and reenter are constantly being formed so that reentry circuits are constantly present to sustain fibrillation.

Mother Rotors Theory

Winfree introduced the term "rotor" to describe a source of rapid activation that creates a spiral wave in two dimensions and a scroll wave in three dimensions (Winfree, 1987). In his theory, although a spiral or scroll wave has a large rotating wavefront, the actual source of activation is the rotor in the center (Chen and Weiss, 2005). Animal study using Langendorff-perfused rabbit heart demonstrated that rotating spiral waves are most likely the underlying mechanism of VF and contribute to its frequency content (Mandapati et al., 1998). These electrical spiral waves or so called "rotors" have been observed on the endocardium and epicardium during VF in animal and human studies of VF (Cha et al., 1994; Zaitsev et al., 2000; Ohara et al., 2001; Masse et al., 2007; Krummen et al., 2014). Other studies have also documented the existence of rotors in the first few seconds of VF (Zipes and Jalife, 2013). Other studies however have failed to prove an existence of mother rotor at VF initiation (Choi et al., 2002) and several studies reported that two types of VF can occur, a type with "wandering wavelets" and another type consisting of a "mother rotor" with daughter wavelets (Choi et al., 2002; Liu et al., 2004; Nanthakumar et al., 2007).

The basic components of the rotor are a curved wave front, a curved wave tail, and a core around which the wave front and tail rotate (Zipes and Jalife, 2013) (Pandit and Jalife, 2013) (**Fig. 5**). The traditional view of a wave propagates in a fixed ring-like path, with an excitable gap separating the wave front from its tail of refractoriness (**Fig. 5A**). Functional reentry was initially described via the leading circle hypothesis (**Fig. 5B**) (Allessie et al., 1977). Different from leading circle hypothesis in a rotor, the curved

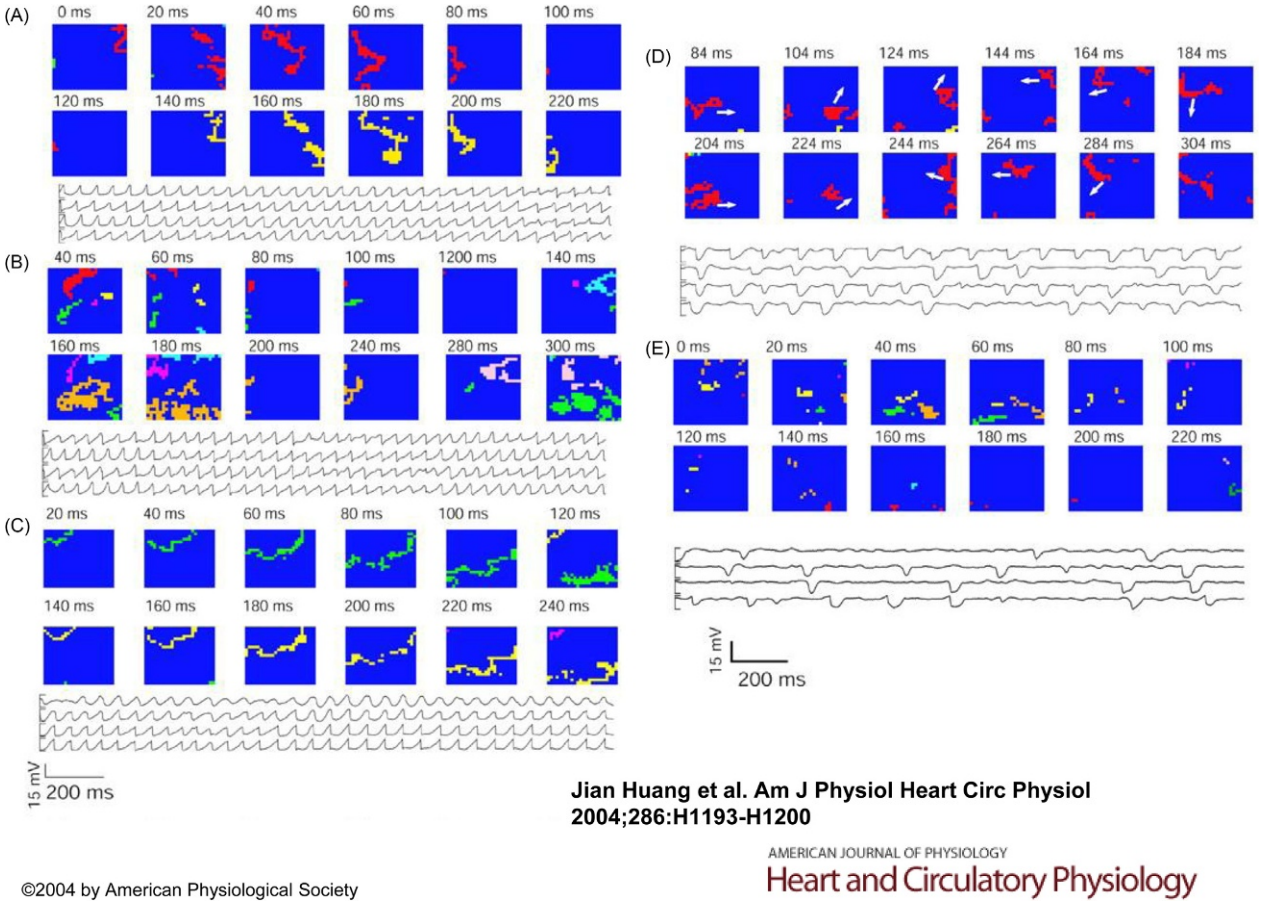

Fig. 4 Snapshots of activation during *stages i–v* of ventricular fibrillation (VF) in one dog. Each colored pixel is an electrode site at which the rate of voltage change (dV/dt) is less than or equal to −0.5 V/s sometime during the 5-ms interval represented by each frame. The numbers show the time from the beginning of each VF stage. Different-colored pixels indicate distinct isolated wavefronts. The arrows in *stage iv* indicate the direction of wavefront movement. Recordings from the same four electrodes are shown below the activation maps for each stage. (A) During *stage i*, the wavefronts are large and coherent and in this particular example repeatedly propagate from right to left. (B) During *stage ii*, there are large spatial and temporal variations in the number of wavefronts and extent of the area swept out by each wavefront. (C) During *stage iii*, the wavefronts become more regular. (D) A reentrant wavefront rotates counterclockwise in this particular example during *stage iv*. (E) There are few wavefronts in *stage v*, which is characterized by slow conduction and small wavefront areas.

wavefront and the wave tail meet each other, at a singularity (white asterisk, Fig. 5C), and the tissue at the center is not refractory (Pandit and Jalife, 2013). The waves might be highly stable and spiral periodically around their generating rotor to activate the ventricles at extremely high frequencies, or might breakup in the rotor peripherally and result in fibrillatory conduction. That can explain complex spatial and temporal pattern of ventricular activation in VF. A rotor is composed of a wave of excitation propagating around a topological defect, which is known as a phase singularity. Rotor termination occurred when rotors of opposite chirality merged or when a single rotor collided with a boundary (Gray et al., 1998).

Defibrillation

Over the last half century, electrical defibrillation has been used clinically as the principal effective treatment for VF (Beck et al., 1947; Zoll et al., 1956) and defibrillation is still the only known effective therapy for VF. ICDs (implantable cardioverter defibrillators) have been used widely for prevention of SCD and have become standard therapy for high-risk patients with cardiac disease. Previously, it was believed that to have a successful defibrillation, a shock must be strong enough to depolarize a large amount of the cardiac tissue. Then if a sufficient amount of the cardiac tissue were changed to unexcitable state by the shock, the wavefronts of VF would be eliminated (Zipes et al., 1975). Although this concept remains as the underlying principle of defibrillation, the process of defibrillation is likely to be much more complex.

In defibrillation, a shock is delivered to the cardiac tissue to uniformly and simultaneously depolarize a critical mass of the excitable myocardium. To defibrillate cardiac muscle, a shock must alter the transmembrane potential so that it terminates the VF

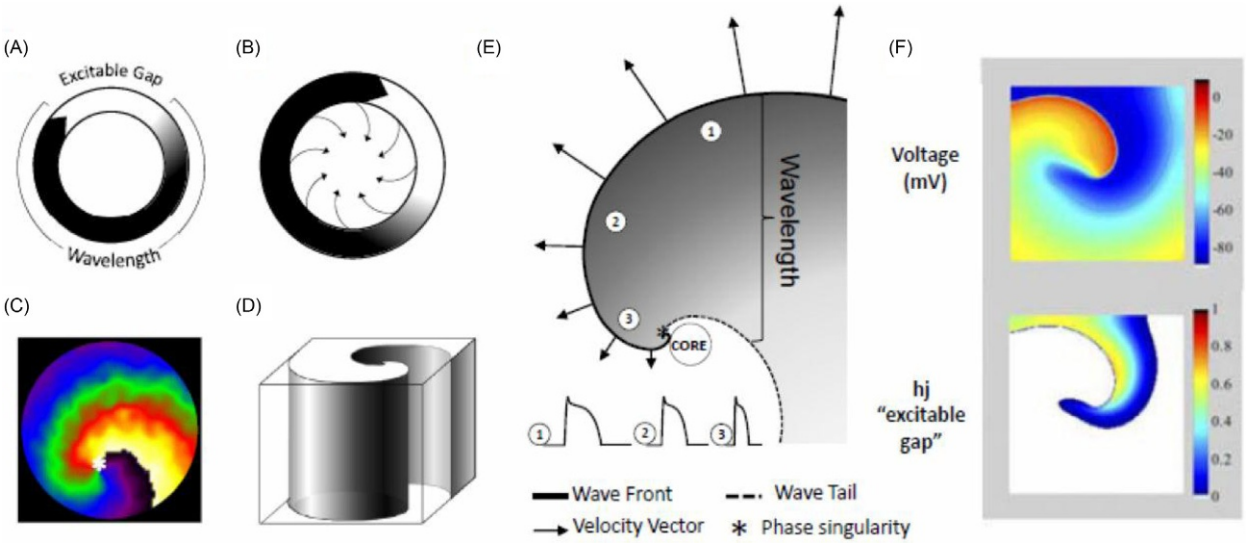

Fig. 5 Rotors and spirals, basic concepts. (A) Schematic representation of reentry around a ring-like anatomical obstacle where the wavelength (*black*) is shorter than the path length allowing for a fully excitable gap (*white*). (B) Leading circle reentry around a functional obstacle, with centripetal forces pointing inward toward a refractory center. (C) Two-dimensional spiral wave, along with the rotor tip at the center "*". (D) Schematic of a three dimensional scroll wave. (E) Snapshot of the spiral wave: electrotonic effects of the core decrease conduction velocity (*arrows*), action potential duration (representative examples shown from positions 1, 2, and 3) and wavelength (the distance from the wave front (*black line*) to the wave tail (*dashed line*)). Conduction velocity (CV) decreases and wavefront curvature becomes more pronounced, near the rotor, which is a phase singularity at the point where the wave front and the wave tail meet "*". (F) Computer simulation of reentry. Top panel: snapshot of the transmembrane voltage distribution during simulated reentry in chronic AF conditions in a 2D sheet incorporating human atrial ionic math models. Bottom panel: snapshot of inactivation variables of sodium current, "h.j"during reentry.

wave fronts but simultaneously must not reinitiate new wave fronts that lead to VF. Therefore, to fully understand the mechanism of defibrillation, it is essential to understand the mechanism of initiation and reinitiation of VF.

Mechanism of Defibrillation

The exact mechanism by which electrical energy defibrillates the cardiac tissue has not been completely elucidated. The electrical shock seem to be electrically synchronize cardiac cells by either activation or change cardiac cells into refractory state by extending refractoriness.

Computer simulations showed that areas close to shocking electrodes produce virtual electrodes that either hyperpolarize or depolarize the surrounding tissue (Roth, 1995; Wikswo et al., 1995; Dosdall et al., 2010).

Defibrillation Waveforms

The electrical shocks delivered from the defibrillator (either ICD or external defibrillator) discharge to the cardiac muscle through the high energy electrodes. The waveform typically shows a voltage that declines exponentially (Fig. 6).

Biphasic Versus Monophasic Wave

Two different types of waveforms are used clinically, monophasic and biphasic (Fig. 6). In monophasic waveforms, the polarity of the shock is unchanged at each duration of the electric shock, while in biphasic forms, the polarity of the shock reverses at each electrode pathway through the defibrillation waveforms. Contemporary ICDs incorporate biphasic waveforms because biphasic defibrillation is superior to monophasic defibrillation, requiring less energy and causing less cardiac damage with higher success rates.

The monophasic shock defibrillates most of the myocardial cells in the ventricle (at least 60%, can be 90%). Therefore, monophasic shocks may be strong enough to terminate the wavefronts of VF. However, the shocks would create virtual electrodes that can generate new wave fronts that can reinitiate VF. Recovered cells are stimulated and simultaneously the action potential will be extended to those cells that are activated already. The required voltage for monophasic shocks are higher and that is consistent with the fact the defibrillation threshold (DFT) with monophasic shock is higher than biphasic shock. Unnecessarily high-energy defibrillation shocks have since been shown to result in more postshock cardiac dysfunction as a result of a direct membrane-depressant effect and electroporation (Jones and Jones, 1984; Jones et al., 1987; Noujaim et al., 2007; Tang et al., 1999).

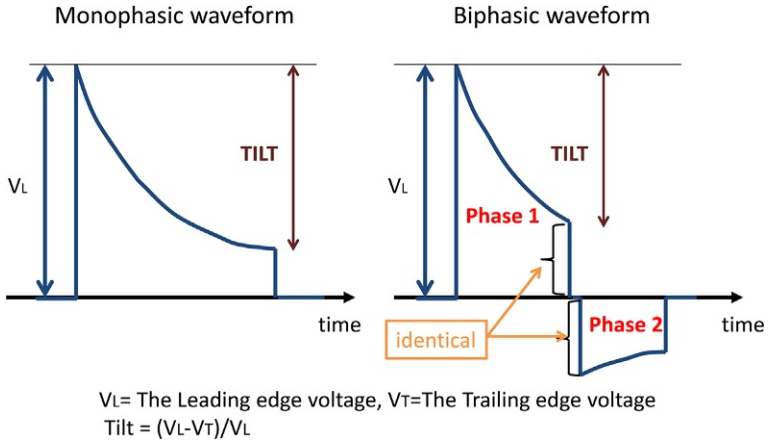

Fig. 6 Monophasic and biphasic waveform in defibrillation.

A biphasic shock has two phases of opposite polarity. After an initial pulse (phase 1), a second pulse with the opposite polarity (phase 2) is delivered after 2 or 3 ms in ICD (Stroobandt et al., 2009). In the middle of the shock, the waveform reverses direction rather than traveling in one direction only. Just as monophasic shock, phase 1 depolarizes and extends the refractory period of most ventricular cells, while phase 2 acts as a powerful catalyst to reduce significantly the electrical requirements of the first phase. Phase 2 removes the charge of cells that were not captured (capture means extending refractory periods of the cardiac cell). This phase 2 shock reduces the number of borderline stimulated cells that would exhibit delayed activation capable of desynchronizing the heart. In fact, as the effectiveness of the single capacitor biphasic waveform has been established, it has successfully been incorporated in clinical use in the ICD as well as the automated external defibrillator (AED) (Stroobandt et al., 2009; Bardy et al., 1996; Schneider et al., 2000).

Truncated Waveform

The waveform terminates prematurely before full capacitor discharge; thus it is called truncated biphasic waveform. Truncated biphasic waveform defibrillation has had a very favorable impact on ICD and AED energy requirements (Bardy et al., 1989, 1996; Schneider et al., 2000; Marks et al., 1994; Mittal et al., 1999; Swartz et al., 1993; Winkle et al., 1989). Mainly there are two merits for truncation. First, truncation can avoid the refibrillatory effects induced by the low-voltage tail of complete capacitor discharge. Secondly, an excessively long shock waveform wastes much energy. At maximum membrane voltage, the largest number of cells will be captured. Extension of the shock duration beyond this point results in the decreasing voltage that can reduce the success rate of defibrillation. It captures the largest number of cells, long before complete discharge of the high voltage capacitor. Unnecessary long shock duration can also cause shortening of the battery life of ICD.

Defibrillation Threshold

The goal of defibrillation is to use the minimum amount of energy required to overcome the threshold of defibrillation. Excessive energy can cause myocardial injury and cardiac arrhythmias. The DFT is the minimum amount of energy required to reliably defibrillate the heart when it is experiencing a hemodynamically unstable VF or VT.

To know the DFT allows the physicians to be sure that the ICD is programmed to deliver energy that is sufficient enough to defibrillate ventricular arrhythmias. Therefore DFT testing requires VF induction. Another reason for the test is to confirm reliable sensing, detection, and redetection of VF.

The DFT does not exhibit a constant value because defibrillation requirements vary as a result of many influencing factors. In fact, a clinical measurement of DFT has only fair reproducibility. In one study of 25 patients, the correlation coefficient between two successful determinations of the DFT was 0.64 (Swerdlow et al., 1996).

DFT Test in Clinical Practice

DFT Testing

DFT testing had been considered an integral part of ICD implantation for many years. This has traditionally been done by VF induction and termination through the device or, less commonly applied, through upper limit of vulnerability (ULV) testing. The DFT testing usually starts by programming a ventricular sensitivity of 1.2 mV, which is higher than nominal setting of 0.3 mv (less sensitive). Defibrillation from ICD might fail and external defibrillation pads are placed before the implantation procedure begins.

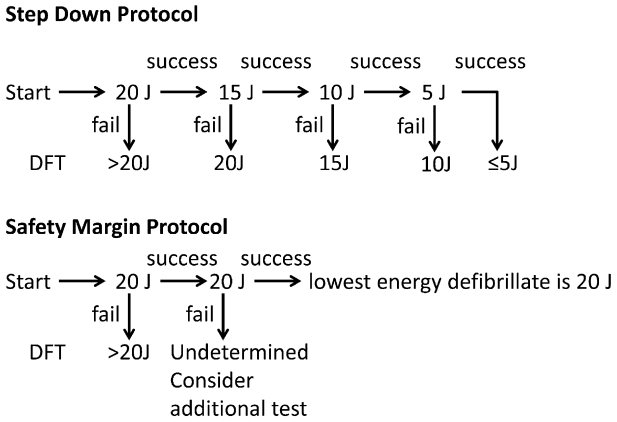

Fig. 7 Two common protocols of defibrillation threshold testing at implant of an ICD with a maximum shock strength of 30 J.

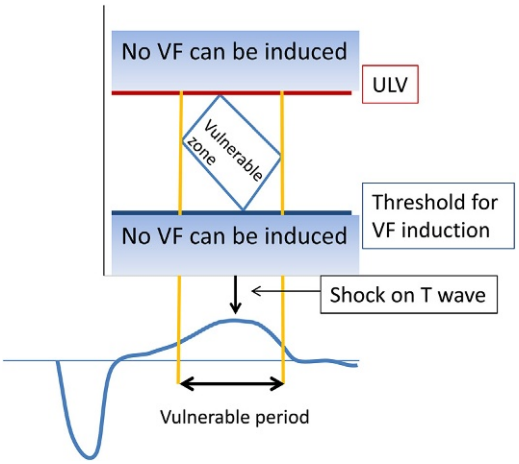

Fig. 8 Vulnerable period and upper limit of vulnerability (ULV).

Testing is done with the device in the surgical pocket and with leads connected. Electrical activity during VF is lower than that during VT and VF and not fast VT has to be induced for the testing. The method of VF induction depends upon the manufacturer, and the capabilities of the device and programmer. VF is induced by rapid pacing, direct current, or T-wave shocks. Given the probabilistic nature of DFT, DFT testing requires multiple shocks to determine with precision. During DFT testing, VF is induced one or more times, and each episode of VF is defibrillated at one or more shock energies. In patient-specific testing, the success or failure of the first test shock usually determines the programmed strength of the next test shock:

There are two common methods for DFT testing, "the safety margin protocol" and "the step down protocols" (**Fig. 7**). In both protocols, the first shock is programmed at least 10 J below the maximal output of the ICD. A safety-margin protocol is preferred when the principal goal is to minimize the risks of shocks, fibrillation and defibrillation testing. After the safety margin testing, the first shock usually is programmed to maximum output.

Upper Limit of Vulnerability

Testing the ULV has been used as an alternative to standard DFT testing as a way to estimate DFT without inducing VF. ULV testing provides an accurate estimate of the probability of defibrillation success and is more reproducible than the DFT testing (Swerdlow et al., 1996). If shock was applied during a certain period of time of a regular cardiac cycle, VF can be induced. This period is called as "vulnerable period." This period is simultaneous with the T wave in the ECG (**Fig. 8**). The ULV is the weakest shock that does not induce VF when delivered during the vulnerable period (Stroobandt et al., 2009; Swerdlow et al., 2003). To successfully defibrillate, the shock strength must reach or exceed the ULV. A shock on the vulnerable period of the T wave can only induce VF if the energy is smaller than a critical value. If a higher energy is delivered, VF will not be induced. It correlates closely with the minimum shock energy that defibrillates reliably (Chen et al., 1986; Swerdlow et al., 2003). The measurement of ULV is performed in regular rhythm (usually during right ventricular pacing at 120–150 beats per minute) providing an estimate of the minimum shock strength required for reliable defibrillation. A shock is delivered on top of the T wave. If VF is not induced, the next shock will be delivered

20 ms before or after the top of the T wave. If you only need to determine a safety margin, the shock strength should be 5–10 J below the maximum output of the device. For example, if VF is not induced by the 20 J shock, the ICD shock can be programmed at 30 J (more than 10 J safety margin). The ULV is more reproducible than the DFT and can provide an accurate patient-specific safety margin with fewer episodes of VF (Swerdlow et al., 1996, 1998). Thus, the ULV testing has been used as a surrogate for the DFT testing, especially patients with high risk for DFT.

The Future of DFT Testing

With developments in the technology of manufacturer of defibrillators, ICDs are now capable of delivering high energy (>35 J) shock and can be programmed to increase the efficacy of biphasic shock. The need for DFT testing has therefore become debatable, given that the incidence of an inadequate safety margin is infrequent. While complications during ICD implantation are also infrequent, many of these can be directly or indirectly related to DFT testing. Adverse effects related to DFT testing include myocardial injury, depression of ventricular systolic function, persistent hypotension, central nervous system injury, stroke, thromboembolic events, or respiratory depression (Mollerus and Naslund, 2007; Schlüter et al., 2001; Toh et al., 2012; Wilkoff et al., 2016a,b,c).

Recently large prospective randomized studies were conducted to evaluate the efficacy of DFT testing (Bänsch et al., 2003; Blatt et al., 2008; Brignole et al., 2012; Healey et al., 2015). Those studies revealed that routine defibrillation testing at the time of ICD implantation does not improve shock efficacy or reduce arrhythmic death in patients with left-sided pectoral implantation. Based on these results, the US guidelines was modified and now advocates that DFT testing can be reasonably omitted in patients undergoing a left pectoral transvenous ICD implantation with a right ventricular apical lead that is functioning appropriately (Wilkoff et al., 2016a,b,c).

Conclusions

VF is an important cause of morbidity and sudden death. Even after many decades of research, the mechanism of VF has not been fully understood. An electrical defibrillation is the only proven effective therapy for VF and ICD has become standard of care for patients with the risk of SCD. The efficacy of the ICD for the primary and secondary prevention of SCD has been well established. The goal of defibrillation is to use the minimum amount of energy required to overcome the threshold of defibrillation while minimizing myocardial injury and cardiac arrhythmias caused by excessive energy. Until recently, DFT testing has been considered the standard of care during ICD implantation procedure.ULV has also been used as alternative to DFT during ICD implantation to minimize the number of induction of VF and defibrillation. With the development of contemporary pectoral ICD systems with biphasic waveforms and higher output shocks, the risk of unsuccessful defibrillation becomes much lower, and DFT is not recommended routinely any more. Further studies are needed to elucidate the mechanism of VF and defibrillation and that would lead to improvement of prognosis of patients with the risk of SCD.

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Ventricular Remodeling in Heart Failure

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Introduction

Heart failure (HF) affects 5.7 million Americans, costs $30.7 billion annually, and is associated with a 50% 5-year mortality (Mozaffarian et al., 2015). The lifetime risk of HF in Americans is approximately 26% (Folsom et al., 2015). The prevalence and incidence of HF increases exponentially with age, disproportionately burdening the elderly who are projected to account for 20% of the U.S. population by 2030 (Ortman et al., 2014). Indeed, using the American College of Cardiology (ACC) and American Heart Association (AHA) HF staging system (Yancy et al., 2013), which emphasizes the continuum of risk for the HF syndrome, contemporary data from an elderly community-based cohort demonstrate that 52% had clinical risk factors for HF without overt structural heart disease (Stage A), 30% had evidence of cardiac structural and functional abnormalities (Stage B), with only 5% having neither (Shah et al., 2017a). These HF trends are partly related to advances in diagnosis and treatment of coronary artery atherosclerosis, including cardiac revascularization therapy, such that greater numbers of patients survive myocardial infarction (MI) and are at heightened risk for development of HF (Armstrong and Committee, 2006).

At the same time, the phenotypic profile of HF is changing, related to both the aging population and secular trends in the prevalence of cardiometabolic HF risk factors including obesity and diabetes. Data from community-based studies have established that HF with preserved ejection fraction (HFpEF) accounts for up to 50% of HF cases and is increasing in prevalence (Bhatia et al., 2006; Owan et al., 2006). HFpEF is associated with rates of HF rehospitalization and functional decline similar to HF with reduced ejection fraction (HFrEF) (Tsutsui et al., 2001; Smith et al., 2003), and carries a significantly higher risk of death compared to age-matched controls (Vasan et al., 1999; Davis et al., 2008). Although outcomes of patients with HFrEF remain poor, the clinician has several evidence-based pharmacologic and device interventions to reduce disease-associated morbidity and mortality. In contrast, no disease-specific therapy has been definitively shown to improve prognosis in HFpEF, despite multiple randomized controlled trials (Yusuf et al., 2003; Cleland et al., 2006; Massie et al., 2008; Pitt et al., 2014).

Left ventricular (LV) remodeling is the culmination of a complex series of transcriptional, signaling, structural, electrophysiological, and functional events occurring within the cardiac myocyte and other elements of the LV. The remodeling process encompasses cellular changes including both cellular and interstitial components of the myocardium. The alteration in ventricular architecture is driven at a cellular level by a combination of myocyte hypertrophy, necrosis (Tan et al., 1991), apoptosis (Sharov et al., 1996; Olivetti et al., 1997), fibrosis (Anderson et al., 1979), increased fibrillar collagen (Brilla et al., 1990), and fibroblastic proliferation (Villarreal et al., 1993). Pathophysiologic changes in myocyte function are related to decreased beta-adrenergic receptor density and signaling, abnormal excitation–contraction coupling, and myosin heavy chain isoform switching (Mann and Bristow, 2005). In addition, reorganization of intercellular matrix components, involving interstitial and perivascular fibrosis, and associated increases in LV stiffness and alterations in chamber geometry, characterize remodeling (Weber, 2000).

This review article will focus on patterns of structural LV remodeling in HF as assessed by noninvasive imaging techniques. Given the burden of HFpEF, and lack of clarity regarding pathophysiology of this heterogeneous syndrome, we will place particular emphasis on what is known—and not known—about ventricular remodeling in HFpEF.

Ventricular Remodeling: Describing LV Size and Shape

The term "ventricular remodeling" was first coined to describe the progressive cavity dilatation following MI (Pfeffer et al., 1985), but has come to be used more generally to refer to changes in LV structure in response to physiologic and pathologic hemodynamic stress. Indeed, alterations in cardiac structure and function have long been recognized as markers of heightened risk for cardiovascular events. Using pathologic samples, Linzbach described two distinct patterns of LVH, reflecting the impact of volume and pressure loading on LV muscle mass and chamber size (Linzbach, 1960): eccentric (volume) hypertrophy characterized by increased chamber size and residual volume, and concentric (pressure) hypertrophy with preserved chamber size and residual volume. Grossman et al. (1975) subsequently articulated this fundamental relationship between myocardial wall stress and ventricular hypertrophy and chamber enlargement: pressure overload associates with sarcomere replication in parallel and increased wall

thickness-to-chamber radius ratio (concentric hypertrophy), allowing for normalization of LV peak systolic meridional wall stress, while volume overload associates with sarcomere replication in series and preserved wall thickness-to-chamber radius ratio (eccentric hypertrophy). The prognostic importance of LV volumes (Hammermeister et al., 1978) and ejection fraction (Hammermeister et al., 1979) for survival in patients with valvular and coronary diseases respectively was them demonstrated by Hammermeister et al. using left ventriculography. These findings were replicated and reinforced by imaging studies of patients with chronic or post-MI LV systolic dysfunction without overt HF in the SOLVD Prevention (SOLVD Investigators et al., 1992; Quinones et al., 2000) and SAVE (Pfeffer et al., 1992; St John Sutton et al., 1994) trials. Subsequent investigations have further demonstrated that both regression of LVH (Devereux et al., 2004) and the prevention or reversal of LV enlargement (Solomon et al., 2010a; Doughty et al., 1997; Merlo et al., 2011) are associated with improved clinical outcomes.

Beyond LV mass and volume, the relationship between LV wall thickness and cavity dimension, which can be assessed by noninvasive imaging modalities and is termed the relative wall thickness (RWT), has been used further refine risk stratification among persons without hypertrophy. Ganau et al. (1992) incorporated RWT with LV mass in a sample of hypertensive persons to identify different patterns of ventricular shape, while Koren et al. (1991) demonstrated the prognostic implications of elevated RWT but normal LV mass for hypertensive patients. These geometric patterns have subsequently been described in patients with cardiovascular risk factors, prevalent coronary disease, and HFpEF, although the prognostic relevance of increased RWT in the absence of LVH (termed concentric remodeling) has been inconsistent (Krumholz et al., 1995; Verdecchia et al., 1996; Ghali et al., 1998; Zile et al., 2011; Apostolakis et al., 2014).

Furthermore, these alterations in LV structure are closely linked to impairments in LV function. LV dilatation and eccentric hypertrophy are associated with a change in the LV from a prolate ellipse to a more spherical shape, reflected in a higher eccentricity index. This change in shape appears to be accompanied by detrimental alterations in myocardial fiber orientation (van Dalen et al., 2010; von Deuster et al., 2016) and associated reduction in the efficiency of myocyte shortening (Sallin, 1969). Concentric remodeling is associated with impaired diastolic function and increased diastolic LV stiffness (Fouad et al., 1984), but may also mask impairments in myocardial systolic function despite preserved or even increased chamber-level measures of systolic function, such as LVEF and fractional shortening (Aurigemma et al., 1995a,b; Palmieri et al., 2001). Quantification of myocardial deformation in persons with preserved LVEF using 2D speckle tracking suggests that progressive concentric remodeling is associated with reduced longitudinal deformation and increased circumferential deformation (Kuznetsova et al., 2008).

Impact of HF Risk Factors on Age-Related Ventricular Remodeling

Longitudinal echocardiographic data from the Framingham Heart Study (FHS) show that HF risk factors modify normal age-related changes in LV structure such that wall thickness increases are more pronounced, cavity size fails to decrease, increases in systolic function (fractional shortening) are attenuated, and increases in LV mass are exaggerated (Cheng et al., 2009, 2010; Lieb et al., 2009). Once present, LV concentric hypertrophy progresses to eccentric hypertrophy in 20%–25% at 5 years (Lieb et al., 2014; Desai et al., 2011), and is associated with a higher risk of subsequent reduced LVEF (Drazner et al., 2004; Kishi et al., 2014). In addition to age-related changes in LV structure and systolic function, LV diastolic function—particularly LV longitudinal relaxation velocities—also appear to decrease with age, in a manner that is accelerated by HF risk factors (Shah et al., 2017b). Indeed, the low rates of LV hypertrophy relative to diastolic dysfunction in population-based studies (Redfield et al., 2003) and clinical trials of hypertension (Solomon et al., 2007, 2010b) and HFpEF (Solomon et al., 2012) suggest that diastolic dysfunction may precede LV remodeling and hypertrophy.

Importantly, the same HF risk factors that modify longitudinal changes in LV structure, including hypertension, diabetes, obesity, and renal dysfunction, correlate with diastolic dysfunction (Abhayaratna et al., 2006; Boyer et al., 2004; Hayashi et al., 2006) and impaired systolic function detected by strain (Narayanan et al., 2009; Fonseca et al., 2004; Andersen et al., 2005; Kosmala et al., 2008; Wong et al., 2004; Tumuklu et al., 2007). Indeed, greater LV mass and cavity size themselves correlate with concomitant abnormalities of LV diastolic function and systolic function detected by strain imaging (Fouad et al., 1984; Hare et al., 2008; Hung et al., 2017), although these measures can be abnormal in the absence of chamber enlargement or hypertrophy.

Ventricular Remodeling in HF With Reduced Ejection Fraction

HFrEF is characterized, by definition, by reduced LVEF and LV dilatation. As previously noted, it is well recognized that both lower LVEF and larger LV volumes—and larger LV end-systolic volumes in particular—predict worse cardiovascular outcomes in HFrEF. Furthermore, imaging substudies of both the SOLVD trial (Konstam et al., 1992, 1993; Greenberg et al., 1995) in HFrEF and the SAVE study (St John Sutton et al., 1994, 1997) in patients with post-MI LV dysfunction demonstrated attenuation of LV enlargement with ACE inhibitor therapy compared to placebo, effects that paralleled the beneficial impact of ACE inhibitor therapy compared to placebo on clinical outcomes. Beyond LVEF and ventricular size, patterns of LV hypertrophy and remodeling, including eccentric and concentric remodeling, influence patient risk. Concentric hypertrophy at baseline predisposes to LV enlargement post-MI (Jilaihawi et al., 2003). Among patients who sustained an MI complicated by LV dysfunction or HF, specific patterns of LV geometry have been associated with death or HF hospitalization, even after adjusting for EF and LV end-diastolic volume (Verma et al., 2008).

Ventricular Remodeling in HF With Preserved Ejection Fraction

Classically, HFpEF has been considered an expression of advanced hypertensive heart disease, with antecedent hypertension present in 60%–90% of HFpEF cases in epidemiologic studies (Aurigemma and Gaasch, 2004; Lam et al., 2011). Biopsy-based pathologic studies in select samples of HFpEF patients demonstrate increased cardiomyocyte stiffness, elevated interstitial myocardial collagen content, and greater cardiomyocyte hypertrophy (van Heerebeek et al., 2006), while invasive hemodynamic studies in select HFpEF patients have demonstrated both impaired early diastolic active relaxation and increased passive stiffness (Zile et al., 2004; Westermann et al., 2008). Further supporting this view, previous studies have described significantly increased LV wall thickness and reduced chamber dimension in HFpEF when compared to healthy controls or hypertensive patients without HF (Kitzman et al., 2002; Melenovsky et al., 2007; Lam et al., 2007). However, in contrast to these findings largely in patients in midlife, data from the Cardiovascular Health Study (CHS) demonstrate that elderly persons with HFpEF have increased LV cavity size and mass compared to hypertensive controls (Maurer et al., 2007)—changes concordant with the observed effects of HF risk factors on longitudinal changes in LV structure from mid- to late-life (Cheng et al., 2009, 2010; Lieb et al., 2009).

Heterogeneity in the cardiac phenotype—including LV structure and function—in HFpEF is increasingly recognized (Shah and Pfeffer, 2012), in parallel with the greater appreciation of the pathophysiologic heterogeneity of this syndrome. Diastolic measures perform poorly in discriminating HFpEF patients from their comorbidity matched symptom-free counterparts (Abhayaratna et al., 2006), and are absent in approximately one-third of HFpEF patients (Zile et al., 2011; Persson et al., 2007; Shah et al., 2014a). Simultaneously, numerous additional pathophysiologic abnormalities have been described in HFpEF, including impaired systolic function despite preserved LVEF (Kraigher-Krainer et al., 2014; Shah et al., 2015), increased ventricular dyssynchrony (Yu et al., 2007; Santos et al., 2014a; Biering-Sorensen et al., 2017), impaired left atrial function (Kurt et al., 2009; Santos et al., 2014b, 2016), pulmonary hypertension (Lam et al., 2009), increased arterial stiffness (Kawaguchi et al., 2003), and impaired peripheral oxygen extraction (Haykowsky et al., 2011). These abnormalities likely underpin the contributions of clinical comorbidities beyond hypertension—including coronary artery disease, atrial fibrillation, and pulmonary dysfunction. Early studies of HFpEF often selected patients based on prior HF hospitalization and/or the presence of concentric structural remodeling which, together with limited sample size, may have limited the cardiac phenotype observed (Kitzman et al., 2002; Melenovsky et al., 2007; Zile et al., 2001).

The least biased phenotypic descriptions of HFpEF originate from community-based epidemiologic cohort studies. However, these studies tend to be limited by relatively small numbers of incident HFpEF cases. Across five epidemiologic studies, mean LV size tended to be normal, with variable degrees of LV wall thickening (Lam et al., 2007; Maurer et al., 2007; Devereux et al., 2000; He et al., 2009; Gupta et al., 2013). Ventricular geometry was only characterized in two (Lam et al., 2007; Gupta et al., 2013). One of the largest and most comprehensive epidemiologic evaluations was in the Olmsted County cohort (Lam et al., 2007), which characterized 244 HFpEF cases. Ventricular hypertrophy was present in only 42% and eccentric in pattern in 16%. LV geometry was normal in 31%. The CHS, an older study of HFpEF cases of similar age and gender, demonstrated similar average LV mass index but somewhat larger ventricles with a lesser degree of concentric remodeling (Maurer et al., 2007).

Hospital-based HFpEF registries facilitate the assessment of larger numbers of HFpEF cases (Klapholz et al., 2004; Tribouilloy et al., 2008; Katz et al., 2013), although referral bias is an important consideration, as frequently only patients admitted with acute decompensation are enrolled. However, these registries obviate many of the inclusion biases inherent in clinical trial populations, particularly with regard to inclusion of patients with advanced comorbidities—such as chronic kidney disease—who are often excluded from therapeutic trials for safety reasons. Unfortunately, uniform implementation, analysis, and reporting of cardiac structure and function have been limited in most large HF registries. Perhaps the most comprehensive assessment of ventricular remodeling in a registry setting to date is The Northwestern HFpEF Registry, which demonstrated greater concentric remodeling and higher prevalence of LV hypertrophy (60%) than many epidemiologic studies (Katz et al., 2013). Interestingly, similar to the Olmsted County cohort (Lam et al., 2007), 12% of patients in this registry demonstrated an eccentric pattern of ventricular hypertrophy. This registry is also one of the few to report on left atrial remodeling and noted a high prevalence of left atrial enlargement with a mean LA volume index of 33.6 mL/m^2 (abnormal >34 mL/m^2).

Much of the data on cardiac structure and function in HFpEF originate from imaging substudies of HFpEF clinical trials, which allow for the accrual of large numbers of cases, robust clinical phenotyping, and prospective assessments. Given the inclusion and exclusion criteria necessarily imposed by such trials, these studies are clearly not reflective of HFpEF epidemiology, and identify specific subsets of patients within the HFpEF syndrome. Across seven major phase 2 and 3 HFpEF clinical trial imaging studies (Cleland et al., 2006; Zile et al., 2011; Solomon et al., 2012; Persson et al., 2007; Shah et al., 2014a; Edelmann et al., 2013; Redfield et al., 2013), mean LV size tended to be normal but with appreciable within trial and between trial variability. Similar variability was noted in LV wall thickness and the prevalence of both increased concentricity (wall thickness relative to chamber diameter) and LV hypertrophy, with values ranging from 14% to 49% and 21% to 59% respectively. For example, in the two largest and most comprehensive studies to date, the echocardiographic substudies of the I-PRESERVE and TOPCAT trials (Zile et al., 2011; Shah et al., 2014a), concentric hypertrophy was present in 29% and 43% of subject respectively, while normal LV geometry was present in 46% and 14% respectively. These observations highlight the marked heterogeneity of ventricular morphology found within this syndrome.

The prognostic relevance of greater LV mass in HFpEF, independent of conventional clinical risk factors, has been well documented in several studies (Zile et al., 2011; Shah et al., 2014b; Burke et al., 2014). Although concentric LVH has classically

characterized HFpEF, no significant differences in event rates have been noted between HFpEF patients with concentric compared to eccentric hypertrophy (Katz et al., 2013; Shah et al., 2014b). In contrast to HFrEF, neither LVEF nor LV size have demonstrated prognostic importance in HFpEF, possibly due to the restricted distribution of values in this syndrome. Importantly, measures of LV diastolic function also provide independent prognostic value in HFpEF (Shah et al., 2014b). These include left atrial enlargement, likely a marker of LV diastolic dysfunction and chronically elevated filling pressure (Zile et al., 2011; Shah et al., 2014b), and E/e′, an index of instantaneous LV filling pressure (Shah et al., 2014b).

Although attention has largely focused on alterations in LV structure and diastolic performance, HFpEF is also characterized by abnormal systolic function, detectable by strain (Kraigher-Krainer et al., 2014; Yip et al., 2011; Tan et al., 2009), but also by M-mode echocardiography (Petrie et al., 2002; Yip et al., 2002) tissue Doppler imaging (Yu et al., 2002; Cioffi et al., 2012; Vinereanu et al., 2005). In the echocardiographic study of the TOPCAT trial in HFpEF, strain was impaired in 52% of participants, was the strongest echocardiographic predictor of cardiovascular death or HF hospitalization, and provided incremental value beyond clinical risk factors, LVEF, and measures of LV structure and diastolic function (Shah et al., 2015).

Conclusions

LV remodeling refers to changes in LV structure in response to physiologic and pathologic hemodynamic stress. Patterns of LV remodeling vary depending on the nature of the insult, but are closely linked to alterations in LV systolic and diastolic performance that underlie the HF syndrome. HFrEF is characterized by ventricular enlargement and spherical remodeling. The cardiac phenotype in HFpEF, while classically associated with LV concentric hypertrophy, is diverse and likely mirrors the clinical and pathophysiologic heterogeneity of this syndrome. Detailed quantification of LV structure and function offers a promising foundation for identifying pathophysiologically relevant subgroups within the HFpEF syndrome.

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Ventricular Tachycardia in Ischemic and Dilated Cardiomyopathy: Mechanisms and Diagnosis

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Introduction

Ventricular tachycardia (VT) is a common tachydysrhythmia. Although the spectrum of its consequences ranges from none to sudden cardiac death, the possibility of the latter demands the practitioner's attention. VT most commonly occurs in patients with significant structural heart disease but can also occur in patients with no detectable structural heart disease, then referred to as idiopathic VT (Kusumoto, 2008). This article will focus on VT that occurs in patients with structural heart disease; specifically, in patients with ischemic cardiomyopathy or nonischemic dilated cardiomyopathy.

Definitions and Classifications

VT is defined as a wide QRS complex (>120 msec) rhythm with a rate exceeding 100 beats per minute. Sustained VT is that which lasts for more than 30 s or that which requires early intervention due to hemodynamic instability (Kusumoto, 2008). Nonsustained VT is sometimes defined as that which lasts for five or more beats at a rate exceeding 100 beats per minute but does not otherwise meet criteria as being sustained. When this definition of nonsustained VT is used, three consecutive QRS complexes of ventricular origin are referred to as a ventricular triplet and four consecutive QRS complexes of ventricular origin are referred to as a ventricular salvo. Others simply define nonsustained VT as that which lasts for three or more beats at a rate exceeding 100 beats per minute but does not otherwise meet criteria as being sustained.

Monomorphic VT presents wide QRS complexes that have a single QRS morphology and usually a stable rate and QRS axis. Polymorphic VT presents wide QRS complexes that have varying QRS morphologies, coupling intervals, and axis (Zipes et al., 2006). Some distinguish monomorphic VT and polymorphic VT from pleomorphic VT that presents wide QRS complexes of two or more morphologies each present for a period of time, including two morphologies that are alternating (bidirectional VT). VTs may also be classification according to the patient's clinical presentation as hemodynamically stable VT (that with minimal symptoms such as palpitations) or as hemodynamically unstable VT (that with symptoms and/or signs of systemic hypotension, pulmonary venous hypertension, myocardial ischemia, cerebral hypoperfusion, or cardiac arrest/death). Three or more episodes of sustained VT within a 24-h period defines a VT storm (Kusumoto, 2008; Israel and Barold, 2007). Approximately 10% of patients who receive an implantable cardioverter defibrillator will experience a VT storm within two years (Raymond et al., 2009). Sustained VT is distinguished from two other sustained ventricular tachydysrhythmias (**Table 1**). Ventricular flutter presents wide QRS complexes without intervening isoelectric periods (like a sine wave) usually at a rate of approximately 300 beats per minute suggesting that the mechanism of the ventricular tachydysrhythmia is macro reentry following a stable reentrant route. Ventricular fibrillation presents wide QRS complexes that are ultrarapid (>300 beats per minute) in the form of baseline undulations varying in morphology and timing.

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Table 1 Classification of Ventricular tachycardia

Classification by electrocardiography			
Nonsustained VT		Three or more beats in duration, terminating spontaneously in less than 30 s VT is a cardiac arrhythmia of three or more consecutive complexes in duration emanating from the ventricles at a rate of greater than 100 bpm (cycle length less than 600 ms)	(7)
	Monomorphic	Nonsustained VT with a single QRS morphology	(7)
	Polymorphic	Nonsustained VT with a changing QRS morphology at cycle length between 600 and 180 ms	(7)
Sustained VT		VT greater than 30 s in duration and/or requiring termination due to hemodynamic compromise in less than 30 s	(7)
	Monomorphic	Sustained VT with a stable single QRS morphology	(7)
	Polymorphic	Sustained VT with a changing or multiform QRS morphology at cycle length between 600 and 180 ms	(7)
Bundle-branch re-entrant tachycardia		VT due to reentry involving the His-Purkinje system, usually with LBBB morphology; this usually occurs in the setting of cardiomyopathy	(7)
Bidirectional VT		VT with a beat-to-beat alternans in the QRS frontal plane axis, often associated with digitalis toxicity	(7)
Torsades de pointes		Characterized by VT associated with a long QT or QTc, and electrocardiographically characterized by twisting of the peaks of the QRS complexes around the isoelectric line during the arrhythmia: ● "Typical," initiated following "short-long-short" coupling intervals. ● Short coupled variant initiated by normal-short coupling	(7)
Ventricular flutter		A regular (cycle length variability 30 ms or less) ventricular arrhythmia approximately 300 bpm (cycle length—200 ms) with a monomorphic appearance; no isoelectric interval between successive QRS complexes	(7)
Ventricular fibrillation		Rapid, usually more than 300 bpm/200 ms (cycle length 180 ms or less), grossly irregular ventricular rhythm with marked variability in QRS cycle length, morphology, and amplitude	(7)

From Zipes et al. (2006).

Mechanisms and Substrates

The characteristics of sustained monomorphic VT indicate that the mechanism of monomorphic VT is either reentry with a stable conduction pathway and exit site or automaticity with a stable origin and subsequent conduction route. The former is most common in patients with structural heart disease that includes a ventricular scar while the latter is most common in patients with idiopathic VT in the absence of identifiable structural heart disease (Roberts-Thomson et al., 2011). The ventricular scar forming the substrate for reentrant VT may be the result of ischemic heart disease, nonischemic dilated cardiomyopathy, prior cardiac surgery (often in the setting of corrected congenital heart disease), arrhythmogenic right ventricular cardiomyopathy, cardiac sarcoidosis, or hypertrophic cardiomyopathy (Roberts-Thomson et al., 2011). The substrate regions usually consist of dense fibrosis with some surviving myocardial bundles that help to create conducting channels (Roberts-Thomson et al., 2011). These conducting channels may have reduced cell-to-cell coupling resulting in slow conduction and may be adjacent to anatomical obstacles due to dense fibrosis (Roberts-Thomson et al., 2011). Accordingly, areas of slow conduction, areas of fixed conduction block due to fibrosis, and areas of functional conduction block due to anisotropy represent ideal physiological conditions for reentry initiated by premature beats or cycle length fluctuations (**Fig. 1**). This VT is monomorphic because ventricular activation outside the scar proceeds from a stable location, which is typically the exit of a group of surviving myocyte bundles, which form a channel for conduction through the scar. A single area of scar is often able to support multiple different circuits. Hence, multiple VTs may be induced with programmed stimulation in patients with structural heart disease (Roberts-Thomson et al., 2011).

Mechanisms for VT in Ischemic Cardiomyopathy

The link between ventricular scar and arrhythmia risk suggests that identification and characterization of scar with imaging may allow prediction of arrhythmia risk. In magnetic resonance images (MRI), scar is identified by delayed washout of gadolinium contrast (Nazarian et al., 2005; Kwong et al., 2006). In myocardial infarction survivors, the scar typically extends along a vascular territory supplied by the infarct-related artery (**Fig. 2**). The central zone of the scar is typically transmural with fewer surviving myocytes. The peripheral part of the scar, the "border zone," is typically more heterogeneous and has a mixture of fibrous tissue and surviving myocytes, and may play a more important role in permitting reentry. This has been confirmed by three-dimensional MRI-derived scar reconstruction (Piers et al., 2014), and the size of the border zone has been shown to be a marker for late mortality (Kwong et al., 2006). The burden of scar and its characteristics has also been shown to be related with the presence of both spontaneous and inducible ventricular tachycardia (Gouda et al., 2015; Schmidt et al., 2007). Computer simulations using MRI-derived scar mapping were recently shown to be feasible to predict the existence of VT circuits in animal models (Ng et al., 2012) and in humans (Arevalo et al., 2013). Furthermore, such maps could also predict optimal VT ablation targets within the scar (Ashikaga et al., 2013).

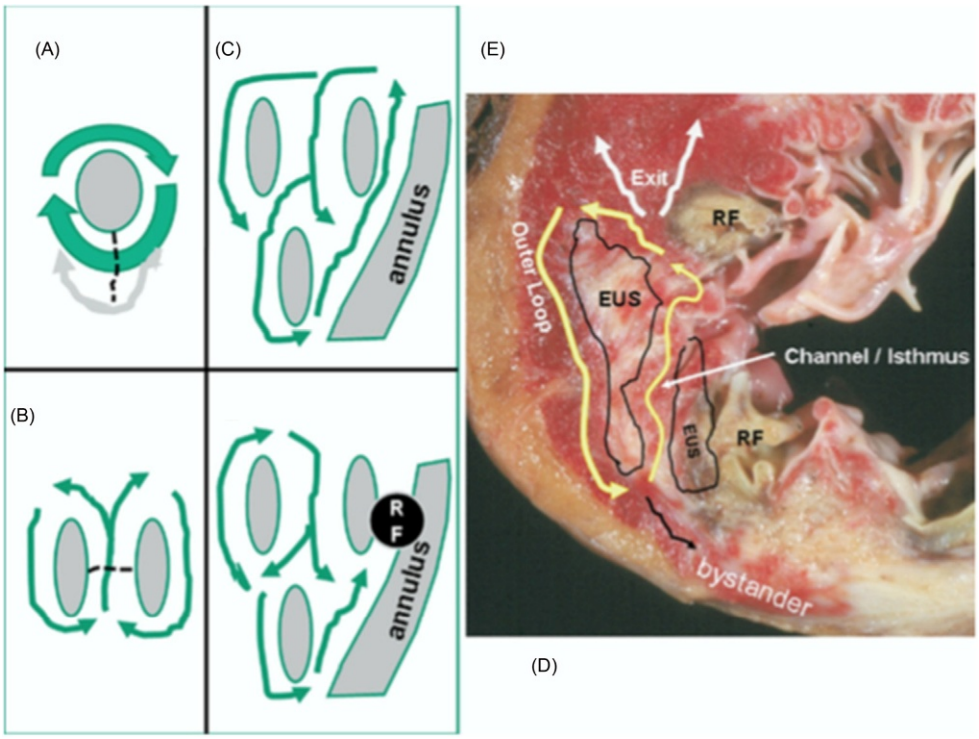

Fig. 1 Theoretical reentry circuits as they relate to areas of block and isthmuses are shown. In the schematics on the left the reentry circuit is indicated by the arrows. Gray regions indicate conduction block that might be from fibrosis, collision of wave fronts, or refractoriness. (A) A simple circuit consisting of a single loop around a region of block. If the loop is broad, ablation in the loop (dotted black line) may increase the reentry path but fail to interrupt reentry. (B) A figure-of-eight type of reentry circuit defined by wave fronts propagating around 2 regions of block and sharing a common isthmus and a common pathway (CP) through which conduction is slowed, creating a "figure-of-eight" configuration. Ablation across the common isthmus (dotted black line) would interrupt reentry. Ablation in either loop alone leaves the other loop to continue reentry. (C) A complex circuit with several regions of conduction block along a valve annulus that creates multiple potential channels. (D) Ablation (RF) that interrupts 1 channel and leaves the other potential channels that may support reentry. (E) Photograph of an explanted heart from a patient who had incessant VT and failed VT ablation with a theoretical reentry circuit (*yellow arrows*) is shown. Areas of dense scar, that may form electrically unexcitable scar (EUS), is indicated. A hypothetical channel/isthmus is present between two EUS areas. The circulating reentry wave front propagates through the channel, emerging at the exit to propagate across the ventricles producing the QRS complex. The circulating wave front propagates along the border of the scar (outer loop) to reenter the channel. A bystander area (*black arrow*) is also shown. RF lesions failed to interrupt VT, likely related to the location of the re-entry circuit deep to the endocardium. From Raymond, J. M., Sacher, F., Winslow, R., Tedrow, U. and Stevenson, W. G. (2009). Catheter ablation for scar-related ventricular tachycardias. *Current Problems in Cardiology* **34**, 225–270.

Mechanisms for VT in Nonischemic Cardiomyopathy

In patients with nonischemic cardiomyopathy (NICM), subendocardial scarring and patchy fibrosis may occur and may contribute to decreased cellular coupling and regional slow conduction, thus providing an anatomic substrate for reentrant VT. However, the extent and degree of fragmented and abnormal endocardial electrograms appear to be significantly less than in patients with ischemic cardiomyopathy (Hsia et al., 2003). In addition, the distribution of scar is more commonly basal, in anatomic relationship with the mitral annulus. In contrast, epicardial patchy fibrosis and abnormal conduction may be detected in 30%–50% of patients with NICM at the time of open heart surgery (de Bakker et al., 1996; Pogwizd et al., 1998) (**Fig. 2**). Several experimental and clinical reports indicate that focal nonreentrant mechanisms may be common in patients with NICM (Pogwizd et al., 1998; Delacretaz et al., 2000). In this population, myocardial macroreentrant circuits may account for only 50%–70% of VT, with focal mechanisms and bundle branch reentry comprising the remainder (Delacretaz et al., 2000). Most patients have multiple distinct QRS morphologies, often with different mechanisms. Given the different mechanisms, it is not surprising that programmed stimulation frequently fails to induce the VT observed clinically.

Bundle Branch Reentry as a Mechanism of VT

Bundle branch reentry VT is a VT that occurs most commonly in patients with nonischemic dilated cardiomyopathy. These patients usually have evidence of conduction system disease that provides the zone of slow conduction. The Purkinje network is integral to this macro reentrant VT (Roberts-Thomson et al., 2011). Most commonly, the right bundle branch is the antegrade limb and the left

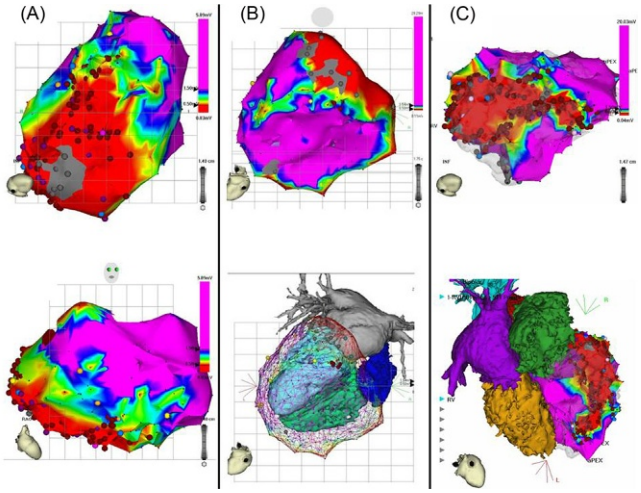

Fig. 2 Representative examples of scar distribution in various types of scar-related ventricular tachycardias. Contact bipolar voltage maps with reduced bipolar voltage (<1.5 mV), representing scar areas are shown in with the provided color scale. Purple areas represent relatively normal bipolar voltage corresponding with normal myocardium. Panel A shows an LV scar extending along the basal and mid inferior wall in a patient with a prior inferior myocardial infarction. Inferior (*top*) and right anterior oblique (*bottom*) views are shown. Panel B shows an epicardial bipolar voltage map of the inferior wall of both LV and RV in a patient with nonischemic dilated cardiomyopathy. The scar location is anatomically related to the basal LV along the inferior mitral annulus (*top*). Integration of the CT-scan image of the cardiac chambers is shown (*bottom*). LV endocardial bipolar voltage map was completely normal in this patient (not shown here). Panel C shows inferior views of the RV in a patient with arrhythmogenic right ventricular cardiomyopathy (ARVC). Notice the scar zone extending along the inferior and lateral RV walls from the tricuspid annulus to the apex (*top*). CT-image integration shows the relatively dilated RV compared to the LV in this patient (*bottom*).

bundle branch is the retrograde limb resulting in a left bundle branch block morphology VT (Roberts-Thomson et al., 2011). Bundle branch reentrant VT can be treated with transcatheter ablation of the right bundle branch; hence recognition of this mechanism is important for treatment.

Mechanisms for Polymorphic VT

The characteristics of polymorphic VT indicate that the mechanism of polymorphic VT is either reentry with varying conduction pathways and/or varying exit sites or abnormal automaticity with varying origins and/or varying subsequent conduction routes. The polymorphic VTs are considered in two groups. Polymorphic VTs without QT-interval prolongation most commonly occur during unstable cardiac disorders such as acute myocardial ischemia or infarction or decompensated congestive heart failure, especially when accompanied by hypoxia, acidosis, or electrolyte disturbances (Zipes et al., 2006). The mechanisms of polymorphic VTs without QT-interval prolongation include spatial and temporal disparities in myocyte excitability, refractory periods, and conduction velocities favoring standard forms of reentry; dispersion of action potential durations allowing phase 2 re-entry; abnormal automaticity; and, altered intracellular calcium handling (Zipes et al., 2006). Polymorphic VTs with QT interval prolongation occur in patients with the congenital QT-interval prolongation syndromes or in patients with acquired QT-interval prolongation (Goldenberg et al., 2008; Zipes et al., 2006; Kuriachan et al., 2015). Although the latter are more common in patients with structural heart disease, they often require hypokalemia, hypomagnesemia, and/or use of QT-interval prolonging drugs. Their mechanisms include prolongation of action potential duration (especially in ventricular M cells), spatial and temporal dispersion of ventricular repolarization, early afterdepolarizations, reactivation or delayed inactivation of L-type calcium channels, and altered intracellular calcium handling (Goldenberg et al., 2008).

Epidemiology

The most common cause of sustained monomorphic VT is that resulting from prior myocardial infarction (Raymond et al., 2009). Other causes of scar-related VT include nonischemic dilated cardiomyopathy, hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, cardiac sarcoidosis, and prior surgical interventions (Raymond et al., 2009). Such VT may be hemodynamically stable, may be hemodynamically unstable, or may degenerate into ventricular fibrillation (VF) or into asystole resulting in sudden cardiac death. Postinfarction ventricular tachydysrhythmias have been better studied than ventricular tachydysrhythmias due to other forms of structural heart disease.

Sudden deaths account for approximately 350,000 deaths per year in the United States (Zipes et al., 2006). When sudden death is defined as that within one hour of onset of symptoms, as many as 13% of all natural deaths may be sudden cardiac death (Zipes et al., 2006). The overall incidence of SCD in the United States is 0.1%–0.2% per year (Zipes et al., 2006). When a patient experiences VT within the first 24–48 h after a transmural myocardial infarction (MI), the subsequent risk of death or VT recurrence

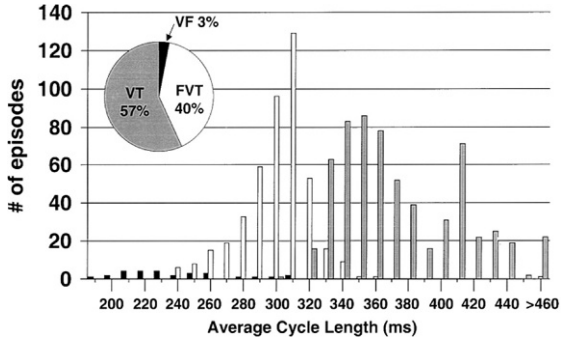

Fig. 3 Distribution of ventricular arrhythmias by detection zone and average cycle length (CL) (derived from last 4 CLs before detection, allowing for irregular or undersensed fast VT rhythms to have CL 320 ms.). Ventricular tachycardia is the predominant dysrhythmia with only 3% ventricular fibrillation. From Wathen, M. S., Sweeney, M. O., Degroot, P. J., Stark, A. J., Koehler, J. L., Chisner, M. B., Machado, C., Adkisson, W. O. and Pain, F. I. (2001). Shock reduction using antitachycardia pacing for spontaneous rapid ventricular tachycardia in patients with coronary artery disease. *Circulation***104**, 796–801.

is not significantly higher than that of a patient with an equivalent MI without early VT (Zipes et al., 2006). The risk of sudden death in the first 30 days after an MI is highest in patients with left ventricular dysfunction and/or heart failure and is in the range of 1.4% (Solomon et al., 2005).

In patients with ischemic cardiomyopathy and in patients with nonischemic dilated cardiomyopathy the probability of experiencing VT is primarily dependent upon the extent of ventricular scar (Zipes et al., 2006) and the degree of the resultant reduction in left ventricular ejection fraction (LVEF). In patients with ischemic cardiomyopathy who received an ICD for standard indications, VT was found to be the initial ventricular tachydysrhythmia much more frequently than was VF (**Fig. 3**) (Wathen et al., 2001). Such observations have led to the hypothesis that, in patients with structural heart disease, VT may lead to sudden death by deteriorating into VF or asystole. However, the details of the relationship between VT and sudden death are not clear and there are many examples of treatments that affect the former without altering the latter.

Clinical Presentation

The presentations of patients with VT range from no symptoms to sudden cardiac death. Patients between these two extremes may present with any cardiac symptom including palpitations, diaphoresis, dyspnea, angina pectoris, presyncope, and syncope. Although patients may initially seem to be hemodynamically stable, careful examination and investigation may reveal otherwise immediately or after a short period of time. For example, significant deterioration in renal function and myocardial necrosis may occur without associated symptoms due to low cardiac output. In general, faster ventricular rates and underlying ventricular systolic or diastolic dysfunction lead to more hemodynamic instability. On the other hand, patients can present in VT with no hemodynamic compromise and with minimal or no symptoms (Morady et al., 1985). When a patient presents with a wide QRS complex tachycardia, regardless of the associated symptoms, a history of known structural heart disease should raise suspicions that the rhythm is VT. Indeed, a history of structural heart disease or symptoms thereof have a positive predictive value of over 95% for a wide QRS complex tachycardia being VT (Roberts-Thomson et al., 2011). Furthermore, in patients approaching the age group of structural heart disease (over age 35 years) who present with a wide QRS complex tachycardia, the positive predictive value of age alone for a diagnosis of VT is 85% (Roberts-Thomson et al., 2011). Accordingly, prior cardiac history and patient age are important features to consider in evaluating the differential diagnosis of a wide QRS complex tachycardia.

Patients who have already received an ICD may also present with symptoms related to antitachycardia pacing or shocks from the device. Of course, if such a patient has VT with a rate below that set for ICD detection, a common scenario in patients after antiarrhythmic drug or ablation therapy, the spectrum of presentations with VT will be similar to that of the patient without an ICD.

On examination, a patient with VT will, by definition, have a heart rate over 100 bpm. The physical examination should focus on assessment of the degree of hemodynamic stability and on the presence of underlying structural heart disease. Relative to the determination that the underlying rhythm is VT, signs of AV dissociation, such as a jugular venous canon A waves and variable intensity of first heart sound, support the diagnosis of VT (Garratt et al., 1994).

ECG Features

The electrocardiogram (ECG) is the key tool in the diagnosis of VT. A wide QRS complex (>120 msec) tachycardia at a rate greater than 100 beats per minute should be considered to be VT unless proven otherwise. This admonition reflects the fact that therapy for supraventricular tachycardia may worsen the state of a patient with VT whereas the converse is unlikely.

Recalling that a narrow QRS complex is the result of simultaneous activation of the two ventricles, wide QRS complex rhythms result from sequential activation of the two ventricles—one ventricle being activated early (a ventricular rhythm or a supraventricular rhythm with ventricular preexcitation) or one ventricle being activated late (a paced ventricular rhythm or a supraventricular rhythm with aberrant AV conduction (right bundle branch block, left bundle branch block, or an intraventricular conduction defect)).

Atrioventricular (AV) dissociation is the only ECG feature that is diagnostic for VT (Wellens, 2001). On an ECG recording VA dissociation is manifest as evident P-waves at a rate that is unrelated to the QRS rate and that is generally slower than the QRS rate. Such P-waves may intermittently conduct to the ventricles resulting in a "capture beat" that has the same QRS morphology as that patient's normal rhythm or a "fusion beat" that has a QRS complex morphology representing the sum of that patient's normal QRS morphology and the QRS morphology of the VT (Wellens, 2001) (**Fig. 4**). Unfortunately, AV dissociation during VT is uncommonly seen on the ECG recording because slower VTs may conduct retrogradely to the atrium, preserving a 1:1 relationship between atrial and ventricular activation, and faster VTs obscure the pattern of background P-waves, precluding recognition of AV dissociation (Roberts-Thomson et al., 2011).

Other ECG findings are not diagnostic but may contribute to the differential diagnosis of a wide QRS complex tachycardia. In general, if the ECG QRS complex pattern is that of typical right or left bundle branch block then a supraventricular tachydysrhythmia with aberrant AV conduction is favored. The less the QRS complex is typical of right or left bundle branch block, the more likely it is a VT. VT is likely if a wide QRS complex tachycardia presents QRS complexes with a right superior axis, precordial concordance (implying early basal or apical activation), and a very wide QRS (left bundle branch block like pattern with a QRS duration >160 ms or a right bundle branch block like pattern with a QRS duration >140 ms) (Roberts-Thomson et al., 2011).

Comparison of the relative time courses of ventricular activation at the beginning of the QRS complex and at the end of the QRS complex may be helpful in the differential diagnosis of a wide QRS complex tachycardia. When ventricular activation involves the Purkinje system, voltage changes are rapid. When ventricular activation depends upon ventricular myocyte conduction, voltage changes are slower. Accordingly, a slurry initial QRS complex associated with a sharp terminal QRS complex suggests VT or ventricular preexcitation while the reverse suggests a supraventricular tachycardia conducted with aberrancy.

These ECG features have been incorporated into formal algorithms designed to assist the differential diagnosis. A four-step algorithm developed by Brugada was reported to have a sensitivity of 98.7% and specificity of 96.5% for the diagnosis of VT (**Fig. 5**) (Brugada et al., 1991). The initial step was to assess the absence of RS complexes in all the precordial leads, a finding that suggests VT. When there was a precordial lead with an RS morphology, an onset of R to a nadir of S interval greater than 100 msec suggests VT provided that the patient was not receiving an antiarrhythmic drug that slows intraventricular conduction. The third step identified AV dissociation. The final step examined morphology criteria in ECG leads V1 and V6 that basically assessed whether the morphology in these leads deviated from a typical left or right bundle branch block pattern as this would also suggest VT. Another algorithm, derived from a patient population that included preexcited supraventricular tachycardias, suggested by Vereckei et al., examined ECG lead aVR for the presence of an initial R wave (Vereckei et al., 2008). This stepwise algorithm suggests that the presence of an initial dominant R wave, the presence of a nondominant r or q wave lasting more than 40 ms, the presence of a notch on the descending limb of a negative onset and predominantly negative QRS, and a ventricular activation velocity ratio, v_i/v_t (v_i is the vertical excursion during the initial part of the QRS and v_t is during the terminal part of the QRS) all suggest VT (Vereckei et al., 2008) (**Fig. 6**).

A VT that emerges from the interventricular septum usually presents QRS complexes that are less wide than those of VT arising from other regions. In patients that already have an underlying wide QRS complex during supraventricular rhythm, a less wide QRS complex during tachycardia should raise suspicion that the tachycardia is VT because a supraventricular tachycardia would have no mechanism to produce a QRS complex less wide than a slower supraventricular rhythm (Wellens, 2001).

The 12-lead ECG may be used to localize the site of origin of VT, useful information for the diagnosis of idiopathic VT (the most common site of origin of which is the right ventricular outflow tract), or when VT ablation is planned. Here the site of origin refers to the site of abnormal automaticity in the case of an automatic VT or the site of exit from the zone of slow conduction in the case of a

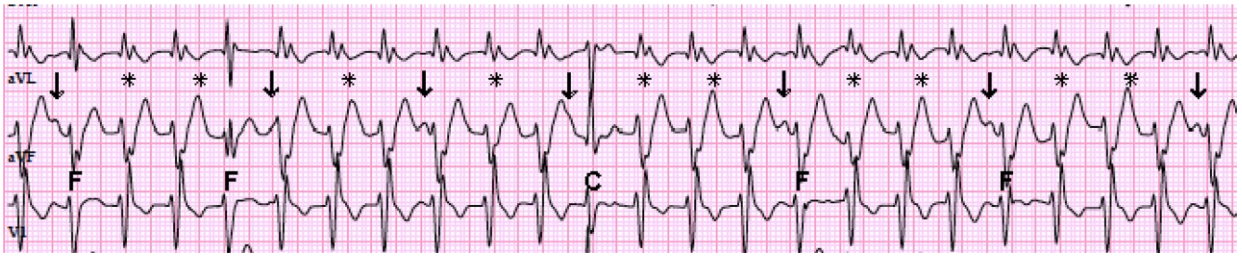

Fig. 4 Simultaneously recorded ECG leads aVL, aVF, and V1 during a regular wide QRS complex tachycardia with left bundle branch block like and superior axis morphology and a ventricular rate of 143 beats per minute with AV dissociation, fusion beats, and a capture beat. P-waves are best seen in lead aVF. The arrows denote evident P-waves including one distorting the onset of the QRS complex (*second arrow*) and one distorting the downslope of the T-wave (*forth arrow*). The asterisks indicate where other P-waves must be although they are obscured by the background rhythm. The atrial rate is 103 beats per minute. C denotes a narrow QRS complex capture beat with normal QRS morphologies in each lead. F denotes three fusion beats with morphologies that are variably intermediate between that of the underlying rhythm and that of the capture beat. The third fusion beat is the least fused with morphology change evident only in V1.

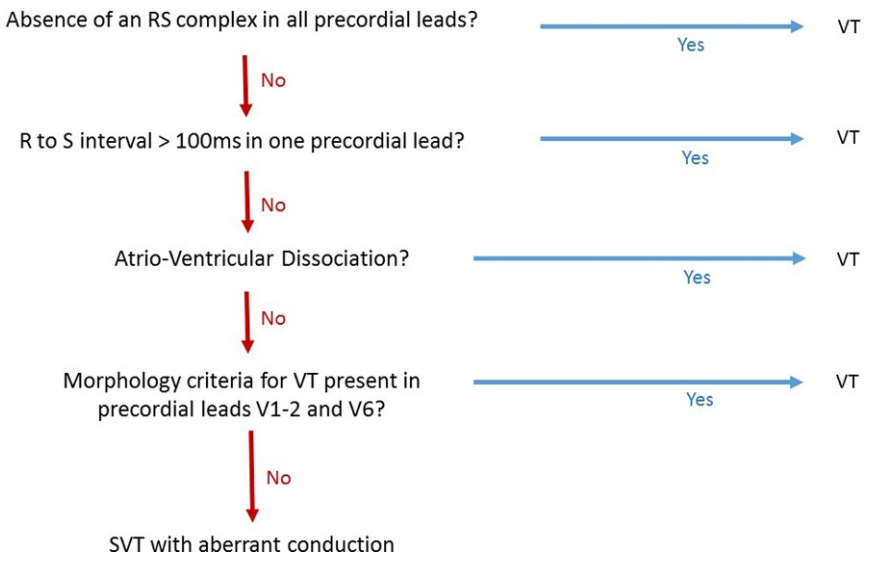

Fig. 5 Algorithm from Brugada et al. for diagnosis of a tachycardia with a widened QRS complex. When an RS complex cannot be identified in any precordial lead, the diagnosis of ventricular tachycardia (VT) is made. If an RS complex is present in one or more precordial leads, the longest RS interval is measured. If the RS interval is longer than 100 msec, the diagnosis of VT is made. If shorter than 100 msec, the next step of the algorithm is considered: whether atrioventricular dissociation is present. If present, the diagnosis of VT is made. If absent, the morphology criteria for VT are analyzed in leads $_{V,}$ and V6. If both leads fulfill the criteria for VT, the diagnosis of VT is made. If not, the diagnosis of supraventricular tachycardia (SVT) with aberrant conduction is made by exclusion of VT. From Brugada, P., Brugada, J., Mont, L., Smeets, J. and Andries, E. W. (1991). A new approach to the differential diagnosis of a regular tachycardia with a wide QRS complex. *Circulation* **83**, 1649–1659.

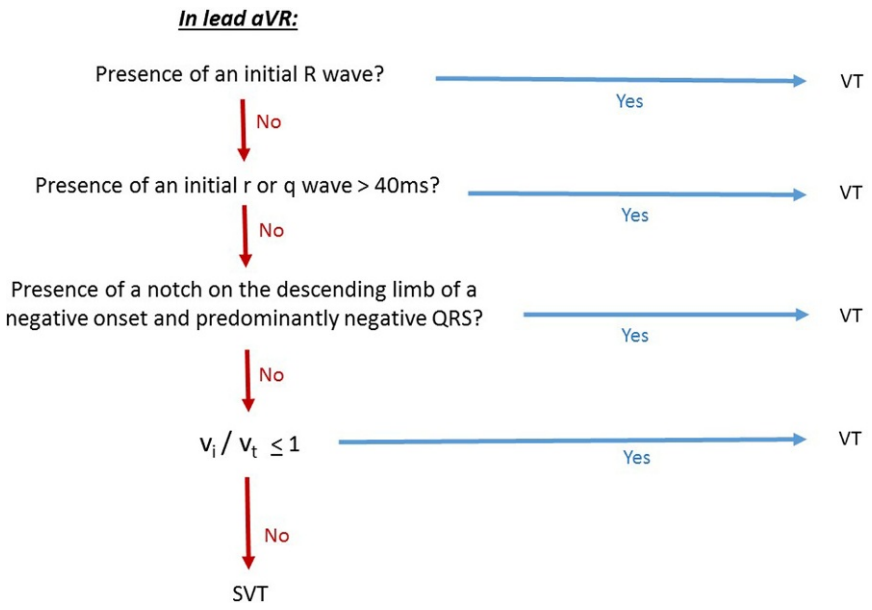

Fig. 6 Algorithm developed by Vereckei et al. for diagnosis of VT from a wide complex tachycardia using aVR. If an initial R wave is seen then VT is diagnosed. If not then presence of r or q wave >40 ms diagnoses VT. If not presence of a notch on the descending limb of a negative onset and predominantly negative QRS suggests VT. Otherwise $v_i/v_t < 1$ suggests VT. From Vereckei, A., Duray, G., Szenasi, G., Altemose, G.T., and Miller, J. M. (2008). New algorithm using only lead aVR for differential diagnosis of wide QRS complex tachycardia. *Heart Rhythm* **5**, 89–98.

reentrant VT. In patients without structural heart disease, using the ECG for localization is reasonably accurate. In patients with structural heart disease, the ECG morphology identifies the zone of epicardial breakthrough, which may be distant from the site of origin when intraventricular conduction is disturbed. VT that originates from the right ventricle or the anterior portion of the interventricular septum usually has a left bundle branch block like morphology in lead V1. VT originating from the left ventricle tends to have a right bundle branch block like morphology in lead V1. VT that presents QRS complexes that are negative in all of the precordial leads (negative concordance) is likely originating from near the apex; VT that presents QRS complexes that are positive in all of the precordial leads (positive concordance) is likely originating from the posterior portion of the left ventricle (Wellens, 2001).

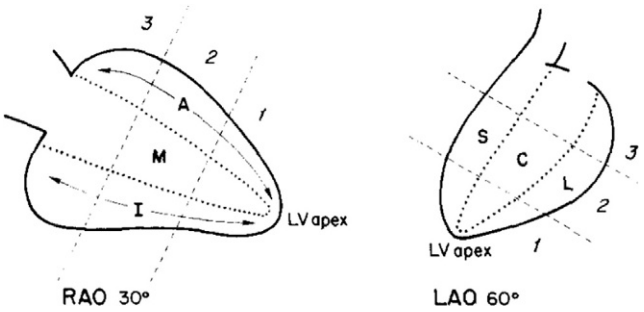

Fig. 7 Left ventricular endocardial mapping grid. The grid is divided into three sections in each of three planes, determined from two fixed fluoroscopic planes. *A*, anterior; *C*, central; *I*, inferior; *L*, lateral; *LAO*, left anterior oblique projection; *M*, middle; *RAO*, right anterior oblique projection; *S*, septal; *1,2,3*, three regions along the long axis of the left ventricle (LV): apical, mid-ventricular and basal. From Kuchar, D. L., Ruskin, J. N. and Garan, H. (1989). Electrocardiographic localization of the site of origin of ventricular tachycardia in patients with prior myocardial infarction. *Journal of the American College of Cardiology* **13**, 893–900.

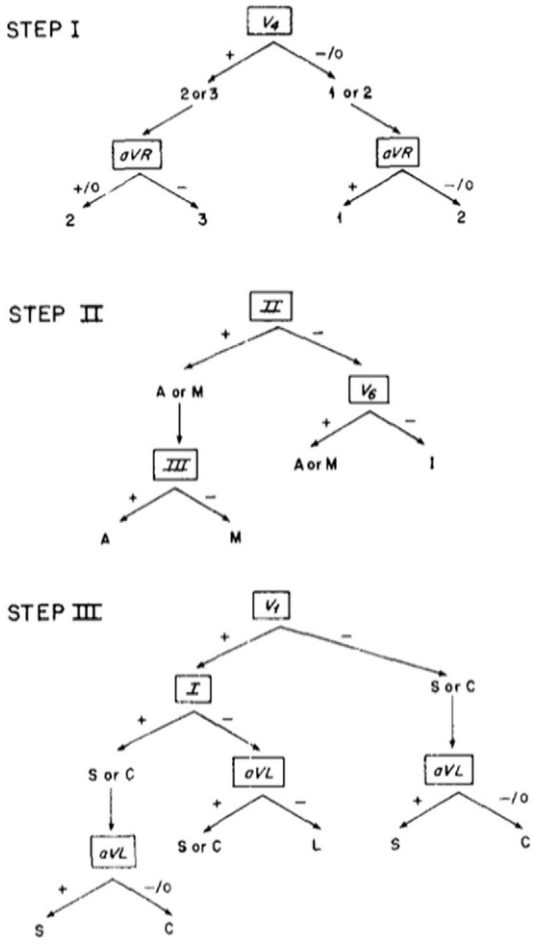

Fig. 8 Flow chart used for predicting site of origin of ventricular tachycardia: a three-step flowchart for predicting each of the three axis coordinates. To determine these sites, begin with the designated electrocardiographic lead and, depending on the QRS morphology in that lead, follow the arrows to the bottom of the diagram. The electrocardiographic leads are enclosed by boxes, whereas endocardial sites are indicated in bold type. Abbreviations as in **Fig. 6**. From From Kuchar, D. L., Ruskin, J. N. and Garan, H. (1989). Electrocardiographic localization of the site of origin of ventricular tachycardia in patients with prior myocardial infarction. *Journal of the American College of Cardiology* **13**, 893–900.

VT with an Inferior axis in the frontal plane suggests an origin in the right or left ventricular outflow tract; VT with a superior axis suggests an origin in the inferior wall.

Kuchar et al. developed an algorithm using the QRS morphology resulting from endocardial pacing (Kuchar et al., 1989) as shown in Figs. 7 and 8. When this algorithm was tested in patients with VT after remote MI the correct location of VT origin was predicted in 39% of patients and an adjacent location of VT origin was predicted in an additional 36% of patients.

An epicardial origin of VT is suggested by a pseudo delta wave (measured from earliest ventricular activation to earliest fast deflection in any precordial lead) with a duration greater than or equal to 34 ms, by an RS complex duration (measured from earliest ventricular activation to nadir of first S wave in any precordial lead) >121 ms, or by an intrinsicoid deflection time (earliest ventricular activation to peak of R wave in V2) greater than or equal to 85 ms (Berruezo et al., 2004). Not infrequently, an epicardial origin of VT is surmised after failed endocardial VT mapping and/or ablation.

Some patients, particularly those with advanced structural heart disease, will already have an ICD when their first VT occurs. In such patients, an ECG documenting the VT will be difficult to obtain and use of device recorded electrograms will be necessary to diagnose VT (Hook et al., 1993).

Investigations

When a patient has been diagnosed as having VT initial investigations are focused on identification of the etiology of and the magnitude of the patient's underlying structural heart disease. These investigations will be guided by the patient's demographics, family history, cardiac risk factors, history, and clinical examination. If no evidence of structural heart disease is found, the VT is likely an idiopathic VT or the result of an inherited channelopathy.

When structural heart disease is documented, it is usually the result of ischemic heart disease or a nonischemic congestive cardiomyopathy. Monomorphic VT in these settings usually originates from a preexisting ventricular myocardial scar. Polymorphic VTs in such patients usually indicate myocardial ischemia and/or decompensated congestive heart failure (Priori et al., 2015). Cardiac enzymes may be elevated in a patient with VT due to a prolonged episode of VT resulting in myocardial supply and demand mismatch, due to resuscitation, and/or due to defibrillator shocks. Accordingly, elevations of cardiac enzymes must be interpreted with caution in a patient presenting with VT.

Patients presenting with VT will usually start their investigations with laboratory investigations and with a transthoracic echocardiogram (Roberts-Thomson et al., 2011). Laboratory investigations include evaluations of electrolytes, renal function, blood counts, liver function, and thyroid function. Abnormalities in these tests are rarely the principle cause for monomorphic VT, but will inform subsequent treatment, particularly with antiarrhythmic drugs. Abnormalities on the transthoracic echocardiogram will prompt further testing which will usually take the form of cardiac catheterization and coronary angiography and cardiac magnetic resonance imaging for infiltrative disorders. If a nonischemic congestive cardiomyopathy is suspected, a workup for cause of such a cardiomyopathy is indicated. The details of this workup will depend on both patient and environmental characteristics and will consider Chagas disease in South America and cardiac sarcoidosis in the presence of coexisting conduction system disease. Arrhythmogenic right ventricular cardiomyopathy is considered when testing reveals no clear left ventricular structural heart disease especially when the VT had a left bundle branch block like morphology (Zipes et al., 2006). Signal-averaged electrocardiography and endomyocardial biopsy may also be necessary in selected patients (Roberts-Thomson et al., 2011).

Patients presenting with VT who already have received an ICD will likely have already had all the required testing performed. Nevertheless, such patients usually have laboratory tests and a transthoracic echocardiogram repeated to assess for contributing electrolyte or metabolic factors and to reassess their structural heart disease, particularly their left ventricular systolic function.

Conclusion

Ventricular tachycardia can portend a poor prognosis in patients with structural heart disease. The mechanism for VT in such patients is usually reentry. Symptoms such as palpitations or syncope should raise suspicion of VT and further investigation and monitoring may need to be performed. Developing technologies include smart phone apps, implantable loop recorders, other ambulatory monitoring devices that will facilitate the diagnosis of VT in patients with structural heart disease, and developments in imaging that may also aid in diagnosis and risk stratification.

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Ventricular Tachycardia in Structurally Normal Hearts

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Introduction

The clinical spectrum of ventricular tachycardia (VT) in structurally normal hearts, "idiopathic VT," ranges from isolated premature ventricular complexes (PVCs) to sustained VT, and in general portends a benign prognosis. The prognosis is unclear at initial presentation as the arrhythmias are indistinguishable from VT with structural heart disease (SHD) or a reversible cardiomyopathy may be present.

The pathophysiology, epidemiology, prognosis, and treatment of VT with SHD have been extensively characterized with well-developed guidelines for risk stratification and treatment that are generalizable to large populations. On the other hand, idiopathic VT has not been systematically assessed with large population-based studies due to low prevalence.

Elucidating the pathophysiology and developing treatment strategies is confounded by the fact that the definition of a structurally normal or abnormal heart is not rigorously defined. The connotation of this classification differs in among subspecialties of cardiovascular medicine. The absence of obvious ischemic or nonischemic cardiomyopathy implies a structurally normal heart but relies on conventional angiographic and echocardiographic assessment. However, this does not account for microvascular, ultrastructural (i.e., fibrosis or inflammation), molecular, or genetic abnormalities that have become increasingly recognized with advanced imaging techniques, biomarkers, and genetic testing.

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The prevailing clinical gestalt suggests that idiopathic VT is benign; however, numerous epidemiologic studies over the previous 50 years have demonstrated that ambient ventricular ectopy is associated with heightened risk of cardiovascular morbidity and mortality and may represent subclinical disease not discernible with conventional assessment.

There are several recognized types of idiopathic VT that have been classified and categorized based on clinical presentation, site of origin, electrocardiographic pattern, response to medication testing, and mechanism. Pharmacologic and ablative treatment strategies targeting ventricular ectopy with or without SHD suggest clinical benefit and also suggests that ventricular ectopy is a modifiable risk factor for cardiovascular morbidity and mortality.

Epidemiology

In 1962 the incidence of PVCs or nonsustained ventricular tachycardia (NSVT) on screening electrocardiograms (ECGs) among apparently healthy United States Air Force flight personnel was estimated at 0.8% (Hiss and LAMB, 1962). Large population studies, such as the Framingham Heart Study (Bikkina et al., 1992) and Atherosclerosis Risk in Communities (Simpson et al., 2002) study, estimate that the prevalence of PVCs or complex ventricular ectopy ranges from 6% to 12% based on ECG. The definition of complex ventricular ectopy includes more than 30 PVCs per hour or multiform PVCs, ventricular couplets, VT, or R-on-T phenomenon (Bikkina et al., 1992). PVC prevalence increases with advancing age, male gender, African-American ethnicity, and hypertension (HTN); HTN is associated with a 23% increase in the prevalence of PVCs (Simpson et al., 2002).

Ambient ventricular ectopy was found in 50% of healthy medical students on 24-hour ambulatory monitoring but only 2% had greater than 50 PVCs (Brodsky et al., 1977). Multiple other studies with 24-h ambulatory monitoring between 1977 and 1982 demonstrated the prevalence of PVCs or complex ventricular ectopy ranging from 40% to 75% (Kennedy et al., 1985). Hingorani et al. recently demonstrated the incidence of PVCs and complex ventricular ectopy as 43% and 16%, respectively, on 24-hour ambulatory monitoring in healthy drug-free participants of phase I clinical trials (Hingorani et al., 2016).

Exercise-induced PVCs has been observed in 2%–27% of healthy patients undergoing exercise stress testing in an assessment of Parisian civil service employees and the Framingham Offspring Study (Jouven et al., 2000; Morshedi-Meibodi et al., 2004). Exercise-induced NSVT occurred in 4% of the Baltimore Longitudinal Study of Aging.

PVCs are commonly encountered in clinical practice; however, the incidence of idiopathic PVCs may be lower since these studies did not fully exclude the presence of SHD.

Prevalence Among Treated VT

Early case series of patients referred for management and treatment of VT estimated the prevalence of idiopathic VT in the range of 7%–38% (Hoffmayer and Gerstenfeld, 2013). More contemporary estimates suggest a prevalence of 10% in the United States (Lerman et al., 1997) and 20% in Japan (Okumura and Tsuchiya, 2002). This may underestimate the true prevalence as the generally benign prognosis of idiopathic VT may limit referral for electrophysiologic evaluation.

Gender Differences

There are well-recognized gender differences in the prevalence and presentation of arrhythmias owing to gender-related variation of ion channels (genetic or molecular), autonomic tone, and modulation of myocardial electrophysiologic properties with sex hormones. The anatomic distribution and presentation differs between men and women.

Idiopathic VT arising from the right ventricular outflow tract (RVOT) has a 1.5–2:1 female predominance, left ventricular outflow tract (LVOT) VT 1.4:1 male predominance, and fascicular VT 3:1 male predominance (Nakagawa et al., 2002; Tanaka et al., 2011).

Marchlinski et al. observed that cyclic and age-related hormonal variation due to menses, pregnancy, and menopause triggers VT from the RVOT (RVOT-VT) in women, whereas exercise or stress more commonly triggers RVOT-VT in men (Marchlinski et al., 2000). Ventricular arrhythmias triggered during pregnancy subside postpartum (M. Nakagawa et al., 2004). Ovulatory peak estradiol and hormone replacement therapy (HRT) both attenuate PVCs (DOGAN et al., 2016; Hu et al., 2011).

Age Distribution

Multiple population studies, as mentioned above, demonstrated that the prevalence of PVCs increase with age (Bikkina et al., 1992; 1993; Hiss and LAMB, 1962; Simpson et al., 2002) (**Fig. 1**).

RVOT-VT frequently occurs between the age of 30 and 50 (M. Nakagawa et al., 2002), whereas VT arising from the LVOT (LVOT-VT) increases with age and frequently occurs between the age of 50 and 70 (Tanaka et al., 2011) (**Fig. 2**). Fascicular VT frequently occurs between the age of 20 and 40 with male predominance; however, women present at an earlier age(M. Nakagawa et al., 2002; Tanaka et al., 2011).

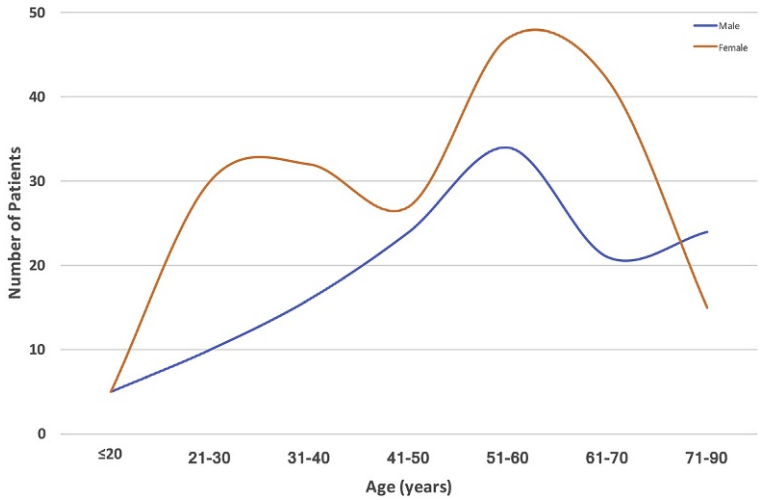

Fig. 1 Age distribution of outflow tract ventricular arrhythmias based on gender. Woman have bimodal age distribution with peaks at 20–30 and 50–60 years of age whereas for men the prevalence linearly increase with age (Tanaka et al., 2011).

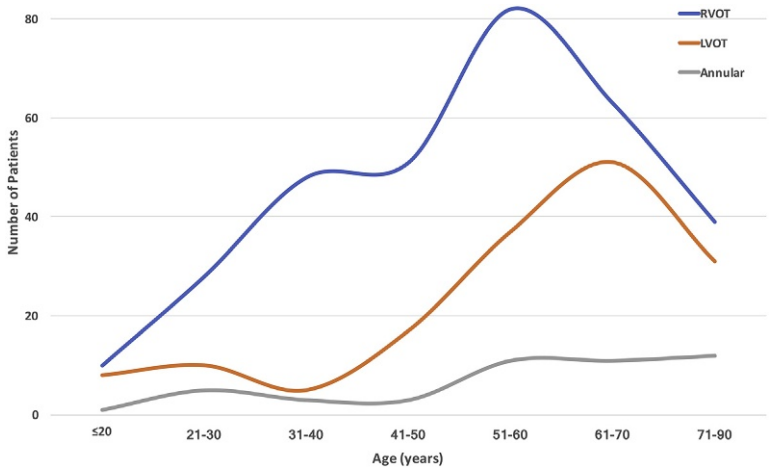

Fig. 2 Age distribution of the most common idiopathic ventricular arrhythmias. Right ventricular outflow tract (RVOT) ventricular arrhythmias are most common and present at an earlier age than either left ventricular outflow tract (LVOT) or annular ventricular arrhythmias (Tanaka et al., 2011).

Prognostic Implications

Initial studies attempted to define the prognostic significance of ventricular ectopy following myocardial infarction (MI) and determined that complex ventricular ectopy after MI increased mortality by a factor of 3 but antiarrhythmic drugs (AADs) worsened outcomes (Kotler et al., 1973; Ruberman et al., 1977).

Multiple population studies demonstrated that PVCs without evidence of cardiac disease similarly increased cardiovascular morbidity and mortality:

- Multiple Risk Factor Intervention Trial found that PVCs conferred a threefold increased risk of sudden cardiac death (SCD) with the effect more pronounced with complex ventricular ectopy (Abdalla et al., 1987).
- Framingham Heart Study found that complex ventricular ectopy without coronary artery disease (CAD) increased MI and mortality in men but not in women (Bikkina et al., 1992). In the same cohort, complex ventricular ectopy with underlying left ventricular hypertrophy (LVH) increased mortality (Bikkina et al., 1993).
- Cardiovascular Health Study found the highest quartile of PVC burden was associated with a 3-fold increase of LV dysfunction over 5 years, 48% increased risk of congestive heart failure (CHF), and 31% increased risk of death (Dukes et al., 2015) (**Fig. 3**).
- Lin et al. demonstrated that PVCs and NSVT conferred a higher risk of mortality and incident CHF while identifying both as independent risk factors for transient ischemic attack and atrial fibrillation (AF) (Lin et al., 2015, 2016).

Exercise-induced PVCs in the absence of SHD is associated with an increased risk of death (Jouven et al., 2000; Morshedi-Meibodi et al., 2004), however, exercise-induced NSVT does not independently increase mortality (Marine et al., 2013) (**Fig. 4**).

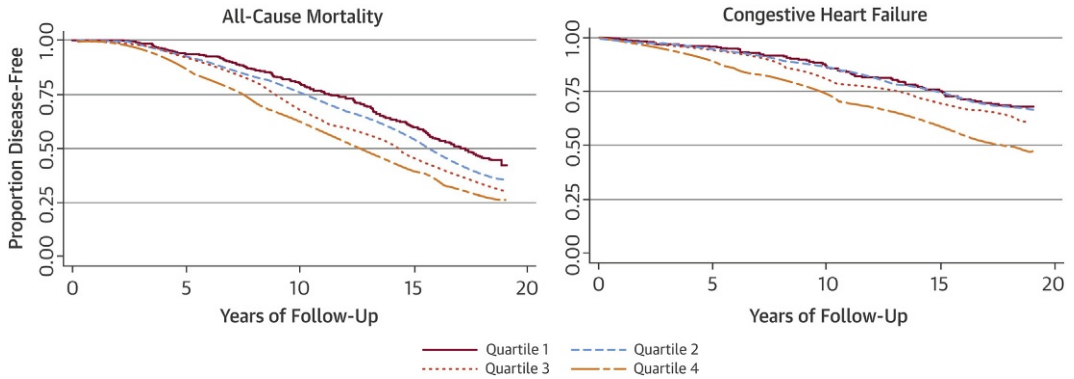

Fig. 3 The population-based risk of disease-free survival from all-cause mortality or incident congestive heart failure (CHF) associated with burden of premature ventricular complexes stratified by quartiles. Quartiles 1–4 represent PVC burden of 0–0.002%, 0.002–0.011%, 0.011–0.123%, and 0.123–17.7%. There is a gradient of risk for all-cause mortality across all four quartiles; however, the risk of CHF similar amongst quartiles 1–3 and lowest with quartile 4.

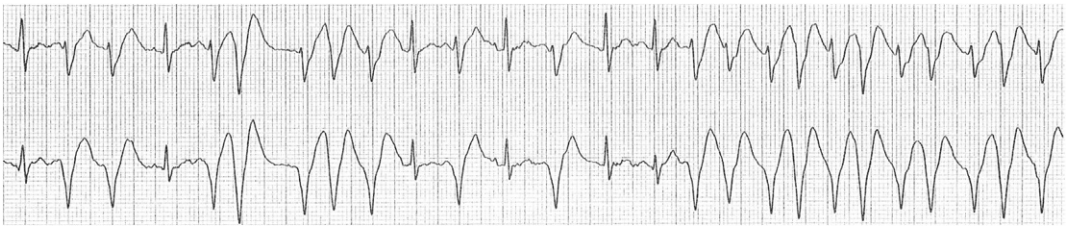

Fig. 4 Exercise-induced idiopathic ventricular tachycardia during exercise tolerance testing in a 26-year old with a structurally normal heart based performed due to exercise-induced chest discomfort and dizziness.

Clinical Presentation

Symptoms

The most frequent reported clinical symptoms in patients presenting for ablation are palpitations (80%), chest pain (26%), and syncope (24%) (Kim et al., 2007). Exercise- or stress-triggered palpitations are more frequent with sustained monomorphic VT (53%) when compared to NSVT (30%) or PVCs (18%). Other common symptoms include presyncope, dyspnea, fatigue, exercise intolerance, cough, or dysphagia (Laplante and Benzaquen, 2016).

A significant proportion are asymptomatic and diagnosed incidentally when an irregular or slow pulse is noted or an ECG is performed for other reasons.

Triggers

The most common triggers for idiopathic VT are exercise, emotional stress, and stimulants (Table 1). Other triggers include hormonal variation, as discussed above, medications, and metabolic abnormalities.

Circadian variation in autonomic tone results in peak sympathetic tone in the morning, which coincides with the observed early morning risk of VT and SCD. Idiopathic PVCs follow the same circadian pattern (Hayashi et al., 1999). These observations implicate sympathetic tone as a trigger or promoter of ventricular arrhythmias and provide mechanistic insight for the common triggers. A small proportion of patients are paradoxically observed to have PVCs intensify during periods of heightened vagal tone (rest or sleep) and attenuate with increase in sympathetic tone, which may portend a benign prognosis.

Arrhythmia Patterns

Idiopathic VT commonly manifests as frequent monomorphic or pleomorphic PVCs, salvos of NSVT also known as repetitive monomorphic VT (MMVT), and sustained VT (Fig. 5).

Kim et al. observed that sustained MMVT occurred in 28%, NSVT in 36%, and PVCs in 35% among patients with idiopathic VT presenting for ablation (Kim et al., 2007). Sustained VT was elicited during exercise testing in 67%, 10%, and 10% of patients presenting with sustained VT, NSVT, and PVCs, respectively. VT was induced during electrophysiologic testing in 78%, 48%, and 4% across the three groups.

Table 1 Common triggers for ventricular arrhythmias without structural heart disease

Clinical history
Physical activity
Exercise
Sexual intercourse
Anxiety
Toxic effects
Caffeine
Alcohol toxicity and withdrawal
Tobacco
Exogenous catecholamines (isoproterenol, dopamine, norepinephrine, etc.)
Drugs (i.e., digoxin, tricyclics, macrolides, fluoroquinolones, etc.
Illicit drugs (i.e., cocaine, cannabis, amphetamine, etc.)
Hormonal effects
Menses
Gestational
Perimenopausal
Hormone replacement therapy or oral contraceptives
Other
Hyperthyroidism
Pheochromocytoma
Hypoxemia
Acidemia
Ischemia
Occult structural heart disease



Fig. 5 Idiopathic ventricular arrhythmias may clinically present as occasional isolated premature ventricular complexes, ventricular bigeminy (A), salvos of repetitive monomorphic ventricular tachycardia (B), and sustained ventricular tachycardia (C).

Anatomic Substrate

Idiopathic VT classically refers to outflow tract or fascicular VT; however, other common endocardial or epicardial sites of origin include the mitral or tricuspid annulus, aortomitral continuity (AMC), and papillary muscles (Table 2).

Understanding the complex anatomy of these sites and the adjacent structures prone to collateral damage is critically important to optimize efficacy and safety of ablation. Real-time integration of multimodality imaging including fluoroscopy, cardiac computed tomography (CCT), cardiac magnetic resonance (CMR) imaging, electroanatomic mapping, and ultrasound further facilitates understanding of the anatomic relationships and procedural outcomes.

RVOT-VT accounts for 50%–80% and LVOT-VT for 15%–25% of all idiopathic VTs (Callans et al., 1997; Kim et al., 2007; Tanaka et al., 2011). The posterior aspect of the RVOT is the most common site for RVOT-VT; LVOT-VT commonly arises from aortic root or coronary cusps (Yamada, 2016).

RVOT or infundibulum is a superiorly located conical structure, extending leftward from the tricuspid annulus to the pulmonic annulus, anteriorly overriding the LVOT (Fig. 6). The distal extent at the pulmonic annulus is superior, leftward, and orthogonal to

Table 2 Anatomic classification of idiopathic ventricular arrhythmias

Outflow tract ventricular arrhythmias
Right ventricular outflow tract
Left ventricular outflow tract
Sinuses of Valsalva or coronary cusps
Fascicular ventricular arrhythmias
Left posterior fascicle
Left anterior fascicle
Septal fascicle
Annular ventricular arrhythmias
Mitral annulus
Aortomitral continuity
Tricuspid annulus
Other ventricular arrhythmias
Epicardial (LV crux and summit)
Papillary muscle
Moderator band

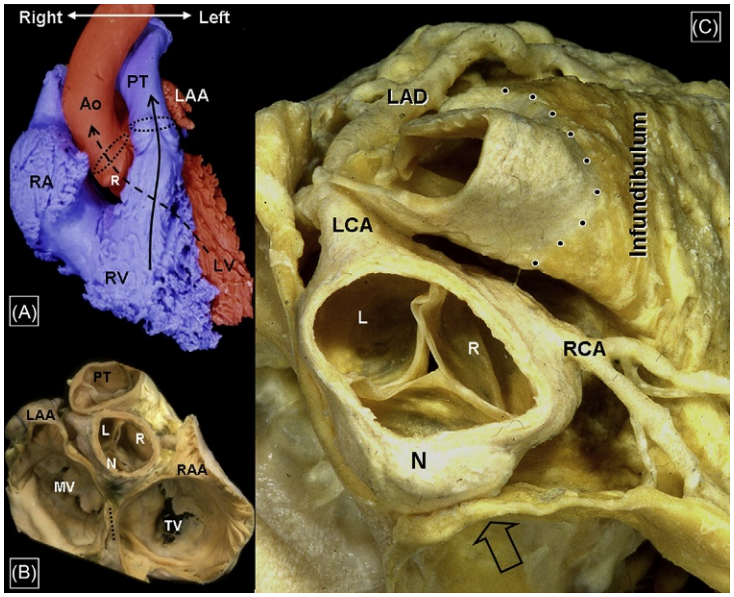

Fig. 6 Anatomic relationship of the right ventricular outflow tract (RVOT) to the left ventricular outflow tract (LVOT): (A) An endocardial cast of the right ventricle (RV) and left ventricle (LV) displayed in an anteroposterior projection. The dotted circles represent the annuli of the aortic and pulmonary valves. RVOT anteriorly overrides the LVOT from right to left. (B) The aortic valve is a central structure that is located anterior and superior to the mitral valve (MV) and tricuspid valve (TV) but posterior to the pulmonary trunk (PT). The dotted line represents the interatrial septum. The non-coronary cusp (N) is posterior and adjacent to the atria and the left (L) and right (R) coronary cusps. (C) The dotted line delineates the junction between the RV and the pulmonary artery; the pulmonic valve is orthogonal and superiorly located to the aortic valve. The septal and posterior RVOT are in proximity to the left main (LCA) coronary artery whereas the right coronary artery (RCA) is separated by more distance and epicardial fat. From Ho, S.Y., 2009. Anatomic insights for catheter ablation of ventricular tachycardia. *Heart Rhythm: The Official Journal of the Heart Rhythm Society* 6(8 Suppl), S77–80, with permission.

the aortic valve (Asirvatham, 2009; Lerman, 2015). The proximal and rightward extent at the level of the tricuspid annulus is adjacent to the His bundle and noncoronary cusp (NCC) of the aortic valve. RVOT is subdivided into septal (posterior and leftward) and free wall (anterior and rightward) segments with the posterior RVOT immediately anterior to the right (RCC) and left (LCC) coronary cusps of the aortic valve and in proximity to the left main coronary artery (LMCA) (**Fig. 7**). The anterior free wall is generally thinner and at increased risk of perforation than posterior or proximal segments. Myocardial sleeves extending up to and beyond the pulmonary artery or sinuses of Valsalva are implicated as the source of automaticity or triggered activity (Gami et al., 2011; Liu et al., 2014) (**Fig. 8**). RVOT and LVOT are intimately involved structures derived from a common embryologic outflow tract with slow conduction properties before incorporating into the embryologic RV and developing rapid conduction properties;

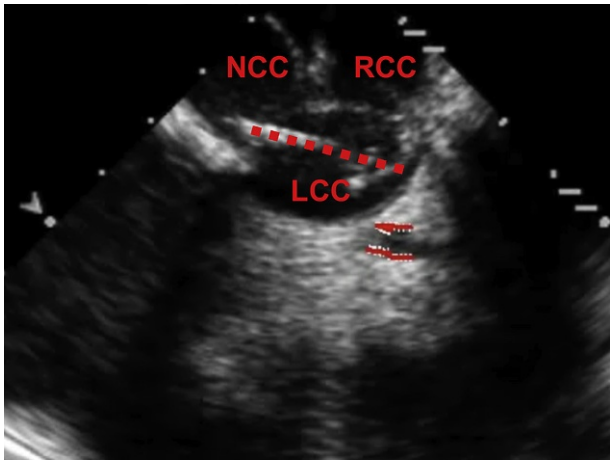

Fig. 7 Intracardiac echocardiography (ICE) of the sinus of Valsalva delineating the non-coronary cusp (NCC), right coronary cusp (RCC), and left coronary cusp (LCC). The two solid lines delineate the course of the left main coronary artery from the LCC and the dotted red line represents a mapping and ablation catheter positioned at the site of earliest ventricular activation in the left coronary cusp.

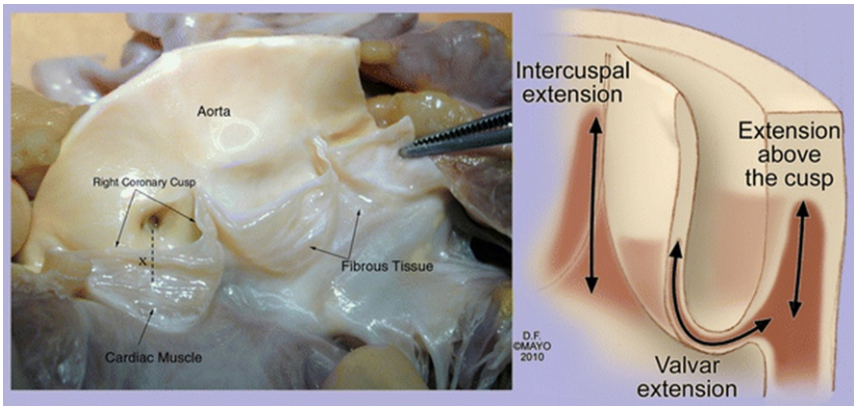

Fig. 8 A longitudinally dissected aortic annulus revealing the cusps of the aortic valve with visible myocardium extending to the right cusp. The adjacent illustration the extension of myocardial tissue above and into the cusps. From Gami, A.S. et al., 2011. Anatomical correlates relevant to ablation above the semilunar valves for the cardiac electrophysiologist: a study of 603 hearts. *Journal of Interventional Cardiac Electrophysiology: An International Journal of Arrhythmias and Pacing* 30(1) 5–15, with permission.

persistent remnants of slow conducting tissue may represent the substrate for arrhythmia, the myocardial sleeves (Boukens et al., 2009).

Fascicular VT accounts for approximately 10%–15% and originates from the left-sided Purkinje network including the left posterior (~90%), left anterior, and septal fascicles. The left anterior fascicle traverses the anterior mitral annulus and arborizes to the anterolateral wall and anterolateral papillary muscle. The left posterior fascicle extends from the His bundle through the inferoseptum and arborizes to the inferolateral wall and posteromedial papillary muscle; a septal fascicle is present in approximately 65%.

VT arising from the mitral or tricuspid annulus accounts for approximately 10% of idiopathic VT (Tanaka et al., 2011). Mitral annular VT most commonly originates from the anterolateral followed by the posteromedial aspects (**Figs. 9** and **10**). PVCs may arise from the confluence of the subaortic apparatus and anteromedial aspect of the mitral annulus below the left and NCC, referred as the AMC (**Fig. 11**). Tricuspid annular VT more commonly originates from the anteroseptal region compared to the free wall (Yamada, 2016).

Idiopathic VT of epicardial origin typically arises from the crux or LV summit, basal structures in proximity to the coronary venous and arterial systems. The crux is the confluence of the atrioventricular and posterior interventricular grooves near the posterior descending artery and middle cardiac vein. The LV summit region is the triangular space delineated by the bifurcation of the LMCA and the great cardiac vein. A significant layer of epicardial fat and risk of coronary artery damage limits mapping and ablation in this region.

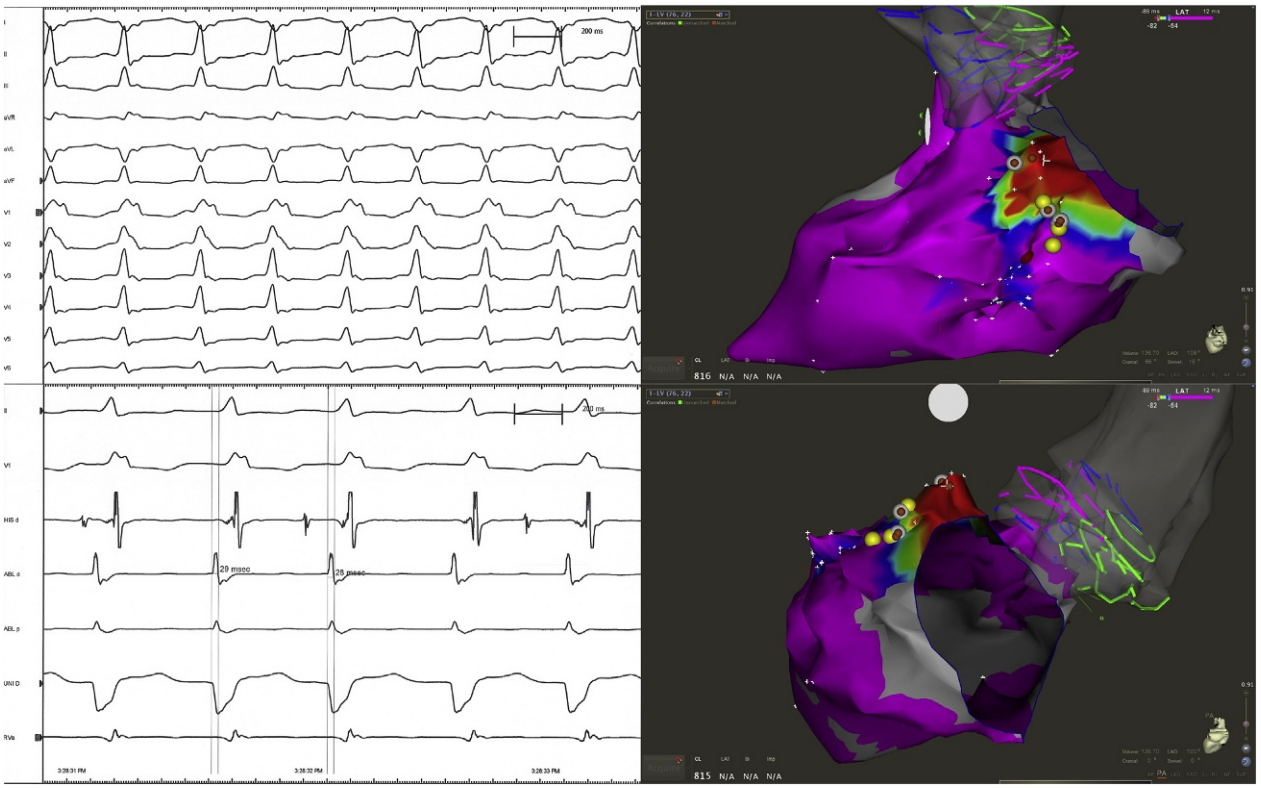

Fig. 9 Sustained monomorphic ventricular tachycardia in a patient with a structurally normal heart with right bundle branch block (RBBB) morphology with inferior and rightward axis. Electroanatomic mapping revealed that the anterolateral mitral annulus was the site of earliest activation with excellent bipolar and unipolar presystolic electrograms where the tachycardia was successfully ablated.

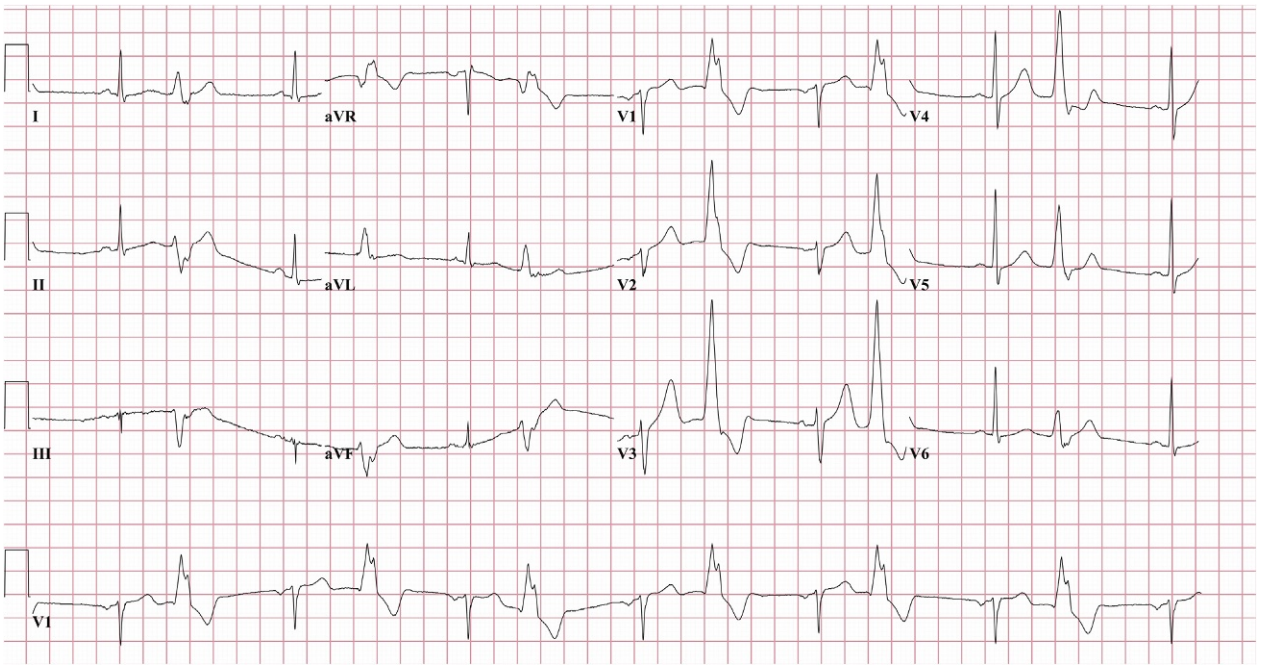

Fig. 10 Bigeminal premature ventricular complexes with right bundle branch block (RBBB) pattern, positive concordance, and superior axis that was mapped and successfully ablated at the inferior mitral annulus.

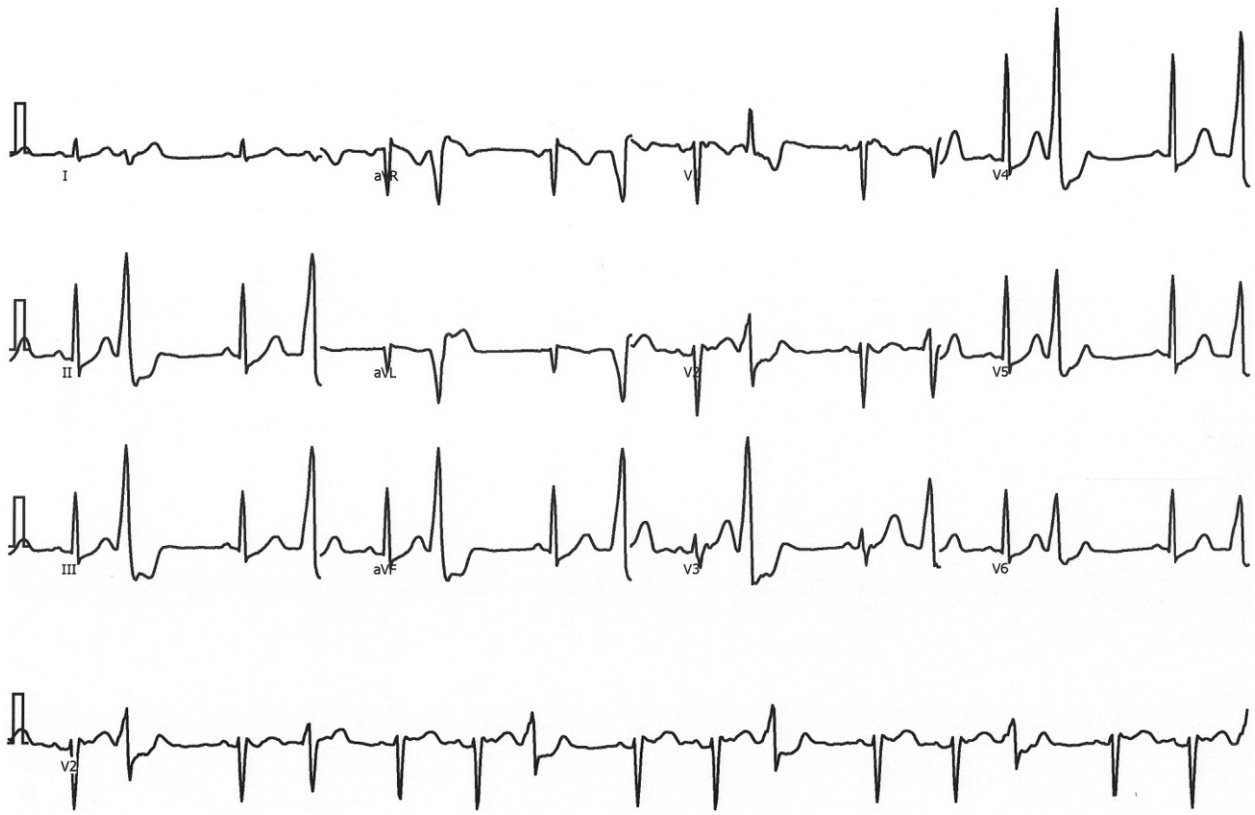

Fig. 11 Premature ventricular complexes with qR complex in lead V1 and Rs in lead I indicative of origin from the aortomitral continuity.

Malignant Consequences

Cardiomyopathy

Development of reversible PVC-induced cardiomyopathy and heart failure was first described by Duffee et al. (1998). The estimated incidence is 7%–8% among all patients with idiopathic VT (Hasdemir et al., 2011; Kim et al., 2007) with a male predominance (Yokokawa et al., 2012a). Differentiating between primary and secondary cardiomyopathies at presentation is difficult and empiric treatment is performed. The time course for developing LV dysfunction is variable with recovery typically in 4–6 months though up to 4 years was reported. Numerous factors including PVC burden, QRS duration (QRSd) and contour, interpolation, coupling interval, symptoms, and site of origin are associated with heightened risk and suggest a complex interplay of factors including an underlying genetic susceptibility.

Multiple studies demonstrated that PVC burden correlates with incidence and severity of LV dysfunction but without a clear threshold for risk. Kanei et al. observed a graded risk of LV dysfunction based on PVC burden: 4% with <1000, 12% with 1000–10,000, and 34% with >10,000 on 24-h ambulatory monitoring (Kanei et al., 2008) (**Fig. 12**). Dukes et al. similarly observed a graded risk of cardiomyopathy and heart failure with PVC burden stratified quartiles (Dukes et al., 2015). More than 20,000 PVCs in 24 h were associated with LV dysfunction (Niwano et al., 2009) but with less than 15%–20% there was neither difference in LV dysfunction nor improvement with ablation (Hasdemir et al., 2011; Takemoto et al., 2005). PVC burden of 24% is 79% sensitive and 78% specific for PVC-induced cardiomyopathy (Baman et al., 2010). In general, >10,000–20,000 or 20% PVCs in 24 h are considered clinically significant but only a minority is affected in the setting of persistent ventricular bigeminy, whereas reversible cardiomyopathy has been described with less frequent PVCs (Shanmugam et al., 2006). Circadian variability of PVCs impacts the long-term burden and less variability predicts cardiomyopathy independent of PVC burden (Bas et al., 2016). NSVT and pleomorphic PVCs increase the risk of cardiomyopathy (del Carpio Munoz et al., 2011; Kanei et al., 2008).

QRSd and contour predicts underlying heart failure and cardiomyopathy: PVCs with QRSd >160 ms and broad (>40 ms) notching are reliable markers of cardiomyopathy (Moulton et al., 1990). Yokokawa et al. established that PVC QRSd >150 predicts reversible cardiomyopathy and the required burden needed to develop cardiomyopathy was lower at this threshold (Yokokawa et al., 2012b). Further increases in QRSd predict irreversible cardiomyopathy (Deyell et al., 2012). Interestingly, fascicular PVCs are narrower but do not have a lower incidence of cardiomyopathy.

PVCs that do not alter the surrounding sinus rate (R-R interval) are interpolated (**Fig. 13**). Interpolation allows more sinus beats and PVCs due to the coupled relationship to the preceding sinus beat. One study suggested that interpolation independently

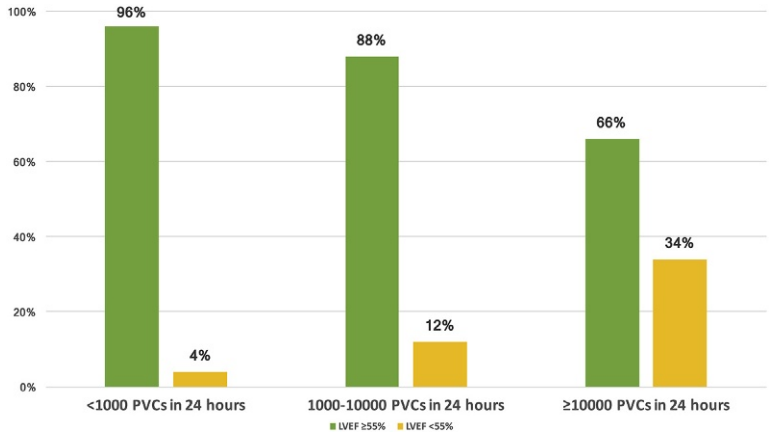

Fig. 12 Association of premature ventricular complex (PVC) frequency during 24-hour ambulatory monitoring with left ventricular systolic function. Adapted from Kanei et al., 2008. Frequent premature ventricular complexes originating from the right ventricular outflow tract are associated with left ventricular dysfunction. *Annals of Noninvasive Electrocardiology* 13: 81–85. https://doi.org/10.1111/j.1542-474X.2007.00204.x.

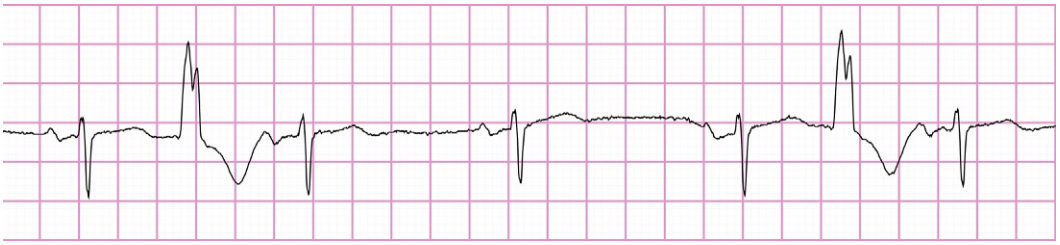

Fig. 13 Interpolated premature ventricular complexes (PVCs) do not alter the underlying sinus rate and are associated with a higher burden of PVCs. Interpolation has been suggested as a marker of risk for developing a PVC-induced cardiomyopathy independent of PVC burden.

predicted PVC induced cardiomyopathy (Olgun et al., 2011); however, other studies did not find relationship (Ban et al., 2013; Bas et al., 2016).

Duration or absence of symptoms correlates with the accumulative PVC burden and both are independently associated with PVC-induced cardiomyopathy (Blaye-Felice et al., 2016; Yokokawa et al., 2012a). Lack of symptoms is associated with a higher 24-hour PVC burden and is 3 times more likely with than without cardiomyopathy (Yokokawa et al., 2012a).

Initially, the mechanism was considered analogous to tachycardia-mediated cardiomyopathy prototypically due to AF but multiple studies demonstrated that heart rate remains unchanged regardless of PVC burden (Olgun et al., 2011; Takemoto et al., 2005). The putative mechanism is considered to be due to dyssynchronous ventricular activation similar to pacing-induced cardiomyopathy or manifest right-sided accessory pathways (Udink ten Cate et al., 2010). Though conceptually appealing, several clinical insights suggest greater complexity. First, LV dysfunction is not triggered by LV only pacing or with left-sided accessory pathways. Second, anatomic origin of PVCs should have differential effects if dyssynchrony was fully explicative; however, both right- and left-sided PVCs trigger cardiomyopathy. The rates of cardiomyopathy are independent of endocardial site of origin (del Carpio Munoz et al., 2011; Yokokawa et al., 2012b) but Del Carpio Munoz et al. observed that fascicular VT was associated with higher LVEF and the critical burden for developing cardiomyopathy was different: >10% for RVOT and >20% for LVOT (del Carpio Munoz et al., 2011). Potfay et al. with multisite pentageminal ventricular pacing demonstrated that markers of dyssynchrony are not associated with anatomic location but rather a longer coupling interval (Potfay et al., 2015). Neither burst ventricular pacing nor pentageminal atrial pacing produced the same degree of dyssynchrony and if causative likely relies on a complex interaction between site of origin and coupling interval. Multiple studies demonstrated that epicardial PVCs are associated with greater QRSd prolongation and PVC-induced cardiomyopathy (Blaye-Felice et al., 2016; Hamon et al., 2016; Yokokawa et al., 2012b).

PVC-induced cardiomyopathy develops after 12 weeks in a canine model of ventricular bigeminy and rapidly reverses in 2–4 weeks upon cessation of PVCs (Huizar et al., 2011). PVCs result in volumetric and electrical remodeling as is evident from developing of systolic dysfunction, dilation, and increased effective refractory period. Interestingly, there was no histopathologic evidence or change in inflammation, fibrosis, apoptosis, or levels of oxidative stress suggesting a functional cardiomyopathic process. The associated electrical remodeling was further characterized by Wang et al. who demonstrated that dispersion of action potential duration (APD) increased with wider beat-to-beat variation establishing a potentially proarrhythmic substrate (Wang et al., 2014). The densities of outward potassium (Ito, IK1) and inward calcium (L-type) currents decreased without change in other potassium currents (IKr or IKs). Furthermore, impairment of sarcoplasmic reticulum (SR) calcium-induced calcium release and

alterations in protein expression (juntophilin-2) responsible for maintaining the optimal interface between the cell membrane and SR may account for contractile dysfunction.

Malignant Arrhythmias

Focal triggers of ventricular fibrillation (VF) arising from the Purkinje network of the RVOT were described by Haïssaguerre et al. (2003) followed by rare case reports of polymorphic ventricular tachycardia (PMVT) or VF triggered by closely coupled RVOT PVCs with ablation mitigating the risk (Noda et al., 2005; Viskin et al., 2005) (**Fig. 14**). The aforementioned electrical remodeling facilitates arrhythmogenesis.

The coupling interval for triggering PVCs is longer than observed in idiopathic VF but shorter than benign PVCs; triggering coupling intervals are longer for MMVT than PMVT (Shimizu, 2009). MMVT with shorter cycle lengths are associated with a more malignant prognosis.

Clinical Evaluation

Idiopathic VT is a diagnosis of exclusion and the principle goal of clinical evaluation is to rule out SHD or heritable arrhythmia syndromes associated with increased risk of SCD or heart failure that persists despite treatment. Initial evaluation should include a comprehensive history and physical examination, 12-lead ECG, and echocardiogram.

History

The history establishes the severity, duration, frequency, and triggers of symptoms and impact on quality of life. High-risk features such as syncope or prior cardiac disease significantly modify the prognosis, diagnostic approach, and treatment. A careful review of symptoms to evaluate for signs or symptoms of angina or CHF as well as excluding secondary causes such as endocrine (thyroid, adrenal, or pituitary) and pulmonary disease is paramount.

Medications including caffeine, prescription drugs, herbal supplements, or illicit drugs need to be reviewed for potential electrophysiologic effects.

Family history should thoroughly evaluate for premature CAD, SCD among first-degree relatives, and heritable cardiomyopathies or electrical disorders.

Physical Examination

The physical examination is focused on evaluating the signs of CHF such as elevated jugular venous pressure, pulmonary rales, and pathologic murmurs or gallops suggestive of elevated filling pressures or valvular disease. Evaluation for other systemic manifestations of thyroid (hyperthyroidism), adrenal (pheochromocytoma), or pituitary (acromegaly) disorders as well as diseases with cardiac manifestations (i.e., muscular dystrophy, hemochromatosis, etc.,) is helpful.

There are numerous other obvious or subtle findings that may suggest occult cardiopulmonary disease such as xanthelasma, abnormal peripheral pulses or bruits, clubbing, psoriasis, or signs for rheumatoid arthritis.

Electrocardiogram

Twelve-lead ECG is generally specific but not sensitive for manifestations of SHD or primary electrical disorders such as q-waves, hypertrophy, conduction delay or block, Brugada pattern, T-wave abnormalities, Epsilon waves, preexcitation, and prolonged QTc. Morphologic assessment of ventricular ectopy allows anatomic localization and assessing complexity of ablation.

Outflow tract VT demonstrates left bundle branch block (LBBB) morphology and inferior axis (positive in lead II, III, and aVF) generally with QRSd $<$140 ms as the Purkinje system is penetrated and allows more rapid ventricular activation. Distinguishing between RVOT and LVOT origin depends on the precordial transition: RVOT-VT inscribe a QS complex in lead V1 and progressively more posterior sites of origin (posterior RVOT to LVOT to mitral annulus) progress from a qR complex then R or Rs complex



Fig. 14 Idiopathic premature ventricular complexes (PVCs) in a bigeminal pattern triggering polymorphic ventricular tachycardia.

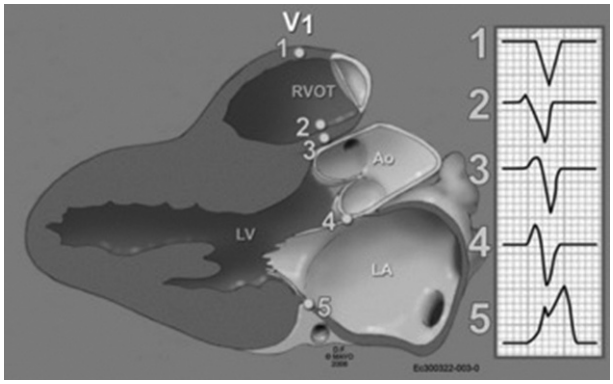

Fig. 15 Transition of V1 morphology from left bundle branch block to right bundle branch block morphology as the site of origin progressed posteriorly from the anterior right ventricular outflow tract to the lateral mitral annulus. From Asirvatham SJ (2009) Correlative anatomy for the invasive electrophysiologist: Outflow tract and supravalvar arrhythmia. *Journal of Cardiovascular Electrophysiology* 20: 955–968. https://doi.org/10.1111/j.1540-8167.2009.01472.x, with permission.

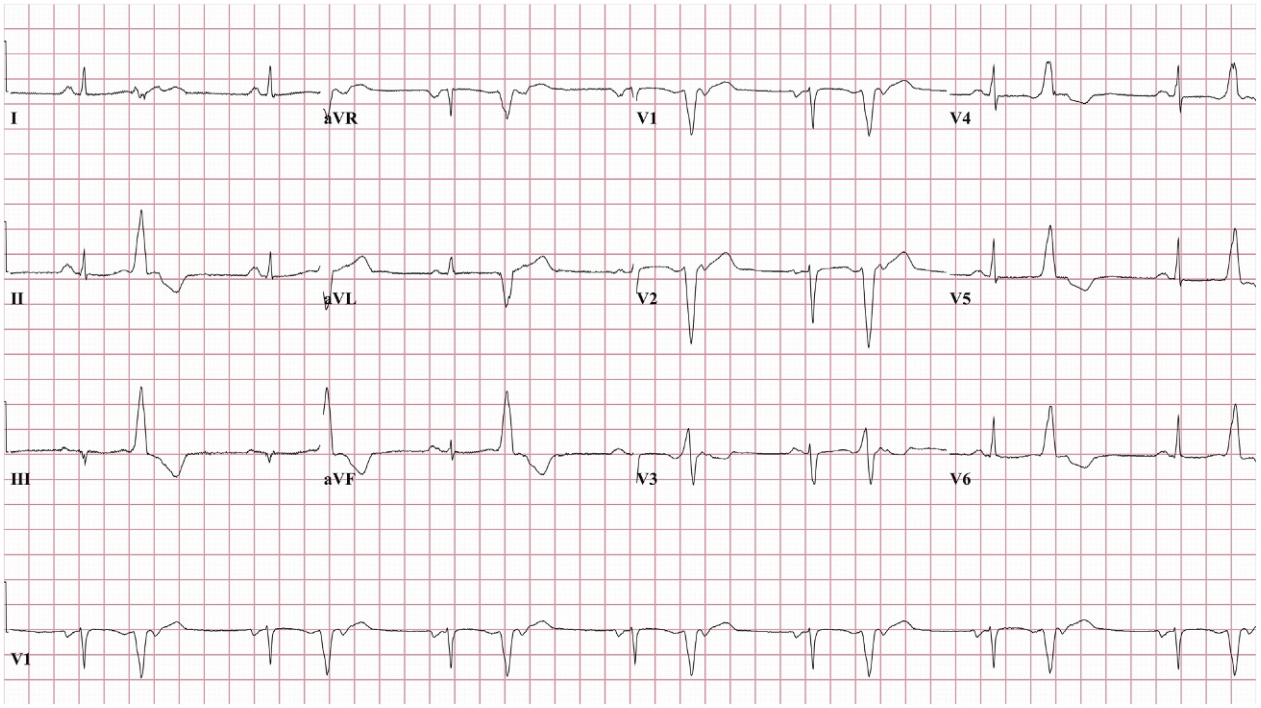

Fig. 16 12-lead electrocardiogram of premature ventricular complexes with left bundle branch block (LBBB) morphology, left and inferior axis, QRSd <140 msec, and precordial transition between leads V3 and V4 suggesting origin from the right ventricular outflow tract (RVOT).

(Jadonath et al., 1995); QRS morphology transitions to a right bundle branch block (RBBB) morphology at the lateral mitral annulus (**Fig. 15**). Accordingly, RVOT-VT typically transitions at or beyond lead V3, mirroring the sinus beat transition; transition earlier than V3 or sinus beats suggests LVOT-VT (Betensky et al., 2011; Callans et al., 1997) (**Figs. 16** and **17**). QS complex in lead aVL and QS or qR in lead I suggest anterior or septal RVOT origin; R or Rs complex in lead I and QS or R in lead aVL suggest posterior RVOT origin (Jadonath et al., 1995). QRSd, presence of fragmentation or notching, and transition differentiate between septal or free wall RVOT-VT: notching, increased QRSd, and delayed transition suggest free wall origin (Dixit et al., 2003).

Multiple electrocardiographic algorithms or signs facilitate localization of VT important for counseling patients regarding the complexity and risk of ablation (Dixit et al., 2003; Jadonath et al., 1995; Kamakura et al., 1998; Ouyang et al., 2002b) (**Table 3**). However, the algorithms are unable to account for variation in anteroposterior chest diameter, cardiac rotation, and lead placement. Betensky et al proposed the V2 transition ratio that reliably distinguishes between RVOT and LVOT origin by comparing the ratio of R to QRS amplitude during VT to sinus, self-controlling for patient-specific variation (Betensky et al., 2011) (**Fig. 18**).

Epicardial VT arises from perivascular structures and depolarization slowly progresses centrifugally from myocyte to myocyte without penetrating the Purkinje network. This manifests as slow or delayed initial forces of the QRS complex (pseudodelta wave). Several different electrocardiographic markers of epicardial origin have been evaluated of which a maximum deflection index (MDI)

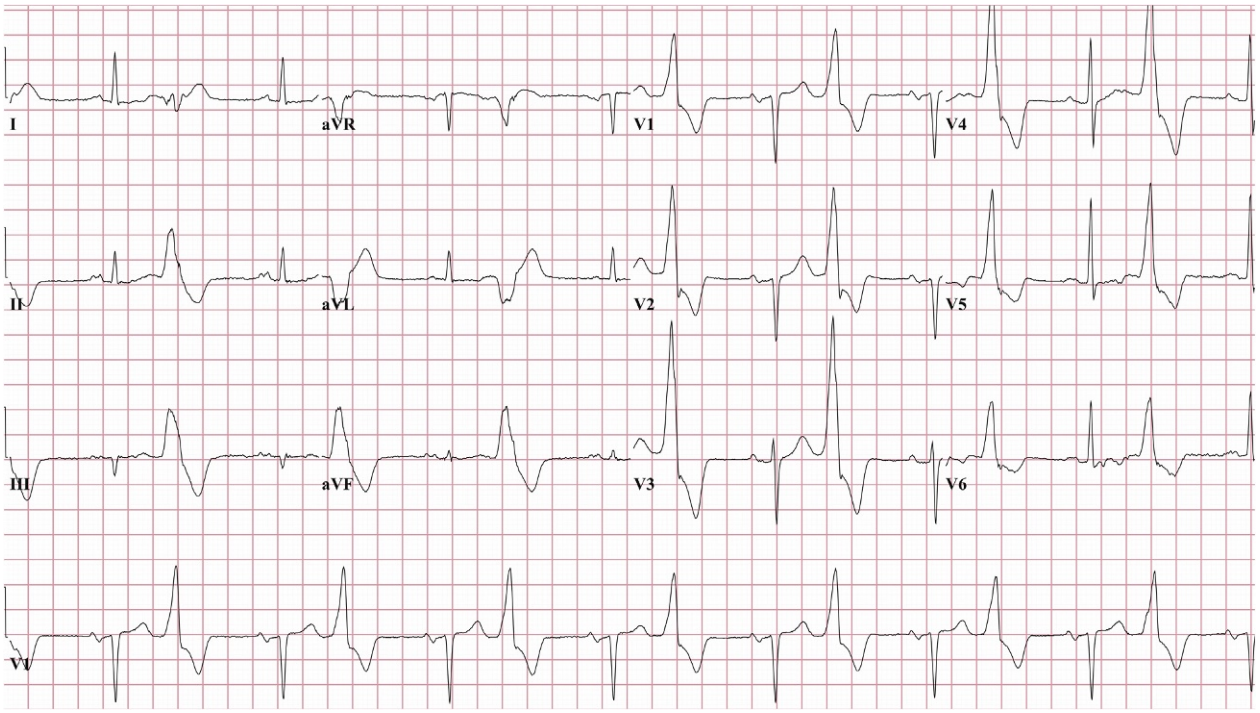

Fig. 17 12-lead electrocardiogram of premature ventricular complexes (PVCs) with inferior axis and R/S ratio ≥ 1 suggestive of an origin from the left ventricular outflow tract (LVOT). PVCs transition much earlier that the sinus beats which also suggests LVOT origin.

greater than 0.55 is considered sensitive and specific (Berruezo et al., 2004; Daniels et al., 2006; Vallès et al., 2010) (**Table 4**). MDI is the time to maximal deflection divided by QRSd (**Fig. 19**). A 3-step ECG algorithm with 100% sensitivity and 98% specificity to discriminate between epicardial and endocardial origin was proposed (Hamon et al., 2016).

Fascicular VT presents with QRSd <140 ms and rapid initial forces due to activation of the Purkinje system. Left posterior fascicular VT presents with right bundle branch block (RBBB) morphology and left superior axis. Left anterior fascicular VT presents with RBBB morphology and right inferior axis (**Fig. 20**).

Echocardiogram

Two-dimensional echocardiography allows a full assessment of cardiac structure, function, and hemodynamics. The presence of left ventricular dysfunction, wall motion abnormalities, hypertrophy, or chamber enlargement such as the right ventricle should prompt further evaluation for nonischemic cardiomyopathy, hypertrophic cardiomyopathy (HCM), or arrhythmogenic right ventricular cardiomyopathy (ARVC).

Evaluation of LV morphology, systolic function, and diastolic function has been rigorously and systematically established. The importance of right ventricular (RV) function has only been recognized recently and lack of normative data and standard assessments for RV function limit full evaluation. Moreover, echocardiography does not reliably diagnose subtle structural abnormalities or allow tissue characterization.

Holter

PVC burden is an important risk factor for cardiomyopathy and quantification with 24-h Holter monitoring remains important to assess prognosis and monitor response to treatment. PVC burden is expressed as percentage or total number of beats with greatest risk for cardiomyopathy when >20% or >20,000 beats. 24-h monitoring at the minimum is needed to assess PVC burden due to diurnal variation. PVC burden varies 5%–40% (Niwano et al., 2009) or clusters, requiring multiple Holters to fully appreciate the problem. Mobile cardiac telemetry systems overcome this limitation but are not a routine part of evaluation.

Exercise Stress Testing

Idiopathic VT is commonly triggered during peak exercise or during recovery but does not provide prognostic value, however, suppression during exercise is reassuring for absence of SHD or a malignant phenotype. Exercise stress testing is helpful when exercise-induced symptoms are present or CAD remains a possibility; myocardial perfusion imaging or echocardiography may be combined to enhance the diagnostic yield.

Table 3 Electrocardiographic characteristics to localize idiopathic ventricular tachycardia to the right ventricular out flow tract (RVOT), left ventricular outflow tract (LVOT), and other locations

	RVOT		*LVOT*			*Other*			
ECG Lead	*Anterior*	*Posterior*	*RCC*	*RCC-LCC Commissure*	*LCC*	*AMC*	*MA*	*Fascicular*	*Papillary*
V1	QS or rS	rS	rS Monophasic R wave: V2/V3	QS or rS (notched)	qS or "M" or "W" sign Monophasic R wave: V1/V2	qR	R		Concordance
rsR′	R (notched)								
I	QS or rS	Rs	R or Rr′ or rS	R or rS	rS or R	Rs		rS or R	
aVL	QS	QS	QS	QS	QS				
II, III, aVF	R	R	R	R II/III R wave ratio $>$ RCC	R II/III R wave ratio $>$ RCC	II/III R wave ratio $>$ 1		Superior axis Inferior axis	Superior axis-posteromedial Inferior axis-anterolateral
Transition	V3 or later Later than sinus rhythm V2 transition ratio $<$0.6	V2-V4	V2-V3 Earlier than sinus rhythm V2 transition ratio $\geq$0.6	V1-V3	Earlier than V2				

RCC, Right coronary cusp; *LCC*, Left coronary cusp; *AMC*, Aortomitral continuity; *MA*, Mitral annulus.

Al'Aref et al. (2015). *Circulation: Arrhythmia and Electrophysiology* 8, 616–624; Yamada et al. (2010). *Circulation: Arrhythmia and Electrophysiology* 3, 324–331; Yamada et al. (2008). *Journal of the American College of Cardiology* 52, 139–147; Kuo et al. (2003). *PACE* 26, 1986–1992; Kamakura et al. (1998). 98, 1525–1533; Betensky et al. (2011). *Journal of the American College of Cardiology* 57, 2255–2262; Jadonath et al. (1995). *American Heart Journal* 130, 1107–1113; Ouyang et al. (2002). *Journal of the American College of Cardiology* 39, 500–508; Steven et al. (2009). *Circulation: Arrhythmia and Electrophysiology* 2, 660–666; Hoffmayer et al. (2013). *Current Problems in Cardiology* 38, 131–158; Dixit, S., et al. (2003). *Journal of Cardiovascular Electrophysiology* 14,1–7.

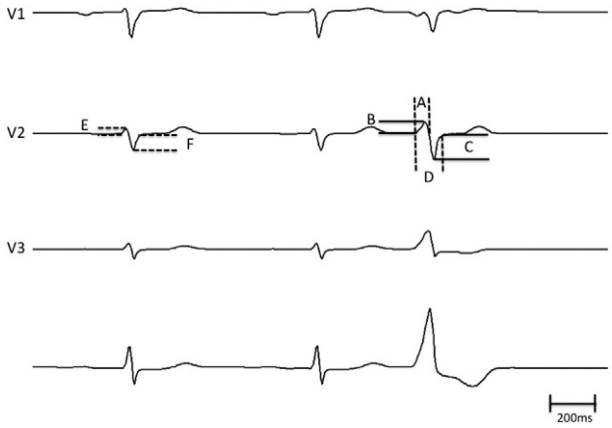

Fig. 18 V2 transition ratio of $\geq$0.60 is a highly sensitive (95%) and specific (100%) marker for origin from the left ventricular outflow tract. The transition ratio is calculated with the following formula: [B/(B + C) ÷ E/(E + F)]. A = PVC R-wave duration (ms); B = PVC R-wave amplitude (mV); C = PVC S-wave amplitude (mV); D = PVC QRS duration (ms); E = sinus rhythm R-wave amplitude (mV); and F = sinus rhythm S-wave amplitude (mV). With permission (Betensky et al. 2011).

Table 4 Electrocardiographic markers indicative of epicardial origin

Criteria	*Sensitivity and Specificity*
Pseudodelta wave $\geq$34 ms	83% and 95%
V2 intrinsicoid deflection $\geq$85	87% and 90%
Shortest RS complex $\geq$121	76% and 85%
Maximum deflection index $\geq$0.55	100% and 98.7%
Q wave in I or inferior leads	88% and 88%

Berruezo et al. (2004). *Circulation*. 109, 1842–1847; Daniels et al. (2006). *Circulation* 113, 1659–1666; Vallès et al. (2010). *Circulation: Arrhythmia and Electrophysiology* 3, 63–71.

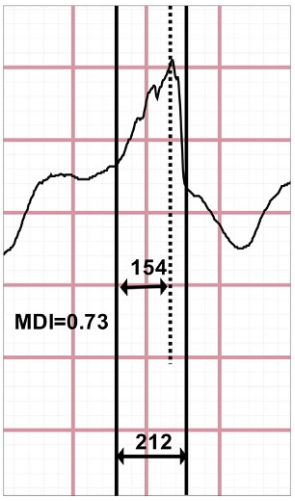

Fig. 19 Maximal deflection index (MDI) is the fraction of the QRS duration represented by the initial forces. The time from onset to maximal deflection (dotted line) is 154 msec and overal QRS duration is 212 msec. MDI is 154/212 or 0.73. MDI $\geq$0.55 is a highly sensitive and specific marker for origin from the epicardium.

Advanced Cardiac Imaging

Advanced cardiovascular imaging techniques such as CCT, CMR, and positron emission tomography (PET) allow for highly detailed anatomic evaluation of cardiac structures and coronary anatomy as well as assessment of microvascular perfusion and tissue characterization for myocardial fibrosis, fibrofatty replacement, and inflammation.

In particular, attention has focused on the relationship between idiopathic outflow tract VT and ARVC. ARVC is a progressive disease with sites for VT from the outflow tract, apex, and sub-tricuspid area (triangle of dysplasia) associated with RV dilation and

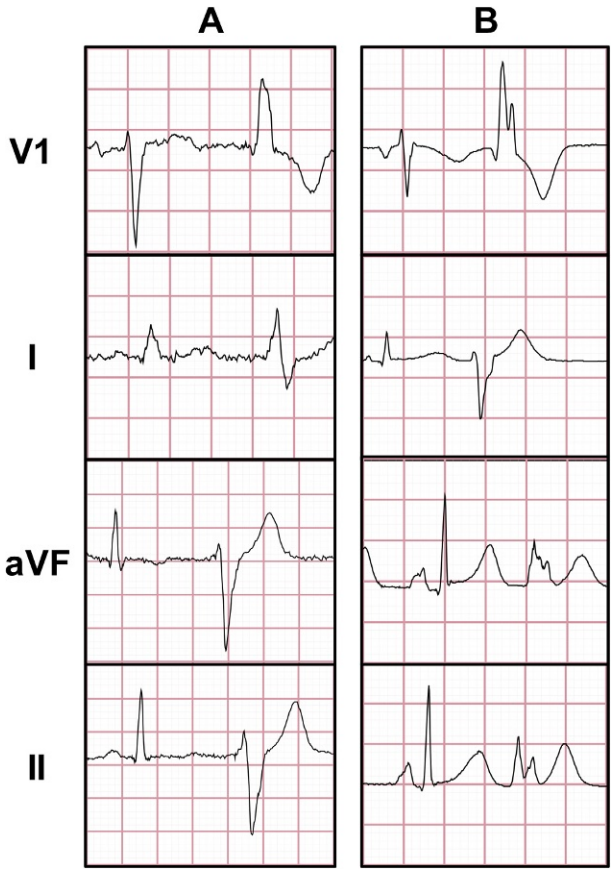

Fig. 20 Fascicular premature ventricular complex (PVC) morphology from both the posterior (A) and anterior (B) fascicles. Left posterior fascicular PVCs have right bundle branch block (RBBB) morphology and left superior axis. Left anterior fascicular PVCs have RBBB morphology and right inferior axis.

aneurysms (**Fig. 21**). The overlap of VT from the outflow tract raises the question of whether idiopathic outflow tract VT is an early manifestation or forme fruste of ARVC.

Mehta et al. demonstrated approximately 40% of patients with idiopathic RVOT-VT had abnormal endomyocardial biopsies of which 73% had abnormal echocardiograms; echocardiogram was abnormal in 6% with a normal biopsy. Sustained VT with LBBB morphology and superior axis suggests occult RV cardiomyopathy (Mehta et al., 1989, 1994) (**Fig. 22**).

Interestingly, Carlson et al. demonstrated that RVOT-VT is associated with focal RV structural abnormalities only detected on CMR including focal wall thinning, decreased systolic thickening, and abnormal wall motion (Carlson et al., 1994). This finding was confirmed by multiple other studies that additionally observed mild fibrofatty infiltration in RVOT-VT (Globits et al., 1997; O'Donnell et al., 2003; Proclemer et al., 1997). Gaita et al. reevaluated 61 patients with idiopathic RVOT-VT after a mean follow-up of 15 years and found that none experienced SCD or developed ARVC but 8 of 11 (73%) patients who underwent CMR displayed focal fibrofatty replacement (Gaita et al., 2001).

18-fluorodeoxyglucose (FDG) PET combines different imaging modalities including myocardial FDG avidity as a marker of metabolic activity, rubidium-82 perfusion imaging, and morphologic assessment with CCT where a mismatch between metabolic and perfusion imaging suggests regions of scar due to active myocardial inflammation. PET is helpful when cardiac sarcoidosis is suspected as there is no specific cardiac presentation pattern and may mimic other diseases such as ARVC. Nery et al. found that 42% of all patients presented with sustained MMVT had abnormal FDG-PET scans suggesting myocardial inflammation and 28% were confirmed to have cardiac sarcoidosis (Nery et al., 2014). FDG-avidity without evidence of sarcoidosis may represent myocarditis or is nonspecific. Variability in QRS morphology and cycle length may distinguish VT associated with cardiac sarcoidosis from idiopathic VT (Panda et al., 2015).

CCT, CMR, or FDG-PET is not routinely indicated in the presence of a normal 12-lead ECG, monomorphic ventricular ectopy, normal echocardiogram, and in the absence of high risk features such as syncope or family history of sudden death. These imaging modalities are generally reserved for case when the history, ECG, and echocardiogram raise the specter of congenital heart disease, infiltrative disease, hypertrophic cardiomyopathy, or ARVC. Other imaging modalities with limited utility are routine coronary angiography, RV angiography, myocardial perfusion scintigraphy, and endomyocardial biopsy.

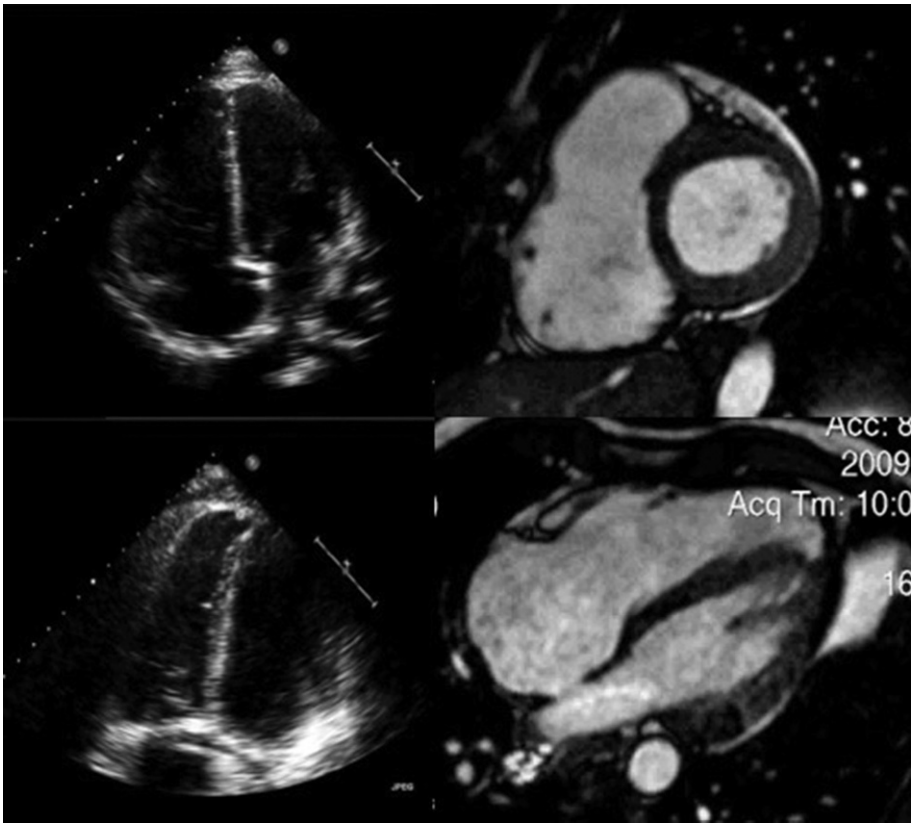

Fig. 21 Morphological changes of the right ventricle consistent with arrhythmogenic right ventricular (RV)cardiomyopathy. The two echocardiographic panels on the left show a dilated RV with a thin walled apical aneurysm wrapping around the left ventricular apex (LV) on parasternal long axis views. The two cardiac magnetic resonance imaging panels on the right confirm severe RV dilation and a RV apical aneurysm.

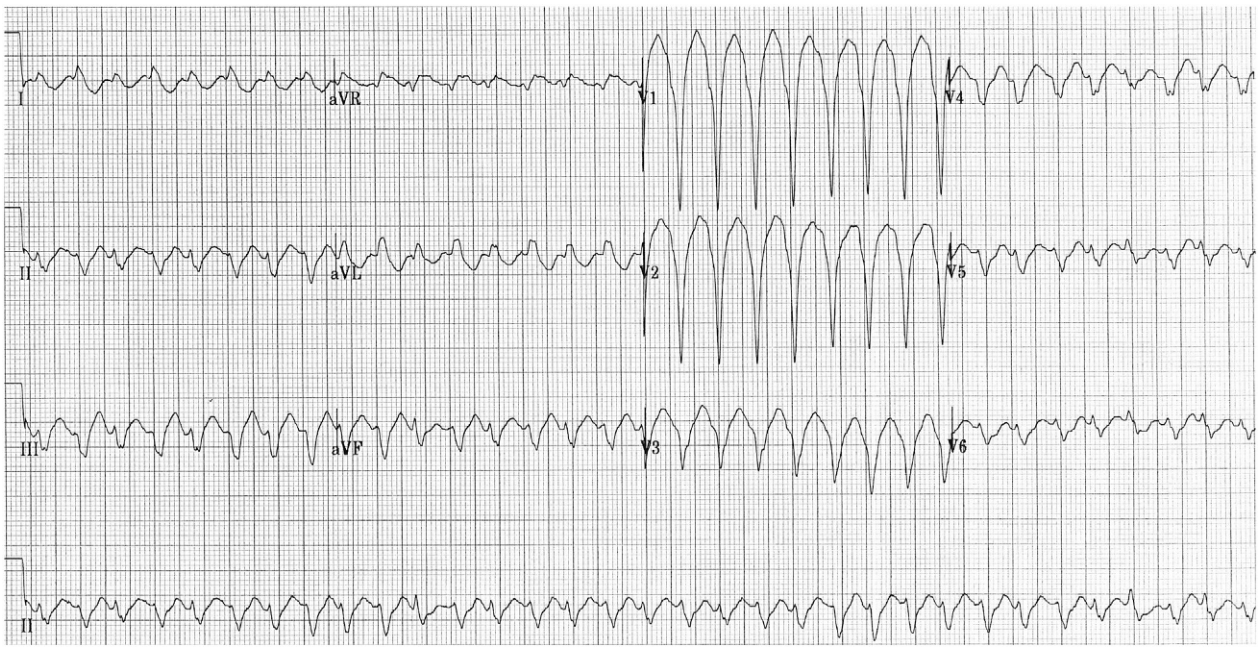

Fig. 22 Sustained monomorphic ventricular tachycardia with left bundle branch block morphology, negative concordance, and superior axis suggestive of origin from the inferoapical right ventricle suggestive of arrhythmogenic right ventricular cardiomyopathy.

Table 5 Electropharmacologic classification of idiopathic ventricular arrhythmias

	Adenosine-sensitive	*Verapamil-sensitive*	*Propranolol-sensitive*
Mechanism	*Triggered activity cAMP-mediated calcium overload leading to DADs*	*Intrafascicular reentry*	*Enhanced automaticity*
Pattern	Monomorphic PVCs, salvos, sustained VT	Monomorphic PVCs, salvos, sustained VT	Monomorphic or polymorphic Incessant
ECG Morphology	LBBB with inferior axis	RBBB with left-superior axis RBBB with right-inferior axis	RBBB, LBBB, polymorphic
Anatomic Origin	RVOT or LVOT	LAF or LPF	RV and LV
Induction			
PES (rapid pacing)	(+)	(+)	(−)
Catecholamines	(+)	(+)	(+)
Exercise	(+)	(+)	(+)
Entrainment	Absent	Present	Absent
Drug response			
Adenosine	Termination	No response	Transient suppression
Verapamil	Termination	Termination	No response
Propranolol	Termination	No response	Termination or transient suppression
Acetylcholine	Termination	No effect	Transient suppression

cAMP, Cyclic adenosine monophosphate; *DADs*, Delayed afterdepolarization; *PVCs*, Premature ventricular complexes; *VT*, Ventricular tachycardia; *LBBB*, Left bundle branch block; *RBBB*, Right bundle branch block; *RVOT*, Right ventricular outflow tract; *LVOT*, Left ventricular outflow tract; *LAFB*, Left anterior fascicle; *LPF*, Left posterior fascicle; *RV*, Right ventricle; *LV*, left ventricle; (+), Induced or facilitates; (−), No effect

Mechanisms of Arrhythmogenesis

The clinical presentation and electropharmacologic profiles of idiopathic VT are variable but classification is possible by differences in electrocardiographic pattern, anatomic origin, response to exercise or catecholamines, response to programmed electrical stimulation (PES), or medications including adenosine, verapamil, or propranolol. The most common classification framework is based on response to adenosine, verapamil, or propranolol as drug response provides mechanistic insight: triggered activity, reentry, and automaticity, respectively (**Table 5**).

Adenosine-Sensitive

Lerman et al. first characterized the response of idiopathic VT with LBBB morphology and inferior axis, now recognized as outflow tract VT, to pharmacologic agents and PES (Lerman, 1993; Lerman et al., 1986; Wilber et al., 1993). Burst pacing reliably induces and terminates VT; isoproterenol facilitates induction; beta-blockade suppresses induction; and VT terminates with adenosine, verapamil, beta-blockers, Valsalva maneuver, or carotid sinus massage. Kim et al. demonstrated that the clinical presentation, electrophysiologic provocation, and drug response are similar across the spectrum of outflow tract VTs suggesting a common mechanism of arrhythmogenesis (Kim et al., 2007).

The sensitivity to adenosine provided the central insight to understand the clinical observations and mechanism. Adenosine (A1) receptors are found in atrial and ventricular myocardium with greater density in atrial versus ventricular myocardium (Lerman and Belardinelli, 1991). A1 receptors couple to an inhibitory G protein with two principal signal transduction pathways: cAMP and phosphatidylinositol. The response of supraventricular tachycardia to adenosine is due to cAMP-independent activation of the outward potassium current with resultant decrease in APD; however, this is not observed in ventricular myocardium. Adenosine inhibits adenylate cyclase- and cAMP-mediated activation of the inward calcium current (L-type) resulting in negative chronotropic and inotropic effects. Acetylcholine, via cardiac muscarinic receptors, yields identical effects explaining the unusual response to vagal maneuvers. Calcium channel blockers (CCBs) directly inhibit the L-type calcium current (**Fig. 23**).

The findings are consistent with a mechanism dependent on cAMP-mediated calcium overload. cAMP activates protein kinase A leading to phosphorylation of the L-type calcium channel and ryanodine receptor resulting in increased inward calcium current and calcium release from the SR that feeds back to further stimulate calcium-induced calcium release. Intracellular calcium overload results in an increase in the transient inward sodium current due to the activation of the sodium-calcium exchanger during phase 4 of the action potential (AP), triggering delayed afterdepolarizations (DADs). DADs reaching the critical threshold will trigger another AP that may result in development of successive APs leading to triggered VT. Accordingly, adenosine sensitivity is the sine qua non of cAMP-mediated triggered activity.

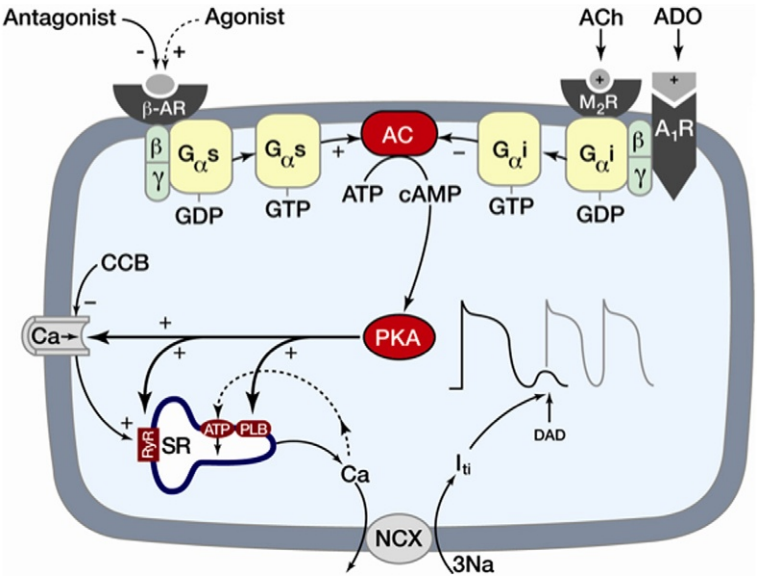

Fig. 23 Signal transduction pathway for cAMP-mediated triggered ventricular tachycardia. AC = adenylate cyclase; Ach = acetylcholine; ADO = adenosine; A1R = A1-adenosine receptor; β-AR = β-adrenergic receptor; CCB = calcium channel blocker; DAD = delayed after depolarization; Iti = transient inward current; M2R = muscarinic receptor; NCX = Naþ/Ca2þ exchanger; PLB = phospholamban; PKA = protein kinase A; RyR = ryanodine receptor; SR = sarcoplasmic reticulum. From Lerman, B.B., 2007. Mechanism of outflow tract tachycardia. *Heart Rhythm : The Official Journal of the Heart Rhythm Society* 4(7) 973–976, with permission.

Verapamil Sensitive

Zipes et al. in 1979 first described idiopathic VT with RBBB morphology and left axis deviation that was induced with atrial pacing (Zipes et al., 1979) and later described by Belhassen et al. to be sensitive to verapamil (Belhassen et al., 1981). Multiple studies suggested intrafascicular reentry as the mechanism due electrocardiographic morphology, response to PES with entrainment, early retrograde His potential suggesting fascicular origin and identification of an area of slow in the region of the fascicles (Belhassen et al., 1981; German et al., 1983; Lin et al., 1983; Ohe et al., 1988; Okumura et al., 1987, 1996; Zipes et al., 1979). Common triggers include exercise or catecholamines.

The antegrade limb extends from the basal to mid apical septum and slowly conducting verapamil-sensitive fascicular fibers with decremental conduction properties are implicated as the retrograde limb of the reentrant circuit (Lin et al., 1983). It was postulated that presystolic Purkinje potentials would be appropriate targets for ablation and was confirmed by Nakagawa et al. (1993) (Fig. 24). Diastolic Purkinje potentials have been observed (Nogami et al., 2000). Targeting Purkinje potentials for ablation during sinus rhythm has proved successful (Ouyang et al., 2002a). Interestingly, successful ablation of the left posterior fascicle for fascicular VT does not result in QRS widening or fascicular block, suggesting that the fascicles are not critical substrate (Kuo et al., 2003). Two studies suggest that the fascicle is in fact a bystander and that the retrograde limb is LV myocardium (Maeda et al., 2014; Morishima et al., 2012). The optimal site of ablation therefore cannot be identified if VT is not induced and an anatomic linear ablation to transect the region of the posterior fascicle is necessary to sufficiently modify the substrate.

Propranolol Sensitive

The clinical characteristics and mechanisms of propranolol-sensitive VT are poorly defined but may present as MMVT or PMVT that is modulated by catecholamines (Lerman et al., 1997; Sung et al., 1988). VT may be induced with exercise or isoproterenol and conversely suppressed with beta-blockers. PES characteristically is unable to initiate or terminate VT. Adenosine suppresses, not terminates, VT. These characteristics suggest an automatic mechanism arising from fully repolarized myocardium (normal, −90 mV) not abnormal automaticity (−60 mV).

Management Strategies

Observation

In the absence of symptoms and underlying cardiomyopathy there is no immediate need for intervention if the burden of PVCs is <20% and NSVT is not present. A monitoring strategy may be employed with a higher burden assuming LV function is normal; however, close clinical and echocardiographic follow-up is required to monitor for incipient LV dysfunction. Niwano et al. validated

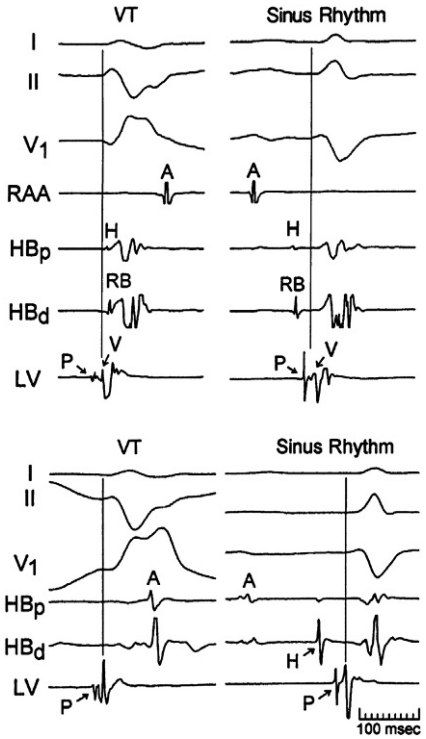

Fig. 24 Electroanatomic mapping of fascicular ventricular tachycardia (VT) revealing a presystolic Purkinje potential (left) during tachycardia and in sinus rhythm (right) at the site of successful ablation in two different patients (upper and lower panels). From Nakagawa, H. et al., 1993. Radiofrequency catheter ablation of idiopathic left ventricular tachycardia guided by a Purkinje potential. *Circulation* 88(6) 2607–2617, with permission.

this approach by following patients over 4 years with greater than 1000 PVCs daily in whom SHD was excluded with echocardiography and cardiac MR (Niwano et al., 2009). SCD, VT, syncope, or heart failure was not observed during follow-up. However, a small but significant decline in LVEF and increase in LV dimension were observed with >20,000 PVCs.

Avoidance of Triggers

A common recommendation is to avoid potential triggering substances, medications, food, or illicit drugs including caffeine, alcohol, tobacco, chocolate, beta-agonists, marijuana, cocaine, amphetamines, and other stimulants. While recommendations to avoid illicit stimulants, alcohol, or tobacco are beneficial for a myriad of reasons, there is little data supporting avoidance or behavioral modification for caffeine, alcohol, and tobacco in regard to ventricular arrhythmias (Glatter et al., 2012). In fact, Debacker et al. did not find any benefit from cessation or reduction of caffeine, tobacco, or alcohol (DeBacker et al., 1979).

Pharmacotherapy: Beta-Blockers and Calcium Channel Blockers

Beta-blockers and CCBs are generally first-line therapy for asymptomatic or symptomatic idiopathic VT but overall efficacy is not well established. Generally, the effectiveness of beta-blockers and CCBs are considered to be modest and limited by potential side effects encountered in doses sufficient for arrhythmia suppression. Beta-blockers inhibit activation of adenylate cyclase thus reduce cAMP-dependent phosphorylation of L-type calcium channels and ryanodine receptors leading to reduced intracellular calcium thus suppressing automaticity and afterdepolarizations.

Atenolol significantly reduced PVC burden by 33% and reduced symptoms but placebo resulted in a similar degree of symptom reduction without change in PVC burden (Krittayaphong et al., 2002). Verapamil reduces PVC burden by 35% (Gill et al., 1992). A retrospective analysis found that beta-blockers and calcium channel blockers resulted in 36% and 43% reduction in PVC burden, respectively (Zhong et al., 2014).

Intravenous adenosine and verapamil both have been used for the acute treatment of idiopathic VT as well as sympatholytic maneuvers (Valsalva or carotid sinus massage). The vasodilatory and electrophysiologic effects of adenosine or verapamil may lead to hemodynamic compromise or rhythm degeneration, especially in the presence of SHD, which is rarely known at the time of presentation.

Pharmacotherapy: Antiarrhythmic Drugs (AADs)

The clinical response to AADs has been evaluated in several small studies and included mexiletine, tocainide, flecainide, disopyramide, and sotalol (Capucci et al., 1989, 1991; Gill et al., 1992; Masotti et al., 1984; Tanabe et al., 1991). Amiodarone, mexiletine, flecainide, sotalol, and propafenone result in 70%–89% reduction in PVC burden (Zhong et al., 2014).

Of these medications, sotalol and flecainide are most commonly used. Sotalol has been used safely in the presence of SHD and appears to be greater than 89% effective for the treatment of RVOT-VT (Gill et al., 1992). These medications are generally well tolerated but care must be taken when administering to patients at risk for or with cardiomyopathy due to negative inotropic effects, QT prolongation, and proarrhythmia. AADs are generally reserved for risk averse or frail patients or for specific anatomic sites of origin (i.e., epicardial) that suggest lower ablation success with higher complication rates.

Catheter Ablation

Chugh et al. first described a patient with cardiomyopathy and high burden of PVCs in whom successful catheter ablation resulted in reversal of cardiomyopathy (Chugh et al., 2000).

Takemoto et al. in 2005 systematically evaluated the role of ablation for RVOT PVCs and found that the success rate was 93% with 3% complication and 3% recurrence rates (Takemoto et al., 2005). Ablation of patients with a high burden (>20%) of PVCs was associated with improvement in LVEF and LV dimensions. Yarlagadda et al. confirmed this finding with an 85% success rate of ablation (Yarlagadda et al., 2005).

Multiple subsequent single-center experiences have demonstrated that ablation is successful in 66%–92% all associated with significant reduction in PVC burden and improvement in LVEF and dimensions (Ban et al., 2013; Bogun et al., 2007; Penela et al., 2013; Sarrazin et al., 2009; Yokokawa et al., 2012a; 2013). Ablation was further associated with improvement in functional class and decrease in brain natriuretic peptide levels (Penela et al., 2013). Ineffective ablation was associated with a further decline in LVEF (Bogun et al., 2007). The time to recovery was generally within 4–6 months (Bogun et al., 2007; Yokokawa et al., 2013) but a significant minority (32%) of patients experienced more delayed improvement (Yokokawa et al., 2013). Epicardial PVCs or wider QRSd are independently associated with delayed recovery (Yokokawa et al., 2013). The recurrence rate of PVCs following an initially successful ablation appears to range from 9% to 17%. Tanaka et al. (2011) found that the success of ablation was 88% for RVOT origin but only 58% for LVOT origin.

Latchamsetty et al. published an extensive retrospective multicenter outcomes study for catheter ablation of idiopathic PVCs from eight high volume ablation centers with mean PVC burden of 20% and LVEF 55% in which the acute success of ablation was 84% (Latchamsetty et al., 2015). The success rate was as high as 93% for RVOT origin and as low as 67% for epicardial origin (**Fig. 25**). 71% experienced a durable decline in PVC burden (80% reduction) over 20 months of follow-up; 19% were on antiarrhythmic medications at follow-up. Among patients with cardiomyopathy, LVEF improved ≥10% in 67%, <10% in 18%,

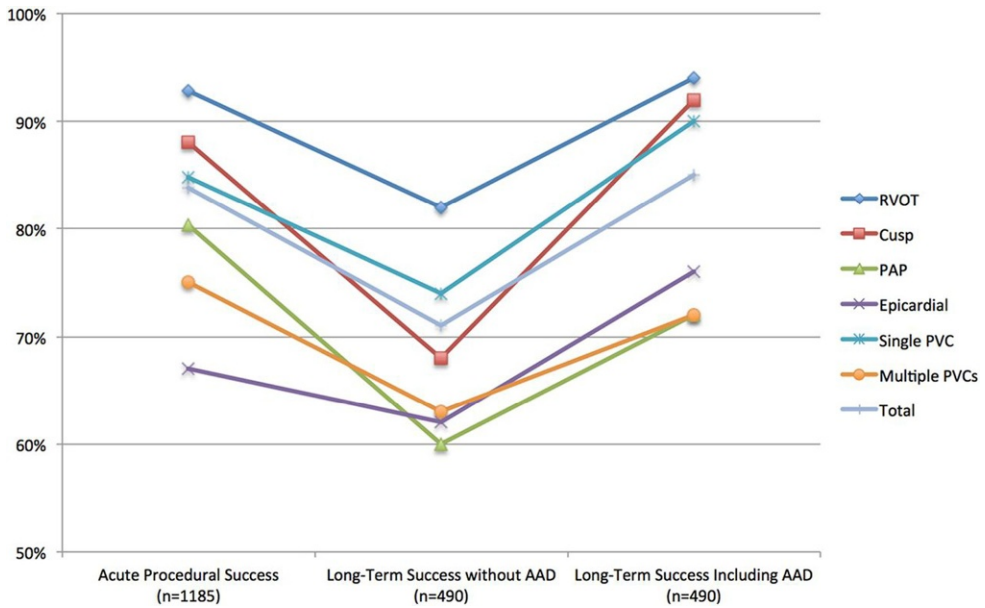

Fig. 25 Acute and long-term success (without and with AAD) of catheter ablation for idiopathic premature ventricular complexes by site of anatomic origin without and with AAD therapy. PAP = papillary muscle; RVOT = right ventricular outflow tract; AAD = antiarrhythmic drugs. From Latchamsetty R, Yokokawa M, and Morady F (2015) Multicenter outcomes for catheter ablation of idiopathic premature ventricular complexes. *JACC: Clinical Electrophysiology* 1(3): 116–123. https://doi.org/10.1016/j.jacep.2015.04.005, with permission.

and was unchanged or worsened in 15%. The overall complication rate was 5% with a near even distribution between major and minor complications; tamponade occurred in 0.8%.

The differential success of ablation based on anatomic location and significant complication rate raises the question of the relative benefits of AADs vs. ablation. Two recent studies sought to compare AADs with catheter ablation:

- Zhong et al. retrospectively analyzed 510 patients of whom 40% underwent ablation and 60% were treated with AADs (Zhong et al., 2014). Ablation was associated with a 93% decrease in the PVC burden that was significantly greater than AADs. LVEF improved significantly after ablation but there was no change with AADs; normalization of LVEF was two times more likely with ablation than AADs (47% vs. 21%).
- Ling et al. prospectively randomized 330 patients with idiopathic RVOT PVCs to catheter ablation or AADs (Ling et al., 2014). Ablation was associated with a greater decrease in PVC burden and lower recurrence at 1 year (19% vs. 89%). LVEF improved in both treatment arms. QS morphology in lead I predicted a more successful ablation.

These results of these studies suggest that while medical therapy is effective, ablation has incremental value over AADs and is more likely to result in durable clinical response and recovery of LVEF.

Conclusion

VT with structurally normal hearts has a diverse clinical presentation with a predominantly benign prognosis. However, epidemiological studies suggest a heightened risk of cardiovascular morbidity and mortality due to malignant presentations with cardiomyopathy and sudden death. Risk stratification is dependent on the careful evaluation for cardiomyopathy or subclinical heart disease, arrhythmia burden, and electrocardiographic characteristics. The anatomic substrate, arrhythmia mechanisms, and treatment strategies can be ascertained from the electrocardiographic and electropharmacologic profiles. Ablation is potentially curative with improved outcomes compared to pharmacotherapy and is increasingly first-line therapy; but careful consideration of the anatomic location and the implications for success and complications need to be factored.

Challenges

Several challenges need further investigation:

- Improving risk stratification for those at risk of developing idiopathic VT or malignant phenotypes.
- Improving evaluation for SHD
- Improving efficacy and safety of antiarrhythmic drugs or catheter ablation.
- Improving understanding of the mechanisms for cardiomyopathy or SCD.

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Wide QRS Complex Tachycardia: What is the Diagnosis?

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Introduction and Definitions

Wide complex tachycardia (WCT, sometimes referred to as "broad complex tachycardia," BCT) is a term used to describe the ECG appearances of an arrhythmia, whose underlying mechanism can be broad and undifferentiated. Correct diagnosis of the mechanism of the WCT has significant implications for the clinical outcomes, acute and chronic management, and prognosis.

The common definition of a WCT is a rhythm with a ventricular rate over 100 beats per minute (bpm) and a QRS duration of $\geq$120 ms (Miller and Das, 2009). However, it is also understood that the term "tachycardia" may be used loosely when patients have structural heart disease or are undergoing treatment with antiarrhythmic drug therapy. In these situations, ventricular tachycardia (VT) may present with rates under 100 bpm.

Several descriptive terms are used to describe the ECG appearances of a WCT and may aid in diagnosing the underlying mechanism. These include:

- *Regularity*: predictability of the rhythm from beat to beat with a fixed cycle length.
- *Capture beats*: QRS complexes during a WCT which match the morphology of the patient's conducted beats (Fig. 1).
- *Fusion beats*: QRS complexes during a WCT which are intermediate in morphology between the WCT and the patient's conducted beats (Fig. 1).
- *Atrioventricular (AV) dissociation*: independent activation of the atria and ventricles (Fig. 1)
- *Positive concordance*: the predominant direction of the QRS in each of the precordial leads (V1–V6) is positive, for example, qR, Rs, or monophasic R waves (Fig. 2).
- *Negative concordance*: the predominant direction of the QRS in each of the precordial leads (V1–V6) is negative, for example, Qr, rS, or monophasic QS waves (Fig. 3).
- QRS Morphology
  - *Polymorphic*: QRS complexes vary in amplitude, duration, and axis (Fig. 4).
  - *Monomorphic*: QRS complexes are uniform in appearance within one episode of WCT (e.g., Figs. 2 and 3).
  - *Pleomorphic (or pleiomorphic)*: more than one morphology of WCT at different times. For example, this may occur when scar-related VT activates the ventricle via a different exit point during different instances of the dysrhythmia.
  - *Alternating, or bidirectional*: two different QRS morphologies present on alternate beats (Fig. 5).

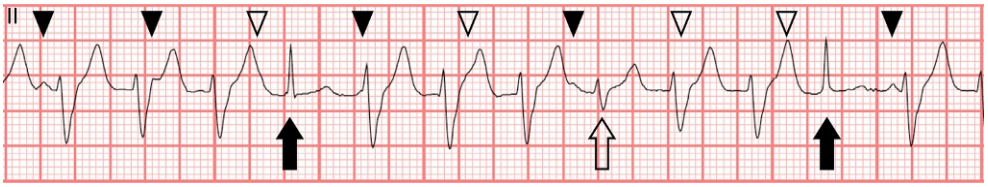


Fig. 1 Lead II rhythm strip, recorded at 25 mm s^{-1}, of a wide complex tachycardia, demonstrating AV dissociation. Independent P waves are seen (black arrowheads) or inferred (open arrowheads). Two capture beats can be identified (black arrows), occurring a suitable interval after the location of a preceding P wave. There is also a fusion beat (open arrow) which is intermediate in morphology between the conducted beats and the wide complex tachycardia.

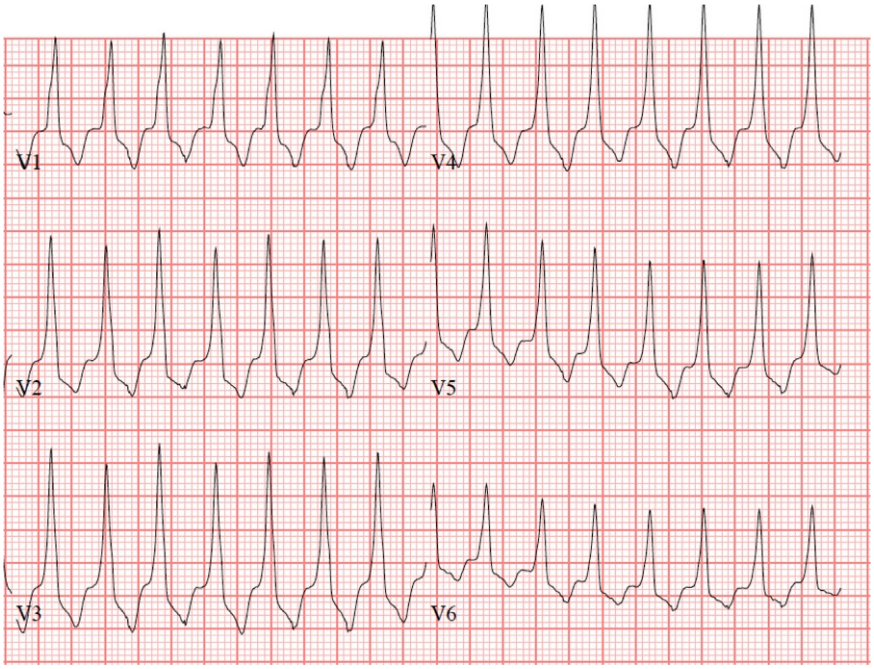


Fig. 2 Chest leads of a WCT showing positive concordance, with upright QRS complexes across all the leads. Since the complexes in V1 are predominantly positive (in this case, monophasic R waves), this tachycardia would be described as having a "right bundle branch block (RBBB) morphology," even though the appearances are in no way typical for actual block in the right bundle. The ECG suggests VT with an exit at the base of the LV. ECG recorded at 25 $mm.s^{-1}$.

- "Right bundle branch block (RBBB) morphology" refers to a predominant positive deflection in V1, for example, qR, Rsr′, rsR′, or monophasic R wave (e.g., **Fig. 2**).
- "Left bundle branch block (LBBB) morphology" refers to a predominant negative deflection in V1, for example, Qr, rS, or monophasic QS wave (e.g., **Fig. 3**). Importantly, the latter two terms are purely descriptive in this context, referring to the ECG morphology alone and do not necessarily imply that either bundle branch is mechanistically linked to the WCT.
- "RBBB morphology" and "LBBB morphology" may further be described as "typical" when they match the expected morphology for block in each respective bundle, and "atypical" when they do not (see later discussion).
- Slurred onset: slow rather than abrupt initial depolarization. This has been defined in several ways, including:
  - Width of the R wave in V1-2 exceeding 60 ms for WCT with a LBBB morphology (Kindwall et al., 1988)).
  - RS interval of >100 ms in one or more precordial leads (Brugada et al., 1991)).
  - V_i/V_t ratio <1 (Vereckei et al., 2008; see later discussion).
  - R-wave peak time >50 ms (Pava et al., 2010).
- Ventricular flutter: continuous, oscillatory monomorphic waveform, with no isoelectric line and T waves may not be clearly visible (**Fig. 6**).

The diagnostic utility of these features will be covered in more detail in the discussion of how they help to differentiate VT from other etiologies.

Differential Diagnosis

When attempting to make a diagnosis of the mechanism of any tachycardia on ECG, it is useful to start by asking two simple questions:

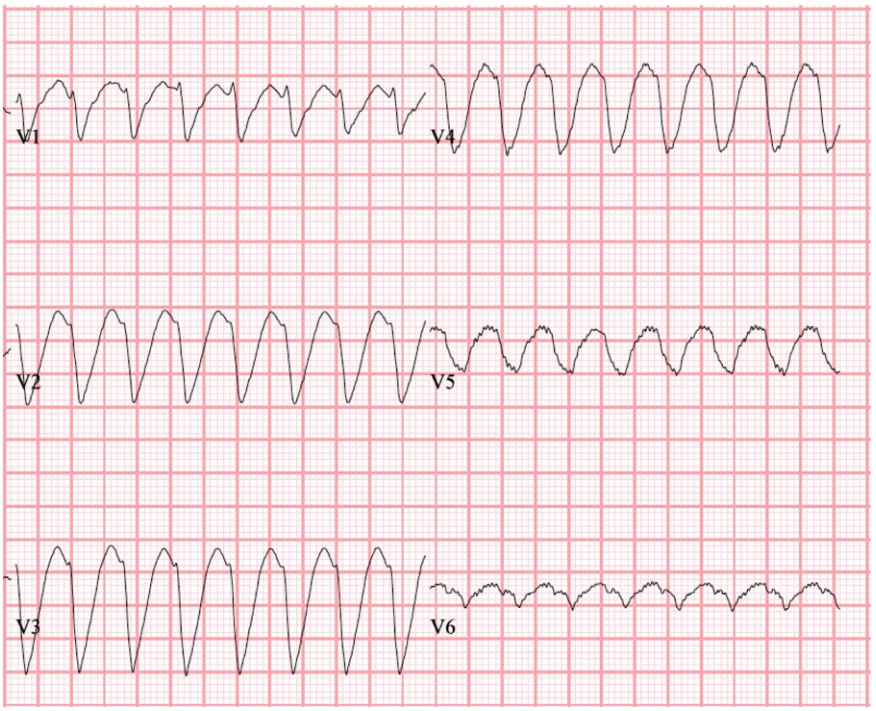


Fig. 3 Chest leads of a WCT showing negative concordance, with predominantly negative QRS complexes across all the leads. Since the complexes in V1 are predominantly negative (in this case, rS waves), this tachycardia would be described as having a "left bundle branch block (LBBB) morphology," even though the appearances are not typical for actual block in the left bundle. The ECG suggests VT with an apical exit. ECG recorded at 25 mm s^{-1}.

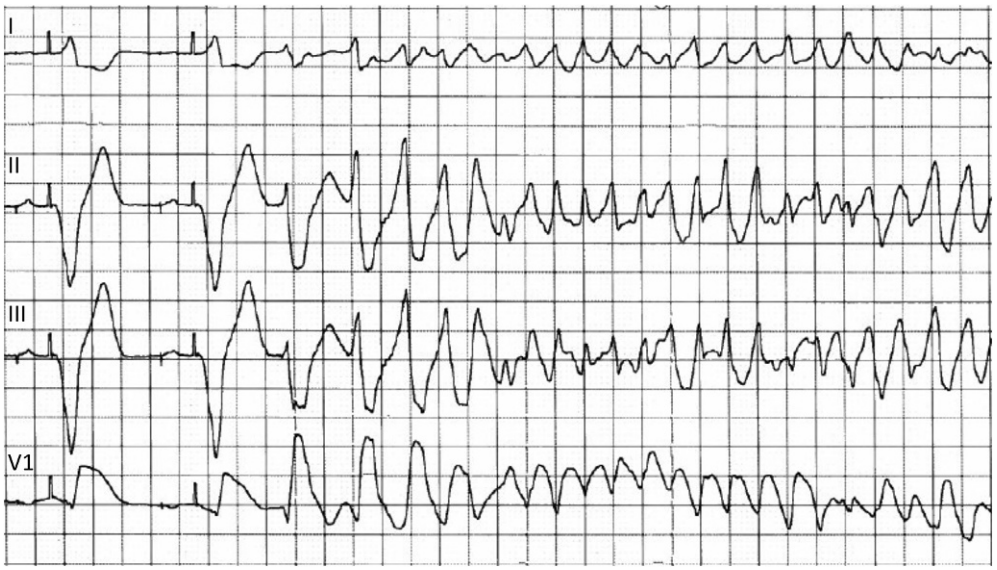


Fig. 4 ECG leads I, II, III, V1, recorded at 25 mm s^{-1}, showing dual-chamber pacing followed by irregular, polymorphic VT. On the first two beats the ST segments appear abnormal in lead I and V1, even given the fact that the rhythm is paced. In this case the polymorphic VT was occurring in the setting of acute ischemia.

- Is the rhythm visibly regular or irregular?
- Are the QRS complexes narrow (<120 ms) or wide (≥120 ms)?

Any tachycardia will then fall into one of four mutually exclusive groups, each with their own differential diagnosis. This article will address irregular and regular WCTs, with the main focus being on the latter since this is the area where most diagnostic doubt may occur.

Before considering the differential diagnosis, it is useful to review first the mechanisms by which the QRS can become wide. In general, to produce a narrow QRS complex (<120 ms) activation of the ventricles must occur via antegrade activation of the His–Purkinje system, including both the left and right bundle branches. This achieves rapid depolarization of the entire ventricular



Fig. 5 Lead II rhythm strip, recorded at 25 mm s^{-1}, showing an alternating (bidirectional) rhythm.

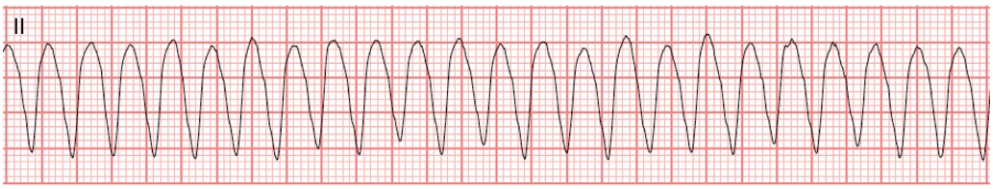


Fig. 6 Lead II rhythm strip, recorded at 25 mm s^{-1}, showing ventricular flutter at 255 bpm.

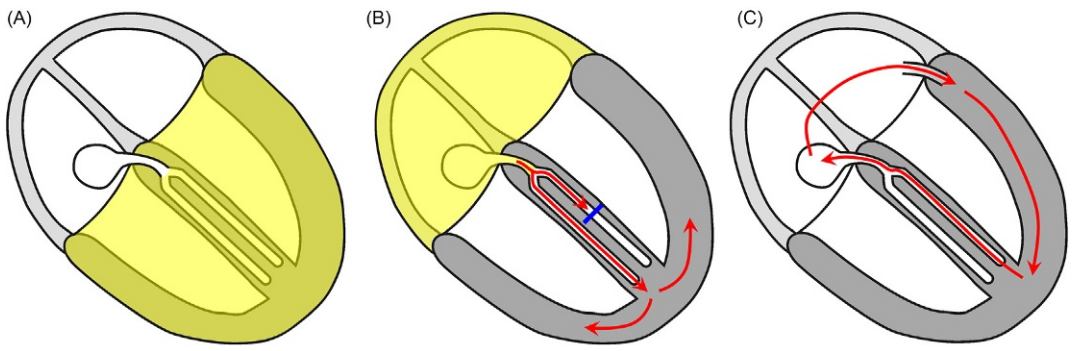


Fig. 7 Schematic diagrams outlining potential mechanisms for producing wide (>120 ms) QRS complexes. Panel A indicates a rhythm originating below the bifurcation of the His bundle (*yellow shading*), with common causes including VT and ventricular pacing. Panel B indicates abnormal conduction of a supraventricular rhythm (yellow shading, including rhythms such as orthodromic AVRT), with QRS widening caused by bundle branch block (e.g., diagram indicates LBBB) or abnormal myocardial conduction. Panel C indicates a preexcited tachycardia, with the diagram showing antidromic AV reciprocation. Other supraventricular rhythms conducted predominantly over an accessory pathway would similarly produce wide QRS complexes. *AFL*: atrial flutter; *AT*: atrial tachycardia; *AVNRT*: atrioventricular nodal reentry tachycardia; *AVRT*: atrioventricular reciprocating tachycardia; *BBB*: bundle branch block; *LVH*: left ventricular hypertrophy; *VT*: ventricular tachycardia.

myocardium in a short space of time (often <100 ms). If the ventricles are not activated via this route, then the QRS complex will generally exceed 120 ms. As outlined in Fig. 7, this includes:

- Rhythms where the ventricles are activated by conduction from myocyte to myocyte, not involving the specialized conduction tissue, for example, VT or ventricular pacing.
- Rhythms where there is antegrade conduction over the His bundle, but there is block or marked conduction delay in one of the bundle branches.

- Rhythms where the ventricle is activated via antegrade conduction over an accessory pathway, that is, preexcited rhythms. This is because subsequent spread of activation to complete depolarization of the ventricles occurs by myocyte-to-myocyte conduction, as in mechanism 1.
- Circumstances where there is generalized abnormal slowing of depolarization in the conducting tissue and/or ventricular myocardium, for example, with marked electrolyte disturbance (such as severe hyperkalemia), ischemia, inherited channelopathies, or presence of drugs or toxins, particularly those which affect sodium channel function.

Differential Diagnosis of an Irregular WCT

This includes:

- Atrial fibrillation (AF) with bundle branch block
- Atrial flutter (AFL) with a variable AV conduction ratio and bundle branch block
- Atrial tachycardia (AT) with a variable AV conduction ratio and bundle branch block
- Multifocal atrial tachycardia (MAT) with bundle branch block
- AF with preexcitation
- AFL with a variable AV conduction ratio and preexcitation
- AT with a variable AV conduction ratio and preexcitation
- MAT with preexcitation
- Polymorphic VT, including torsades de pointes VT
- Ventricular fibrillation (VF)
- Artifact

Differential Diagnosis of a Regular WCT

This includes:

- VT
- "Supraventricular" tachycardia (SVT) with bundle branch block, where in this context "SVT" refers to:
  - sinus tachycardia
  - AT with a fixed AV conduction ratio
  - AFL with a fixed AV conduction ratio
  - AV nodal reentrant tachycardia (AVNRT)
  - orthodromic (i.e., with antegrade conduction over the normal conduction system) AV reentant tachycardia (AVRT).
- SVT with preexcitation, where in this context "SVT" refers to:
  - sinus tachycardia
  - AT with a fixed AV conduction ratio
  - AFL with a fixed AV conduction ratio
  - AVNRT with conduction over a bystander accessory pathway
  - antidromic (i.e., with retrograde conduction over the normal conduction system) AVRT
- Ventricular pacing, including tracking of atrial rates >100 bpm and pacemaker-mediated tachycardia
- Artifact

Ventricular pacing (Fig. 8) should be suspected in a patient who has had a pacemaker or defibrillator and ventricular rates at, or below, the upper tracking rate of the device (130 bpm is a common default upper tracking rate of many devices). If previous ECGs are available, the WCT can be compared with any previously documented paced beats. Pacing spikes themselves may not be readily apparent on the ECG, especially with bipolar pacing. The QRS morphology will often indicate activation originating from the right ventricular apex, with negative QRS complexes in the inferior leads and negative concordance in the chest leads.

Artifact can appear regular or irregular and should be suspected if a narrow QRS can be seen superimposed at regular intervals upon what appears to be a WCT (Fig. 9). This can be correlated with a lack of clinical symptoms (e.g., palpitation, hypotension, and syncope) along with other signs such as shivering, tremor, or regular muscular activity. Occasionally it can be very difficult to



Fig. 8 Pacemaker-mediated tachycardia, with a ventricular rate of 146 bpm. Pacing spikes are seen in front of the QRS complexes. In this case the pacemaker was tracking retrograde P waves in the atrium (not clearly shown on this strip), triggering ventricular pacing. The paced rate was below the programmed maximum tracking rate (150 bpm in this case). ECG recorded at 25 mm s^{-1}.

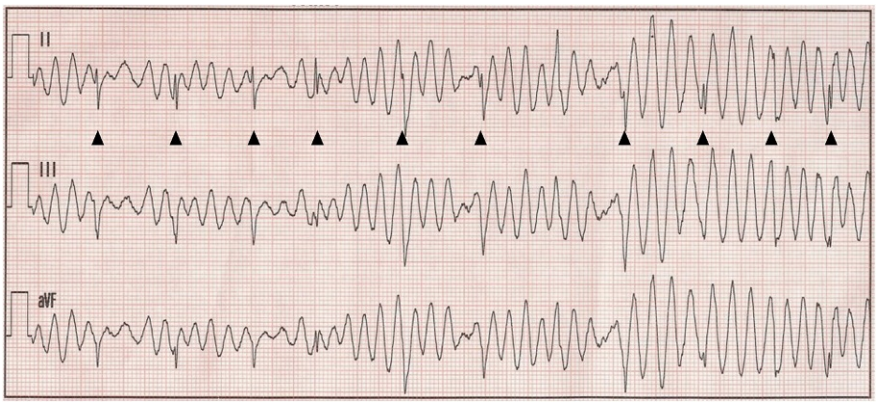


Fig. 9 ECG leads II, III, and aVF from a 77-year-old female with dizziness, nausea, and shaking, treated as torsades de pointes VT with intravenous magnesium sulfate. Closer inspection reveals sharply inscribed complexes occurring across the strip, best seen in Lead II (black arrowheads). The other apparent complexes are due to artifact caused by her muscle tremor. The intrinsic rhythm is slightly irregular, making it even harder to distinguish. The rhythm was later seen to be sinus with premature atrial beats. ECG recorded at 25 mm s^{-1}.

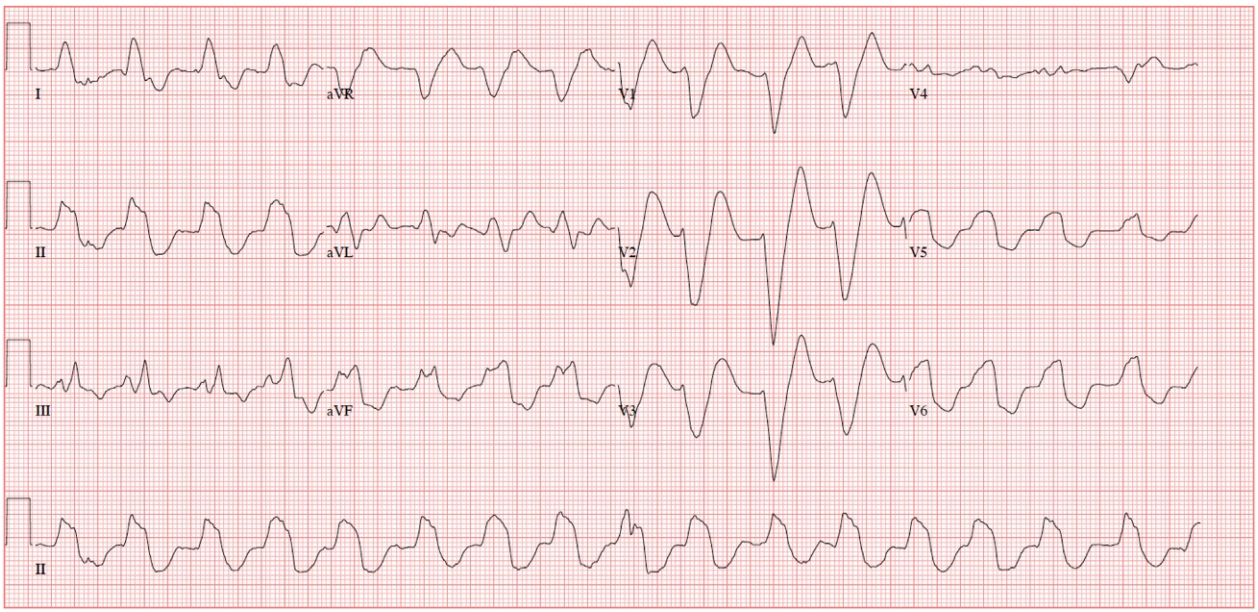


Fig. 10 ECG showing wide complex rhythm at around 100 bpm, with markedly widened, bizarre QRS complexes, in the context of hyperkalemia ($K^+ = 6.4$ mmol L^{-1}) and systemic illness. ECG recorded at 25 mm s^{-1}.

distinguish artifact from a genuine WCT, particularly if only one or two ECG leads are available, for example, on telemetry or Holter monitoring (Knight et al., 2001), and there is a danger of instituting inappropriate therapy.

For both irregular and regular WCT, electrolyte disturbance (Fig. 10), generalized ischemia, drugs, and toxins should also be considered as a cause for widening of the QRS. This mechanism may be obvious from the circumstances (e.g., cardiac arrest, antiarrhythmic drug overdose, renal failure) and/or initial blood test results. One particular circumstance worth noting is bidirectional VT in the context of digoxin overdose, since this is a "classic" finding.

Approach to Regular WCT

Prevalence and Likelihood

Before applying any diagnostic reasoning, it is important to review the most likely causes for a regular WCT presenting to medical attention, since this will help determine the pretest probability of each potential cause. Akhtar et al. performed a prospective assessment of 150 patients presenting with a regular WCT. One hundred and twenty-two patients (81%) had VT, 21 (14%) had SVT

with aberrant conduction, and 7 (5%) had preexcited tachycardias (Akhtar et al., 1988). Of those with SVT, 6 had preexisting BBB, while 15 had rate-related (functional) BBB. Similarly, Steinman et al. found that 17 of 20 patients (85%) presenting with WCT had VT, while 3 (15%) had SVT (Steinman et al., 1989). Miller et al. evaluated 650 WCTs, with the final diagnosis confirmed by electrophysiology (EP) study (Miller et al., 2006). They found VT in 73% of cases, SVT with aberrancy in 20%, and preexcited tachycardia in 6%. Two patients had SVT with abnormal baseline QRS complexes and a further two had paced ventricular rhythms. Thus, even before obtaining any clinical information, or analyzing the ECG, around 75–85% of WCT presenting for emergency care will turn out to have VT.

This section will focus on distinguishing between the two most common causes for regular WCT: VT and SVT with aberrant conduction. Clues to the presence of a preexcited tachycardia will be addressed briefly in a later section.

Diagnosis

A diagnostician has several tools at his disposal to make the proper diagnosis. These include:

- Clinical history
- Physical examination and bedside maneuvers
- ECG
- Cardiac imaging
- Cardiac implantable device Interrogation
- Invasive EP studies

Clinical history

Many of the studies looking at clinical history as an aid to the diagnosis of WCT were conducted in the late 1980s and early 1990s (Baerman et al., 1987; Tchou et al., 1988; Griffith et al., 1991). Common variables examined include age, sex, history of myocardial infarction (MI), congestive heart failure (CHF), cardiac surgery, smoking, syncope, and chest pain. Univariate analysis suggested that history of MI, age, CHF, and prior cardiac surgery were significant predictors for VT. However, history of MI remained the only independent variable on multivariate analysis, with a positive predictive value (PPV) for VT of over 95%. The PPV of age >35 years as a predictor of VT was lower at 85%. There were no strong predictors of SVT, with the best predictor being age <35 years with a PPV of 70% (Griffith et al., 1991).

Therefore, a careful history should be taken, with a focus on any known cardiac disease, such as prior MI, known heart failure and/or reduced LV function, cardiac surgery, valvular or congenital heart disease, and presence of a pacemaker or implantable cardioverter defibrillator (ICD). Any family history of cardiac disease or sudden cardiac arrest should also be determined. Any recent increase in angina-type symptoms or change in New York Heart Association functional class should also be noted. A full list of current medication should be obtained, noting any of which could cause widening of the QRS, either at therapeutic dosage or with overdose. Such agents are generally those with sodium channel-blocking effects, for example, Class 1A or 1C antiarrhythmic agents, psychiatric drugs (including tricyclic antidepressants, carbamazepine, citalopram), some antihistamines (e.g., diphenhydramine), chloroquine and hydroxychloroquine, and illicit drugs such as cocaine (Hollowell et al., 2005).

Physical examination and bedside maneuvers

Findings on physical examination of the patient may be helpful but generally nondiagnostic. Some have made the incorrect supposition that a patient with a hemodynamically stable WCT must be in SVT, but this has been shown to be incorrect. Hypotension was seen in only 36% of VT in one study, with hemodynamically stable VT being more common than SVT due to higher numbers of VT in general (Griffith et al., 1991). This was consistent with a prior study showing 30% of patients with VT present with significant clinical symptoms of poor tissue perfusion as evidenced by presyncope and syncope (Morady et al., 1985). The 2006 ACC/AHA/ESC guidelines on ventricular arrhythmias remind us that hemodynamic stability is a function of "the rate of the tachycardia, presence of retrograde conduction, ventricular function, and compensatory mechanisms" and should not be presumed to indicate absence of heart disease (Zipes et al., 2006). Neither does stable WCT reflect a benign prognosis as it can be a cause of hemodynamic deterioration. Other physical signs thought to be helpful in differentiating between SVT and VT include (Marriott, 1970; Harvey and Ronan, 1966):

- Examination of the JVP for cannon A waves, which are intermittent pulsations in the JVP, typically of varying amplitude and timing
- Variable intensity of the first heart sound (S1) on breath hold
- Variation in systolic blood pressure (unrelated to respiration)

The underlying mechanism common to these physical findings is AV dissociation. Cannon A waves are caused by right atrial contraction against a closed tricuspid valve which occurs with AV dissociation or intermittent retrograde activation of the atria. Similarly, variable intensity of S1 suggests variable degrees of opening of the mitral and tricuspid valve at the time of ventricular systole and variation in systolic blood pressure with different degrees of ventricular filling. However, though AV dissociation is quite specific for VT, it is not sensitive as VT may be associated with 1:1 retrograde activation of the atria through the conduction system. Thus, absence of these findings does not exclude VT.

Termination of a WCT with maneuvers that increase vagal tone (such as a Valsalva maneuver or carotid sinus massage) or administration of adenosine suggests that the AV node is part of the circuit and thus favors SVT over VT as the mechanism of the tachycardia. However, some uncommon forms of VT may be adenosine-sensitive or influenced by vagal tone (e.g., outflow tract VT and idiopathic left ventricular VT). The Advanced Cardiac Life Support (ACLS) Guidelines from 2015 include a class IIb indication for using adenosine if the etiology of a WCT cannot be determined, the rate is regular, and the QRS is monomorphic (Link et al., 2015). Evidence includes a small retrospective observational study of patients who had adenosine administered for WCT (Marill et al., 2009). There were 116 patients with SVT and 81 with VT. 104/116 (90%) of SVTs responded to adenosine versus 2/81 (2%) of VTs. Adenosine was felt to be safe, with only two patients experiencing angina after adenosine, and one becoming hypotensive but not requiring therapy. Please note that there is also a contraindication (Class III) in those with unstable, irregular, or polymorphic WCT as adenosine may cause degeneration of the rhythm into VF. The concern is that adenosine can cause a coronary artery "steal" syndrome through vasodilation which can lead to hypotension and myocardial ischemia in patients with coronary disease. Therefore, while termination of a WCT with adenosine favors SVT, it is not specific enough to be used for diagnostic purposes. Vagal maneuvers and adenosine may be considered for termination of a WCT in patients where the physician is certain of the presence of SVT or adenosine-sensitive VT (e.g., when the diagnosis has previously been determined with an EP study).

ECG

Examination of the ECG during the tachycardia and in baseline rhythm often yields clues regarding the mechanism of the WCT.

Baseline ECG

A prior ECG, or an ECG after termination of the WCT, will often be available and should be examined for:

- Pathological Q waves, indicating prior MI.
- ST and T wave changes, indicating acute infarction or ischemia.
- Baseline QRS duration, including whether there is preexisting BBB. If aberrant conduction is present, then it should be determined whether this closely matches the QRS morphology of the WCT. This may indicate a supraventricular origin for the WCT although there are important exceptions, such as bundle branch reentry VT. If the baseline ECG and the WCT show "contralateral" block, this suggests VT. For example, a patient with underlying RBBB would not be expected to conduct with a LBBB pattern during SVT. If the QRS duration of the WCT is narrower than the baseline QRS duration (e.g., in the presence of BBB) then this strongly suggests VT. Although extremely rare, this would be considered to be quite specific for VT.
- Preexcitation, noting that for some accessory connections (e.g., left lateral pathways) the delta wave may be quite subtle (**Fig. 11**).
- Ectopic beats. Even if the baseline ECG has narrow (<120 ms) QRS complexes, premature atrial contractions (PACs) may be seen to conduct with BBB (Ashman's phenomenon). The morphology of these beats should be compared with the WCT, since they may suggest that functional BBB could be present at higher heart rates. If frequent premature ventricular contractions (PVCs) are seen, these too can be compared to the morphology of the WCT.
- Paced beats.

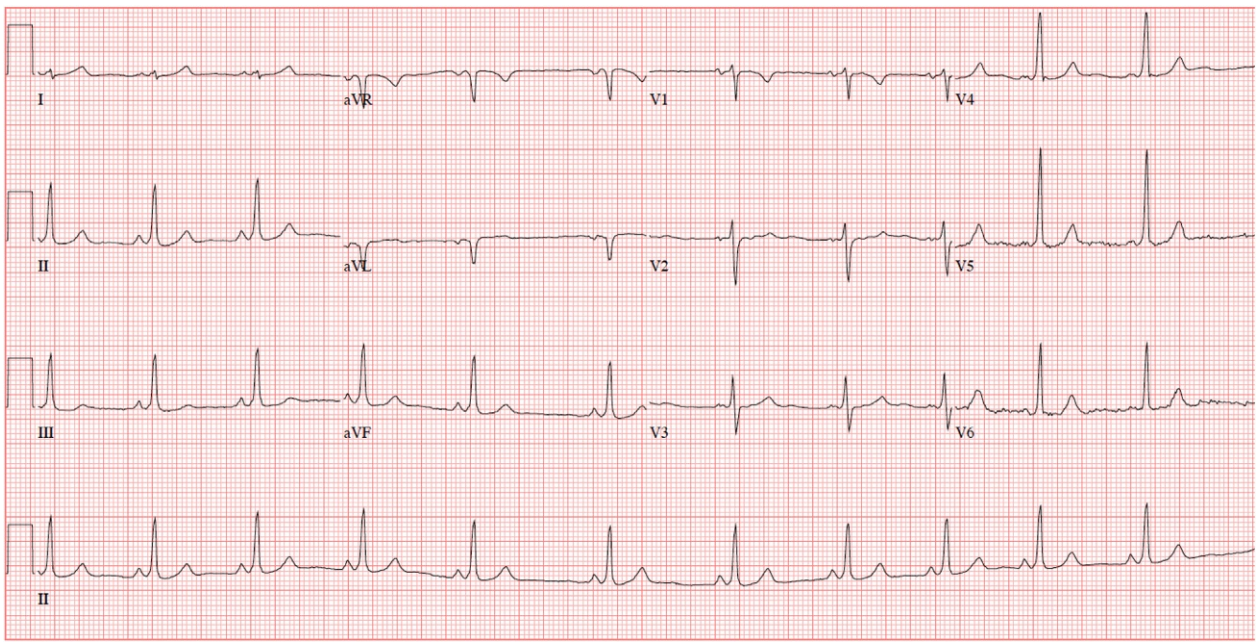


Fig. 11 ECG in sinus rhythm showing subtle preexcitation, with small delta waves seen most clearly in the inferolateral leads. ECG recorded at 25 mm s^{-1}.

- Evidence of channelopathies such as Brugada syndrome or long-QT syndrome; and other cardiomyopathies such as hypertrophic cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy (ARVC). The presence of these conditions would increase the likelihood of VT, although, of course, other supraventricular dysrhythmias could coexist.
- The baseline atrial rhythm. If sinus rhythm is present then it should be noted which ECG leads have the largest P wave amplitude, since this may help to identify dissociated atrial activity during the WCT. If AF is the baseline rhythm, then AV dissociation is unlikely to be seen during the WCT. If AFL or AT is the baseline rhythm, then it should be determined whether the cycle length of the atrial rhythm is related to the cycle length of the WCT (e.g., in a 1:1 or 2:1 relationship).
- The overall QRS morphology should be noted and can help when examining the WCT for capture or fusion beats.

ECG during tachycardia

A systematic approach to the WCT ECG may help to discern diagnostic clues. The following aspects should be examined.

AV relationship

The ECG should be carefully examined for any evidence of atrial activity, including P waves or flutter waves. If AV dissociation is seen, where independent rhythms are identified in the ventricle and atria (usually sinus rhythm) then this is highly specific for VT. Capture and fusion beats (see **Fig. 1**) are further evidence of AV dissociation and will generally also indicate VT (although note that during SVT with BBB, an appropriately timed PVC ipsilateral to the side of the BBB could "fuse" with the conducted rhythm, causing that QRS complex to be narrower). It should be noted too that AV relationships other than complete dissociation can also be diagnostically useful. Any time more ventricular beats than atrial beats are seen, it is highly likely that the WCT is VT. In addition to a (slower) dissociated atrial rhythm, this also includes 2:1 retrograde atrial activation (**Fig. 12**) or retrograde Wenckebach conduction. Conversely, if more atrial beats than ventricular beats are seen then the WCT most likely has a supraventricular origin (usually AFL [**Fig. 13**] or AT).

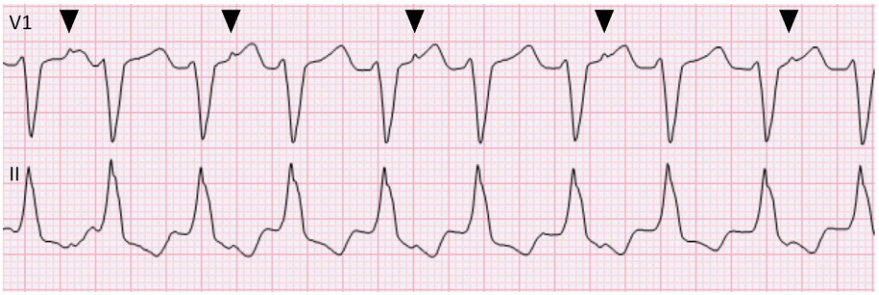


Fig. 12 ECG rhythm strip, recorded at 25 mm s^{-1}, showing a wide complex tachycardia with 2:1 retrograde conduction. P waves are visible after every other QRS (black arrowheads). This finding is highly specific for ventricular tachycardia.

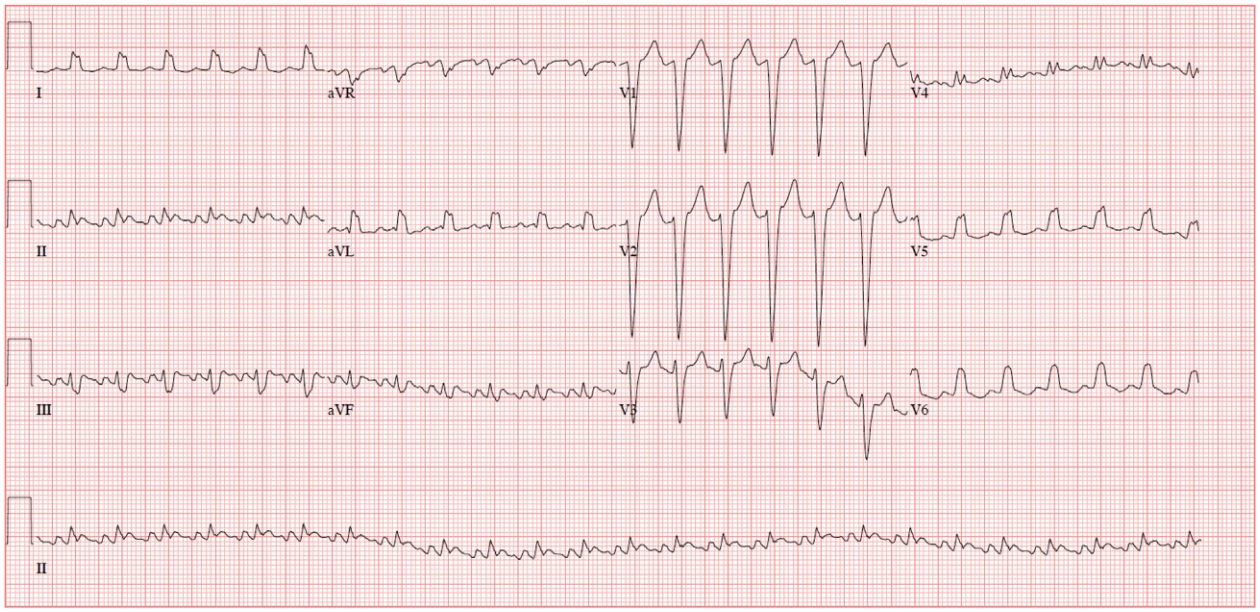


Fig. 13 ECG from a 48-year-old female with a wide complex tachycardia at 150 bpm. In the inferior leads there is the suggestion of atrial activity at twice the ventricular rate. When the rhythm was later slowed pharmacologically, the atrial rhythm was found to be typical atrial flutter. The QRS morphology is typical for left bundle branch block. ECG recorded at 25 mm s^{-1}.

The presence of AV dissociation in VT ranges from 21% to 31%. Brugada et al. noted in their prospective study that only 21% of 172 VTs had readily apparent AV dissociation (Brugada et al., 1991). Miller et al. examined the AV relationship in 473 cases of VT and 132 cases of SVT with aberrant conduction (Miller et al., 2006). The first thing to note is that the AV relationship could not be determined in around half of all cases. A 1:1 AV relationship was more likely to be seen in SVT cases (52%) than VT cases (8%). In the cases proven to be VT, AV dissociation was seen in 31%, 2:1 VA conduction in 4% (see **Fig. 12**), and VA Wenckebach in 1%, making the VA relationship diagnostically useful in 36% of VT cases. These three findings were never seen in SVT, making them 100% specific for VT. However, given the discussion earlier, the absence of these findings does not rule out VT.

QRS width

The width of the QRS during tachycardia also yields clues regarding the nature of the tachycardia. The typical QRS duration during RBBB is 120–130 ms with LBBB being longer at around 140 ms. It is unusual for SVT to have a QRS with RBBB morphology and a QRS duration >140 ms or LBBB morphology and a QRS duration >160 ms (Wellens et al., 1978). Therefore, in these cases, VT should be suspected. Some caveats are that: (1) in the presence of drugs which prolong the QRS duration (particularly Class 1C agents like flecainide) the aberrantly conducted beats can be wider than usual; (2) preexcited tachycardias can have markedly widened QRS complexes, especially when conduction is over a right or left free wall accessory pathway; (3) some patients with congenital heart disease may have RBBB with a markedly widened QRS (**Fig. 14**).

QRS axis

Analysis of the QRS in the frontal plane (I, II, III, aVL, aVR, aVF) can also be helpful in differentiating SVT from VT. The QRS axis can easily be measured and can be quite useful if the axis is between −90 and −180 degrees otherwise known as the "northwest quadrant" or "right superior" axis (**Fig. 15**). Typical fascicular blocks range between −30 and −90 degrees for left anterior fascicular block, and 110 and 150 degrees for left posterior fascicular block. Therefore, a marked deviation from these suggests VT (Wellens et al., 1978). This observation is true for VT with a RBBB configuration but has been questioned in VT with a LBBB configuration (Kindwall et al., 1988).

QRS concordance

Analysis of the precordial leads (V1–V6) is useful as it may show abnormal progression of the QRS complexes. If the QRS is predominantly positive or negative in leads V1–V6, it is said to have positive or negative concordance, respectively, which favors VT with a specificity of 90%–95% (Miller et al., 2006), see **Figs. 2** and **3**). A rhythm with negative concordance suggests an origin at the apex of the heart (e.g., consider the morphology of RV apical pacing), while positive concordance suggests an origin from the base or posterior wall of the left ventricle. The latter would include VT from this site or conduction over a left posterior accessory pathway, so it is not 100% specific for VT.

QRS morphology

Morphological criteria suggesting VT or BBB can be subtle and depend largely on a clinician's familiarity with typical bundle branch block patterns. In general, these criteria rely on identifying how closely the QRS morphology of the tachycardia matches the

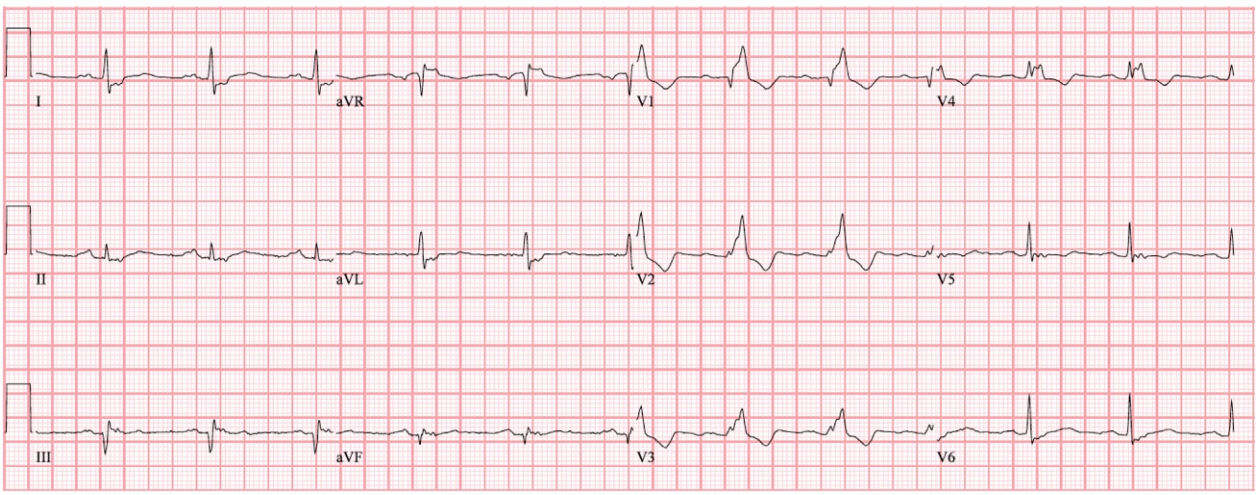


Fig. 14 Baseline ECG in a patient with congenital heart disease (Uhl's anomaly) demonstrating right bundle branch block with markedly widened QRS complexes (QRS duration 200 ms). ECG recorded at 25 mm s^{-1}.

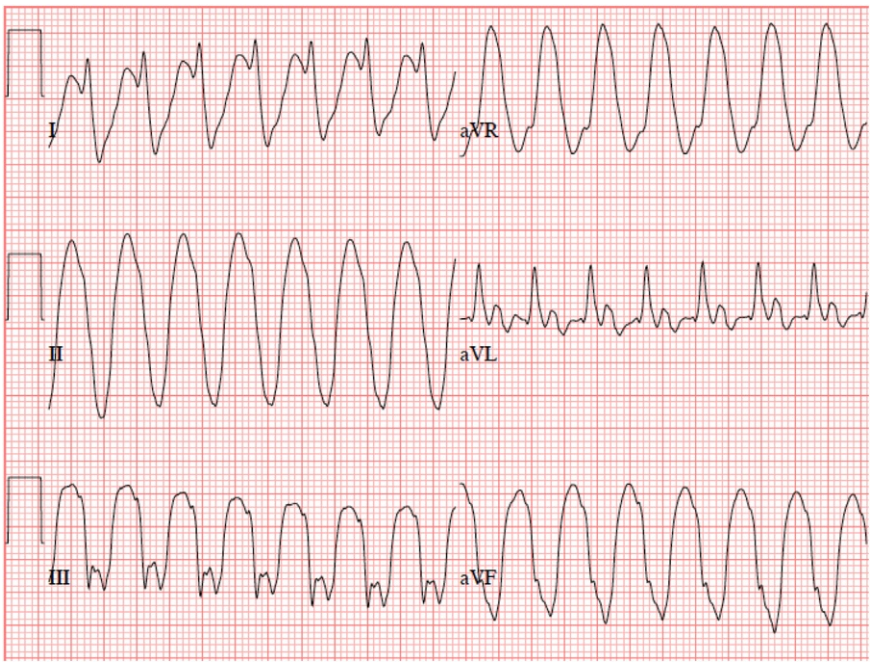


Fig. 15 ECG limb leads, recorded at 25 mm s^{-1}, showing right superior or "Northwest" axis deviation, and wide, bizarre QRS complexes, in a patient with ventricular tachycardia.

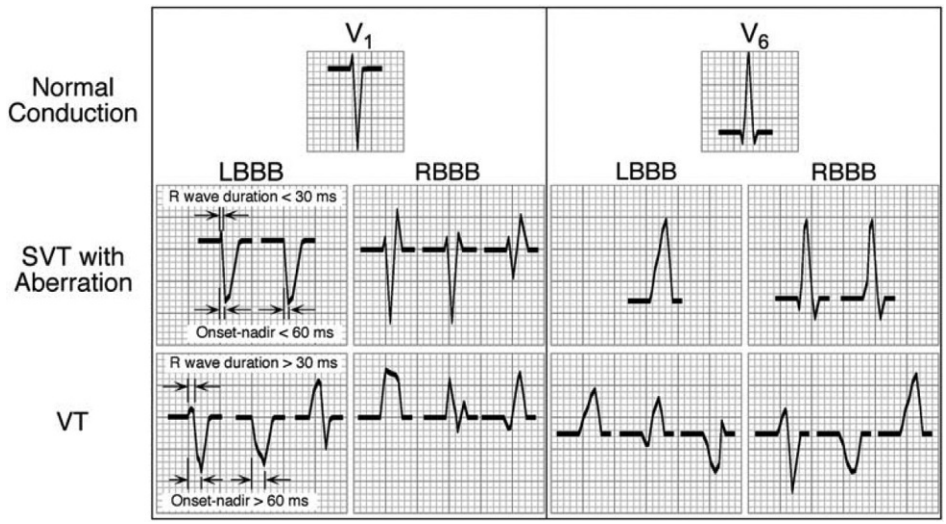


Fig. 16 QRS morphology patterns in leads V1 and V6 which may help differentiate SVT with aberrant conduction from VT. In the case of SVT with aberration, the QRS morphology shows the typical appearances for left or right bundle branch block, generally with rapid initial forces and delay later in the QRS. In the case of VT, the QRS morphology is more atypical, often with delay at the start of the QRS complex. *LBBB*: left bundle branch block; *RBBB*: right bundle branch block; *SVT*: supraventricular tachycardia; *VT*: ventricular tachycardia. Reproduced, with permission, from Miller, J. M., Das, M. K., Yadav, A. V., Bhakta, D., Nair, G., Alberte, C. (2006). Value of the 12-lead ECG in wide QRS tachycardia. *Cardioliology Clinics* **24**, 439–451 (their Fig. 5).

expected typical findings for BBB, where the closer the match, the more likely it is to be SVT with BBB, and the more disparate the appearances, the more likely it is to be VT. Within these criteria, attention is also focused on the initial portion of the QRS since in BBB the activation here is generally rapid (since it uses the normal conduction system of the working bundle) while in VT it is slowed (since it occurs by cell-to-cell conduction). Fig. 16 summarizes some of these criteria.

WCT with "RBBB-morphology" QRS In an SVT with typical RBBB, one should note:

- The QRS duration exceeds 120 ms.
- The QRS in V1 is described as triphasic with an rsr′, rsR′, or rSR′ pattern. The initial r reflects normal septal activation; the S reflects LV activation and the R′ reflects late activation of the RV. Whereas in leads I, aVL and the left precordial leads, the S wave is wider than the preceding R wave due to the late depolarization of the right ventricle via slow conduction from myocyte-to-myocyte (Wellens and Conover, 2006; Mirvis and Goldberger, 2015).
- In the absence of coexisting left fascicular block, the axis is typically normal in the limb leads.

"RBBB" QRS patterns that are highly suggestive of VT include a monophasic (R), or biphasic (qR, QR) in V1 (Marriott, 1970). It is important to note that there are limitations to these generalizations as large Q waves on baseline ECGs can make an SVT look biphasic. When these characteristics were assessed by Brugada et al., they noted 84% specificity for VT for the monophasic R, 98% specificity for a QR or RS, and 91% specificity for the triphasic configuration (Brugada et al., 1991). Therefore, looking at additional leads can help provide consistency in findings. The QRS morphology in lead V6 suggests VT if the R:S ratio is <1 (Wellens et al., 1978). However, the specificity of this finding was found to be only moderately useful with a value of 76%–79% in other studies (Miller et al., 2006; Brugada et al., 1991).

WCT with "LBBB-morphology" QRS WCTs with a LBBB morphology are more complicated and careful examination of leads V1, V2, and V6 are critical. In an SVT with typical LBBB, one would expect (Wellens and Conover, 2006; Mirvis and Goldberger, 2015):

- The QRS duration exceeds 120 ms
- Broad, notched, or slurred R waves in leads I, aVL, V5, and V6. The initial R from septal activation is reversed from that seen in a RBBB or narrow QRS, as septal depolarization occurs from right to left. Depolarization then occurs from the RV followed by LV activation.
- There is a small or absent initial r in V1 and V2 (less than 30 ms) followed by a deep S wave.
- The downstroke of the S wave is rapid without slurring or notching.

ECG criteria for distinguishing VT from SVT with BBB Kindwall et al. suggested four ECG criteria to differentiate VT from SVT in LBBB-morphology WCT (Kindwall et al., 1988). These are:

1. The width of the r in V1–V2. Durations of longer than 30 ms are suggestive of VT.
2. Presence of a Q wave in V6. As described earlier, septal depolarization in a typical LBBB occurs from right to left due to the LBBB. Thus, an initial R would be expected.
3. Onset of QRS to the nadir of the S wave in V1 or V2 being >60 ms.
4. Notching on the downstroke of the S wave in V1 or V2.

All four criteria were found to be specific for VT in the original paper (ranging from 94% to 100%), but less so in subsequent papers, including Miller et al. who reported that the specificity of QRS onset to nadir of the S wave >60 ms was 85% (Miller et al., 2006).

Where Kindwall et al. had suggested that onset of the R to the deepest part of the S in V1 and V2 in LBBB WCT could be used to differentiate VT from SVT, Brugada et al. suggested that the longest RS interval in any precordial lead exceeding 100 ms would be suggestive of VT (Brugada et al., 1991). This removes the complexity of having the observers be comfortable with recognizing the bundle branch block morphology and their individual nuances. Brugada et al. observed that RS intervals of >100 ms were only seen in VT, and that absence of any RS complex in any of the precordial leads was seen only in VT. They concluded these findings were 100% specific. Absence of RS was only seen in 21% of VTs, and an RS interval >100 ms was seen in 66% of VTs in the original report. Subsequent reports found these to be 93% specific (Vereckei et al., 2007; Vereckei et al., 2008).

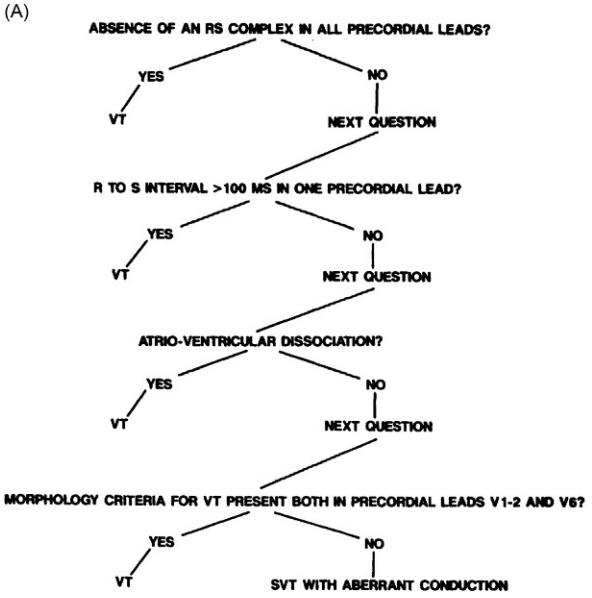
As a measure of slow conduction through myocardium, Verekei et al. proposed that a ratio of the amplitude of the initial 40 ms of the QRS to the terminal 40 ms of the QRS (V_i/V_t) of <1 would be specific to VT (Vereckei et al., 2007). These could be measured in any lead where the QRS was best visualized. They noted the $V_i/V_t < 1$ criteria had a specificity of 82% for VT.

In the subsequent year, Verekei et al. published another paper focusing on lead aVR, using the width of the initial q or r wave >40 ms as a marker for VT (Vereckei et al., 2008). While this was insensitive, with only 28% of VTs having a q or r wave in aVR, it was fairly specific with a reported specificity of approximately 92%.

Another group proposed using lead II to assess slow ventricular depolarization in VT, using the R-wave peak time (RWPT) >50 ms to detect VT (Pava et al., 2010). RWPT was defined as onset of the QRS to the first change of polarity, independent of whether the QRS deflection was positive or negative. They looked at 218 patients who had undergone EP studies for different reasons and reported an initial sensitivity of 93% and specificity of 99%. Its main benefit over the previous algorithms is simplicity. RWPT was assessed by another group, comparing it to the Verekei aVR algorithm and found it did surprisingly well despite its apparent simplicity, with 74%–85% specificity (Szelényi et al., 2013). However, its sensitivity was quite low at 62%–79% versus the Verekei aVR algorithm which had sensitivities in the 91%–93% range.

The presence of multiple WCT morphologies also suggests VT. Scar tissue can result in circuits with different exit points and thus different morphologies in VT. Miller and Das analyzed 861 patients assessed for WCT with an EP study and found that 266/524 (50%) of patients with VT had multiple WCT morphologies whereas only 31/392 (8%) of patients in SVT did (Miller and Das, 2009).

(A)



(B)

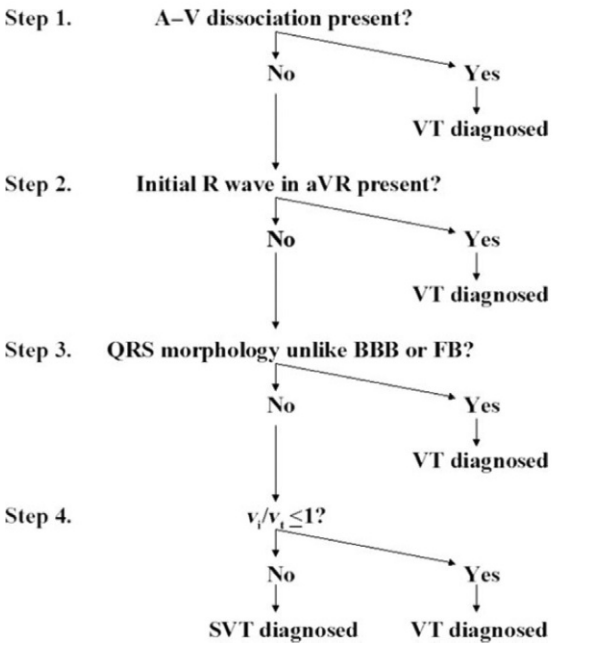
Table 1. Morphology Criteria in This Study for Ventricular Tachycardia

	SVT	VT	Sensitivity SN	Specificity SP	Predictive +Value	Predictive −Value
Tachycardia with a right bundle branch block–like QRS						
Lead V_1						
Monophasic R	11/69	39/65	0.60	0.84	0.78	0.69†
QR or RS	1/69	20/65	0.30	0.98	0.95	0.60†
Triphasic	57/69	6/65	0.82	0.91	0.90	0.83‡
Lead V_6						
R to S ratio <1	4/69	27/65	0.41	0.94	0.87	0.63†
QS or QR	0/69	19/65	0.29	1.0	1.0	0.60†
Monophasic R	0/69	1/65	0.01	1.0	1.0	0.52†
Triphasic	44/69	3/65	0.64	0.95	0.93	0.71‡
R to S ratio >1	21/69	15/65	0.30	0.76	0.58	0.51‡
Tachycardia with a left bundle branch block–like QRS						
Lead V_1 or V_2						
Any of following:*						
R >30 msec,						
>60 msec to nadir S, notched S	3/24	91/91	1.0	0.89	0.96*	
Lead V_6						
QR or QS	0/31	6/35	0.17	1.0	1.0	0.52*
Monophasic R	31/31	29/35	1.0	0.17	0.51	1.0‡

Data obtained from References 5 and 6.
*Including any Q wave in lead V_6.
†Values for the diagnosis of VT.
‡Values for the diagnosis of SVT with aberrant conduction.
SVT, supraventricular tachycardia; VT, ventricular tachycardia; +Value, predictive positive value; −Value, predictive negative value; R to S ratio, ratio of R wave to S wave voltage.

Fig. 17 (A) Stepwise algorithm from Brugada et al for differentiating VT from SVT with aberrant conduction. (B) Table outlining the criteria referred to in the last step of the algorithm. Reproduced, with permission, from Brugada, P., Brugada, J., Mont, L., Smeets, J., Andries, E. W. (1991). A new approach to the differential diagnosis of a regular tachycardia with a wide QRS complex. *Circulation* **84**,1649-59 (their Fig. 1 and Table 1).

(A)



(B)

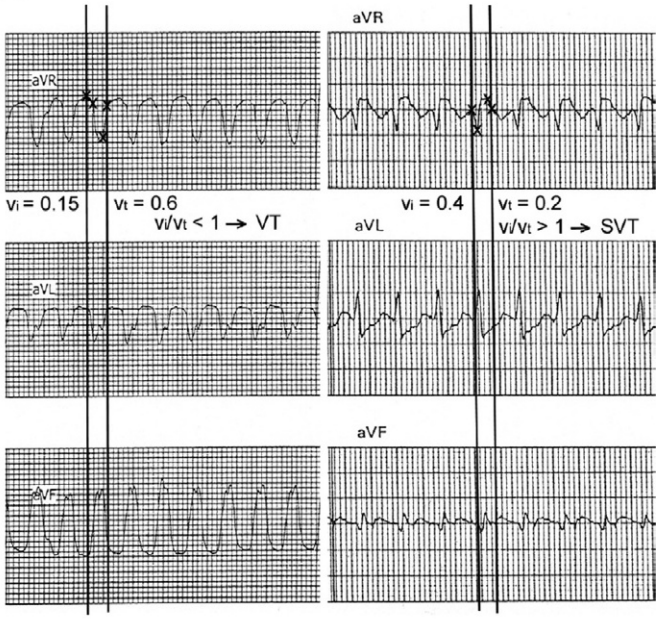


Fig. 18 (A) Stepwise algorithm from Vereckei et al for differentiating VT from SVT with aberrant conduction. (B) Method for calculating V_i/V_t for step 4 of the algorithm. V_i is the voltage change, in mV, of the initial 40 ms of the QRS complex, while V_t is the same measurement made for the terminal 40 ms of the QRS complex. *A-V*: atrioventricular; *BBB*: bundle branch block; *FB*: fascicular block; *SVT*: supraventricular tachycardia; *VT*: ventricular tachycardia. Reproduced, with permission, from Vereckei, A., Duray, G., Szénási, G., Altemose, G. T., Miller, J. M. (2007). Application of a new algorithm in the differential diagnosis of wide QRS complex tachycardia. *European Heart Journal* **28**, 589–600 (their Fig. 3); Vereckei, A., Duray, G., Szénási, G., Altemose, G. T., Miller, J.M. (2008). New algorithm using only lead aVR for differential diagnosis of wide QRS complex tachycardia. *Heart Rhythm* **5**, 89–98 (their Figs. 1 and 3).

Stepwise algorithms for distinguishing VT from SVT with BBB Several algorithms have been published to improve the sensitivity and specificity by combining multiple ECG findings. The most popular of these were published by Brugada et al. (1991), see Fig. 17) and the two algorithms by Verekei et al. (2007); Vereckei et al. (2008), see Figs. 18 and 19). Initial sensitivities and specificities were >90%, but real-world application by cardiologists and emergency doctors tends to yield poorer results (Isenhour et al., 2000; Jastrzebski et al., 2012; Szelényi et al., 2013).

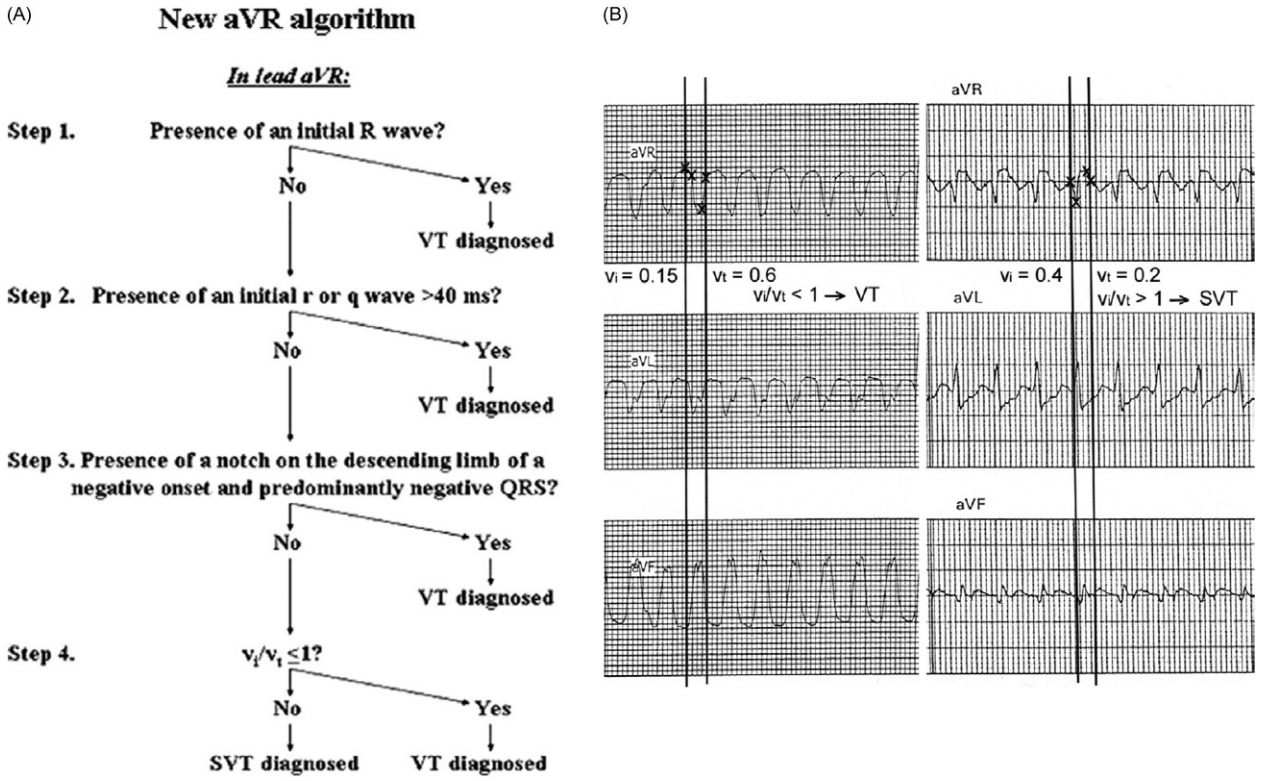


Fig. 19 (A) Simplified stepwise algorithm from Vereckei et al for differentiating VT from SVT with aberrant conduction, using Lead aVR alone. (B) Method for calculating V_i/V_t for step 4 of the algorithm. V_i is the voltage change, in mV, of the initial 40 ms of the QRS complex, while V_t is the same measurement made for the terminal 40 ms of the QRS complex. SVT: supraventricular tachycardia; VT: ventricular tachycardia. Reproduced, with permission, from Vereckei, A., Duray, G., Szénási, G., Altemose, G. T., Miller, J. M. (2008). New algorithm using only lead aVR for differential diagnosis of wide QRS complex tachycardia. *Heart Rhythm* **5**, 89–98 (their Figs. 1 and 3).

One of these studies assessed the performance of several algorithms/methods: Brugada, Bayesian, Griffith, Verekei aVR and RWPT (Jastrzebski et al., 2012). All five methods had only moderate accuracy (69%–77%). Overall, the Brugada algorithm had the best balance of sensitivity (89%) and specificity (59%) compared to the other criteria. A Bayesian approach involving 19 features (Lau et al., 2000) did not perform better than the much simpler Brugada algorithm. The Griffith approach yields slightly better sensitivity (94%) as it presumes all WCT to be VT until proven otherwise. However, this is at the expense of specificity (39%). The lead II RWPT had the highest specificity (83%) but at the expense of sensitivity (60%).

Despite the advances and further refinements in ECG interpretation, there is no single algorithm that can reliably differentiate SVT from VT. The most specific finding for VT, AV dissociation, is found only in a small proportion of VTs (approximately 30%). Attempts to increase the sensitivity unfortunately decrease the specificity. The more tools a clinician has in his toolbox, the greater the chance of an accurate diagnosis, although sometimes assuming that the WCT is VT can be the safest approach, particularly if the diagnostic criteria give disparate results.

Cardiac imaging Most of the literature on the differentiation of SVT from VT aims at interpretation of the ECG. However, imaging may also prove useful in assessing the likelihood the arrhythmia is VT, by providing an assessment for structural heart disease. While a normal echo or magnetic resonance imaging (MRI) cannot rule out VT, an abnormal study would increase the likelihood of VT (Tandri et al., 2004). Reduced LV ejection fraction is a crude measure which correlates with the risk of VT and sudden cardiac arrest (Vest and Gold, 2010). Some imaging studies have tried to provide a more refined estimate of the risk of ventricular arrhythmias. Cardiac MRI studies suggest that the extent of the ventricular scar, as well as tissue heterogeneity may be correlated to an increased likelihood of ventricular arrhythmia in ischemic heart disease (Schmidt et al., 2007). Cardiac MRI has also been shown to predict VT in other patient groups including hypertrophic cardiomyopathies (Nojiri et al., 2011; O'Hanlon et al., 2010), dilated cardiomyopathies (Gao et al., 2012), ARVC (Mavrogeni et al., 2012; Jain et al., 2008; Sen-Chowdhry et al., 2007), Tetralogy of Fallot (Tsai et al., 2010)), and chronic Chagas' disease (Rochitte et al., 2005).

Cardiac implantable device interrogation It is becoming more and more common for patients to present with an undifferentiated arrhythmia in the presence of a cardiac device. Proper interpretation of stored device episodes and electrograms may aid in the differentiation of VT from SVT and lead to the appropriate clinical management. The amount of information available will depend on the type of device (implantable loop recorder, pacemaker, or ICD), manufacturer of the device, and number of leads (single versus dual chamber).

The first consideration is that any patient with an ICD in place will either have had prior ventricular arrhythmias (secondary prevention ICDs) or have been judged to be at higher risk of ventricular arrhythmias (primary prevention ICDs). Thus, their pretest probability of VT will already be significantly elevated. However, such patients may also have BBB and coexisting arrhythmias, including SVT and other atrial arrhythmias, so it is not unusual for them to have other mechanisms for a WCT. Some device findings may be diagnostically useful.

The ventricular rate of an episode alone is a fairly poor discriminator and can lead to inappropriate therapy for SVT in up to 45% of cases at 1 year (Dorian et al., 1999). The MADIT-RIT trial noticed a very high rate of inappropriate therapies with frequent atrial tachyarrhythmias occurring in the range of 170–199 bpm and failure of the device to discriminate between atrial and ventricular tachyarrhythmias (Moss et al., 2012). ATs were significantly less frequent at higher rates (over 200 bpm). Changes in local or far-field electrogram morphology compared to the baseline rhythm are also poor discriminators for VT versus SVT with BBB, since differences may occur in both cases. In a dual-chamber device, the most useful diagnostic information can come from examining the AV relationship during an episode of WCT. In contrast to ECG interpretation, where P waves can be difficult to discern, up to 90% of VTs have been reported to show AV dissociation on dual-chamber ICDs (Swerdlow, 2001). In approximately 3% of patients with ICDs, VT occurs in the setting of AF (Stein et al., 1999). The combination of regularity of the ventricular rhythm and AF (not AFL) in the atrium is highly successful in distinguishing VT.

Invasive EP study For many of the published studies on differentiation of SVT from VT, the EP study was used as the gold standard for diagnosis, and this can also be the case in clinical practice. Pacing protocols can allow the induction of tachycardia, which can be compared to the clinical WCT. Intracardiac recordings and pacing maneuvers can identify substrates for SVT or confirm that the rhythm is ventricular. There is also the opportunity for curative ablation of SVT circuits. Ablation would not typically be employed as first-line therapy for VT in the setting of ischemic or nonischemic cardiomyopathy but could be considered for outflow tract VT, fascicular (Belhassen) VT, and bundle branch reentry VT.

Particular Types of WCT With Classic Electrocardiographic Features

Table 1 outlines several forms of WCT which may be recognized from their classic ECG appearances. Identification of these mechanisms is important since they may be treated with ablation (also reviewed in Issa et al., 2012).

Identification of Preexcited Tachycardias

Access to an ECG in sinus rhythm greatly aids in the identification of preexcited tachycardias, especially when the preexcitation is readily apparent and can be compared to the WCT being considered. It is important to remember that ventricular activation in sinus rhythm will usually involve fusion between activation over the normal conduction system and activation over the accessory pathway. Often in a regular preexcited tachycardia, ventricular activation occurs exclusively over the accessory pathway (the ECG shows maximal preexcitation), so there can be significant differences in the overall QRS morphology between these two situations. ECG clues may be used to help differentiate between VT and SVT with antegrade conduction over an accessory pathway. Brugada and coworkers developed some criteria for distinguishing preexcited tachycardias (Antunes et al., 1994). These focused on the following features:

- Preexcited tachycardias activate from base to apex, therefore leads V4–V6 are predominantly positive. Negative complexes in V4–V6 therefore favor the diagnosis of VT.
- In the absence of structural heart disease, QR complexes should not be observed during preexcited tachycardia; therefore this favors the diagnosis of VT.
- AV dissociation, as this is nearly 100% specific for VT.

Based on these criteria, they found that preexcited tachycardias were all appropriately identified. However, 25% of VTs were misdiagnosed as preexcited tachycardias, signifying caution when applying these criteria. This is not surprising, since VT with an exit near the mitral or tricuspid annulus could look identical to a preexcited tachycardia with the ventricular insertion of the accessory pathway at this site.

Approach to Irregular WCT

As listed earlier, the main differential diagnosis of an irregular WCT includes atrial dysrhythmias (AF, AFL with variable AV conduction, AT with variable AV conduction, MAT) conducted with BBB or over an accessory pathway, polymorphic VT, VF, and artifact. In keeping with the approach to regular WCT, the patient's prior medical history (including medications) and current clinical context can aid in making the rhythm diagnosis. Baseline ECGs should be examined in particular for the atrial rhythm, any evidence of ischemia, infarction, or preexcitation, and any QT prolongation or other abnormalities suggesting a cardiomyopathy. The serum potassium level should be checked since hypokalemia can induce or exacerbate QT prolongation and increase the risk of torsades de pointes VT.

Table 1 Selected wide complex tachycardia diagnoses with classic electrocardiographic features

Tachycardia	*WCT type*	*Classic ECG findings*	*Notes and references*
Right ventricular outflow tract (RVOT) VT	VT	• LBBB-morphology QRS • Inferior frontal axis • V3 R/S <1	Lerman (2015)
Left ventricular outflow tract (LVOT) VT	VT	• LBBB-morphology QRS • Inferior frontal axis • V3 R/S >1 • V3 R $=$ S	Origin can be below or above the aortic valve. Nogami (2002) and Lerman (2015)
Idiopathic LV VT (also referred to as "Belhassen VT" and LV fascicular VT)	VT	• RBBB-morphology QRS • QRS duration 100–140 ms • Left axis deviation • R/S <1 in V1–V2	"Common type" of idiopathic LV VT has left-axis deviation; other types can have rightward or normal axis Nogami (2002)
Bundle branch reentry VT	VT	• BBB pattern matching conducted rhythm • AV dissociation may be apparent	Generally seen in the context of significant baseline conduction system disease $\pm$ structural heart disease LBBB morphology is the most common (normal or left-axis deviation) Bundle branch reentry with a RBBB morphology usually has a normal or right axis deviation
Bidirectional VT	VT	• Alternating QRS axis	Associated with CPVT, digitalis toxicity, and Anderson–Tawil syndrome Wellens and Canover (2006) and Smith et al. (2006)
Atriofascicular reentry tachycardia	SVT	• LBBB-morphology QRS • Frontal axis can be normal, horizontal, or leftward • QRS duration often minimally prolonged or normal	Gandhavadi et al. (2013)

AV: atrioventricular; *BBB*: bundle branch block; *CPVT*: catecholaminergic polymorphic ventricular tachycardia; *LBBB*: left bundle branch block; *LV*: left ventricular; *RBBB*: right bundle branch block; *SVT*: supraventricular tachycardia; *VT*: ventricular tachycardia; *WCT*: wide complex tachycardia.

References: Gandhavadi, M., Sternick, E. B., Jackman, W. M., Wellens, H. J., Josephson, M. E. (2013). Characterization of the distal insertion of atriofascicular accessory pathways and mechanisms of QRS patterns in atriofascicular antidromic tachycardia. *Heart Rhythm* **10**, 1385–1392; Lerman, B. B. (2015). Mechanism, diagnosis, and treatment of outflow tract tachycardia. *Nature Reviews Cardiology* **12**, 597–608; Nogami, A. (2002). Idiopathic left ventricular tachycardia: assessment and treatment. *Cardiac Electrophysiology Review* **6**, 448–457; Smith, A. H., Fish, F. A., Kannankeril, P. J. (2006). Andersen-Tawil syndrome. *Indian Pacing and Electrophysiology Journal* **6**, 32–43; Wellens, H. J. J., Conover, M. (2006). *The ECG in emergency decision making.* (2nd edn.). Philadelphia, PA: Saunders Elsevier.

Some types of irregular WCT have very characteristic ECG appearances and are often easily recognized. Irregular VT with continuously varying QRS morphology is termed polymorphic VT. When this is associated with baseline QT prolongation and displays a typical "twisting" appearance of the QRS axis around the baseline, it is termed torsades de pointes VT (**Fig. 20**). The other circumstance where polymorphic VT is typically seen is in the context of acute ischemia (**Fig. 21**). In this case the baseline QT interval is generally normal and the QRS morphology of the tachycardia tends to vary in a more unpredictable manner than it does with torsades de pointes VT. Ventricular fibrillation (VF) also displays irregularity of ventricular activity in terms of cycle length, amplitude and axis, with a chaotic continuous waveform on the ECG (**Fig. 22**). "Coarse" VF, with larger average amplitudes, may be indistinguishable from polymorphic VT, and both can have the same hemodynamic consequences.

In cases where the QRS complexes are uniform in appearance but occur at an irregularly irregular rate, it should be suspected that the underlying rhythm is AF and that the widening of the QRS complex is due to BBB (**Fig. 23**). The ECG should then be analyzed for the typical appearances of left or right bundle branch block, as described earlier. In the presence of tachycardia and wide QRS complexes it can be hard to determine on the ECG whether the atrial rhythm is AF, AFL, or MAT; however the relative prevalence of each condition means that AF is by far the most likely rhythm. Often the atrial activity can only be determined with certainty when the ventricular rate has been slowed, for example, by medication. Also, if the bundle branch block is functional (rate-related), it may resolve at slower ventricular rates or not be present with every beat, facilitating diagnosis of the atrial rhythm.

AF with preexcitation (**Fig. 24**) is an important diagnosis to make since it has immediate consequences for the acute care of the patient and also strongly suggests that subsequent management should include an ablation procedure. The hallmarks of this dysrhythmia are:

- An irregularly irregular rhythm (which should always raise the suspicion of AF, irrespective of the QRS morphology)
- Variable QRS morphology, typically with differing QRS duration and often the presence of some narrow QRS complexes
- The wide QRS beats show a slurred onset and do not resemble typical left or right bundle branch block



Fig. 20 (A) Lead II rhythm strip, recorded at 25 mm s^{-1}, from a 51-year-old female with chronic nausea and diarrhea, after bowel preparation for colonoscopy. After an initial conducted sinus beat there is an irregular wide complex tachycardia with a characteristic "twisting" of the axis, indicating torsades de pointes VT. (B) Baseline rhythm strip, showing marked QT prolongation (around 600 ms). Her serum potassium was 2.6 mmol L^{-1}, and her medications included fluoxetine and hydrochlorothiazide.



Fig. 21 ECG recorded from a 60-year-old male with chest pain. There are repeated bursts of irregular, polymorphic VT, and the inferior leads show around 1 mm of ST elevation. This ECG was taken shortly after reperfusion of an inferior ST elevation myocardial infarction. ECG recorded at 25 mm s^{-1}.

- Very short coupling intervals may be seen (<250 ms), and when present this increases the risk of sudden cardiac arrest (Attoyan et al., 1994; Orczykowski et al., 2013)
- There is often significant left or right axis deviation.

Bayesian Approach to Diagnostic Testing

Diagnostic tests are rarely, if ever, performed on a homogeneous population of subjects, all with the same probability of having the condition and identical to the population in which the test was originally developed. When applying any diagnostic test in

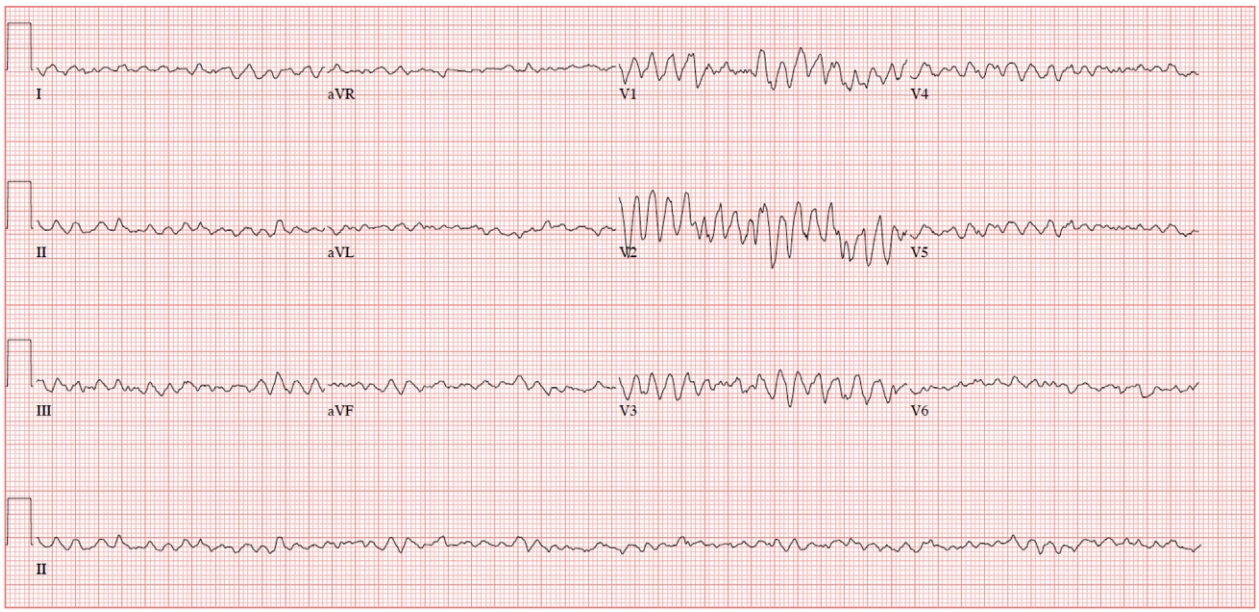


Fig. 22 12-lead ECG showing ventricular fibrillation, recorded from a 60-year-old male with a left ventricular assist device. ECG recorded at 25 mm s^{-1}.

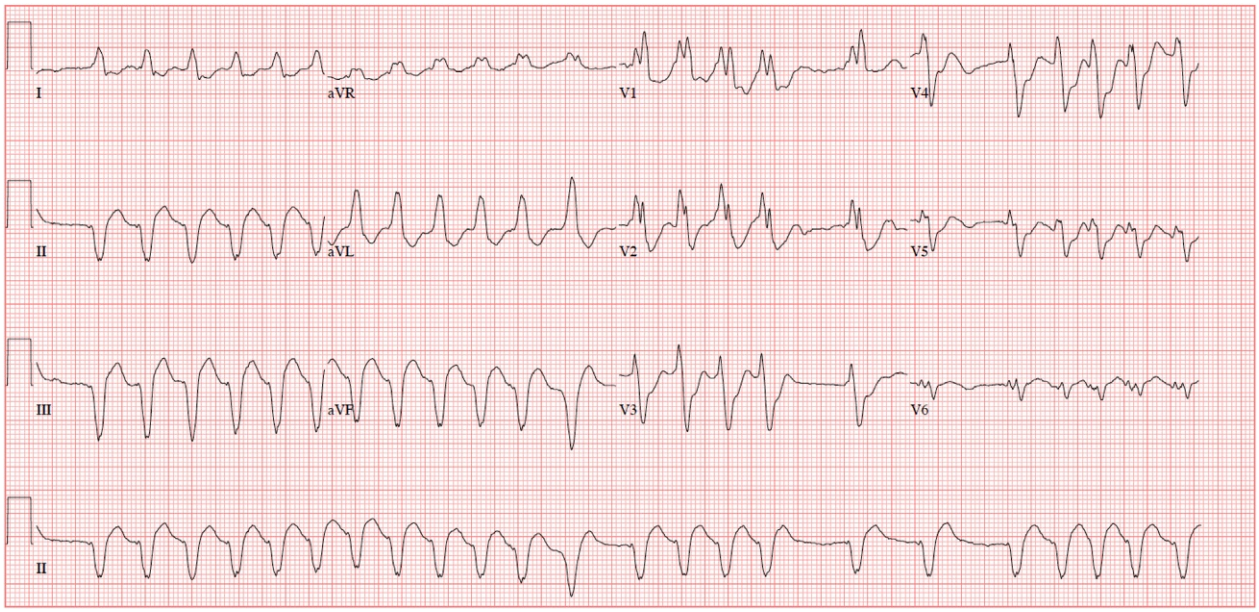


Fig. 23 ECG showing an irregular wide complex tachycardia, recorded from a 66-year-old male with ischemic cardiomyopathy. The ventricular rhythm is irregularly irregular, with no P waves visible during the longer R–R intervals, in keeping with atrial fibrillation. This is conducted with right bundle branch block and left anterior fascicular block, with significant QRS widening (QRS duration around 200 ms). ECG recorded at 25 mm s^{-1}.

medicine, it is important to consider a number of factors when interpreting the test result (or even when deciding to perform the test at all):

- How closely does the patient undergoing the test resemble the population in which the test was derived? For example, if a test was developed in a predominantly male population of subjects over the age of 60, how likely is it to perform well in an 18-year-old female? If there is a large discrepancy in patient demographics and/or comorbidities between a proposed test subject and the test derivation cohort, then there is an increased risk that the test result will be misleading. In these circumstances, it may be better not to perform the test.



Fig. 24 ECG from a 39-year-old female with presyncope. The rhythm is irregularly irregular, with variable QRS morphology, including some narrow QRS complexes. The wide beats show a slurred onset, and there are some very short R–R intervals present (160 ms). These characteristics are typical for preexcited atrial fibrillation. ECG recorded at 25 mm s^{-1}.

- What is the pretest probability that the patient undergoing the test has the condition being tested for? This should take into account the overall prevalence of the condition (is it rare or common?) and any individual patient factors which influence their baseline chance of having the condition. For example, considering a screening test for lung cancer, a smoker will have a higher pretest probability of having the condition than a nonsmoker. As we will shortly see, the pretest probability of the condition can have a critical influence on how the test result should be interpreted. Counterintuitive results can be encountered when the pretest probability is very low or very high, such that for a very rare condition a positive test result can be more likely to be a false positive than a true positive, and, conversely, for a very common condition a negative test result can be more likely to be a false negative than a true negative.
- How well does the test perform? A perfect test would correctly identify all the subjects with and without the condition 100% of the time. In clinical medicine, it is rare for a diagnostic test to perform at this level. Thus, we should always consider how diagnostically accurate the test is when interpreting the results. The performance characteristics of a test can be reported in a number of ways, including the sensitivity (the proportion of subjects with the condition correctly identified by the test, or true positive rate), specificity (the proportion of subjects without the condition correctly identified by the test, or true negative rate), positive predictive value (the proportion of subjects with a positive test result that truly have the condition), and negative predictive value (the proportion of subjects with a negative test result that truly do not have the condition). Another useful measure with regard to diagnostic testing is the likelihood ratio (LR), where the positive-likelihood ratio (LR+) is the true positive rate divided by the false positive rate, and the negative LR (LR−) is the false negative rate divided by the true negative rate. LR+ can also be calculated as sensitivity/(1-specificity) and LR− as (1-sensitivity)/specificity. A likelihood ratio of 1 means that the test has no discriminating ability, a LR >1 means that a positive test increases the chance that the condition is present, while a LR <1 means that a negative test decreases the chance that the condition is present.

The practical application of these factors to diagnostic testing was facilitated by the publication of a nomogram by Fagan in 1975 to aid in calculating the posttest probability of a condition based on the pretest probability and likelihood ratios of the test (Fagan, 1975; Caraguel and Vanderstichel, 2013). This can be applied to the diagnosis of WCT using published ECG algorithms, as shown in **Fig. 25**. For example, if we use the Vereckei aVR algorithm (Vereckei et al., 2008), then the LR+ and LR− for the diagnosis of VT can be calculated from their paper as 3.86 and 0.05, respectively (accepting that in "real-world" circumstances the LRs may not be as good) (Szelényi et al., 2013). Considering a hypothetical patient with a pretest probability of VT of 50% (i.e., VT and SVT+BBB are both felt to be equally likely) then if the algorithm is "positive" for VT, the posttest probability of VT increases to around 80%, and if the algorithm is "negative" then the probability of VT drops to around 5% (**Fig. 25A**). In this case the test (algorithm) provides a good dichotomy between the likelihood of VT or SVT+BBB depending on whether it is positive or negative. Note, however, that a negative test still means there is a 5% chance that the WCT is actually VT. A practitioner should consider whether this is an acceptable "miss rate" for a potentially life-threatening condition. We can now consider a hypothetical patient aged 70 years with prior MI and LV dysfunction, where we judge the pretest probability of VT to be 98% (**Fig. 25B**). If the algorithm is positive then the posttest probability of VT is 99.5%, however if the test is negative then the posttest probability of VT is 70%. This last point is worth stressing:

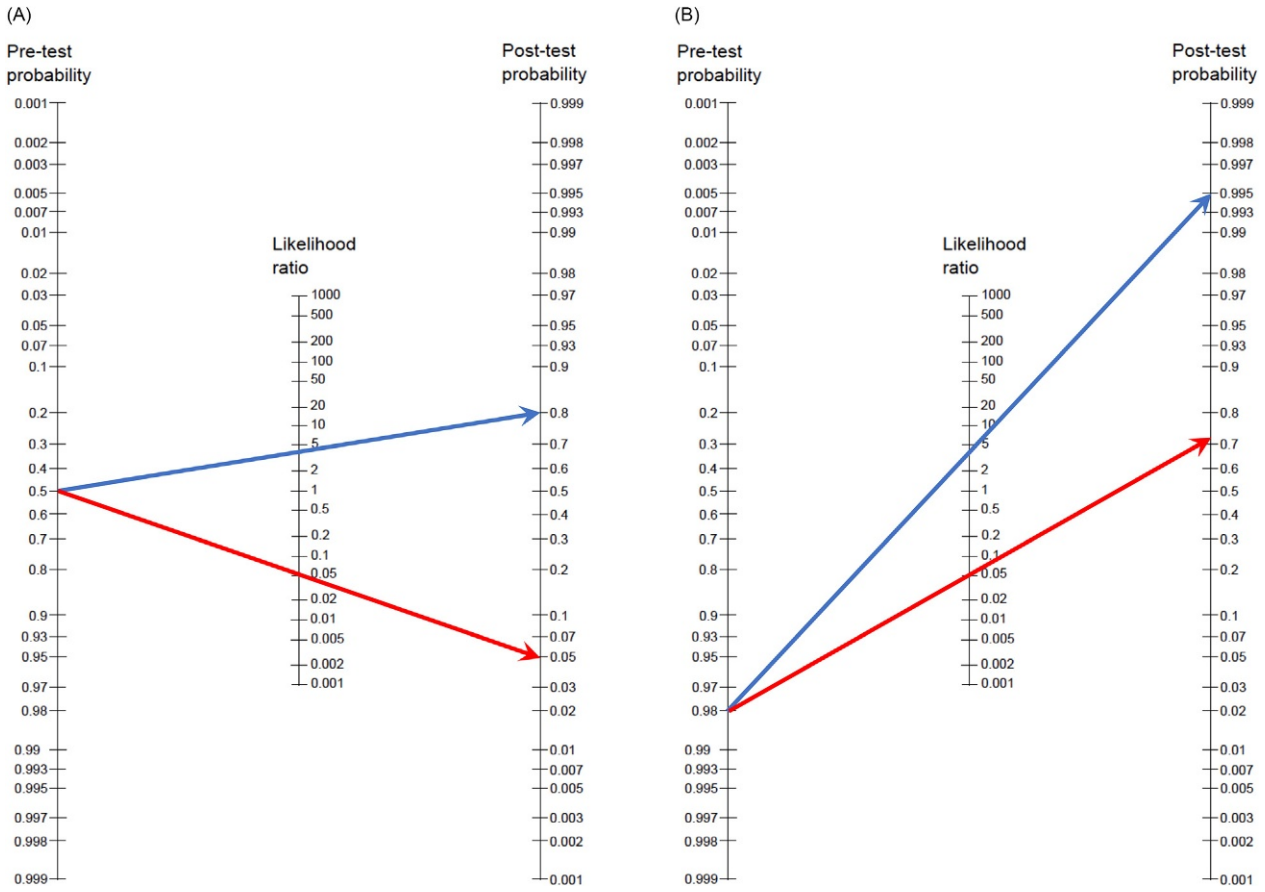


Fig. 25 Illustrative examples of the use of a nomogram for calculating the posttest probability of a condition, based on the pretest probability and performance characteristics (positive- and negative-likelihood ratio) of the diagnostic test. In Panel A, the pretest probability of the condition (in this case VT) is estimated at 0.5 (50%). The diagnostic algorithm of Vereckei et al. is applied, with positive- and negative-likelihood ratios of 3.86 and 0.05, respectively (Vereckei et al., 2008). Each arrow will then indicate the posttest probability for VT if the algorithm is "positive" (*blue arrow*) or "negative" (*red arrow*). In this case the posttest probabilities of VT are 0.8 (80%) for a positive test and 0.05 (5%) for a negative test. Panel B applies the same approach, but for a hypothetical patient with a pretest probability of VT of 98%. In this case the posttest probabilities of VT are around 0.995 (99.5%) for a positive test and 0.7 (70%) for a negative test, the latter indicating a probable false negative result. See text for full discussion. Nomograms from Wikimedia Commons.

in these circumstances a negative test (i.e., the algorithm suggests SVT+BBB) still means there is a 70% chance that the true diagnosis is VT (i.e., a false negative result). Practitioners should bear this counterintuitive fact in mind before deciding to manage the patient according to the test result alone. This reinforces the maxim: if in doubt, treat as VT.

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Wolff–Parkinson–White and Preexcitation Syndromes

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Abbreviations

AF Atrial fibrillation
AP Accessory pathway
AV Atrioventricular
WPW Wolff–Parkinson–White syndrome

Preexcitation syndrome is detected in 0.15%–0.5% of the general population. It can be asymptomatic or revealed by a paroxysmal tachycardia and in this case is called Wolff–Parkinson–White syndrome (WPW) with a variable frequency of 4%–90% according to the mode of recruitment. Atrial fibrillation (AF) is rarer. Preexcitation syndrome generally is benign, but ventricular fibrillation may be the first event of this syndrome in 0.1%–0.6% of cases per year. At the time of the curative treatment of preexcitation syndrome by radiofrequency ablation (Warin et al., 1988; Jackman et al., 1991), it is particularly important to detect the forms at risk of sudden death (Klein et al., 1979). Sudden death in preexcitation syndrome is related to the degeneration of AF with high ventricular rates into ventricular fibrillation.

If the prognosis evaluation actually is known, the management of preexcitation syndrome remains dependent on several factors as symptoms, age, sport activity, electrophysiological data, and location of the accessory pathway (AP).

Mechanism of Preexcitation Syndrome

Ventricular preexcitation syndrome is defined by the premature activation of all or part of the ventricles by atrial activity and is related to the presence of an atrioventricular (AV) AP. APs are present during fetal development and should disappear during the first 3 months of fetal development. The bypass of AV node, a physiologic barrier of AV conduction, explains the possible severity of the disease. Bypass tracts that involve the AV node are relatively rare and do not have the same adverse consequences that can occur with bypass of the AV node. Examples include atrionodal APs (called James or Brechenmacher pathways) and nodoventricular APs (also called Mahaim fibers).

Preexcitation can be associated with a congenital heart disease. The frequency (7.5%–17%) is higher in children and mainly concerns Ebstein's anomaly. All heart diseases have been associated with the preexcitation syndrome with a debatable significance but a higher risk of AF.

ECG Diagnosis of Preexcitation Syndrome

The surface ECG in sinus rhythm diagnoses preexcitation with a typical pattern associating a short PR interval (<0.12 s in adults) and a widening of QRS complex with a delta wave (Fig. 1). It is often associated with disorders of repolarization. The pattern is

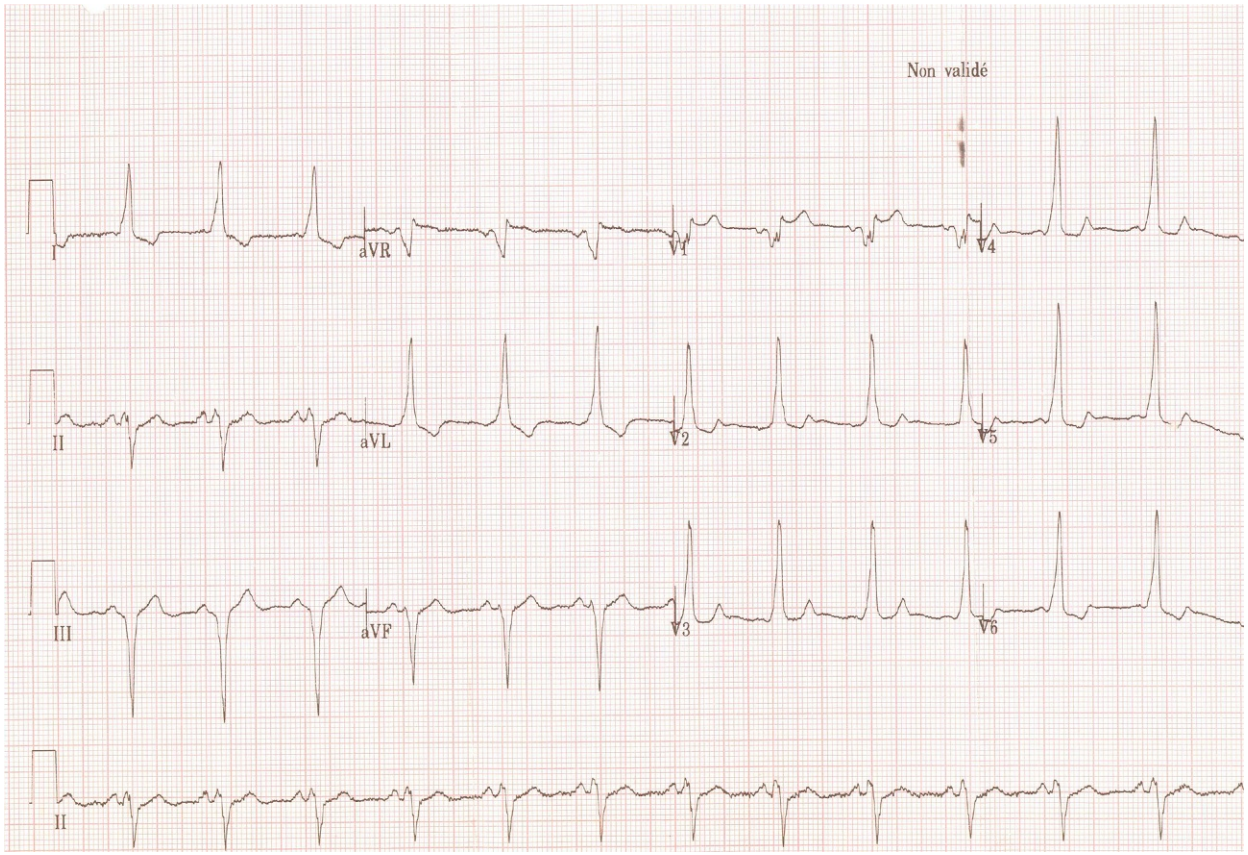

Fig. 1 Typical pattern of a preexcitation syndrome: short PR interval, large QRS complex, delta wave.

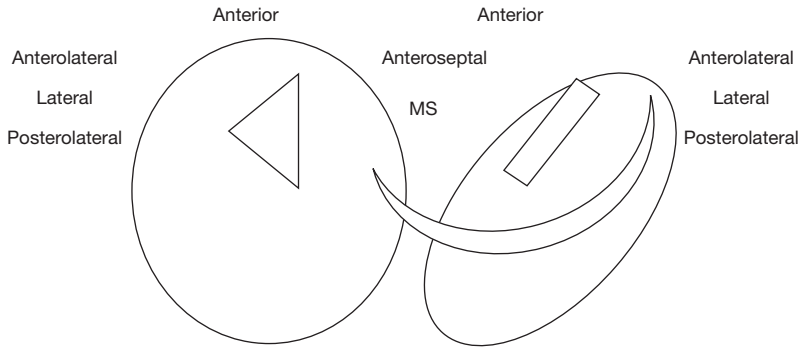

Fig. 2 Schematic cartoon figure with the different pathway locations.

dependent on the location of AP (different AP locations are shown in **Fig. 2**) but also of the properties of AV node and His Purkinje system and there is a wide spectrum of types encountered ECG.

The importance of ventricular preexcitation on ECG in sinus rhythm depends on the quality of the nodohisian conduction and the distance between the AP and the normal conduction system (Brembilla-Perrot et al., 2013a). Ventricular preexcitation is less visible the further away that the AP is from the sinus node and the normal AV system. Therefore, classical electrocardiographic criteria of preexcitation are sometimes not visible on ECG recorded in sinus rhythm (**Fig. 3**).

Spontaneous normalization of ECG with intermittent preexcitation is reported in 20%–30% of cases (Teo et al., 1991; Fujiki et al., 1990). An explanation for the intermittent loss of the delta wave in the preexcitation syndrome may result from precarious conduction over the AP and, as such, would predict a benign prognosis when AF occurs.

Several pharmacological tests were proposed to enhance the pattern of preexcitation when it is doubtful. Under the influence of exercise or emotion, or after isoproterenol infusion, the facilitation of anterograde conduction occurs only in those capable of spontaneous conduction (Easley et al., 1988; Yamamoto et al., 1990; Przybylski et al., 1980). The method was used to differentiate the concealed AP, which has lost its anterograde conduction, from the masked or intermittent preexcitation. Adenosine by blocking

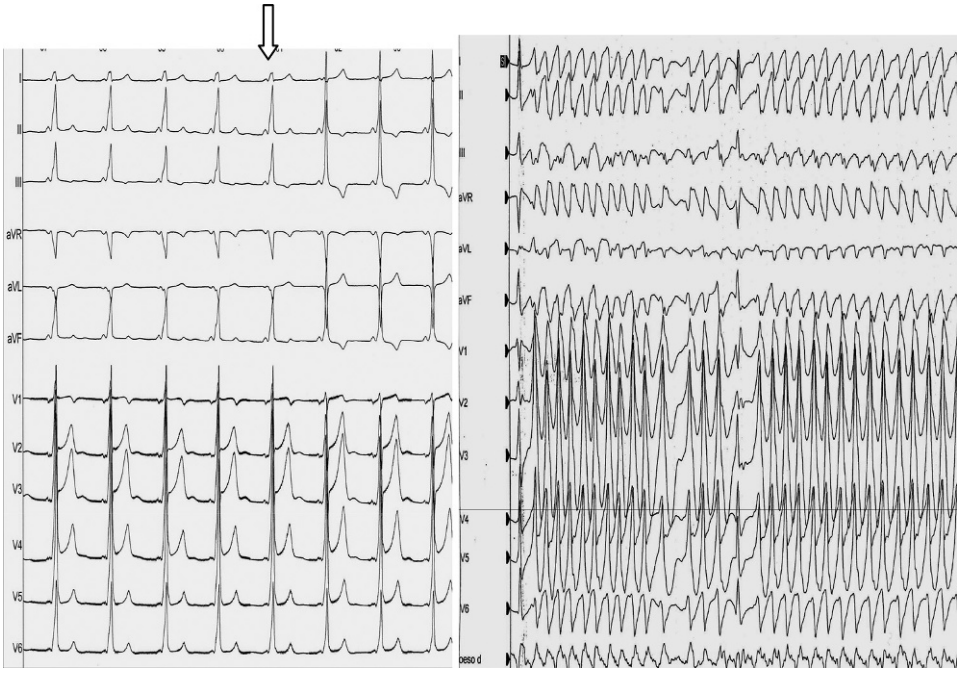

Fig. 3 Masked preexcitation; the minor signs in sinus rhythm only are visible after ablation of AP (the *arrow* indicates the beginning of ablation). However this patient presented with a spontaneous malignant form (AF with minimal cycle conducted by AP $\leq$250 ms).

the conduction in the AV node was used to unmask preexcitation (Garratt et al., 1990; Morgan-Hughes et al., 1993), but the test rarely is used and it is not specific.

Atrial pacing remains the most reliable technique to identify anterograde conduction over AP.

Natural History of Preexcitation Syndrome

A pattern of preexcitation is noted among 0.1%–0.5% of the population (Pelliccia et al., 2007). The frequency is higher in young children; AP tends to degenerate earlier than the normal AV conduction system, but the disappearance occurs early generally before the age of 1 year (50%) and more rarely before the age of 12 years (Vignati et al., 2000) and in this last case only when the refractory period of AP is long (Perry and Garson, 1990). Despite a normalization of ECG, the AP still can conduct by the retrograde way explaining the reentrant tachycardia in patients with a normal ECG. When the refractory period of AP noted before the age of 12 years is short, no disappearance can be expected (Chen et al., 1996).

Detected after the age of 5 years, spontaneous regression is rare since the appearance ECG remains unchanged in 75% after a mean follow-up of 7 years (Perry and Garson, 1990). However, electrophysiological data change with an increase in the refractory period of AP but an increased risk of AF (Chen et al., 1996).

Clinical Signs and Prognosis

The presence of AP exposes to the occurrence of two tachycardias: the most common is the reciprocal tachycardia rhythm called orthodromic AV reentrant tachycardia that usually characterizes the symptomatic forms of the disease (WPW syndrome). This tachycardia uses AV node for the anterograde conduction and AP for the retrograde conduction (**Fig. 4**). Tachycardia presents as a narrow-regular QRS tachycardia. Antidromic tachycardia, related to the anterograde conduction over AP and the retrograde conduction over AV node, is rare and generally a finding of electrophysiological study (**Fig. 5**). The tachycardia presents as a wide QRS tachycardia mimicking a ventricular tachycardia.

The less common is AF (10%) but represents the possible severity of the condition when the AP has a short refractory period: arrhythmia is conducted in 1/1 to the ventricles and can degenerate into ventricular fibrillation, leading to sudden death (**Fig. 6**). The sudden death presentation has mainly been reported in athletes. This is likely because they are subject to a high level of catecholaminergic influence that facilitates the occurrence of arrhythmias and improves conduction in the AP.

However, the prognosis of preexcitation syndrome is discussed according to the type of study population and recruitment. If this is a population detected in preventive medicine, the risk of sudden death is low. However, this event may occur in a subject completely asymptomatic until then, with a similar impact to that of symptomatic subjects: 26%–56% of patients with a



Fig. 4 Induction of an orthodromic AV reentrant tachycardia, when the refractory period of AP is obtained.

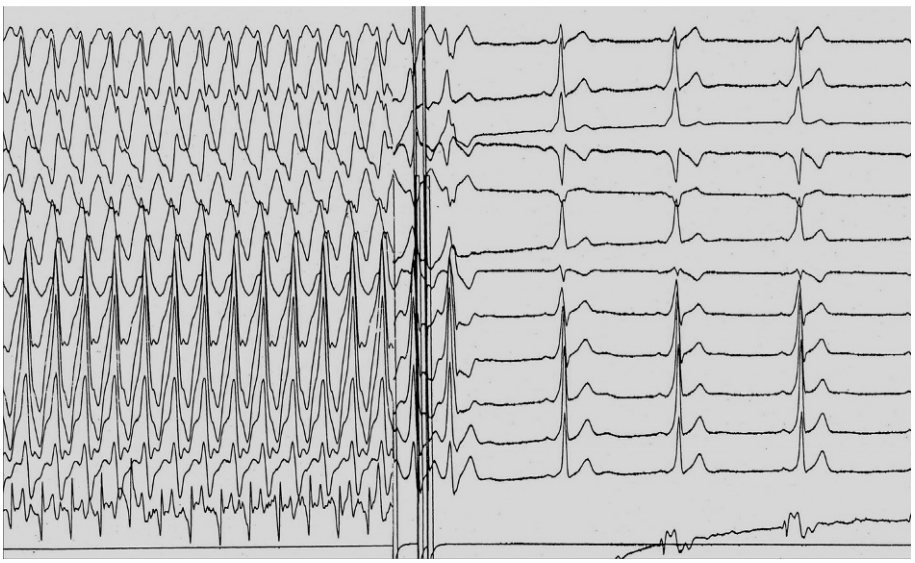

Fig. 5 Antidromic tachycardia (stopped by atrial stimulation): wide QRS tachycardia presenting as a ventricular tachycardia).

preexcitation syndrome were asymptomatic before sudden death (Klein et al., 1979; Timmermans et al., 1995; Torner Montoya et al., 1991). Sudden death incidence however is low and rare in children (0.02%–0.6% per year) (Timmermans et al., 1995; Munger et al., 1993; Beckman and Lamb, 1968; Fitzsimmons et al., 2001; Pappone et al., 2003; Brembilla-Perrot et al., 2010). The most recent study (Fitzsimmons et al., 2001) reports a sudden cardiac death risk of 0.02%/patient/year, while the supraventricular tachycardia incidence was 1% per patient-year. In addition, adverse events sometimes have been reported in very old patients or very early but exceptionally in young children between 6 days and 12 years, especially in cases of associated congenital heart disease. In symptomatic subjects, adverse event may be due to a prolonged supraventricular tachycardia responsible for ischemic events or heart failure. Sudden death also can be related to the use of drugs that promote conduction in the AP as calcium channel blockers (Brembilla-Perrot et al., 2010) or more rarely digoxin. The risk of sudden death is low, but these sudden deaths are very regrettable, concerning patients with normal heart but the presence of AP and easy to cure. Therefore, the detection of patients with preexcitation syndrome at risk of sudden death is fundamental.

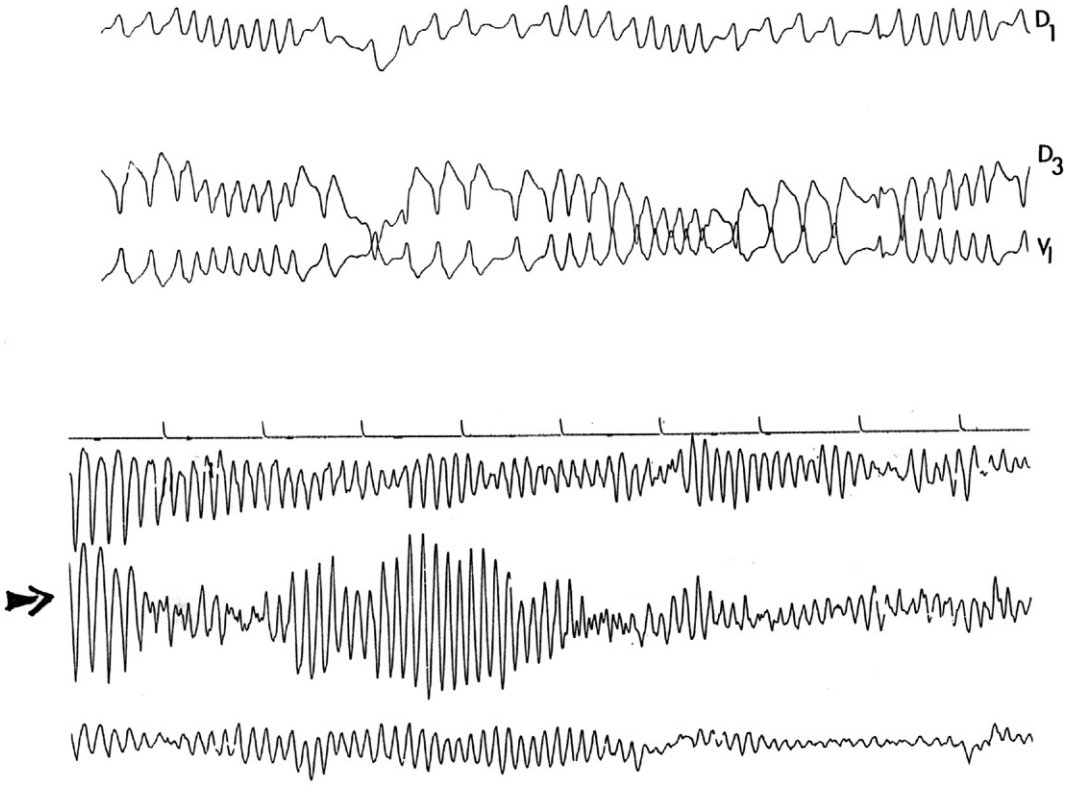

Fig. 6 AF conducted by AP and degenerating into ventricular fibrillation, classical (but rare cause of sudden death in preexcitation syndrome).

Methods of Evaluation

Several noninvasive methods have a low sensitivity and specificity:

ECG: The pattern of preexcitation on 12 lead surface ECG, the permanent or intermittent feature are not specific enough, although posteroseptal location is more frequently noted in patients at risk of rapid arrhythmias (Wellens, 2005) and the intermittent preexcitation is often associated with a benign form. False tests were reported and other tests are required in subjects at risk of arrhythmias as athletes.

Exercise testing was reported at first by Levy et al. (1979) as an important test of prognosis: the abrupt disappearance of preexcitation during an exercise stress testing was proposed as a sign of long refractory period of AP (Levy et al., 1979; Bricker et al., 1985; Strasberg et al., 1980; Sharma et al., 1987; Czosek et al., 2011) (**Fig. 7**) and argues that the AP is benign. In subjects who are not of athletic competition, this disappearance is sufficient to stop the investigations (class I level B grade of recommendation) (Page et al., 2016). While specific, this finding is seldom observed because exercise improves the conduction in the normal AV node conduction system, which can just mask the AP. The gradual loss of preexcitation can be noted in malignant form (Daubert et al., 1986). Therefore, the sensitivity is low (20%) and preexcitation can persist in benign form. Exercise testing is not useful as a provocative test for arrhythmia. However, occasionally the preexcitation will become manifest preexcitation during exercise.

Holter monitoring is rarely useful but can detect spontaneous asymptomatic tachycardias in children who rarely complain of tachycardia as in study of Paponne et al. (2004).

Pharmacological tests (class I antiarrhythmic drug injection) was used several years ago: the disappearance of the pattern of preexcitation was reported as a sign of long refractory period of the AP (Wellens et al., 1980); however, many false positive tests were reported (Critelli et al., 1984) and the test is no longer used.

Electrophysiological study appears as the most reliable method to establish the prognosis of the preexcitation syndrome.

Methods: Patients might be studied by transesophageal (Brembilla-Perrot et al., 2007) or intracardiac route. The first route is indicated in asymptomatic patients and the second in symptomatic patients to perform catheter ablation of AP in a second time. Several advantages concern esophageal route: (1) the patient is not hospitalized and is leaving hospital after study. Only those with induced atrial arrhythmias are awaiting and followed until sinus rhythm is restored. (2) No radiation is used (Bacher et al., 2005) and catheter-related risks are avoided (Paponne et al., 2004; Vitello et al., 1998). (3) The method is feasible from the age of 5 years without sedation (and in newborns) while general anesthesia is required in young children for an intracardiac catheter study. (4)

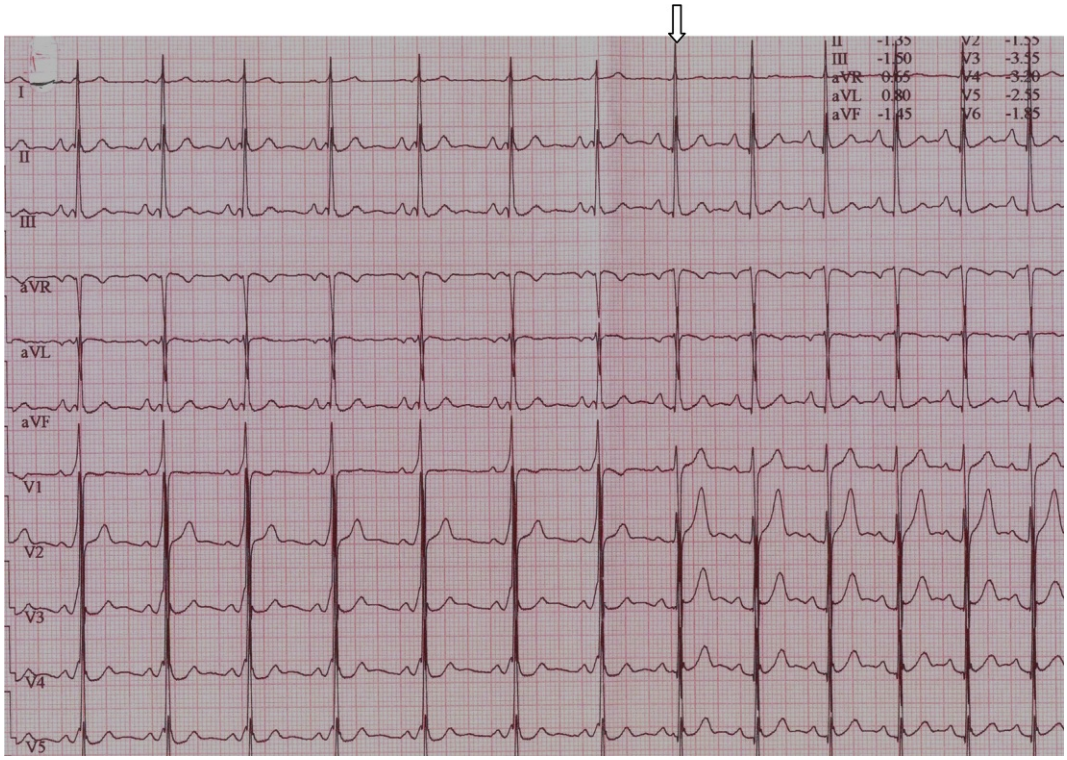

Fig. 7 Abrupt disappearance of preexcitation, sign of a benign form.

There is an overestimation of AF induction in the young patients when intracardiac study is used (Brembilla-Perrot et al., 2001). However, there also some disadvantages. (1) The main problem is related to the appropriate placement of the esophageal electrode complicated by an important gag reflex in children younger than 10 years. (2) The method is associated with chest pain and anxiety related to pacing; the presence of the parents recommended in young children and rapid rates of pacing should be avoided in children. (3) Finally, it is not possible to pace atria in 5%–10% of patients.

It should be noted that all electrophysiological studies in preexcitation syndromes may be dangerous and should be performed with an external defibrillator ready to be used, because a poorly tolerated arrhythmia can be induced in asymptomatic or symptomatic patients.

Cardiac stimulation is performed with a biphasic stimulator. Pulses of 1 ms duration and 0.5–3 mA are used for intracardiac study and pulses of 10 ms duration, output from 8 to 18 mA are used for esophageal study. For a simple intracardiac electrophysiological study, only one catheter is needed: a bipolar silicone esophageal lead or one bipolar intracardiac catheter.

The classical protocol is as following:

Incremental atrial pacing is performed until second degree AV block occurred. The maximal rate of 1:1 conduction over AP is noted.

Programmed atrial stimulation at a basic cycle length of 600 and 400 ms with the introduction of one and two extrastimuli is performed: the disappearance of preexcitation syndrome indicates that the refractory period of AP is longer than that of the AV node. The coupling interval (S1S2 or S2S3) at which the AP becomes refractory will depend on the drive train cycle length (S1S1 interval), with shorter drive trains associated with shorter refractory periods.

The method also is used to induce a supraventricular tachycardia, generally an orthodromic tachycardia, rarely an antidromic tachycardia, or an atrial tachycardia or fibrillation.

These data should be studied under adrenergic situations, except in patients who have a form at risk of sudden death in control state. Two methods are used: atrial pacing might be repeated during an exercise testing which is the most physiological situation, but which is difficult to perform. Many authors prefer the infusion of isoproterenol, a beta-adrenergic drug and which is simpler to use during an electrophysiological study (Crick et al., 1985; Mabo et al., 1992). Isoproterenol (0.02–1 μg min^{-1}) is infused to increase the sinus rate to >130 min^{-1} and the pacing protocol is repeated (Wellens et al., 1981). Beta-adrenergic stimulation results in shortening of the anterograde AP refractory period and in increasing in ventricular rates during atrial pacing and during AF. Isoproterenol test was also previously used to verify the efficacy of antiarrhythmic drug before the time of catheter ablation of AP. The loss of efficacy of some antiarrhythmic drugs was demonstrated after isoproterenol administration (Brembilla-Perrot et al., 1985), although the use of isoproterenol infusions in this setting can be controversial.

At the end of the protocol, using the following definitions, the prognosis of the preexcitation syndrome can be established:

- Sustained AF or reciprocating tachycardia is defined as a tachycardia that is longer than 1 min. The exact duration of induced tachycardia to be considered as pathological is controversial and varies from 30 s up to 5 min.
- Conduction over the AP evaluated by the measurement of the shortest atrial cycle length at which there is 1 to 1 conduction over the AP and the shortest atrial tachycardia cycle length at which there is 1 to 1 conduction over the AP.

The preexcitation syndrome is considered as presenting a risk of sudden death when the following association is observed (Brembilla-Perrot et al., 2010; Milstein et al., 1986; Klein et al., 1989): sustained AF is induced and the shortest RR interval between preexcited beats is ≤250 ms in the control state in adults, ≤220 ms in children (Bricker et al., 1985) or ≤200 ms during isoproterenol infusion (Strasberg et al., 1980; Brembilla-Perrot et al., 2007, 2014). These criteria were those classically retained before the recommendations of 2012 (Cohen et al., 2012). It should be noted that for Klein et al. (1979) have broken up the risk of sudden death on the basis of the shortest RR interval during AF as follows: (1) definite risk, shortest preexcited RR interval <220 ms; (2) probable risk, shortest preexcited RR interval <250 but >220 ms; (3) possible risk, shortest preexcited RR interval <300 but >250 ms; and (4) negligible risk, shortest preexcited RR interval >300 ms. Electrophysiological study now was considered as negative if no tachycardia was induced and a long refractory period of AP (>250 ms in control state and >200 ms after isoproterenol) was noted. Recent papers have looked at labeling a patient as being considered high-risk (malignant) only using minimal cycle length of preexcitation in the absence of inducible AF: in the PACES/HRS 2012 Management of Asymptomatic Preexcitation Recommendations it was recommended that in the absence of inducible AF the minimal cycle length of preexcitation should be used as an alternative (Cohen et al., 2012).

General Results of Electrophysiological Studies

The exact nature of the preexcitation syndrome is assessed. Most preexcitations are related to an AV AP (Kent bundle): the degree of preexcitation increases during premature atrial stimulation until the refractory period of AP is reached, because the conduction does not change in AP with the shortening of atrial cycle length and increases in the AV node. Rarely preexcitation is related to a nodoventricular AP or Mahaim bundle; the degree of preexcitation remains unchanged during premature atrial stimulation. Furthermore, atrial pacing also is an important method to detect or confirm or infirm the presence of a preexcitation syndrome when delta wave is not evident.

In symptomatic patients it is easy to reproduce the spontaneous tachycardia. Reentrant tachycardias are easily induced by stimulation (**Fig. 3**): orthodromic tachycardia which is a reciprocating tachycardia using the normal AV conduction system for the anterograde conduction and the AP for the retrograde conduction is rarely induced in asymptomatic patients (<10%) (Critelli et al., 1984; Brembilla-Perrot et al., 2007) but represents the most frequent tachycardia of symptomatic patients complaining of tachycardia and palpitations (90%) (Rinne et al., 1987).

In patients with spontaneous adverse events the sensitivity of electrophysiologic study to induce sustained AF with the shortest RR interval between preexcited beats ≤250 ms in the control state or ≤200 ms after isoproterenol infusion also is excellent (Brembilla-Perrot et al., 2010). Therefore, electrophysiological study is considered as the most sensitive and specific method to assess the prognosis of preexcitation (Wellens, 2005; Milstein et al., 1986; Klein et al., 1989).

AF easily is induced during intracardiac studies by salvos of rapid atrial stimulation and is not specific (Brembilla-Perrot et al., 2001). AF induction by intracardiac programmed stimulation is obtained in 27%–75% according to the studies, in asymptomatic patients and in patients with only documented reentrant tachycardia (Rinne et al., 1987); AF is induced in 95% of those with documented AF (Rinne et al., 1987). The important variations of the incidence of induced AF depends on the technique of programmed stimulation, on the interpretation of the duration of induced arrhythmia and on the use of isoproterenol infusion or other means to reproduce the effects of adrenergic stimulation. In our experience, the induction of AF during transesophageal pacing is more clinically significant than with intracardiac pacing (Brembilla-Perrot et al., 2001): AF induction is rarer with TEE pacing from 10% to 30% according to the age in patients without documented AF (less false positives) and remained sensitive to induce AF in those with documented AF (95%). The incidence of induced AF also depends on the presence of associated heart disease and on the age of the patient: AF is rarely induced in children younger than 10 years (Brembilla-Perrot et al., 2007), is induced in 20% of teenagers and adults without heart disease and becomes relatively frequent in elderly (31%) (Brembilla-Perrot et al., 2016).

Ventricular tachyarrhythmias also are easily used in asymptomatic or symptomatic patients by programmed ventricular stimulation and are not specific in preexcitation syndrome: ventricular fibrillation is induced in 4% syndrome and nonsustained multiform ventricular tachycardia in 37% of them (Brembilla-Perrot et al., 1987).

Antidromic tachycardia which is a reciprocating tachycardia using the AP for the anterograde conduction and the normal AV conduction system is a rare finding (5%), more frequently noted in young patients with a good retrograde normal VA conduction or in patients with several APs and seems more frequent in patients at risk of rapid arrhythmias.

- The presence of syncope does not increase the risk to find a potential dangerous form in adults (Auricchio et al., 1991); in young patients (<25 years) the significance of syncope seems different and associated with occurrence of AF with a rapid ventricular response (Paul et al., 1990; Brembilla-Perrot et al., 2013b). Reentrant tachycardia remains the main cause of syncope (Brembilla-Perrot et al., 2013b).

- The incidence of forms considered at risk of rapid arrhythmias is similar in symptomatic and asymptomatic patients and concerns 10% of the total population with preexcitation (Milstein et al., 1986; Auricchio et al., 1991; Brembilla-Perrot and Ghawi, 1993; Michelucci et al., 1988; Fan et al., 1991), independently on the age of the patient (Auricchio et al., 1991).
- In asymptomatic patients important variations of results also are reported. The percentage of induction of reentrant tachycardias varies from 11% to 50%. The percentage of AF induction varies from 0% in children <11 years (Brembilla-Perrot et al., 2007) to 20%–50% in adults. The percentage of induction of AF induction with a short RR interval between preexcited beats varies from 15% to 25%. These variations depend on the age of patient (Michelucci et al., 1988; Fan et al., 1991). Children considered as asymptomatic sometimes have spontaneous tachycardia without a clear feeling. In our experience, AVRT induction is not rare (most children recognize the tachycardia usually well tolerated). In the study of Paponne et al. (2004) five apparently asymptomatic patients in the control group had documented silent episodes of sustained AF at Holter monitoring. They remained asymptomatic despite extremely rapid ventricular responses over AP. High incidences of events (one sudden death and two ventricular fibrillations) were reported in these "asymptomatic" children, who had documented rapid AF and were not treated.

The exact clinical significance of the electrophysiological form at risk of sudden death in asymptomatic patients remained controversial. A low incidence of adverse events during a mean follow-up of 4 years was reported in previous studies using the intracardiac evaluation of preexcitation (Klein et al., 1989; Brembilla-Perrot and Ghawi, 1993; Leitch et al., 1990). However several studies of Pappone and Santinelli recently have shown that electrophysiological study could predict sudden death of asymptomatic patients (Pappone et al., 2003; Paponne et al., 2004; Santinelli et al., 2009). In 2009 (Santinelli et al., 2009) they consider that a short AP ERP <240 ms, the tachycardia inducibility and the presence of multiple APs are the main factors of risk in children.

Besides the serious forms, 75%–80% of asymptomatic patients have a benign form of preexcitation.

Indications of Electrophysiological Studies in Preexcitation Syndrome

The indications are now large in symptomatic patients to perform in a second time the catheter ablation of patients complaining frequent sustained tachycardias. The study should be performed using intracardiac catheterization (Page et al., 2016).

In patients with syncope, but no documented tachycardia, electrophysiological study is necessary and might be performed by transesophageal route because the role of the AP in the occurrence of syncope remains rare in adults (Auricchio et al., 1991).

In patients who have a documented rapid or syncopal AF, electrophysiological study is not indicated, because direct AP catheter ablation is recommended (Page et al., 2016). The induction of AF should be avoided.

In asymptomatic patients, the indications are more debatable (Page et al., 2016).

- In asymptomatic patients who are not competitive athletes, the abrupt disappearance of preexcitation is sufficient to stop the investigations (class I, level B grade of recommendation) (Czosek et al., 2011; Page et al., 2016).
- In other patients it is recommended to use techniques with a low risk of adverse events but a good diagnostic value is mandatory. Esophageal route when possible should be the preferred means of evaluation, because the probability to find a form at potential risk of sudden death remains rare (10%).

Some indications of a systematic electrophysiological are actually recommended:

1. Most of sudden deaths have the peculiarity to occur during exercise (Wiedermann et al., 1987). Because of the important development of sports from the infancy to the elderly, it is important to detect those patients with a preexcitation at risk of sudden death who practice a sportive activity; the indication generally begins after 10 years, because the risk of induction of a rapid AF is very small and the level of sport still low. The competitive athlete should be studied in all ranges of age (Cohen et al., 2012; Heidbüchel et al., 2006). The indications are also recommended in professions with a high level of sportive activity (policemen, soldier, fireman, . . .).
2. Electrophysiological study is recommended in patients with high responsibility profession such as professional pilot (plane, truck, bus, train).
3. These indications are largely accepted in teenagers and adults up to middle age. The indications in children or elderly are more controversial.

In children, it is rare to indicate an electrophysiological study <10 years of age because AF is unusual in the absence of associated heart disease (Vignati et al., 2000; Brembilla-Perrot et al., 2007). The recommendations of 2005 (Wiedermann et al., 1987) reported that "in children <12 years of age, the risk of AF and/or SCD is considered virtual, and assessment of the risk may be postponed." However the age of 5 years is the recommended age for Pappone (Pappone et al., 2003; Santinelli et al., 2009). Furthermore, conduction over AP and normal AV conduction system are more rapid without probably a clinical significance: in the study of Bromberg (Bromberg et al., 1996; Sarubbi et al., 2005) a cycle length <220 ms in basal state is considered at risk of severe arrhythmias in children <18 years; in adults, the value of ≤250 ms is admitted as a sign of a dangerous form. However, because rare sudden deaths were reported in children (Santinelli et al., 2009; Sarubbi et al., 2005), the indications should be large in young children practicing sports competition and in all children >10 years.

In elderly, the shortest atrial pacing cycle length with 1:1 anterograde conduction via the AP increased progressively with age (Brembilla-Perrot et al., 2016; Michelucci et al., 1988; Fan et al., 1991; Rosenfeld et al., 1991). However, the propensity for AF was shown to be higher in older patients compared to younger patients (Brembilla-Perrot et al., 2016; Michelucci et al., 1984). While the exact mechanism is uncertain, degenerative changes associated is the most commonly proposed mechanism and the dispersion of

atrial refractoriness increases progressively with age (Michelucci et al., 1984). The risk to have a severe arrhythmia as the first manifestation of preexcitation syndrome in an old patient was previously reported (Brembilla-Perrot et al., 2003, 2010). High-level sporting activity is rare in elderly, but other causes for adrenergic tone increase might be encountered, as important surgery.

Therefore, because of the increase of the sport in all ranges of age and in particularly in young children or after 60 years, the risk of occurrence of a potentially severe arrhythmia in an asymptomatic patient should be not underestimated.

Management of Preexcitation Syndrome

Acute Treatment of Arrhythmias (Page et al., 2016)

In the case of hemodynamically stable narrow-complex QRS tachycardia, after failure of sinocarotid massage, adenosine triphosphate injection is the recommended treatment (class I, level B). Rarely other treatments are required (class IIA): Intravenous diltiazem, verapamil (level of evidence: B-R), or beta blockers (level of evidence C-LD) can be effective for acute treatment in patients with orthodromic AV reentrant tachycardia who do not have preexcitation on their resting ECG during sinus rhythm. Intravenous amiodarone preferentially is used in Europa after failure of other drugs.

In the case of antidromic tachycardia or AF conducted by the AP, the treatment depends on tolerance. In poorly tolerated tachycardias, urgent cardioversion after sedation is the recommended treatment (class I, level B).

In other cases calcium inhibitors and digoxin are prohibited because they facilitate the conduction over AP and are the causes of ventricular fibrillation (Brembilla-Perrot et al., 2010).

Amiodarone or preferentially intravenous flecainide (1 mg kg^{-1}) when associated heart disease is excluded are the recommended treatments.

Preventive Treatment (Page et al., 2016)

Ablation of AP, which is a curative treatment, now is recommended in preexcitation syndrome requiring a treatment. However, the results and risks of the method still need to be compared with the symptoms and preexcitation-related risks. Age of the patient, competitive sport activity, particular jobs (fireman, military, train, or airplane pilot), and location of AP are some of several factors that should be considered before the indications of treatment.

Risks and benefits related to the ablation of the AP

Introduced in 1990, the ablation of AP is able to permanently cure the subject with a ventricular preexcitation syndrome (Warin et al., 1988; Jackman et al., 1991). The immediate success of ablation has increased since 1990 and is currently around 93/95%. Nevertheless, it is lower in case of right lateral location (78%), in very young children, and in the presence of heart disease, particularly in cases of Ebstein's anomaly.

Also it is necessary to consider the risk of recurrence of preexcitation, which averages 10% and is dependent on the location of AP (right sided 24%, midseptal 17%, left AP 5%). The phenomenon usually occurs early (first 2 months). In addition, the risks of complications also have decreased from 4.3% to 2.9% (average 3%). It remains most common in young children: Mortality fortunately is exceptional (0%–0.2%). The complications are mainly related to radiation exposure (Bacher et al., 2005) (less important if using a mapping system is used), vascular access (e.g., hematoma, pneumothorax, arteriovenous fistula), catheter manipulation (coronary dissection, perforation of the coronary sinus, microemboli), and the application of radio frequency (risk of complete AV block, coronary stenosis) (Vitello et al., 1998; Fujiki et al., 1990; Schuffer et al., 1996). Complete AV block is a known complication of the ablation of a parahisian AP and has been reported for posteroseptal AP. The risk is exceptional when using cryoablation in place of radiofrequency ablation, but this technique is not used in all the teams because of a risk of higher recurrence of preexcitation.

Indications of ablation in 2016 (Page et al., 2016) (Table 1)

The recommendations are based on the 2012 publications in children (Cohen et al., 2012), on the 2015/2016 data in the symptomatic or asymptomatic adults (Page et al., 2016). Several elements are to be considered:

A. The patient is symptomatic or not;
B. What is the patient's age?
C. Does the patient participate in a competitive sport or is does their profession prohibit the presence of an AP (e.g., pilot)?
D. What is the location of AP?

A—The subject is symptomatic (reentrant tachycardia or AF with rapid conduction over the AP): Ablation is recommended (class I level B), especially in adults. Drug treatment is a class I level C recommendation only when AP is concealed (Page et al., 2016).

It must be remembered that successful ablation prevents the risk of AF than in subjects <50 years (Dagres et al., 2001) and especially when it was proved that AF was due to reciprocal tachycardia rhythm. Otherwise ablation is used to prevent too rapid conduction through the AP during AF. This fact has been confirmed by Bunch et al. (2015) who also showed a lower mortality in patients who underwent ablation.

Table 1 Indications of ablation of AP

Class	*Level of proof*	*Indications*
I	B	• Symptoms (tachycardia) • No symptoms: malignant form at electrophysiology • No symptoms: sport competition, job at risk
II a	B	Asymptomatic: malignant form at electrophysiology Symptomatic; child
III		Asymptomatic, benign form at electrophysiology

Malignant form at electrophysiology (AF induced with minimal cycle conducted by AP $\leq$250 ms).
Benign form: no inducible tachycardia, refractory period of the AP >250 ms.

B—The subject is asymptomatic: Ablation is recommended (class IIa level B) in the case of detection of a form to high risk at electrophysiology study (AF with a minimum cycle length conducted by AP $\leq$250 ms) but also systematically in the subject exercising a profession at risk (e.g., pilots or drivers) (Page et al., 2016).

It should be noted that the simple monitoring of asymptomatic individuals also has the same class of recommendation (class IIa level B) and a recent study of Papponne and Santinelli (Pappone et al., 2014) the prognosis of preexcitation syndrome essentially depends on intrinsic electrophysiological properties of AP rather than on symptoms. For these authors ablation performed during the same procedure after electrophysiological testing is of benefit in improving the long-term outcomes.

C—In young children, the indications are discussed because of radiation risks and catheterization complications (Bacher et al., 2005; Vitello et al., 1998). They should be discussed with parents (Cohen et al., 2012).

AF risk is low unless associated with congenital heart disease. In addition, the involution of AP is possible before the age of 12 years if the AP refractory period is long and finally the sports in competition do not apply to young children. Drug treatment is usually effective in young symptomatic children.

In practice, the ablation can be exceptionally indicated before the age of 12 if antiarrhythmic drugs or beta-blockers are ineffective or not tolerated but requires general anesthesia.

In 2012, ablation is recommended (class II level A) in asymptomatic children ($\geq$8 years) if AF with rapid conduction is induced and if reciprocal tachycardia is inducible (class II, level B) (Cohen et al., 2012). However the recommendation is the same (class II level B) in the absence of inducible tachycardia in special cases (sports) and in the absence of parahisian location. The absence of induction of tachycardia and the induction of AF with a driving cycle >250 ms leads to a simple monitoring (recommendation of class II (level A).

D—Athletic competition: Evaluation and ablation indications are wider. Sport is authorized without ablation if the subject is asymptomatic and if systematic electrophysiological study shows that this is a benign form (refractory period >250 ms and no inducible tachycardia) (Heidbüchel et al., 2006).

E—Location of AP: In the case of parahisian location, the risk of complete AV block is important except when cryoablation is used, but there is a high risk of recurrence (Swissa et al., 2015). This location is particularly important to consider in asymptomatic subjects where ablation indications remain questionable.

In conclusion, several data should be considered in patients with a preexcitation syndrome

- Confirmation of the diagnosis.
- Evaluation of the prognosis. Electrophysiological study is the best means to define this prognosis. The method is easily performed in the out-patient clinic by transesophageal route. The indications should be large to avoid the misdiagnosis of a form at risk of rapid arrhythmias. This dangerous form is relatively rare in asymptomatic patients, symptomatic patients, or those with unexplained syncope. Most of these patients (>85%) would be allowed to continue their activities, without specific treatment, because they have a benign form of preexcitation syndrome.
- Discussion of the treatment. Ablation of AP is required in symptomatic subject. It remains controversial in asymptomatic subject except in the case of sport competition or in the case of a profession at risk and in the in case of detection of a potentially serious form at electrophysiology (induction of AF conducted by the AP with a $\leq$250 ms cycle length). The recommendations are contradictory because the risks are low and ablation does not have a success rate of 100%. The indications rarely are needed in children before 12 years.

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Z

Zebrafish

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Introduction

Research History

The zebrafish (*Danio rerio*) is an important and widely used vertebrate model organism in scientific research. It is a freshwater fish, native to the streams of the tropical Asia region (Mayden et al., 2007), and is found in parts of India, Pakistan, Bangladesh, Nepal, Myanmar, and Burma (retrieved information from U.S. Geological Survey on April 4, 2017). The male is torpedo-shaped, with gold stripes between blue stripes (**Fig. 1B**); the female has a larger, whitish belly and silver stripes instead of gold (**Fig. 1A**). The zebrafish can grow to 6.4 cm (2.5 in.) in length, and its lifespan is around 2–3 years (Gerhard et al., 2002).

Use of zebrafish as a laboratory animal was pioneered by the American biologist Dr. George Streisinger, he and his colleagues initiated genetic studies of zebrafish at the University of Oregonin in the 1970s and 1980s (Stahl, 1985). The approximate generation time for *Danio rerio* is 3 months; this particular feature made it an appealing genetic system to Dr. Streisinger. A male must be present for ovulation and spawning to occur. Females can spawn at intervals of 2–3 days, laying hundreds of eggs in each clutch, making it an ideal system for genetic studies. Upon release, eggs are fertilized, and embryonic development begins. Fertilized eggs almost immediately become transparent, a characteristic that makes *Danio* rerio an ideal research model species for imaging and observation of developing internal organs.



Fig. 1 A typical adult female (A) and male (B) zebrafish at 3-month old. The characteristic stripes running along the body and the fins gave this species its name.

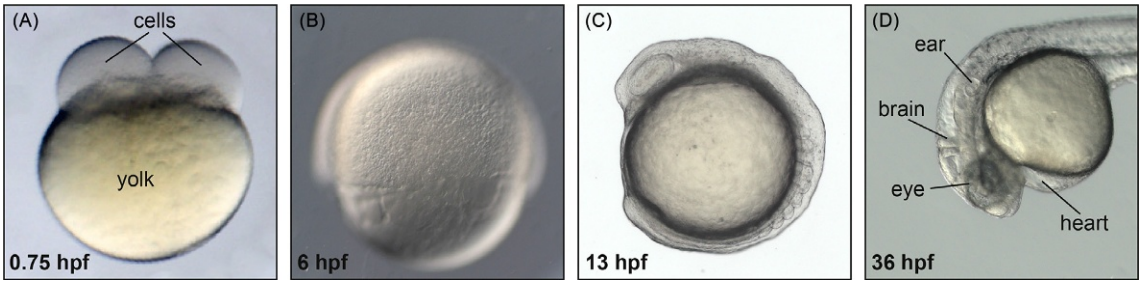

Fig. 2 The embryonic development of zebrafish. The zebrafish embryo begins as a yolk with a single enormous cell on top (0 hpf, not shown), which divides into two (0.75 hpf) (A) and continues dividing until there are thousands of small cells. The cells then migrate down the sides of the yolk during gastrulation movements (B) and begin forming a head and tail (C). The 36 hpf old zebrafish has major organs being formed in the body (D).

Research Advantage

The zebrafish embryo develops rapidly, and precursors to all major organs appear within 36 h postfertilization (hpf) (**Fig. 2D**). Upon egg activation, the cell cytoplasm separates from the yolk. The first cell cleavage occurs at 0.75 hpf (**Fig. 2A**) and divisions continue synchronously and without cell growth until there are thousands of small cells that form the blastoderm at 3.25 hpf. After genome activation, the cells migrate down the sides of the yolk around 6 hpf (**Fig. 2B**). The germ layers are specified at the shield stage, and formation of the head and tail begins at 13 hpf (**Fig. 2C**). The tail then extends away from the yolk at 24 hpf. The yolk shrinks over time as the embryo uses the yolk as food during the first few days. After a few months, the adult fish reaches reproductive maturity (Kimmel et al., 1995).

Today, as an established model biological system, the zebrafish possesses numerous advantages for scientists. Its genome has been fully sequenced, and it has well-understood, readily observable, and testable developmental behaviors. As mentioned, embryonic development is very rapid, and the embryos are relatively large and transparent, and importantly develop outside of the mother. Furthermore, well-characterized mutant strains are readily available or are easy to characterize. Significantly, the Zebrafish Information Network (ZFIN: http://zfin.org) provides a dedicated online database of genetic, genomic, and developmental information that is a vital resource for zebrafish scientists globally.

Research with *Danio rerio* has yielded advances in the fields of developmental biology, genetics, cardiovascular biology, stem cell research and regenerative medicine, oncology, toxicology, reproductive studies, teratology, neurobiology, environmental sciences, and evolutionary theory. In the field of cardiovascular research, due to a unique combination of visual access to the early embryonic cardiovascular system and established tools and methods for genetic analysis, the zebrafish is recognized as an ideal model organism for investigating the molecular mechanisms underlying heart and blood vessel formation (Stainier and Fishman, 1994; Liu and Stainier, 2012; Evans et al., 2010; Gore et al., 2012; Covassin et al., 2009; Lawson and Weinstein, 2002; Vogel and Weinstein, 2000; Isogai et al., 2001; Yelon and Stainier, 1999). Besides, the zebrafish embryo does not require a functional cardiovascular system for its survival through 5 dpf (Alexander et al., 1998; Stainier et al., 1996; Pelster and Burggren, 1996), thus cardiovascular defects can be analyzed in detail throughout embryogenesis.

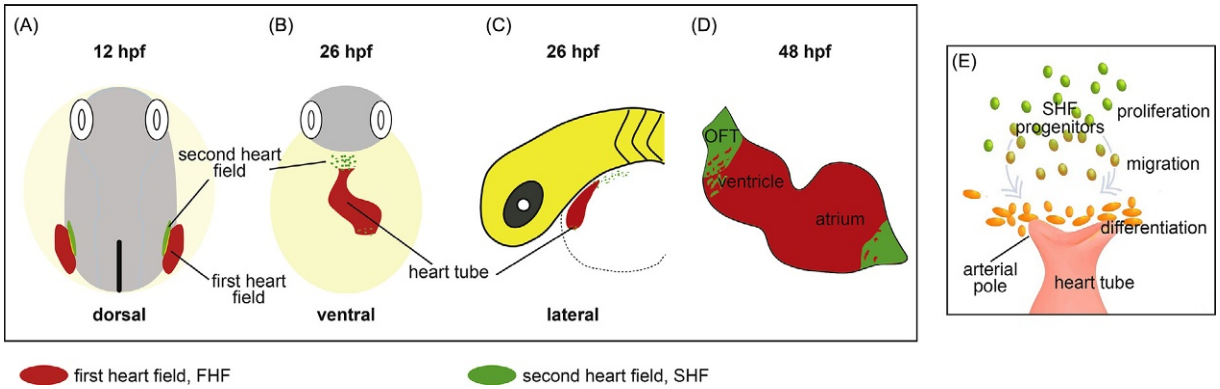

Fig. 3 The formation of the embryonic vertebrate heart involves two temporally distinct phases of cardiomyocyte differentiation. Schematics depict locations of cells derived from the first heart field (FHF in red) and the second heart field (SHF in green) in the zebrafish embryo. (A) Initially, the SHF is located medially adjacent to the FHF at 12 hpf. (B, C) A pool of progenitor cells is located adjacent to the arterial pole at the ventral (B) and lateral view (C) at 26 hpf. (D) The SHF-derived cardiomyocytes contribute to both poles of the heart at 48 hpf. (E) A schematic dorsal view of the arterial pole of the heart tube around 26 hpf. A pool of proliferative progenitor cells (green) is detected in a distal region to the arterial pole. They initially exhibit a dispersed pattern, before actively moving toward the arterial pole and accumulating proximal to the primitive heart tube, and they seem to express different combinations of molecular markers as they initiate the cardiac differentiation process. Eventually, these cells are matured into cardiomyocytes and are incorporated into the forming the cardiac outflow tract (OFT).

In this article, we will highlight the advantages of zebrafish in cardiovascular and biomedical research, including the sophisticated methods in genetic screening, gene manipulation, genome editing, and implementation of these technologies toward the study of regeneration biology and small-molecule drug discoveries.

Zebrafish Cardiovascular System

Heart Development

As early as the late blastula stage (~5 hpf), myocardial progenitor cells are already spatially organized in bilateral zones near the embryonic margin (Keegan et al., 2004). When gastrulation is complete (~12 hpf), both ventricular and atrial myocardial progenitors reside in bilateral heart fields within the anterior lateral plate mesoderm (ALPM) (**Fig. 3A**), and retain their spatial organization as the gastrula forms (Schoenebeck et al., 2007), and myocardial differentiation initiates. Subsequently, cardiomyocytes move to the embryonic midline and form a cardiac cone; characteristic molecular markers *ventricular myosin heavy chain* (*vmhc*) and *atrial myosin heavy chain* (*amhc*) are expressed around this stage (~19 hpf) (Berdougo et al., 2003). At 26 hpf, cardiomyocytes migrate and elongate the cardiac cone to form the heart tube (**Fig. 3B, C**). By 48 hpf, further growth and morphogenesis of the heart tube results in a morphologically distinct ventricle and atrium, with a constriction at the atrioventricular canal separating both chambers (**Fig. 3D**). In the adult zebrafish heart, blood enters the sinus venosus and passes through a single atrium and ventricle. The ventricle then pumps venous blood through the bulbus arteriosus to the ventral aorta for distribution to the gills for oxygenation.

Despite apparent anatomic differences between the two-chambered zebrafish heart and four-chambered mammalian heart, many studies have highlighted similarities in the genes and regulatory networks that specify cardiac fates, extensively reviewed (Evans et al., 2010). Work in birds and mammals has demonstrated that the multichambered vertebrate heart is built by two waves of cardiac progenitors: first heart field progenitors and second heart field progenitors, which differ in their timing of differentiation and in their contributions to specific regions of the heart (Buckingham et al., 2005; Dyer and Kirby, 2009; Abu-Issa and Kirby, 2007). Initially, an early wave of cardiomyocytes contributes to the formation of the primitive heart tube; these cells arise from a set of cardiac progenitor cells located in the ALPM, termed the first heart field (**Fig. 3A**). Subsequently, the gradually controlled addition of cardiomyocytes to the arterial poles of the maturing heart occurs via a second and late-differentiating cell lineage originating from the second heart field (**Fig. 3A,E**) (Buckingham et al., 2005). In zebrafish, assays using fluorescent reporters to capture the timing of differentiation have confirmed the existence of two waves of cardiac differentiation (de Pater et al., 2009). Further, lineage-tracing and cell-labeling analyses have validated the existence of distinct pools of progenitor populations similar to those seen in mammals, and deepened the concept of the first and second heart fields in lower vertebrates (Zhou et al., 2011; Zeng and Yelon, 2014; Hami et al., 2011).

Since many of the genes and signaling pathways essential to embryogenesis are conserved between zebrafish and mammals, the characterization of two distinct phases of cardiomyocyte differentiation in the zebrafish heart have significant implications.

Vascular Development

Vascular development includes a series of cellular and molecular events, from endothelial cell differentiation to blood vessel assembly and morphogenesis. During the last several decades, extensive research utilizing model animals has demonstrated that the

embryonic vasculature develops in a conserved manner in all vertebrates: endothelial progenitor cells differentiate from mesodermal cells, then migrate and assemble into the dorsal aorta and the cardinal vein. This primitive circulatory loop undergoes sprouting and branching to form the trunk vascular network (Risau and Flamme, 1995).

In the vertebrate embryo, the dorsal aorta and the posterior cardinal vein form in the trunk to establish the circulatory loop prior to the first heartbeat. The endothelial tubes of the great vessels in the embryo proper assemble through migration and de novo coalescence of endothelial progenitors (angioblasts), a process termed vasculogenesis (Risau and Flamme, 1995; Risau, 1996). Subsequent angiogenesis occurs, where intersomitic vessels sprout from the dorsal aorta and the cardinal vein to form anastomotic arterial and venous systems, allowing blood to circulate in the developing embryo. In higher vertebrates, the embryonic yolk sac also forms a vascular network. First, angioblasts assemble and form a complex capillary plexus via vasculogenesis. This primitive plexus undergoes pruning and remodeling to develop into the vitelline artery and vitelline vein. In zebrafish embryos, vascular development occurs in a conserved manner compared with other vertebrates (Isogai et al., 2001; Zhong, 2005). The zebrafish yolk sac does not develop a separate vascular system. Instead, part of the cardinal venous system, the duct of Cuvier, comes together to form the common cardinal vein.

Genetic Manipulation in Zebrafish

Forward Genetic Screening Identifies Novel Genes and Pathways in Cardiovascular System

Genetic factors contribute to the pathogenesis of cardiovascular diseases. Due to disease heterogeneity, variable penetrance, and high mortality, human genetic studies have been combined with animal models to elucidate the genetic basis of cardiovascular diseases. Notably, animal models have emerged as powerful tools for identifying novel genes that are involved in cardiovascular pathology, and further to verify the effect of disease gene candidates on cardiovascular function.

Enabling the unbiased identification of novel cardiovascular genes, zebrafish are exceptionally suitable for forward genetics approaches as an animal model system. Large-scale ENU-based mutagenesis screens have been conducted in zebrafish since 1996, and several hundred mutants with cardiovascular defects have been identified (Stainier et al., 1996; Alexander et al., 1998). Many of these mutations have now been characterized in detail and positionally cloned. According to the disease-related genes listed in the Online Mendelian Inheritance in Man (OMIM) database, 82% have at least one zebrafish ortholog (Howe et al., 2013).

The spectrum of cardiac phenotypes that were isolated in zebrafish mutants has demonstrated close correlation with human disease, such as congenital heart disease, cardiomyopathies, and arrhythmias. For example, several important genes encoding key transcription factors that have been shown to cause the pathogenesis associated with human congenital heart defects are also critical for heart development in zebrafish, such as *gata4/5/6* (Peterkin et al., 2007; Garg et al., 2003; Wei et al., 2013; Holtzinger and Evans, 2005; Reiter et al., 1999; Holtzinger and Evans, 2007), *tbx5* (Li et al., 1997; Garrity et al., 2002), *hand2* (Lu et al., 2016; Schoenebeck et al., 2007; Yelon et al., 2000), and *nkx2.5* (Schott et al., 1998; Tu et al., 2009) genes. Prostaglandin signaling has been recently discovered to regulate heart laterality and ciliogenesis through zebrafish genetics studies (Jin et al., 2014).

Similarly, components of the molecular signaling pathways (such as TGF-β, BMP, VEGF, etc.) involved in embryonic vascular development have been implicated in adult human disease, including pulmonary arterial hypertension, hereditary hemorrhagic telangiectasia, and preeclampsia (Venkatesha et al., 2006; The International PPH Consortium et al., 2000; Johnson et al., 1996). Although studies in many vertebrate systems, including chick, frog, and mouse, have contributed significantly to our understanding of embryonic vascular development, genetic approaches in zebrafish have led to the identification of novel mutations and molecules that are responsible for specification of endothelial progenitor cells, differentiation of arterial and venous cells, and patterning of the dorsal aorta and intersegmental vessels. These studies highlight the benefits of the zebrafish system for studying the development of embryonic blood vessels. For example, the discovery of the *gridlock* (*grl*) mutation and identification of *grl*/*hey2* gene as a novel hairy-related basic helix–loop–helix (bHLH) transcription factor in arterial endothelial development proves the strength of zebrafish as an excellent genetic system studying vascular development (Weinstein et al., 1995; Zhong et al., 2001; Zhong et al., 2000). Another good example was the discovery that Semaphorin–Plexin signaling guides patterning of the developing vasculature (Torres-Vazquez et al., 2004). Studies in zebrafish revealed that proper blood vessel path finding requires the endothelial receptor PlexinD1 and semaphorin signals, and indicates a fundamental conservation of repulsive patterning mechanisms between axonal migration in the central nervous system and vascular endothelium during angiogenesis.

Morpholino Antisense Technology Continues to Serve as a Powerful Tool for Functional Analysis

Zebrafish mutants identified in small- and large-scale forward genetic screens were primarily the foundation for genetic analysis of zebrafish development and physiology (Chakrabarti et al., 1983; Driever et al., 1996; Haffter and Nusslein-Volhard, 1996). While forward genetics was instrumental in establishing zebrafish as vertebrate model system, a reliable technology to conduct reverse genetics in this model was sought by the community. The use of antisense oligonucleotides also known as morpholinos (MOs) was first introduced in frogs (Heasman et al., 2000) and then in zebrafish (Nasevicius and Ekker, 2000). This technology has spread rapidly and widely through both research communities for transiently knocking down gene function.

The MO antisense technology is based on nucleic acid bases that are linked to morpholine rings and a noncharged phosphorodiamidate backbone (Summerton and Weller, 1997). The rationale for this design was that MOs would not bind electrostatically to protein, hence would cause less toxicity, while at the same time would be resistant to nucleases (Summerton, 2007). MOs are

injected into one-cell stage zebrafish embryos using standard microinjection techniques, and it makes them convenient, easy, and accessible to even small-sized research groups.

Commonly, MOs are ~25-mers designed to be an exact antisense match against the region surrounding the first translated ATG start codon to block translation; or against a splice donor or acceptor site to interfere with precursor mRNA splicing (Summerton and Weller, 1997). To date, countless studies using this technology have been published, including some using MOs to knock down proteins produced from maternally deposited transcripts to generate maternal-zygotic mutants (Ciruna et al., 2002), and others using caged MOs (Griepenburg et al., 2015; Wang et al., 2012; Shestopalov and Chen, 2011; Ouyang et al., 2009), which allow for an inducible release of these antisense reagents and result in a spatially and temporally controlled gene function regulation (Shestopalov et al., 2012).

Recently, serious concerns have been raised as to the specificity of MO effects. There are several examples in which the developmental delays, defects in organ asymmetry, and pericardial edema are associated with nonspecific MO artifacts (Shmukler et al., 2016; Shmukler et al., 2015; Kok et al., 2015). After rounds of discussion in scientific journals and at conferences, the zebrafish community concludes that adequately controlled MOs should still continue to be accepted as useful loss-of-function approach with careful evaluation guidelines being implemented (Lawson, 2016; Schulte-Merker and Stainier, 2014; Stainier et al., 2015; Rossi et al., 2015).

New Era in Genome Editing: TALENs and CRISPR/Cas Systems

The introduction of new genome editing tools such as ZFNs (zinc finger nucleases), TALENs (transcription activator-like effector nucleases), and more recently, the CRISPR (clustered regularly interspaced short palindromic repeats)/CRISPR-associated (Cas) system has greatly expanded the ability to knockout genes in different animal models with surprisingly high efficiency (Beumer et al., 2008; Tesson et al., 2011; Yang et al., 2013), including zebrafish (Chang et al., 2013; Sander et al., 2011a; Sander et al., 2011b; Hwang et al., 2013a; Hwang et al., 2013b; Zu et al., 2013; Gagnon et al., 2014). Particularly, CRISPR/Cas systems have been providing exciting new opportunities for high-efficiency mutagenesis (Hwang et al., 2013a; Shah et al., 2016; Shah et al., 2015), and have been reviewed extensively (Auer and Del Bene, 2014).

In bacteria, exogenous foreign nucleic acids are silenced by CRISPR–Cas systems (Barrangou, 2015; Morange, 2015; Mojica et al., 2005). Originally, bacterial type II CRISPR systems were adapted to create guide RNAs that direct site-specific DNA cleavage by the Cas9 endonuclease in cultured cells (Cong et al., 2013; Mali et al., 2013). Owing to their effectiveness as a genome editing tool, several efficient protocols have been generated in the zebrafish field, and numerous studies have shown that the CRISPR–Cas system functions in vivo to induce targeted genetic modifications in zebrafish embryos with efficiencies similar to those obtained using ZFNs and TALENs (Talbot and Amacher, 2014; Gagnon et al., 2014; Yin et al., 2016).

By using the CRISPR–Cas genome editing system, both research time and costs required for the screening of a huge number of zebrafish animals, aimed to identify first founder fishes (F0), and then carriers (F1) are relatively affordable. Currently, high-resolution melting (HRM) analysis, real-time PCR system, and even traditional restriction enzyme-based assays resolved using agarose gel electrophoresis are common and efficient technologies for large-scale InDels detection that are easily adapted in research laboratories to distinguish between the mutant and the wild-type genotypes.

Taking advantage of the simplicity, rapidity, and cheapness of these methods, one can set up a pilot approach for massive screening of F0-injected founders. The scope can then be restricted to a limited number of F1 embryos for the next step, which is sequencing the affected gene to identify the induced mutation.

Zebrafish Model in Cardiac Regeneration Biology

Cardiac Regeneration in Zebrafish

Myocardial infarction and ischemic heart disease are caused by an acute reduction of blood to the myocardium, which has the direct effect of diminishing oxygen supply. This results in a loss of a vast number of cardiomyocytes, leading to hypertrophy and heart failure. Care of such patients places enormous burdens on the health care system, requiring 1 million hospitalizations every year at an annual cost of $35 billion in the United States (Bernstein and Srivastava, 2012). Development of therapeutics that can facilitate new cardiomyocyte generation would be of enormous medical and economic impact. Given the limited treatment options for advanced human heart failure, there is considerable interest in identifying ways to regenerate cardiomyocytes to restore myocardial function. For instance, treatments that can stimulate endogenous cardiomyocyte regeneration in areas of infarction could be the new frontier of regenerative therapy. Eventually, an ideal therapeutic option would be to employ small-molecules that have the capacity to stimulate and accelerate endogenous cardiomyocyte regeneration after injury.

In contrast to adult mammals, adult zebrafish can regenerate heart tissue following partial surgical resection (**Fig. 4A**) (Poss et al., 2002). In experimental settings, approximately 3% of cardiomyocytes in the compact myocardium of adult zebrafish hearts incorporated the thymidine analog bromodeoxyuridine (BrdU) during a 7-day pulse-labeling experiment. Two weeks after amputation of the cardiac apex, the fraction of BrdU-positive cardiomyocytes had increased 10-fold, and this parameter remained as high as 20% 1 month after injury. Strikingly, the authors found that by 60 days postamputation the lost cardiomyocytes had completely regenerated. Of functional and therapeutic significance, the study showed that the cardiac muscle supplanted the fibrin clot without generating scar tissue, leading to restoration of vascularized cardiac muscle and normal electrical coupling with adjacent cells.

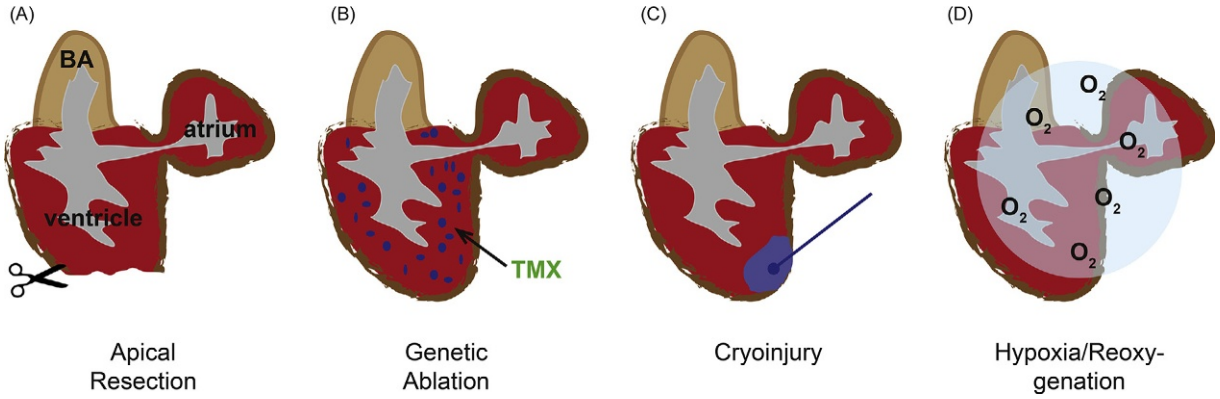

Fig. 4 Methods to induce cardiac injury in zebrafish adult hearts. The adult zebrafish heart consists of one ventricle, one atrium, and the balbus artieriosis (BA). Since the adult zebrafish heart regenerates spontaneously after injury, current models to study mechanisms of cardiac regeneration include resection of the ventricular apex (A), ventricular cryoinjury (B), genetic ablation of cardiomyocytes (C), and cardiac injury induced by hypoxia/reoxygenation (D).

The cellular and molecular basis underlying cardiac regeneration is of significant interest. In the case of zebrafish, the regeneration process predominantly occurred through dedifferentiation of proliferating cardiomyocytes at the injury site based on the observed reactivation of cardiac developmental genes as well as disassembly of sarcomeric structure of existing cardiomyocytes. A stem/progenitor cell-mediated mechanism was probably not involved in this zebrafish cardiac regeneration model (Jopling et al., 2010).

Three additional unrelated injury models—genetic ablation (Wang et al., 2011), cryocauterization (Gonzalez-Rosa et al., 2011), and hypoxia/reoxygenation (Parente et al., 2013) (**Fig. 4B, C, D**)—also stimulated robust myocardial regeneration in zebrafish. In these studies, the wounded heart had entirely regenerated after 30, 130, and 30 days, respectively. Heart tissue throughout the organ is responsive to injury during zebrafish heart regeneration, not just tissue near the site of trauma. All major cardiac tissues, including epicardium, endocardium, and myocardium, appear to employ this strategy, in which epicardium and endocardial cells show the early responses (Kikuchi et al., 2011a). Over different time courses and depending on the cell type, these initial widespread injury-activated expression signatures disappear globally and eventually seem to localize to the injury site. Factors released during cardiac inflammation may help trigger an organ-wide injury response, which might be a key to regenerative success. Signaling pathways that participate in zebrafish heart regeneration have been identified, including fibroblast growth factor (Lepilina et al., 2006), platelet-derived growth factor (Kim et al., 2010), and retinoic acid pathways (Kikuchi et al., 2011b).

Cardiac Regeneration is Conserved between Zebrafish and Mammals

In mammals, the heart of 1-day-old neonatal mice can regenerate after partial surgical resection or left anterior descending artery (LAD) ligation (Porrello et al., 2011; Haubner et al., 2012). Similar to the injured zebrafish adult heart, local injury induces global changes in cardiomyocyte morphology and proliferation in the neonatal mouse heart. This regenerative capacity is lost 7 days after birth when resection or ligation surgery leads to the formation of a fibrotic scar. The timeline coincides with the withdrawal of cardiomyocytes from the cell cycle through the completion of mitosis without cytokinesis, resulting in the formation of binucleated cells. This postnatal switch in cardiomyocyte proliferation leads to the transient presence of regenerative capacity in the neonatal mouse heart. Scar formation may also cause loss of regeneration capacity by 7 days.

Several hypotheses speculate why the mammalian heart only has a short period in regenerative capacity after birth (Foglia and Poss, 2016). One of the interesting models posits that a transition to the oxygen-rich postnatal environment results in the cell-cycle arrest of cardiomyocytes. Studies in mice have shown that postnatal hypoxemia, reactive oxygen species (ROS) scavenging, or inhibition of DNA damage response (DDR) all extend the postnatal proliferative status of cardiomyocytes, in contrast hyperoxemia and ROS conditions can reduce its length (Puente et al., 2014).

Following this rationale, Hesham Sadek and colleagues show that chronic severe hypoxia enhances the regeneration capacity of injured hearts in adult mice (Nakada et al., 2017). After myocardial infarction treatment, experimental mice that were placed in a hypoxic environment for a prolonged period could make new cardiomyocytes and restore partial cardiac function, through a mechanism involving a reduction of ROS concentration and cardiomyocyte cell-cycle reentry. Thus, targeting components in this hypoxia relevant pathway have potential to induce myocardial regeneration and eventually might provide a viable therapeutic strategy for use in humans.

In humans, over the course of an average lifetime, almost 45% of cardiomyocytes slowly turnover in adulthood, while 55% remain after birth (Bergmann et al., 2009). This turnover is very low and decreases significantly with age. Both the integration of radioactive ^{14}C (generated by nuclear bomb tests during the Cold War) into DNA and the infusion of iododeoxy uridine (IdU, a thymidine analog used as a radio sensitizer for therapy (Kajstura et al., 2010)) provide evidence for this measurable ability in the adult human heart.

Compared with the zebrafish heart, the mammalian heart has more fibroblasts. Mammalian cardiomyocytes have a higher DNA content (binucleated cells with a diploid nucleus in rodents, mononucleated cells with a polyploid nucleus in humans). These cardiac cell properties are thought to result in poor regenerative capacity when the mammalian heart is injured.

Cardiac Regeneration Involves Dedifferentiation and Proliferation of Existing Cardiomyocytes

Lineage mapping of the heart during regeneration using inducible genetic markers has demonstrated that zebrafish heart regeneration occurs through cardiomyocyte dedifferentiation and proliferation (Jopling et al., 2010; Kikuchi et al., 2010). The 4-hydroxytamoxifen (4-HT)-inducible Cre/lox system in zebrafish has been established to prelabel cardiomyocytes with enhanced green fluorescent protein (EGFP) prior to regeneration tests. After amputation of the apex, the new apical myocardium is 100% EGFP+, indicating that new cardiomyocytes arise from an existing cardiomyocyte lineage rather than through recruitment of cardiomyogenic precursors. A subpopulation of cardiomyocytes within the ventricular wall expresses Gata4 to reactivate an embryonic program that facilitates dedifferentiation and proliferation and contributes substantially to local muscle regeneration. This is consistent with the results of a cardiac sarcomere study, indicating that proliferating cardiomyocytes detach from one another and acquire a less organized sarcomeric structure during regeneration.

In accordance with this notion, recent studies suggest that mammalian cardiomyocytes regenerate through the division of preexisting cardiomyocytes (Senyo et al., 2013). By combining genetic line age mapping with stable isotope labeling, the nonradioactive tracer [^{15}N] thymidine and DNA synthesis can be measured using multiisotope imaging mass spectrometry (MIMS) in the adult heart of double-transgenic MerCreMer/ZEG mice. During normal mammalian myocardial homeostasis, mature resident cardiomyocytes are the dominant source of cardiomyocyte replacement, at a rate of about 0.76% per year. This process is increased fivefold during recovery from myocardial infarction. Adult cardiomyocytes retain some capacity to re-enter the cell cycle, but after injury, most DNA synthesis occurs in preexisting cardiomyocytes, without completion of cell division. In contrast, cardiac progenitor cells do not make a significant contribution to myocardial homeostasis in mammals, and their roles after injury are also limited. The epicardium and endocardium also appear to play vital signaling roles in heart regeneration. Some of the activated epicardial cells can transdifferentiate into cardiomyocytes at a low rate when preconditioned with thymosin β4 injections (Smart et al., 2011). Together, these findings provide strong evidence that the adult human heart is capable of regenerating cardiomyocytes to preserve cardiac function, although the cell turnover is extremely low throughout the life.

Studying the cellular and molecular regenerative mechanisms in lower vertebrate animals, such as zebrafish, could greatly contribute to developing strategies for promoting endogenous cardiac regeneration. In the future, an ideal therapeutic option would be to employ small-molecules that have the capacity to stimulate and accelerate endogenous cardiomyocyte regeneration after injury.

Chemical Biology and Drug Discovery in Zebrafish

Embryo-Based Small-Molecule Screens Identify Novel Chemical Probes and Drug Candidates

The zebrafish has emerged as a model organism used in multiple steps of the drug discovery process through their use in in vivo phenotypic screens (Wiley et al., 2017; Tan and Zon, 2011). Whole-embryo screens offer considerable advantages in drug discovery by evaluating complex target cell populations in organs, within a relevant physiological context. The identified compounds normally have efficacy with little toxicity, which can be further developed as medicines. Given the ease and rapidity with which the zebrafish embryos can be manipulated, small-molecule screens in zebrafish have contributed to identifying novel suppressors of disease phenotypes and characterization of the associated molecular pathways. For instances, several compounds have been shown to disrupt cardiac valve morphogenesis, vessel formation and others have been shown to influence cardiac action potential.

Grl/Hey2 inhibitor

Direct visualization of vascular phenotypes in zebrafish has enabled high-throughput small-molecule screening to elucidate the mechanisms behind congenital vascular defects. For example, zebrafish expressing the *gridlock* mutation *grl* in the *hey2* gene exhibit malformation of the aorta (Weinstein et al., 1995). As a result, there is disruption of circulation to the tail and trunk and compromised development of collateral vessels, analogous to human aortic coarctation. Using this model, a chemical screen of 5000 candidate small-molecules identified two structurally similar compounds that rescued the *gridlock* phenotype in a dose-dependent manner, resulting in normal flow through the aorta (Peterson et al., 2004). Subsequent experiments demonstrated that these drug candidates acted by upregulating expression of vascular endothelial growth factor (VEGF), which plays a crucial role in the formation of the aorta. These types of chemical suppressor screens offer insight into the molecular pathways responsible for developmental vascular defects such as aortic coarctation.

BMP inhibitor

Dorsomorphin was initially discovered as a BMP inhibitor that regulates dorsal–ventral axis formation in zebrafish embryos (Yu et al., 2008). Dorsomorphin selectively inhibits the BMP type I receptors ALK2, ALK3, and ALK6 and thus blocks BMP-mediated SMAD1/5/8 phosphorylation. Further studies demonstrated that the administration of dorsomorphin promotes

myocardial differentiation in mouse pluripotent embryonic stem (ES) cells (Hao et al., 2008). In addition, dorsomorphin derivatives were developed through initial medicinal chemistry optimization and prepared for clinical testing in patients with fibrodysplasia ossificans progressiva (FOP), which is a rare, fatal disease marked by inappropriate BMP signaling that causes growth of bone fragments within the muscles, ligaments, and other connective tissues, causing pain and progressive immobility (Martelli and Santos, 2014; Shore et al., 2007; Shore et al., 2006).

PGE2 agonist

Another drug with therapeutic potential identified through studies using zebrafish is Prohema. Currently being developed by fate therapeutics, the drug was discovered in a zebrafish screen (in the laboratory of Dr. Leonard Zon at Boston Children's Hospital) for compounds that increased or decreased the numbers of hematopoietic stem cells (HSCs). Prohema's therapeutic potential was first demonstrated in adult zebrafish, where it was shown to enhance the rate of marrow recovery after sublethal irradiation (North et al., 2007). As a stabilized derivative of prostaglandin E2 (PGE2), Prohema is currently in clinical trials in patients undergoing umbilical cord blood (UCB) transplantation for leukemia or lymphoma.

Embryo-Based Small-Molecule Screens Focusing on Cardiomyocyte Generation

Although small-molecule screens focused on heart regeneration are challenging in adult zebrafish, they can be readily carried out in zebrafish embryos. Based on the prior work showing that developmental programs are activated and similar signaling molecules and pathways are employed during regeneration of the injured adult heart (Kikuchi, 2014; Laflamme and Murry, 2011), it is highly likely that the compounds identified from developmental heart screens may have the capacity to induce heart regeneration in adults.

IGF, TGFβ, and Hh agonists

A newly developed fluorescent ubiquitylation-based cell-cycle indicator (FUCCI) screening system employs two fusion proteins (mCherry-zCdt1 and Venus-hGeminin) that are expressed during the G1 and S/G2/M phases. The FUCCI system enables the visualization of cell proliferation events in live animals. Transgenic *mylc7:FUCCI* embryos were exposed to a panel of compounds targeting various developmental signaling pathways, revealing that small-molecules that activate Hedgehog (Hh), insulin-like growth factor (IGF), and TGFβ signaling pathways can be seen to regulate cardiomyocyte proliferation (Choi et al., 2013). Direct examination of zebrafish heart regeneration after mechanical or genetic ablation injuries confirmed that Hh, IGF, and TGFβ signaling pathways are activated in regenerating cardiomyocytes.

Wnt inhibitor

Since the embryonic heart size primarily reflects cardiomyocyte generation, the size of a fluorescence-expressing heart can also be used as a visual indicator to screen for cardiomyocyte phenotypes. Three structurally related compounds with the same pharmacophore, namedcardionogen-1 (CDNG1) to -3 (CDNG3), have been found to enlarge heart size by causing myocardial hyperplasia (Ni et al., 2011). The increased cardiomyocyte number in CDNG1-treated embryos is due to an expansion of the cardiac progenitor cell population, and it may also promote cardiac cell proliferation. Mechanistically, CDNG functions as an inhibitor of the Wnt/β-catenin signaling pathway and can rescue cardiomyocyte deficiency and heart-specific phenotypes induced by Wnt8 overexpression during development. Expanding the panel of pharmacological manipulations that regulate this process will help reveal mechanisms that induce myocardial development and regeneration. Compounds identified from the unbiased screens in zebrafish are likely to represent promising tools for probing and potentially enhancing cardiomyocyte proliferation and regeneration in the injured mammalian heart.

Mammalian cells have also been used to screen for small-molecules that enhance cardiomyogenesis. The small-molecules IWR1 (inhibitor of Wnt response) and IWP3 (inhibitor of Wnt production) were identified as inducers of cardiogenesis, and demonstrated that Wnt inhibition is sufficient to drive human ESCs to form cardiomyocytes (Willems et al., 2011). However, treating zebrafish embryos with IWR1 results in truncation of the posterior axis and disruption of cardiac chamber formation. Notably, CDNG treatment induces cardiac cell formation without causing tail truncation and atrium disruption in zebrafish, indicating that CDNG has less toxicity compared to IWR1. Consistent with the in vivo activities, CDNG blocks Wnt/β-catenin-dependent transcription in embryos and ESCs but not in HEK293 cells, while IWR1 blocks Wnt signaling in embryos, ESCs, and HEK293 cells. These findings suggest that CDNG selectively reduces Wnt signaling associated with certain embryonic or cardiac factors, revealing the benefits of whole-embryo screens by targeting cardiac cell types. This may ultimately aid in design of therapeutic approaches for cardiac regeneration and repair.

Conclusions

We highlight the advantages of the zebrafish as a model animal for cardiovascular research. Zebrafish have a closed cardiovascular system and a cardiac cycle that is highly reminiscent of human physiology. Zebrafish can be easily genetically manipulated, and their genome has been fully sequenced. Transparent zebrafish embryos are particularly suitable for high-resolution phenotypic analyses of cardiovascular morphology as well as high content small-molecule screens for identification of chemical probes or

potential drug candidates. Zebrafish have two distinct phases of cardiomyocyte differentiation that are analogous to the differentiation of the two cardiac progenitor cell populations in mammals, termed as the first and second heart fields. The unique ability of zebrafish cardiomyocytes to regenerate offers a particularly exciting opportunity to develop much-needed therapies for cardiac injuries and heart failure following myocardial infarction. Future studies will undoubtedly take advantage of the zebrafish model to pair human genetic mutations with their molecular functions.

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Zebrafish as a Tool to Study Congenital Heart Diseases

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Introduction

Congenital heart diseases (CHDs) are heart defects present at birth caused by abnormal embryonic development. In the US alone, CHD affects approximately 0.8% of live births, and approximately 40% of those affected babies die in infancy (Reller et al., 2008) making CHD the most prevalent type of birth defect. Therefore, understanding the molecular mechanisms that underpin CHD is vitally important to improve diagnostics and therapeutic opportunities.

After decades of research it has become evident that CHD is defined by a complex genetic architecture. Two types of CHD exist, syndromic and nonsyndromic. Patients that fall in the latter category present only isolated defects of the heart and constitute approximately 90% of all CHD patients. The further 10% of patients, in addition to defects in the heart, have other developmental problems such as defects in other organs or an intellectual disability. Until recently, it was accepted that both the syndromic and nonsyndromic variations of the disease resulted from spontaneous de novo mutations. Recently, though, Sifrim et al. (2016) showed that patients suffering from syndromic CHD often had noninherited mutations which are likely to result in defects in the heart. Interestingly, they also showed that nonsyndromic CHD was likely caused by mutant gene variants inherited from healthy parents.

Accumulation of de novo mutations and copy number variation (CNV) of genes involved in cardiac developmental are found to contribute to the majority of CHD cases, with most mutations responsible for CHD identified in a small set of developmental genes. For example in Homeobox containing transcription factor 2.5, NKX2-5 (Schott et al., 1998), GATA4 transcription factor (Garg et al., 2003), and Notch homolog1, NOTCH1 (Garg et al., 2005).

Two back-to-back landmark studies by Iafrate et al. (2004) and Sebat et al. (2004) revealed that CNVs commonly occur in the population. CNVs are manifested throughout the genome as both deletions and duplications which may disrupt none, one or several genomic elements. CNVs that are common (i.e., present at >1%–5% in the population) may be phenotypically neutral or function as regulators of disease susceptibility. On the other hand, rare CNVs (i.e., at a frequency of <1%) are more likely than common CNVs to cause disease, especially developmental disorders like CHD (Costain et al., 2016; Edwards and Gelb, 2016; Lee and Scherer, 2010). However, as CNVs are expected to show some variable expression and/or incomplete penetrance, differentially identified CNVs between unaffected and CHD affected human genomes may not always result in a phenotype. Moreover, CNVs encompass large genomic regions that can contain over 100 genes, making the task of identifying the causative gene more difficult without proper validation in animal models. Currently, CNVs are thought to be involved in ~10% of CHD cases.

The bulk of CHD is expected to be caused by de novo point mutations. NGS has been pivotal in identifying CHD mutations at high read depths ($\geq$20 reads per base) in the exome or the genome. Indeed recent whole exome sequencing (WES) studies have identified hundreds of possible mutations associated with CHD, suggesting that the major part of genes and genetic lesions associated with CHD pathogenesis remain unidentified. Furthermore, the targeted mutation of over 500 genes in mice resulted in heart defects (Andersen et al., 2014). This highlights the scope for the discovery of novel CHD-causing mutations in humans.

Identifying deleterious de novo mutations is a daunting task, as they do not follow Mendelian inheritance and large cohorts of patients and controls are required to have enough statistical power to call disease variants. Therefore, in order to uncover the genetic mechanisms that underlie CHD, the National Heart, Lung, and Blood Institute (NHLBI) created the Pediatric Cardiac Genomics Consortium (PCGC) (https://benchtobassinet.com/About/AboutPCGC/PCGCResearch.aspx) in 2013. As a multicenter initiative, the PCGC has the capacity to enlist the large number of patients and relatives that these studies require. To understand the link between genetic factors and pathological phenotypes, the PCGC established the Congenital Heart Disease Genetic Network Study (CHD GENES) (Pediatric Cardiac Genomics et al., 2013). CHD GENES analyzed a combined total of 1575 CHD trios (pediatric patients who had unaffected parents) and 1164 control trios by employing WES (Homsy et al., 2015; Zaidi et al., 2013). Nonconserved missense, conserved missense, protein changing, and damaging protein altering mutations were identified in both patient and control sets. Notably, a total of 1202 and 894 mutations were discovered in the patient and control sets, respectively

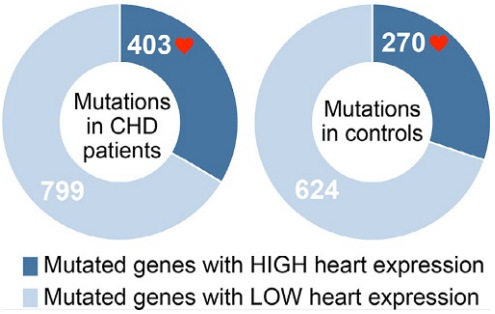

Fig. 1 Pie charts indicating the combined numbers of de novo mutations identified by WES from Homsy et al. (2015) and Zaidi et al. (2013). These studies yielded a total of 1202 and 894 mutations in patient and control sets, respectively, from 1575 CHD trios and 1164 control trios. 403 and 270 mutations were identified in high heart expression genes in the patient and control sets, respectively.

(Homsy et al., 2015; Zaidi et al., 2013). These WES studies uncovered mutations in high heart expressed (HHE) genes in both patient and control sets. 403 (33%) of mutations were identified in HHE genes from the patient cohort. Surprisingly, 270 (30%) mutations were also identified in HHE genes from the control set (**Fig. 1**). Assuming that mutations identified in the control cohort do not contain false positives, these results suggest that mutations in heart genes, even those that occur in highly expressed genes, are not necessarily disease causing. The contribution of de novo CNVs to CHD cases was also investigated by Glessner et al. (2014). They identified 57 and 242 novel CNVs within high heart and low heart expression, respectively, from 538 CHD trios using both genome-wide dense single nucleotide polymorphism (SNP) arrays and WES.

Despite these spearheaded efforts to uncover the genetic basis of CHD, future research should be aimed at addressing the causal relationship between the genes uncovered by the consortium and congenital heart malformations. This need for a casual validation can be also extended to other cardiac diseases, like atrial fibrillation (Tucker et al., 2016). However, the high number of candidate gene variants unearthed to date makes it difficult to validate them all using traditional methods and demands a more nimble approach. Overall, these studies iterate the need for a robust, high-throughput, and fast validation tool. Targeted genome editing in zebrafish using CRISPR/Cas9 knockout technology has the potential to fulfill this requirement to quickly screen through gene variants and become the gold standard for validating candidate disease-causing genes.

Several features of zebrafish development make for an exceptional model organism to study heart development. First, high fertility rates and external fertilization means that hundreds of embryos are available daily for microinjection experiments. Second, complete optical clarity of the embryo coupled to transgenic fluorescent reporters in different tissues provides an excellent platform to visualize in vivo heart development and function with cellular resolution. Third, as oxygen readily diffuses passively into the embryo, the zebrafish can survive without a cardiovascular system for several days, giving researchers ample time to analyze even the most deleterious mutations (Pelster and Burggren, 1996). Fourth, the two-chamber organization of the zebrafish heart (ventricle and atrium) lined with endocardium, myocardium, and pericardium simplifies the morphological and functional analysis. And finally, thanks to the rapid development of zebrafish embryos, heart beating and blood circulation are evident 30 h after fertilization, significantly accelerating the discovery process.

Most recent work is targeted at coupling the accessibility of zebrafish with the new mutagenic technologies, as a systematic and translational strategy, to uncover causative genes of CHD.

Using Zebrafish to Study Cardiovascular Development and Disease

Identifying disease-causing sequence variants from the many nondeleterious gene variants using NGS alone is challenging (Richards et al., 2015). For example, Narasimhan et al. (2016) show that even rare homozygous loss-of-function variants can be detected in unaffected individuals using NGS, suggesting such variants may not be clinically significant. Clearly, falsely assigning variant pathogenicity would hamper our understanding of disease. Thus, it is of the utmost importance that candidate disease-causing gene variants are validated for pathogenic causality. Typically, this is achieved by generating a loss-of-function mutant. The presence of an abnormal phenotype is strong evidence to assign pathogenicity to the particular gene variant (MacArthur et al., 2014). Traditionally, mouse is the model of choice to study disease mechanisms and the efficacy of new therapies (Dow and Lowe, 2012). However, the mouse model is not conducive for high-throughput analyses because of their small number of progeny and higher costs to maintain. Zebrafish may represent a more advantageous alternative for translational research (Gama Sosa et al., 2012).

From the genomic point of view, the zebrafish model is particularly amenable to studying human cardiac development and disease. For example, there is striking similarity between zebrafish and human genomes: 70% of protein-coding human genes and 84% of genes associated with human disease have an analog in zebrafish (Howe et al., 2013). Also, the transcription programs that direct cardiogenesis and maintenance of cardiac physiology are conserved between zebrafish and humans. Furthermore, mutations in genes encoding transcription factors and cell signaling molecules in zebrafish have been linked to CHD in humans. For example,

the human transcription factors: GATA4/5/6, NKX2.5, TBX5, HAND2; the human cell signaling molecules and growth factors: NOTCH1, SMAD6, FLK1, SEMA3 are also important for heart development in zebrafish (Brown et al., 2016). Indeed, because of the high degree of conservation between zebrafish and humans, the structural and functional defects that cause human heart disease also lead to the same defects in heart size, shape, or function in the zebrafish model. Lastly, despite the zebrafish heart contains only two chambers (atrium and ventricle) compared to the human heart, both human and fish hearts are assembled using comparable cell types and both function in similar manner. Thus zebrafish has rapidly become the organism of choice to study the effect of gene variants in human heart disease.

Thanks to the rapid embryonic development of zebrafish, the embryonic heart starts contracting just 24 h postfertilization, and is developmentally equivalent to a 3-week-old human heart. Furthermore, zebrafish and human share a similar cardiac physiology. Though, the optical transparency of zebrafish embryos provides arguably the greatest advantage to study cardiac development. For example, structures such as blood vessels are easily seen using a light microscope. Also the optical clarity of zebrafish embryos allows the use of tissue-specific transgenic reporters in which cells are labeled with fluorescent protein markers, such as GFP (Lawson and Weinstein, 2002; Shafizadeh et al., 2002). Zebrafish reporter lines that label specifically the endocardium, myocardium, or epicardium are readily available in the community (Poon et al., 2010). This allows for defects to be directly observed in live zebrafish embryos. Importantly, even the most severe cardiovascular defects, incompatible with life in other model organisms, can be studied in zebrafish, as embryos receive oxygen by diffusion and do not require an active blood flow during the first week of life (Bakkers, 2011; Pelster and Burggren, 1996).

The ability to produce transgenic zebrafish lines is a further attraction to use this organism to model human CHD. For example, transgenic zebrafish have been generated to overexpress defective candidate proteins linked to human cardiac diseases. Huttner et al. (2013) established a transgenic zebrafish line that stably expresses a human arrhythmogenic SCNA5A channel variant. Phenotypically, these modified zebrafish show bradycardia and conduction abnormalities. This approach of expressing a human protein in zebrafish further highlights the power of using zebrafish as a means to study human CHD in vivo.

There is also an outstanding record of unbiased forward genetic screens that have taken advantage of these zebrafish features to make significant contributions to our understanding of heart tube formation (Alexander et al., 1998; Chen et al., 1996; Stainier et al., 1996) and cardiac function (Chen et al., 1996; Stainier et al., 1996; Warren et al., 2000). More recently, targeted knockdown with morpholinos (MOs) (Thienpont et al., 2010) or mRNA overexpression (Smith et al., 2009) has been used to validate mutations found in human patients and uncover the molecular mechanisms that underlie cardiac development and disease.

Cofunctional Networks to Identify Candidate Disease-Causing Genes

As alluded to above, recently, several large-scale population studies coupled to NGS have provided evidence indicating that a significant subset of these identified gene variants were in fact either polymorphisms or are also present in unaffected individuals. This notion was further supported by the fact that disease-relevant phenotypes were not observed following knockdown of the predicted causal gene variants. Despite this, such variant-driven approaches have been pivotal in the identification of a number of genes potentially implicated in CHD. However to increase the stringency with which candidate genes are identified, cofunctional networks can be used. A cofunctional network is a way to represent sequencing data as gene functional information (van Dam et al., 2017). This is a proven platform to study gene functions as most genes exert their function through their molecular interactions. These networks are assembled by the transfer of functional gene annotations based on the "closeness" of genes. Recently, applying network-based gene prioritization approaches has identified many disease-causing genes. The idea involves the identification of candidate genes based on "relatedness" to a gene variant with a disease annotation. Ultimately this allows for novel predicted functions to be assigned to genes. Generally these cofunctional networks are achieved by either guilt-by-association (GBA) or guilt-by-profiling (GBP) approaches. GBA works on the principle that genes with related functions are connected by genetic or physical interactions, while GBP exploits the relationship between function and other gene characteristics.

Musso et al. (2014) combined GBA and GBP approaches for the de novo discovery of gene functions implicated in CHD. This method, accurately predicted known mutant phenotypes, suggesting robustness in the approach taken. Using targeted MO knockdown, Musso et al. (2014) validated 16 high-confidence cardiac predictions in zebrafish embryos using transient knockdown mediated by MOs, observing a significant enrichment for cardiac phenotypes as compared to controls. Notably, they identify *tmem88a* as an effector of the intercellular coupling pattern in zebrafish cardiac epithelium.

Zebrafish combines the amenability and wide extensive genetic toolkit typically available in invertebrate model organisms like *C. elegans* and *D. melanogaster* with a developmental ontology closer to humans as both are vertebrates. This genomic similarity between zebrafish and human was recently exploited by Shim et al. (2016) to compile a cofunctional network of zebrafish genes as an integrated genomics resource. They selected a total of 18,971 coding genes, with reliable Ensembl annotation, for network modeling. The networks were established from 18 genomics datasets using a Bayesian statistical framework in a database named DanioNet (http://www.inetbio.org/danionet/) (Shim et al., 2016). DanioNet can be used as a means to predict pathway/phenotype information for a given gene, or to predict particular pathway/phenotypes affected by new genes. For example, pathway/phenotype information can be predicted for a gene of interest by analyzing pathway/phenotype annotations of network neighbors. Notably, information gained from DanioNet can easily be extended to humans as human orthologs of zebrafish genes are also included. Future updates of DanioNet will include the compilation of RNA-seq datasets, which will provide gene expression information. This will provide a useful resource for the identification candidate genes implicated in CHD.

Genetic Toolkit to Validate the Causal Link Between Candidate Genes and Disease in Zebrafish

Overexpression

To uncover CHD causative genes embedded in de novo CNVs or to test the role of a putative dominant negative mutant, transient overexpression and monitoring of cardiac development is required (**Fig. 2**). Transient overexpression is easily achieved in zebrafish by injecting an in vitro transcribed mRNA (in the range of 100 pg per embryo) encoding the protein of interest into one-cell stage embryos. Due to the reductive nature of embryonic cell divisions, any product injected at one-cell stage will be present in all cells of the embryo. While this feature will ensure a robust overexpression of the candidate gene, in some cases it may impede expression of test genes that require precise tissue specificity or have other gain-of-function when expressed ectopically.

Using the approach of overexpressing a mutant gene variant in zebrafish embryos, Smith et al. (2009) identified a dominant negative mutation of the bone morphogenetic protein receptor (ALK2) being responsible for a particular CHD. More specifically, Smith et al. (2009) identified 11 SNPs in genes involved in atrioventricular septum development. These SNPs were expected to detrimentally affect protein function and therefore present high-confidence candidates as drivers of atrioventricular septum defects. Overexpression of the mutant form of ALK2, by microinjection in zebrafish, induced improper atrioventricular canal formation. In this way, Smith et al. (2009) were able to demonstrate the casual link between a mutant variant of ALK2 and CHD.

Morpholinos

Traditionally, loss-of-function mutants in zebrafish were achieved by microinjection of short antisense modified oligonucleotides, or MOs, in early zebrafish embryos resulting in transient knockdown of the gene of interest (**Fig. 2**) (Shestopalov and Chen, 2010; Yuan and Sun, 2009). Partial to full loss-of-function is observed leading to a hypomorphic phenotype in a reverse genetics approach. Mechanistically, MOs function by either binding to the complementary sequence surrounding the first translated ATG to block mRNA translation, or by binding to splice or donor sites to block precursor mRNA splicing.

MOs have been used successfully to faithfully identify genes implicated in CHD. For example, MO knockdown of *nphp4* gene in zebrafish was linked to a fault in the looping of the heart. This was further supported by partial rescue of the mutant phenotype following injection of wild type but not mutant gene variants of NPHP4 in zebrafish (French et al., 2012).

Wang et al. (2013) identified that loss of fatty acid-binding protein 3 (FABP3) causes CHD through MO knockout in zebrafish. Loss of FABP3 resulted in multiple phenotypes including a linear heart tube, incomplete cardiac loop formation, and abnormal positioning of the ventricles and atria. FABP3-MO injected zebrafish (morphants) also exhibited delayed development, down-regulated expression of cardiac-specific markers, and decreased heart rate. The authors noted the increased expression of the retinoic acid (RA) catabolizing enzyme Cyp26a1 in FABP3-MO zebrafish. However, there was no significant change in levels of

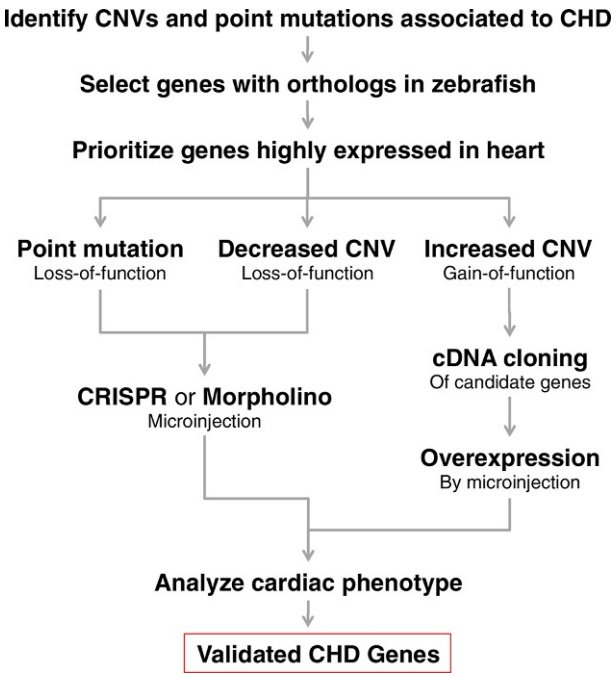

Fig. 2 A schematic outlining the methods to validate candidate CNVs and point mutations associated with CHD. Point mutations or loss-of-function CNVs can be validated by injecting CRISPRs into zebrafish embryos to knockout the gene of interest and observing the resulting phenotype. Gain-of-function CNVs can be validated with overexpression studies by injecting the mRNA of interest into zebrafish embryos and observing the resulting phenotype.

the RA synthesizing enzyme Raldh2 between mutant and wild-type zebrafish. That is, FABP3 is a critical regulator of RA signaling and is required for proper cardiac development. Ultimately, this study identifies that the loss of FABP3 underpins the etiology of CHD.

More recently, Schuster et al. (2015) showed that by injecting MOs against the cohesin protein Rad21 into zebrafish, similar types of heart defects were observed to Cornelia de Lange Syndrome (CdLS) affected patients. CdLS is a rare developmental syndrome that is manifested as both physical and cognitive defects, with up to 70% of patients having CHD (Chatfield et al., 2012). Broadly, mutations in the subunits or regulators of the cohesin proteins are responsible for CdLS. As the morphant zebrafish embryos only showed evidence of heart structural abnormalities, this suggested that the observed phenotype was caused by knockdown of Rad21 rather than an off-target effect. Furthermore, they showed that cells involved in developing heart structures failed to migrate to the heart upon depletion of Rad21. Significantly, this study suggests that the amount of Rad21 and cohesin is a critical regulator of heart development, and that mild mutations in cohesin may cause some fraction of CHD.

The transcription factor NKX2.5 is required for cardiac differentiation (Targoff et al., 2008). NKX2.5 is the earliest marker of heart development, driving development of the ventricle over the atrium. Zebrafish embryos were injected with MOs against NKX2.5 and p53; mutants exhibited less ventricular cardiomyocytes and more atrial cardiomyocytes in comparison to wild type (Targoff et al., 2013). In humans nkx2.5 mutations are associated with congenital heart defects (Abou Hassan et al., 2015).

Despite these successful MOs examples, there is a rising concern in the zebrafish field regarding the false positive rates of MOs. This has been recently emphasized in a study by Kok et al. (2015) where they applied site-specific nucleases (zinc finger, TALENs, CRISPRs) in zebrafish targeting more than 20 genes. They find that 10 different mutant zebrafish lines do not recapitulate published MO-induced phenotypes. The authors also confirm that the absence of phenotype was not influenced by the maternal contribution or the failure to eliminate gene function. In addition, the authors compare published morphant defects with the Sanger Zebrafish Mutation Project (Kettleborough et al., 2013) and conclude that approximately 80% of morphant phenotypes were not observed in mutant embryos. Therefore, it is of the utmost importance that MOs experiments shall be complemented with careful titration and rescue. The rescue is achieved by injecting mRNA of the wild-type candidate gene with the MO-binding sites mutated to prevent repression. Only when the rescue restores the wild-type phenotype can the absence of MO-mediated toxicity or off-target effects be confirmed. This strategy is even more relevant in the case of cardiac analysis. It was shown that MOs result in the upregulation of the apoptosis factor p53 (Robu et al., 2007) and can induce a phenotype with a cardiovascular component. Thus, this off-target effect may conceal the actual phenotype, complicating the phenotype analysis. To overcome this problem, co-injection of a p53-specific MO is important (Bedell et al., 2011).

CRISPR

Genome editing using CRISPR/Cas9 system has taken the zebrafish field by landslide. Their high mutagenic efficiency (Hwang et al., 2013) coupled with the fast development of zebrafish embryos allows for phenotype screening to be performed 24–48 h postinjection of CRISPRs (Moreno-Mateos et al., 2015; Ota et al., 2014; Shah et al., 2015).

CRISPRs were first noted in 2005 as a bacterial host defense system against viruses (Mojica et al., 2005). However it was not until 2010 when Cas9 was shown to cleave DNA. Garneau et al. (2010) demonstrated that CRISPR–Cas9 creates double-stranded breaks in target DNA. More specifically, they confirm that Cas9 protein is required for cleavage and has a domain that binds to a short DNA sequence termed the protospacer adjacent motif (PAM). Cas9 cleaves at precisely three nucleotides upstream of the PAM sequence. It was then in 2013 that CRISPR–Cas9 was successfully adapted for genome editing in eukaryotic cells (Cong et al., 2013). They engineered two different Cas9 orthologs (from *S. thermophilus* and *S. pyogenes*) and showed site-specific genome editing in both human and mouse cells. Mechanistically, genome editing is accomplished by complementarity of the guide RNA to a specific DNA sequence, directing Cas9 endonuclease activity at that specific site. Currently, the CRISPR/Cas9 system is widely regarded as a specific, efficient tool to manipulate the genome.

sgRNAs can vary in their activity; thus it is critical to uncover the rules that instruct efficient targeting in vivo. To this end, several groups tested the cleavage efficiency of 122 (Gagnon et al., 2014), 168 (Varshney et al., 2015), and 1280 (Moreno-Mateos et al., 2015) sgRNAs in zebrafish embryos and established the rules that orchestrate efficient CRISPR/Cas9-mediated mutagenesis.

Gagnon et al. (2014) showed that injection of recombinant Cas9 protein precomplexed with sgRNAs increased the mutagenic efficiency. In addition, they tested the strategy of knocking in an oligonucleotide with multiple stop codons in all three reading frames to avoid nonframe shifting recurrent repairs and ensure protein truncations. Varshney et al. (2015) targeted 168 loci with a stunning efficiency of 99%. However, only 28% show germ line transmission to the next generation. Moreno-Mateos et al. (2015) determined the characteristics of stability, activity, and loading into Cas9 in vivo for 1280 sgRNA. They observed that sgRNA stability and activity were increased with guanine enrichment and adenine depletion. Furthermore, they found that differential sgRNA loading, nucleosome positioning, and Cas9 off-target binding did not influence sgRNA stability and activity. Based on these observations they developed a predictive sgRNA-scoring algorithm, CRISPRscan, that allows the user to design sgRNAs in a host of organisms including zebrafish. More recently, Varshney et al. (2016) complied CRISPR targets derived from a large-scale mutagenesis project and published validated targets into a database, CRISPRz (https://research.nhgri.nih.gov/CRISPRz/?mode=search).

Regarding the specific use of CRISPR/Cas9 to study zebrafish development, Shah et al. (2015) used engineered CRISPRs to efficiently knockout genes of interest to identify genes that affect synaptogenesis during zebrafish development. They achieved high mutagenic activity by injecting a codon-optimized Cas9 mRNA with two nuclear localization sequences (Jao et al., 2013). Significantly, their high levels of biallelic disruption enabled the phenotyping of the CRISPR-injected embryos (F0 screening)

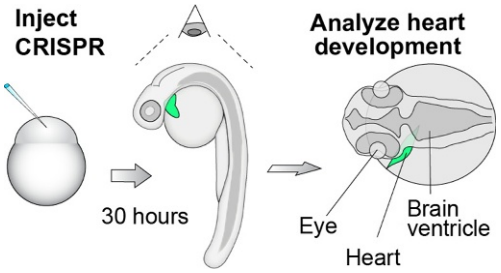

Fig. 3 Cartoon that illustrates the experimental strategy to manipulate gene expression in zebrafish. CRISPRs are injected in one-cell stage zebrafish embryos. 30 h postinjection, cardiac phenotypes are already visible.

within 48 h postinjection (**Fig. 3**). These results demonstrate that phenotype analyses can be performed in as little as 24–48 h postinjection.

The definitive proof of principle that F0 screenings can tackle CHD in zebrafish was delivered by Ota et al. (2014). The authors injected sgRNAs directed against s1pr2 (sphingosine 1′phosphate receptor 2) whose zebrafish mutant *miles apart* manifests its characteristic *spina bifida* phenotype caused by the failure of the cardiac precursor cells to fuse in the midline after migrating from the anterior lateral plate mesoderm (Kupperman et al., 2000). One day after injection of the CRISPRs, the authors were able to observe the *cardia bifida* in the CRISPR-injected embryos even though they were chimeric for the mutation (**Figs. 2** and **3**). These results provide evidence that accurate sgRNA selection and injection allows the analysis of complex cardiac phenotypes in as little as 24 h after injection. Thus, the power of CRISPRs coupled with the range of observable phenotypes in zebrafish translates into a broadly valuable approach as a means to validate candidate genes associated with many biological processes in a timely manner.

Although this approach is a rapid and powerful tool for the initial validation of CHD candidate genes, there are important caveats to note. CRISPR animals are mosaic, in part, as a result of the delay in Cas9 mRNA translation and protein folding. Thus, each CRISPR-injected animal (F0 generation) can display many different allele variants; mosaicism will inevitably complicate genotyping and phenotyping analyses. Furthermore, it is expected that one-third of indels would be in frame and therefore potentially hypomorphic or silent. To confirm the phenotype of CRISPR-injected F0 embryos, they should be rescued by co-injection of the wild-type mRNA. In addition, the mutant fish obtained from these screenings can be raised to adulthood to establish a mutant line to study, in detail, the molecular mechanisms driving cardiac disease.

Finally, these mutant zebrafish lines provide an invaluable resource to the community, as they have the potential to be used in small molecule screenings to uncover novel drugs. Peterson et al. (2004) screened over 5000 small molecules for their ability to rescue the hey2 zebrafish mutant, which exhibit malformation of the aorta and is a model for human aortic coarctation. They were successful in isolating two compounds that were able to restore blood flow through the aorta. This highlights the potential of zebrafish to not only discover mutations that are causative of the CHD but also to uncover novel therapies that would ameliorate the burden of CHD in the affected children.

Conclusions

Zebrafish is emerging as a model organism of choice to understand the etiology of human CHD. The genetic underpinnings of heart development in zebrafish are highly similar to that in humans, while zebrafish presents many advantages that allow for rapid screening of candidate CHD-associated gene variants. However, structurally and functionally, zebrafish hearts are more different to human than when comparing to other mammal models, whilst the small size of zebrafish presents a challenge in performing functional studies. Despite these limitations, the advancement of genome editing technologies and their application in zebrafish have provided an avenue to effectively study the causal link between mutant gene variants and various cardiovascular diseases, especially CHD.

Fundamental processes associated with heart functions appear to be conserved in zebrafish. This strongly supports the use of zebrafish as a model to study cardiovascular disease. Indeed, zebrafish has been successfully used to study CHD, but other emerging zebrafish models are beginning to answer fundamental questions in atherosclerosis (e.g., Fang et al., 2014), cardiomyopathy (e.g., Hassel et al., 2009) and arrhythmias (e.g., Milan et al., 2003).

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Further Reading

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